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Discovery of selective diacylglycerol lipase β inhibitors

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Discovery of selective diacylglycerol lipase β inhibitors

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There is only one heroism in the world: to see the world as it is and to love it.

Romain Rolland

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Chapter 1

General introduction

Zhu, N., Janssen, A.P.A. & van der Stelt, M. Understanding and Targeting the Endocannabinoid System with Activity-Based Protein Profiling. *Isr. J. Chem.* **63**, e202200115 (2023).

In 1995, Cravatt and colleagues reported the identification of a sleep-inducing lipid, oleamide (*N*-oleoylamine), which was isolated from the cerebrospinal fluid of sleep-deprived cats.[1] Oleamide was found to be rapidly hydrolyzed to oleic acid by an unidentified membrane-bound enzyme, whose activity was completely inhibited by phenylmethylsulfonyl fluoride (PMSF, **3**). They used this information to isolate the enzyme by making use of affinity beads modified with a covalent reversible inhibitor to purify the enzyme from rat liver plasma membranes, followed by amino acid sequencing and genomic analysis.[2] The enzyme identified was fatty-acid amide hydrolase (FAAH). FAAH also proved to be responsible for the hydrolysis of the amide bond of the endocannabinoid *N*-arachidonoylethanolamine (AEA, anandamide)[3], which is an endogenous agonist of the cannabinoid receptors type 1 and 2 (CB_1R and CB_2R). FAAH thereby effectively terminates the signaling of AEA. FAAH belongs to the enzyme family of serine hydrolases, which employ a catalytic serine residue to hydrolyze their substrates. A large number of serine hydrolases had been identified, but the biological function of many members was unknown due to the lack of specific chemical tools. Inhibitors containing fluorophosphonates (FP) were previously shown to be highly reactive to serine hydrolases.[4,5] Inspired by this observation, Cravatt and his team designed and synthesized FP-biotin, a broad-spectrum inhibitor containing a fluorophosphonate warhead and a biotin as reporter tag, thereby creating the very first activity-based probe (ABP), to study the activity of serine hydrolases in complex biological systems.[6] FP-biotin labeled FAAH as well as other serine hydrolases in an activity-dependent manner in cell and tissue lysates by covalent binding to the nucleophilic serine residue. This seminal paper can be considered the foundation of activity-based protein profiling (ABPP) for the identification and investigation of the metabolic enzymes and their inhibitors. In follow-up studies, an avidin-based isolation method was generated which enabled the rapid and simultaneous identification of multiple FP-biotinylated serine hydrolases after trypsin digestion and mass spectrometry (MS) analysis.[7,8] These early papers showed the potential of ABPP for the efficient discovery and profiling of inhibitors for serine hydrolases. In this Chapter, we will focus on the role of ABPP in the discovery of inhibitors for the biosynthesis and metabolism of ana and 2-arachidonoylglycerol (2-AG), another endogenous agonist of CB_1R and CB_2R. We will discuss the biological effects of the inhibitors in disease models and their therapeutic applications, but first we will start with a brief introduction of the endocannabinoid system (ECS).

1.1 The endocannabinoid system

AEA and 2-AG are the two main endocannabinoids that activate CB_1R and CB_2R to modulate various biological processes, such as neurotransmission, synaptic plasticity, memory formation and learning, locomotion, mood, pain sensation and immune response.[9,10] Both endocannabinoids are produced 'on demand' from membrane phospholipids and rapidly inactivated by metabolic enzymes after actions.[11] The biosynthesis and metabolism of each endocannabinoid is mediated by multiple enzymes (Figure 1.1).[12] The endocannabinoids, their biosynthetic and metabolic enzymes and cannabinoid receptors constitute the ECS. In the first step of AEA biosynthesis, phospholipase A2 group IVE (PLA2G4E)[13] or phospholipase A1/2

acyltransferase 1-5 (PLAAT1-5)[14] transfer the arachidonoyl moiety at the *sn*1 position from phosphatidylcholine (PC) to phosphatidylethanolamine (PE) to form *N*-arachidonoyl-phosphatidylethanolamine. PLA2G4E is a Ca^{2+}-dependent serine hydrolase, whereas PLAAT1-5 are cysteine hydrolases. Subsequently, *N*-arachidonoyl-phosphatidylethanolamine is hydrolyzed to AEA by *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD)[15] in one step or by the combined actions of other enzymes, including α/β-hydrolase domain-containing 4 (ABHD4)[16], GDE1[17], GDE4[18] or GDE7.[19] AEA is degraded to arachidonic acid (AA) and ethanolamine by FAAH[2] and to minor extent by *N*-acylethanolamine acid amidase (NAAA).[20] The biosynthesis of 2-AG from phosphatidylinositol-4,5-biphosphate (PIP_2) is mediated by the consecutive actions of phospholipase C-β (PLCβ) and *sn*-1 specific diacylglycerol lipase α and β (DAGLs).[21] 2-AG is metabolized to AA and glycerol, predominantly by monoacylglycerol lipase (MAGL) and to a lesser extent by ABHD6 and ABHD12.[22] Inhibitors are required to study the biological effects of these enzymes in an acute and spatiotemporal manner. In the sections below, we will discuss how ABPP enabled the discovery of highly potent and selective inhibitors for the ECS enzymes.

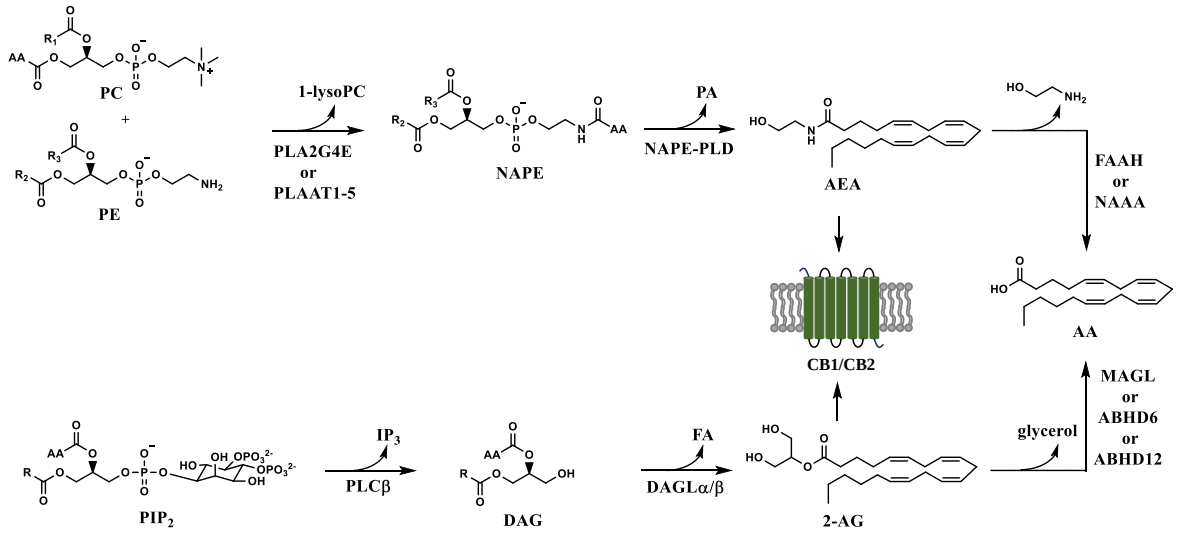


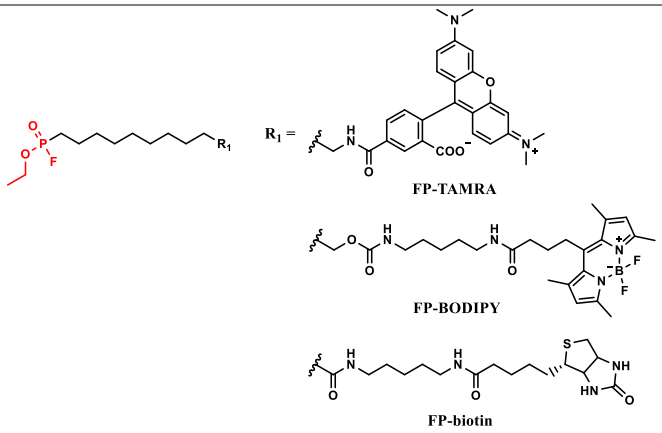
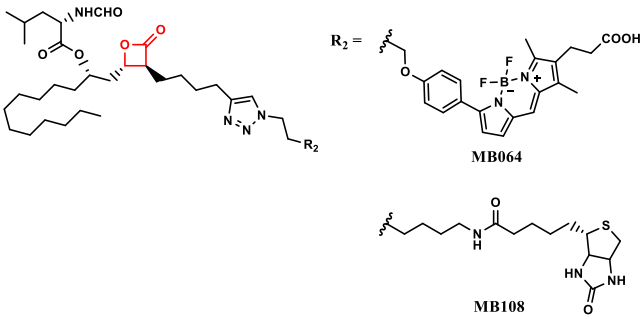
Figure 1.1 Overview of major metabolic pathways of the endocannabinoids AEA and 2-AG that activate the cannabinoid receptors type 1 and 2. PLA2G4E or PLAAT1-5 transfer the arachidonoyl group from PC to the amine of PE to produce NAPEs, which are subsequently hydrolyzed mainly by NAPE-PLD to form AEA. AEA is metabolized by FAAH and to minor extent by NAAA to AA and ethanolamine. PLCβ converts PIP_2 to diacylglycerol (DAG). DAG is hydrolyzed by DAGLs to 2-AG, which is in turn degraded by MAGL, ABHD6 or ABHD12. PLA2G4E, FAAH, DAGLs, MAGL, ABHD6 and ABHD12 are serine hydrolases.

1.2 Development of broad-spectrum ABPs for ECS enzymes

ABPs can be classified into two categories: broad-spectrum ABPs and tailored ABPs.[23] Broad-spectrum ABPs enable target identification and parallel profiling of protein families to evaluate potency and selectivity of inhibitors. Tailored ABPs, based on specific inhibitors, are ideal for identifying potential off-targets *in situ* and *in vivo*, visualizing and evaluating the localization of proteins in cells, tissues and organisms. Two types of broad-spectrum ABPs have been

developed to study serine hydrolases, i.e. FP-based probes[6,24] and β-lactone-based probes[25,26] (Table 1.1).

Table 1.1 Overview of broad-spectrum activity-based probes (ABPs) for ECS enzymes.

Chemotype	Probe	Structure	Labeling profile
Fluorophosphonates (FP)	FP-based probes	 FP-TAMRA; FP-BODIPY; FP-biotin	Serine hydrolases, including FAAH, MAGL, DAGLα, DAGLβ, ABHD6, ABHD12, PLA2G4E, CES enzymes
β-lactones	β-lactone-based probes	 MB064; MB108	Serine and cysteine hydrolases, including DAGLα/β, ABHD6, ABHD12, ABHD16a, DDHD2, PLAATs

As discussed in the introduction, FP-TAMRA[24] (also known as FP-rhodamine) and FP-biotin[6] label multiple serine hydrolases of the ECS, such as FAAH, MAGL, DAGLα, DAGLβ, ABHD6 and ABHD12. This allowed assessment of ECS inhibitor activity and selectivity in cells and brain proteomes. For example, oleoyl trifluoromethyl ketone (TFMK, **2**) was revealed to react with multiple brain membrane serine hydrolases by ABPP with FP-biotin.[7] By combining ABPP with targeted lipidomics, MAGL was demonstrated to be the most prominent 2-AG hydrolase in mouse brain followed by ABHD12 and ABHD6, accounting for ~85%, ~9% and ~4% of the total hydrolase activity respectively.[22] In addition, PLA2G4E was identified by using FP-biotin as the canonical *N*-acetyltransferase (NAT), which is responsible for the Ca^{2+}-dependent formation of NAPEs.[13] To extend the chemical toolbox for serine hydrolases, Janssen *et al.* synthesized and characterized a new fluorescent FP-probe, FP-BODIPY.[27] Despite containing the same FP warhead, FP-BODIPY was able to label different serine hydrolases in mouse brain lysate compared to FP-TAMRA, which may be due to its higher lipophilicity. This would promote its interaction with membrane proteins resulting in enhanced labeling of several membrane-bound serine hydrolases including DAGLα.

Next to FP-based probes, β-lactone-based probes MB064[25] and MB108[26], have been used to label serine hydrolases of the ECS. These probes were based on tetrahydrolipstatin (THL, Orlistat, **1**), which is an FDA-approved drug for the treatment of obesity and was initially reported to covalently inhibit pancreatic and gastric lipases.[28–30] In 2003, Bisogno *et al.* reported the identification of DAGLα and DAGLβ and found that they were potently inhibited by THL.[21] A series of THL derivatives was synthesized and investigated as DAGL inhibitors, which led to the discovery of OMDM-188.[31] Biochemical assays using radiolabeled natural substrates showed good selectivity of OMDM-188 for DAGL over FAAH and MAGL. However, competitive ABPP studies for THL and OMDM-188 were unavailable at that time due to the lack of broad-spectrum ABPs targeting DAGLα. To develop ABPs for DAGLα, Baggelaar *et al*. designed a fluorescent probe MB064[25] and a biotinylated probe MB108.[26] These β-lactone-based probes have a more restricted labeling profile in comparison with FP-based probes and they were proven to label the serine hydrolases ABHD6, ABHD12 and cysteine hydrolases PLAATs.[32] The selectivity of THL and OMDM-188 was evaluated in ABPP studies by using these probes and the results revealed their cross reactivity to several ABHD enzymes and phospholipase DDHD2. A probe cocktail of MB064 and FP-BODIPY was developed to visualize most ECS enzymes in tissue samples in a single experiment, which promoted the efficiency of selectivity assessments.[27] A combination of MB108 and FP-biotin was employed in label-free quantitative proteomics to assess the protein interaction landscape of DAGL inhibitor DH376 in diverse organs.[33]

1.3 Development of selective inhibitors and tailored ABPs for ECS enzymes

Early serine hydrolase inhibitors, such as THL (**1**), TFMK (**2**), PMSF (**3**) and RHC80267 (**4**), lacked selectivity required for *in vivo* investigation of FAAH, MAGL and DAGL (Figure 1.2). The discovery of highly potent and selective inhibitors has benefited greatly from the application of ABPP. Numerous inhibitors for ECS enzymes have been reported and most of them can be classified into 3 main chemotypes: α-ketoheterocycles, carbamates and ureas. There are also specific chemotypes for several ECS enzymes, such as glycine sulfonamides for DAGLs, thioureas for ABHD12, α-ketoamides for PLAATs, and pyrimidine-4-carboxamides for NAPE-PLD. In addition, various tailored ABPs have been developed based on selective inhibitors, which were used to study their selectivity across the entire proteome *in situ* and *in vivo* and to visualize target activity in cells and intact tissues. In the following sections we will discuss the various inhibitor chemotypes profiled with ABPP.

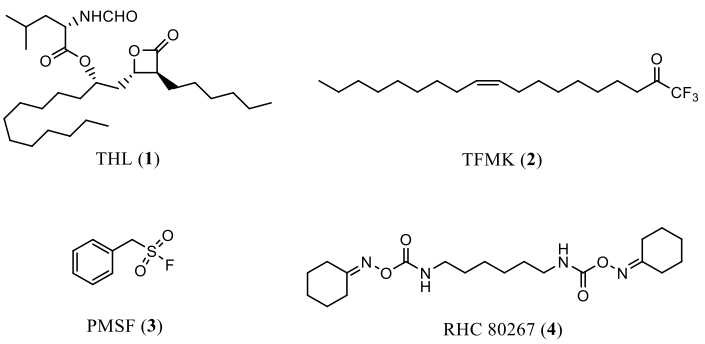


Figure 1.2 Non-selective serine hydrolase inhibitors.

1.3.1 α-ketoheterocycle-based inhibitors for FAAH and DAGL

α-ketoheterocycles were initially disclosed by Edwards *et al.* as effective protease inhibitors.[34] The electrophilic carbonyl of α-ketoheterocycles reversibly reacts with the catalytic serine to form a hemiketal intermediate. Boger *et al.* reported the development of α-ketoheterocycles as highly active FAAH inhibitors in 2000 (Figure 1.3).[35] Leung *et al.* employed ABPP with FP-based probes to assess both potency and selectivity of this chemotype for multiple serine hydrolases and identified two off-targets, namely triacylglycerol hydrolase (TGH) and neutral cholesterol ester hydrolase 1 (KIAA1363).[8] Structure-activity relationship (SAR) studies for α-ketoheterocycles afforded OL-135 (**5**) as a potent FAAH inhibitor with nanomolar activity and excellent selectivity over TGH (>10000-fold) and KIAA1363 (300-fold).[36] Moreover, OL-135 elevated brain AEA levels and promoted CB2-dependent analgesia in spinal nerve ligation model of neuropathic pain.[37] However, OL-135 showed a relatively short *in vivo* duration of action. Structural studies of FAAH with OL-135 derivatives[38–40] revealed that the adjacent Cys269 could be covalently and irreversibly trapped by a cysteine warhead to improve potency and *in vivo* half-life. Therefore, a series of OL-135 analogues (*e.g.* **6**) with different types of cysteine-targeted warheads were developed.[41,42] ABPP studies showed that the most promising compounds exhibited higher potency than OL-135 and good selectivity for FAAH over TGH, KIAA1363, MAGL and ABHD6. Moreover, these compounds increased brain *N*-acylethanolamine (NAE) levels and exhibited sustained (> 6 h) antinociceptive activity in a model of neuropathic pain.[42]

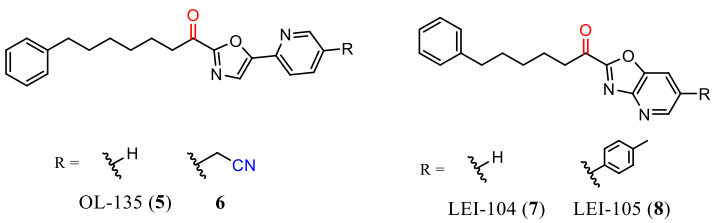


Figure 1.3 Covalent reversible FAAH and DAGL inhibitors based on α-ketoheterocycle warhead.

Using pharmacophore models, Baggelaar *et al.* discovered an α-ketoheterocycle compound, LEI-104 (**7**), an OL-135 analogue, as the first covalent reversible inhibitor for

DAGLα.[25] Competitive ABPP using MB064 and FP-TAMRA confirmed FAAH as an off-target of LEI-104. Screening a large focused library (>1000 compounds) of α-ketohetercycles established the SAR.[43] Structure-guided design led to the development of LEI-105 (**8**) as a highly potent inhibitor for DAGLα and DAGLβ.[26] Competitive ABPP using β-lactone-based probes and FP-based probes indicated that LEI-105 was more selective than LEI-104, because it did not target FAAH or any other serine hydrolase. Biological studies showed that LEI-105 dose-dependently decreased 2-AG levels without modulating AEA levels in Neuro2A cells. LEI-105 treatment significantly suppressed depolarization-induced suppression of inhibition (DSI) in mouse hippocampal slices, thereby confirming the 'on demand' model[11] of endocannabinoid production.

1.3.2 Carbamate-based inhibitors for FAAH and MAGL

Another frequently encountered chemotype in serine hydrolase inhibitors are the carbamates. For example, URB597 (**9**), based on *O*-aryl carbamate, was reported as a potent, selective and *in vivo* active FAAH inhibitor in 2003 (Figure 1.4B).[44] *In vivo* studies showed that administration of URB597 elevated brain AEA levels without modifying 2-AG levels and exerted anti-nociceptive and anxiolytic effects in animal models. Kinetics and dialysis studies indicated the irreversible interaction of URB597 with FAAH, possibly via its active serine residue. Alexander and Cravatt confirmed the carbamylation of the catalytic residue Ser241 using MS analysis (Figure 1.4A).[45] To directly identify *in vivo* targets of FAAH-directed carbamates, a click-chemistry probe JP104 (**10**) was synthesized and employed.

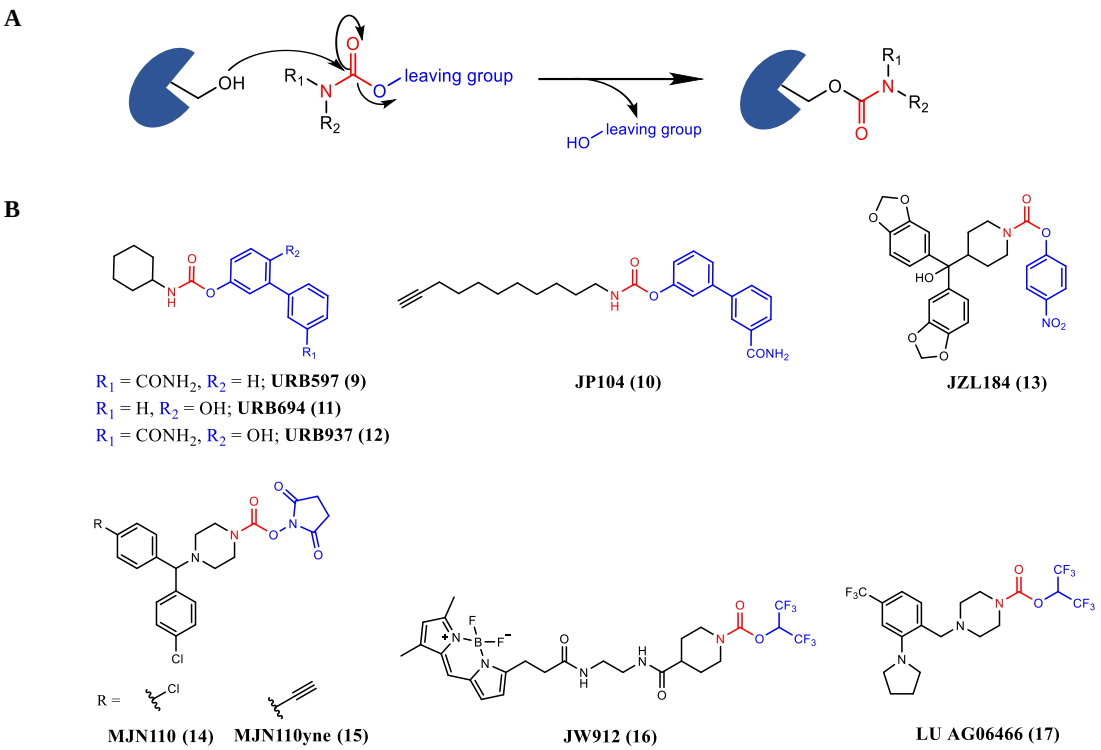


Figure 1.4 (A) Mechanism of carbamate-based serine hydrolases inhibitors. (B) Covalent irreversible inhibitors and probes based on carbamates targeting MAGL and FAAH.

ABPP studies identified FAAH as the primary target of JP104 in brain. Several carboxylesterases (CES) were identified enzymes as the off-targets in peripheral organs.[45,46] To improve selectivity and *in vivo* stability, a second generation of carbamate-based FAAH inhibitors was developed.[47] The representative compound URB694 (**11**) showed attenuated activity to CES enzymes and improved *in vivo* metabolic stability. URB937 (**12**) was peripherally restricted and exhibited antinociceptive effects in rat models of peripheral nerve injure and inflammation.[48,49]

In an extensive competitive ABPP screening of carbamate-based compounds, WWL98 was identified as an inhibitor for several serine hydrolases, including FAAH, MAGL and ABHD6.[50] SAR studies led to the development of JZL184 (**13**) as a potent, selective and brain active MAGL inhibitor.[51] *In vitro* and *in vivo* ABPP studies indicated the selectivity of JZL184 for MAGL over ~40 other serine hydrolases. JZL184 dose-dependently elevated 2-AG levels and reduced AA levels in the brain. JZL184 exhibited selectivity across different tissues, but it showed tissue-dependent effects on monoacylglycerols (MAGs) and AA levels.[52] These results indicated a more general metabolic function of MAGL. Of note, JZL184 was shown to inhibit CES in the periphery and to elevate AEA levels by inhibiting FAAH in chronic and high-dosing treatments.[52–54] In subsequent studies, the effect of the carbamate leaving group on MAGL inhibitory potency and selectivity was studied, leading to the development of KML29. This compound, with a hexafluoroisopropanol (HFIP) leaving group, exhibited good potency and improved selectivity over FAAH and CES.[54] Modification of KML29 on the staying group resulted in JW651, which was highly active and selective for MAGL with ABHD6 as the only identified off-target at high concentrations.[55] Substituting the HFIP moiety in JW651 with an *N*-hydroxylsuccinimidyl (NHS) afforded inhibitor MJN110 (**14**). To identify potential off-targets alkynylated analogues JW651yne and MJN110yne (**15**) were developed. Competitive ABPP studies indicated that both inhibitors were highly selective across the entire proteome. Probe JW912 (**16**), containing an HFIP carbamate warhead and a BODIPY fluorophore, was developed for *in situ* labeling and visualization of MAGL and ABHD6.[55] JW912 selectively labeled MAGL in H29 cells and ABHD6 in Neuro2A cells. The activity of the probe was blocked by MAGL inhibitor JW651 and ABHD6 inhibitor KT195, respectively. Based on JW651, another fluorescent probe DH463 was developed and used to visualize and quantify MAGL activity in cells at the nanoscale level by using PharmacoSTORM, a super-resolution imaging method.[56] Application of FP-TAMRA and JW912 enabled the simultaneous optimization of potency and selectivity of MAGL inhibitors in a medicinal chemistry program, which led to the discovery of Lu AG06466 (previously ABX-1431, **17**) as a highly potent, selective and orally available MAGL inhibitor.[57] A single dose of Lu AG06466 was well-tolerated and showed a positive effect for patients with Tourette syndrome in a phase 1b study.[58] However, it failed in a phase 2 clinical trial for Tourette syndrome.[59] Pfizer also disclosed a series of carbamate-based MAGL inhibitors with PF-06795071 as a representative compound containing a [3.1.0] pyrazole core system and a trifluoromethyl glycol leaving group.[60] PF-06795071 exhibited comparable potency and selectivity to inhibitors containing the HFIP leaving group but significantly increased solubility. PF-06795071 elevated brain 2-AG levels with a concomitant reduction in the levels of AA and inflammatory markers PGE_2, IL-1β and

TNF-α. No clinical trial studies have been reported for PF-06795071 to date.

1.3.3 Urea-based inhibitors for FAAH, DAGL and ABHD6

The tetrazole urea LY2183240 was initially reported as an AEA reuptake inhibitor[61], but its mechanism-of-action was later shown to be dependent on the carbamylation of FAAH's catalytic serine residue and releasing the tetrazole moiety.[62] In 2009, benzothiophene piperazine and piperidine ureas were reported as selective FAAH inhibitors and some of them showed anti-hyperalgesic effects in a rat model of inflammatory pain.[63] Janssen and Pfizer disclosed several urea-based compounds as FAAH inhibitors.[64–66] JNJ-42165279 (**18**) was highly potent and selective and exhibited beneficial effects in rat models of neuropathic pain and inflammation (Figure 1.5).[67] JNJ-42165279 was well-tolerated in both single and multiple-ascending dose clinical studies.[68,69] JNJ-42165279 (25 mg daily) elicited a moderate anxiolytic effect in subjects with social anxiety disorder and a greater effect was observed in individuals with more complete inhibition of FAAH.[70] JNJ-42165279 is currently under investigation for the treatment of post-traumatic stress disorder (PTSD) at a dose of 25 mg twice daily. PF-04457845 (**19**) was originally developed for treatment of osteoarthritis pain, but failed in phase 2 clinical trials due to a lack of efficacy.[71] In a phase 2a study of cannabis use disorder, PF-04457845 reduced symptoms of cannabis withdrawal and self-reported cannabis use in men.[72] PF-04457845 was also reported to enhance the recall of fear extinction but not to influence within-session fear extinction.[73]

In 2016, one healthy volunteer who received 50 mg per day of the FAAH inhibitor BIA 10-2474 (**20**) died in a phase 1 clinical trial due to brain damage.[74] Another five participants who received the same dose were hospitalized. This tragedy resulted in the halt of all clinical trials with FAAH inhibitors. Off-target activities of BIA 10-2474 and/or its metabolites were hypothesized to be the cause of the serious adverse events. Van Esbroeck *et al.* employed gel-based and MS-based ABPP with FP-based probes and β-lactone-based probes to reveal the protein interaction landscape of BIA 10-2474.[75] ABPP studies in human cells and human brain revealed numerous off-targets of BIA 10-2474, including FAAH2, ABHD6, ABHD11, LIPE, CTSA, PLA2G15, PNPLA6 and CES. In contrast, PF-04457845, taken along as a safe control compound, displayed good selectivity. Many of the off-targets regulate lipid metabolism. Lipidomic analysis confirmed that BIA 10-2474 altered the levels of NAEs and several other lipid classes, whereas PF-04457845 predominantly elevated the levels of NAEs. This study highlighted the application of ABPP as a versatile method to assess on-target engagement and off-target activities of covalent drugs to guide drug discovery and development processes.

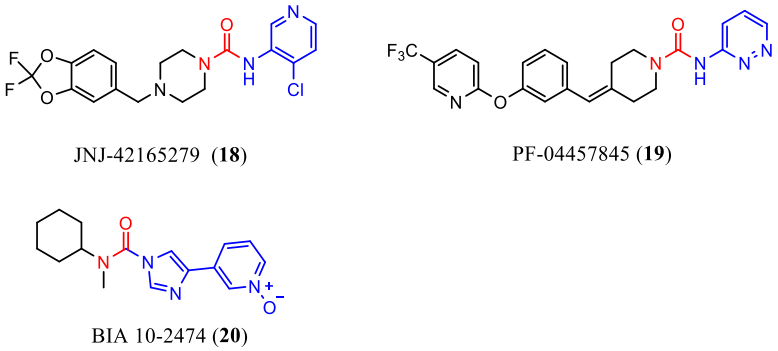


Figure 1.5 Clinical FAAH inhibitors based on a urea scaffold.

Urea-based compounds, especially 1,2,3-triazole ureas, are widely investigated as DAGL inhibitors (Figure 1.6). Initially, KT117 (**21**) was identified to inhibit DAGLβ in an ABPP-based screening of a library of thirty 1,2,3-triazole ureas by using FP-TAMRA.[76] KT116 (**22**), a 1,4-regioisomer, was 10-fold more potent than the 2,4-regioisomer KT117. However, KT116 inhibited several other serine hydrolases, including FAAH, MAGL, ABHD6, ABHD11 and PLA2G7. Lead optimization led to the discovery of two DAGL inhibitors KT109 and KT172 (**23** and **24**). Competitive ABPP studies identified ABHD6 as the common off-target of both KT109 and KT172. DAGL-tailored probe HT-01 (**25**) was generated from these 1,2,3-triazole ureas. Competitive ABPP with HT-01 using recombinant DAGLs indicated that KT109 and KT172 were 55-fold and 2-fold selective for DAGLβ over DAGLα, respectively. However, KT109 was reported to inhibit DAGLα with high potency in other assays.[77,78] KT109 and KT172, but not ABHD6 inhibitor KT195 (**26**), reduced the levels of 2-AG, AA, and prostaglandin E2 and D2 (PGE_2 and PGD_2) in macrophages and suppressed the production of proinflammatory cytokine TNFα in LPS-treated mice. Using KT109 as a starting point, DH376 and DO34 (**27** and **28**) were developed as highly potent, and centrally active dual DAGL inhibitors.[78,79] DH376 is a 2,4-substituted 1,2,3-triazole urea, which was shown to have a higher binding affinity for DAGLα than its 1,4-substituted isomer in a structure-kinetics relationship study.[80] Via a click reaction between the alkyne of DH376 and the azide of a BODIPY-based fluorophore, DH379 (**29**) was generated as another DAGL-tailored probe. DH379 labeled both DAGLα and DAGLβ in mouse brain. ABPP studies with FP-based probes, HT-01 and DH379 revealed that DH376 and DO34 dose-dependently inhibited DAGLs *in vitro* and *in vivo* with cross reactivity to ABHD6 and CES1C for both inhibitors. In addition, DO34 also inhibited PLA2G7, ABHD2, and PAFAH2. In contrast, DO53 (**30**), a control compound derived from KT195, inhibited all off-targets without affecting DAGLs activity. Interestingly, DAGLα was revealed to have a short *in vivo* half-life of 2-4 h, which indicated a tight regulation of 2-AG levels by DAGL protein synthesis and breakdown. Acute blockade of DAGL by DH376 and DO34, but not by DO53, elevated 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG) levels and reduced the levels of 2-AG, AEA, AA, PGE_2 and PGD_2. The reduction in AEA levels might suggest an *in vivo* crosstalk of AEA and 2-AG. Moreover, both inhibitors completely blocked cerebellar GABAergic (DSI) and hippocampal glutamatergic (DSE) neurotransmissions and attenuated LPS-induced neuroinflammation.

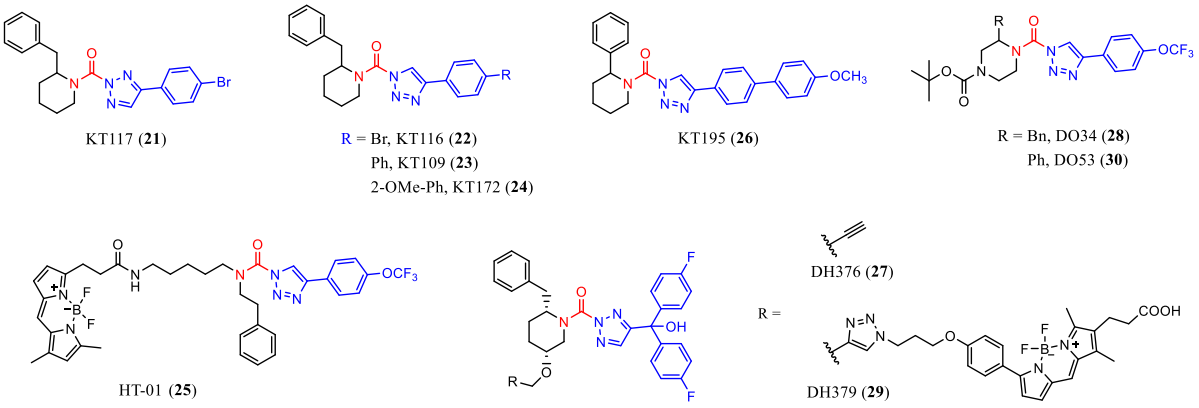


Figure 1.6 DAGL inhibitors and probes, and control compounds based on 1,2,3-triazole ureas.

KT195 was initially developed as a control compound to address the selectivity problem of KT109 and KT172, but proved to be a good ABHD6 inhibitor. Further SAR studies led to the development of KT182 (as a systemic inhibitor), KT203 (as a peripherally restricted inhibitor), and KT185 (as an orally active inhibitor).[81] Competitive ABPP with FP-TAMRA and HT-01 revealed that these three compounds were potent ABHD6 inhibitors and selective up to 1 µM. *In vivo* ABPP studies confirmed the high potency and selectivity of these inhibitors in brain and liver. KT182 was recently evaluated in rodent models of multiple sclerosis (MS), but showed limited efficacy.[82,83]

1.3.4 Specific chemotypes

1.3.4.1 Glycine sulfonamides for DAGL

Glycine sulfonamides (Figure 1.7) were identified as non-covalent DAGL inhibitors in a high-throughput screening (HTS) by researchers from Bristol-Myers Squibb.[84] SAR studies by Janssen *et al.* led to the development of LEI-106 (**31**) as a DAGLα inhibitor with good potency.[85] Gel-based ABPP with FP-TAMRA and MB064 identified ABHD6 as the off-target of LEI-106, which was further confirmed by the biochemical ABHD6 activity assay. In 2016, another extensive SAR study of glycine sulfonamides was published, in which several compounds were developed as highly potent, peripherally restricted and orally available DAGL inhibitors.[86]

1.3.4.2 Thioureas for ABHD12

Due to the limited cross-reactivity of ABHD12 to previously described inhibitors, novel chemotypes of ABHD12 inhibitors were needed. To this end, an enzyme-coupled assay for ABHD12 was used in a HTS, which led to the identification of a thiourea-based compound DO130 as a novel ABHD12 inhibitor.[87] To optimize this chemotype, JJH350[88], a tailored ABP, originating from an *N*-hydroxyhydantoin (NHH) carbamate, was employed in competitive ABPP studies to evaluate the potency of the compounds. After a series of modifications,

compound DO264 (**32**) was discovered as a highly potent ABHD12 inhibitor. Competitive ABPP studies with FP-based probes and JJH350 revealed the excellent selectivity of DO264 for ABHD12 over other serine hydrolases in either *in vitro* or *in vivo* models. DO264 elevated lyso-PS and 20:4 PS as well as the cytokines TNF-α, IL-1β, and chemokines CCL3 and CCL4 levels in human monocytic cells.[87,89] Moreover, chronic treatment of DO264 significantly increased lyso-PS and 20:4 PS contents and heightened immunopathological responses to CLMV clone 13 (Cl13) infection in mice, which indicated an immunosuppressive function of ABHD12. In a screening of serine hydrolase inhibitors for potentiating ferroptosis, ABHD12 inhibitor DO264 was found to enhance ferroptosis death caused by a lipid peroxidase GPX4 inhibitor RSL1.[90]

1.3.4.3 α-ketoamides for PLAATs

Recently, it was discovered that β-lactone probe MB064 not only labeled serine hydrolases, but also several cysteine hydrolases, including PLAATs.[32] Using competitive ABPP with MB064, an α-ketoamide derivative was identified as a PLA2G16 (also known as PLAAT3) inhibitor in a screening of 50 lipase inhibitors. Hit optimization led to the development of LEI-110 as a potent pan-PLAAT inhibitor. ABPP studies with FP-based probes and β-lactone-based probes revealed the high selectivity of LEI-110 for PLAATs over other enzymes in mouse brain membrane and cytosol proteomes. Further SAR studies led to the development of LEI-301 (**33**) as a selective pan-PLAAT inhibitor which was more potent than LEI-110.[91] LEI-301 exhibited a good selectivity profile within the ECS with minimal affinity for CB_1R and CB_2R and no inhibition for other enzymes, including FAAH, MAGL, ABHD6, PLA2G4E, NAPE-PLD, and DAGLs. Overexpression of PLAAT2 and PLAAT5 elevated NAEs levels in U2OS cells, which was reduced by LEI-301 treatment.

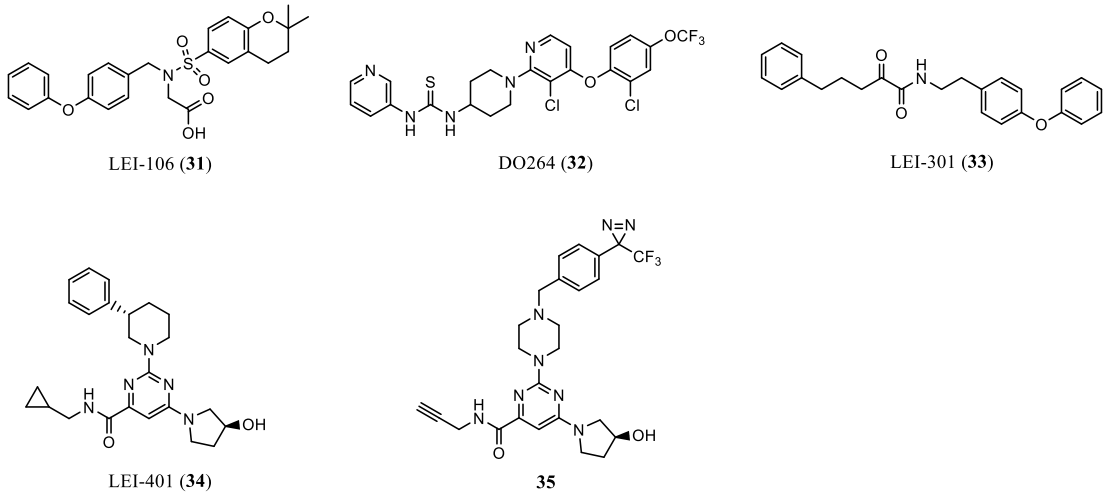


Figure 1.7 Inhibitors and probes based on specific chemotypes.

1.3.4.4 Pyrimidine-4-carboxamides for NAPE-PLD

NAPE-PLD is a metallo-β-lactamase, which was identified as the principal enzyme producing NAEs in 2004.[15] Due to the lack of a catalytic serine or cysteine residue, NAPE-PLD cannot be targeted by previously described chemotypes. Recently, a pyrimidine-4-carboxamide was identified as a novel scaffold for NAPE-PLD inhibition in a HTS using a library of ~350,000 compounds.[92,93] Hit-to-lead optimization afforded compound LEI-401 (**34**), which showed good potency and selectivity for NAPE-PLD with no inhibitory activity for other metabolic enzymes in ECS. Based on LEI-401, a click-chemistry photoprobe (**35**) was synthesized containing a UV-active trifluoromethyl phenyl diazirine and an alkynyl handle. Affinity-based protein profiling (AfBPP) studies showed that recombinant NAPE-PLD could be labeled by the probe, which was concentration-dependently inhibited by LEI-401. In MS-based AfBPP studies, LEI-401 selectively inhibited NAPE-PLD without outcompeting other proteins labeled by the photoprobe. LEI-401 reduced mouse brain NAE levels in a NAPE-PLD dependent manner. Moreover, LEI-401 activated the hypothalamus–pituitary–adrenal (HPA) axis and reduced fear extinction, which were both reversed by the FAAH inhibitor URB597.[44]

1.4 Summary

Endocannabinoids are involved in various physiological functions and disease processes. The development of selective and *in vivo* active ABPs and inhibitors for ECS enzymes was a vital step to dissect the various endocannabinoid functions and established the therapeutic potential of these proteins. Various drug discovery strategies, such as HTS, structure-based design, and *de novo* design, have been employed to identify highly potent and selective inhibitors for ECS enzymes. ABPP efficiently guided the optimization of the hits by profiling their activity and selectivity in their native biological context. ABPP coupled with advanced imaging techniques such as PharmacoSTORM and CATCH[94] enables the visualization of enzymatic activity in tissue slices at nanometer resolution. Although FAAH and MAGL inhibitors have entered clinical studies, selective inhibitors are still lacking for PLA2G4E, DAGLα and DAGLβ. In addition, the enzymes involved in the alternative NAE biosynthesis routes are understudied. Selective inhibitors for these enzymes will be valuable for elucidating their biological functions. To conclude, ABPP is a valuable chemical biology technology that aids the design and evaluation of inhibitors. The combination of ABPP with advanced imaging technologies and lipidomics leads to a better understanding of physiological and pathological processes involving serine hydrolases. The generation of novel molecular therapies based on the ECS enzymes will certainly benefit from these advances.

1.5 Aim and outline of this thesis

Selective DAGLβ inhibitors are important for elucidating the physio(patho)logical functions of this enzyme and could be potential treatment of inflammation with reduced CNS side effects mediated by DAGLα inhibition. In view of this, the aim of the research described in this thesis is to develop DAGLβ selective inhibitors for evaluating the biological functions of DAGLβ.

In **Chapter 2**, an EnzChek lipase substrate assay for DAGL is optimized and miniaturized into a 384-well plate format to screen a library of 12,587 compounds using purified catalytic domain of DAGLβ. After the primary screening, confirmed screening, deselection, and dose-response evaluation, eight hits classified into four chemotypes are obtained. Hit **1** based on glycine sulfonamide, also known as LEI-106, exhibits the highest potency for DAGLβ and favorable physiological properties for hit optimization.

In **Chapter 3**, a comprehensive structure-activity relationship of glycine sulfonamides as DAGL inhibitors is explored through systematic modifications on different parts of this chemotype. While most of compounds discussed in this Chapter demonstrate comparable potency for both DAGL isoforms or slight selectivity for DAGLα, three compounds varying on the sulfonyl substituent display some selectivity for DAGLβ. This suggests the sulfonyl group as a modification hotspot for achieving selectivity for DAGLβ.

In **Chapter 4**, an in-depth structure-activity relationship study (SAR) is conducted to enhance DAGLβ selectivity, with a focus on optimizing the sulfonyl substituent of the glycine sulfonamide chemotype. This effort leads to the identification of 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine as the optimal substituent on this position. Further optimization of potency and selectivity involves exploring other components of this chemotype, resulting in the discovery of six compounds with promising potency, selectivity and physiochemical properties. These findings position these compounds as the first-in-class DAGLβ selective inhibitors deserving further profiling and exploration.

In **Chapter 5**, a fluorescence-based plate reader assay using a GPCR activation-based endocannabinoid sensor ($GRAB_{eCB2.0}$) is established to assess the cellular activity of DAGL inhibitors. The sensor, expressed in mouse neuroblastoma cells (Neuro2A), demonstrates exquisite sensitivity to 2-AG and can be indirectly activated by adenosine triphosphate (ATP). In this assay, 23 DAGL inhibitors with diverse activities and isoform selectivities are evaluated. This profiling reveals that 2-AG production in Neuro2A upon ATP stimulation is primarily mediated by DAGLα.

In **Chapter 6**, representative DAGLβ selective inhibitors, **LEI-130** and **LEI-131**, along with a negative control compound, **LEI-132**, are profiled in *in vitro* and *in situ* studies. Mode-of-inhibition studies reveal a noncompetitive mechanism for LEI-130 and LEI-131 against DAGLβ, suggesting their binding to an allosteric pocket. *In vitro* and *in situ* ABPP studies of LEI-130 and LEI-131 confirm their activity against DAGLβ over a panel of proteins in mouse brain proteomes and living cells. Lipidomics analysis of LEI-130, LEI-131 and LEI-132 in N9 microglia and J774A.1 macrophage cells demonstrate that DAGLβ regulates the levels of 2-AG as well as its downstream lipids in a cell type-dependent manner. Furthermore, LEI-130, LEI-131 and LEI-132 all attenuate the LPS-stimulated cytokine production, suggesting the involvement of an unknown off-target in regulating this process.

In **Chapter 7**, the work presented in this thesis is summarized and new directions for future research are provided.

Reference

1. Cravatt, B. F. *et al.* Chemical Characterization of a Family of Brain Lipids That Induce Sleep. *Science* **268**, 1506–1509 (1995).
2. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
3. Devane, W. A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–9 (1992).
4. Creighton, T. E. Proteins: Structures and Molecular Properties. *W. H. Freeman* (1992). doi:10.1002/jctb.280620121.
5. Christopher T. Walsh. Enzymatic Reaction Mechanisms. *W. H. Freeman* (1979). doi:10.1093/oso/9780195122589.001.0001.
6. Liu, Y., Patricelli, M. P. & Cravatt, B. F. Activity-based protein profiling: The serine hydrolases. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14694–14699 (1999).
7. Kidd, D., Liu, Y. & Cravatt, B. F. Profiling Serine Hydrolase Activities in Complex Proteomes. *Biochemistry* **40**, 4005–4015 (2001).
8. Leung, D., Hardouin, C., Boger, D. L. & Cravatt, B. F. Discovering potent and selective reversible inhibitors of enzymes in complex proteomes. *Nat. Biotechnol.* **21**, 687–691 (2003).
9. Baggelaar, M. P., Maccarrone, M. & van der Stelt, M. 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. *Prog. Lipid Res.* **71**, 1–17 (2018).
10. Mock, E. D., Gagestein, B. & van der Stelt, M. Anandamide and other N-acylethanolamines: A class of signaling lipids with therapeutic opportunities. *Prog. Lipid Res.* **89**, 101194 (2022).
11. Alger, B. E. & Kim, J. Supply and demand for endocannabinoids. *Trends Neurosci.* **34**, 304–315 (2011).
12. Punt, J. M., van der Vliet, D. & van der Stelt, M. Chemical Probes to Control and Visualize Lipid Metabolism in the Brain. *Acc. Chem. Res.* **55**, 3205–3217 (2022).
13. Ogura, Y., Parsons, W. H., Kamat, S. S. & Cravatt, B. F. A calcium-dependent acyltransferase that produces N-acyl phosphatidylethanolamines. *Nat. Chem. Biol.* **12**, 669–671 (2016).
14. Uyama, T. *et al.* Generation of N-Acylphosphatidylethanolamine by Members of the Phospholipase A/Acyltransferase (PLA/AT) Family. *J. Biol. Chem.* **287**, 31905–31919 (2012).
15. Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T. & Ueda, N. Molecular Characterization of a Phospholipase D Generating Anandamide and Its Congeners. *J. Biol. Chem.* **279**, 5298–5305 (2004).

16. Simon, G. M. & Cravatt, B. F. Endocannabinoid Biosynthesis Proceeding through Glycerophospho-N-acyl Ethanolamine and a Role for α/β-Hydrolase 4 in This Pathway. *J. Biol. Chem.* **281**, 26465–26472 (2006).

17. Simon, G. M. & Cravatt, B. F. Anandamide Biosynthesis Catalyzed by the Phosphodiesterase GDE1 and Detection of Glycerophospho-N-acyl Ethanolamine Precursors in Mouse Brain. *J. Biol. Chem.* **283**, 9341–9349 (2008).

18. Tsuboi, K. *et al.* Glycerophosphodiesterase GDE4 as a novel lysophospholipase D: a possible involvement in bioactive N-acylethanolamine biosynthesis. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **1851**, 537–548 (2015).

19. Rahman, I. A. S. *et al.* Calcium-dependent generation of N-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **1861**, 1881–1892 (2016).

20. Sun, Y.-X. *et al.* Involvement of N-acylethanolamine-hydrolyzing acid amidase in the degradation of anandamide and other N-acylethanolamines in macrophages. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **1736**, 211–220 (2005).

21. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163**, 463–468 (2003).

22. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356 (2007).

23. Deng, H., Lei, Q., Wu, Y., He, Y. & Li, W. Activity-based protein profiling: Recent advances in medicinal chemistry. *Eur. J. Med. Chem.* **191**, 112151 (2020).

24. Patricelli, M. P., Giang, D. K., Stamp, L. M. & Burbaum, J. J. Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *Proteomics* **1**, 1067–1071 (2001).

25. Baggelaar, M. P. *et al.* Inside Back Cover: Development of an Activity-Based Probe and In Silico Design Reveal Highly Selective Inhibitors for Diacylglycerol Lipase-α in Brain. *Angew. Chem. Int. Ed.* **52**, 12081–5 (2013).

26. Baggelaar, M. P. *et al.* Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J. Am. Chem. Soc.* **137**, 8851–8857 (2015).

27. Janssen, A. P. A. *et al.* Development of a Multiplexed Activity-Based Protein Profiling Assay to Evaluate Activity of Endocannabinoid Hydrolase Inhibitors. *ACS Chem. Biol.* **13**, 2406–2413 (2018).

28. Hadváry, P., Lengsfeld, H. & Wolfer, H. Inhibition of pancreatic lipase *in vitro* by the covalent inhibitor tetrahydrolipstatin. *Biochem. J.* **256**, 357–361 (1988).

29. Hadváry, P., Sidler, W., Meister, W., Vetter, W. & Wolfer, H. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. *J. Biol. Chem.* **266**, 2021–2027 (1991).

30. Gargouri, Y., Chahinian, H., Moreau, H., Ransac, S. & Verger, R. Inactivation of pancreatic and gastric lipases by THL and C12:0-TNB: a kinetic study with emulsified tributyrin. *Biochim. Biophys. Acta, Mol. Lipids Lipid Metab.* **1085**, 322–328 (1991).

31. Ortar, G. *et al.* Tetrahydrolipstatin Analogues as Modulators of Endocannabinoid 2-Arachidonoylglycerol Metabolism. *J. Med. Chem.* **51**, 6970–6979 (2008).

32. Zhou, J. *et al.* Activity-Based Protein Profiling Identifies α-Ketoamides as Inhibitors for Phospholipase A2 Group XVI. *ACS Chem. Biol.* **14**, 164–169 (2019).

33. van Rooden, E. J. *et al.* Mapping in vivo target interaction profiles of covalent inhibitors using chemical proteomics with label-free quantification. *Nat. Protoc.* **13**, 752–767 (2018).

34. Edwards, P. D. *et al.* Design, synthesis, and kinetic evaluation of a unique class of elastase inhibitors, the peptidyl .alpha.-ketobenzoxazoles, and the x-ray crystal structure of the covalent complex between porcine pancreatic elastase and Ac-Ala-Pro-Val-2-benzoxazole. *J. Am. Chem. Soc.* **114**, 1854–1863 (1992).

35. Boger, D. L. *et al.* Exceptionally potent inhibitors of fatty acid amide hydrolase: The enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 5044–5049 (2000).

36. Boger, D. L. *et al.* Discovery of a Potent, Selective, and Efficacious Class of Reversible α-Ketoheterocycle Inhibitors of Fatty Acid Amide Hydrolase Effective as Analgesics. *J. Med. Chem.* **48**, 1849–1856 (2005).

37. Chang, L. *et al.* Inhibition of fatty acid amide hydrolase produces analgesia by multiple mechanisms. *Br. J. Pharmacol.* **148**, 102–113 (2006).

38. Saghatelian, A. *et al.* Assignment of Endogenous Substrates to Enzymes by Global Metabolite Profiling. *Biochemistry* **43**, 14332–14339 (2004).

39. McKinney, M. K. & Cravatt, B. F. Structure-Based Design of a FAAH Variant That Discriminates between the N -Acyl Ethanolamine and Taurine Families of Signaling Lipids. *Biochemistry* **45**, 9016–9022 (2006).

40. Saghatelian, A., McKinney, M. K., Bandell, M., Patapoutian, A. & Cravatt, B. F. A FAAH-Regulated Class of N-Acyl Taurines That Activates TRP Ion Channels. *Biochemistry* **45**, 9007–9015 (2006).

41. Otrubova, K., Cravatt, B. F. & Boger, D. L. Design, Synthesis, and Characterization of α-Ketoheterocycles That Additionally Target the Cytosolic Port Cys269 of Fatty Acid Amide Hydrolase. *J. Med. Chem.* **57**, 1079–1089 (2014).

42. Otrubova, K. *et al.* Rational Design of Fatty Acid Amide Hydrolase Inhibitors That Act by Covalently Bonding to Two Active Site Residues. *J. Am. Chem. Soc.* **135**, 6289–6299 (2013).

43. Janssen, F. J. *et al.* Comprehensive Analysis of Structure–Activity Relationships of α-Ketoheterocycles as sn -1-Diacylglycerol Lipase α Inhibitors. *J. Med. Chem.* **58**, 9742–9753 (2015).

44. Kathuria, S. *et al.* Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* **9**, 76–81 (2003).

45. Alexander, J. P. & Cravatt, B. F. Mechanism of Carbamate Inactivation of FAAH: Implications for the Design of Covalent Inhibitors and In Vivo Functional Probes for Enzymes. *Chem. Biol.* **12**, 1179–1187 (2005).

46. Zhang, D. *et al.* Fatty acid amide hydrolase inhibitors display broad selectivity and inhibit multiple carboxylesterases as off-targets. *Neuropharmacology* **52**, 1095–1105 (2007).

47. Clapper, J. R. *et al.* A Second Generation of Carbamate-Based Fatty Acid Amide Hydrolase Inhibitors with Improved Activity in vivo. *ChemMedChem* **4**, 1505–1513 (2009).

48. Clapper, J. R. *et al.* Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nat. Neurosci.* **13**, 1265–1270 (2010).

49. Greco, R. *et al.* Characterization of the peripheral FAAH inhibitor, URB937, in animal models of acute and chronic migraine. *Neurobiol. Dis.* **147**, 105157 (2021).

50. Li, W., Blankman, J. L. & Cravatt, B. F. A Functional Proteomic Strategy to Discover Inhibitors for Uncharacterized Hydrolases. *J. Am. Chem. Soc.* **129**, 9594–9595 (2007).

51. Long, J. Z. *et al.* Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat. Chem. Biol.* **5**, 37–44 (2009).

52. Long, J. Z., Nomura, D. K. & Cravatt, B. F. Characterization of Monoacylglycerol Lipase Inhibition Reveals Differences in Central and Peripheral Endocannabinoid Metabolism. *Chem. Biol.* **16**, 744–753 (2009).

53. Schlosburg, J. E. *et al.* Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat. Neurosci.* **13**, 1113–1119 (2010).

54. Chang, J. W. *et al.* Highly Selective Inhibitors of Monoacylglycerol Lipase Bearing a Reactive Group that Is Bioisosteric with Endocannabinoid Substrates. *Chem. Biol.* **19**, 579–588 (2012).

55. Chang, J. W., Cognetta, A. B., Niphakis, M. J. & Cravatt, B. F. Proteome-Wide Reactivity Profiling Identifies Diverse Carbamate Chemotypes Tuned for Serine Hydrolase Inhibition. *ACS Chem. Biol.* **8**, 1590–1599 (2013).

56. Prokop, S. *et al.* PharmacoSTORM nanoscale pharmacology reveals cariprazine binding on Islands of Calleja granule cells. *Nat. Commun.* **12**, 6505 (2021).

57. Cisar, J. S. *et al.* Identification of ABX-1431, a Selective Inhibitor of Monoacylglycerol Lipase and Clinical Candidate for Treatment of Neurological Disorders. *J. Med. Chem.* **61**, 9062–9084 (2018).

58. Müller-Vahl, K. R. *et al.* Endocannabinoid Modulation Using Monoacylglycerol Lipase Inhibition in Tourette Syndrome: A Phase 1 Randomized, Placebo-Controlled Study. *Pharmacopsychiatry* **55**, 148–156 (2022).

59. Müller-Vahl, K. R. *et al.* Monoacylglycerol Lipase Inhibition in Tourette Syndrome: A 12-Week, Randomized, Controlled Study. *Mov. Disord.* **36**, 2413–2418 (2021).

60. McAllister, L. A. *et al.* Discovery of Trifluoromethyl Glycol Carbamates as Potent and Selective Covalent Monoacylglycerol Lipase (MAGL) Inhibitors for Treatment of Neuroinflammation. *J. Med. Chem.* **61**, 3008–3026 (2018).

61. Moore, S. A. *et al.* Identification of a high-affinity binding site involved in the transport of endocannabinoids. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 17852–17857 (2005).

62. Alexander, J. P. & Cravatt, B. F. The Putative Endocannabinoid Transport Blocker LY2183240 Is a Potent Inhibitor of FAAH and Several Other Brain Serine Hydrolases. *J. Am. Chem. Soc.* **128**, 9699–9704 (2006).

63. Johnson, D. S. *et al.* Benzothiophene piperazine and piperidine urea inhibitors of fatty acid amide hydrolase (FAAH). *Bioorg. Med. Chem. Lett.* **19**, 2865–2869 (2009).

64. Janssen Pharmaceutica N.V. US7598249B2. (2009).

65. Pfizer Inc. WO2009127949. (2009).

66. Pfizer Inc. US 2010/0113465 A1. (2010).

67. Keith, J. M. *et al.* Preclinical Characterization of the FAAH Inhibitor JNJ-42165279. *ACS Med. Chem. Lett.* **6**, 1204–1208 (2015).

68. NCT01650597. *Clinicaltrial.gov*.

69. Postnov, A. *et al.* Fatty Acid Amide Hydrolase Inhibition by JNJ-42165279: A Multiple-Ascending Dose and a Positron Emission Tomography Study in Healthy Volunteers. *Clin. Transl. Sci.* **11**, 397–404 (2018).

70. Schmidt, M. E. *et al.* The effects of inhibition of fatty acid amide hydrolase (FAAH) by JNJ-42165279 in social anxiety disorder: a double-blind, randomized, placebo-controlled proof-of-concept study. *Neuropsychopharmacology* **46**, 1004–1010 (2021).

71. Huggins, J. P., Smart, T. S., Langman, S., Taylor, L. & Young, T. An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain* **153**, 1837–1846 (2012).

72. D'Souza, D. C. *et al.* Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: a double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. *Lancet Psychiatry* **6**, 35–45 (2019).

73. Mayo, L. M. *et al.* Elevated Anandamide, Enhanced Recall of Fear Extinction, and Attenuated Stress Responses Following Inhibition of Fatty Acid Amide Hydrolase: A Randomized, Controlled Experimental Medicine Trial. *Biol. Psychiatry* **87**, 538–547 (2020).

74. Bonini, S. & Rasi, G. First-in-Human Clinical Trials — What We Can Learn from Tragic Failures. *N. Engl. J. Med.* **375**, 1788–1789 (2016).

75. van Esbroeck, A. C. M. *et al.* Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science* **356**, 1084–1087 (2017).

76. Hsu, K.-L. *et al.* DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat. Chem. Biol.* **8**, 999–1007 (2012).

77. van der Wel, T. *et al.* A natural substrate-based fluorescence assay for inhibitor screening on diacylglycerol lipase α. *J. Lipid. Res.* **56**, 927–935 (2015).

78. Deng, H. *et al.* Triazole Ureas Act as Diacylglycerol Lipase Inhibitors and Prevent Fasting-Induced Refeeding. *J. Med. Chem.* **60**, 428–440 (2017).

79. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 26–33 (2016).

80. Janssen, A. P. A. *et al.* Structure Kinetics Relationships and Molecular Dynamics Show Crucial Role for Heterocycle Leaving Group in Irreversible Diacylglycerol Lipase Inhibitors. *J. Med. Chem.* **62**, 7910–7922 (2019).

81. Hsu, K.-L. *et al.* Discovery and Optimization of Piperidyl-1,2,3-Triazole Ureas as Potent, Selective, and in Vivo-Active Inhibitors of α/β-Hydrolase Domain Containing 6 (ABHD6). *J. Med. Chem.* **56**, 8270–8279 (2013).

82. Manterola, A. *et al.* Deregulation of the endocannabinoid system and therapeutic potential of ABHD6 blockade in the cuprizone model of demyelination. *Biochem. Pharmacol.* **157**, 189–201 (2018).

83. Manterola, A. *et al.* Re-examining the potential of targeting ABHD6 in multiple sclerosis: Efficacy of systemic and peripherally restricted inhibitors in experimental autoimmune encephalomyelitis. *Neuropharmacology* **141**, 181–191 (2018).

84. Appiah, K. K. *et al.* Identification of Small Molecules That Selectively Inhibit Diacylglycerol Lipase–α Activity. *SLAS Discov.* **19**, 595–605 (2014).

85. Janssen, F. J. *et al.* Discovery of Glycine Sulfonamides as Dual Inhibitors of sn -1-Diacylglycerol Lipase α and α/β-Hydrolase Domain 6. *J. Med. Chem.* **57**, 6610–6622 (2014).

86. Chupak, L. S. *et al.* Structure activity relationship studies on chemically non-reactive glycine sulfonamide inhibitors of diacylglycerol lipase. *Bioorg. Med. Chem.* **24**, 1455–1468 (2016).

87. Ogasawara, D. *et al.* Selective blockade of the lyso-PS lipase ABHD12 stimulates immune responses in vivo. *Nat. Chem. Biol.* **14**, 1099–1108 (2018).

88. Cognetta, A. B. *et al.* Selective *N*-Hydroxyhydantoin Carbamate Inhibitors of Mammalian Serine Hydrolases. *Chem. Biol.* **22**, 928–937 (2015).

89. Ogasawara, D. *et al.* Discovery and Optimization of Selective and in Vivo Active Inhibitors of the Lysophosphatidylserine Lipase α/β-Hydrolase Domain-Containing 12 (ABHD12). *J. Med. Chem.* **62**, 1643–1656 (2019).

90. Kathman, S. G., Boshart, J., Jing, H. & Cravatt, B. F. Blockade of the Lysophosphatidylserine Lipase ABHD12 Potentiates Ferroptosis in Cancer Cells. *ACS Chem. Biol.* **15**, 871–877 (2020).

91. Zhou, J. *et al.* Structure–Activity Relationship Studies of α-Ketoamides as Inhibitors of the Phospholipase A and Acyltransferase Enzyme Family. *J. Med. Chem.* **63**, 9340–9359 (2020).

92. Mock, E. D. *et al.* Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. *Nat. Chem. Biol.* **16**, 667–675 (2020).

93. Mock, E. D. *et al.* Structure–Activity Relationship Studies of Pyrimidine-4-Carboxamides as Inhibitors of N -Acylphosphatidylethanolamine Phospholipase D. *J. Med. Chem.* **64**, 481–515 (2021).

94. Pang, Z. *et al. In situ* identification of cellular drug targets in mammalian tissue. *Cell* **185**, 1793-1805 (2022).

Chapter 2

A fluorescent surrogate substrate assay for high-throughput screening for DAGLβ

2.1 Introduction

The endocannabinoids anandamide (AEA)[1] and 2-arachidonoylglycerol (2-AG)[2] are endogenous signalling lipids that play a crucial role in human health and disease. The amount of 2-AG was found to be 170-fold higher than AEA in the brain, and its production is predominantly mediated by *sn*-1 specific diacylglycerol lipases (DAGLs).[3] There are two known isoforms of DAGL: DAGLα and DAGLβ.[4] DAGLα is the principal isoform to produce 2-AG in the central nervous system (CNS), where 2-AG functions as a retrograde messenger to activate cannabinoid receptor type 1 (CB_1R) on the presynaptic sites to inhibit neurotransmitter release on GABAergic and glutamatergic neurons.[5–7] By contrast, DAGLβ is the dominant isoform to produce 2-AG in the peripheral system during inflammation.[8,9] Selective DAGLβ inhibitors have been proposed as a potential treatment for inflammatory diseases with reduced potential for CNS mediated side effects, but they are currently lacking.[5]

Assay development is pivotal in drug discovery to identify compounds with desired activity for the drug target. A high-quality assay can alleviate the potential issues that might arise in later stages. Depending on the substrates employed in the assay, DAGL activity assays can be categorized into two groups: natural substrate-based assays and surrogate substrate-based assays. In 2003, Bisogno *et al.* developed a radiometric assay using *sn*-1-stearoyl-2-[^{14}C]arachidonoyl-glycerol ([^{14}C]-SAG) in the identification of DAGLs.[4] Although it is highly sensitive, the assay is labor intensive requiring the synthesis of the [^{14}C]-SAG substrate, lipid extraction, TLC separation, and quantification of the 2-[^{14}C]-AG product. An alternative way to avoid using radiolabeled SAG is to detect the formation of 2-AG by employing LC-MS analysis.[10] However, this method is still labor intensive. Moreover, neither of these two methods can monitor the formation of product in real-time. Inspired by the success of coupled enzyme glycerol assays for α/β-hydrolase domain ABHD6 and ABHD12[11], Van der Wel *et al.* developed a natural substrate-based fluorescence assay for DAGLα/β.[12] This assay can be used to monitor the reaction process in real-time and to determine the kinetic parameters, as well as to evaluate inhibitors for DAGL. However, the assay window for DAGLβ was narrow, which limited its application to evaluate the selectivity of DAGL inhibitors.

Surrogate substrate-based assays using either *para*-nitrophenol butyrate (PNPB)[13,14] or 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) octanoate[15] are widely used in hit identification and initial SAR studies for DAGLα due to their cost-effectiveness. The progress of the reaction can be easily detected in real-time by measuring the absorbance in PNPB assay or fluorescence in DiFMU assay. However, the chemical structures of PNPB and DiFMU-octanoate are quite different from the structure of the natural substrate DAG, which may lead to distorted results of inhibitory activity, because of their different binding affinity.[12] Moreover, the assay window is relatively limited for DAGLβ.[16]

Recently, another commercially available lipase substrate, EnzChek™ lipase substrate was employed in a DAGLβ assay and showed an improved signal window.[16] The EnzChek lipase substrate (Figure 2.1) is a fluorescence-quenched substrate composed of a glycerol-like backbone, a green fluorophore BODIPY-C12 on the *sn*1 position, and a DABCYL-C12

quencher on the *sn*2 position. DAGL cleaves the *sn*1 ester bond to release the BODIPY fluorophore, whose fluorescence can be measured in real-time. It was envisioned that the EnzChek lipase assay could provide the opportunity to screen a library of compounds in a high throughput fashion and to evaluate their DAGL selectivity. In this Chapter, the EnzChek lipase substrate assay was, therefore, optimized and adapted for a high-throughput screening using recombinant human DAGLα and DAGLβ. A library of 12,587 compound was screened, resulting in the identification of eight confirmed hits.

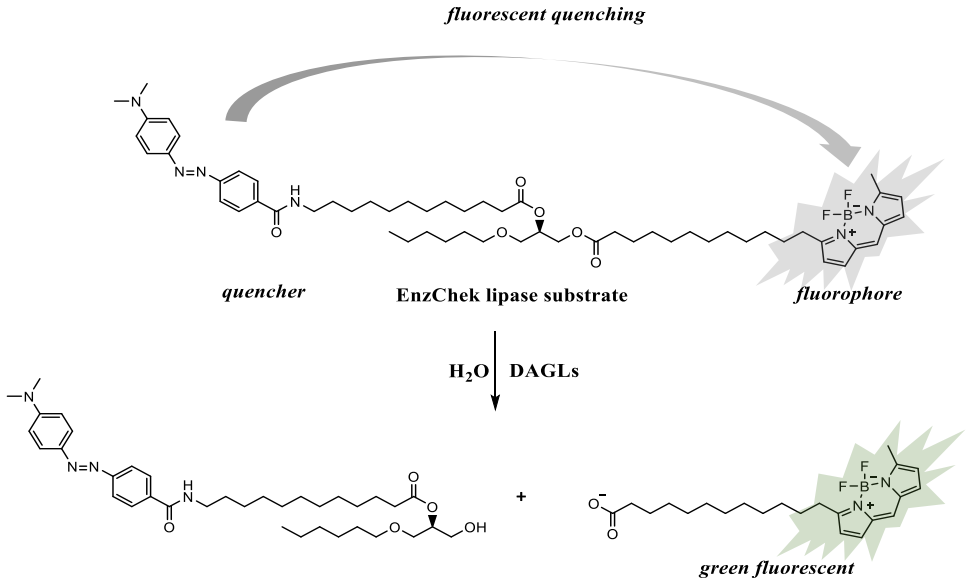


Figure 2.1 Schematic representation of the principle of EnzChek lipase substrate assay for DAGL.

2.2 Results and discussion

2.2.1 Optimization of EnzChek lipase substrate assay for DAGLα and DAGLβ

The EnzChek lipase substrate assay was adapted and optimized for recombinant human DAGLα and DAGLβ which were transiently overexpressed in human embryonic kidney (HEK293T) cells. The conditions of EnzChek lipase substrate assay were optimized in dark, flat-bottom 96-well plates. Initially, an assay buffer with 50 mM HEPES and 5% DMSO at pH 7.5 was used according to published methods.[16] It was observed that the fluorescence intensity increased in the first 5 minutes and then slowed down, resulting in the first linear part of the product-progression curve being too short to accurately calculating the initial velocity. Detergents are commonly used in biochemical assays to solubilize membrane proteins and hydrophobic substrates. DAGLα activity was previously reported to be observed in a narrow range of Triton X-100 concentrations, between 0.0075% to 0.075% in a natural substrate-based assay.[12] Therefore, diverse concentrations of Triton X-100 from 0% to 0.1% were assessed. 0.0025% Triton X-100 extended the linear part to at least 60 min for both DAGLs. This low concentration of Triton X-100 increased the overall activity of DAGLα about threefold

compared with that without Triton X-100, while the overall activity of DAGLβ remained unchanged. However, higher concentrations of Triton X-100 were intolerable, as they dramatically decreased the activity of DAGLα/β (Figure 2.2A, B). Previous studies observed that Ca^{2+} increased the activity of DAGL in a radiometric assay using substrate [^{14}C]-SAG.[4] To study the effects of cations on DAGL activity, $CaCl_2$ as well as other salts widely used in assay buffers, were investigated (Figure 2.2C, D). Ca^{2+} significantly decreased the activity of DAGLα but slightly increased the activity of DAGLβ when the concentrations of Ca^{2+} were at 5 mM and 10 mM. Mg^{2+} showed similar influence as Ca^{2+} on DAGL activities. However, monovalent ions Na^+ and K^+ had no significant influence on DAGLβ activity but decreased DAGLα activity at high concentrations of 100 mM and 150 mM. Due to the conflicting impacts on DAGL activity, no salt was added in the assay buffer. Subsequently, different protein concentrations were assessed (Figure 2.2E). The enzyme activity of DAGLα and DAGLβ increased up to 1 μg/mL and then declined. The signal-to-background (S/B) ratio was positively associated with enzyme activity. Therefore, the optimal concentration of protein used in the assay was determined to be 0.5 μg/mL for both DAGLα and DAGLβ.

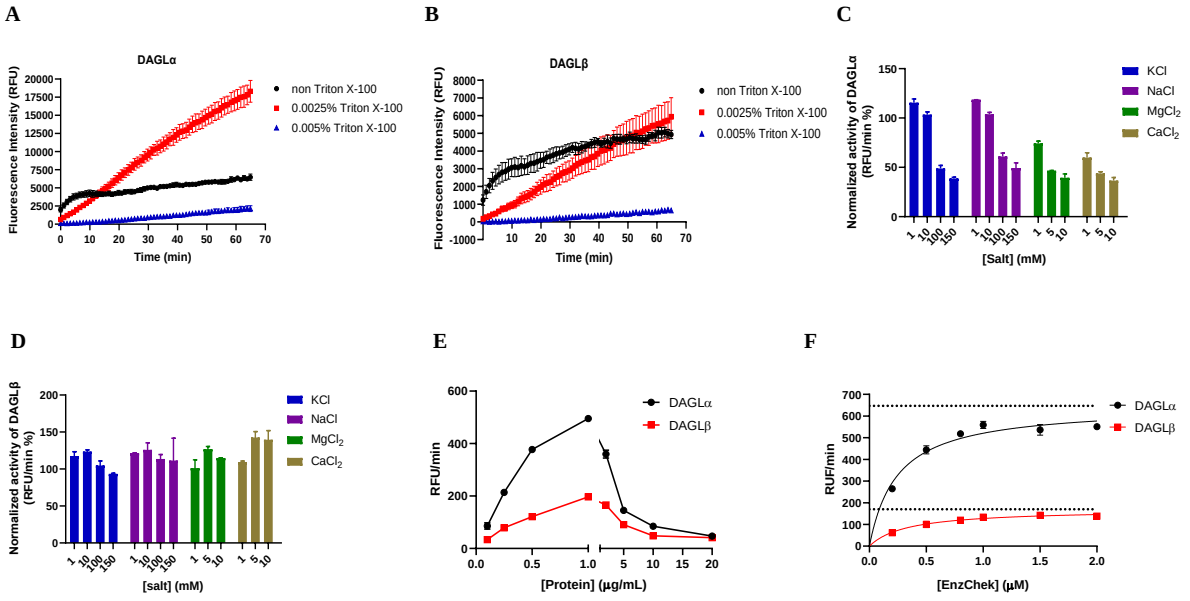


Figure 2.2 Optimization of EnzChek lipase substrate assay for DAGL. (A, B) Time course of EnzChek hydrolysis by DAGLα (A) and DAGLβ (B) as measured by fluorescence in assay buffers without and with different concentrations of Triton X-100. (C, D) The influence of salts in enzyme activity of DAGLα (C) and DAGLβ (D). (E) Rate of EnzChek hydrolysis as a function of protein concentration. (F) Michaelis-Menten kinetic curves. Rate of EnzChek hydrolysis by DAGLα (black) and DAGLβ (red) as a function of substrate concentration. All data were corrected for background fluorescence with mock membrane fraction observed at the same condition. Rates were determined in the linear interval of t = 10 min to t = 20 min. Data shown are mean ± SD (n = 2-4).

After optimizing the conditions of the assay, the Michaelis-Menten constant (K_M) was determined by measuring DAGLα/β activity at different substrate concentrations. Nonlinear regression analysis (Michaelis-Menten model) was used to calculate a V_{max} of 647 ± 38 RFU/min and a K_M of 0.24 ± 0.06 μM for DAGLα and a V_{max} of 170 ± 14 RFU/min and a K_M of 0.33 ± 0.09 μM for DAGLβ (Figure 2.2F). The EnzChek lipase substrate is highly

hydrophobic and has limited solubility in the aqueous assay buffer, leading to decreased enzyme activity when the substrate concentration exceeds 2 µM.

Subsequently, the assay was miniaturized to a dark flat-bottom 384-well plate to enhance the throughput. The concentration of EnzChek lipase substrate was increased from 0.2 µM in 96-well plate to 0.5 µM in 384-well plate, while the total assay volume was reduced from 100 µL to 30 µL. A time course of EnzChek hydrolysis was recorded for 180 min (Figure 2.3A), and similar product progression curves were observed as in 96-well plate. Endpoint measurement at 180 min, rather than kinetic measurements, were ultimately used. DAGL inhibitors, KT109[8] and DH376[7], were dose-dependently tested in the optimized assay, yielding pIC_{50} values of 8.90 ± 0.04 and 8.84 ± 0.08 for DAGLα, and 8.79 ± 0.08 and 8.66 ± 0.09 for DAGLβ, respectively (Figure 2.3B, C).

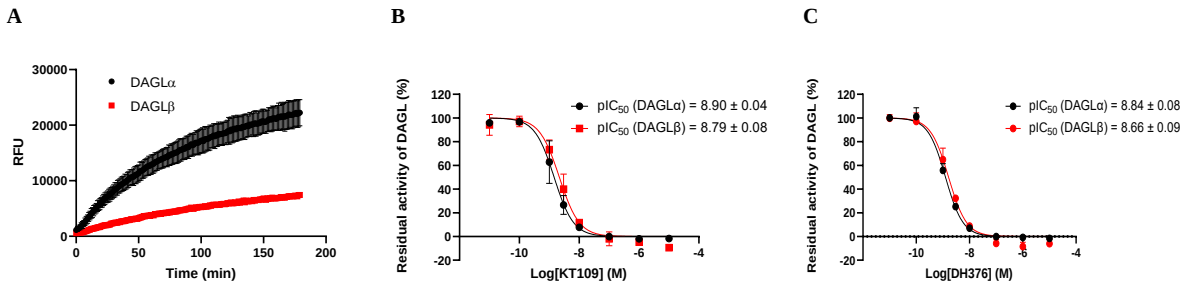


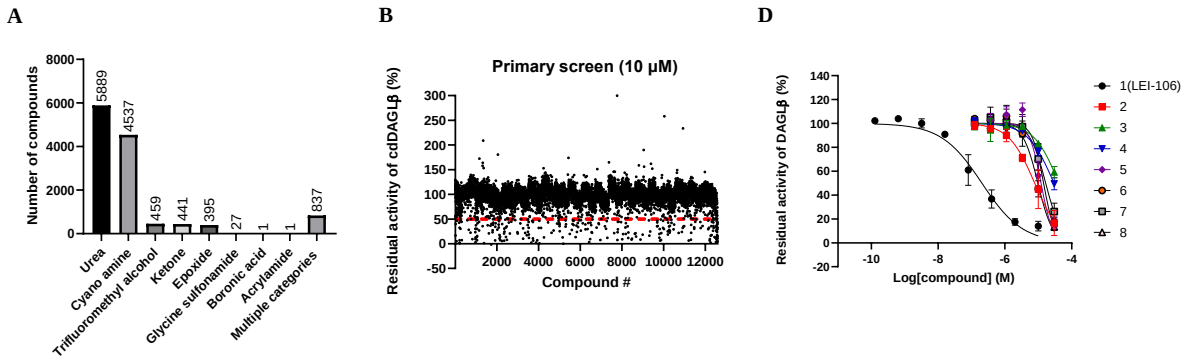
Figure 2.3 Miniaturization of DAGL EnzChek lipase substrate assay to 384-well plate format. (A). Time course of EnzChek hydrolysis by DAGL (Data shown are mean ± SD, n =3). (B, C). Dose-dependent curves of DAGL inhibitors KT109 (B) and DH376 (C) for DAGLα and DAGLβ (Data shown are mean ± SD, n = 1, N = 3).

2.2.2 High-throughput screening

EnzChek lipase substrate is commercially available but expensive. A large batch of EnzChek lipase substrate was, therefore, synthesized according to Scheme 2.1. The synthesized EnzChek lipase substrate displayed a K_M of 0.15 ± 0.05 µM for DAGLα and a K_M of 0.36 ± 0.13 µM for DAGLβ (Supplementary Figure S2.1), which was consistent with that of the commercial substrate. The S/B ratio of EnzChek lipase substrate assay for recombinant DAGLβ was around 2-3, which was not high enough for a HTS assay. Therefore, the catalytic domain (cd) of DAGLβ containing a His-MBP tag on the N-terminal (cdDAGLβ) was purified (Supplementary Figure S2.2) and applied in a HTS assay, which increased the S/B ratio more than 10-fold.

In total, 12,560 compounds from Enamine and 27 glycine sulfonamide analogs[13] synthesized previously in-house, were screened. An overview of chemotypes in the screened library is shown in Figure 2.4A. All compounds were screened at a single concentration of 10 µM in 384-well plates using 0.5 µg/mL cdDAGLβ. The concentration of EnzChek lipase substrate was adjusted to 0.25 µM, because it was sufficient for purified cdDAGLβ. The Z'-factor varied between 0.61 to 0.88, indicating a good screening quality (Z' > 0.6). Residual activity of cdDAGLβ (Figure 2.4B) was calculated for each compound, resulting in 277 primary actives showing > 50% inhibition at 10 µM, which reflects a hit rate of 2.2% (Figure 2.4C). All primary actives were screened again at 10 µM, which afforded 157 confirmed actives with a hit

rate of 1.2%. A deselection assay was performed to remove the compounds which may directly quench the fluorescence signal. EnzChek lipase substrate was incubated with cdDAGLβ to obtain the maximum fluorescence signal (after 5 h incubation), followed by adding the confirmed actives at a final concentration of 10 µM. Compounds decreasing the fluorescence signal by more than 30% were deselected, resulting in 156 remaining hits. Based on effect size, chemotype and clustering, 32 compounds were selected for dose-response determination. A list of eight hit compounds (**1**-**8**) was obtained based on potency, chemotype and the slope of dose-response curves.



C

	No. of compounds	Criterion	% Total
Total screened	12,587	-	100
Primary assay	277	> 50% inhibition at 10 µM	2.2
Confirmed assay	157	> 50% inhibition at 10 µM	1.2
Deselection assay	156	< 30% quenching at 10 µM	1.2
Selected for dose-response	32	efficacy, chemotype, clustering	0.25
Qualified hits	8	IC_{50}, hill slope	0.06

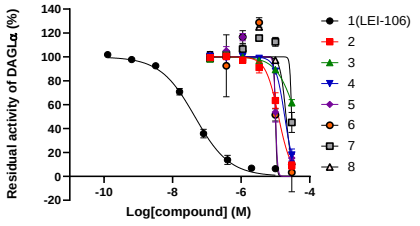


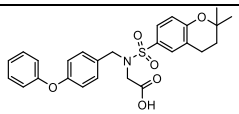
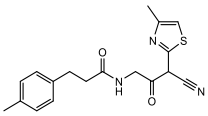
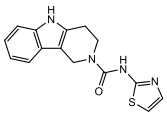
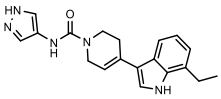
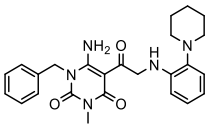
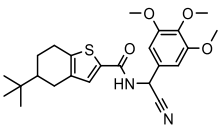
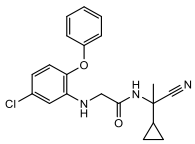
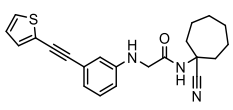
Figure 2.4 Overview of the high-throughput screening. (A) Overview of chemotypes of the screened library. (B) Residual activity of cdDAGLβ in primary screening. (C) Summary of the high-throughput screening for cdDAGLβ. (D, E) Dose-response curves of hits 1-8 for inhibiting recombinant human DAGLβ (D) and DAGLα (E) (Data shown are mean ± SD, n = 1, N = 3).

To confirm these hits, hit **1**, previously published as LEI-106[13], was resynthesized following the reported synthetic route.[13] Hit compounds **2**-**8** were purchased from Enamine. Molecular structure and purity of hits **1**-**8** were confirmed by ^{1}H NMR and HRMS. The potency of the eight hits for recombinant human DAGLβ (Figure 2.4D) and DAGLα (Figure 2.4E) were determined and summarized in Table 2.1.

Hit **1** (LEI-106), which is a glycine sulfonamide, displayed the highest potency for DAGL. It inhibited DAGL with a pIC_{50} of 6.69 ± 0.14 for DAGLβ and 7.35 ± 0.06 for DAGLα, respectively. Its potency for DAGLα in EnzChek lipase substrate assay fell within the same nanomolar range as the potency (pIC_{50} = 7.74) previously reported in a *para*-nitrophenyl butyrate (PNPB) assay[13], further confirming the reliability of EnzChek lipase substrate assay for evaluating inhibitor activity against DAGL. Hit **1** also exhibited the most optimal physicochemical properties, including molecular weight (MW), lipophilicity (cLogD) and topological polar surface area (tPSA).

Hits **2** and **5**, **3** and **4**, and **6-8** belong to the class of ketones, ureas, and cyano amides, respectively. They inhibited DAGLβ with pIC_{50} values ranging from 4.4 to 5.1. The activity of hits **5-8** for DAGLα could not be accurately determined due to the steepness of their dose-response curves, likely caused by aggregation or denaturation of the protein promoted by these compounds. Although less active compared to hit **1**, hit **2** could also be a potential starting point for developing a selective DAGLβ inhibitor in view of its potency, low molecular weight, and reasonable lipophilicity.

Table 2.1 Qualified hit list and corresponding physiochemical properties.[a]

ID	Structure	pIC_{50} (DAGLβ)	pIC_{50} (DAGL*α*)	MW (Da)	cLogP	tPSA (Å^2)	LipE (DAGLβ)	LipE (DAGL*α*)
1		6.69 ± 0.14	7.35 ± 0.06	481.56	1.9[b]	104	4.8	5.5
2		5.11 ± 0.10	4.91 ± 0.04	341.43	2.1	111	3.0	2.8
3		4.39 ± 0.10	4.38 ± 0.05	298.36	2.6	89	1.8	1.8
4		4.53 ± 0.06	4.73 ± 0.02	335.41	2.2	77	2.3	2.5
5		4.92 ± 0.09	n.d.	447.54	2.1	99	2.8	n.d.
6		5.01 ± 0.08	n.d.	442.57	4.5	109	0.5	n.d.
7		4.78 ± 0.08	n.d.	369.85	3.1	74	1.7	n.d.
8		4.84 ± 0.06	n.d.	377.51	3.9	93	0.9	n.d.

[a]The negative logarithm of half maximal inhibitory concentration (pIC_{50}) was determined with EnzChek lipase substrate assay. Molecular weight (MW), the calculated logarithm of the *n*-octanol-water partition coefficient (cLogP) and topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was calculated by formula LipE = pIC_{50} – cLogP. [b]cLogD equal to cLogP at pH 7.4 was used for Hit **1**.

2.3 Conclusion

In this Chapter, an EnzChek lipase substrate assay for DAGLα/β was successfully optimized and miniaturized into a 384-well plate format to screen a library of 12,587 compounds using purified catalytic domain of DAGLβ. This resulted in a hit list of eight compounds, which could be classified into four chemotypes, including glycine sulfonamide (hit **1**), ketones (hits **2**, **5**), ureas (hits **3**, **4**) and cyano amides (hits **6**-**8**). Hit **1**, also known as LEI-106, demonstrated the highest potency for DAGLβ and displayed favorable physiological properties. LEI-106 was, therefore, selected as a starting point for hit optimization in Chapter 3.

2.4 Acknowledgements

Dr. Hedwich Vlieg is kindly acknowledged for purifying the catalytic domain of DAGLβ. Julia Pols is acknowledged for making the running protocols and the training of Opentrons OT-2 in screening. Frans ter Brake is acknowledged for assisting in diluting the compounds in the screened library. Hans van den Elst is kindly acknowledged for HRMS measurements.

2.5 Experimental methods

Biology

Cell culture

HEK293T cells were cultured at 37 °C under 7% CO_2 in Dulbecco′s modified Eagle′s medium (DMEM) containing phenol red, Glutamax (2 mM), penicillin/streptomycin (200 µg/mL) and 10% newborn calf serum (Thermo Fischer). Cells were passaged twice a week by resuspension in fresh medium to appropriate confluence.

Transient Transfection

One day before transfection, HEK293T cells (~10^7) were seeded to 15 cm petri dishes. Before transfection, the medium was refreshed with a minimal amount of medium (13 mL). A mixture (3:1) of polyethyleneimine (PEI, 60 µg/dish) and plasmid DNA (20 µg/dish, human DAGLα-FLAG or DAGLβ-FLAG) was prepared in serum-free culture medium (2 mL/dish) and incubated at rt for 15 min. Transfection was done by dropwise addition of the PEI/DNA mixture to the cells. Transfection with the empty pcDNA3.1 vector was used for the mock control. After 24 h, the medium was refreshed and cells were harvested after 72 h by suspension in cold PBS. The suspension was centrifuged (200 g, 10 min, 4 °C) and supernatant was discarded. The cell pellets were flash frozen in liquid N_2 and stored at -80 °C until use.

Membrane preparation

Cell pellets were thawed on ice and suspended in cold lysis buffer (20 mM HEPES pH 7.2, 250 mM sucrose, 2 mM DTT, 1 mM $MgCl_2$, 2.5 U/mL Benzonase). The suspension was pipetted up and down and incubated on ice for 30 min. The cytosolic fraction and the membrane fraction were separated by ultra-centrifugation (93,000 g, 45 min, 4 °C, Beckman Coulter, Ti 70.1 rotor) or normal centrifugation (30,130 g, 3 h, 4 °C, Eppendorf Centrifuge 5430R). The pellet was washed with cold storage buffer (20 mM HEPES pH 7.2, 2 mM DTT) and suspended in cold storage buffer by pipetting through insulin needles. Protein concentrations were determined by Qubit® 2.0 fluorometer and the samples were diluted in cold storage buffer to 2.0 µg/µL. All samples were flash frozen in liquid N_2 and stored in small aliquots at -80 °C until use.

Cloning for insect cell expression and BACmid generation

For recombinant expression of the soluble DAGLβ catalytic domain with N-terminal 3C-cleavable His-MBP tags, the cDNA of human DAGLβ residues 265 to 627 was cloned into the pFastBac derived vector pCPF2.15 using the IVA cloning strategy.[17] Bacmids were generated by transforming this vector in to EmBacY cells (Geneva Biotech).

Protein expression in Sf9 cells

A P0 stock of baculovirus was generated by transfecting 8×10^5 Spodoptera frugiperda 9 (Sf9) cells with 10 µg of bacmid using CelFectin (Invitrogen) in SFM-II medium (Gibco) in a 6-well

plate. After at least 72 h of incubation at 28 °C, cell fluorescence was checked. The medium was harvested when at least 90% of cells showed YFP expression. This medium was used to generate a P1 viral stock by infecting 50 mL of Sf9 cells at 10^6 in Insect-Express medium (Lonza). The cells were incubated for 27 h at 28 °C while shaking. The medium was harvested and used to infect larger P2 cultures at 1 mL of P1 viral stock per 500 mL culture at 10^6 cells/mL. P2 cells were harvested 48-60 hours after infection by centrifugation (500 g, 10 min). Cell pellets were stored at -20 °C until use.

Purification of cdDAGLβ

The purification was performed at 4 °C. The cell pellets were resuspended in lysis buffer (25 mM HEPES pH 7.8, 300 mM NaCl, 1 mM $MgCl_2$, 2 mM DTT, 0.1% Triton X-100 and 25 U/mL Benzonase) and lysed by two cycles of freeze thaw. Lysates were cleared by centrifugation for 30 min at 15,000 g. The supernatant was incubated with amylose resin (New England Biolabs) for 1 h. Beads were washed extensively with wash buffer (25 mM HEPES pH 7.8, 300 mM NaCl, 2 mM DTT) followed by elution of the protein with elution buffer (25 mM HEPES pH 7.8, 300 mM NaCl, 2 mM DTT, 20 mM maltose and 0.025% octyl glucoside). The purified protein was concentrated to 1-2 μg/μL using a centrifugal filter (Millipore) and stored in small aliquots at -80 °C until use.

EnzChek lipase substrate assay for DAGLα and DAGLβ in 96-well plate

The membrane fractions of HEK293T cells transiently overexpressing human DAGLα or DAGLβ were diluted in assay buffer (50 mM HEPES pH 7.5, 4.5% DMSO, 0.0025% Triton X-100) to 2 μg/mL. 25 μL of protein solution was added to a black 96-well plate (Greiner Bio-One, REF 655076) and then 65 μL assay buffer was added. The membrane fraction from HEK293T cells transfected with empty pcDNA3.1 vector was used for the negative control (mock). The substrate EnzChek (1 mM in DMSO) was consecutively diluted in DMSO to 20 μM (50× dilution) and in assay buffer to 2 μM (10× dilution). 10 μL of substrate solution was added to the assay plate and the measurement was started immediately in CLARIOstar® (excitation 477-14 nm, emission 525-30 nm, gain = 1600, 60 or 72 sec/cycle for 61 cycles). The assay was performed in a final volume of 100 μL with 0.5 μg/mL protein and 0.2 μM EnzChek lipid substrate in 50 mM HEPES pH 7.5 with 5% DMSO and 0.0025% Triton X-100. Mock control was used for background subtraction. The initial linear part (t = 10 min to t = 20 min) was used to calculate the enzymatic rate (RFU/min). The assays varying in salts concentration, protein concentration and EnzChek concentration were adapted from this.

EnzChek lipase substrate assay for DAGLα and DAGLβ in 384-well plate

The membrane fractions from HEK293T cells transiently overexpressing human DAGLα and DAGLβ were diluted to 1.5 μg/mL in assay buffer 1 (50 mM HEPES pH 7.5, 5% DMSO, 0.0025% Triton X-100) and 10 μL was pipetted into a black 384-well plate (Greiner Bio-One, REF 781076). The membrane fraction from HEK293T cells transfected with empty pcDNA3.1 vector was used for the negative control (mock). Inhibitors were consecutively diluted in

DMSO to 600 µM and in assay buffer 2 (50 mM HEPES pH 7.5, 0.0025% Triton X-100) to 30 µM. A dilution series (8 concentrations) was prepared in assay buffer 1. 10 µL of inhibitor solution or assay buffer 1 was transferred to the enzyme samples in the assay plate. The plate was spun down at 1000 rpm for 1 min in Eppendorf Centrifuge 5810R and incubated at rt for 30 min. The EnzChek lipase substrate was consecutively diluted in DMSO to 30 µM and in assay buffer 2 to 1.5 µM. 10 µL of EnzChek solution (final concentration 0.5 µM) was added to each well. The plate was spun down at 1000 rpm for 1 min and incubated for 3 h at rt in the dark. The final concentrations of protein and substrate were 0.5 µg/mL and 0.5 µM, respectively. Endpoint fluorescence was measured in CLARIOstar® (excitation 477-14 nm, emission 525-30 nm, gain = 1600). DAGL and mock membrane fractions with DMSO were used as positive and negative controls respectively to calculate the windows of the assay. The mock membrane fraction with inhibitors at each concentration was used for background correction. Assay performance was assessed using Z' factor, which was calculated with the formula: $Z' = 1 - 3(\sigma_{pc} + \sigma_{nc})/(\mu_{pc} - \mu_{nc})$, where pc represents the positive control, nc represents the negative control, σ represents the standard deviation, and µ represents the mean value. Residual activity of DAGL was calculated using the equation: Residual activity (%) = $(\mu_{DAGL} - \mu_{mock})/(\mu_{pc} - \mu_{nc}) \times 100\%$, where μ_{DAGL} and μ_{mock} represent the fluorescence intensities of DAGL and mock with inhibitors, respectively. Residual activities were used to generate the dose-response curves using GraphPad Prism 9.0.0 (log(inhibitor) vs. normalized response with variable slope). All measurements were performed three times independently (n = 1, N = 3 or n = 4, N = 3 for controls, with $Z' \geq 0.6$).

EnzChek lipase substrate assay for cdDAGLβ in 384-well plate

All compounds (10 mM) in the screened library were diluted in DMSO to 300 µM, of which 1 µL was transferred to a black 384-well plate (Greiner Bio-One, REF 781076) by an Opentrons OT-2 robot. DMSO and 10 µM KT109 (n=8 for each assay plate) were added as positive and negative controls, respectively. Next, 19 µL of cdDAGLβ solution diluted in assay buffer (50 mM HEPES pH 7.5, 0.0025% Triton X-100, 0.79 µg/mL) was added. The plate was spun down at 1000 rpm for 1 min in Eppendorf Centrifuge 5810R and incubated at rt for 30 min. The EnzChek lipase substrate was consecutively diluted in DMSO to 15 µM and in assay buffer to 0.75 µM. 10 µL of EnzChek solution was added to each well. The plate was spun down at 1000 rpm for 1 min and incubated at rt for 3 h in the dark. The final concentrations of screened compound, cdDAGLβ, and EnzChek lipase substrate were 10 µM, 0.5 µg/mL, 0.25 µM, respectively. Endpoint fluorescence was measured in CLARIOstar® (excitation 477-14 nm, emission 525-30 nm, gain = 1600). Assay performance was assessed using Z' factor, which was calculated with the formula: $Z' = 1 - 3(\sigma_{pc} + \sigma_{nc})/(\mu_{pc} - \mu_{nc})$, where pc represents the positive control, nc represents the negative control, σ represents the standard deviation, and µ represents the mean value. Residual activity of DAGL was calculated via the equation: Residual activity (%) = $(\mu_{cdDAGL\beta} - \mu_{nc})/(\mu_{pc} - \mu_{nc}) \times 100\%$, where $\mu_{cdDAGL\beta}$ represents the fluorescence intensity of cdDAGLβ with inhibitors. The compounds which inhibited cdDAGLβ activity over 50% were considered as hits. The primary screening gave 277 hits with a hit rate of 2.2%. The

primary hits were screened again at 10 µM, resulting in 157 confirmed hits with a hit rate of 1.2%. For deselection, 19 µL of cdDAGLβ solution and 10 µL of EnzChek solution were incubated together at rt for 5 h in the dark before measuring the fluorescence. Subsequently, 1 µL of compound solution was added and the fluorescence was measured again. The compounds which inhibited the fluorescence over 30% were deselected, resulting in 156 remaining hits.

Chemistry

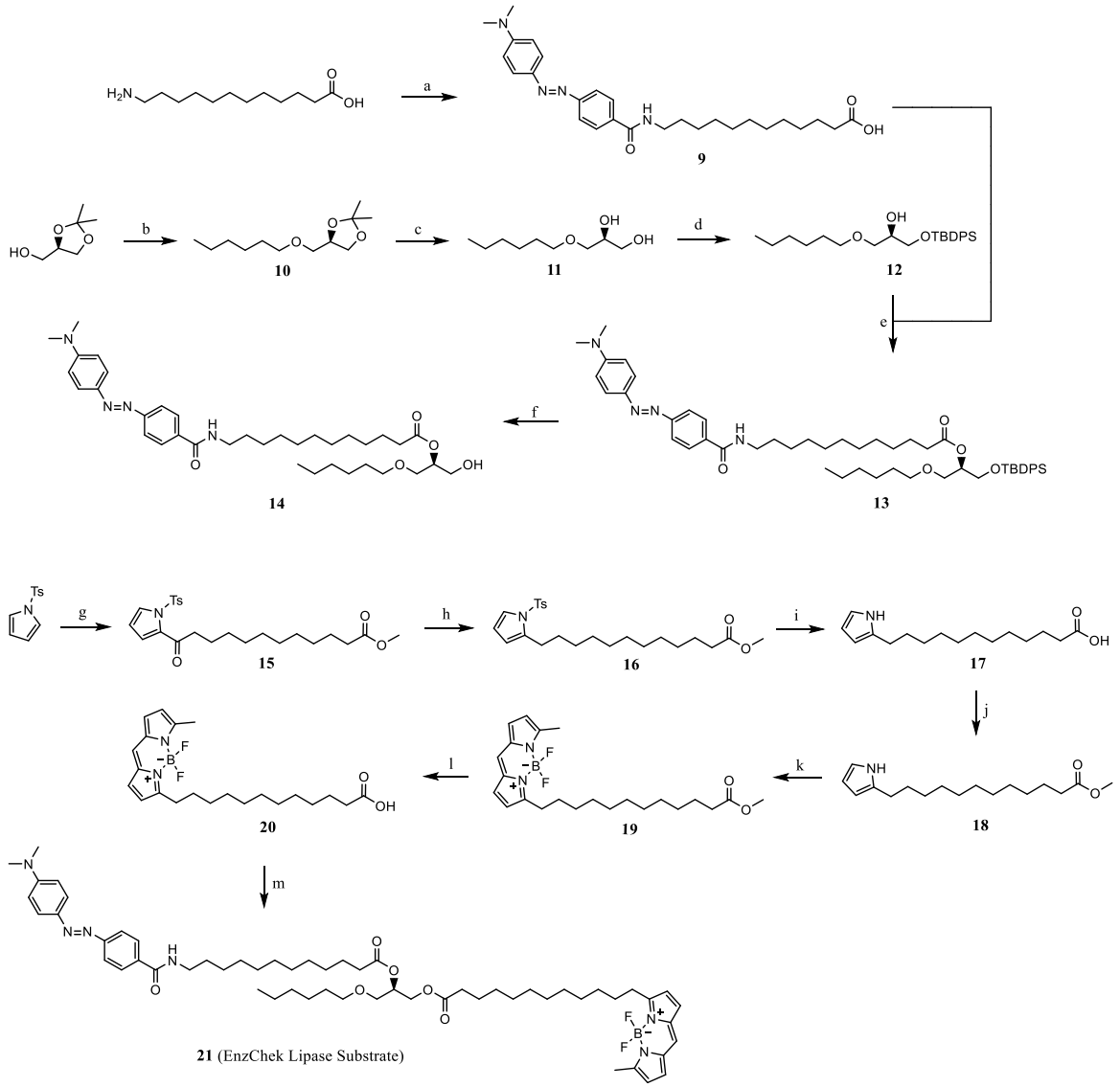
General remarks

All purchased chemicals were used without purification unless stated otherwise. All reactions were performed in oven-dried or flame-dried glassware. Anhydrous solvents were dried by activated 3 Å or 4 Å molecular sieves. Traces of water in starting materials were removed by co-evaporation with toluene if necessary. Thin layer chromatography (TLC) analysis was performed on Merck silica gel 60 F_{254} aluminium sheets and the compounds were visualized by using UV absorption at 254 nm and 366 nm and/or $KMnO_4$ staining (5 g/L $KMnO_4$ and 25 g/L K_2CO_3 in water). TLC plates were analysed with the Advion CMS Plate Express® connected to the Advion Expression® L-MS using 90% MeOH in H_2O with 0.1% formic acid as the solvent. Liquid chromatography-mass spectrometry (LC-MS) analyses were performed on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI^+) coupled to a Surveyor HPLC system equipped with a C18 column (50 × 4.6 mm, 3 µm particle size, Macherey-Nagel) or a Thermo Finnigan LCQ Fleet ion-trap mass spectrometer (ESI^+) coupled to a Vanquish UHPLC system using H_2O, CH_3CN and 0.1% aq. TFA as eluents. Purification was performed on manual silica gel column chromatography (40-63 µm, 60 Å silica gel, Macherey-Nagel) or automated silica gel column chromatography (40-63 µm, 60 Å pre-packed silica gel, Screening Devices) on a Biotage Isolera™ Four 3.0 system. ^1H and ^{13}C spectra were recorded on a Bruker AV 400 MHz (400 MHz for ^1H and 101 MHz for ^{13}C) or AV 500 MHz spectrometer (500 MHz for ^1H and 126 MHz for ^{13}C) in deuterated solvents. Chemical shifts are reported in ppm with tetramethylsilane (TMS) or solvent resonance as the internal standard ($CDCl_3$: δ 7.26 for ^1H, δ 77.16 for ^{13}C; CD_3OD: δ 3.31 for ^1H, 49.00 for ^{13}C; DMSO-*d6*: δ 2.50 for ^1H, δ 39.52 for ^{13}C). Data is reported as follows: chemical shifts δ (ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, t = triplet, td = triplet of doublets, tt = triplet of triplets, q = quartet, quintet = p, bs = broad singlet, m = multiplet), coupling constants *J* (Hz) and integration. High resolution mass spectrometry (HRMS) analysis was performed on a Thermo Finnigin LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctyl phthalate (m/z = 391.28428) as a lock mass.

Synthesis of EnzChek lipase substrate

EnzChek lipase substrate was synthesized as depicted in Scheme 2.1. Building block **9** was synthesized from dabcyl succinimidyl ester and 12-aminododecanoic acid and building block

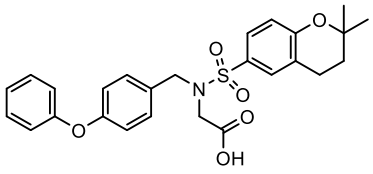
12 was obtained from (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol via sequential nucleophilic substitution (**10**), hydrolysis (**11**) and TBDPS protection[18]. Next, building block **9** was coupled to **12** to obtain ester **13**, whose TBDPS group was deprotected to gain *sn*2 ester **14**. During TBDPS deprotection, acetic acid was used to adjust the pH to avoid shifting of the *sn*2 ester bond towards the primary hydroxyl group. The synthesis of BODIPY green **20** started with the condensation of 1-tosyl-1*H*-pyrrole and 12-methoxy-12-oxododecanoic acid under the agency of TFAA[19] to obtain compound **15**. Next, the chemo-selective ketone reduction using ZnI_2 and $NaBH_3CN$[20] gained compound **16**. Intermediate **18** was obtained from **16** via sequential tosyl deprotection, saponification and esterification. **18** was condensed with 5-methyl-1*H*-pyrrole-2-carbaldehyde using $POCl_3$ and $BF_3{\cdot}Et_2O$[21] to obtain compound **19**, which was hydrolyzed under acidic condition to form bodipy **20**. Finally, dabcyl **14** and bodipy **20** were coupled to obtain EnzChek lipase substrate.



Scheme 2.1 Synthesis of EnzChek lipase substrate. Reagents and conditions: a) DABCYL NHS, DIPEA, DMF, rt; 79%; b) 1-bromohexane, NaH, DMF, rt, 71%; c) $TsOH{\cdot}H_2O$, MeOH, 45 °C, 74%; d) TBDPSCl, Et_3N, DMAP, DCM, rt, 80%; e) **9**, DIC, DMAP, DCM, rt, 51%; f) TBAF, AcOH, THF, rt, 66%; g) TFAA, 12-methoxy-12-oxododecanoic acid, DCM, reflux, 70%; h) $NaBH_3CN$, ZnI_2, DCE, 80 °C, 71%; i) 2 M aq. NaOH, MeOH, 80 °C,

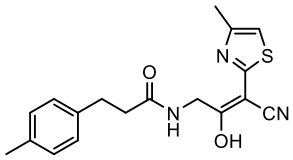
79%; j) DIC, DMAP, MeOH, DCM, rt, 81%; k) *i.* 5-methyl-1*H*-pyrrole-2-carbaldehyde, $POCl_3$, DCM, rt; *ii.* DIPEA, $BF_3 \cdot Et_2O$, rt, 64%; l) 4 M aq. HCl in THF, rt, 79%; m) **14**, DIC, DMAP, DCM, rt, 72%.

N-((2,2-Dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (1, LEI-106)



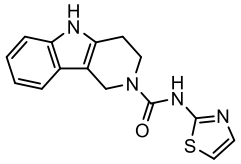
The compound was synthesized according to previously reported procedure.[13] ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.59 – 7.51 (m, 2H), 7.35 – 7.25 (m, 2H), 7.18 – 7.10 (m, 2H), 7.12 – 7.04 (m, 1H), 6.99 – 6.91 (m, 2H), 6.92 – 6.83 (m, 2H), 6.85 – 6.77 (m, 1H), 4.41 (s, 2H), 3.86 (s, 2H), 2.80 (t, *J* = 6.7 Hz, 2H), 1.82 (t, *J* = 6.8 Hz, 2H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 171.62, 158.54, 157.75, 157.31, 130.64, 130.46, 130.31, 130.22, 129.92, 127.40, 123.98, 122.03, 119.45, 119.08, 118.19, 76.25, 51.12, 47.15, 32.60, 27.01, 22.72. HRMS $[C_{26}H_{27}NO_6S+H]^+$: 482.16318 calculated, 482.16313 found.

N-(3-Cyano-3-(4-methylthiazol-2-yl)-2-oxopropyl)-3-(*p*-tolyl)propenamide (2)



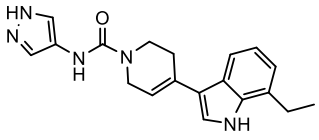
The compound was purchased from Enamine. ^{1}H NMR (500 MHz, $CDCl_3$+MeOD) δ 7.15 – 7.07 (m, 4H), 6.46 (t, *J* = 1.3 Hz, 1H), 4.35 (s, 2H), 2.98 – 2.91 (m, 2H), 2.59 – 2.54 (m, 2H), 2.36 (s, 3H), 2.31 (s, 3H). HRMS $[C_{18}H_{19}N_3O_2S+H]^+$: 342.12707 calculated, 342.12714 found.

N-(Thiazol-2-yl)-1,3,4,5-tetrahydro-2*H*-pyrido[4,3-*b*]indole-2-carboxamide (3)



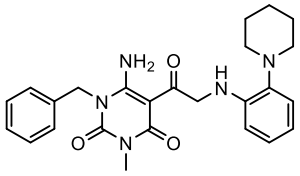
The compound was purchased from Enamine. ^{1}H NMR (500 MHz, MeOD+$CDCl_3$) δ 7.44 – 7.38 (m, 1H), 7.34 – 7.24 (m, 2H), 7.14 – 7.00 (m, 2H), 6.81 (t, *J* = 4.4 Hz, 1H), 4.75 (s, 2H), 3.92 (t, *J* = 5.5 Hz, 2H), 2.88 (t, *J* = 5.7 Hz, 2H). HRMS $[C_{15}H_{14}N_4OS+H]^+$: 299.09611 calculated, 299.09600 found.

4-(7-Ethyl-1*H*-indol-3-yl)-*N*-(1*H*-pyrazol-4-yl)-3,6-dihydropyridine-1(2*H*)-carboxamide (4)



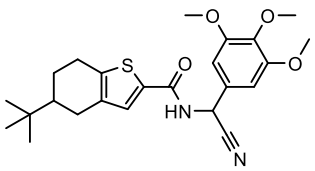
The compound was purchased from Enamine. ^{1}H NMR (500 MHz, $CDCl_3$+MeOD) δ 7.73 (d, *J* = 8.0 Hz, 1H), 7.66 (s, 2H), 7.23 (s, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.06 (d, *J* = 7.1 Hz, 1H), 6.19 (s, 1H), 4.28 – 4.15 (m, 2H), 3.77 (t, *J* = 5.5 Hz, 2H), 2.89 (q, *J* = 7.6 Hz, 2H), 2.73 – 2.56 (m, 2H), 1.37 (t, *J* = 7.5 Hz, 3H). HRMS $[C_{19}H_{21}N_5O+H]^+$: 336.18189 calculated, 336.18168 found.

6-Amino-1-benzyl-3-methyl-5-((2-(piperidin-1-yl)phenyl)glycyl)pyrimidine-2,4(1*H*,3*H*)-dione (5)



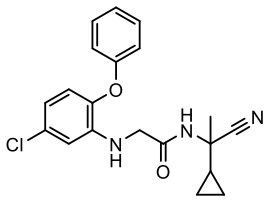
The compound was purchased from Enamine. ^{1}H NMR (500 MHz, $CDCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.39 (m, 2H), 7.39 – 7.34 (m, 1H), 7.29 – 7.25 (m, 4H), 7.02 – 6.95 (m, 2H), 6.70 – 6.63 (m, 2H), 5.88 (t, *J* = 4.0 Hz, 1H), 5.27 (s, 2H), 4.65 (d, *J* = 4.0 Hz, 2H), 3.45 (s, 3H), 3.17 – 2.50 (m, 4H), 1.74 (p, *J* = 5.5 Hz, 4H), 1.57 (s, 2H). LC-MS: HRMS $[C_{25}H_{29}N_5O_3+H]^+$: 448.23432 calculated, 448.23417 found.

5-(*tert*-Butyl)-*N*-(cyano(3,4,5-trimethoxyphenyl)methyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-carboxamide (6)



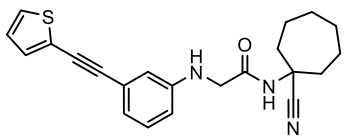
The compound was purchased from Enamine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.73 (s, 2H), 6.32 – 6.19 (m, 2H), 3.88 (s, 6H), 3.85 (s, 3H), 2.97 – 2.88 (m, 1H), 2.77 – 2.66 (m, 2H), 2.38 – 2.27 (m, 1H), 2.13 – 2.04 (m, 1H), 1.52 – 1.34 (m, 2H), 0.94 (s, 9H) (There is water in $CDCl_3$.). HRMS $[C_{24}H_{30}N_2O_4S+H]^+$: 443.19990 calculated, 443.19993 found.

2-((5-Chloro-2-phenoxyphenyl)amino)-*N*-(1-cyano-1-cyclopropylethyl)acetamide (7)



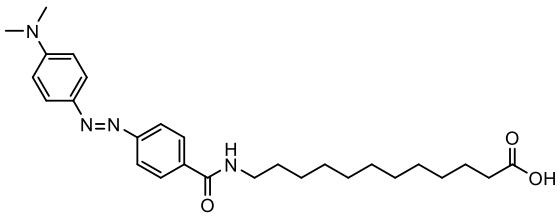
The compound was purchased from Enamine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 2H), 7.16 – 7.10 (m, 1H), 6.98 – 6.94 (m, 2H), 6.83 – 6.72 (m, 3H), 6.61 (d, *J* = 2.3 Hz, 1H), 4.86 (t, *J* = 5.7 Hz, 1H), 3.88 – 3.78 (m, 2H), 1.77 (s, 3H), 1.25 – 1.18 (m, 1H), 0.76 – 0.55 (m, 4H). HRMS $[C_{20}H_{20}ClN_3O_2+H]^+$: 370.13168 calculated, 370.13167 found.

***N*-(1-Cyanocycloheptyl)-2-((3-(thiophen-2-ylethynyl)phenyl)amino)acetamide (8)**



The compound was purchased from Enamine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.28 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.03 – 6.99 (m, 2H), 6.77 (dd, *J* = 2.6, 1.4 Hz, 1H), 6.72 (s, 1H), 6.60 (ddd, *J* = 8.1, 2.5, 0.9 Hz, 1H), 4.29 (t, *J* = 5.4 Hz, 1H), 3.83 (d, *J* = 5.4 Hz, 2H), 2.32 (ddd, *J* = 14.2, 8.4, 2.2 Hz, 2H), 1.98 (ddd, *J* = 14.3, 9.6, 2.2 Hz, 2H), 1.75 – 1.47 (m, 8H). HRMS $[C_{22}H_{23}N_3OS+H]^+$: 378.16346 calculated, 378.16323 found.

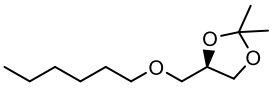
(*E*)-12-(4-((4-(Dimethylamino)phenyl)diazenyl)benzamido)dodecanoic acid (9)



To a stirred mixture of 2,5-dioxopyrrolidin-1-yl-4-((4-(dimethylamino)phenyl)diazenyl)benzoate (30 mg, 0.082 mmol, 1 eq) and 12-aminododecanoic acid (23 mg, 0.11 mmol, 1.3 eq) in anhydrous DMF (1.3 mL, 0.06 M) was

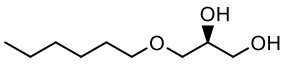
added DIPEA (43.0 µL, 0.246 mmol, 3 eq) and the mixture was stirred at rt for 3 days. The mixture was diluted in EtOAc and washed 1× with 0.05 M aq. HCl and 1× with H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-3% MeOH in DCM) to afford the product as a yellow solid (30 mg, 0.064 mmol, 79%). ^{1}H NMR (400 MHz, MeOD) δ 7.91 – 7.84 (m, 3H), 7.83 – 7.78 (m, 3H), 6.78 – 6.72 (m, 2H), 3.37 (t, *J* = 7.3 Hz, 2H), 3.09 (s, 6H), 2.25 (t, *J* = 7.5 Hz, 2H), 1.65 – 1.52 (m, 4H), 1.39 – 1.19 (m, 14H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 177.20, 168.71, 155.38, 153.51, 143.89, 135.16, 128.44, 125.80, 122.38, 111.94, 40.63, 40.43, 34.54, 29.92, 29.90, 29.81, 29.75, 29.65, 29.52, 27.45, 25.35. LC-MS $[C_{27}H_{38}N_4O_3+H]^+$: 467.30 calculated, 467.40 found.

(*R*)-4-((Hexyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (10)



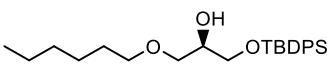
(*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methanol (50 mg, 0.38 mmol, 1 eq) was dissolved in anhydrous DMF (0.95 mL, 0.4 M) and cooled to 0 °C. NaH (60% w/w in mineral oil, 22.7 mg, 0.567 mmol, 1.5 eq) was added portion-wise and the mixture was stirred at 0 °C for 15 min. Subsequently, 1-bromohexane (63.7 µL, 0.454 mmol, 1.2 eq) was added and the mixture was stirred at 0 °C for 30 min after which it was allowed to warm to rt for 4 h. The reaction was quenched with MeOH (114 µL). The mixture was then diluted in water and extracted 3× with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-10% Et_2O in *n*-pentane) to afford the product as a colorless liquid (58 mg, 0.27 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.27 (p, *J* = 6.0 Hz, 1H), 4.06 (dd, *J* = 8.2, 6.4 Hz, 1H), 3.73 (dd, *J* = 8.2, 6.4 Hz, 1H), 3.56 – 3.39 (m, 4H), 1.62 – 1.53 (m, 2H), 1.42 (s, 3H), 1.36 (s, 3H), 1.35 – 1.24 (m, 6H), 0.88 (t, *J* = 6.9, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 109.44, 74.85, 71.98, 71.91, 67.03, 31.76, 29.62, 26.86, 25.83, 25.52, 22.71, 14.13.

(*S*)-3-(Hexyloxy)propane-1,2-diol (11)



(*R*)-4-((Hexyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (**10**, 58 mg, 0.27 mmol, 1 eq) and TsOH·H_2O (2.6 mg, 0.013 mmol, 0.05 eq) was dissolved in MeOH (0.67 mL, 0.4 M) at rt. The reaction mixture was stirred at 45 °C for 2 days. The reaction mixture was quenched with solid $NaHCO_3$ (4.5 mg, 0.053 mmol, 0.2 eq) and concentrated. The residue was purified by silica gel column chromatography (50-80% EtOAc in *n*-pentane) to afford the product as a colorless oil (35 mg, 0.20 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 – 3.81 (m, 1H), 3.76 – 3.56 (m, 2H), 3.53 – 3.40 (m, 4H), 3.26 (d, *J* = 4.5 Hz, 1H), 2.98 (t, *J* = 5.9 Hz, 1H), 1.57 (p, *J* = 6.6 Hz, 2H), 1.39 – 1.20 (m, 6H), 0.89 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 72.44, 71.92, 70.72, 64.28, 31.74, 29.60, 25.81, 22.68, 14.12.

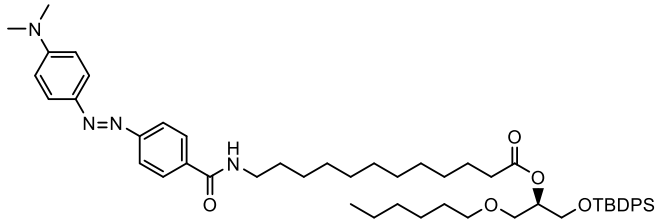
(*R*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3-(hexyloxy)propan-2-ol (12)



To a solution of (*S*)-3-(hexyloxy)propane-1,2-diol (**11**, 53 mg, 0.30 mmol, 1 eq, co-evaporated with toluene twice before the reaction)

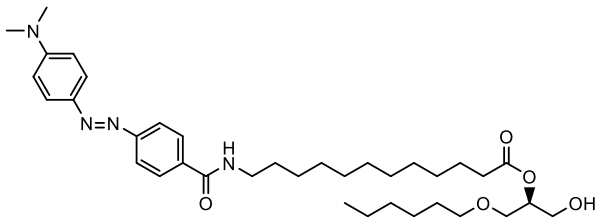
and 1*H*-imidazole (31 mg, 0.45 mmol, 1.5 eq) in anhydrous DMF (1.5 mL, 0.2 M) at 0 °C was added *tert*-butylchlorodiphenylsilane (86 µL, 0.33 mmol, 1.1 eq). The mixture was stirred at rt for 2 h. The mixture was diluted in water and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-5% EtOAc in *n*-pentane) to afford the product as a colorless oil (100 mg, 0.241 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.64 (m, 4H), 7.45 – 7.34 (m, 6H), 3.88 (h, *J* = 5.4 Hz, 1H), 3.71 (d, *J* = 5.4 Hz, 2H), 3.54 – 3.45 (m, 2H), 3.43 (t, *J* = 6.8 Hz, 2H), 2.54 (d, *J* = 5.1 Hz, 1H), 1.59 – 1.51 (m, 2H), 1.34 – 1.23 (m, 6H), 1.06 (s, 9H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.67, 133.33, 129.88, 127.85, 71.76, 71.55, 70.85, 64.91, 31.81, 29.72, 26.95, 25.89, 22.72, 19.38, 14.18.

(*R*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3-(hexyloxy)propan-2-yl (*E*)-12-(4-((4-(dimethylamino)phenyl)diazenyl)benzamido)dodecanoate (13)



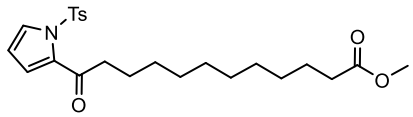
To a solution of (*E*)-12-(4-((4-(dimethylamino)phenyl)diazenyl)benzamido)dodecanoic acid (**9**, 68.0 mg, 0.146 mmol, 1 eq) and (*R*)-1-((*tert*-butyldiphenylsilyl)oxy)-3-(hexyloxy)propan-2-ol (**12**, 60.4 mg, 0.146 mmol, 1 eq) in anhydrous DCM (2 mL, 0.07 M) at 0 °C was added DIC (24.8 µL, 0.160 mmol, 1.1 eq) and DMAP (7.1 mg, 0.058 mmol, 0.4 eq). The mixture was allowed to warm to rt for overnight. The mixture was concentrated with Celite. The residue was purified by silica gel column chromatography (10-30% EtOAc in *n*-pentane) to afford the product as a red powder (64.5 mg, 75.0 µmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.84 (m, 6H), 7.69 – 7.63 (m, 4H), 7.46 – 7.34 (m, 6H), 6.77 – 6.71 (m, 2H), 6.26 (t, *J* = 5.7 Hz, 1H), 5.12 (p, *J* = 5.1 Hz, 1H), 3.79 (d, *J* = 5.1 Hz, 2H), 3.60 (dd, *J* = 5.2, 1.8 Hz, 2H), 3.49 – 3.35 (m, 4H), 3.09 (s, 6H), 2.37 – 2.21 (m, 2H), 1.66 – 1.57 (m, 4H), 1.56 – 1.48 (m, 2H), 1.42 – 1.20 (m, 20H), 1.04 (s, 9H), 0.87 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.42, 167.14, 155.05, 152.87, 143.74, 135.70, 135.64, 134.98, 133.47, 133.42, 129.81, 129.79, 127.84, 127.79, 125.48, 122.32, 111.55, 72.84, 71.69, 69.00, 62.70, 40.39, 40.32, 34.57, 31.79, 29.81, 29.69, 29.64, 29.53, 29.46, 29.39, 29.25, 27.15, 26.84, 25.85, 25.09, 22.73, 19.36, 14.18.

(*S*)-1-(Hexyloxy)-3-hydroxypropan-2-yl (*E*)-12-(4-((4-(dimethylamino)phenyl)diazinyl)benzamido)dodecanoate (14)



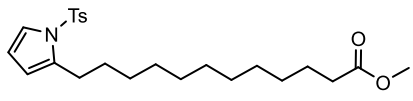
To a solution of (*R*)-1-((*tert*-butyldiphenylsilyl)oxy)-3-(hexyloxy)propan-2-yl(*E*)-12-(4-((4-(dimethylamino)phenyl)diazenyl)benzamido)dodecanoate (**13**, 65 mg, 0.075 mmol, 1 eq) in anhydrous THF (2.5 mL, 0.03 M) was added acetic acid (8.6 µL, 0.15 mmol, 2 eq) and TBAF (1 M in THF, 149 µL, 0.149 mmol, 2 eq) at 0 °C. The mixture was stirred at rt for overnight. The mixture was concentrated with Celite. The residue was purified by silica gel column chromatography (10-50% EtOAc in *n*-pentane) to afford the product as an orange-red oil (31 mg, 0.050 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.84 (m, 6H), 6.78 – 6.72 (m, 2H), 6.31 (t, *J* = 5.6 Hz, 1H), 5.00 (p, *J* = 4.8 Hz, 1H), 3.80 (t, *J* = 4.6 Hz, 2H), 3.66 – 3.57 (m, 2H), 3.50 – 3.38 (m, 4H), 3.10 (s, 6H), 2.48 (t, *J* = 6.1 Hz, 1H), 2.35 (t, *J* = 7.9 Hz, 2H), 1.68 – 1.50 (m, 6H), 1.44 – 1.17 (m, 20H), 0.88 (m, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.84, 167.19, 155.04, 152.87, 143.72, 134.96, 127.84, 125.48, 122.31, 111.55, 72.95, 71.97, 70.06, 62.98, 40.39, 40.31, 34.48, 31.73, 29.78, 29.60, 29.57, 29.55, 29.47, 29.40, 29.30, 29.15, 27.10, 25.80, 25.06, 22.70, 14.24.

Methyl 12-oxo-12-(1-tosyl-1*H*-pyrrol-2-yl)dodecanoate (15)



To a solution of 1-tosyl-1*H*-pyrrole (300 mg, 1.36 mmol, 1 eq) in anhydrous DCM (2.7 mL, 0.5 M) at 0 °C was added trifluoroacetic anhydride (942 µL, 6.78 mmol, 5 eq) followed by 12-methoxy-12-oxododecanoic acid (331 mg, 1.36 mmol, 1 eq). The reaction was heated to reflux for 40 h. The reaction was quenched with sat. $NaHCO_3$ and extracted 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (5-20% Et_2O in *n*-pentane) to afford the product as a white solid (425 mg, 0.950 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.87 (m, 2H), 7.79 (dd, *J* = 3.2, 1.7 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.03 (dd, *J* = 3.8, 1.7 Hz, 1H), 6.32 (t, *J* = 3.5 Hz, 1H), 3.66 (s, 3H), 2.68 – 2.63 (m, 2H), 2.41 (s, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.67 – 1.53 (m, 4H), 1.33 – 1.21 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.18, 174.37, 144.71, 136.06, 133.45, 130.07, 129.37, 128.32, 123.25, 110.26, 51.49, 39.54, 34.14, 29.39, 29.36, 29.26, 29.20, 29.16, 24.99, 24.94, 21.73. LC-MS $[C_{24}H_{33}NO_5S+H]^+$: 448.22 calculated, 448.27 found.

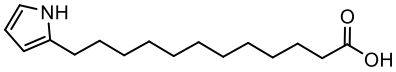
Methyl 12-(1-tosyl-1*H*-pyrrol-2-yl)dodecanoate (16)



To a solution of methyl 12-oxo-12-(1-tosyl-1*H*-pyrrol-2-yl)dodecanoate (**15**, 0.13 g, 0.29 mmol, 1 eq) in DCE (1.5

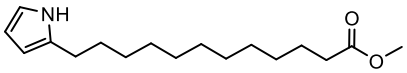
mL, 0.2 M) was added ZnI_2 (139 mg, 0.436 mmol, 1.5 eq) and $NaBH_3CN$ (137 mg, 2.18 mmol, 7.5 eq). The mixture was stirred at 80 °C for 4.5 h. The reaction was quenched with a solution of sat. aq. NH_4Cl and 6 M HCl (9:1, v:v, 10 mL), followed by extraction 3× with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (5-20% Et_2O in *n*-pentane) to afford the product as a white solid (89 mg, 0.21 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.61 (m, 2H), 7.32 – 7.23 (m, 3H), 6.19 (t, *J* = 3.3 Hz, 1H), 6.00 – 5.96 (m, 1H), 3.67 (s, 3H), 2.63 (t, *J* = 7.7 Hz, 2H), 2.40 (s, 3H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.61 (p, *J* = 7.1 Hz, 2H), 1.51 (p, *J* = 7.8 Hz, 2H), 1.35 – 1.19 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.49, 144.78, 136.68, 136.13, 130.05, 126.86, 122.26, 111.81, 111.34, 51.59, 34.24, 29.68, 29.64, 29.56, 29.51, 29.40, 29.39, 29.28, 28.75, 27.24, 25.09, 21.74. LC-MS $[C_{24}H_{35}NO_4S+H]^+$: 434.24 calculated, 434.27 found.

12-(1*H*-Pyrrol-2-yl)dodecanoic acid (17)



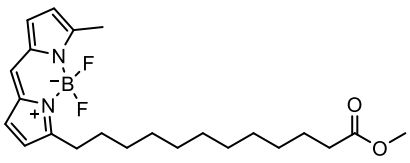
To a solution of methyl 12-(1-tosyl-1*H*-pyrrol-2-yl)dodecanoate (**16**, 289 mg, 0.667 mmol, 1 eq) in MeOH (2 mL, 0.33 M) was added 2 M aq. NaOH solution (1.67 mL, 3.33 mmol, 5 eq) and the mixture was stirred at 80 °C for 3 days. The mixture was diluted in 0.2 M aq. HCl solution and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (40-50% EtOAc in *n*-pentane) to afford the product as a white powder (140 mg, 0.527 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (bs, 1H), 7.93 (s, 1H), 6.68 – 6.59 (m, 1H), 6.12 (q, *J* = 2.8 Hz, 1H), 5.95 – 5.84 (m, 1H), 2.58 (t, *J* = 7.4 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.62 (h, *J* = 7.4 Hz, 4H), 1.38 – 1.19 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.38, 133.00, 116.08, 108.21, 104.93, 34.21, 29.78, 29.65, 29.63, 29.54, 29.50, 29.49, 29.33, 29.15, 27.84, 24.78. LC-MS $[C_{16}H_{27}NO_2+H]^+$: 266.21 calculated, 266.27 found.

Methyl 12-(1*H*-pyrrol-2-yl)dodecanoate (18)



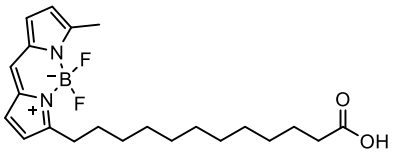
To a solution of 12-(1*H*-pyrrol-2-yl)dodecanoic acid (**17**, 76 mg, 0.29 mmol, 1 eq) and MeOH (116 µL, 2.86 mmol, 10 eq) in DCM (2.9 mL, 0.1 M) at 0 °C was added DIC (53.2 µL, 0.344 mol, 1.2 eq) and DMAP (21 mg, 0.17 mol, 0.6 eq). The mixture was allowed to warm to rt for overnight. The mixture was diluted water and extracted 3× with DCM. Combined organic layers were washed with 0.01 M aq. HCl solution, brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (5-10% EtOAc in *n*-pentane) to afford the product as a white solid (65 mg, 0.23 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 6.67 – 6.60 (m, 1H), 6.12 (q, *J* = 2.9 Hz, 1H), 5.94 – 5.84 (m, 1H), 3.66 (s, 3H), 2.58 (t, *J* = 7.8 Hz 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.67 – 1.55 (m, 4H), 1.39 – 1.21 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.49, 132.94, 116.04, 108.21, 104.86, 51.54, 34.19, 29.76, 29.61, 29.59, 29.50, 29.48, 29.45, 29.31, 29.21, 27.80, 25.03.

Methyl 12-(5,5-difluoro-7-methyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)dodecanoate (19)



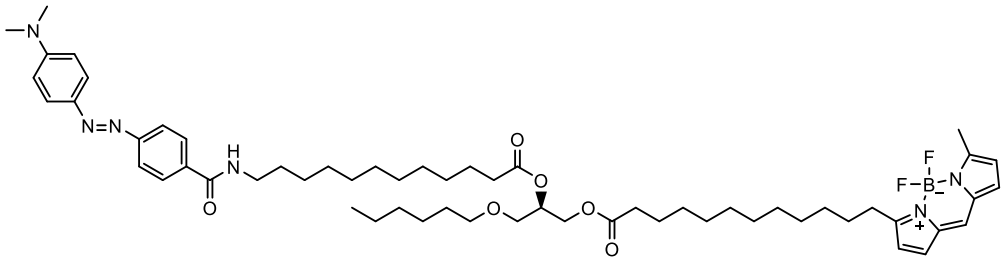
To a solution of methyl 12-(1*H*-pyrrol-2-yl)dodecanoate (**18**, 30.4 mg, 0.109 mmol, 1 eq) and 5-methyl-1*H*-pyrrole-2-carbaldehyde (13 mg, 0.12 mmol, 1.1 eq) in anhydrous DCM (1.1 mL, 0.1 M) was dropwise added $POCl_3$ (11.2 µL, 0.120 mmol, dissolved in anhydrous DCM) at 0 °C. The mixture was stirred at rt for 5 h. DIPEA (114 µL, 0.653 mmol, 6 eq) was added dropwise followed by stirring 20 min at 0 °C. Boron trifluoride diethyl etherate (81 µL, 0.65 mmol, 6 eq) was added subsequently at 0 °C. The reaction mixture was stirred at rt for overnight. More DIPEA (86 µL, 0.49 mmol, 4.5 eq) was added dropwise at 0 °C and stirred for 20 min. Subsequently, additional boron trifluoride diethyl etherate (60 µL, 0.49 mmol, 4.5 eq) were added at 0 °C. The mixture was stirred at rt for another overnight. The mixture diluted in brine and extracted 3× with DCM. Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (10% Et_2O in *n*-pentane) to afford the product as a red oil (29 mg, 0.069 mmol, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (s, 1H), 6.95 (d, *J* = 4.2 Hz, 1H), 6.92 (d, *J* = 4.1 Hz, 1H), 6.33 (d, *J* = 4.2 Hz, 1H), 6.25 (d, *J* = 4.0 Hz, 1H), 3.66 (s, 3H), 2.99 (t, *J* = 7.9 Hz, 2H), 2.61 (s, 3H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.73 (p, *J* = 7.5 Hz, 2H), 1.65 – 1.56 (m, 2H), 1.46 – 1.39 (m, 2H), 1.36 – 1.20 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.48, 163.52, 158.07, 134.71, 134.60, 130.27, 129.93, 126.94, 119.54 (d, $J_{C\text{-}F}$ = 2.9 Hz), 118.19 (d, $J_{C\text{-}F}$ = 3.0 Hz), 51.55, 34.25, 29.83, 29.70, 29.65, 29.61, 29.54, 29.36, 29.27, 28.98, 28.69, 25.09, 15.03.

12-(5,5-Difluoro-7-methyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)dodecanoic acid (20)



To a solution of methyl 12-(5,5-difluoro-7-methyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)dodecanoate (**19**, 38 mg, 0.091 mmol, 1 eq) was added 4 M aq. HCl solution in THF (4.5 mL, 198 eq). The mixture was stirred at rt for overnight. The mixture was diluted in water and extracted 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-3% MeOH in DCM) to afford the product as a red solid (29 mg, 0.072 mmol, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (s, 1H), 6.95 (d, *J* = 4.2 Hz, 1H), 6.92 (d, *J* = 4.1 Hz, 1H), 6.33 (d, *J* = 4.2 Hz, 1H), 6.25 (d, *J* = 4.1 Hz, 1H), 2.98 (t, *J* = 7.9 Hz, 2H), 2.61 (s, 3H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.73 (p, *J* = 7.6 Hz, 2H), 1.63 (p, *J* = 7.5 Hz, 2H), 1.46 – 1.38 (m, 2H), 1.36 – 1.23 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.08, 163.53, 158.07, 134.71, 134.60, 130.28, 129.94, 126.95, 119.55 (d, $J_{C\text{-}F}$ = 3.4 Hz), 118.19 (d, $J_{C\text{-}F}$ = 3.0 Hz), 34.17, 29.70, 29.65, 29.60, 29.53, 29.35, 29.18, 28.98, 28.68, 24.81, 15.04.

(*R*)-1-((12-(5,5-Difluoro-7-methyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)dodecanoyl)oxy)-3-(hexyloxy)propan-2-yl (*E*)-12-(4-((4-(dimethylamino)phenyl)diazinyl)benzamido)dodecanoate (21, EnzChek lipase substrate)



To a solution of (*S*)-1-(hexyloxy)-3-hydroxypropan-2-yl (*E*)-12-(4-((4-(dimethylamino)phenyl)diazenyl)benzamido)dodecanoate (**14**, 31 mg, 0.050 mmol, 1 eq) and 12-(5,5-difluoro-7-methyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-3-yl)dodecanoic acid **(20**, 22 mg, 0.055 mmol, 1.1 eq) in anhydrous DCM (2.5 mL, 0.02 M) at 0 °C was added DIC (8.5 µL, 0.055 mmol, 1.1 eq) and DMAP (2.4 mg, 0.020 mmol, 0.4 eq). The mixture was allowed to warm to rt for overnight. The mixture was diluted in water and extracted 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (20-30 % EtOAc in *n*-heptane) to afford the product as an orange-red powder (36 mg, 0.036 mmol, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 – 7.87 (m, 2H), 7.85 (s, 4H), 7.05 (s, 1H), 6.95 (d, *J* = 4.2 Hz, 1H), 6.92 (d, *J* = 4.1 Hz, 1H), 6.78 – 6.73 (m, 2H), 6.32 (d, *J* = 4.2 Hz, 1H), 6.25 (d, *J* = 4.1 Hz, 1H), 6.21 (t, *J* = 5.7 Hz, 1H), 5.24 – 5.16 (m, 1H), 4.33 (dd, *J* = 11.9, 3.7 Hz, 1H), 4.16 (dd, *J* = 11.9, 6.4 Hz, 1H), 3.57 – 3.50 (m, 2H), 3.49 – 3.37 (m, 4H), 3.10 (s, 6H), 2.98 (t, *J* = 7.9 Hz, 2H), 2.60 (s, 3H), 2.36 – 2.26 (m, 4H), 1.72 (p, *J* = 7.7 Hz, 2H), 1.63 – 1.50 (m, 6H), 1.46 – 1.21 (m, 36H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.61, 173.29, 167.14, 163.50, 158.07, 155.07, 152.91, 143.78, 135.01, 134.71, 134.60, 130.29, 129.95, 127.84, 126.95, 125.50, 122.34, 119.57 (d, $J_{C\text{-}F}$ = 3.4 Hz), 118.20 (d, $J_{C\text{-}F}$ = 3.4 Hz), 111.59, 71.87, 70.19, 69.06, 62.89, 40.42, 40.33, 34.48, 34.29, 31.76, 29.83, 29.73, 29.71, 29.65, 29.63, 29.60, 29.57, 29.48, 29.41, 29.39, 29.26, 29.20, 28.98, 28.69, 27.17, 25.82, 25.09, 25.05, 22.74, 15.04, 14.18. HRMS $[C_{58}H_{85}BF_2N_6O_6 + H]^+$: 1011.66741 calculated, 1011.66683 found.

References

1. Devane, W. A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
2. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**, 83–90 (1995).
3. Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773–777 (1997).
4. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163**, 463–468 (2003).
5. Janssen, F. J. & van der Stelt, M. Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders. *Bioorg Med Chem Lett* **26**, 3831–3837 (2016).
6. Baggelaar, M. P. *et al.* Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J Am Chem Soc* **137**, 8851–8857 (2015).
7. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 26–33 (2016).
8. Hsu, K. -L. *et al.* DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat Chem Biol* **8**, 999–1007 (2012).
9. Shin, M., Buckner, A., Prince, J., Bullock, T. N. J. & Hsu, K. -L. Diacylglycerol Lipase-β Is Required for TNF-α Response but Not $CD8^+$ T Cell Priming Capacity of Dendritic Cells. *Cell Chem. Biol.* **26** 1036–1041 (2019).
10. Chupak, L. S. *et al.* Structure activity relationship studies on chemically non-reactive glycine sulfonamide inhibitors of diacylglycerol lipase. *Bioorg Med Chem* **24**, 1455–1468 (2016).
11. Navia-Paldanius, D., Savinainen, J. R. & Laitinen, J. T. Biochemical and pharmacological characterization of human α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). *J Lipid Res* **53**, 2413–2424 (2012).
12. van der Wel, T. *et al.* A natural substrate-based fluorescence assay for inhibitor screening on diacylglycerol lipase α. *J Lipid Res* **56**, 927–935 (2015).
13. Janssen, F. J. *et al.* Discovery of Glycine Sulfonamides as Dual Inhibitors of sn -1-Diacylglycerol Lipase α and α/β-Hydrolase Domain 6. *J Med Chem* **57**, 6610–6622 (2014).
14. Deng, H. *et al.* Triazole Ureas Act as Diacylglycerol Lipase Inhibitors and Prevent Fasting-Induced Refeeding. *J Med Chem* **60**, 428–440 (2017).
15. Appiah, K. K. *et al.* Identification of Small Molecules That Selectively Inhibit Diacylglycerol Lipase–α Activity. *SLAS Discovery* **19**, 595–605 (2014).

16. Singh, P. K. *et al.* Assay and Inhibition of the Purified Catalytic Domain of Diacylglycerol Lipase Beta. *Biochemistry* **55**, 2713–2721 (2016).

17. García-Nafría, J., Watson, J. F. & Greger, I. H. IVA cloning: A single-tube universal cloning system exploiting bacterial *In Vivo* Assembly. *Sci Rep* **6**, 1–12 (2016).

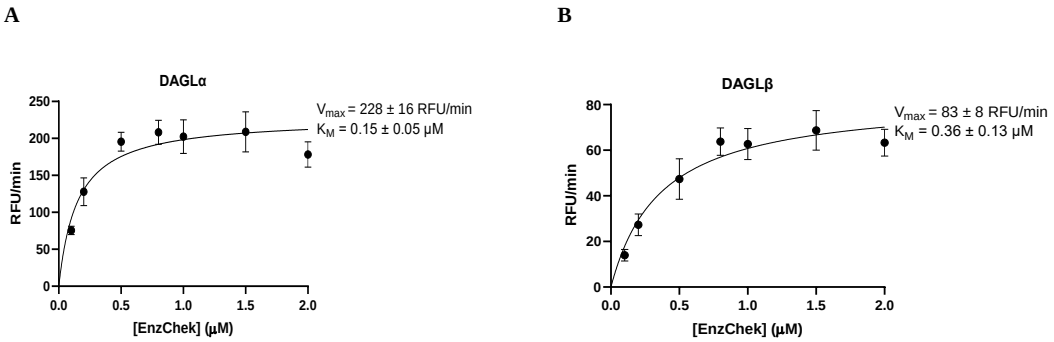
18. Aiba, A. T. *et al.* Employing BINOL-phosphoroselenoyl chloride for selective inositol phosphorylation and chemical synthesis of glycosyl inositol phospholipid from Entamoeba histolytica. *Chem. Eur. J.* **23**, 8304–8308 (2017).

19. Song, C., Knight, D. W. & Whatton, M. A. A new method for the acylation of pyrroles. *Tetrahedron Lett* **45**, 9573–9576 (2004).

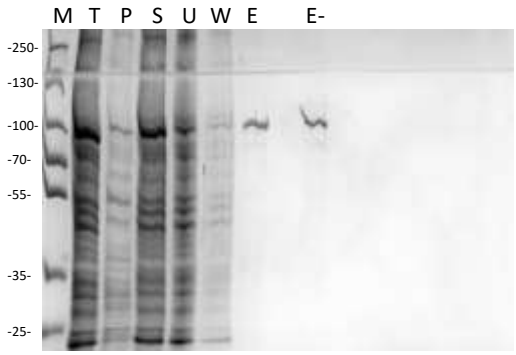
20. Jagtap, P. Formal Synthesis of (±)-Roseophilin. *Org Lett* **11**, 49–52 (2009).

21. Gao, M. *et al.* Neutrophil-Selective Fluorescent Probe Development through Metabolism-Oriented Live-Cell Distinction. *Angew. Chem. Int. Ed.* **60**, 23743–23749 (2021).

Supplementary Figures



Supplementary Figure S2.1 Michaelis-Menten kinetic curves. Rate of the synthesized EnzChek lipase substrate hydrolysis as a function of substrate concentration for DAGLα (A) and DAGLβ (B). All data were corrected for background fluorescence with mock membrane fraction observed at the same condition. Rates were determined in the linear interval of t = 10 min to t = 20 min. Data shown are mean ± SD (n = 5-6).



Supplementary Figure S2.2 Coomassie of different fractions during the purification of His-MBP-cdDAGLβ. Fractions on gel are **T**otal lysate, **P**ellet and **S**oluble fraction from lysate clearing, **U**nbound, the proteins that did not bind to the amylose resin, beads **W**ash fraction, the **E**luent with and without (**E-**) 2-mercaptoethanol in the sample buffer.

Chapter 3

Structure-activity relationship study of glycine sulfonamides as DAGLα/β inhibitors

3.1 Introduction

Diacylglycerol lipases (DAGLα and DAGLβ) are enzymes responsible for the biosynthesis of 2-arachidonoylglycerol (2-AG) in the brain and immune system. 2-AG is the most abundant endocannabinoid in tissues which can activate cannabinoid receptors type 1 and 2 (CB_1R and CB_2R) as a full agonist.[1] Due to the important role of 2-AG in regulating physio(patho)logical processes, DAGL inhibitors have been studied as a potential therapy for metabolic disorders[2,3], addiction[4], pathological pain, and (neuro)inflammation.[5–7] The current DAGL inhibitors can be divided into three classes: 1,2,3-triazole ureas (*e.g.* KT109[8], DH376 and DO34[9]), α-ketoheterocycles (*e.g.* LEI-105[10]), and glycine sulfonamides (*e.g.* LEI-106[11]).

KT109, DH376 and DO34 are widely used to investigate the function of DAGL in cellular and animal models.[12–14] These irreversible inhibitors exhibit high potency *in vitro* and *in vivo*[15], but they show relatively moderate selectivity among the serine hydrolase family.[8,9] KT195 and DO53, serving as negative control compounds lacking DAGL inhibitory activity, are necessary to assess off-target involvement in biological studies. Importantly, triazole ureas function as dual DAGLα and DAGLβ inhibitors. LEI-105 stands as the most selective dual DAGL inhibitor to date, with no known off-targets.[10] However, it encounters challenges related to low solubility and high metabolic clearance, resulting in poor bioavailability.

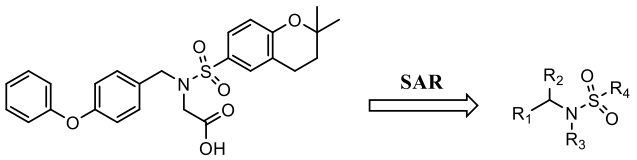
Glycine sulfonamides have been reported as DAGLα inhibitors and exhibit interactions with α/β-hydrolase domain containing 6 (ABHD6) along with a few unknown off-targets in the mouse brain membrane proteome.[11] These compounds lack an obvious serine-targeting warhead, but contain a carboxyl essential for activity.[11,15] Despite concerns about their poor cell permeability due to ionization under physiological pH, glycine sulfonamides have demonstrated good cellular activity and promising pharmacokinetics.[15] Notably, they do not easily cross the blood-brain barrier, reducing the potential for central nervous system (CNS)-mediated side effects observed with centrally acting CB_1R antagonists and DAGLα inhibitors.

Currently, subtype-selective DAGL inhibitors are still lacking, and systematic structure-activity relationships for DAGLα and DAGLβ are scarcely available. In Chapter 2, LEI-106 was identified as a hit for DAGLβ in a high-throughput screening. In this Chapter, the first structure-activity relationship (SAR) study of glycine sulfonamides and their analogs for DAGLβ, as well as their selectivity over DAGLα is described. Most of the compounds exhibited equal potency for both isoforms or slight selectivity for DAGLα, except three compounds (**49**, **51** and **52**) which demonstrated some selectivity for DAGLβ.

3.2 Results and discussion

3.2.1 Design and synthesis of DAGL inhibitors 2-52

To explore the structure-activity relationship (SAR), the scaffold of hit **1** (LEI-106) was divided into four parts, designated as R_1-R_4 (Figure 3.1), resulting in the design and synthesis of compounds **2-52**.



Hit **1** (LEI-106)

pIC_{50} (DAGLβ): 6.69 ± 0.14
pIC_{50} (DAGLα): 7.35 ± 0.06
cLogD: 1.9
LipE (DAGLβ): 4.8
Selectivity: 4-fold for DAGLα

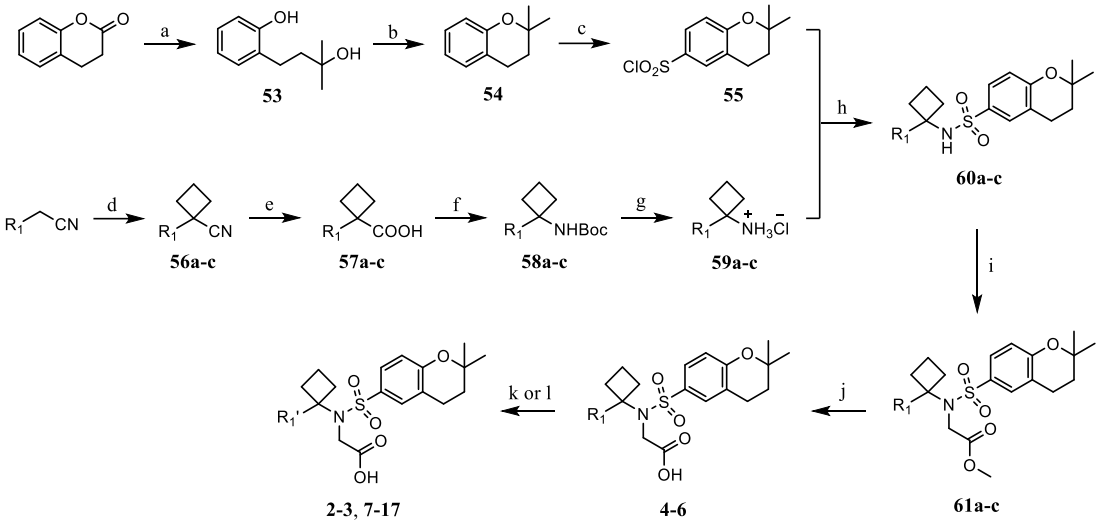
Figure 3.1 Chemical structure and biochemical activity and selectivity of Hit **1** (LEI-106) and the core structure with R_1-R_4 substituents in SAR study.

Glycine sulfonamides **2-17** featuring various modifications on R_1 were synthesized according to Scheme 3.1. The synthesis started with the addition of methyl magnesium bromide at chroman-2-one to afford diol **53**. Subsequent intramolecular ring-closure afforded ether **54**,[16] which underwent electrophilic aromatic substitution using chlorosulfonic acid to form regioselective sulfonyl chloride **55**.[17] The synthesis of amines **59a-c** started from the nitriles via sequential nucleophilic substitution[18], hydrolysis, Curtius rearrangement and *tert*-butyloxycarbamate formation[19], and finally acidolysis. Sulfonyl chloride **55** was condensed with **59a-c** to afford sulfonamides **60a-c**, which were followed by alkylating with methyl 2-bromoacetate to form sulfonamide-glycinates **61a-c**. Essential to the reaction was the use of 2-(tert-butylimino)-*N*,*N*-diethyl-1,3-dimethyl-1,3,2λ^5-diazaphosphinan-2-amine (BEMP) as a strong organic base which facilitated the alkylation.[15] Saponification of the methyl esters gave compounds **4-6**. Compounds **2-3** and **9-17** were synthesized through Suzuki-Miyaura coupling, starting from compounds **4** and **6**, respectively. A palladium-catalysed coupling in the presence of $K_4Fe(CN)_6{\cdot}3H_2O$[20] afforded final compound **7**, whose cyano group was subsequently hydrolyzed to an amide, yielding final compound **8**.

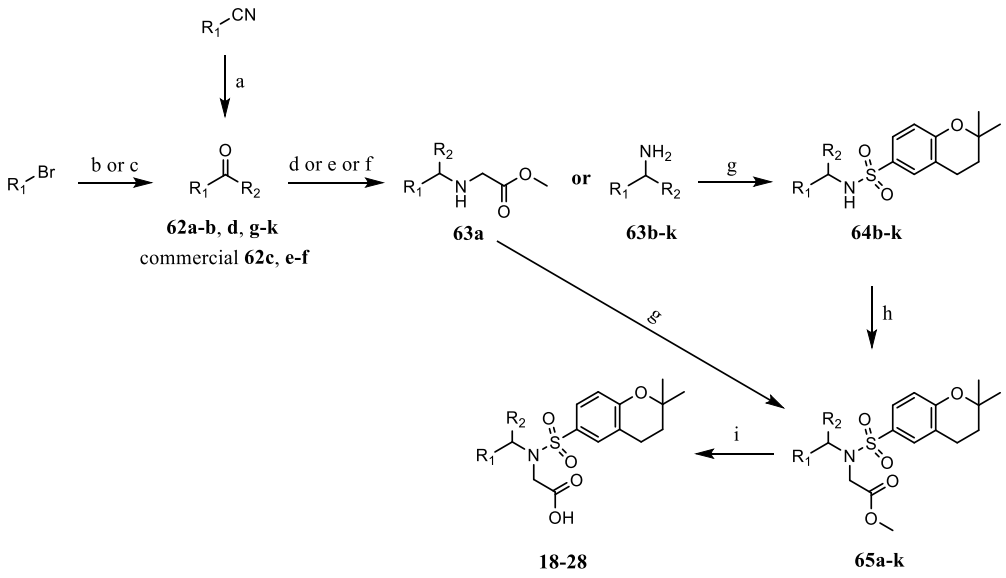
The synthesis of compounds **18-28** varying on R_2 is depicted in Scheme 3.2. When available, commercially ketones (**62c**, **e-f**) were used. Ketones not commercially available were synthesized from nitriles via nucleophilic addition and subsequent hydrolysis[21] (**62a-b**), or from bromides via nucleophilic addition of their lithium intermediates to the aldehydes and subsequent oxidation[22] (**62g-k**). Ketone **62d** was formed from 1,4-dibromobenzene and *N*-methoxy-*N*-methylcyclopropanecarboxamide via nucleophilic addition and elimination.[23] Reductive aminations[24] obtained secondary amine **63a** and primary amines **63b-k**. The final compound **18** was obtained from amine **63a** and sulfonyl chloride **55** via condensation and saponification, whereas final compounds **19-28** were obtained from amines **63b-k** and sulfonyl chloride **55** via condensation, alkylation and saponification.

Compounds **29-34**, with different substituents on R_3, were synthesized according to Scheme 3.3. Compound **60c** was *N*-alkylated with different alkyl bromides to afford compounds **34** and **66a-c** which were subsequently transformed, after saponification, into the final compounds **29**, **30**, and **32**. Compound **31** was synthesized from **30** via Jones oxidation.[25] Compound **33** was obtained through amide coupling of **6** and hydroxylamine.

Compounds **35-52** featuring various sulfonyl substituents were synthesized according to Scheme 3.4. Compound **67** was synthesized from 4-bromobenzaldehyde and glycine methyl ester via reductive amination and subsequently coupled with various sulfonyl chlorides to afford sulfonamides **68a-r**. Saponification of the methyl esters gained final compounds **35-52**.

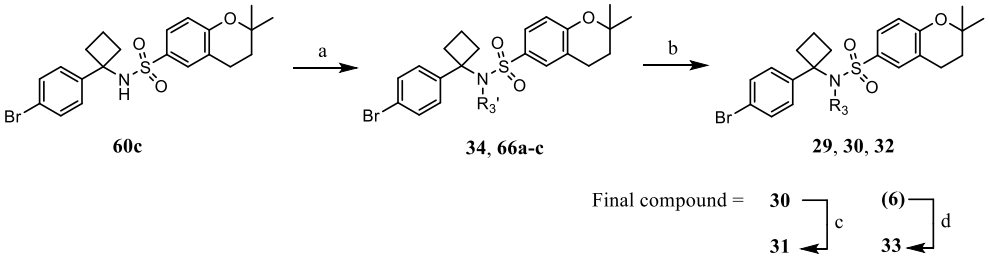


Scheme 3.1 Synthesis of glycine sulfonamides **2-17**. Reagents and conditions: a) CH_3MgBr, anhydrous THF, 0 °C-rt, 84%; b) 15% aq. H_2SO_4, toluene, reflux, 72%; c) HSO_3Cl, anhydrous DCM, 0 °C-rt, 63%; d) 1,3-dibromopropane, TBABr, KOH, toluene, H_2O, reflux, 33-61%; e) KOH, ethylene glycol, reflux, 50-92%; f) DPPA, Et_3N, anhydrous *t*-BuOH, 30 °C-reflux, 42-85%; g) 3 M aq. HCl in MeOH, rt, 93%-quant.; h) Et_3N, anhydrous DCM, rt, 46-89%; i) methyl 2-bromoacetate, BEMP, anhydrous DMF, 80 °C, 90%-quant.; j) 1 M or 2 M aq. NaOH, MeOH/THF, rt, 47-quant.; k) corresponding boronic acid, K_2CO_3, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, 1,4-dioxane/$H_2O$, 80 °C, 12-75% for **2-3**, **9-17**; l) $Pd(OAc)_2$, $K_4Fe(CN)_6 \cdot 3H_2O$, Na_2CO_3, *i*-PrOH, H_2O, DMF, 100 °C, 45% for **7**; 30% H_2O_2, 2 M aq. NaOH, MeOH, rt, 39% for **7** → **8**.

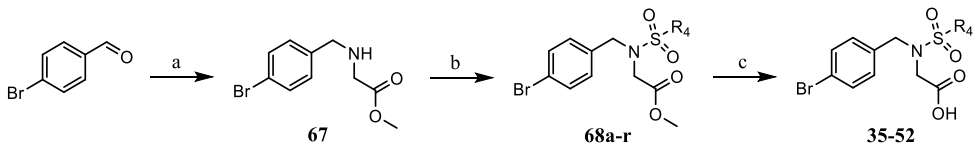


Scheme 3.2 Synthesis of glycine sulfonamides **18-28**. Reagents and conditions: a) R_2-MgX, anhydrous THF, reflux, 91% for **62a**, 86% for **62b**; b) *i.* 1,4-dibromobenzene, *n*-BuLi, anhydrous THF, -78 °C; *ii.* *N*-methoxy-*N*-methylcyclopropanecarboxamide, rt, 13% for **62d**; c) *i.* *n*-BuLi, anhydrous THF, -78 °C; *ii.* corresponding benzaldehyde, -78 °C; *iii.* I_2, K_2CO_3, *t*-BuOH, reflux, 39-54% for **62g-k**; d) 2-methoxy-2-oxoethan-1-aminium

chloride, Et_3N, AcOH, $NaBH_3CN$, anhydrous MeOH, reflux, 74% for **63a**; e) ammonium acetate, $NaBH_3CN$, anhydrous MeOH or EtOH, reflux, 15%-quant. for **63b-c**, **f-k**; f) *i. O*-methylhydroxylammonium chloride, pyridine, rt; *ii.* BH_3·THF, anhydrous THF, reflux; *iii.* aq. NaOH, 85 °C, 77% for **63d**; *i.* hydroxyl ammonium chloride, EtOH, reflux; *ii.* $LiAlH_4$, anhydrous THF, reflux, 22% for **63e**; g) 2,2-dimethylchromane-6-sulfonyl chloride (**55**), Et_3N, anhydrous DCM, 0 °C-rt, 22-99% for **64b-k**, 28% for **65a**; h) methyl 2-bromoacetate, BEMP, anhydrous DMF, 80 °C, 72%-quant. for **65b-k**; i) 1 M or 2 M aq. NaOH, THF/MeOH, rt, 22-70%.



Scheme 3.3 Synthesis of compounds **29-34**. Reagents and conditions: a) R_3'-Br, BEMP, anhydrous DMF, 80 °C, 10-90%; b) 2 M aq. NaOH, THF/MeOH, rt, 40-99%; c) Jones reagent, acetone, 0 °C, 56%; d) NH_2OH·HCl, EDCI, HOBt, DIPEA, anhydrous DMF, rt, 27%.



Scheme 3.4 Synthesis of glycine sulfonamides **35-52**. Reagents and conditions: a) *i.* glycine methyl ester hydrochloride, Et_3N, AcOH, anhydrous MeOH, rt; *ii.* $NaBH_3CN$, rt, 76%; b) sulfonyl chlorides, Et_3N, pyridine, anhydrous DCM, rt, 19%-quant.; c) 2 M aq. NaOH, THF/MeOH, rt, 21-100%.

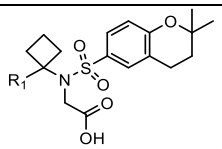
3.2.2 Biochemical evaluation and structure-activity-relationship of compounds 2-52

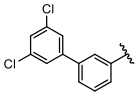
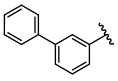
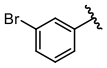
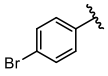
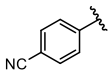
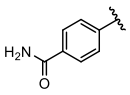
3.2.2.1 Modification on R_1

LEI-106 exhibited inhibition of both DAGLα and DAGLβ with a 4-fold selectivity for DAGLα. Additionally, compound **2** was previously reported as a potent DAGLα inhibitor with nanomolar potency and 67-fold selectivity for DAGLα over DAGLβ.[15] This compound was synthesized and its activity was confirmed with a negative logarithm half-maximal inhibitory concentration (pIC_{50}) of 7.22 ± 0.17 for DAGLβ and a pIC_{50} of 7.60 ± 0.08 for DAGLα in the EnzChek lipase substrate assay as detailed in Chapter 2 (Table 3.1). The selectivity was lower than previously reported, likely due to the utilization of different biochemical assays.[15] Although compound **2** is highly potent, it exhibits a low lipophilic efficiency (LipE) attributed to its high lipophilicity. To reduce lipophilicity, either the removal of the two chlorines in compound **2** (resulting in compound **3**) or the replacement of the 3,5-dichlorophenyl ring with a bromine (yielding compound **4**) was undertaken. Eliminating the two chlorines demonstrated no influence on potency for DAGL, whereas substituting the 3,5-dichlorophenyl ring with a bromine significantly reduced potency for DAGLα but not for DAGLβ. Both **3** and **4** exhibited higher LipE than **2**, benefiting from their decreased lipophilicity.

To investigate the impact of substituent position, compounds **5** and **6**—featuring a bromine on the *ortho* and *para* positions—were synthesized and assessed. The *para* bromine proved preferable over the *meta* and *ortho* ones for DAGL, aligning with the results of prior SAR studies involving LEI-106 counterparts.[11] Additionally, compound **6** exhibited higher potency and LipE compared to LEI-106. As a result, subsequent modifications in the SAR study primarily centered around compound **6**. Efforts to further reduce lipophilicity involved replacing the para bromine with nitrile (**7**), amide (**8**), and methyl groups (**9**). However, these analogs demonstrated lower potency than **6**. Generally, compounds tended to be less potent if the substituent introduced was less lipophilic. Compounds **7** and **8** also exhibited high topological polar surface area (tPSA), potentially resulting in reduced cellular permeability. Substituting the *para* bromine with a phenyl ring containing electron-withdrawing or donating moieties (**10-17**) maintained potency. These SAR findings strongly suggest the existence of a substantial binding pocket on DAGL for the R_1 group, with the primary determinant of potency being the compound's lipophilicity.

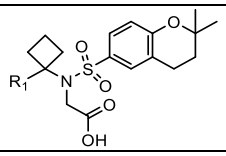
Table 3.1 Biochemical results and physicochemical properties of glycine sulfonamides **2-17**.[a]

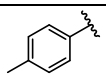
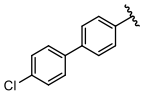
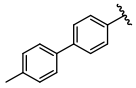
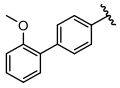
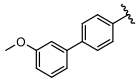
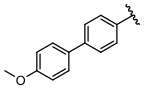
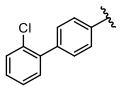
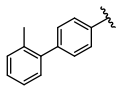
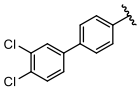


ID	R_1	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
2		7.22 ± 0.17	7.60 ± 0.08	0.4	4.5	95	2.7
3		7.24 ± 0.09	7.52 ± 0.04	0.5	3.2	95	4.0
4		7.17 ± 0.10	7.20 ± 0.03	0.9	2.3	95	4.9
5		6.66 ± 0.07	6.91 ± 0.05	0.6	2.3	95	4.4
6		7.34 ± 0.18	7.89 ± 0.11	0.3	2.3	95	5.0
7		6.14 ± 0.06	6.47 ± 0.12	0.5	1.4	119	4.7
8		< 5	5.36 ± 0.05	n.d.	0.7	138	n.d.

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

Table 3.1 (continued) Biochemical results and physicochemical properties of glycine sulfonamides **1-17**.[a]



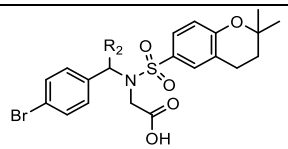
ID	R_1	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
9		6.88 ± 0.07	7.27 ± 0.14	0.4	1.9	95	5.0
10		7.22 ± 0.12	8.17 ± 0.21	0.1	3.8	95	3.4
11		7.51 ± 0.08	7.86 ± 0.04	0.4	3.6	95	3.9
12		7.15 ± 0.13	7.57 ± 0.06	0.4	3.2	104	4.0
13		7.52 ± 0.08	7.70 ± 0.06	0.7	3.2	104	4.3
14		7.36 ± 0.10	7.75 ± 0.11	0.4	3.2	104	4.2
15		7.01 ± 0.09	7.41 ± 0.09	0.4	3.8	95	3.2
16		6.94 ± 0.10	7.44 ± 0.08	0.3	3.6	95	3.3
17		7.18 ± 0.10	7.27 ± 0.05	0.8	4.5	95	2.7

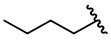
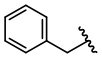
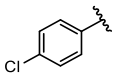
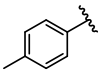
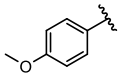
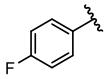
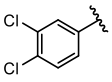
[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

3.2.2.2 Modification on R_2

Exploration of the impact of the R_2 group on potency and selectivity was conducted based on compound **6**. Substituting the fused cyclobutyl ring in **6** with an ethyl group in **18** significantly reduced potency for DAGL (Table 3.2). Analogs **19-20**, featuring longer *n*-propyl and *n*-butyl groups, displayed increased potency and lipophilicity, resulting in a lower LipE. Compound **21**, with a cyclopropyl ring, demonstrated good potency and slightly higher LipE for DAGLβ compared to compound **6**.

Table 3.2 Biochemical results and physicochemical properties of glycine sulfonamides **6**, and **18-28**.[a]



ID	R_2	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
6		7.34 ± 0.18	7.89 ± 0.11	0.3	2.3	95	5.0
18		6.70 ± 0.10	6.98 ± 0.07	0.5	2.0	95	4.7
19		7.11 ± 0.09	7.44 ± 0.05	0.5	2.5	95	4.6
20		7.37 ± 0.09	7.60 ± 0.04	0.6	2.9	95	4.5
21		7.06 ± 0.09	7.23 ± 0.03	0.7	1.9	95	5.2
22		7.07 ± 0.11	7.19 ± 0.04	0.8	3.0	95	4.1
23		7.46 ± 0.11	7.64 ± 0.07	0.7	3.0	95	4.5
24		7.07 ± 0.14	7.34 ± 0.07	0.5	3.6	95	3.5
25		7.14 ± 0.11	7.29 ± 0.07	0.7	3.3	95	3.8
26		7.04 ± 0.11	7.11 ± 0.09	0.9	2.9	104	4.1
27		7.06 ± 0.11	7.24 ± 0.06	0.7	3.1	95	4.0
28		6.96 ± 0.10	7.36 ± 0.14	0.4	4.2	95	2.8

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

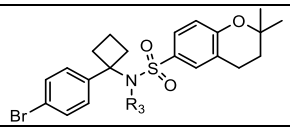
Introducing a phenyl group in **22**, a benzyl in **23**, and various substituted phenyl groups in **24-28** proved well-tolerated, indicating a substantial binding pocket for the R_2 group. Compound **23** exhibited greater potency than compound **22**, likely due to the higher lipophilicity and flexibility of the benzyl group. Analogs **24-28**, featuring electron-donating or withdrawing substituents, demonstrated similar potency, suggesting that the electronegativity

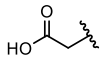
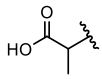
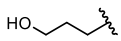
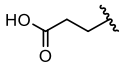
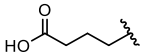
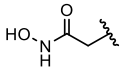
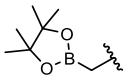
of the phenyl ring had no discernible effect on potency. Notably, these analogs were synthesized and evaluated as racemic mixtures, leaving uncertainty regarding whether the two enantiomers might differ in potency or selectivity. Since none of the variations exhibited significantly higher LipE than compound 6, further pursuit of these modifications was not undertaken.

3.2.2.3 Modification on R_3

To evaluate the significance of the acetic acid moiety, analogs **29-34** (Table 3.3) featuring different substituents on R_3 were examined. Introducing a methyl group on the Cα of the carboxyl and replacing the carboxyl with an alcohol resulted in inactive compounds **29** and **30**, respectively. The length of the linker between the carboxyl and the sulfonamide proved crucial for potency. Extended linkers led to diminished potency (**31**, **32**). Substituting the carboxyl with an *N*-hydroxylamide in **33** or a boronic acid pinacol ester in **34** drastically reduced potency. Although the analog featuring a boronic acid moiety was speculated to exhibit greater potency than its pinacol ester **34**, its synthesis was unsuccessful.

Table 3.3 Biochemical results and physicochemical properties of sulfonamides **6**, and **29-34**.[a]



ID	R_3	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å²)	LipE DAGLβ
6		7.34 ± 0.18	7.89 ± 0.11	0.3	2.3	95	5.0
29		< 5	< 5	n.d.	2.7	95	n.d.
30		< 5	< 5	n.d.	5.2	75	n.d.
31		5.04 ± 0.15	5.24 ± 0.10	0.6	2.8	95	2.2
32		< 5	< 5	n.d.	3.2	95	n.d.
33		5.16 ± 0.16	5.72 ± 0.06	0.3	3.9	104	1.3
34		5.41 ± 0.10	5.30 ± 0.05	1.3	6.2	73	< 0

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

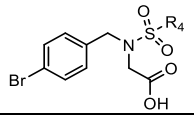
In summary, the acetic acid moiety emerged as a crucial functional group on R_3 for achieving high potency, indicating the presence of a small binding pocket in DAGL that forms specific interactions with the carboxyl, such as hydrogen bonding and/or ionic interactions.

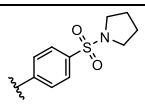
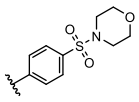
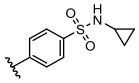
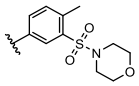
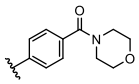
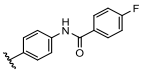
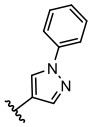
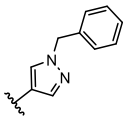
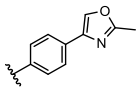
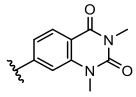
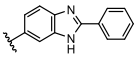
3.2.2.4 Modification on R_4

The influence of the R_4 group on potency and activity was insufficient in previous studies.[11,15] To extend the SAR study on R_4, a library containing 18 glycine sulfonamides (compounds **35-52**) was synthesized and evaluated (Table 3.4). The substituents on R_4 were selected based on the diversity, lipophilicity (cLogD < 5), polarity (tPSA < 140 $Å^2$), and molecular weight (MW < 600) of the corresponding final compounds. The cyclobutyl group on R_2 was removed to simplify the synthesis. Compound **35**, with a sulfonyl pyrrolidine on the *para* position, showed low potency, whereas the other analogs **36-38** with a sulfonamide on the phenyl ring were completely inactive. Compounds **39** and **40**, with an amide, **41** and **42** with a pyrazole, and **43-48** with different substituents on the phenyl group, were all inactive for DAGLβ, whereas compound **49**, with a quinoline ring, showed some activity for DAGLβ but not for DAGLα. Compounds **50-52** were active for both DAGL enzymes, and among them, **51** and **52** showed a selectivity of around 3-fold for DAGLβ over DAGLα. Most of the tested analogs were inactive for DAGL, indicating that the binding pocket of the R_4 substituents is quite restricted.

While most modifications on R_4 were not tolerated, the SAR analysis of this segment resulted in the development of several inhibitors (**49**, **51**, and **52**) that were the first to selectively target DAGLβ over DAGLα by a factor of ~3. Among these inhibitors, **52** exhibited the highest potency for DAGLβ, with a pIC_{50} of 6.72 ± 0.15 and a LipE of 4.9.

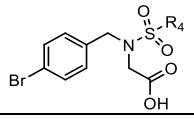
Table 3.4 Biochemical results and physicochemical properties of glycine sulfonamides **35-52**.[a]

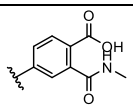
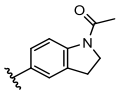
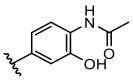
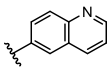
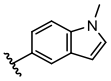
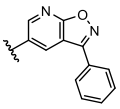
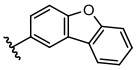


ID	R_4	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
35		5.16 ± 0.13	5.10 ± 0.08	1.1	-0.1	132	5.3
36		< 5	< 5	n.d.	-0.9	141	n.d.
37		< 5	< 5	n.d.	-0.3	140	n.d.
38		< 5	< 5	n.d.	-0.5	141	n.d.
39		< 5	< 5	n.d.	-0.3	115	n.d.
40		< 5	< 5	n.d.	1.3	115	n.d.
41		< 5	< 5	n.d.	-0.8	104	n.d.
42		< 5	< 5	n.d.	-0.5	104	n.d.
43		< 5	5.21 ± 0.07	n.d.	0.6	112	n.d.
44		< 5	< 5	n.d.	-0.5	127	n.d.
45		< 5	5.03 ± 0.36	n.d.	1.2	115	n.d.

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

Table 3.4 (continued) Biochemical results and physicochemical properties of glycine sulfonamides **35-52**.[a]



ID	R_4	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
46		< 5	5.08 ± 0.08	n.d.	-3.1	155	n.d.
47		< 5	5.36 ± 0.14	n.d.	0.2	106	n.d.
48		< 5	< 5	n.d.	-0.6	135	n.d.
49		5.45 ± 0.14	< 5	> 2.8	0.4	99	5.1
50		5.58 ± 0.06	6.04 ± 0.04	0.3	0.3	91	5.3
51		5.55 ± 0.11	5.02 ± 0.05	3.4	0.8	125	4.7
52		6.72 ± 0.15	6.29 ± 0.07	2.7	1.8	99	4.9

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

3.3 Conclusion

To summarize, this Chapter explored the structure-activity relationship (SAR) of glycine sulfonamides by systematically modifying the R_1-R_4 positions. A total of 51 analogs were synthesized and assessed for their biochemical potency against both DAGL enzymes using the EnzChek lipase substrate assay.

Notably, modifications in the R_1 position significantly affected potency, revealing a preference for *para* substituents on the phenyl ring over *meta* and *ortho* counterparts. It was observed that lipophilic substitutions were better tolerated compared to hydrophilic ones. Moreover, while replacing the cyclobutyl ring on the R_2 position with alkyl groups or phenyl rings retained potency, it did not enhance it. The acetic acid moiety (at R_3) emerged as a crucial substituent, presumably due to its pivotal ionic interactions with DAGL. On the other hand, modifications on the R_4 position were only moderately accepted. However, these modifications

proved instrumental in conferring selectivity, resulting in the first selective DAGLβ inhibitors (**49**, **51**, and **52**) in the glycine sulfonamide series. Out of the 51 compounds investigated in this Chapter, compound **52** stood out as the most potent and selective DAGLβ inhibitor.

3.4 Acknowledgements

Jonathan de Ruiter and Robin van der Woude are acknowledged for their contribution to the synthesis and biochemical evaluation of final compounds. Hans van den Elst is kindly acknowledged for preparative HPLC purification and HRMS measurements.

3.5 Experimental methods

Biology

EnzChek lipase substrate assay for DAGLα and DAGLβ in 384-well plate

The membrane fractions from HEK293T cells transiently overexpressing human DAGLα and DAGLβ were diluted to 1.5 µg/mL in assay buffer 1 (50 mM HEPES pH 7.5, 5% DMSO, 0.0025% Triton X-100) and 10 µL was pipetted into the dark flat-bottom 384-well plate (Greiner Bio-One, REF 781076). The membrane fraction from HEK293T cells transfected with empty pcDNA3.1 vector was used for the negative control (mock). Inhibitors were consecutively diluted in DMSO to 600 µM and in assay buffer 2 (50 mM HEPES pH 7.5, 0.0025% Triton X-100) to 30 µM. A dilution series (8 concentrations, 5× dilution each time) was prepared in assay buffer 1. 10 µL of inhibitor solution or assay buffer 1 was transferred to the enzyme samples in the assay plate. The plate was spun down at 1000 rpm for 1 min and incubated at rt for 30 min. The EnzChek lipase substrate was consecutively diluted in DMSO to 30 µM and in assay buffer 2 to 1.5 µM. 10 µL of EnzChek solution was added to each well. The plate was spun down at 1000 rpm for 1 min and incubated at rt for 3 h in the dark. The final concentrations of protein, EnzChek lipase substrate and inhibitors were 0.5 µg/mL, 0.5 µM and 10 µM -> 0.128 nM, respectively. Endpoint fluorescence was measured in CLARIOstar® (excitation 477-14 nm, emission 525-30 nm, gain = 1600). DAGL and mock membrane fractions with DMSO were used as positive and negative controls, respectively, to calculate the assay window. The mock membrane fraction with inhibitors at each concentration was used for background correction. Assay performance was assessed using Z' factor, which was calculated with the formula: $Z' = 1 - 3(\sigma_{pc} + \sigma_{nc})/(\mu_{pc} - \mu_{nc})$, where pc represents the positive control, nc represents the negative control, σ represents the standard deviation, and µ represents the mean value. Residual activity of DAGL was calculated using the equation: Residual activity (%) = $(\mu_{DAGL} - \mu_{mock})/(\mu_{pc} - \mu_{nc}) \times 100\%$, where μ_{DAGL} and μ_{mock} represent the fluorescence intensities of DAGL and mock with inhibitors, respectively. Residual activities were used to generate the dose-response curves using GraphPad Prism 9.0.0 (log(inhibitor) vs. normalized response with variable slope). All measurements were performed three times independently (n = 1, N = 3 or n = 4, N = 3 for controls, with $Z' \geq 0.6$).

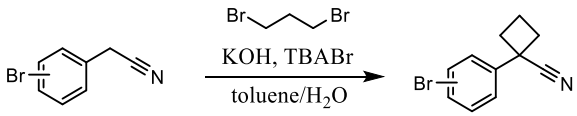
Chemistry

General remarks

All purchased chemicals were used without purification unless stated otherwise. All reactions were performed in oven-dried or flame-dried glassware. Anhydrous solvents were dried by activated 3 Å or 4 Å molecular sieves. Thin layer chromatography (TLC) analysis was performed on Merck silica gel 60 F_{254} aluminium sheets and the compounds were visualized by using UV absorption at 254 nm and/or $KMnO_4$ staining (5 g/L $KMnO_4$ and 25 g/L K_2CO_3 in water). TLC plates were analysed with the Advion CMS Plate Express® connected to the Advion Expression® L-MS using 90% MeOH in H_2O with 0.1% formic acid as the solvent.

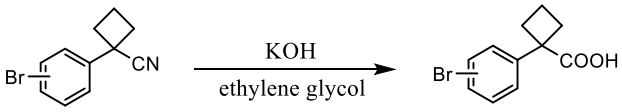
Liquid chromatography-mass spectrometry (LC-MS) analysis was performed on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI^+) coupled to a Surveyor HPLC system equipped with a C18 column (50 × 4.6 mm, 3 µm particle size, Macherey-Nagel) or a Thermo Finnigan LCQ Fleet ion-trap mass spectrometer (ESI^+) coupled to a Vanquish UHPLC system using H_2O, CH_3CN and 0.1% aq. TFA as eluents. Purification was performed on manual silica gel column chromatography (40-63 µm, 60 Å silica gel, Macherey-Nagel) or automated silica gel column chromatography (40-63 µm, 60 Å pre-packed silica gel, Screening Devices) on a Biotage Isolera™ Four 3.0 system. Alternatively, purification was performed using preparative HPLC on a Waters Acquity Ultra performance LC equipped with a C18 column (21 × 150 mm, 5 µm particle size, Phenomenex). ^1H and ^{13}C spectra were recorded on a Bruker AV 400 MHz (400 MHz for ^1H and 101 MHz for ^{13}C) or AV 500 MHz (500 MHz for ^1H and 126 MHz for ^{13}C) or AV 850 MHz spectrometer (850 MHz for ^1H and 214 MHz for ^{13}C) in deuterated solvents. Chemical shifts are reported in ppm with tetramethylsilane (TMS) or solvent resonance as the internal standard ($CDCl_3$: δ 7.26 for ^1H, δ 77.16 for ^{13}C; CD_3OD: δ 3.31 for ^1H, 49.00 for ^{13}C; DMSO-*d6*: δ 2.50 for ^1H, δ 39.52 for ^{13}C). Data is reported as follows: chemical shifts δ (ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, t = triplet, td = triplet of doublets, tt = triplet of triplets, q = quartet, quintet = p, bs = broad singlet, m = multiplet), coupling constants *J* (Hz) and integration. High resolution mass spectrometry (HRMS) analysis was performed on a Thermo Finnigin LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctyl phthalate (m/z = 391.28428) as a lock mass.

General procedure A



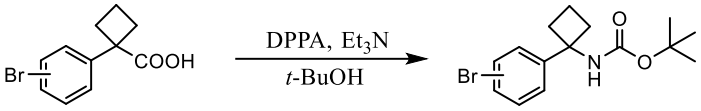
A mixture of corresponding phenyl acetonitrile (1 eq), 1,3-dibromopropane (1 eq) and TBABr (0.1 eq) in toluene (0.35 M) and solid KOH (8 eq) in H_2O (75% w/w) was heated to 100 °C with occasionally slow stirring to facilitate liquification of the aqueous phase. The reaction was then refluxed with continuous vigorous stirring for 1-2 h. The mixture was diluted in water and extracted 3× with EtOAc. Combined organic layers were washed with sat. NH_4Cl, brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure B



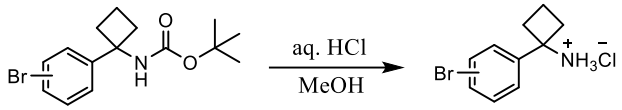
The mixture of corresponding nitrile (1 eq) and KOH (6 eq) in ethylene glycol (0.4-0.8 M) was refluxed for 2 h. The mixture was diluted in water and washed 1× with Et_2O. The pH of water layer was adjusted by 2 M aq. HCl solution to 2, which was then extracted 3× with EtOAc. Combined organic layers were dried by anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure C



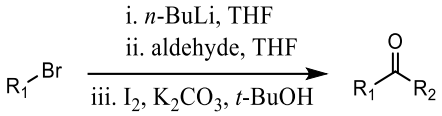
To a solution of corresponding carboxylic acid (1 eq) in anhydrous *t*-BuOH (0.08 M) with 4 Å molecular sieves was added diphenylphosphoryl azide (1 eq) and Et_3N (1.1 eq). The reaction was stirred at 30 °C for 1 h and then refluxed for overnight. The mixture was filtered and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography to afford the product.

General procedure D



The corresponding Boc-protected amine (1 eq) was dissolved in 3 M aq. HCl in MeOH (0.04-0.3 M) and the reaction was stirred at rt for 24 h. The solvent was removed and the residue was washed 2× with Et_2O and filtered to afford the product.

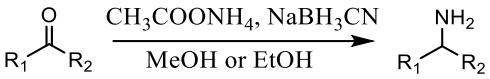
General procedure E



n-Butyllithium (2.5 M in hexane, 1.1 eq) was added dropwise to a solution of corresponding bromide (1 eq) in anhydrous THF (0.8 M) at -78 °C. The solution was allowed to stir for 30 min at -78 °C. After 30 min, corresponding aldehyde (1.05 eq) diluted in anhydrous THF (0.5 mL) was slowly added at -78 °C. The obtained mixture was warmed to rt and stirred at rt for 1 h. The mixture was concentrated under vacuum and subsequently I_2 (1.6 eq), K_2CO_3 (3 eq) and *t*-BuOH (0.67 M) were added. The mixture was allowed to reflux for 3 h. After completion, the reaction was quenched with sat. Na_2SO_3 and extracted with 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The

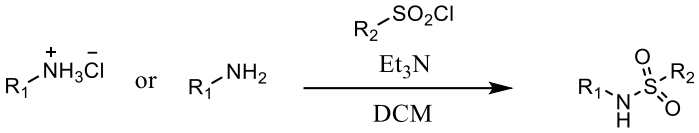
residue was purified by silica gel column chromatography to afford the product or directly used without purification.

General procedure F



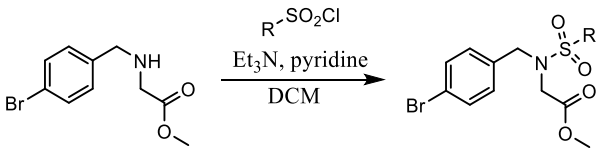
To a stirred solution of corresponding ketone (1 eq) in anhydrous MeOH or EtOH (5.0 mL) was added ammonium acetate (10-20 eq) and $NaBH_3CN$ (1.5-3 eq) at rt. The obtained mixture was refluxed for overnight. After completion, the mixture was concentrated under vacuum. The residue was quenched with 1 M aq. NaOH and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was used in subsequent reactions without further purification.

General procedure G



To a solution of corresponding ammonium chloride or amine (1 eq) in anhydrous DCM at 0 °C was added Et_3N (3-5 eq) and corresponding sulfonyl chloride (1.1-2 eq). The reaction was stirred at rt for overnight. The mixture was diluted in 0.2 M aq. HCl and extracted 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure H

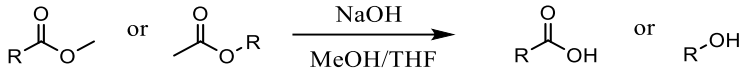


To a solution of methyl (4-bromobenzyl)glycinate (1 eq) in anhydrous DCM (0.1 M) was added Et_3N (4 eq), a drop of pyridine and corresponding sulfonyl chloride (1 eq). The reaction was stirred at rt until it was finished. The mixture was diluted in water or 0.2 M aq. HCl and extracted 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure I

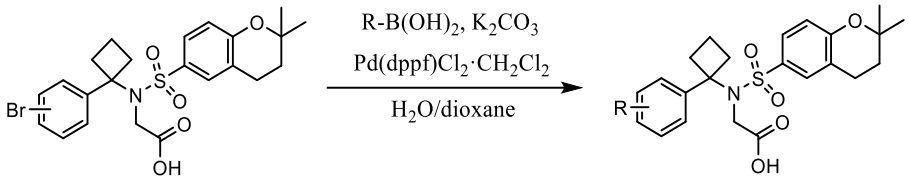
To a solution of corresponding sulfonamide (1 eq) in anhydrous DMF (0.1 M) was added corresponding alkyl bromide (1.5-3 eq) and 2-(*tert*-butylimino)-*N*,*N*-diethyl-1,3-dimethyl-1,3,2λ^5-diazaphosphinan-2-amine (BEMP, 1 M in hexane, 1.5-3 eq). The reaction was heated to 80 °C until the reaction was completed. The reaction mixture was diluted in EtOAc and washed with water or 0.2 M aq. HCl and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product or directly used without purification.

General procedure J



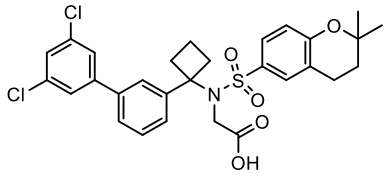
To a solution of corresponding ester (1 eq) in MeOH/THF (1:1, 0.1 M) was added 1 M or 2 M aq. NaOH (2-5 eq) and the reaction was stirred at rt until completion. The mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography or preparative HPLC to afford the product.

General procedure K



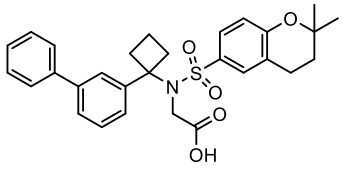
In a microwave vial containing glycine sulfonamide (1 eq) was added corresponding boronic acid (1.2-1.5 eq), K_2CO_3 (5 eq) and degassed H_2O/dioxane (1:1, 0.1 M). The mixture was degassed with N_2 and Pd(dppf)Cl_2·CH_2Cl_2 (0.05 eq) was added. The reaction was heated to 80-85 °C until completion. The mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography or preparative HPLC to afford the product.

_N_-(1-(3',5'-Dichloro-[1,1'-biphenyl]-3-yl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (2)



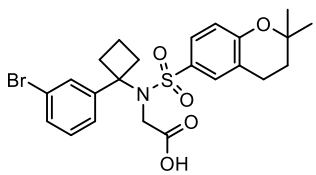
The title compound was synthesized according to general procedure **K** using _N_-(1-(3-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**4**, 30 mg, 0.059 mmol, 1 eq), (3,5-dichlorophenyl)boronic acid (17 mg, 0.089 mmol, 1.5 eq), K_2CO_3 (40.8 mg, 0.295 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (2.40 mg, 2.95 µmol, 0.05 eq). Total time: 4 h at 80 °C. Silica gel column chromatography (1-7% MeOH in DCM) and preparative HPLC afforded the product as a white powder (17 mg, 0.030 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.53 (m, 1H), 7.47 – 7.37 (m, 3H), 7.33 (t, _J_ = 1.8 Hz, 1H), 7.24 (dd, _J_ = 8.7, 2.5 Hz, 1H), 7.22 (d, _J_ = 1.9 Hz, 2H), 6.86 (d, _J_ = 2.4 Hz, 1H), 6.65 (d, _J_ = 8.7 Hz, 1H), 4.16 (s, 2H), 2.94 – 2.82 (m, 2H), 2.66 – 2.57 (m, 2H), 2.49 (t, _J_ = 6.7 Hz, 2H), 1.91 – 1.81 (m, 1H), 1.72 – 1.57 (m, 3H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.62, 157.90, 143.89, 143.52, 138.77, 135.46, 130.70, 129.30, 128.95, 127.44, 127.10, 126.92, 126.49, 126.21, 125.67, 121.01, 117.46, 75.71, 65.80, 48.38, 35.28, 32.18, 26.87, 22.30, 14.85. HRMS $[C_{29}H_{29}Cl_2NO_5S+Na]^+$: 596.10357/598.10059 calculated, 596.10330/598.10039 found.

_N_-(1-([1,1'-Biphenyl]-3-yl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (3)



The title compound was synthesized according to general procedure **K** using _N_-(1-(3-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**4**, 37 mg, 0.073 mmol, 1 eq)**,** phenylboronic acid (13.3 mg, 0.109 mmol, 1.5 eq), K_2CO_3 (50.5 mg, 0.365 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (2.98 mg, 3.65 µmol, 0.05 eq). Total time: 4 h at 80 °C. Silica gel column chromatography (1-7% MeOH in DCM) and preparative HPLC afforded the product as a white powder (12 mg, 0.024 mmol, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (t, _J_ = 1.8 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.44 – 7.31 (m, 6H), 7.23 (dd, _J_ = 8.7, 2.5 Hz, 1H), 6.80 (d, _J_ = 2.4 Hz, 1H), 6.61 (d, _J_ = 8.7 Hz, 1H), 4.10 (s, 2H), 2.94 – 2.80 (m, 2H), 2.73 – 2.57 (m, 2H), 2.45 (t, _J_ = 6.7 Hz, 2H), 1.92 – 1.77 (m, 1H), 1.71 – 1.61 (m, 1H), 1.58 (t, _J_ = 6.7 Hz, 2H), 1.19 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.14, 157.90, 142.96, 141.41, 140.81, 130.14, 129.43, 128.96, 128.70, 127.60, 127.17, 127.11, 126.37, 126.32, 125.92, 121.15, 117.29, 75.68, 65.95, 48.48, 35.40, 32.13, 26.90, 22.24, 14.95. HRMS $[C_{29}H_{31}NO_5S+Na]^+$: 528.18151 calculated, 528.18139 found.

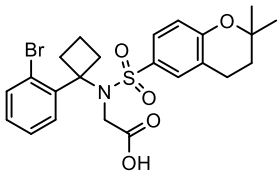
_N_-(1-(3-Bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (4)



The title compound was synthesized according to general procedure **J** using methyl _N_-(1-(3-bromophen-yl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**61a**, 68 mg, 0.13 mmol, 1 eq) and 2 M aq. NaOH (0.26 mL, 0.52 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (1-10% MeOH in DCM) afforded the product as a white powder (43 mg, 0.085 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.36

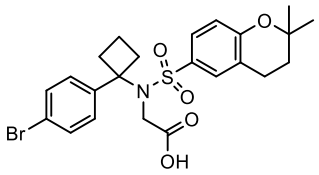
(d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.10 (t, *J* = 7.9 Hz, 1H), 6.88 (s, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 4.10 (s, 2H), 2.98 – 2.81 (m, 2H), 2.66 – 2.42 (m, 4H), 1.87 – 1.69 (m, 3H), 1.64 – 1.49 (m, 1H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.76, 157.74, 145.15, 130.83, 130.60, 130.34, 129.74, 129.21, 126.88, 125.83, 122.45, 121.07, 117.35, 75.69, 65.31, 48.65, 35.16, 32.36, 27.03, 22.30, 14.82. HRMS $[C_{23}H_{26}BrNO_5S+Na]^+$: 530.06073/532.05863 calculated, 530.06064/532.05847 found.

_N_-(1-(2-Bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (5)



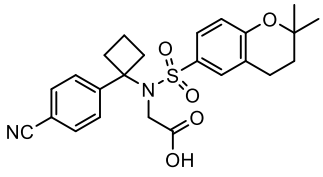
The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(2-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**61b**, 22 mg, 0.042 mmol, 1 eq) and 2 M aq. NaOH (83.0 µL, 0.166 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (1-7% MeOH in DCM) afforded the product as a white powder (10 mg, 0.020 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.9 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 1H), 6.58 (s, 1H), 6.49 (d, *J* = 8.7 Hz, 1H), 4.46 (s, 2H), 3.19 – 2.99 (m, 2H), 2.78 (bs, 2H), 2.49 (t, *J* = 6.6 Hz, 2H), 1.90 – 1.78 (m, 1H), 1.72 (t, *J* = 6.7 Hz, 2H), 1.54 – 1.41 (m, 1H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.36, 157.27, 139.45, 134.43, 130.98, 128.76, 128.51, 126.82, 126.29, 124.85, 120.37, 116.97, 75.42, 66.85, 51.62 (br), 35.41, 32.41, 26.83, 22.27, 15.16. HRMS $[C_{23}H_{26}BrNO_5S+Na]^+$: 530.06073/532.05863 calculated, 530.06062/532.05844 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (6)



The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**61c**, 438 mg, 0.838 mmol, 1 eq) and 1 M aq. NaOH (3 mL, 3 mmol, 3.6 eq). Total time: 30 min at 90 °C. The product was afforded as a white solid (426 mg, 0.838 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.35 (m, 2H), 7.34 – 7.24 (m, 3H), 6.86 (d, *J* = 2.3 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 1H), 4.06 (s, 2H), 2.87 – 2.76 (m, 2H), 2.62 (t, *J* = 6.7 Hz, 2H), 2.57 – 2.49 (m, 2H), 1.88 – 1.77 (m, 3H), 1.65 – 1.52 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.85, 157.89, 141.51, 131.35, 130.69, 129.31, 129.22, 126.89, 121.64, 121.30, 117.50, 75.88, 65.41, 48.12, 35.14, 32.37, 26.97, 22.34, 14.76. HRMS $[C_{23}H_{26}BrNO_5S+NH_4]^+$: 525.10533/527.10323 calculated, 525.10553/527.10339 found.

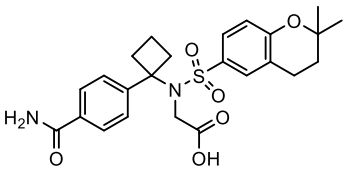
_N_-(1-(4-Cyanophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (7)



i-PrOH (0.14 mL), H_2O (0.35 mL), Na_2CO_3 (52 mg, 0.49 mmol, 2.5 eq), $Pd(OAc)_2$ (4.5 mg, 0.020 mmol, 0.1 eq), Potassium hexacyanoferrate(II) trihydrate (25 mg, 0.059 mmol, 0.3 eq) and *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 100 mg, 0.197 mmol, 1 eq) were sequentially added to a microwave tube

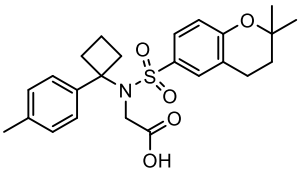
equipped with DMF (2.4 mL, 0.08 M). The mixture was degassed under argon and heated to 100 °C overnight. The mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by preparative HPLC to afford the product as a white solid (40 mg, 0.088 mmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 4H), 7.26 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 1H), 4.13 (s, 2H), 2.90 – 2.80 (m, 2H), 2.62 (t, *J* = 6.7 Hz, 2H), 2.58 – 2.49 (m, 2H), 1.90 – 1.77 (m, 3H), 1.65 – 1.53 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.97, 158.05, 148.11, 132.01, 130.93, 129.19, 128.16, 126.71, 121.20, 118.67, 117.71, 111.23, 75.98, 65.50, 48.09, 34.82, 32.26, 26.93, 22.35, 14.63. HRMS $[C_{24}H_{26}N_2O_5S+Na]^+$: 477.14546 calculated, 477.14539 found.

_N_-(1-(4-Carbamoylphenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (8)



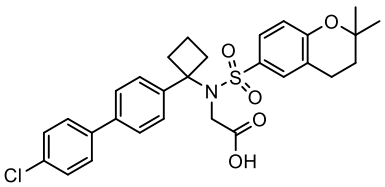
A solution of *N*-(1-(4-cyanophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**7**, 20 mg, 0.044 mmol, 1 eq) in MeOH (3.0 mL, 0.015 M) was treated with 30% H_2O_2 (13 µL, 0.12 mmol, 2.7 eq) and 2 M aq. NaOH (50 µL, 0.10 mmol, 2.3 eq). The reaction was stirred at rt until completion. The mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by preparative HPLC to afford the product as a white solid (8.0 mg, 0.017 mmol, 39%). ^{1}H NMR (400 MHz, DMSO) δ 7.94 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.35 (s, 1H), 7.17 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.64 – 6.58 (m, 2H), 4.05 (s, 2H), 2.81 – 2.70 (m, 2H), 2.49 – 2.41 (m, 4H), 1.78 – 1.61 (m, 3H), 1.48 – 1.37 (m, 1H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 171.74, 167.38, 156.65, 145.64, 132.77, 131.10, 128.58, 127.00, 126.84, 126.22, 120.86, 116.57, 75.50, 64.66, 48.03, 34.68, 31.49, 26.54, 21.40, 14.25. HRMS $[C_{24}H_{28}N_2O_6S+Na]^+$: 495.15603 calculated, 495.15569 found.

_N_-((2,2-Dimethylchroman-6-yl)sulfonyl)-_N_-(1-(*p*-tolyl)cyclobutyl)glycine (9)



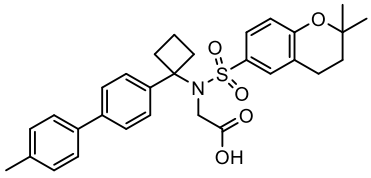
The title compound was synthesized according to general procedure **K** using *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 38 mg, 0.075 mmol, 1 eq), methylboronic acid (6.7 mg, 0.12 mmol, 1.5 eq), K_2CO_3 (31 mg, 0.22 mmol, 3 eq) and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (6.0 mg, 7.4 µmol, 0.098 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20-40% EtOAc in *n*-pentane) afforded the product as a white solid (25 mg, 0.056 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 2H), 7.24 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.66 (d, *J* = 8.7 Hz, 1H), 3.98 (s, 2H), 2.87 – 2.75 (m, 2H), 2.64 – 2.53 (m, 4H), 2.34 (s, 3H), 1.85 – 1.75 (m, 3H), 1.63 – 1.53 (m, 1H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.62, 157.78, 139.29, 137.32, 130.65, 129.59, 129.05, 127.21, 127.19, 121.12, 117.35, 75.71, 65.77, 48.12, 35.12, 32.37, 26.93, 22.30, 21.21, 14.92. HRMS $[C_{24}H_{29}NO_5S+Na]^+$: 466.16586 calculated, 466.16591 found.

_N_-(1-(4'-Chloro-[1,1'-biphenyl]-4-yl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (10)



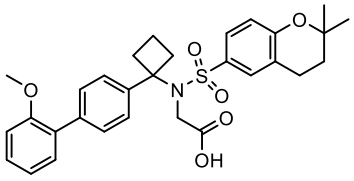
The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), (4-chlorophenyl)boronic acid (15 mg, 0.094 mmol, 1.2 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (12 mg, 0.022 mmol, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.35 (m, 8H), 7.21 (d, _J_ = 8.1 Hz, 1H), 7.00 (s, 1H), 6.61 (d, _J_ = 8.6 Hz, 1H), 4.06 (s, 2H), 2.97 – 2.80 (m, 2H), 2.60 (t, _J_ = 8.7 Hz, 2H), 2.51 (t, _J_ = 6.2 Hz, 2H), 1.91 – 1.74 (m, 1H), 1.70 – 1.54 (m, 3H), 1.24 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.83, 157.72, 141.86, 138.96, 138.86, 133.71, 130.85, 129.44, 129.14, 128.31, 127.90, 127.04, 126.65, 121.11, 117.40, 75.70, 65.62, 48.39, 35.15, 32.21, 26.86, 22.28, 14.93. HRMS $[C_{29}H_{30}ClNO_5S+Na]^+$: 562.14254 calculated, 562.14264 found.

_N_-((2,2-Dimethylchroman-6-yl)sulfonyl)-_N_-(1-(4'-methyl-[1,1'-biphenyl]-4-yl)cyclobutyl)glycine (11)



The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), _p_-tolylboronic acid (13 mg, 0.094 mmol, 1.2 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (19 mg, 0.037 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.42 (m, 6H), 7.31 – 7.23 (m, 2H), 7.21 (dd, _J_ = 8.6, 2.1 Hz, 1H), 6.97 (d, _J_ = 2.4 Hz, 1H), 6.62 (d, _J_ = 8.7 Hz, 1H), 4.05 (s, 2H), 2.85 (q, _J_ = 10.6 Hz, 2H), 2.61 (t, _J_ = 8.4 Hz, 2H), 2.51 (t, _J_ = 6.6 Hz, 2H), 2.40 (s, 3H), 1.84 (q, _J_ = 9.8 Hz, 1H), 1.70 – 1.59 (m, 3H), 1.24 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.83, 157.74, 140.98, 140.23, 137.48, 137.42, 130.64, 129.68, 129.51, 127.71, 127.06, 126.92, 126.61, 121.19, 117.36, 75.69, 65.70, 48.27, 35.21, 32.19, 26.86, 22.26, 21.27, 14.94. HRMS $[C_{30}H_{33}NO_5S+Na]^+$: 542.19716 calculated, 542.19702 found.

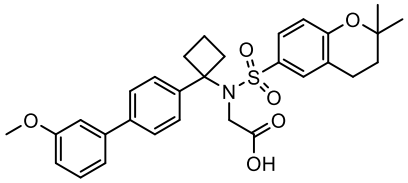
_N_-((2,2-Dimethylchroman-6-yl)sulfonyl)-_N_-(1-(2'-methoxy-[1,1'-biphenyl]-4-yl)cyclobutyl)glycine (12)



The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), (2-methoxyphenyl)boronic acid (17.9 mg, 0.118 mmol, 1.5 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (14 mg, 0.026 mmol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.42 (m, 4H), 7.38 – 7.30 (m, 2H), 7.15 – 7.09 (m, 2H), 7.04 (td, _J_ = 7.5, 1.0 Hz, 1H), 7.00 (d, _J_ = 8.6 Hz, 1H), 6.66 – 6.59 (m, 1H), 4.03

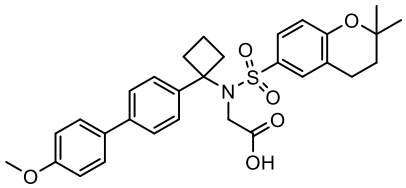
(s, 2H), 3.84 (s, 3H), 2.93 – 2.81 (m, 2H), 2.68 – 2.57 (m, 4H), 1.90 – 1.79 (m, 1H), 1.73 – 1.61 (m, 3H), 1.26 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.76, 157.76, 156.57, 140.76, 137.79, 130.97, 130.63, 129.84, 129.66, 129.44, 128.91, 127.14, 126.86, 121.14, 121.00, 117.50, 111.30, 75.68, 65.88, 55.57, 48.29, 35.14, 32.29, 26.89, 22.25, 14.98. HRMS $[C_{30}H_{33}NO_6S+Na]^+$: 558.19208 calculated, 558.19175 found.

_N_-((2,2-Dimethylchroman-6-yl)sulfonyl)-_N_-(1-(3'-methoxy-[1,1'-biphenyl]-4-yl)cyclobutyl)glycine (13)



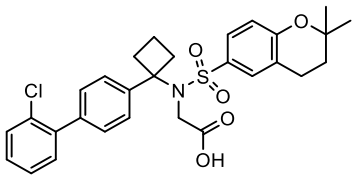
The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), (3-methoxyphenyl)boronic acid (15.5 mg, 0.102 mmol, 1.3 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (5.0 mg, 9.3 µmol, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.45 (m, 4H), 7.37 (t, _J_ = 7.9 Hz, 1H), 7.20 – 7.14 (m, 2H), 7.13 – 7.11 (m, 1H), 6.95 (d, _J_ = 1.7 Hz, 1H), 6.91 (dd, _J_ = 8.5, 2.8 Hz, 1H), 6.61 (d, _J_ = 8.7 Hz, 1H), 4.05 (s, 2H), 3.88 (s, 3H), 2.85 (q, _J_ = 10.6 Hz, 2H), 2.63 (t, _J_ = 8.3 Hz, 2H), 2.50 (t, _J_ = 6.7 Hz, 2H), 1.85 (q, _J_ = 9.6 Hz, 1H), 1.70 – 1.58 (m, 3H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.94, 160.13, 157.84, 141.89, 141.47, 140.19, 130.30, 129.98, 129.54, 127.72, 127.08, 126.92, 121.22, 119.61, 117.42, 112.94, 112.91, 75.74, 65.74, 55.47, 48.40, 35.29, 32.18, 26.85, 22.27, 14.97. HRMS $[C_{30}H_{33}NO_6S+Na]^+$: 558.19208 calculated, 558.19171 found.

_N_-((2,2-Dimethylchroman-6-yl)sulfonyl)-_N_-(1-(4'-methoxy-[1,1'-biphenyl]-4-yl)cyclobutyl)glycine (14)



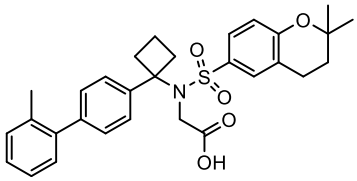
The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), (4-methoxyphenyl)boronic acid (15.5 mg, 0.102 mmol, 1.3 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (8.0 mg, 0.015 mmol, 19%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.51 (m, 2H), 7.45 (s, 4H), 7.22 (dd, _J_ = 8.7, 2.4 Hz, 1H), 7.01 – 6.95 (m, 3H), 6.63 (d, _J_ = 8.7 Hz, 1H), 4.05 (s, 2H), 3.86 (s, 3H), 2.90 – 2.79 (m, 2H), 2.65 – 2.56 (m, 2H), 2.51 (t, _J_ = 6.7 Hz, 2H), 1.89 – 1.79 (m, 1H), 1.70 – 1.57 (m, 3H), 1.23 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.65, 159.42, 157.74, 140.59, 139.88, 132.88, 130.66, 129.52, 128.11, 127.73, 127.05, 126.33, 121.20, 117.37, 114.39, 75.70, 65.69, 55.51, 48.27, 35.20, 32.19, 26.85, 22.26, 14.93. HRMS $[C_{30}H_{33}NO_6S+Na]^+$: 558.19208 calculated, 558.19175 found.

_N_-(1-(2'-Chloro-[1,1'-biphenyl]-4-yl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (15)



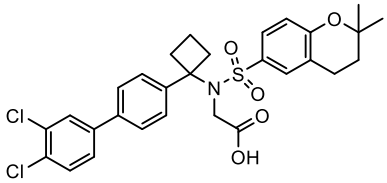
The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), (2-chlorophenyl)boronic acid (18.5 mg, 0.118 mmol, 1.5 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (28 mg, 0.052 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.46 (m, 3H), 7.39 (d, _J_ = 8.4 Hz, 2H), 7.36 – 7.27 (m, 4H), 7.05 (dd, _J_ = 8.7, 2.1 Hz, 1H), 6.62 (d, _J_ = 8.7 Hz, 1H), 4.03 (s, 2H), 2.94 – 2.82 (m, 2H), 2.71 – 2.60 (m, 4H), 1.91 – 1.80 (m, 1H), 1.73 (t, _J_ = 6.7 Hz, 2H), 1.69 – 1.62 (m, 1H), 1.28 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.71, 157.87, 141.73, 139.87, 138.60, 132.50, 131.59, 130.62, 130.26, 129.76, 129.49, 128.79, 127.11, 127.07, 126.93, 121.12, 117.69, 75.77, 65.86, 48.24, 35.08, 32.30, 26.94, 22.38, 15.01. HRMS $[C_{29}H_{30}ClNO_5S+Na]^+$: 562.14254 calculated, 562.14210 found.

_N_-((2,2-Dimethylchroman-6-yl)sulfonyl)-_N_-(1-(2'-methyl-[1,1'-biphenyl]-4-yl)cyclobutyl)glycine (16)



The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), _o_-tolylboronic acid (11 mg, 0.079 mmol, 1 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (24 mg, 0.046 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.45 (m, 2H), 7.33 (d, _J_ = 2.4 Hz, 1H), 7.30 – 7.20 (m, 6H), 7.11 (dd, _J_ = 8.7, 2.4 Hz, 1H), 6.62 (d, _J_ = 8.7 Hz, 1H), 4.03 (s, 2H), 2.95 – 2.82 (m, 2H), 2.71 – 2.56 (m, 4H), 2.31 (s, 3H), 1.89 – 1.79 (m, 1H), 1.74 (t, _J_ = 6.7 Hz, 2H), 1.70 – 1.59 (m, 1H), 1.29 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.93, 157.83, 141.26, 141.20, 140.79, 135.43, 130.90, 130.60, 129.98, 129.77, 129.20, 127.49, 127.10, 126.94, 125.96, 121.11, 117.57, 75.77, 65.87, 48.16, 34.99, 32.30, 26.94, 22.39, 20.75, 14.98. HRMS $[C_{30}H_{33}NO_5S+Na]^+$: 542.19716 calculated, 542.19694 found.

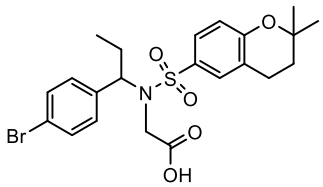
_N_-(1-(3',4'-Dichloro-[1,1'-biphenyl]-4-yl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (17)



The title compound was synthesized according to general procedure **K** using _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq), (3,4-dichlorophenyl)boronic acid (22.5 mg, 0.118 mmol, 1.5 eq), K_2CO_3 (54.4 mg, 0.393 mmol, 5 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (3.2 mg, 3.9 µmol, 0.05 eq). Total time: 2 h at 85 °C. Preparative HPLC afforded the product as a white solid (24 mg, 0.042 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, _J_ = 2.1 Hz, 1H), 7.55 – 7.47

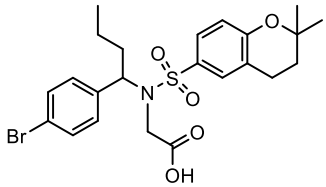
(m, 3H), 7.47 – 7.38 (m, 3H), 7.22 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.06 (d, *J* = 2.3 Hz, 1H), 6.63 (d, *J* = 8.7 Hz, 1H), 4.08 (s, 2H), 2.91 – 2.79 (m, 2H), 2.64 – 2.50 (m, 4H), 1.91 – 1.78 (m, 1H), 1.67 (t, *J* = 6.7 Hz, 2H), 1.65 – 1.57 (m, 1H), 1.27 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.95, 157.78, 142.43, 140.48, 137.80, 133.07, 131.78, 130.98, 130.93, 129.40, 128.88, 128.01, 127.00, 126.69, 126.33, 121.13, 117.46, 75.77, 65.60, 48.20, 35.04, 32.25, 26.87, 22.33, 14.85. HRMS $[C_{29}H_{29}Cl_2NO_5S+Na]^+$: 596.10357 calculated, 596.10313 found.

N-(1-(4-Bromophenyl)propyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (18)



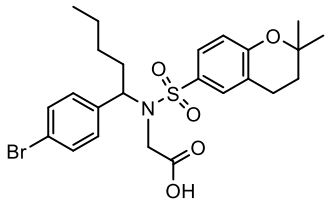
The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(4-bromophenyl)propyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65a**, 10 mg, 0.020 mmol, 1 eq) and 2 M aq. NaOH (39 µL, 0.078 mmol, 4 eq). Total time: 2 h at rt. Preparative HPLC afforded the product as a white powder (5 mg, 0.01 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.45 (s, 1H), 7.39 – 7.33 (m, 2H), 6.97 (d, *J* = 8.1 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 1H), 4.72 (t, *J* = 7.7 Hz, 1H), 3.99 – 3.71 (m, 2H), 2.74 (t, *J* = 6.7 Hz, 2H), 1.90 (p, *J* = 7.5 Hz, 2H), 1.83 (t, *J* = 6.7 Hz, 2H), 1.36 (s, 6H), 0.80 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.34, 136.70, 131.76, 130.14, 130.00, 129.82, 127.40, 122.27, 121.64, 117.96, 75.97, 62.27, 45.39, 32.34, 26.93, 25.01, 22.40, 11.37. HRMS $[C_{22}H_{26}BrNO_5S+Na]^+$: 518.06073/520.05862 calculated, 518.06075/520.05854 found.

N-(1-(4-Bromophenyl)butyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (19)



The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(4-bromophenyl)butyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65b**, 55.0 mg, 0.105 mmol, 1 eq) and 2 M aq. NaOH (0.21 mL, 0.42 mmol, 4 eq). Total time: 2 h at rt. Preparative HPLC afforded the product as a white powder (28 mg, 0.055 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.52 (d, *J* = 2.4 Hz, 1H), 7.41 – 7.33 (m, 2H), 7.04 – 6.97 (m, 2H), 6.83 (d, *J* = 8.7 Hz, 1H), 4.81 (dd, *J* = 9.3, 6.0 Hz, 1H), 4.01 – 3.72 (m, 2H), 2.76 (t, *J* = 6.7 Hz, 2H), 1.92 – 1.80 (m, 3H), 1.77 – 1.67 (m, 1H), 1.36 (s, 6H), 1.29 – 1.12 (m, 2H), 0.83 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.98, 158.28, 136.76, 131.71, 130.26, 130.12, 129.92, 127.44, 122.27, 121.56, 117.90, 75.93, 60.00, 44.79, 33.58, 32.34, 26.91, 22.41, 19.74, 13.81. HRMS $[C_{23}H_{28}BrNO_5S+Na]^+$: 532.07638/534.07428 calculated, 532.07636/534.07413 found.

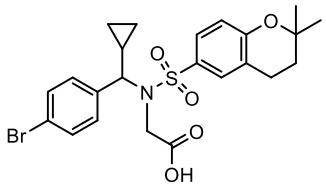
N-(1-(4-Bromophenyl)pentyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (20)



The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(4-bromophenyl)pentyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65c**, 39 mg, 0.072 mmol, 1 eq) and 2 M aq. NaOH (139 µL, 0.278 mmol, 3.9 eq). Total time: 2 h at rt. Silica gel column chromatography (2-10% MeOH in DCM) afforded the product as a white powder (10.7 mg, 20.4 µmol, 29%). ^{1}H NMR (400 MHz,

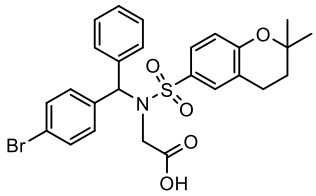
$CDCl_3$) δ 7.57 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.35 (m, 2H), 7.07 – 7.01 (m, 2H), 6.84 (d, *J* = 8.6 Hz, 1H), 4.83 (dd, *J* = 9.3, 6.1 Hz, 1H), 3.98 – 3.73 (m, 2H), 2.77 (t, *J* = 6.8 Hz, 2H), 1.91 – 1.69 (m, 4H), 1.36 (s, 6H), 1.30 – 1.06 (m, 4H), 0.80 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.88, 158.42, 136.79, 133.59, 131.83, 130.13, 129.94, 127.50, 122.38, 121.62, 118.00, 75.99, 60.46, 44.89, 32.35, 31.21, 28.76, 26.96, 26.93, 22.46, 14.02. HRMS $[C_{24}H_{30}BrNO_5S+Na]^+$: 546.09203/548.08993 calculated, 546.09222/548.09010 found.

_N_-((4-Bromophenyl)(cyclopropyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (21)



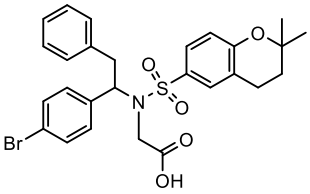
The title compound was synthesized according to general procedure **J** using methyl *N*-((4-bromophenyl)(cyclopropyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65d**, 38 mg, 0.073 mmol, 1 eq) and 2 M aq. NaOH (141 µL, 0.282 mmol, 3.9 eq). Total time: 2 h at rt. Silica gel column chromatography (2-10% MeOH in DCM) afforded the product as a white powder (8.2 mg, 0.016 mmol, 22%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.41 – 7.31 (m, 3H), 7.12 (d, *J* = 8.3 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 1H), 4.17 (d, *J* = 3.8 Hz, 1H), 4.16 – 3.86 (m, 2H), 2.77 – 2.65 (m, 2H), 1.83 (t, *J* = 6.7 Hz, 2H), 1.36 (s, 3H), 1.35 (s, 3H), 1.23 – 1.17 (m, 1H), 0.79 – 0.68 (m, 1H), 0.68 – 0.58 (m, 1H), 0.46 – 0.36 (m, 1H), 0.33 – 0.24 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.43, 138.03, 131.59, 129.94, 129.75, 127.37, 122.02, 121.60, 117.97, 114.17, 76.02, 66.17, 46.36, 32.32, 26.94, 22.41, 14.35, 6.37, 5.88. HRMS $[C_{23}H_{26}BrNO_5S+Na]^+$: 530.06073/532.05863 calculated, 530.06076/532.05860 found.

_N_-((4-Bromophenyl)(phenyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (22)



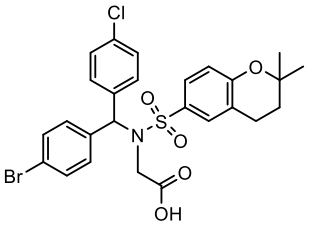
The title compound was synthesized according to general procedure **J** using methyl *N*-((4-bromophenyl)(phenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)-glycinate (**65e**, 34 mg, 0.061 mmol, 1 eq) and 1 M aq. NaOH (237 µL, 0.237 mmol, 3.9 eq). Total time: 4 h at rt. Preparative HPLC afforded the product as a white powder (9.5 mg, 0.017 mmol, 29%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.55 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.39 – 7.32 (m, 3H), 7.26 – 7.19 (m, 3H), 7.05 – 6.99 (m, 2H), 6.98 – 6.92 (m, 2H), 6.77 (d, *J* = 8.7 Hz, 1H), 6.20 (s, 1H), 4.04 (s, 2H), 2.63 (t, *J* = 6.7 Hz, 2H), 1.80 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.46, 158.33, 137.45, 137.31, 131.54, 130.67, 130.28, 129.51, 129.08, 128.66, 128.30, 127.53, 122.04, 121.41, 117.77, 75.93, 64.61, 46.74, 32.32, 26.94, 26.90, 22.33. HRMS $[C_{26}H_{26}BrNO_5S+Na]^+$: 566.06073/568.05864 calculated, 566.06065/568.05846 found.

_N_-(1-(4-Bromophenyl)-2-phenylethyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (23)



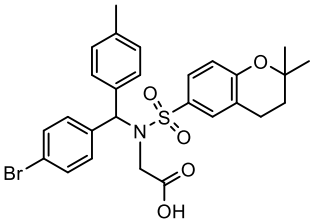
The title compound was synthesized according to general procedure **J** using methyl _N_-(1-(4-bromophenyl)-2-phenylethyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65f**, 83.2 mg, 0.145 mmol, 1 eq) and 2 M aq. NaOH (291 µL, 0.582 mmol, 4 eq). Total time: 4 h at rt. Silica gel column chromatography (30-70% dist. EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product as a white powder (35 mg, 0.063 mmol, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, _J_ = 8.7, 2.4 Hz, 1H), 7.48 (d, _J_ = 2.3 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.20 – 7.09 (m, 3H), 7.07 – 6.99 (m, 4H), 6.80 (d, _J_ = 8.7 Hz, 1H), 5.14 (dd, _J_ = 9.8, 5.6 Hz, 1H), 4.15 – 3.74 (m, 2H), 3.28 – 3.15 (m, 2H), 2.73 (t, _J_ = 6.7 Hz, 2H), 1.81 (t, _J_ = 6.7 Hz, 2H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.01, 158.34, 137.24, 135.82, 131.67, 130.53, 130.01, 129.94, 129.13, 128.61, 127.41, 126.70, 122.45, 121.57, 118.00, 75.96, 61.79, 45.39, 37.97, 32.30, 27.00, 26.87, 22.41. HRMS $[C_{27}H_{28}BrNO_5S+Na]^+$: 580.07638/582.07430 calculated, 580.07598/582.07388 found.

_N_-((4-Bromophenyl)(4-chlorophenyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (24)



The title compound was synthesized according to general procedure **J** using methyl _N_-((4-bromophenyl)(4-chlorophenyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65g**, 63.2 mg, 0.107 mmol, 1 eq) and 2 M aq. NaOH (213 µL, 0.426 mmol, 4 eq). Total time: 4 h at rt. Silica gel column chromatography (30-70% dist. EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product as a white powder (37 mg, 0.064 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, _J_ = 8.7, 2.4 Hz, 1H), 7.38 – 7.31 (m, 3H), 7.23 – 7.16 (m, 2H), 7.02 – 6.96 (m, 2H), 6.96 – 6.90 (m, 2H), 6.77 (d, _J_ = 8.7 Hz, 1H), 6.14 (s, 1H), 4.03 (s, 2H), 2.63 (t, _J_ = 6.7 Hz, 2H), 1.80 (t, _J_ = 6.7 Hz, 2H), 1.35 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.36, 158.34, 136.75, 136.13, 134.15, 131.66, 130.67, 130.42, 130.15, 129.54, 128.72, 127.43, 122.30, 121.44, 117.81, 75.97, 64.01, 46.72, 32.28, 26.90, 26.87, 22.32. HRMS $[C_{26}H_{25}BrClNO_5S+Na]^+$: 600.02176/602.01948 calculated, 600.02139/602.01905 found.

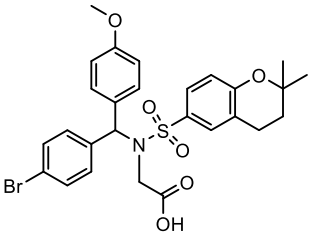
_N_-((4-Bromophenyl)(_p_-tolyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (25)



The title compound was synthesized according to general procedure **J** using methyl _N_-((4-bromophenyl)(_p_-tolyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65h**, 67.5 mg, 0.118 mmol, 1 eq) and 2 M aq. NaOH (236 µL, 0.472 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (30-70% dist. EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product as a white powder (46 mg, 0.083 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, _J_ = 8.6, 2.4 Hz, 1H), 7.38 (d, _J_ = 2.3 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.03 (d, _J_ = 7.9 Hz, 2H), 6.99 – 6.94 (m, 2H), 6.92 – 6.86

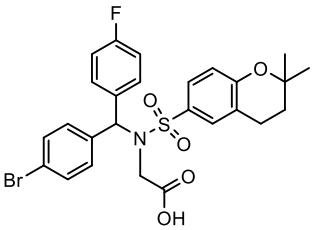
(m, 2H), 6.76 (d, *J* = 8.6 Hz, 1H), 6.14 (s, 1H), 4.02 (s, 2H), 2.63 (t, *J* = 6.7 Hz, 2H), 2.29 (s, 3H), 1.80 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.62, 158.20, 138.14, 137.58, 134.35, 131.40, 130.54, 130.24, 129.62, 129.30, 129.05, 127.49, 121.83, 121.34, 117.68, 75.86, 64.36, 46.68, 32.30, 26.90, 26.83, 22.30, 21.16. HRMS $[C_{27}H_{28}BrNO_5S+Na]^+$: 580.07638/582.07430 calculated, 580.07598/582.07388 found.

_N_-((4-Bromophenyl)(4-methoxyphenyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (26)



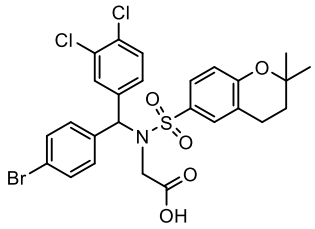
The title compound was synthesized according to general procedure **J** using methyl *N*-((4-bromophenyl)(4-methoxyphenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)-glycinate (**65i**, 96.8 mg, 0.164 mmol, 1 eq) and 2 M aq. NaOH (329 µL, 0.658 mmol, 4 eq). Total time: 4 h at rt. The obtained residue was purified by silica gel column chromatography (30-70% dist. EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product as a white powder (61.0 mg, 0.106 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.37 (d, *J* = 2.4 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.01 – 6.89 (m, 4H), 6.80 – 6.70 (m, 3H), 6.12 (s, 1H), 4.10 – 3.94 (m, 2H), 3.75 (s, 3H), 2.70 – 2.57 (m, 2H), 1.80 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.55, 159.44, 158.18, 137.69, 131.40, 130.55, 130.36, 130.21, 129.67, 129.34, 127.43, 121.73, 121.34, 117.68, 113.91, 75.87, 64.14, 55.36, 46.64, 32.27, 26.89, 26.83, 22.28. HRMS $[C_{27}H_{28}BrNO_6S+Na]^+$: 596.07129/598.06923 calculated, 596.07091/598.06877 found.

_N_-((4-Bromophenyl)(4-fluorophenyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (27)



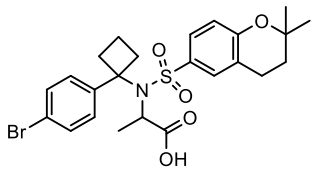
The title compound was synthesized according to general procedure **J** using methyl *N*-((4-bromophenyl)(4-fluorophenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)-glycinate (**65j**, 36 mg, 0.062 mmol, 1 eq) and 2 M aq. NaOH (125 µL, 0.250 mmol, 4 eq). Total time: 4 h at rt. Silica gel column chromatography (30-70% dist. EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product as a white powder (22 mg, 0.040 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.38 – 7.31 (m, 3H), 7.08 – 6.97 (m, 2H), 6.99 – 6.86 (m, 4H), 6.78 (d, *J* = 8.6 Hz, 1H), 6.17 (s, 1H), 4.15 – 3.93 (m, 2H), 2.64 (t, *J* = 6.8 Hz, 2H), 1.80 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.19, 162.49 (d, $J_{C\text{-}F}$ = 248.1 Hz), 158.35, 137.03, 133.36 (d, $J_{C\text{-}F}$ = 3.2 Hz), 131.64, 130.97 (d, $J_{C\text{-}F}$ = 8.2 Hz), 130.50, 130.18, 129.56, 127.45, 122.17, 121.44, 117.81, 115.53 (d, $J_{C\text{-}F}$ = 21.6 Hz), 75.97, 63.95, 46.61, 32.29, 26.91, 26.86, 22.32. HRMS $[C_{26}H_{25}BrFNO_5S+Na]^+$: 584.05131/586.04923 calculated, 584.05116/586.04896 found.

_N_-((4-Bromophenyl)(3,4-dichlorophenyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (28)



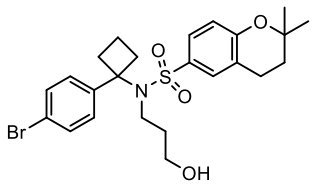
The title compound was synthesized according to general procedure **J** using methyl _N_-((4-bromophenyl)(3,4-dichlorophenyl)methyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (**65k**, 22 mg, 0.036 mmol, 1 eq) and 2 M aq. NaOH (71 µL, 0.14 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (30-70% dist. EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product as a white powder (13.4 mg, 21.8 µmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, _J_ = 8.6 Hz, 1H), 7.39 (d, _J_ = 7.2 Hz, 3H), 7.31 (d, _J_ = 8.2 Hz, 1H), 7.09 (d, _J_ = 1.9 Hz, 1H), 6.97 – 6.89 (m, 3H), 6.79 (d, _J_ = 8.5 Hz, 1H), 6.12 (s, 1H), 4.04 (s, 2H), 2.66 (t, _J_ = 6.0 Hz, 2H), 1.81 (t, _J_ = 6.6 Hz, 2H), 1.35 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.14, 158.57, 137.97, 136.05, 132.82, 132.37, 131.92, 130.81, 130.50, 130.07, 129.31, 128.17, 127.48, 122.75, 121.61, 117.93, 76.08, 63.64, 46.66, 32.27, 26.95, 22.38. HRMS $[C_{26}H_{24}BrCl_2NO_5S+NH_4]^+$: 629.02739/631.02520 calculated, 629.02721/631.02471 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)alanine (29)



The title compound was synthesized according to general procedure **J** using methyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)alaninate (**66a**, 23 mg, 0.043 mmol, 1 eq) and 2 M aq. NaOH (86 µL, 0.17 mmol, 4 eq). Total time: overnight at rt. Preparative HPLC afforded the product (9.0 mg, 0.017 mmol, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, _J_ = 8.7, 2.5 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.37 – 7.30 (m, 2H), 7.17 (d, _J_ = 2.4 Hz, 1H), 6.76 (d, _J_ = 8.7 Hz, 1H), 4.24 (q, _J_ = 7.1 Hz, 1H), 3.04 – 2.90 (m, 2H), 2.69 (t, _J_ = 6.7 Hz, 2H), 2.48 – 2.38 (m, 2H), 1.83 (t, _J_ = 6.8 Hz, 2H), 1.80 – 1.71 (m, 1H), 1.54 (d, _J_ = 7.2 Hz, 3H), 1.53 – 1.41 (m, 1H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.27, 157.84, 142.22, 132.55, 131.25, 129.72, 129.67, 127.01, 121.56, 121.21, 117.65, 75.89, 65.83, 56.22, 35.33, 34.89, 32.41, 27.58, 26.96, 22.42, 18.02, 15.12. HRMS $[C_{24}H_{28}BrNO_5S+Na]^+$: 544.07638/546.07428 calculated, 544.07626/546.07410 found.

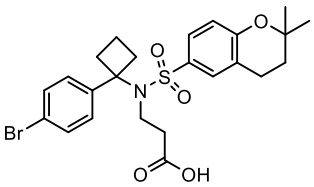
_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-(3-hydroxypropyl)-2,2-dimethylchromane-6-sulfonamide (30)



The title compound was synthesized according to general procedure **J** using 3-((_N_-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane)-6-sulfonamido)propyl acetate (**66b**, 58.0 mg, 0.105 mmol, 1 eq) and 2 M aq. NaOH (0.2 mL, 0.4 mmol, 3.8 eq). Total time: 4 h at rt. Silica gel column chromatography (20-30 % EtOAc in _n_-pentane) afforded the product (48 mg, 0.094 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 4H), 7.33 (dd, _J_ = 8.7, 2.5 Hz, 1H), 6.99 (d, _J_ = 2.5 Hz, 1H), 6.73 (d, _J_ = 8.6 Hz, 1H), 3.61 (q, _J_ = 4.7 Hz, 2H), 3.37 (t, _J_ = 7.1 Hz, 2H), 2.75 – 2.63 (m, 4H), 2.57 – 2.46 (m, 2H), 2.08 (t, _J_ = 4.5 Hz, 1H), 1.81 (t, _J_ = 6.7 Hz, 2H), 1.77 – 1.69 (m, 3H), 1.60 – 1.50 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (101 MHz,

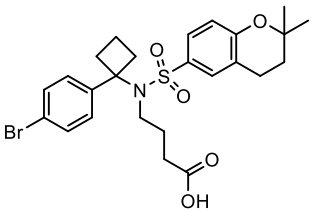
$CDCl_3$) δ 157.47, 142.95, 131.95, 131.31, 129.11, 128.90, 126.49, 121.33, 117.48, 75.75, 65.23, 59.66, 44.20, 35.14, 33.99, 32.41, 26.97, 22.41, 14.52. HRMS $[C_{24}H_{30}BrNO_4S+Na]^+$: 530.09711/532.09501 calculated, 530.09721/532.09496 found.

3-((*N*-(1-(4-Bromophenyl)cyclobutyl)-2,2-dimethylchromane)-6-sulfonamido)propanoic acid (31)



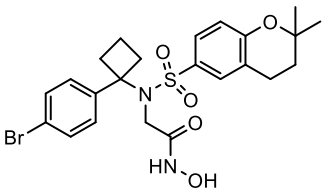
Jones reagent was added dropwise to a solution of *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(3-hydroxypropyl)-2,2-dimethylchromane-6-sulfonamide (**30**, 10 mg, 0.020 mmol, 1 eq) in acetone (0.60 mL, 0.33 M) at 0 °C until the color remained orange. 2-propanol was added until the orange color disappeared and only green suspension remained. The mixture was filtered and the filtrate was diluted in water and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (50% EtOAc in *n*-pentane) to afford the product (6.0 mg, 0.011 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.39 (m, 2H), 7.39 – 7.34 (m, 2H), 7.30 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 1H), 3.51 – 3.42 (m, 2H), 2.78 – 2.70 (m, 2H), 2.69 – 2.60 (m, 4H), 2.59 – 2.52 (m, 2H), 1.81 (t, *J* = 6.7 Hz, 2H), 1.78 – 1.71 (m, 1H), 1.63 – 1.51 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.33, 157.66, 142.32, 131.50, 131.13, 129.03, 126.70, 121.58, 121.40, 117.50, 75.81, 65.46, 42.39, 36.02, 35.35, 32.40, 26.99, 22.40, 14.46. HRMS $[C_{24}H_{28}BrNO_5S+Na]^+$: 544.07638/546.07428 calculated, 544.07639/546.07415 found.

4-((*N*-(1-(4-Bromophenyl)cyclobutyl)-2,2-dimethylchromane)-6-sulfonamido)butanoic acid (32)



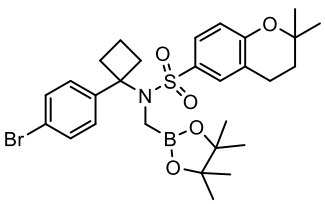
The title compound was synthesized according to general procedure **J** using methyl 4-((*N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane)-6-sulfonamido)butanoate (**66c**, 22 mg, 0.040 mmol, 1 eq) and 2 M aq. NaOH (0.1 mL, 0.2 mmol, 5 eq). Total time: 4 h at rt. Silica gel column chromatography (20-40% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (12 mg, 0.022 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.33 (m, 4H), 7.28 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.89 (d, *J* = 2.4 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 1H), 3.26 – 3.19 (m, 2H), 2.76 – 2.66 (m, 2H), 2.64 (t, *J* = 6.8 Hz, 2H), 2.58 – 2.49 (m, 2H), 2.35 (t, *J* = 6.9 Hz, 2H), 2.01 – 1.91 (m, 2H), 1.80 (t, *J* = 6.7 Hz, 2H), 1.77 – 1.73 (m, 1H), 1.62 – 1.52 (m, 1H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.52, 157.46, 142.66, 131.64, 131.31, 129.14, 129.00, 126.65, 121.33, 121.28, 117.36, 75.74, 65.38, 46.52, 35.49, 32.41, 31.12, 26.97, 26.26, 22.39, 14.67. HRMS $[C_{25}H_{30}BrNO_5S+Na]^+$: 558.09203/560.08994 calculated, 558.09202/560.08982 found.

2-((*N*-(1-(4-Bromophenyl)cyclobutyl)-2,2-dimethylchromane)-6-sulfonamido)-*N*-hydroxyacetamide (33)



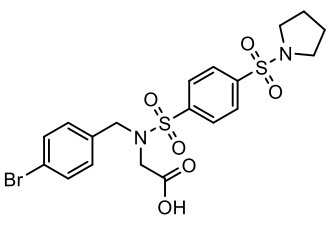
To a solution of *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycine (**6**, 40 mg, 0.079 mmol, 1 eq) in anhydrous DMF (1 mL, 0.08 M) was added EDCI (22.6 mg, 0.118 mmol, 1.5 eq), HOBt (15.9 mg, 0.118 mmol, 1.5 eq), DIPEA (41 µL, 0.236 mmol, 3 eq) and hydroxylammonium chloride (8.2 mg, 0.12 mmol, 1.5 eq). The reaction was stirred at rt for overnight. The mixture was diluted in water and extracted 3× with EtOAc. Combined organic layers were washed with sat. $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by preparative HPLC to afford the product (11 mg, 0.021 mmol, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (bs, 1H), 7.42 – 7.36 (m, 2H), 7.31 – 7.25 (m, 2H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.66 – 6.55 (m, 2H), 3.91 (s, 2H), 2.82 – 2.67 (m, 2H), 2.65 – 2.51 (m, 4H), 1.88 – 1.80 (m, 1H), 1.77 (t, *J* = 6.7 Hz, 2H), 1.61 – 1.51 (m, 1H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.19, 158.25, 140.98, 131.53, 129.36, 129.05, 128.34, 127.18, 121.92, 121.60, 117.53, 76.01, 65.66, 48.68, 35.75, 32.27, 26.96, 22.28, 14.87. HRMS $[C_{23}H_{27}BrN_2O_5S+H]^+$: 523.08968/525.08758 calculated, 523.08964/525.08757 found.

***N*-(1-(4-Bromophenyl)cyclobutyl)-2,2-dimethyl-*N*-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methyl)chromane-6-sulfonamide (34)**



The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (**60c**, 92 mg, 0.20 mmol, 1 eq), 2-(bromomethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (90 mg, 0.41 mmol, 2 eq) and BEMP (1 M in hexane, 0.41 mL, 0.41 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (105 mg, 0.178 mmol, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.39 – 7.34 (m, 4H), 7.16 (d, *J* = 2.4 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 1H), 2.78 – 2.71 (m, 2H), 2.66 (t, *J* = 6.6 Hz, 2H), 2.64 (s, 2H), 2.48 – 2.42 (m, 2H), 1.80 (t, *J* = 6.8 Hz, 2H), 1.73 – 1.67 (m, 1H), 1.54 – 1.47 (m, 1H), 1.33 (s, 6H), 1.26 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.25, 141.74, 131.83, 130.99, 129.33, 127.32, 121.14, 120.83, 117.10, 84.06, 75.53, 65.43, 34.60, 32.50, 31.93, 26.98, 24.95, 22.37, 14.38. HRMS $[C_{28}H_{37}BBrNO_5S+Na]^+$: 612.15661/614.15415 calculated, 612.15545/614.15335.

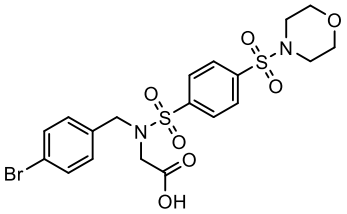
***N*-(4-Bromobenzyl)-*N*-((4-(pyrrolidin-1-ylsulfonyl)phenyl)sulfonyl)glycine (35)**



The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((4-(pyrrolidin-1-ylsulfonyl)phenyl)sulfonyl)glycinate (**68a**, 10 mg, 0.019 mmol, 1 eq) and 2 M aq. NaOH (38 µL, 0.076 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (2-4% MeOH in DCM) afforded the product (2.0 mg, 3.9 µmol, 21%). ^{1}H NMR (400 MHz, DMSO) δ 8.11 – 8.03 (m,

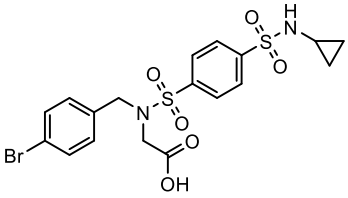
2H), 7.98 – 7.91 (m, 2H), 7.54 – 7.47 (m, 2H), 7.25 – 7.17 (m, 2H), 4.47 (s, 2H), 3.78 (s, 2H), 3.21 – 3.13 (m, 4H), 1.72 – 1.61 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 169.56, 143.79, 139.59, 135.55, 131.34, 130.40, 128.15, 127.97, 120.81, 50.37, 48.49, 47.95, 24.81. HRMS $[C_{19}H_{21}BrN_2O_6S_2+H]^+$: 517.00972/519.00754 calculated , 517.00956/519.00730 found.

***N*-(4-Bromobenzyl)-*N*-((4-(morpholinosulfonyl)phenyl)sulfonyl)glycine (36)**



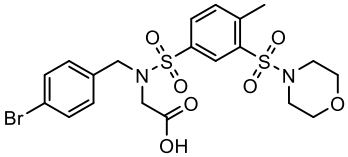
The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((4-(morpholinosulfonyl)-phenyl)sulfonyl)glycinate (**68b**, 10 mg, 0.018 mmol, 1 eq) and 2 M aq. NaOH (36 µL, 0.072 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (30-70% dist. EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (4.2 mg, 7.9 µmol, 43%). ^{1}H NMR (400 MHz, DMSO) δ 8.14 – 8.07 (m, 2H), 7.93 – 7.87 (m, 2H), 7.55 – 7.47 (m, 2H), 7.24 – 7.18 (m, 2H), 4.46 (s, 2H), 3.96 (s, 2H), 3.69 – 3.62 (m, 4H), 2.95 – 2.88 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 169.54, 143.81, 138.15, 135.21, 131.37, 130.45, 128.50, 128.19, 120.98, 65.31, 50.85, 48.04, 45.88. HRMS $[C_{19}H_{21}BrN_2O_7S_2+H]^+$: 533.00463/535.00247 calculated, 533.00438/535.00232 found.

***N*-(4-Bromobenzyl)-*N*-((4-(*N*-cyclopropylsulfamoyl)phenyl)sulfonyl)glycine (37)**



The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((4-(N-cyclopropylsulfamoyl)-phenyl)sulfonyl)glycinate (**68c**, 15 mg, 0.028 mmol, 1 eq) and 2 M aq. NaOH (56.0 µL, 0.112 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (30-70% dist. EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (8.0 mg, 0.016 mmol, 56%). ^{1}H NMR (400 MHz, DMSO) δ 8.18 (d, *J* = 2.7 Hz, 1H), 8.12 – 8.05 (m, 2H), 8.01 – 7.93 (m, 2H), 7.55 – 7.47 (m, 2H), 7.26 – 7.19 (m, 2H), 4.44 (s, 2H), 3.94 (s, 2H), 2.18 – 2.08 (m, 1H), 0.56 – 0.45 (m, 2H), 0.43 – 0.35 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.53, 143.95, 142.97, 135.25, 131.36, 130.46, 128.06, 127.69, 120.97, 50.83, 47.95, 24.10, 5.20. HRMS $[C_{18}H_{19}BrN_2O_6S_2+H]^+$: 502.99407/504.99189 calculated, 502.99354/504.99125 found.

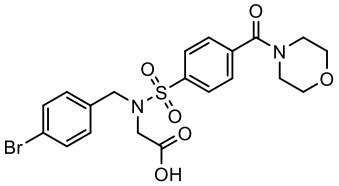
***N*-(4-bromobenzyl)-*N*-((4-methyl-3-(morpholinosulfonyl)phenyl)sulfonyl)glycine (38)**



The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((4-methyl-3-(morpholinosulfonyl)phenyl)sulfonyl)glycinate (**68d**, 9.2 mg, 0.016 mmol, 1 eq) and 2 M aq. NaOH (32 µL, 0.064 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (30-50 % dist. EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (8.6 mg, 0.016 mmol, 96%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 8.31 (d, *J* = 2.0 Hz, 1H), 7.97 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.56 – 7.53 (m, 1H), 7.48 – 7.41 (m, 2H), 7.21 – 7.13 (m, 2H), 4.45 (s, 2H), 3.92 (s, 2H), 3.75 – 3.68 (m, 4H), 3.22 – 3.15 (m, 4H), 2.70 (s, 3H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 170.52, 143.90, 139.00,

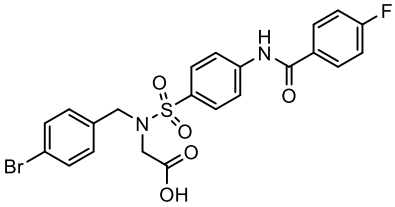
137.00, 134.52, 134.38, 132.45, 132.10, 130.83, 129.70, 122.76, 66.83, 51.18, 47.12, 45.89, 21.10. HRMS $[C_{20}H_{23}BrN_2O_7S_2+H]^+$: 547.02028/549.01813 calculated, 547.02015/549.01787 found.

_N_-(4-Bromobenzyl)-_N_-((4-(morpholine-4-carbonyl)phenyl)sulfonyl)glycine (39)



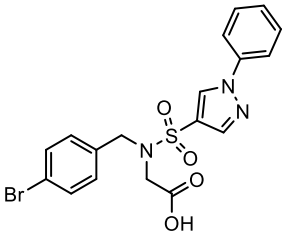
The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((4-(morpholine-4-carbonyl)phenyl)sulfonyl)glycinate (**68e**, 27 mg, 0.054 mmol, 1 eq) and 2 M aq. NaOH (113 µL, 0.216 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (30-70% dist. EtOAc in *n*-heptane) afforded the product (14 mg, 0.027 mmol, 51%). ^{1}H NMR (400 MHz, DMSO) δ 7.89 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.50 (d, *J* = 7.8 Hz, 2H), 7.22 (d, *J* = 7.8 Hz, 2H), 4.41 (s, 2H), 3.92 (s, 2H), 3.75 – 3.18 (m, 8H). ^{13}C NMR (101 MHz, DMSO) δ 169.67, 167.67, 140.21, 139.70, 135.36, 131.25, 130.46, 127.68, 127.30, 120.84, 65.99, 50.85, 48.46, 47.53, 41.96. HRMS $[C_{20}H_{21}BrN_2O_6S+H]^+$: 497.03765/499.03554 calculated, 497.03737/499.03520 found.

_N_-(4-Bromobenzyl)-_N_-((4-(4-fluorobenzamido)phenyl)sulfonyl)glycine (40)



The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((4-(4-fluorobenzamido)phenyl)sulfonyl)glycinate (**68f**, 101 mg, 0.188 mmol, 1 eq), 2 M aq. NaOH (564 µL, 0.564 mmol, 3 eq). Total time: 2 h at rt. Silica gel column chromatography (20-40% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (23 mg, 0.044 mmol, 23%). ^{1}H NMR (400 MHz, DMSO) δ 10.65 (s, 1H), 8.11 – 8.02 (m, 2H), 8.02 – 7.95 (m, 2H), 7.88 – 7.80 (m, 2H), 7.56 – 7.49 (m, 2H), 7.49 – 7.35 (m, 2H), 7.28 – 7.20 (m, 2H), 4.38 (s, 2H), 3.84 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.75, 165.01, 164.34 (d, J_{C-F} = 251.1), 143.17, 135.68, 133.59, 131.30, 130.94 (d, J_{C-F} = 2.8 Hz), 130.68 (d, J_{C-F} = 9.2 Hz), 130.50, 128.23, 120.81, 119.83, 115.51 (d, J_{C-F} = 22.1 Hz), 50.81, 47.96. HRMS $[C_{22}H_{18}BrFN_2O_5S+H]^+$: 521.01766/523.01556 calculated, 521.01750/523.01528 found.

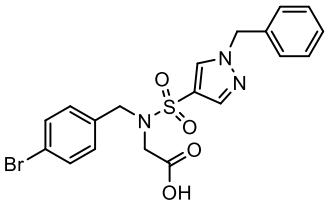
_N_-(4-Bromobenzyl)-_N_-((1-phenyl-1_H_-pyrazol-4-yl)sulfonyl)glycine (41)



The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((1-phenyl-1*H*-pyrazol-4-yl)sulfonyl)glycinate (**68g**, 74.5 mg, 0.160 mmol, 1 eq), 2 M aq. NaOH (481 µL, 0.481 mmol, 3 eq). Total time: 2 h at rt. Silica gel column chromatography (20-40% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (58.3 mg, 0.129 mmol, 81%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 8.46 (d, *J* = 0.7 Hz, 1H), 7.99 (d, *J* = 0.7 Hz, 1H), 7.73 – 7.61 (m, 2H), 7.52 – 7.34 (m, 5H), 7.24 – 7.16 (m, 2H), 4.42 (s, 2H), 3.94 (s, 2H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 170.98, 140.42, 139.37, 134.53, 132.23, 130.68, 130.06, 130.01, 128.50, 123.94, 122.54, 120.27, 51.04,

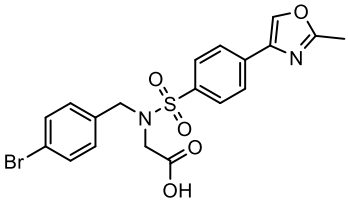
47.40. HRMS $[C_{18}H_{16}BrN_3O_4S+H]^+$: 450.01177/452.00965 calculated, 450.01152/452.00927 found.

N-((1-Benzyl-1*H*-pyrazol-4-yl)sulfonyl)-*N*-(4-bromobenzyl)glycine (42)



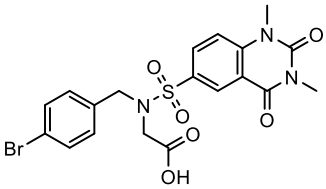
The title compound was synthesized according to general procedure **J** from methyl *N*-((1-benzyl-1*H*-pyrazol-4-yl)sulfonyl)-*N*-(4-bromobenzyl)glycinate (**68h**, 18 mg, 0.038 mmol, 1 eq) and 2 M aq. NaOH (56.5 µL, 0.113 mmol, 3 eq). Total time: 2 h at rt. Silica gel column chromatography (10-40% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (13 mg, 0.028 mmol,74%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.95 – 7.82 (m, 2H), 7.50 – 7.11 (m, 9H), 5.32 (s, 2H), 4.38 (s, 2H), 3.90 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 170.65, 139.21, 134.65, 134.10, 131.86, 131.80, 130.23, 129.09, 128.73, 128.03, 122.13, 122.00, 56.60, 50.51, 46.84. HRMS $[C_{19}H_{18}BrN_3O_4S+H]^+$: 464.02742/ 466.02530 calculated, 464.02752/466.02538 found.

N-(4-Bromobenzyl)-*N*-((4-(2-methyloxazol-4-yl)phenyl)sulfonyl)glycine (43)



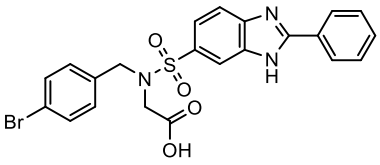
The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((4-(2-methyloxazol-4-yl)phenyl)sulfonyl)glycinate (**68i**, 19 mg, 0.039 mmol, 1 eq) and 2 M aq. NaOH (78.0 µL, 0.156 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (20-50% dist. EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (8.8 mg, 0.019 mmol, 48%). ^{1}H NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 7.99 – 7.82 (m, 4H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.23 (d, *J* = 7.8 Hz, 2H), 4.40 (s, 2H), 3.88 (s, 2H), 2.50 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.65, 161.99, 138.36, 138.08, 136.73, 135.49, 135.24, 131.27, 130.45, 127.74, 125.44, 120.82, 50.80, 47.84, 13.55. HRMS $[C_{19}H_{17}BrN_2O_5S+H]^+$: 465.01143/467.00932 calculated, 465.01124/467.00905 found.

N-(4-Bromobenzyl)-*N*-((1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)sulfonyl)glycine (44)



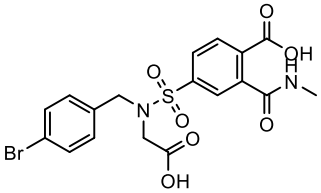
The title compound was synthesized according to general procedure **J** using methyl *N*-(4-bromobenzyl)-*N*-((1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)sulfonyl)-glycinate (**68j**, 31 mg, 0.061 mmol, 1 eq) and 2 M aq. NaOH (122 µL, 0.244 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (20-80% dist. EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (19 mg, 0.039 mmol, 63%). ^{1}H NMR (400 MHz, DMSO) δ 8.29 (d, *J* = 2.3 Hz, 1H), 8.16 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.24 – 7.16 (m, 2H), 4.40 (s, 2H), 3.95 (s, 2H), 3.56 (s, 3H), 3.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.79, 160.50, 150.45, 143.18, 135.25, 133.50, 133.23, 131.33, 130.48, 127.16, 120.88, 115.83, 114.60, 50.88, 48.19, 31.14, 28.39. HRMS $[C_{19}H_{18}BrN_3O_6S+H]^+$: 496.01725/498.01515 calculated, 496.01697/498.01467 found.

_N_-(4-Bromobenzyl)-_N_-((2-phenyl-1_H_-benzo[_d_]imidazol-6-yl)sulfonyl)glycine (45)



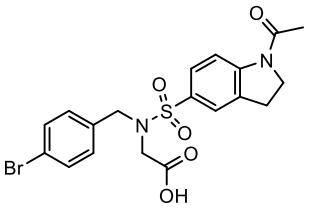
The title compound was synthesized according to general procedure **J** using methyl _N_-(4-bromobenzyl)-_N_-((2-phenyl-1_H_-benzo[_d_]imidazol-6-yl)sulfonyl)glycinate (**68k**, 17 mg, 0.034 mmol, 1 eq) and 2 M aq. NaOH (68.0 µL, 0.136 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (4-10% MeOH in DCM) afforded the product (12 mg, 0.024 mmol, 70%). ^{1}H NMR (400 MHz, MeOD) δ 8.18 – 8.08 (m, 3H), 7.83 – 7.70 (m, 2H), 7.63 – 7.52 (m, 3H), 7.43 – 7.35 (m, 2H), 7.19 – 7.11 (m, 2H), 4.52 (s, 2H), 3.87 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 156.46, 136.64, 135.74, 132.63, 132.16, 131.59, 130.33, 130.31, 128.16, 123.12, 122.64, 116.91, 116.03, 52.00, 49.07. HRMS $[C_{22}H_{18}BrN_3O_4S+H]^+$: 500.02742/502.02531 calculated, 500.02718/502.02500 found.

4-(_N_-(4-Bromobenzyl)-_N_-(carboxymethyl)sulfamoyl)-2-(methylcarbamoyl)benzoic acid (46)



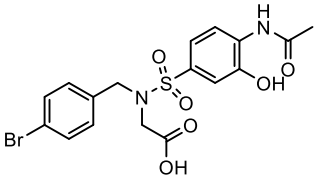
The title compound was synthesized according to general procedure **J** using methyl _N_-(4-bromobenzyl)-_N_-((2-methyl-1,3-dioxoisoindolin-5-yl)sulfonyl)glycinate (**68l**, 15 mg, 0.032 mmol, 1 eq) and 2 M aq. NaOH (64.0 µL, 0.128 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (50-80% dist. EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product (4.0 mg, 8.2 µmol, 27%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 8.06 (d, _J_ = 8.2 Hz, 1H), 7.95 (d, _J_ = 8.1 Hz, 1H), 7.88 (s, 1H), 7.46 (d, _J_ = 8.0 Hz, 2H), 7.18 (d, _J_ = 8.0 Hz, 2H), 4.46 (s, 2H), 3.91 (s, 2H), 2.91 (s, 3H). HRMS $[C_{18}H_{17}BrN_2O_7S+H]^+$: 485.00126/486.99916 calculated, 485.00106/486.99887 found.

_N_-((1-Acetylindolin-5-yl)sulfonyl)-_N_-(4-bromobenzyl)glycine (47)



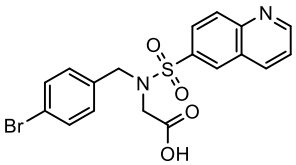
The title compound was synthesized according to general procedure **J** using methyl _N_-((1-acetylindolin-5-yl)sulfonyl)-_N_-(4-bromobenzyl)glycinate (**68m**, 35 mg, 0.072 mmol, 1 eq) and 2 M aq. NaOH (143 µL, 0.286 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (30-80% EtOAc in _n_-pentane with a drop of conc. HCl) afforded the product (22 mg, 0.047 mmol, 66%). ^{1}H NMR (400 MHz, DMSO) δ 8.13 (d, _J_ = 8.4 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.55 – 7.47 (m, 2H), 7.26 – 7.19 (m, 2H), 4.34 (s, 2H), 4.17 (t, _J_ = 8.7 Hz, 2H), 3.84 (s, 2H), 3.19 (t, _J_ = 8.6 Hz, 2H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.85, 169.60, 135.68, 133.08, 131.24, 131.12, 130.53, 127.40, 123.84, 120.77, 115.22, 50.95, 48.68, 48.10, 27.03, 24.13. HRMS $[C_{19}H_{19}BrN_2O_5S+H]^+$: 467.02708/469.02497 calculated, 467.02731/469.02518 found.

_N_-((4-Acetamido-3-hydroxyphenyl)sulfonyl)-_N_-(4-bromobenzyl)glycine (48)



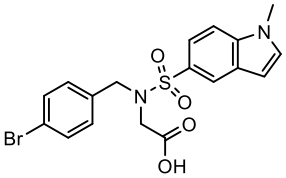
The title compound was synthesized according to general procedure **J** using methyl _N_-(4-bromobenzyl)-_N_-((2-methylbenzo[_d_]oxazol-6-yl)sulfonyl)glycinate (**68n**, 28 mg, 0.062 mmol, 1 eq) and 2 M aq. NaOH (124 µL, 0.247 mmol, 4 eq). Total time: 2 h at rt. Preparative HPLC afforded the product (18 mg, 0.039 mmol, 64%). ^{1}H NMR (400 MHz, MeOD) δ 8.14 – 8.10 (m, 1H), 7.43 – 7.38 (m, 2H), 7.34 – 7.29 (m, 2H), 7.14 – 7.09 (m, 2H), 4.41 (s, 2H), 3.82 (s, 2H), 2.21 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.45, 171.27, 147.57, 135.07, 135.02, 132.31, 131.45, 130.90, 122.54, 121.26, 119.75, 114.46, 51.40, 47.45, 24.19. HRMS $[C_{17}H_{17}BrN_2O_6S+H]^+$: 457.00635/459.00432 calculated, 457.00606/459.00392 found.

_N_-(4-Bromobenzyl)-_N_-(quinolin-6-ylsulfonyl)glycine (49)



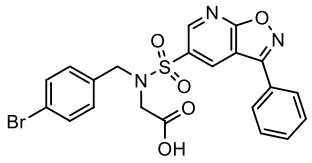
The title compound was synthesized according to general procedure **J** using methyl _N_-(4-bromobenzyl)-_N_-(quinolin-6-ylsulfonyl)glycinate (**68o**, 24 mg, 0.053 mmol, 1 eq) and 2 M aq. NaOH (106 µL, 0.212 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (3-10% MeOH in DCM) afforded the product (18 mg, 0.043 mmol, 80%). ^{1}H NMR (400 MHz, DMSO) δ 9.05 (dd, _J_ = 4.2, 1.7 Hz, 1H), 8.60 (d, _J_ = 2.0 Hz, 1H), 8.57 (dd, _J_ = 8.2, 2.1 Hz, 1H), 8.18 – 8.07 (m, 2H), 7.67 (dd, _J_ = 8.3, 4.2 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.26 – 7.19 (m, 2H), 4.50 (s, 2H), 3.80 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 170.21, 153.12, 148.65, 137.61, 137.57, 135.71, 131.29, 130.43, 130.11, 128.64, 126.95, 126.65, 122.74, 120.74, 50.39, 48.52. HRMS $[C_{18}H_{15}BrN_2O_4S+H]^+$: 435.00087/436.99874 calculated, 435.00082/436.99860 found.

_N_-(4-Bromobenzyl)-_N_-((1-methyl-1_H_-indol-5-yl)sulfonyl)glycine (50)



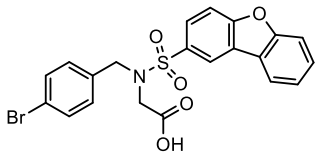
The title compound was synthesized according to general procedure **J** from methyl _N_-(4-bromobenzyl)-_N_-((1-methyl-1_H_-indol-5-yl)sulfonyl)glycinate (**68p**, 29 mg, 0.064 mmol, 1 eq) and 2 M aq. NaOH (96.0 µL, 0.192 mmol, 3 eq). Total time: 2 h at rt. Silica gel column chromatography (3-10% MeOH in DCM) afforded the product (28 mg, 0.064 mmol, 100%). ^{1}H NMR (400 MHz, MeOD) δ 8.15 (d, _J_ = 1.8 Hz, 1H), 7.65 (dd, _J_ = 8.7, 1.8 Hz, 1H), 7.46 (d, _J_ = 8.7 Hz, 1H), 7.40 – 7.33 (m, 2H), 7.26 (d, _J_ = 3.1 Hz, 1H), 7.11 – 7.05 (m, 2H), 6.59 (d, _J_ = 3.1 Hz, 1H), 4.41 (s, 2H), 3.85 (s, 2H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.56, 139.29, 135.38, 132.27, 132.17, 130.98, 130.23, 128.61, 122.44, 122.25, 120.70, 110.43, 103.21, 51.57, 47.65, 33.26. HRMS $[C_{18}H_{17}BrN_2O_4S+H]^+$: 437.01652/439.01439 calculated, 437.01633/439.01414 found.

_N_-(4-Bromobenzyl)-_N_-((3-phenylisoxazolo[5,4-_b_]pyridin-5-yl)sulfonyl)glycine (51)



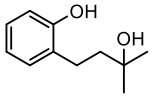
The title compound was synthesized according to general procedure **J** using methyl _N_-(4-bromobenzyl)-_N_-((3-phenylisoxazolo[5,4-_b_]pyridin-5-yl)sulfonyl)glycinate (**68q**, 29 mg, 0.056 mmol, 1 eq) and 2 M aq. NaOH (112 µL, 0.224 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (20-50% EtOAc in _n_-pentane with a drop of conc. HCl) afforded the product (21 mg, 0.042 mmol, 74%). ^{1}H NMR (500 MHz, MeOD+$CDCl_3$) δ 9.10 (d, _J_ = 2.2 Hz, 1H), 8.84 (d, _J_ = 2.2 Hz, 1H), 7.98 – 7.91 (m, 2H), 7.63 – 7.55 (m, 3H), 7.46 – 7.39 (m, 2H), 7.22 – 7.15 (m, 2H), 4.45 (s, 2H), 4.02 (s, 2H). ^{13}C NMR (126 MHz, MeOD+$CDCl_3$) δ 171.27, 170.54, 158.57, 150.38, 134.34, 133.82, 133.78, 132.33, 131.82, 130.64, 129.89, 128.14, 127.55, 122.78, 112.43, 51.07, 47.23. HRMS [$C_{21}H_{16}BrN_3O_5S$+H]$^+$: 502.00668/504.00458 calculated, 502.00654/504.00443 found.

_N_-(4-bromobenzyl)-_N_-(dibenzo[_b_,_d_]furan-2-ylsulfonyl)glycine (52)



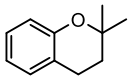
The title compound was synthesized according to general procedure **J** using methyl _N_-(4-bromobenzyl)-_N_-(dibenzo[_b_,_d_]furan-3-ylsulfonyl)glycinate (**68r**, 40 mg, 0.081 mmol, 1 eq), 1 M aq. NaOH (244 µL, 0.244 mmol, 3 eq). Total time: 2 h at rt. Silica gel column chromatography (20-40% EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product (17 mg, 0.035 mmol, 44%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 8.49 (d, _J_ = 2.0 Hz, 1H), 8.02 (dt, _J_ = 7.8, 1.0 Hz, 1H), 7.97 (dd, _J_ = 8.7, 2.0 Hz, 1H), 7.68 (d, _J_ = 8.7 Hz, 1H), 7.64 – 7.57 (m, 1H), 7.57 – 7.49 (m, 1H), 7.45 – 7.36 (m, 3H), 7.18 – 7.11 (m, 2H), 4.49 (s, 2H), 3.94 (s, 2H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 171.00, 158.75, 157.56, 134.78, 132.28, 130.82, 129.03, 127.00, 125.35, 124.13, 123.60, 122.55, 121.71, 121.40, 112.70, 112.41, 51.35, 47.32. HRMS [$C_{21}H_{16}BrNO_5S$+H]$^+$: 474.00053/475.99842 calculated, 474.00033/475.99815 found.

2-(3-Hydroxy-3-methylbutyl)phenol (53)



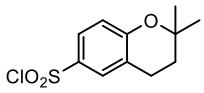
To a solution of dihydrocoumarin (1.2 g, 8.1 mmol, 1 eq) in anhydrous THF (12 mL, 0.7 M) at 0 °C was added CH_3MgBr (3 M in Et_2O, 9.0 mL, 27 mmol, 3.3 eq) dropwise. The reaction was slowly warmed to rt and stirred overnight. The mixture was treated with ice chips containing 5 mL of 1 M aq. H_2SO_4 and extracted 3× with Et_2O. Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (10-30% EtOAc in _n_-pentane) to afford the product (1.22 g, 6.77 mmol, 84%). ^{1}H NMR (400 MHz, MeOD) δ 7.05 (dd, _J_ = 7.3, 1.4 Hz, 1H), 6.97 (td, _J_ = 8.0, 1.7 Hz, 1H), 6.76 – 6.69 (m, 2H), 2.70 – 2.62 (m, 2H), 1.77 – 1.70 (m, 2H), 1.25 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 156.10, 130.78, 130.27, 127.72, 120.58, 115.85, 71.59, 44.92, 29.13, 26.20.

2,2-Dimethylchromane (54)



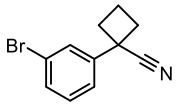
A solution of 2-(3-hydroxy-3-methylbutyl)phenol (**53**, 1.13 g, 6.27 mmol, 1 eq) in 15% aq. H_2SO_4 (10 mL) and toluene (3 mL) was refluxed for 2 h. After completion, the mixture was cooled to rt and extracted 3× with Et_2O. Combined organic layers were washed with water, 2 M aq. NaOH and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-pentane) to afford the product as a white solid (0.73 g, 4.5 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 – 6.99 (m, 2H), 6.84 – 6.73 (m, 2H), 2.80 – 2.72 (m, 2H), 1.83 – 1.70 (m, 2H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.08, 129.55, 127.34, 120.99, 119.70, 117.33, 74.16, 32.89, 26.99, 22.55.

2,2-Dimethylchromane-6-sulfonyl chloride (55)



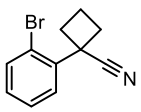
To a solution of 2,2-dimethylchromane (**54**, 560 mg, 3.45 mmol, 1 eq) in anhydrous DCM (15 mL) was added HSO_3Cl (230 µL, 3.45 mmol, 1 eq) dropwise at 0 °C. The reaction was stirred for 2 h and more HSO_3Cl (230 µL, 3.45 mmol, 1 eq) was added dropwise at 0°C. The mixture was slowly warm to rt and stirred overnight. The DCM layer was collected and concentrated to afford the product (570 mg, 2.19 mmol, 63%). ^{1}H NMR (400 MHz, DMSO) δ 7.34 (d, *J* = 2.1 Hz, 1H), 7.28 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.63 (d, *J* = 8.4 Hz, 1H), 2.71 (t, *J* = 6.9 Hz, 2H), 1.74 (t, *J* = 6.8 Hz, 2H), 1.25 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 154.27, 138.52, 127.21, 124.92, 120.08, 116.05, 74.60, 32.08, 26.64, 21.91.

1-(3-Bromophenyl)cyclobutane-1-carbonitrile (56a)



The title compound was synthesized according to general procedure **A** using 2-(3-bromophenyl)acetonitrile (1.20 g, 6.12 mmol 1 eq), 1,3-dibromopropane (1.24 g, 6.12 mmol, 1 eq), TBABr (0.02 g, 0.06 mmol, 0.01 eq) and KOH (2.75 g, 49.0 mmol, 8 eq). Total time: 1.5 h at 100 °C to reflux. Silica gel column chromatography (4-5% Et_2O in *n*-pentane) afforded the product (880 mg, 3.73 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (t, *J* = 1.9 Hz, 1H), 7.45 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H), 7.35 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 2.87 – 2.76 (m, 2H), 2.66 – 2.54 (m, 2H), 2.51 – 2.36 (m, 1H), 2.14 – 2.01 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.01, 131.10, 130.58, 128.88, 124.40, 123.83, 123.09, 39.85, 34.62, 17.11.

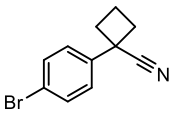
1-(2-Bromophenyl)cyclobutane-1-carbonitrile (56b)



To a suspension of KOH (600 mg, 10.7 mmol, 4.2 eq) in DMSO (4.5 mL, 0.57 M) was added a solution of 2-(2-bromophenyl)acetonitrile (500 mg, 2.55 mmol, 1 eq) and 1,3-dibromopropane (520 mg, 2.55 mmol, 1 eq) dropwise. The reaction was stirred at rt for 5 h. The mixture was diluted in water and extracted 3× with EtOAc. Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (4-5% Et_2O in *n*-pentane) to afford the product (0.20 g, 0.85 mmol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.37

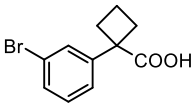
– 7.31 (m, 1H), 7.28 – 7.23 (m, 1H), 7.19 (ddd, *J* = 7.9, 7.3, 1.7 Hz, 1H), 3.03 – 2.93 (m, 2H), 2.70 – 2.60 (m, 2H), 2.55 – 2.41 (m, 1H), 2.00 – 1.89 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.59, 134.23, 129.78, 127.96, 127.71, 123.06, 122.69, 41.71, 34.22, 17.13.

1-(4-Bromophenyl)cyclobutane-1-carbonitrile (56c)



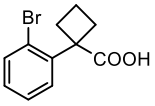
The title compound was synthesized according to general procedure **A** using 2-(4-bromophenyl)acetonitrile (1.0 g, 5.1 mmol, 1 eq), 1,3-dibromopropane (1.0 g, 5.1 mmol, 1 eq), TBABr (20 mg, 0.051 mmol, 0.1 eq) and KOH (2.30 g, 40.8 mmol, 8 eq). Total time: 1.5 h at 100 °C to reflux. Silica gel column (4-5% Et_2O in *n*-pentane) afforded the product (620 mg, 2.63 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.44 (m, 2H), 7.33 – 7.22 (m, 2H), 2.87 – 2.75 (m, 2H), 2.63 – 2.51 (m, 2H), 2.48 – 2.34 (m, 1H), 2.14 – 2.00 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.78, 131.99, 127.35, 123.82, 121.82, 39.70, 34.55, 17.00.

1-(3-Bromophenyl)cyclobutane-1-carboxylic acid (57a)



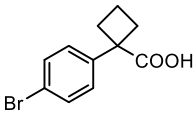
The title compound was synthesized according to general procedure **B** using 1-(3-bromophenyl)cyclobutane-1-carbonitrile (**56a**, 460 mg, 1.93 mmol, 1 eq) and KOH (650 mg, 11.6 mmol. 6 eq). Total time: overnight at reflux. Silica gel column chromatography (30% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (450 mg, 1.75 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.92 (bs, 1H), 7.43 (t, *J* = 1.8 Hz, 1H), 7.37 (dt, *J* = 7.1, 1.8 Hz, 1H), 7.26 – 7.14 (m, 2H), 2.91 – 2.76 (m, 2H), 2.60 – 2.43 (m, 2H), 2.18 – 2.01 (m, 1H), 1.94 – 1.80 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.25, 145.46, 130.14, 130.00, 129.83, 125.28, 122.59, 52.08, 32.36, 16.76.

1-(2-Bromophenyl)cyclobutane-1-carboxylic acid (57b)



The title compound was synthesized according to general procedure **B** using 1-(2-bromophenyl)cyclobutane-1-carbonitrile (**56b,** 0.12 g, 0.52 mmol, 1 eq) and KOH (0.18 g, 3.1 mmol, 6 eq). Total time: 2 h at reflux. Silica gel column chromatography (30% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (66 mg, 0.26 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.37 – 7.29 (m, 2H), 7.12 (ddd, *J* = 7.9, 6.5, 2.6 Hz, 1H), 2.97 – 2.87 (m, 2H), 2.64 – 2.53 (m, 2H), 2.37 – 2.23 (m, 1H), 1.91 – 1.79 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.16, 142.47, 133.63, 128.67, 128.63, 127.27, 123.30, 53.86, 32.18, 16.85.

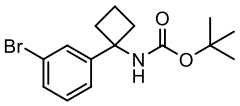
1-(4-Bromophenyl)cyclobutane-1-carboxylic acid (57c)



The title compound was synthesized according to general procedure **B** using 1-(4-bromophenyl)cyclobutane-1-carbonitrile (**56c**, 1.19 g, 5.04 mmol, 1 eq) and KOH (1.70 g, 30.2 mmol, 6 eq). Total time: 2 h at reflux. Silica gel column chromatography (20% EtOAc in *n*-pentane with 0.1% TFA) afforded the product (1.18 g, 4.63 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.46 (bs, 1H), 7.48 – 7.41 (m, 2H), 7.20 – 7.14 (m, 2H), 2.88 – 2.78 (m, 2H), 2.53 – 2.41 (m, 2H), 2.14 – 2.01 (m, 1H), 1.92 – 1.80 (m,

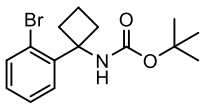
1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.35, 142.21, 131.54, 128.39, 121.04, 51.92, 32.33, 16.70.

tert-Butyl (1-(3-bromophenyl)cyclobutyl)carbamate (58a)



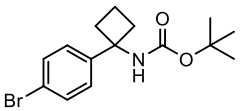
The title compound was synthesized according to general procedure **C** using 1-(3-bromophenyl)cyclobutane-1-carboxylic acid (**57a**, 200 mg, 0.784 mmol, 1 eq), diphenylphosphoryl azide (216 mg, 0.784 mmol, 1 eq) and Et_3N (120 µL, 0.862 mmol, 1.1 eq) in *t*-BuOH (10 mL, 0.08 M). Total time: 1 h at 30 °C and overnight at reflux. Silica gel column chromatography (5-7% EtOAc in *n*-pentane) afforded the product (178 mg, 0.546 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (t, *J* = 1.9 Hz, 1H), 7.43 – 7.29 (m, 2H), 7.20 (t, *J* = 7.8 Hz, 1H), 5.17 (bs, 1H), 2.60 – 2.28 (m, 4H), 2.17 – 2.03 (m, 1H), 1.94 – 1.80 (m, 1H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.24, 148.58, 129.86, 129.67, 128.85, 124.25, 122.44, 79.72, 58.84, 34.42, 28.42, 15.29. LC-MS $[C_{15}H_{20}BrNO_2+H]^+$: 326.08/328.07 calculated, 325.87/327.87 found.

tert-Butyl (1-(2-bromophenyl)cyclobutyl)carbamate (58b)



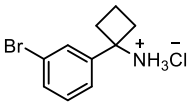
The title compound was synthesized according to general procedure **C** using 1-(2-bromophenyl)cyclobutane-1-carboxylic acid (**57b,** 55.0 mg, 0.216 mmol, 1 eq), diphenylphosphoryl azide (59.3 mg, 0.216 mmol, 1 eq) and Et_3N (33.0 µL, 0.237 mmol, 1.1 eq) in *t*-BuOH (2.75 mL, 0.08 M). Total time: 1 h at 30 °C and overnight at reflux. Silica gel column chromatography (5-7% EtOAc in *n*-pentane) afforded the product (29.4 mg, 0.112 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.47 (m, 2H), 7.35 – 7.24 (m, 1H), 7.08 (td, *J* = 7.6, 1.7 Hz, 1H), 5.56 (bs, 1H), 2.76 – 2.51 (m, 4H), 2.25 – 2.10 (m, 1H), 1.87 – 1.67 (m, 1H), 1.33 (s, 9H). LC-MS $[C_{15}H_{20}BrNO_2+H]^+$: 326.08/328.07 calculated, 325.80/327.80 found.

tert-Butyl (1-(4-bromophenyl)cyclobutyl)carbamate (58c)



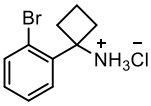
The title compound was synthesized according to general procedure **C** using 1-(4-bromophenyl)cyclobutane-1-carboxylic acid (**57c**, 1.2 g, 4.7 mmol, 1 eq), diphenylphosphoryl azide (1.0 mL, 4.7 mmol, 1 eq) and Et_3N (0.72 mL, 5.2 mmol, 1.1 eq) in *t*-BuOH (60 mL, 0.08 M) Total time: 1 h at 30 °C and overnight at reflux. Silica gel column chromatography (5-7% EtOAc in *n*-pentane) afforded the product (1.30 g, 3.98 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.41 (m, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 5.14 (bs, 1H), 2.58 – 2.35 (m, 4H), 2.16 – 2.06 (m, 1H), 1.92 – 1.77 (m, 1H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.34, 145.32, 131.31, 127.45, 120.48, 79.56, 58.77, 34.29, 28.45, 15.30. LC-MS $[C_{15}H_{20}BrNO_2+H]^+$: 326.08/328.07 calculated, 325.60/327.87 found.

1-(3-Bromophenyl)cyclobutan-1-aminium chloride (59a)



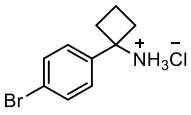
The title compound was synthesized according to general procedure **D** using *tert*-butyl (1-(3-bromophenyl)-cyclobutyl)carbamate (**58a**, 180 mg, 0.552 mmol, 1 eq) in 3 M aq. HCl in MeOH (5.5 mL, 0.1 M). Total time: 24 h at rt. The product was afforded after concentration (135 mg, 0.513 mmol, 93%). ^{1}H NMR (400 MHz, MeOD) δ 7.71 (t, *J* = 1.7 Hz, 1H), 7.58 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.56 – 7.52 (m, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 2.81 – 2.61 (m, 4H), 2.35 – 2.22 (m, 1H), 2.00 – 1.88 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 143.73, 133.02, 132.03, 130.46, 126.22, 123.93, 59.79, 33.19, 14.86. LC-MS $[C_{10}H_{12}BrN+H]^{+}$: 226.02/228.02 calculated, 226.00/228.00 found.

1-(2-Bromophenyl)cyclobutan-1-aminium chloride (59b)



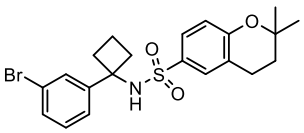
The title compound was synthesized according to general procedure **D** using *tert*-butyl (1-(2-bromophenyl)cyclobutyl)carbamate (**58b**, 29 mg, 0.089 mmol, 1 eq) in 3 M aq. HCl in MeOH (2.4 mL, 0.04 M). Total time: 24 h at rt. The product was afforded after concentration (26 mg, 0.098 mmol, quant.). ^{1}H NMR (400 MHz, MeOD) δ 7.70 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.35 (ddd, *J* = 8.0, 6.8, 2.3 Hz, 1H), 3.01 – 2.90 (m, 2H), 2.80 – 2.62 (m, 2H), 2.44 – 2.29 (m, 1H), 2.04 – 1.90 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 139.70, 135.66, 132.25, 130.47, 129.36, 122.71, 62.95, 34.14, 15.32. LC-MS $[C_{10}H_{12}BrN+H]^{+}$: 226.02/228.02 calculated, 226.00/227.93 found.

1-(4-Bromophenyl)cyclobutan-1-aminium chloride (59c)



The title compound was synthesized according to general procedure **D** using *tert*-butyl (1-(4-bromophenyl)cyclobutyl)carbamate (**58c**, 100 mg, 0.307 mmol) in 3 M aq. HCl in MeOH (1.2 mL, 0.26 M). Total time: 24 h at rt. The solvent was removed and the residue was washed with Et_2O to afford the product (80 mg, 0.31 mmol, 100%). ^{1}H NMR (400 MHz, MeOD) δ 7.68 – 7.60 (m, 2H), 7.52 – 7.44 (m, 2H), 2.81 – 2.59 (m, 4H), 2.35 – 2.20 (m, 1H), 2.01 – 1.87 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 140.50, 133.22, 129.38, 123.87, 59.84, 33.23, 14.81. LC-MS $[C_{10}H_{12}BrN+H]^{+}$: 226.02/228.02 calculated, 225.87/227.87 found.

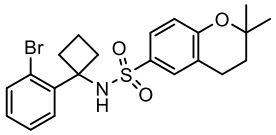
***N*-(1-(3-Bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (60a)**



The title compound was synthesized according to general procedure **G** using 1-(3-bromophenyl)-cyclobutan-1-aminium chloride (**59a**, 65 mg, 0.25 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonyl chloride (**55**, 129 mg, 0.495 mmol, 2 eq) and Et_3N (104 µL, 0.743 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-25% EtOAc in *n*-pentane) afforded the product (53.7 mg, 0.119 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.18 (m, 3H), 7.18 – 7.12 (m, 1H), 6.98 (t, *J* = 7.8 Hz, 1H), 6.92 (dd, *J* = 2.3, 1.1 Hz, 1H), 6.61 (d, *J* = 8.7 Hz, 1H), 5.45 (s, 1H), 2.62 – 2.46 (m, 6H), 2.13 – 2.02 (m, 1H), 1.80 – 1.67 (m, 3H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.38, 144.62, 131.66, 130.47, 129.81, 129.37, 129.01, 126.29, 125.65,

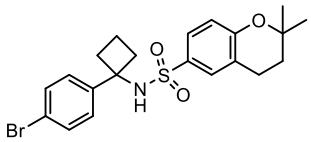
122.17, 120.65, 117.43, 75.58, 61.15, 35.72, 32.39, 27.06, 22.25, 15.36. LC-MS $[C_{21}H_{24}BrNO_3S+H]^+$: 450.07/452.07 calculated, 450.07 /452.00 found.

_N_-(1-(2-Bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (60b)



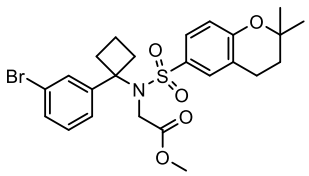
The title compound was synthesized according to general procedure **G** using 1-(2-bromophenyl)cyclobutan-1-aminium chloride (**59b**, 26 mg, 0.099 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonyl chloride (**55**, 51.6 mg, 0.198 mmol, 2 eq) and Et_3N (41.0 µL, 0.297 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-25% EtOAc in *n*-pentane) afforded the product (21 mg, 0.046 mmol, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.18 (td, *J* = 7.5, 1.3 Hz, 1H), 7.11 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.06 (dd, *J* = 7.9, 1.3 Hz, 1H), 6.93 (dt, *J* = 2.1, 0.9 Hz, 1H), 6.89 (ddd, *J* = 7.9, 7.3, 1.7 Hz, 1H), 6.46 (d, *J* = 8.6 Hz, 1H), 5.63 (s, 1H), 2.85 – 2.72 (m, 2H), 2.67 – 2.56 (m, 2H), 2.53 (t, *J* = 6.8 Hz, 2H), 2.41 – 2.26 (m, 1H), 1.82 – 1.75 (m, 1H), 1.72 (t, *J* = 6.8 Hz, 2H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.08, 140.13, 133.47, 131.02, 130.02, 128.65, 128.59, 126.70, 126.36, 122.23, 120.28, 117.02, 75.38, 63.63, 35.12, 32.40, 26.89, 22.20, 15.97. LC-MS $[C_{21}H_{24}BrNO_3S+H]^+$: 450.07/452.07 calculated, 450.07/452.07 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (60c)



The title compound was synthesized according to general procedure **G** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (**59c**, 300 mg, 1.15 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonyl chloride (**55**, 300 mg, 1.15 mmol, 1 eq) and Et_3N (1.0 mL, 5.7 mmol, 5 eq). Total time: overnight at rt. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (457 mg, 1.02 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.23 – 7.18 (m, 2H), 7.08 – 7.03 (m, 2H), 6.86 (d, *J* = 2.7 Hz, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 5.74 (s, 1H), 2.62 – 2.46 (m, 6H), 2.10 – 2.01 (m, 1H), 1.80 (t, 2H), 1.74 – 1.66 (m, 1H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.29, 141.35, 131.97, 130.78, 129.00, 126.25, 120.91, 120.63, 117.51, 75.63, 61.09, 35.73, 32.41, 26.94, 22.22, 15.32. LC-MS $[C_{21}H_{24}BrNO_3S+H]^+$: 450.07/452.07 calculated, 449.60/451.60 found.

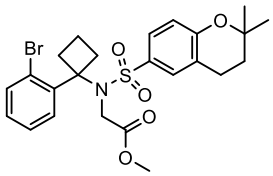
Methyl _N_-(1-(3-bromophenyl)cyclobutyl)-_N_-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (61a)



The title compound was synthesized according to general procedure **I** using *N*-(1-(3-bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (**60a**, 53.7 mg, 0.119 mmol, 1 eq), methyl 2-bromoacetate (23.0 µL, 0.238 mmol, 2 eq) and BEMP (1 M in hexane, 238 µL, 0.238 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (68 mg, 0.13 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, *J* = 1.9 Hz, 1H), 7.40 (ddd, *J* = 7.9, 2.0, 1.0 Hz, 1H), 7.36 – 7.31 (m, 2H), 7.13 (t, *J* = 7.9 Hz, 1H), 7.03 (dd, *J* = 2.3, 1.1 Hz, 1H), 6.69 (d, *J* = 8.7 Hz, 1H), 4.07 (s, 2H), 3.74 (s, 3H), 2.89 – 2.77 (m,

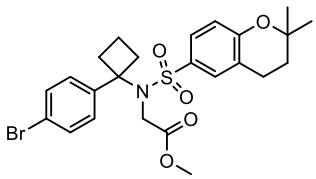
2H), 2.65 (t, *J* = 6.7 Hz, 2H), 2.51 – 2.43 (m, 2H), 1.89 – 1.74 (m, 3H), 1.64 – 1.52 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.83, 157.67, 145.19, 131.65, 130.72, 130.33, 129.67, 129.26, 126.81, 125.87, 122.40, 121.01, 117.40, 75.68, 65.30, 52.53, 48.00, 34.83, 32.38, 27.03, 22.36, 14.66. LC-MS $[C_{24}H_{28}BrNO_5S+Na]^+$: 544.08/546.07 calculated, 544.40/546.33 found.

Methyl *N*-(1-(2-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (61b)



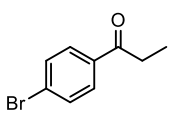
The title compound was synthesized according to general procedure **I** using *N*-(1-(2-bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (**60b**, 21 mg, 0.046 mmol, 1 eq), methyl 2-bromoacetate (8.7 µL, 0.092 mmol, 2 eq) and BEMP (1 M in hexane, 92 µL, 92 µmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (22 mg, 0.042 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.42 – 7.37 (m, 1H), 7.20 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.06 (td, *J* = 7.6, 1.6 Hz, 1H), 6.92 – 6.85 (m, 1H), 6.55 (dd, *J* = 2.3, 1.2 Hz, 1H), 6.49 (d, *J* = 8.7 Hz, 1H), 4.40 (s, 2H), 3.88 (s, 3H), 3.10 – 2.98 (m, 2H), 2.84 – 2.71 (m, 2H), 2.49 (t, *J* = 6.7 Hz, 2H), 1.85 – 1.75 (m, 1H), 1.72 (t, *J* = 6.8 Hz, 2H), 1.54 – 1.44 (m, 1H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.01, 157.17, 139.34, 134.42, 131.42, 130.94, 128.78, 128.44, 126.86, 126.20, 124.78, 120.28, 116.93, 75.79, 66.65, 52.55, 50.97, 35.36, 32.41, 26.82, 22.28, 15.06. LC-MS $[C_{24}H_{28}BrNO_5S+Na]^+$: 544.08/546.07 calculated, 544.33/546.33 found.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (61c)



The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (**60c**, 371 mg, 0.824 mmol, 1 eq), methyl 2-bromoacetate (161 µL, 1.65 mmol, 2 eq) and BEMP (1 M in hexane, 1.24 mL, 1.24 mmol, 1.5 eq). Total time: overnight at 80 °C. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product (416 mg, 0.796 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 5H), 6.97 (dd, *J* = 2.3, 1.1 Hz, 1H), 6.71 (d, *J* = 8.7 Hz, 1H), 4.05 (s, 2H), 3.73 (s, 3H), 2.86 – 2.74 (m, 2H), 2.64 (t, *J* = 6.7 Hz, 2H), 2.52 – 2.42 (m, 2H), 1.86 – 1.72 (m, 3H), 1.61 – 1.48 (m, 1H), 1.34 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.86, 157.60, 141.78, 131.74, 131.18, 129.30, 129.24, 126.80, 121.41, 121.05, 117.40, 75.72, 65.28, 52.50, 47.86, 34.85, 32.40, 26.95, 22.34, 14.67. LC-MS $[C_{24}H_{28}BrNO_5S+Na]^+$: 544.08/546.07 calculated, 544.07/546.07 found.

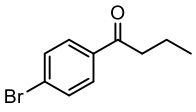
1-(4-Bromophenyl)propan-1-one (62a)



To a solution of 4-bromobenzonitrile (300 mg, 1.65 mmol, 1 eq) in anhydrous THF (5 mL, 0.3 M) under N_2 was added ethylmagnesium bromide (1 M in THF, 5 mL, 5 mmol, 3 eq) dropwise and the reaction was refluxed for 2 h. After

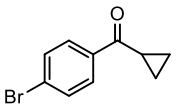
completion, the mixture was cooled down to rt and quenched with cold 2 M aq. HCl. The mixture was extracted 3× with EtOAc and combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (1% EtOAc in *n*-pentane) to afford the product (320 mg, 1.50 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.78 (m, 2H), 7.63 – 7.54 (m, 2H), 2.96 (q, *J* = 7.2 Hz, 2H), 1.21 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.69, 135.62, 131.88, 129.56, 128.01, 31.81, 8.16.

1-(4-Bromophenyl)butan-1-one (62b)



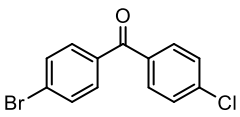
To a solution of 4-bromobenzonitrile (300 mg, 1.65 mmol, 1 eq) in anhydrous THF (5 mL, 0.3 M) under N_2 was added propylmagnesium bromide (2 M in THF, 2.5 mL, 5.0 mmol, 3 eq) dropwise and the reaction was refluxed for 2 h. After completion, the mixture was cooled down to rt and quenched with cold 2 M aq. HCl. The mixture was extracted 3× with EtOAc and combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (1% EtOAc in *n*-pentane) to afford the product (323 mg, 1.42 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.76 (m, 2H), 7.63 – 7.54 (m, 2H), 2.90 (t, *J* = 7.3 Hz, 2H), 1.75 (h, *J* = 7.4 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.23, 135.77, 131.84, 129.59, 127.99, 40.87, 17.66, 13.87.

(4-Bromophenyl)(cyclopropyl)methanone (62d)



To a solution of the 1,4-dibromobenzene (500 mg, 2.12 mmol, 1 eq) in anhydrous THF (5 mL, 2.4 M) at -78 °C was added *n*-BuLi (2.5 M in hexane, 890 µL, 2.23 mmol, 1.05 eq) dropwise and the mixture was stirred for 30 min. A solution of *N*-methoxy-*N*-methylcyclopropanecarboxamide (287 mg, 2.23 mmol, 1.05 eq) in anhydrous THF (2 mL) was added. The mixture was warmed to rt and stirred for 2 h. The reaction was quenched by H_2O (10 mL) and sat. NH_4Cl (10 mL). The aqueous layer was extracted 3× with EtOAc and combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (1% EtOAc in *n*-pentane) to afford the product (63 mg, 0.28 mmol, 13%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.85 (m, 2H), 7.63 – 7.58 (m, 2H), 2.61 (tt, *J* = 7.8, 4.5 Hz, 1H), 1.27 – 1.22 (m, 2H), 1.10 – 1.03 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.67, 136.74, 131.87, 129.65, 127.93, 17.22, 12.04.

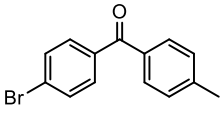
(4-Bromophenyl)(4-chlorophenyl)methanone (62g)



The title compound was synthesized according to general procedure **E** using 1-bromo-4-chlorobenzene (383 mg, 2.00 mmol, 1 eq), *n*-BuLi (2.5 M in hexane, 0.88 mL, 2.2 mmol, 1.1 eq), 4-bromobenzaldehyde (389 mg, 2.10 mmol, 1.05 eq), I_2 (812 mg, 3.20 mmol, 1.6 eq) and K_2CO_3 (829 mg, 6.00 mmol, 3 eq). Total time: 30 min at -78 °C, 1 h at rt and 3 h at reflux. Silica gel column chromatography (1-3% Et_2O in *n*-pentane) afforded the product (0.23 g, 0.77 mmol, 39%). ^{1}H NMR (850 MHz,

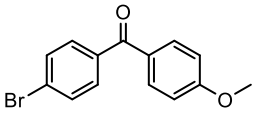
$CDCl_3$) δ 7.74 – 7.70 (m, 2H), 7.66 – 7.60 (m, 4H), 7.48 – 7.45 (m, 2H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 194.44, 139.26, 135.99, 135.48, 131.83, 131.51, 131.43, 128.86, 127.86. LC-MS $[C_{13}H_8BrClO+H]^+$: 294.95/296.95 calculated, 295.00/297.08 found.

(4-Bromophenyl)(*p*-tolyl)methanone (62h)



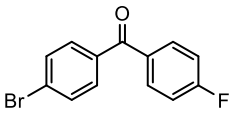
The title compound was synthesized according to general procedure **E** using 1-bromo-4-methylbenzene (342 mg, 2.00 mmol, 1 eq), *n*-BuLi (2.5 M in hexane, 0.88 mL, 2.2 mmol, 1.1 eq), 4-bromobenzaldehyde (389 mg, 2.10 mmol, 1.05 eq), I_2 (812 mg, 3.20 mmol, 1.6 eq) and K_2CO_3 (829 mg, 6.00 mmol, 3 eq). Total time: 30 min at -78 °C, 1 h at rt and 3 h at reflux. Silica gel column chromatography (0.5-1% Et_2O in *n*-pentane) afforded the product (0.24 g, 0.87 mmol, 43%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.66 (m, 3H), 7.66 – 7.61 (m, 3H), 7.31 – 7.27 (m, 2H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.47, 143.69, 136.75, 134.55, 131.64, 131.57, 130.29, 129.21, 127.29, 21.79. LC-MS $[C_{14}H_{11}BrO+H]^+$: 275.01/277.00 calculated, 275.08/277.08 found.

(4-Bromophenyl)(4-methoxyphenyl)methanone (62i)



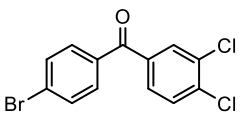
The title compound was synthesized according to general procedure **E** using 1-bromo-4-methoxybenzene (374 mg, 2.00 mmol, 1 eq), *n*-BuLi (2.5 M in hexane, 0.88 mL, 2.2 mmol, 1.1 eq), 4-bromobenzaldehyde (389 mg, 2.10 mmol, 1.05 eq), I_2 (812 mg, 3.20 mmol, 1.6 eq) and K_2CO_3 (829 mg, 6.00 mmol, 3 eq). Total time: 30 min at -78 °C, 1 h at rt and 3 h at reflux. Silica gel column chromatography (0-20% Et_2O in *n*-pentane) afforded the product (317 mg, 1.09 mmol, 54%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.76 (m, 2H), 7.66 – 7.58 (m, 4H), 6.99 – 6.94 (m, 2H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.50, 163.51, 137.09, 132.57, 131.60, 131.39, 129.81, 126.93, 113.79, 55.64. LC-MS $[C_{14}H_{11}BrO_2+H]^+$: 291.00/293.00 calculated, 291.08/293.08 found.

(4-Bromophenyl)(4-fluorophenyl)methanone (62j)



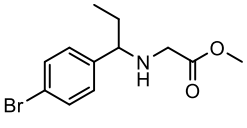
The title compound was synthesized according to general procedure **E** using 1,4-dibromobenzene (472 mg, 2.00 mmol, 1 eq), *n*-BuLi (2.5 M in hexane, 0.88 mL, 2.2 mmol, 1.1 eq), 4-fluorobenzaldehyde (261 mg, 2.10 mmol, 1.05 eq), I_2 (812 mg, 3.20 mmol, 1.6 eq) and K_2CO_3 (829 mg, 6.00 mmol, 3 eq). Total time: 30 min at -78 °C, 1 h at rt and 3 h at reflux. The crude was used without purification. LC-MS $[C_{13}H_8BrFO+H]^+$: 278.98/280.98 calculated, 279.00/281.08 found.

(4-Bromophenyl)(3,4-dichlorophenyl)methanone (62k)



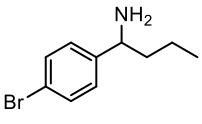
The title compound was synthesized according to general procedure **E** using 1,4-dibromobenzene (472 mg, 2.00 mmol, 1 eq), *n*-BuLi (2.5 M in hexane, 0.88 mL, 2.2 mmol, 1.1 eq), 3,4-dichlorobenzaldehyde (368 mg, 2.10 mmol, 1.05 eq), I_2 (812 mg, 3.20 mmol, 1.6 eq) and K_2CO_3 (829 mg, 6.00 mmol, 3 eq). Total time: 30 min at -78 °C, 1 h at rt and 3 h at reflux. The crude was used without purification.

Methyl (1-(4-bromophenyl)propyl)glycinate (63a)



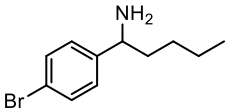
To a mixture of 1-(4-bromophenyl)propan-1-one (**62a**, 100 mg, 0.469 mmol, 1 eq) and 2-methoxy-2-oxoethan-1-aminium chloride (236 mg, 1.88 mmol, 4 eq) in anhydrous MeOH with 3 Å molecular sieves was added Et_3N (262 µL, 1.88 mmol, 4 eq) and AcOH (134 µL, 2.35 mmol, 5 eq). After 1 h, $NaBH_3CN$ (88.0 mg, 1.41 mmol, 3 eq) was added and the mixture was stirred at rt for overnight and refluxed for 24 h. The mixture was diluted in sat. $NaHCO_3$ and EtOAc and filtered through Celite. The filtrate was separated through funnel. The aqueous layer was extracted 2× with EtOAc and combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (20-30% EtOAc in *n*-pentane) to afford the product (100 mg, 0.349 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.41 (m, 2H), 7.21 – 7.13 (m, 2H), 3.69 (s, 3H), 3.49 (dd, *J* = 7.7, 5.9 Hz, 1H), 3.27 (d, *J* = 17.5 Hz, 1H), 3.16 (d, *J* = 17.5 Hz, 1H), 1.95 (s, 1H), 1.81 – 1.69 (m, 1H), 1.68 – 1.55 (m, 1H), 0.81 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 173.11, 142.26, 131.57, 129.36, 120.95, 63.93, 51.85, 48.59, 31.06, 10.62. LC-MS $[C_{12}H_{16}BrNO_2+H]^+$: 286.04/288.04 calculated, 286.00/288.00 found.

1-(4-Bromophenyl)butan-1-amine (63b)



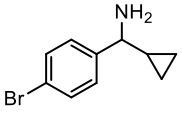
The title compound was synthesized according to general procedure **F** using 1-(4-bromophenyl)butan-1-one (**62b**, 0.10 g, 0.44 mmol, 1 eq), ammonium acetate (0.34 mg, 4.4 mmol, 10 eq) and $NaBH_3CN$ (41.5 mg, 0.661 mmol, 1.5 eq). Total time: over-weekend at reflux. The product was afforded without purification (35 mg, 0.15 mmol, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.33 (m, 2H), 7.23 – 7.10 (m, 2H), 3.90 – 3.77 (m, 1H), 1.74 – 1.47 (m, 4H), 1.41 – 1.10 (m, 2H), 0.95 – 0.78 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.72, 131.54, 128.26, 120.55, 55.56, 41.85, 19.71, 14.09. LC-MS $[C_{10}H_{14}BrN+H]^+$: 228.04/230.04 calculated, 227.93/229.87 found.

1-(4-Bromophenyl)pentan-1-amine (63c)



The title compound was synthesized according to general procedure **F** using 1-(4-bromophenyl)pentan-1-one (commercial **62c**, 0.20 g, 0.83 mmol, 1 eq), ammonium acetate (1.28 g, 16.6 mmol, 20 eq) and $NaBH_3CN$ (89.0 mg, 1.41 mmol, 1.7 eq). Total time: overnight at reflux. The product was afforded without purification (30.2 mg, 0.125 mmol, 15%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.41 (m, 2H), 7.25 – 7.16 (m, 2H), 3.92 – 3.80 (m, 1H), 1.83 – 1.52 (m, 4H), 1.38 – 1.05 (m, 4H), 0.94 – 0.81 (m, 3H). LC-MS $[C_{11}H_{16}BrN+H]^+$: 242.05/244.05 calculated, 242.00/243.87 found.

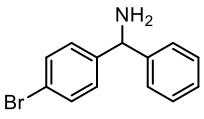
(4-Bromophenyl)(cyclopropyl)methanamine (63d)



To a solution of (4-bromophenyl)(cyclopropyl)methanone (**62d**, 59 mg, 0.26 mmol, 1 eq) in pyridine (1 mL, 0.26 M) was added *O*-methylhydroxylammonium chloride (32.8 mg, 0.393 mmol, 1.5 eq) at rt. The

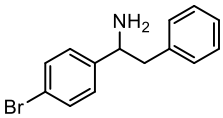
mixture was stirred at rt for over-weekend. Pyridine was removed under vacuum and the residue was extracted with Et_2O, filtered and concentrated. The remaining was dissolved in anhydrous THF (2 mL, 0.13 M) and borane tetrahydrofuran complex (1 M, 1.3 mL, 1.3 mmol, 5 eq) was added dropwise under N_2. The mixture was refluxed for 5 h and then cooled to rt. H_2O (1 mL) and aq. NaOH (20% w/v, 2 mL) were slowly added to the mixture under ice bath and it was stirred at 0 °C for 10 min. The mixture was then heated to 85 °C for overnight. The reaction mixture was diluted in water and extracted 3× with EtOAc. Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The product was afforded without purification (45 mg, 0.20 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.43 (m, 2H), 7.33 – 7.27 (m, 2H), 3.16 (d, *J* = 8.6 Hz, 1H), 1.32 – 1.19 (m, 2H), 1.10 – 1.01 (m, 1H), 0.66 – 0.41 (m, 2H), 0.40 – 0.20 (m, 2H). LC-MS $[C_{10}H_{12}BrN+H]^+$: 226.02/228.02 calculated, 225.80/227.80 found.

(4-Bromophenyl)(phenyl)methanamine (63e)



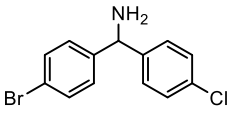
To a solution of 4-bromophenyl(phenyl)-methanone (commercial **62e**, 400 mg, 1.53 mmol, 1 eq) in EtOH (10 mL, 0.15 M) was added hydroxyl ammonium chloride (319 mg, 4.60 mmol, 3 eq). The mixture was refluxed for overnight. After completion the solvent was evaporated, and the residue was dissolved in DCM. The organic layer was washed with water and sat. $NaHCO_3$, dried with Na_2SO_4 and concentrated. The remaining was dissolved in anhydrous THF (5 mL, 0.3 M) and $LiAlH_4$ (1 M in THF, 1.5 mL, 1.5 mmol, 1 eq) was added slowly at reflux. The reaction mixture was allowed to stir overnight. After completion, the reaction was slowly quenched with aq. NaOH over 1 h. The mixture was extracted 3× with DCM and combined organic layers were concentrated. Silica gel column chromatography (3-5% EtOAc in *n*-pentane) afforded the product (44 mg, 0.17 mmol, 22%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.39 (m, 2H), 7.39 – 7.18 (m, 7H), 5.15 (s, 1H), 1.77 (s, 2H). LC-MS $[C_{13}H_{12}BrN+H]^+$: 262.02/264.02 calculated, 262.13/264.00 found.

1-(4-Bromophenyl)-2-phenylethan-1-amine (63f)



The title compound was synthesized according to general procedure **F** using 1-(4-bromophenyl)-2-phenylethan-1-one (commercial **62f**, 0.15 g, 0.55 mmol, 1 eq), ammonium acetate (0.84 g, 11 mmol, 20 eq) and $NaBH_3CN$ (93 mg, 1.5 mmol, 2.7 eq). Total time: overnight at reflux. The product was afforded without purification (0.16 g, 0.56 mmol, quant.). LC-MS $[C_{14}H_{14}BrN+H]^+$: 276.04/278.04 calculated, 275.92/277.92 found.

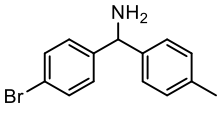
(4-Bromophenyl)(4-chlorophenyl)methanamine (63g)



The title compound was synthesized according to general procedure **F** using (4-bromophenyl)(4-chlorophenyl)methanone (**62g**, 115 mg, 0.390 mmol, 1 eq), ammonium acetate (600 mg, 7.78 mmol, 20 eq) and $NaBH_3CN$ (66.0 mg, 1.05 mmol, 2.7 eq). Total time: overnight at reflux. The product was

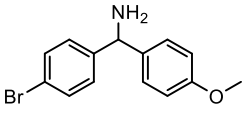
afforded without purification (116 mg, 0.390 mmol, quant.). LC-MS $[C_{13}H_{11}BrClN–NH_2]^+$: 278.96/280.96 calculated, 279.08/281.08 found.

(4-Bromophenyl)(*p*-tolyl)methanamine (63h)



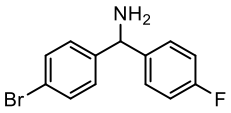
The title compound was synthesized according to general procedure **F** using (4-bromophenyl)(*p*-tolyl)methanone (**62h**, 115 mg, 0.420 mmol, 1 eq), ammonium acetate (644 mg, 8.36 mmol, 20 eq) and $NaBH_3CN$ (71.0 mg, 1.13 mmol, 2.7 eq). Total time: overnight at reflux. The product was afforded without purification (112 mg, 0.406 mmol, 97%). LC-MS $[C_{14}H_{14}BrN–NH_2]^+$: 259.01/261.01 calculated, 259.08/261.08 found.

(4-Bromophenyl)(4-methoxyphenyl)methanamine (63i)



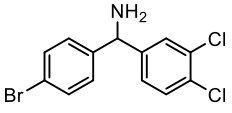
The title compound was synthesized according to general procedure **F** using (4-bromophenyl)(4-methoxyphenyl)methanone (**62i**, 115 mg, 0.400 mmol, 1 eq), ammonium acetate (609 mg, 7.90 mmol, 20 eq) and $NaBH_3CN$ (67.0 mg, 1.07 mmol, 2.7 eq). Total time: overnight at reflux. The product was afforded without purification (113.8 mg, 0.39 mmol, 99%). LC-MS $[C_{14}H_{14}BrNO–NH_2]^+$: 275.01/277.00 calculated, 275.08/277.08 found.

(4-Bromophenyl)(4-fluorophenyl)methanamine (63j)



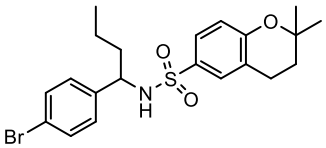
The title compound was synthesized according to general procedure **F** using (4-bromophenyl)(4-fluorophenyl)methanone (**62j**, 200 mg, 0.720 mmol, 1 eq), ammonium acetate (1.10 g, 14.3 mmol, 20 eq) and $NaBH_3CN$ (122 mg, 1.94 mmol, 2.7 eq). Total time: overnight at reflux. The product was afforded without purification (173 mg, 0.618 mmol, 86%). LC-MS $[C_{13}H_{11}BrF–NH_2]^+$: 262.99/264.98 calculated, 263.06/265.00 found.

(4-Bromophenyl)(3,4-dichlorophenyl)methanamine (63k)



The title compound was synthesized according to general procedure **F** using (4-bromophenyl)(3,4-dichlorophenyl)methanone (**62k**, 80 mg, 0.24 mmol, 1 eq), ammonium acetate (374 mg, 4.85 mmol, 20 eq) and $NaBH_3CN$ (41 mg, 0.66 mmol, 2.7 eq). Total time: overnight at reflux. The product was afforded without purification (75 mg, 0.23 mmol, 93%). LC-MS $[C_{13}H_{10}BrCl_2N–NH_2]^+$: 312.92/314.92 calculated, 312.93/314.93 found.

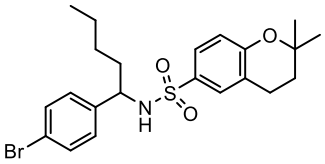
***N*-(1-(4-Bromophenyl)butyl)-2,2-dimethylchromane-6-sulfonamide (64b)**



The title compound was synthesized according to general procedure **G** using 1-(4-bromophenyl)butan-1-amine (**63b**, 34 mg, 0.15 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonyl chloride (**55**, 46.6 mg,

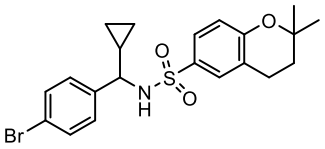
0.179 mmol, 1.2 eq) and Et_3N (62 µL, 0.45 mmol, 3 eq). Total time: overnight at rt. The product was afforded without purification (67 mg, 0.15 mmol, 99%).

N-(1-(4-Bromophenyl)pentyl)-2,2-dimethylchromane-6-sulfonamide (64c)



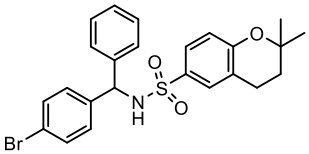
The title compound was synthesized according to general procedure **G** using 1-(4-bromophenyl)-pentan-1-amine (**63c**, 30.0 mg, 0.124 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 48.5 mg, 0.186 mmol, 1.5 eq) and Et_3N (52 µL, 0.37 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-25% EtOAc in *n*-pentane) afforded the product (32 mg, 0.068 mmol, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.30 – 7.22 (m, 2H), 7.13 (dt, *J* = 2.4, 1.1 Hz, 1H), 6.92 – 6.85 (m, 2H), 6.69 (d, *J* = 8.6 Hz, 1H), 4.83 (d, *J* = 7.0 Hz, 1H), 4.25 (q, *J* = 7.2 Hz, 1H), 2.67 – 2.52 (m, 2H), 1.79 (td, *J* = 6.8, 2.3 Hz, 2H), 1.74 – 1.67 (m, 1H), 1.64 – 1.55 (m, 1H), 1.34 (s, 3H), 1.32 (s, 3H), 1.27 – 1.20 (m, 4H), 0.81 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.71, 140.35, 131.38, 130.82, 129.29, 128.54, 124.98, 121.14, 121.11, 117.68, 75.74, 57.80, 37.49, 32.34, 28.03, 27.02, 26.85, 22.31, 13.97. LC-MS $[C_{22}H_{28}BrNO_3S+H]^+$: 466.10/468.10 calculated, 465.87/467.80 found.

N-((4-Bromophenyl)(cyclopropyl)methyl)-2,2-dimethylchromane-6-sulfonamide (64d)



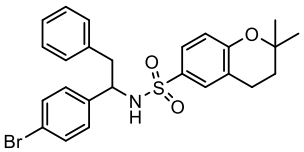
The title compound was synthesized according to general procedure **G** using (4-bromophenyl)(cyclopropyl)-methanamine (**63d**, 45 mg, 0.20 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 104 mg, 0.398 mmol, 2 eq) and Et_3N (83 µL, 0.60 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-25% EtOAc in *n*-pentane) afforded the product (30 mg, 0.066 mmol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.32 – 7.24 (m, 2H), 7.13 (dt, *J* = 2.3, 1.1 Hz, 1H), 7.01 – 6.93 (m, 2H), 6.71 (d, *J* = 8.6 Hz, 1H), 4.97 (d, *J* = 5.0 Hz, 1H), 3.64 (dd, *J* = 8.7, 5.0 Hz, 1H), 2.70 – 2.51 (m, 2H), 1.86 – 1.73 (m, 2H), 1.35 (s, 3H), 1.32 (s, 3H), 1.05 (qt, *J* = 8.1, 4.9 Hz, 1H), 0.62 – 0.43 (m, 2H), 0.34 – 0.24 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.77, 139.54, 131.29, 130.75, 129.44, 128.90, 126.71, 121.33, 121.06, 117.74, 75.77, 62.28, 32.35, 27.03, 26.84, 22.30, 18.37, 4.61, 3.90. LC-MS $[C_{21}H_{24}BrNO_3S+H]^+$: 450.07/452.07 calculated, 449.87/451.73 found.

N-((4-Bromophenyl)(phenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (64e)



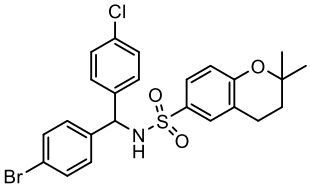
The title compound was synthesized according to general procedure **G** using (4-bromophenyl)-(phenyl)methanamine (**63e**, 44 mg, 0.17 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 52.5 mg, 0.201 mmol, 1.2 eq) and Et_3N (70 µL, 0.50 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-13% EtOAc in *n*-pentane) afforded the product (41 mg, 0.085 mmol, 51%). LC-MS $[C_{24}H_{24}BrNO_3S+H]^+$: 486.07/488.07 calculated, 485.67/487.80 found.

_N_-(1-(4-Bromophenyl)-2-phenylethyl)-2,2-dimethylchromane-6-sulfonamide (64f)



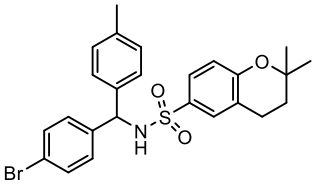
The title compound was synthesized according to general procedure **G** using (4-bromophenyl)-2-phenylethan-1-amine (**63f**, 75 mg, 0.27 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 142 mg, 0.398 mmol, 2 eq) and Et_3N (114 µL, 0.815 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (74.1 mg, 0.148 mmol, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.25 (m, 3H), 7.23 – 7.18 (m, 3H), 7.07 (dt, *J* = 2.3, 1.1 Hz, 1H), 6.97 – 6.88 (m, 4H), 6.66 (d, *J* = 8.6 Hz, 1H), 4.81 (d, *J* = 5.6 Hz, 1H), 4.50 (q, *J* = 6.8 Hz, 1H), 3.00 – 2.87 (m, 2H), 2.67 – 2.50 (m, 2H), 1.83 – 1.76 (m, 2H), 1.35 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.81, 139.62, 135.94, 131.35, 130.19, 129.41, 129.30, 128.81, 128.77, 127.21, 126.72, 121.37, 121.13, 117.71, 75.76, 58.64, 44.14, 32.34, 27.12, 26.79, 22.31. LC-MS $[C_{25}H_{26}BrNO_3S+H]^+$: 500.09/502.09 calculated, 499.58/501.50 found.

_N_-((4-Bromophenyl)(4-chlorophenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (64g)



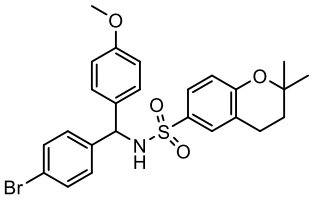
The title compound was synthesized according to general procedure **G** using (4-bromophenyl)(4-chlorophenyl)methanamine (**63g**, 58 mg, 0.20 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 102 mg, 0.391 mmol, 2 eq) and Et_3N (82 µL, 0.59 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-15% EtOAc in *n*-pentane) afforded the product (53.7 mg, 0.103 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.34 – 7.29 (m, 2H), 7.19 (dt, *J* = 2.3, 1.1 Hz, 1H), 7.18 – 7.14 (m, 2H), 7.05 – 7.00 (m, 2H), 6.99 – 6.94 (m, 2H), 6.70 (d, *J* = 8.7 Hz, 1H), 5.63 (d, *J* = 7.7 Hz, 1H), 5.48 (d, *J* = 7.7 Hz, 1H), 2.56 (t, *J* = 6.7 Hz, 2H), 1.78 (t, *J* = 6.7 Hz, 2H), 1.34 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.97, 139.26, 138.68, 133.74, 131.75, 130.21, 129.39, 129.17, 128.85, 128.80, 126.77, 121.87, 121.33, 117.79, 75.85, 60.31, 32.26, 26.91, 26.89, 22.27.

_N_-((4-Bromophenyl)(*p*-tolyl)methyl)-2,2-dimethylchromane-6-sulfonamide (64h)



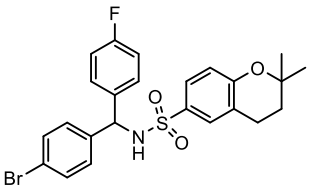
The title compound was synthesized according to general procedure **G** using (4-bromophenyl)(*p*-tolyl)methanamine (**63h**, 112 mg, 0.406 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 106 mg, 0.406 mmol, 1 eq) and Et_3N (170 µL, 1.22 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-15% EtOAc in *n*-pentane) afforded the product (79.7 mg, 0.159 mmol, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.23 (d, *J* = 2.5 Hz, 1H), 7.04 – 6.98 (m, 4H), 6.97 – 6.91 (m, 2H), 6.69 (d, *J* = 8.6 Hz, 1H), 5.52 (d, *J* = 7.5 Hz, 1H), 5.47 (d, *J* = 7.4 Hz, 1H), 2.57 (t, *J* = 7.2 Hz, 2H), 2.26 (s, 3H), 1.77 (t, *J* = 6.8 Hz, 2H), 1.33 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.77, 139.93, 137.60, 137.33, 131.47, 130.47, 129.44, 129.39, 129.23, 127.29, 126.77, 121.40, 121.18, 117.67, 75.69, 60.67, 32.30, 26.90, 26.84, 22.24, 21.14. LC-MS $[C_{25}H_{26}BrNO_3S+NH_4]^+$: 517.12/519.11 calculated, 516.50/518.50 found.

_N_-((4-Bromophenyl)(4-methoxyphenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (64i)



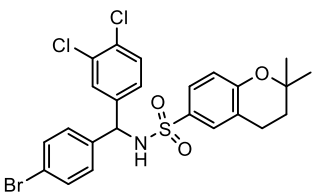
The title compound was synthesized according to general procedure **G** using (4-bromophenyl)(4-methoxyphenyl)methanamine (**63i**, 114 mg, 0.389 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 132 mg, 0.506 mmol, 1.3 eq) and Et_3N (163 µL, 1.17 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (82.5 mg, 0.160 mmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.32 – 7.27 (m, 2H), 7.21 (dt, *J* = 2.3, 1.1 Hz, 1H), 7.03 – 6.99 (m, 2H), 6.99 – 6.94 (m, 2H), 6.73 – 6.67 (m, 3H), 5.52 (d, *J* = 7.5 Hz, 1H), 5.46 (d, *J* = 7.5 Hz, 1H), 3.73 (s, 3H), 2.57 (t, *J* = 6.8 Hz, 2H), 1.77 (t, *J* = 6.8 Hz, 2H), 1.33 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.12, 157.77, 139.97, 132.35, 131.47, 130.47, 129.46, 129.20, 128.66, 126.74, 121.39, 121.19, 117.66, 113.53, 75.70, 60.38, 55.31, 32.26, 26.91, 26.84, 22.24. LC-MS $[C_{25}H_{26}BrNO_4S+NH_4]^+$: 533.11/535.11 calculated, 532.50/534.58 found.

_N_-((4-Bromophenyl)(4-fluorophenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (64j)



The title compound was synthesized according to general procedure **G** using (4-bromophenyl)(4-fluorophenyl)methanamine (**63j**, 80 mg, 0.29 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 74.5 mg, 0.286 mmol, 1 eq) and Et_3N (119 µL, 0.857 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (34 mg, 0.067 mmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.20 (dt, *J* = 2.3, 1.1 Hz, 1H), 7.09 – 7.02 (m, 2H), 7.01 – 6.95 (m, 2H), 6.93 – 6.84 (m, 2H), 6.71 (d, *J* = 8.6 Hz, 1H), 5.50 (s, 2H), 2.58 (t, *J* = 6.7 Hz, 2H), 1.78 (t, *J* = 6.8 Hz, 2H), 1.34 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.23 (d, $J_{C\text{-}F}$ = 247.2 Hz), 157.95, 139.47, 136.03 (d, $J_{C\text{-}F}$ = 3.4 Hz), 131.71, 130.29, 129.34 (d, $J_{C\text{-}F}$ = 16.0 Hz), 129.18, 126.77, 121.78, 121.29, 117.78, 115.58 (d, $J_{C\text{-}F}$ = 21.7 Hz), 75.83, 61.23, 32.26, 26.94, 26.84, 21.86.

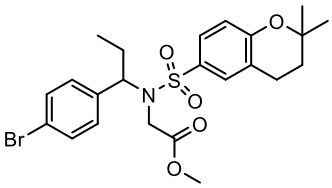
_N_-((4-Bromophenyl)(3,4-dichlorophenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (64k)



The title compound was synthesized according to general procedure **G** using (4-bromophenyl)(3,4-dichlorophenyl)methanamine (**63k**, 74.7 mg, 0.226 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 64.7 mg, 0.248 mmol, 1.1 eq) and Et_3N (94 µL, 0.68 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (28 mg, 50 µmol, 22%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.38 – 7.33 (m, 2H), 7.26 (d, *J* = 1.8 Hz, 1H), 7.23 (dt, *J* = 2.1, 0.9 Hz, 1H), 7.19 – 7.15 (m, 1H), 6.99 – 6.92 (m, 3H), 6.73 (d, *J* = 8.6 Hz, 1H), 5.47 (d, *J* = 7.4 Hz, 1H), 5.41 (d, *J* = 7.4 Hz, 1H), 2.60 (t, *J* = 6.9, 2H), 1.80 (t, *J*

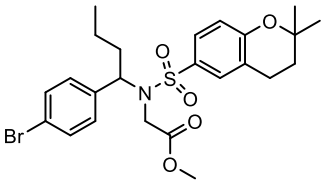
= 6.7 Hz, 2H), 1.34 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.19, 140.29, 138.67, 132.85, 132.00, 130.64, 129.97, 129.48, 129.35, 129.14, 128.36, 126.85, 126.83, 122.28, 121.47, 117.91, 75.95, 59.97, 32.25, 26.97, 26.95, 22.33.

Methyl *N*-(1-(4-bromophenyl)propyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65a)



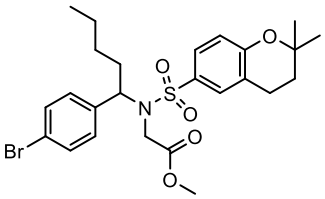
The title compound was synthesized according to general procedure **G** using methyl (1-(4-bromophenyl)propyl)glycinate (**63a**, 20 mg, 0.070 mmol, 1 eq), 2,2-dimethylchromane-6-sulfonylchloride (**55**, 30 mg, 0.12 mmol, 1.65 eq) and Et_3N (19 µL, 0.14 mmol, 2 eq). Total time: over-weekend at rt. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (10 mg, 0.020 mmol, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.58 – 7.55 (m, 1H), 7.41 – 7.34 (m, 2H), 7.03 – 6.97 (m, 2H), 6.84 (d, *J* = 8.6 Hz, 1H), 4.69 (dd, *J* = 9.0, 6.4 Hz, 1H), 3.98 – 3.74 (m, 2H), 3.57 (s, 3H), 2.79 (t, *J* = 6.7 Hz, 2H), 1.94 – 1.78 (m, 4H), 1.37 (s, 6H), 0.80 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.47, 158.12, 136.67, 131.63, 130.86, 130.26, 129.98, 127.13, 122.15, 121.38, 117.86, 75.85, 61.84, 52.24, 44.84, 32.41, 26.96, 26.92, 24.60, 22.47, 11.36. LC-MS $[C_{23}H_{28}BrNO_5S+H]^+$: 510.09/512.09 calculated, 510.00/511.93 found.

Methyl *N*-(1-(4-bromophenyl)butyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65b)



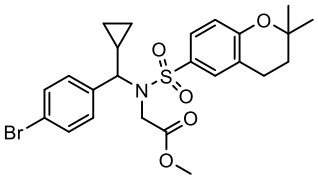
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)butyl)-2,2-dimethylchromane-6-sulfonamide (**64b**, 67 mg, 0.15 mmol, 1 eq), methyl 2-bromoacetate (28 µL, 0.30 mmol, 2 eq) and BEMP (1 M in hexane, 296 µL, 0.296 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (55.0 mg, 0.105 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.59 – 7.56 (m, 1H), 7.40 – 7.34 (m, 2H), 7.05 – 6.99 (m, 2H), 6.84 (d, *J* = 8.6 Hz, 1H), 4.78 (dd, *J* = 9.3, 6.0 Hz, 1H), 3.98 – 3.75 (m, 2H), 3.57 (s, 3H), 2.79 (t, *J* = 6.8 Hz, 2H), 1.91 – 1.79 (m, 3H), 1.73 – 1.67 (m, 1H), 1.37 (s, 3H), 1.36 (s, 3H), 1.24 – 1.12 (m, 2H), 0.82 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.40, 158.09, 136.84, 131.56, 130.74, 130.18, 129.96, 127.51, 122.08, 121.34, 117.81, 75.80, 59.75, 52.18, 44.74, 33.35, 32.36, 26.90, 26.84, 22.42, 19.71, 13.80. LC-MS $[C_{24}H_{30}BrNO_5S+H]^+$: 524.11/526.11 calculated, 523.80/525.93 found.

Methyl *N*-(1-(4-bromophenyl)pentyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65c)



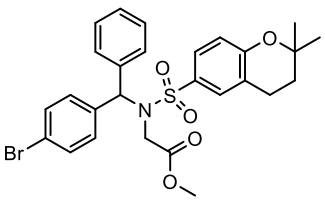
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)pentyl)-2,2-dimethylchromane-6-sulfonamide (**64c**, 32 mg, 0.069 mmol, 1 eq), methyl 2-bromoacetate (13 µL, 0.14 mmol, 2 eq) and BEMP (1 M in hexane, 137 µL, 0.137 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (39 mg, 0.072 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.59 (d, *J* = 2.4 Hz, 1H), 7.42 – 7.33 (m, 2H), 7.07 – 7.00 (m, 2H), 6.85 (d, *J* = 8.6 Hz, 1H), 4.75 (dd, *J* = 9.3, 6.1 Hz, 1H), 3.99 – 3.74 (m, 2H), 3.56 (s, 3H), 2.79 (t, *J* = 6.8 Hz, 2H), 1.84 (t, *J* = 6.8 Hz, 3H), 1.77 – 1.68 (m, 1H), 1.37 (s, 3H), 1.36 (s, 3H), 1.30 – 1.05 (m, 4H), 0.79 (t, *J* = 7.2 Hz, 3H). LC-MS $[C_{25}H_{32}BrNO_5S+Na]^+$: 560.11/562.11 calculated, 560.27/562.20 found.

Methyl *N*-((4-bromophenyl)(cyclopropyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65d)



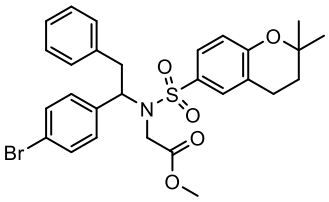
The title compound was synthesized according to general procedure **I** using *N*-((4-bromophenyl)-(cyclopropyl)methyl)-2,2-dimethylchromane-6-sulfonamide (**64d**, 30 mg, 0.067 mmol, 1 eq), methyl 2-bromoacetate (12.6 µL, 0.133 mmol, 2 eq) and BEMP (1 M in hexane, 133 µL, 0.133 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (38 mg, 0.073 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.50 (m, 1H), 7.47 (s, 1H), 7.39 – 7.30 (m, 2H), 7.23 – 7.16 (m, 2H), 6.81 (d, *J* = 8.6 Hz, 1H), 4.25 – 3.87 (m, 3H), 3.62 (s, 3H), 2.79 – 2.62 (m, 2H), 1.83 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 6H), 0.94 – 0.77 (m, 1H), 0.68 (tt, *J* = 9.8, 4.6 Hz, 1H), 0.54 (tt, *J* = 8.5, 4.9 Hz, 1H), 0.30 – 0.13 (m, 2H). LC-MS $[C_{24}H_{28}BrNO_5S+Na]^+$): 544.08/546.07 calculated, 544.27/546.20 found.

Methyl *N*-((4-bromophenyl)(phenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65e)



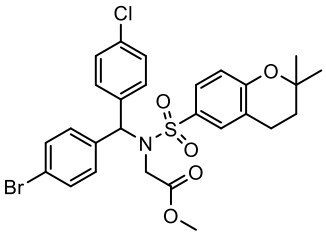
The title compound was synthesized according to general procedure **I** using *N*-((4-bromophenyl)-(phenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (**64e**, 41 mg, 0.084 mmol, 1 eq), methyl 2-bromoacetate (16 µL, 0.17 mmol, 2 eq) and BEMP (1 M in hexane, 169 µL, 0.169 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (3-10% EtOAc in *n*-pentane) afforded the product (34 mg, 0.062 mmol, 73%). LC-MS $[C_{27}H_{28}BrNO_5S+Na]^+$: 580.08/582.07 calculated, 580.20/582.13 found.

Methyl *N*-(1-(4-bromophenyl)-2-phenylethyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65f)



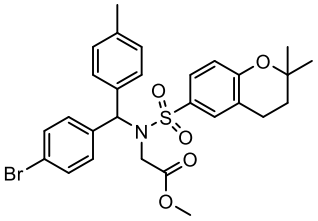
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)-2-phenylethyl)-2,2-dimethylchromane-6-sulfonamide (**64f**, 74.1 mg, 0.148 mmol, 1 eq), methyl 2-bromoacetate (28 µL, 0.30 mmol, 2 eq) and BEMP (1 M in hexane, 296 µL, 0.296 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (83.2 mg, 0.145 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.53 – 7.50 (m, 1H), 7.35 – 7.30 (m, 2H), 7.19 – 7.10 (m, 3H), 7.09 – 7.04 (m, 2H), 7.03 – 6.98 (m, 2H), 6.81 (d, *J* = 8.7 Hz, 1H), 5.09 (dd, *J* = 9.4, 5.9 Hz, 1H), 4.10 – 3.80 (m, 2H), 3.59 (s, 3H), 3.24 – 3.18 (m, 2H), 2.75 (t, *J* = 6.7 Hz, 2H), 1.82 (t, *J* = 6.8 Hz, 2H), 1.35 (d, *J* = 2.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.35, 158.10, 137.40, 135.88, 131.46, 130.59, 130.50, 129.92, 129.06, 128.50, 127.43, 126.56, 122.22, 121.33, 117.87, 75.79, 61.68, 52.24, 45.44, 37.79, 32.30, 26.98, 26.82, 22.38. LC-MS $[C_{28}H_{30}BrNO_5S+Na]^+$: 594.09/596.09 calculated, 594.00/596.00 found.

Methyl *N*-((4-bromophenyl)(4-chlorophenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65g)



The title compound was synthesized according to general procedure **I** using *N*-((4-bromophenyl)(4-chlorophenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (**64g**, 53.7 mg, 0.103 mmol, 1 eq), methyl 2-bromoacetate (20 µL, 0.21 mmol, 2 eq) and BEMP (1 M in hexane, 206 µL, 0.206 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (63.2 mg, 0.107 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.40 (dt, *J* = 2.3, 1.1 Hz, 1H), 7.38 – 7.33 (m, 2H), 7.23 – 7.17 (m, 2H), 7.04 – 6.98 (m, 2H), 6.97 – 6.92 (m, 2H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.10 (s, 1H), 4.02 (s, 2H), 3.50 (s, 3H), 2.67 (t, *J* = 6.7 Hz, 2H), 1.81 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.66, 158.21, 136.88, 136.26, 134.02, 131.55, 130.71, 130.44, 130.19, 129.87, 128.59, 127.51, 122.16, 121.29, 117.77, 75.86, 63.92, 52.17, 46.78, 32.29, 26.87, 26.86, 22.33.

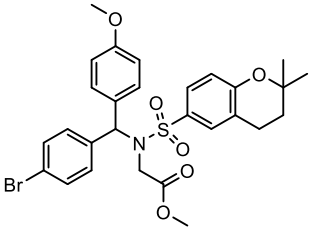
Methyl *N*-((4-bromophenyl)(*p*-tolyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65h)



The title compound was synthesized according to general procedure **I** using *N*-((4-bromophenyl)(*p*-tolyl)methyl)-2,2-dimethylchromane-6-sulfonamide (**64h**, 79.7 mg, 0.159 mmol, 1 eq), methyl 2-bromoacetate (30 µL, 0.32 mmol, 2 eq) and BEMP (1 M in hexane, 319 µL, 0.319 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (5-15% EtOAc in *n*-pentane) afforded the product (67.5 mg, 0.118 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.45 (dt, *J* = 2.4, 1.1 Hz, 1H), 7.38 – 7.30 (m, 2H), 7.07 – 6.95 (m, 4H), 6.92 – 6.86 (m, 2H), 6.78 (d, *J* = 8.6

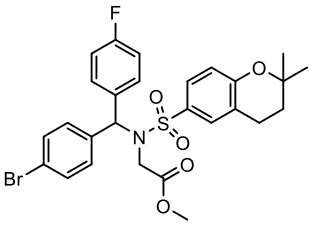
Hz, 1H), 6.11 (s, 1H), 4.02 (s, 2H), 3.47 (s, 3H), 2.67 (t, *J* = 6.6 Hz, 2H), 2.29 (s, 3H), 1.80 (t, *J* = 6.8 Hz, 2H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.84, 158.07, 137.91, 137.72, 134.54, 131.29, 130.61, 130.26, 130.03, 129.15, 129.09, 127.58, 121.69, 121.18, 117.67, 75.75, 64.22, 52.04, 46.69, 32.32, 26.88, 26.82, 22.32, 21.15. LC-MS $[C_{28}H_{30}BrNO_5S+NH_4]^+$: 589.14/591.13 calculated, 588.75/590.75 found.

Methyl *N*-((4-bromophenyl)(4-methoxyphenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65i)



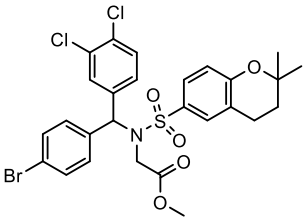
The title compound was synthesized according to general procedure **I** using *N*-((4-bromophenyl)(4-methoxyphenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (**64i**, 82.5 mg, 0.160 mmol, 1 eq), methyl 2-bromoacetate (30 µL, 0.32 mmol, 2 eq) and BEMP (1 M in hexane, 319 µL, 0.319 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (96.8 mg, 0.164 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.43 (dt, *J* = 2.3, 1.1 Hz, 1H), 7.37 – 7.31 (m, 2H), 7.03 – 6.97 (m, 2H), 6.96 – 6.91 (m, 2H), 6.80 – 6.70 (m, 3H), 6.10 (s, 1H), 4.01 (d, *J* = 5.3 Hz, 2H), 3.76 (s, 3H), 3.49 (s, 3H), 2.67 (td, *J* = 6.8, 1.8 Hz, 2H), 1.80 (t, *J* = 6.8 Hz, 2H), 1.35 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.83, 159.31, 158.03, 137.81, 131.27, 130.52, 130.41, 130.20, 130.02, 129.47, 127.49, 121.59, 121.16, 117.63, 113.72, 75.74, 63.98, 55.30, 52.04, 46.64, 32.26, 26.84, 26.80, 22.28. LC-MS $[C_{28}H_{30}BrNO_6S+NH_4]^+$: 605.13/607.13 calculated, 604.75/606.75 found.

Methyl *N*-((4-bromophenyl)(4-fluorophenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65j)



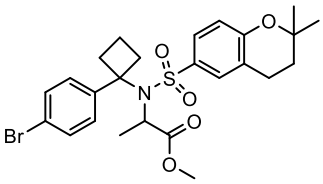
The title compound was synthesized according to general procedure **I** using *N*-((4-bromophenyl)(4-fluorophenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (**64j**, 34 mg, 0.067 mmol, 1 eq), methyl 2-bromoacetate (13.0 µL, 0.135 mmol, 2 eq) and BEMP (1 M in hexane, 319 µL, 0.319 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (36 mg, 0.062 mmol, 93%). LC-MS $[C_{27}H_{27}BrFNO_5S+NH_4]^+$: 593.11/595.11 calculated, 592.83/594.75 found.

Methyl *N*-((4-bromophenyl)(3,4-dichlorophenyl)methyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)glycinate (65k)



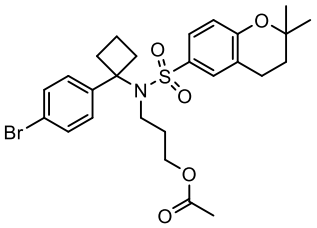
The title compound was synthesized according to general procedure **I** using *N*-((4-bromophenyl)(3,4-dichlorophenyl)methyl)-2,2-dimethylchromane-6-sulfonamide (**64k**, 27.5 mg, 0.050 mmol, 1 eq), methyl 2-bromoacetate (9.4 µL, 0.099 mmol, 2 eq) and BEMP (1 M in hexane, 99 µL, 0.099 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded without purification (22 mg, 0.036 mmol, 72%). LC-MS $[C_{27}H_{26}BrCl_2NO_5S+NH_4]^+$: 643.04/645.04 calculated, 642.75/644.75 found.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethylchroman-6-yl)sulfonyl)alaninate (66a)



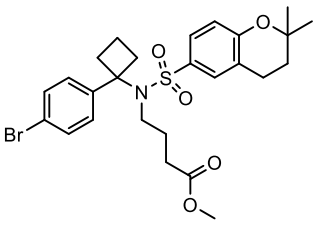
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (**60c**, 200 mg, 0.444 mmol, 1 eq), methyl 2-bromopropanoate (59 µL, 0.53 mmol, 1.2 eq) and BEMP (1 M in hexane, 0.488 mL, 0.488 mmol, 1.1 eq). Total time: 3 days at 80 °C. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (23 mg, 0.043 mmol, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.41 – 7.32 (m, 4H), 7.25 (d, *J* = 2.4 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 1H), 3.71 (s, 3H), 3.03 – 2.85 (m, 2H), 2.71 (t, *J* = 6.7 Hz, 2H), 2.47 – 2.40 (m, 1H), 2.38 – 2.29 (m, 1H), 1.84 (t, *J* = 6.7 Hz, 2H), 1.78 – 1.68 (m, 1H), 1.55 (d, *J* = 7.3 Hz, 3H), 1.52 – 1.46 (m, 1H), 1.36 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.62, 157.55, 142.42, 133.45, 131.07, 129.70, 129.59, 126.91, 121.39, 121.00, 117.56, 75.75, 65.67, 56.38, 52.55, 34.98, 34.72, 32.44, 27.01, 26.92, 22.43, 18.46, 14.91. LC-MS $[C_{25}H_{30}BrNO_5S+Na]^+$: 558.09/560.09 calculated, 558.00/560.00 found.

3-((*N*-(1-(4-Bromophenyl)cyclobutyl)-2,2-dimethylchromane)-6-sulfonamido)propyl acetate (66b)



The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (**60c**, 60.0 mg, 0.133 mmol, 1 eq), 3-bromopropyl acetate (50 mg, 0.28 mmol, 2.1 eq) and BEMP (1 M in hexane, 280 µL, 0.280 mmol, 2.1 eq). Total time: 3 days at 80 °C. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (58 mg, 0.095 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.32 (m, 4H), 7.28 (d, *J* = 5.6 Hz, 1H), 6.88 (s, 1H), 6.71 (d, *J* = 8.6 Hz, 1H), 4.04 (t, *J* = 6.0 Hz, 2H), 3.27 – 3.19 (m, 2H), 2.76 – 2.60 (m, 4H), 2.57 – 2.49 (m, 2H), 2.05 (s, 3H), 2.02 – 1.94 (m, 2H), 1.85 – 1.70 (m, 3H), 1.65 – 1.51 (m, 1H), 1.33 (s, 6H). LC-MS $[C_{26}H_{32}BrNO_5S+NH_4]^+$: 567.15/569.15 calculated, 566.83/568.83 found.

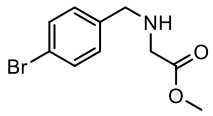
Methyl 4-((*N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane)-6-sulfonamido)butanoate (66c)



The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethylchromane-6-sulfonamide (**60c**, 20 mg, 0.044 mmol, 1 eq), methyl 4-bromobutanoate (10 µL, 0.079 mmol, 1.8 eq) and BEMP (1 M in hexane, 89 µL, 0.089 mmol, 2 eq). Total time: 3 days at 80 °C. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product (22 mg, 0.040 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.34 (m, 4H), 7.27 (dd, *J* = 8.7, 2.4 Hz 1H), 6.88 (d, *J* = 2.4 Hz, 1H), 6.70 (d, *J* = 8.7 Hz, 1H), 3.67 (s, 3H), 3.24 – 3.18 (m, 2H), 2.77 – 2.67 (m, 2H), 2.64 (t, *J* = 6.7 Hz, 2H), 2.59 – 2.50 (m, 2H), 2.30 (t, *J* = 7.0 Hz, 2H), 2.00 – 1.91 (m,

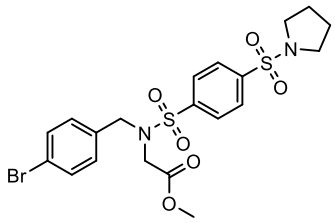
2H), 1.82 – 1.72 (m, 3H), 1.61 – 1.52 (m, 1H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.55, 157.38, 142.72, 131.70, 131.25, 129.13, 128.97, 126.62, 121.25, 121.21, 117.30, 75.68, 65.34, 51.77, 46.64, 35.48, 32.40, 31.24, 26.95, 26.58, 22.36, 14.66.

Methyl (4-bromobenzyl)glycinate (67)



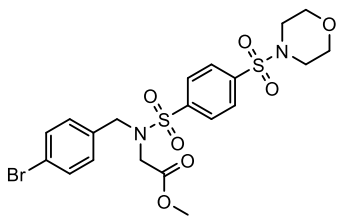
To a stirred solution of 4-bromobenzaldehyde (1.0 g, 5.4 mmol, 1 eq) in anhydrous MeOH (50 mL, 0.1 M) with 3 Å molecular sieves was added glycine methyl ester hydrochloride (0.81 g, 6.5 mmol, 1.2 eq), Et_3N (0.90 mL, 6.5 mmol, 1.2 eq) and AcOH (1.24 mL, 21.6 mmol, 4 eq) at rt. The mixture was stirred at rt for 1 h. $NaBH_3CN$ (0.41 g, 6.5 mmol, 1.2 eq) was added and the reaction was stirred at rt for another 2 h. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was diluted in EtOAc, washed with sat. $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (20-30% EtOAc in *n*-pentane) to afford the product (1.06 g, 4.11 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.41 (m, 2H), 7.23 – 7.18 (m, 2H), 3.75 (s, 2H), 3.72 (s, 3H), 3.39 (s, 2H), 1.95 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.78, 138.53, 131.50, 129.94, 120.94, 52.51, 51.81, 49.78. LC-MS $[C_{10}H_{12}BrNO_2+H]^+$: 258.01/260.01 calculated, 258.00/259.92 found.

Methyl *N*-(4-bromobenzyl)-*N*-((4-(pyrrolidin-1-ylsulfonyl)phenyl)sulfonyl)glycinate (68a)



The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 25 mg, 0.097 mmol, 1.2 eq), 4-(pyrrolidin-1-ylsulfonyl)benzenesulfonyl chloride (25 mg, 0.081 mmol, 1 eq), Et_3N (45 µL, 0.32 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. The product was afforded without purification (10 mg, 0.019 mmol, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 7.92 (m, 4H), 7.51 – 7.44 (m, 2H), 7.19 – 7.12 (m, 2H), 4.47 (s, 2H), 3.96 (s, 2H), 3.56 (s, 3H), 3.34 – 3.23 (m, 4H), 1.86 – 1.75 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.71, 143.67, 141.30, 133.53, 132.21, 130.40, 128.20, 128.12, 122.69, 52.33, 51.01, 48.19, 46.63, 25.47. LC-MS $[C_{20}H_{23}BrN_2O_6S_2+H]^+$: 531.03/533.02 calculated , 531.08/533.00 found.

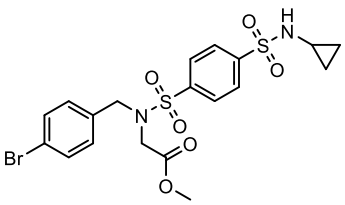
Methyl *N*-(4-bromobenzyl)-*N*-((4-(morpholinosulfonyl)phenyl)sulfonyl)glycinate (68b)



The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 24 mg, 0.092 mmol, 1.2 eq), 4-(morpholinosulfonyl)benzenesulfonyl chloride (25 mg, 0.077 mmol, 1 eq), Et_3N (43 µL, 0.31 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (10 mg, 0.018 mmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.02 (m, 2H), 7.94 – 7.86 (m, 2H), 7.51 – 7.47 (m, 2H), 7.19 – 7.13 (m, 2H), 4.47 (s, 2H), 3.97 (s, 2H), 3.80 – 3.74 (m, 4H), 3.57 (s, 3H), 3.08 – 3.01

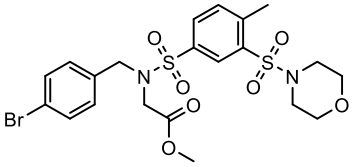
(m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.71, 144.30, 139.52, 133.46, 132.23, 130.40, 128.50, 128.30, 122.73, 66.16, 52.41, 51.02, 46.65, 46.10.

Methyl *N*-(4-bromobenzyl)-*N*-((4-(*N*-cyclopropylsulfamoyl)phenyl)sulfonyl)glycinate (68c)



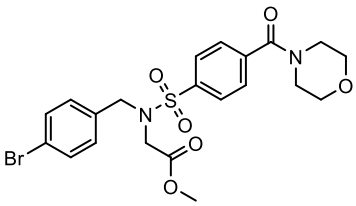
The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 26.2 mg, 0.101 mmol, 1.2 eq), 4-(*N*-cyclopropylsulfamoyl)benzenesulfonyl chloride (25 mg, 0.085 mmol, 1eq), Et_3N (47 µL, 0.34 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (15 mg, 0.028 mmol, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 – 7.99 (m, 4H), 7.51 – 7.44 (m, 2H), 7.21 – 7.13 (m, 2H), 5.09 (s, 1H), 4.47 (s, 2H), 3.97 (s, 2H), 3.56 (s, 3H), 2.33 – 2.27 (m, 1H), 0.70 – 0.58 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.74, 143.92, 143.87, 133.50, 132.21, 130.41, 128.24, 122.69, 52.36, 51.01, 46.66, 24.43, 6.42.

Methyl *N*-(4-bromobenzyl)-*N*-((4-methyl-3-(morpholinosulfonyl)phenyl)sulfonyl)glycinate (68d)



The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 22.8 mg, 0.088 mmol, 1.2 eq), 4-methyl-3-(morpholinesulfonyl)benzenesulfonyl chloride (25 mg, 0.074 mmol, 1 eq), Et_3N (41 µL, 0.30 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (9.2 mg, 0.016 mmol, 22%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 2.0 Hz, 1H), 7.96 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.51 (d, *J* = 8.1 Hz, 1H), 7.49 – 7.44 (m, 2H), 7.20 – 7.14 (m, 2H), 4.44 (s, 2H), 3.97 (s, 2H), 3.80 – 3.70 (m, 4H), 3.58 (s, 3H), 3.27 – 3.19 (m, 4H), 2.73 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.92, 143.43, 138.40, 136.86, 133.75, 133.63, 132.18, 131.53, 130.41, 129.50, 122.63, 66.45, 52.39, 50.87, 46.65, 45.48, 21.14.

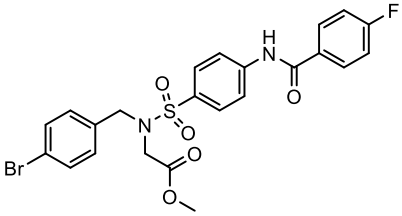
Methyl *N*-(4-bromobenzyl)-*N*-((4-(morpholine-4-carbonyl)phenyl)sulfonyl)glycinate (68e)



The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 26.7 mg, 0.104 mmol, 1.2 eq), 4-(morpholine-4-carbonyl)benzenesulfonyl chloride (25 mg, 0.086 mmol, 1 eq), Et_3N (48 µL, 0.34 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. The product was afforded without purification (27 mg, 0.054 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 – 7.90 (m, 2H), 7.59 – 7.53 (m, 2H), 7.48 – 7.43 (m, 2H), 7.19 – 7.12 (m, 2H), 4.44 (s, 2H), 3.93 (s, 2H), 3.88 – 3.59 (m, 6H), 3.56 (s, 3H), 3.44 – 3.35 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.89, 168.71, 141.00, 139.80, 133.74, 132.09, 130.41,

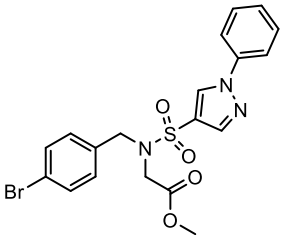
127.91, 127.82, 122.52, 66.90, 52.27, 51.00, 48.19, 46.74, 42.68. LC-MS $[C_{21}H_{23}BrN_2O_6S+H]^+$: 511.05/513.05 calculated, 511.08 / 513.00 found.

Methyl *N*-(4-bromobenzyl)-*N*-((4-(4-fluorobenzamido)phenyl)sulfonyl)glycinate (68f)



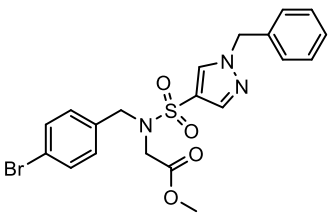
The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 40 mg, 0.16 mmol, 1 eq), 4-(4-fluorobenzamido)benzenesulfonyl chloride (97 mg, 0.31 mmol, 2 eq), Et_3N (86 µL, 0.62 mmol, 4 eq) and a drop of pyridine. Total time: 4 h at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (101 mg, 0.188 mmol, quant.). ^{1}H NMR (400 MHz, MeOD) δ 8.01 – 7.91 (m, 4H), 7.85 – 7.79 (m, 2H), 7.44 – 7.39 (m, 2H), 7.21 – 7.10 (m, 4H), 4.41 (s, 2H), 3.90 (s, 2H), 3.54 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 169.96, 167.13, 165.76 (d, $J_{C\text{-}F}$ = 252.6 Hz), 143.84, 134.83, 134.50, 132.37, 131.27 (d, $J_{C\text{-}F}$ = 3.1 Hz), 130.90, 130.81 (d, $J_{C\text{-}F}$ = 9.0 Hz), 128.99, 122.66, 120.92, 116.09 (d, $J_{C\text{-}F}$ = 21.9 Hz), 52.51, 51.66, 47.64. LC-MS $[C_{23}H_{20}BrFN_2O_5S+H]^+$: 535.03/537.03 calculated, 535.08/536.83 found.

Methyl *N*-(4-bromobenzyl)-*N*-((1-phenyl-1*H*-pyrazol-4-yl)sulfonyl)glycinate (68g)



The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 40 mg, 0.16 mmol, 1 eq), 1-phenyl-1*H*-pyrazole-4-sulfonyl chloride (75 mg, 0.31 mmol, 2 eq), Et_3N (86 µL, 0.62 mmol, 4 eq) and a drop of pyridine. Total time: 4 h at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (74.5 mg, 0.160 mmol, quant.). ^{1}H NMR (400 MHz, MeOD) δ 8.48 (d, *J* = 0.7 Hz, 1H), 7.98 (d, *J* = 0.7 Hz, 1H), 7.74 – 7.65 (m, 2H), 7.53 – 7.35 (m, 5H), 7.23 – 7.14 (m, 2H), 4.40 (s, 2H), 3.96 (s, 2H), 3.61 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 169.91, 140.34, 139.38, 134.41, 132.30, 130.73, 130.15, 130.02, 128.60, 123.71, 122.66, 120.27, 52.55, 51.39, 47.72. LC-MS $[C_{19}H_{18}BrN_3O_4S+H]^+$: 464.03/466.03 calculated, 464.17/466.08 found.

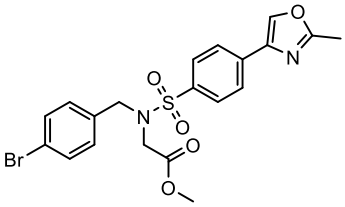
Methyl *N*-((1-benzyl-1*H*-pyrazol-4-yl)sulfonyl)-*N*-(4-bromobenzyl)glycinate (68h)



The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 25 mg, 0.097 mmol, 1 eq), 1-benzyl-1*H*-pyrazole-4-sulfonyl chloride (25 mg, 0.097 mmol, 1 eq), Et_3N (54 µL, 0.39 mmol, 4 eq) and a drop of pyridine. Total time: 2 h at rt. Silica gel column chromatography (5-40% EtOAc in *n*-pentane) afforded the product (38 mg, 0.079 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 0.7 Hz, 1H), 7.81 (d, *J* = 0.7 Hz, 1H), 7.46 – 7.42 (m, 2H), 7.41 – 7.34 (m, 3H), 7.29 – 7.25 (m, 2H), 7.17 – 7.13 (m, 2H), 5.32 (s, 2H), 4.37 (s, 2H), 3.91 (s, 2H), 3.57 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.23, 139.26, 134.80, 134.11, 132.01, 131.51,

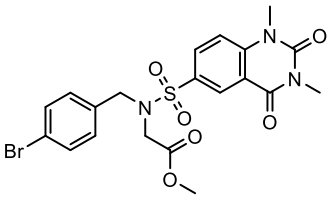
130.33, 129.22, 128.86, 128.25, 122.33, 122.03, 56.89, 52.17, 50.73, 46.95. LC-MS $[C_{20}H_{20}BrN_3O_4S+H]^+$: 478.04/480.04 calculated, 478.25/480.00 found.

Methyl *N*-(4-bromobenzyl)-*N*-((4-(2-methyloxazol-4-yl)phenyl)sulfonyl)glycinate (68i)



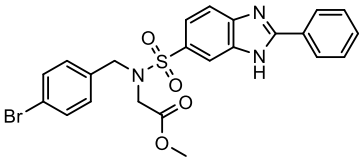
The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 30 mg, 0.12 mmol, 1.2 eq), 4-(2-methyloxazol-4-yl)benzenesulfonyl chloride (25 mg, 0.085 mmol, 1 eq), Et_3N (47 µL, 0.34 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (19 mg, 0.039 mmol, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.92 – 7.83 (m, 4H), 7.49 – 7.41 (m, 2H), 7.18 – 7.11 (m, 2H), 4.47 (s, 2H), 3.93 (s, 2H), 3.54 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.05, 162.52, 139.39, 138.57, 135.83, 134.98, 134.08, 132.05, 130.42, 128.07, 125.83, 122.40, 52.23, 50.94, 46.74, 14.11. LC-MS $[C_{20}H_{19}BrN_2O_5S+H]^+$: 479.03/481.03 calculated, 479.00/480.93 found.

Methyl *N*-(4-bromobenzyl)-*N*-((1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)sulfonyl)glycinate (68j)



The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 26.8 mg, 0.104 mmol, 1.2 eq), 1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride (25 mg, 0.087 mmol, 1 eq), Et_3N (49 µL, 0.35 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (31 mg, 0.061 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 2.3 Hz, 1H), 8.10 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.32 (d, *J* = 8.9 Hz, 1H), 7.20 – 7.12 (m, 2H), 4.43 (s, 2H), 3.99 (s, 2H), 3.67 (s, 3H), 3.60 (s, 3H), 3.51 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.12, 160.83, 150.95, 143.30, 134.55, 133.71, 133.68, 132.10, 130.39, 129.08, 122.50, 115.46, 114.35, 52.39, 50.86, 46.81, 31.34, 28.91. LC-MS $[C_{20}H_{20}BrN_3O_6S+H]^+$: 510.03/512.03 calculated, 509.67/511.80 found.

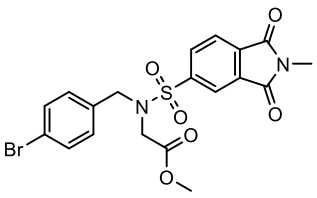
Methyl *N*-(4-bromobenzyl)-*N*-((2-phenyl-1*H*-benzo[*d*]imidazol-6-yl)sulfonyl)glycinate (68k)



The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 26 mg, 0.085 mmol, 1.2 eq), 2-phenyl-1*H*-benzo[*d*]imidazole-6-sulfonyl chloride (25 mg, 0.085 mmol, 1 eq), Et_3N (47.4 µL, 0.340 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (17 mg, 0.034 mmol, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.28 (s, 1H), 8.16 – 8.08 (m, 3H), 7.77 – 7.70 (m, 1H),

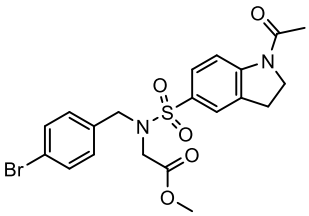
7.50 – 7.43 (m, 4H), 7.41 – 7.36 (m, 2H), 7.11 – 7.04 (m, 2H), 4.41 (s, 2H), 3.91 (s, 2H), 3.48 (s, 3H). LC-MS $[C_{23}H_{20}BrN_3O_4S+H]^+$: 514.04/516.04 calculated, 514.13/516.07 found.

Methyl *N*-(4-bromobenzyl)-*N*-((2-methyl-1,3-dioxoisoindolin-5-yl)sulfonyl)glycinate (68l)



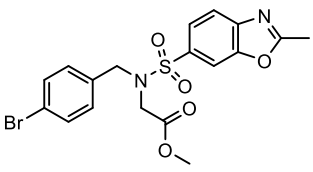
The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 29.8 mg, 0.116 mmol, 1.2 eq) and 2-methyl-1,3-dioxoisoindoline-5-sulfonyl chloride (25 mg, 0.096 mmol, 1 eq), Et_3N (54 µL, 0.38 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (15 mg, 0.032 mmol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (dd, *J* = 1.6, 0.7 Hz, 1H), 8.21 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.99 (dd, *J* = 7.8, 0.7 Hz, 1H), 7.52 – 7.42 (m, 2H), 7.21 – 7.13 (m, 2H), 4.47 (s, 2H), 4.00 (s, 2H), 3.58 (s, 3H), 3.24 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.79, 167.01, 166.92, 145.76, 135.37, 133.31, 133.04, 132.97, 132.24, 130.41, 123.92, 122.75, 122.35, 52.47, 51.01, 46.73, 24.53.

Methyl *N*-((1-acetylindolin-5-yl)sulfonyl)-*N*-(4-bromobenzyl)glycinate (68m)



The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 100 mg, 387 mmol, 1 eq), 1-acetylindoline-5-sulfonyl chloride (151 mg, 0.775 mmol, 1.5 eq), Et_3N (215 µL, 1.55 mmol, 4 eq) and a drop of pyridine. Total time: 4 h at rt. Silica gel column chromatography (20-70% EtOAc in *n*-pentane) afforded the product (34 mg, 0.072 mmol, 19%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 8.24 (d, *J* = 8.6 Hz, 1H), 7.67 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.61 (d, *J* = 1.9 Hz, 1H), 7.44 – 7.38 (m, 2H), 7.16 – 7.06 (m, 2H), 4.38 (s, 2H), 4.18 (t, *J* = 8.6 Hz, 2H), 3.89 (s, 2H), 3.56 (s, 3H), 3.27 (t, *J* = 8.6 Hz, 2H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 170.96, 169.79, 147.09, 134.52, 134.29, 133.25, 132.24, 130.73, 128.29, 124.33, 122.54, 117.04, 52.46, 51.48, 49.69, 47.48, 27.92, 24.29. LC-MS $[C_{20}H_{21}BrN_2O_5S+H]^+$: 481.04/483.04 calculated, 481.00/482.92 found.

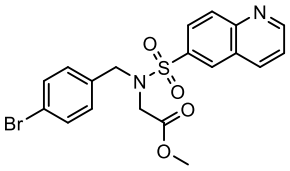
Methyl *N*-(4-bromobenzyl)-*N*-((2-methylbenzo[*d*]oxazol-6-yl)sulfonyl)glycinate (68n)



The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 25 mg, 0.097 mmol, 1 eq), 2-methylbenzo[*d*]oxazole-6-sulfonyl chloride (22 mg, 0.097 mmol, 1 eq), Et_3N (54 µL, 0.39 mmol, 4 eq) and a drop of pyridine. Total time: 2 h at rt. Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product (28 mg, 0.062 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 1.7, 0.6 Hz, 1H), 7.85 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.77 (dd, *J* = 8.4, 0.6 Hz, 1H), 7.47 – 7.41 (m, 2H), 7.17 – 7.11 (m, 2H), 4.46 (s, 2H), 3.96 (s, 2H), 3.54 (s, 3H), 2.72 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.05, 167.52, 150.43, 145.38, 136.04, 133.93, 132.05, 130.40, 123.88, 122.42,

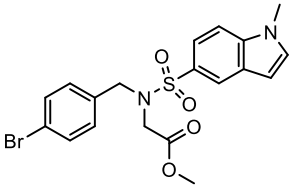
119.92, 110.50, 52.23, 50.98, 46.77, 14.91. LC-MS $[C_{18}H_{17}BrN_2O_5S+H]^+$: 453.01/455.01 calculated, 452.93/454.93 found.

Methyl *N*-(4-bromobenzyl)-*N*-(quinolin-6-ylsulfonyl)glycinate (68o)



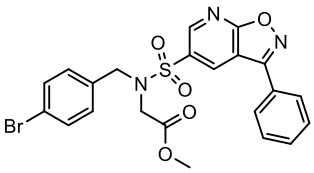
The title compound was synthesized according to general procedure **H** using methyl(4-bromobenzyl)glycinate (**67**, 40.8 mg, 0.158 mmol, 1.2 eq), quinoline-6-sulfonyl chloride (30 mg, 0.13 mmol, 1 eq), Et_3N (74 µL, 0.53 mmol, 4 eq) and a drop of pyridine. Total time: overnight at rt. Silica gel column chromatography (20-100% EtOAc in *n*-pentane) afforded the product (24 mg, 0.053 mmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.43 (d, *J* = 2.1 Hz, 1H), 8.29 (ddd, *J* = 8.3, 1.8, 0.7 Hz, 1H), 8.26 (dt, *J* = 9.0, 0.7 Hz, 1H), 8.09 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.56 (dd, *J* = 8.3, 4.3 Hz, 1H), 7.48 – 7.40 (m, 2H), 7.20 – 7.12 (m, 2H), 4.52 (s, 2H), 4.01 (s, 2H), 3.50 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.01, 153.25, 149.54, 137.56, 137.35, 133.84, 132.09, 131.13, 130.43, 128.92, 127.31, 126.54, 122.74, 122.51, 52.27, 51.08, 46.83. LC-MS $[C_{19}H_{17}BrN_2O_4S+H]^+$: 449.02/451.01 calculated, 449.33/451.17 found.

Methyl *N*-(4-bromobenzyl)-*N*-((1-methyl-1*H*-indol-5-yl)sulfonyl)glycinate (68p)



The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 23 mg, 0.089 mmol, 1 eq), 1-methyl-1*H*-indole-5-sulfonyl chloride (20 mg, 0.087 mmol, 1 eq), Et_3N (49 µL, 0.35 mmol, 4 eq) and a drop of pyridine. Total time: 2 h at rt. Silica gel column chromatography (5-40% EtOAc in *n*-pentane) afforded the product (29 mg, 0.064 mmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (d, *J* = 1.8 Hz, 1H), 7.69 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.44 – 7.38 (m, 3H), 7.19 (d, *J* = 3.2 Hz, 1H), 7.15 – 7.11 (m, 2H), 6.61 (dd, *J* = 3.2, 0.9 Hz, 1H), 4.44 (s, 2H), 3.91 (s, 2H), 3.85 (s, 3H), 3.49 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.38, 138.53, 134.62, 131.88, 131.30, 130.46, 129.97, 127.92, 122.11, 122.00, 120.43, 109.74, 102.90, 52.10, 51.07, 46.99, 33.30. LC-MS $[C_{19}H_{19}BrN_2O_4S+H]^+$: 451.03/453.03 calculated, 451.00/452.92 found.

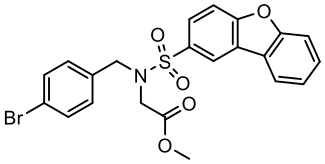
Methyl *N*-(4-bromobenzyl)-*N*-((3-phenylisoxazolo[5,4-*b*]pyridin-5-yl)sulfonyl)glycinate (68q)



The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 26.3 mg, 0.102 mmol, 1 eq), 3-phenylisoxazolo[5,4-*b*]pyridine-5-sulfonyl chloride (30 mg, 0.10 mmol, 1 eq), Et_3N (57 µL, 0.41 mmol, 4 eq) and a drop of pyridine. Total time: 2 h at rt. Silica gel column chromatography (2-20% EtOAc in *n*-pentane) afforded the product (29 mg, 0.056 mmol, 55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.13 (d, *J* = 2.2 Hz, 1H), 8.83 (d, *J* = 2.2 Hz, 1H), 8.00 – 7.96 (m, 2H), 7.64 – 7.60 (m, 3H), 7.49 – 7.45 (m, 2H), 7.21 – 7.17 (m, 2H), 4.45 (s, 2H), 4.07 (s, 2H), 3.60 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.29, 169.15, 158.09, 150.17, 133.74, 133.19, 133.10, 132.26, 131.57, 130.38, 129.68,

127.94, 127.51, 122.82, 112.15, 52.55, 50.92, 46.90. LC-MS $[C_{22}H_{18}BrN_3O_5S+H]^+$: 516.02/518.02 calculated, 516.00/518.00 found.

Methyl *N*-(4-bromobenzyl)-*N*-(dibenzo[*b*,*d*]furan-2-ylsulfonyl)glycinate (68r)



The title compound was synthesized according to general procedure **H** using methyl (4-bromobenzyl)glycinate (**67**, 34.8 mg, 0.135 mmol, 1.2 eq), dibenzo[*b*,*d*]furan-2-sulfonyl chloride (30.0 mg, 0.112 mmol, 1 eq), Et_3N (62.4 µL, 0.450 mmol, 4 eq) and a drop of pyridine. Total time: 4 h at rt. Silica gel column chromatography (0-40% EtOAc in *n*-pentane) afforded the product (40 mg, 0.081 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (dd, *J* = 2.0, 0.5 Hz, 1H), 8.04 – 7.95 (m, 2H), 7.70 (dd, *J* = 8.6, 0.6 Hz, 1H), 7.64 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.56 (ddd, *J* = 8.4, 7.2, 1.3 Hz, 1H), 7.50 – 7.40 (m, 3H), 7.20 – 7.13 (m, 2H), 4.50 (s, 2H), 3.98 (s, 2H), 3.51 (s, 3H). LC-MS $[C_{22}H_{18}BrNO_5S+H]^+$: 488.02/490.01 calculated, 487.83/489.83 found.

Reference

1. Janssen, F. J. & van der Stelt, M. Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders. *Bioorg. Med. Chem. Lett.* **26**, 3831–3837 (2016).
2. Bisogno, T. *et al.* A novel fluorophosphonate inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol with potential anti-obesity effects. *Br. J. Pharmacol.* **169**, 784–793 (2013).
3. Deng, H. *et al.* Triazole Ureas Act as Diacylglycerol Lipase Inhibitors and Prevent Fasting-Induced Refeeding. *J. Med. Chem.* **60**, 428–440 (2017).
4. Winters, N. D. *et al.* Targeting diacylglycerol lipase reduces alcohol consumption in preclinical models. *J. Clin. Invest.* **131**, e146861 (2021).
5. Wilkerson, J. L. *et al.* Diacylglycerol lipase β inhibition reverses nociceptive behaviour in mouse models of inflammatory and neuropathic pain. *Br. J. Pharmacol.* **173**, 1678–1692 (2016).
6. Wilkerson, J. L. *et al.* Investigation of diacylglycerol lipase alpha inhibition in the mouse lipopolysaccharide inflammatory pain model. *J. Pharmacol. Exp. Ther.* **363**, 394–401 (2017).
7. Khasabova, I. A. *et al.* Inhibition of DAGLβ as a therapeutic target for pain in sickle cell disease. *Haematologica* **108**, 859-869 (2023).
8. Hsu, K.-L. *et al.* DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat. Chem. Biol.* **8**, 999–1007 (2012).
9. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 26–33 (2016).
10. Baggelaar, M. P. *et al.* Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J. Am. Chem. Soc.* **137**, 8851–8857 (2015).
11. Janssen, F. J. *et al.* Discovery of Glycine Sulfonamides as Dual Inhibitors of *sn*-1-Diacylglycerol Lipase α and α/β-Hydrolase Domain 6. *J. Med. Chem.* **57**, 6610–6622 (2014).
12. Cavener, V. S. *et al.* Inhibition of Diacylglycerol Lipase Impairs Fear Extinction in Mice. *Front. Neurosci.* **12**, 1–10 (2018).
13. Levine, A. *et al.* DAGLα Inhibition as a Non-invasive and Translational Model of Episodic Headache. *Front. Pharmacol.* **11**, 1–15 (2021).
14. Berger, N. *et al.* Inhibition of diacylglycerol lipase β modulates lipid and endocannabinoid levels in the *ex vivo* human placenta. *Front. Endocrinol.* **14**, 1–12 (2023).

15. Chupak, L. S. *et al.* Structure activity relationship studies on chemically non-reactive glycine sulfonamide inhibitors of diacylglycerol lipase. *Bioorg. Med. Chem.* **24**, 1455–1468 (2016).

16. Jetson, R. *et al.* Practical synthesis of a chromene analog for use as a retinoic acid receptor alpha antagonist lead compound. *Eur. J Med. Chem.* **63**, 104–108 (2013).

17. Zhang, H. *et al.* Synthesis and biological evaluation of novel benzofuroxan-based pyrrolidine hydroxamates as matrix metalloproteinase inhibitors with nitric oxide releasing activity. *Bioorg. Med. Chem.* **26**, 4363–4374 (2018).

18. Barbasiewicz, M., Marciniak, K. & Fedoryński, M. Phase transfer alkylation of arylacetonitriles revisited. *Tetrahedron Lett.* **47**, 3871–3874 (2006).

19. Zhang, D., Zheng, H. & Wang, X. General and cost-effective synthesis of 1-heteroaryl/arylcycloalkylamines and their broad applications. *Tetrahedron* **72**, 1941–1953 (2016).

20. Ren, Y. *et al.* Development of an open-air and robust method for large-scale palladium-catalyzed cyanation of aryl halides: The use of *i*-PrOH to prevent catalyst poisoning by oxygen. *Org. Process Res. Dev.* **13**, 764–768 (2009).

21. Rogacki, M. K. *et al.* Identification and Profiling of Hydantoins – A Novel Class of Potent Antimycobacterial DprE1 Inhibitors. *J. Med. Chem.* **61**, 11221–11249 (2018).

22. Ushijima, S., Dohi, S., Moriyama, K. & Togo, H. Facile preparation of aromatic ketones from aromatic bromides and arenes with aldehydes. *Tetrahedron* **68**, 1436–1442 (2012).

23. He, T., Wang, G., Bonetti, V., Klare, H. F. T. & Oestreich, M. Silylium-Ion-Promoted (5+1) Cycloaddition of Aryl-Substituted Vinylcyclopropanes and Hydrosilanes Involving Aryl Migration. *Angew. Chem. Int. Ed.* **59**, 12186–12191 (2020).

24. Addie, M. *et al.* Discovery of 4-Amino-*N*-[(1*S*)-1-(4-chlorophenyl)-3-hydroxypropyl]-1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidine-4-carboxamide (AZD5363), an Orally Bioavailable, Potent Inhibitor of Akt Kinases. *J. Med. Chem.* **56**, 2059–2073 (2013).

25. Tung, C. L., Wong, C. T. T., Fung, E. Y. M. & Li, X. Traceless and Chemoselective Amine Bioconjugation via Phthalimidine Formation in Native Protein Modification. *Org. Lett.* **18**, 2600–2603 (2016).

Chapter 4

Optimization of glycine sulfonamides as DAGLβ selective inhibitors

4.1 Introduction

2-Arachidonoylglycerol (2-AG) is one of the two main endocannabinoids that play a crucial role in diverse physiological processes, such as synaptic plasticity, memory formation, pain sensation and immune response.[1,2] The synthesis of 2-AG initiates from phosphatidylinositol-4,5-biphosphate (PIP_2), which undergoes sequential transformations by phospholipase C (PLC) and *sn*-1-specific diacylglycerol lipases (DAGL).[1,3] Subsequently, monoacylglycerol lipase (MAGL), along with α/β-hydrolase domain-containing 6 and 12 (ABHD6 and ABHD12), hydrolyzes 2-AG to generate arachidonic acid (AA).[4] Both AA and 2-AG can be oxidized by cyclooxygenase-2 (COX2), leading to the formation of pro-inflammatory prostaglandins and glyceryl prostaglandins, respectively, contributing to inflammation.[5,6] There are two known isoforms of DAGL, namely DAGLα and DAGLβ[3], whose expression levels vary based on tissue and cell types. DAGLα is the dominant isoform in neurons of the central nervous system (CNS), where it produces 2-AG to activate cannabinoid receptor type 1 (CB_1R) to modulate neurotransmitter release.[7–9] In contrast, DAGLβ takes precedence in periphery and immune cells.[8,10,11]

Endocannabinoid signaling, mediated by DAGLα and CB_1R, is closely linked to anxiety, stress and fear responses.[12–14] Knocking out DAGLα resulted in an 80% reduction in brain 2-AG levels, adversely influencing the emotional state of mice and inducing various negative behavioral changes including maternal neglect, fear extinction deficit, reduced hippocampal neurogenesis, and enhanced anxiety-related behaviors.[12] Pharmacological inhibition of DAGLα by DO34 similarly impaired fear extinction learning.[13] Conversely, inhibiting the hydrolysis of 2-AG by MAGL inhibitor JZ184 demonstrated antidepressant and anxiolytic effects.[15,16] These findings underscore the crucial role of 2-AG signaling in the brain and the potential of causing neuropsychiatric side effects by disruption brain 2-AG levels.

DAGLβ was identified as the predominant DAGL in microglia[10] in the brain, and macrophages[8] and dendritic cells[11] of the periphery. DAGLβ deletion notably reduced the basal level of prostaglandin E2 (PGE_2) in the brain without altering 2-AG and AA.[10] Moreover, disrupting DAGLβ protected microglia and macrophages from LPS-induced inflammation by inhibiting inflammatory cytokine production[8,10] and attenuated inflammatory signaling in dendritic cells while preserving its antigen presentation function in adaptive immune responses.[11] Animal studies targeting DAGLβ yielded promising results for anti-inflammation. DAGLβ deletion attenuated LPS-induced hypothermia[10], a profound reduction in core body temperature mediated by neuroinflammatory processes.[17] KT109 treatment, inhibiting DAGLβ, effectively reduced allodynia in chronic constriction injury (CCI) neuropathic pain models and chemotherapy-induced neuropathic pain (CINP) models without inducing side effects such as catalepsy, hypothermia, thermal hypoalgesia and hypomotility.[18] Subtype specific DAGL inhibitors have therefore emerged as a promising strategy to finely tune 2-AG levels while avoiding CNS side effects.

During the structure-activity relationship (SAR) study of glycine sulfonamides for DAGLα/β, as described in Chapter 3, three compounds with different modifications on the

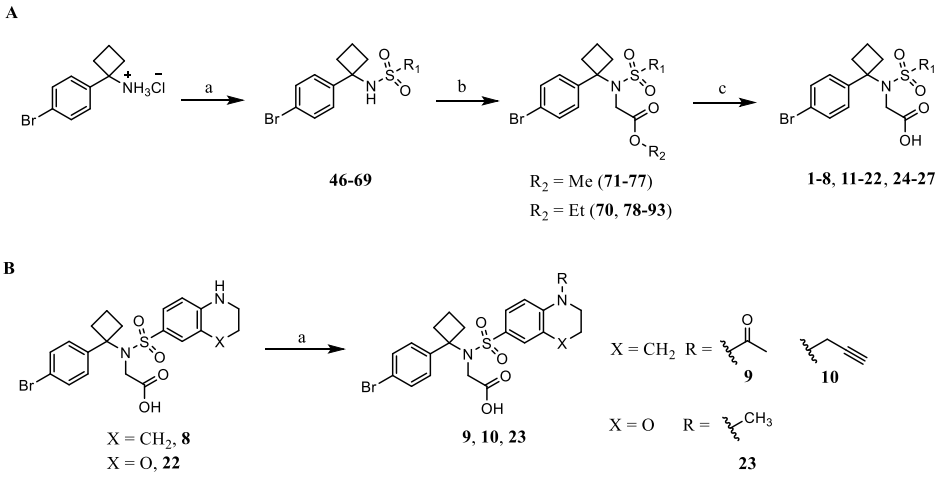
sulfonyl group exhibited subtle yet notable selectivity for DAGLβ. Building upon these initial findings, an extensive SAR study ensued, focusing on further optimizing the sulfonyl group to enhance selectivity. This effort led to the identification of a 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine moiety as the optimal substituent on the sulfonyl group. Subsequent to this discovery, a series of compounds containing a 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine on the sulfonyl group were developed and assessed. Overall, this yielded compounds **27**, **32** and **42-45**, characterized by high potency, good selectivity for DAGLβ, and promising physicochemical properties for further studies.

4.2 Results and discussion

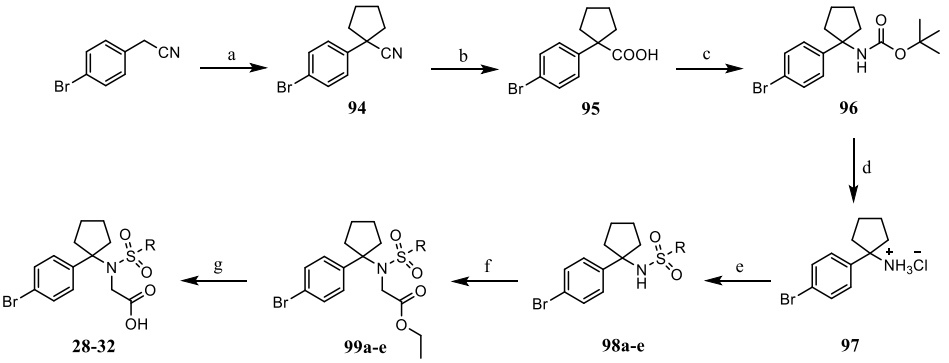
4.2.1 Design and synthesis of glycine sulfonamides 1-45

In Chapter 3, three compounds, featuring different modifications on the sulfonyl, exhibited moderate selectivity for DAGLβ. This observation encouraged an in-depth SAR investigation with a specific emphasis on this region, resulting in the design and synthesis of compounds **1-27**. To further optimize the potency and selectivity, the most selective substituents on the sulfonyl group were combined with modifications in the other regions of the glycine sulfonamide, leading to compounds **28-45**.

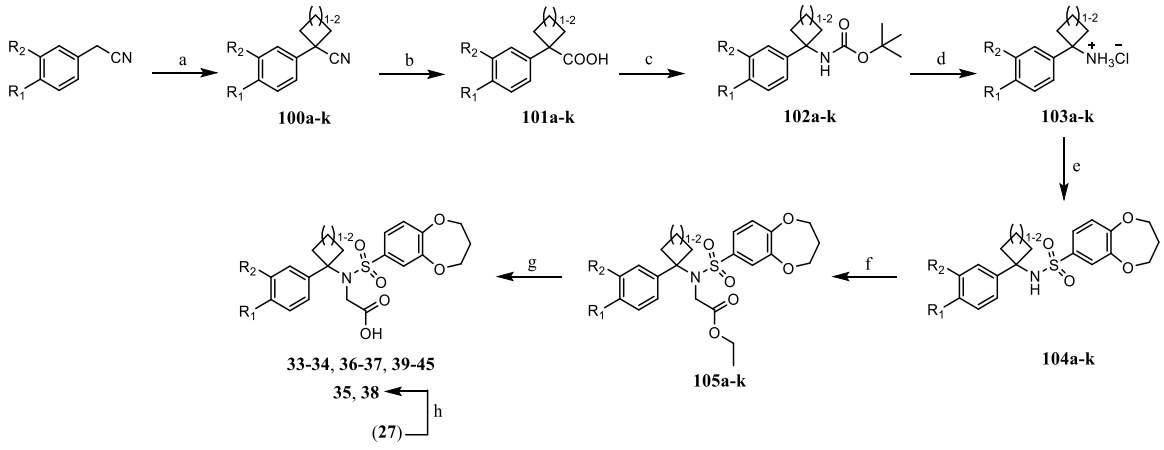
The synthetic route of final compounds **1-27** is depicted in Scheme 4.1, which contains three general steps as described in Chapter 3: coupling between the aminium chloride and sulfonyl chlorides yielded sulfonamides **46-69**; subsequent *N*-alkylation resulted in esters **70-93**, followed by saponification affording glycine sulfonamides **1-8**, **11-22**, and **24-27** (Scheme 4.1A). Compounds **9** and **10** were synthesized from compound **8** via acetylation and alkylation, respectively (Scheme 4.1B). Methylation of **22** yielded **23**. The synthesis of compounds **28-32** (Scheme 4.2) followed a similar procedure as their cyclobutyl counterparts. In brief, cyclopentyl formation through nucleophilic substitution (**94**), followed by nitrile hydrolysis (**95**), diphenylphosphoryl azide-induced Curtius rearrangement and *tert*-butyloxycarbamate formation, yielded compound **96**. Acidolysis (**96** → **97**) followed by sulfonamidation with sulfonyl chlorides formed sulfonamides **98a-e**, which were transformed to glycine sulfonamides **28-32** via alkylation and saponification. Finally, compounds **33-45** were synthesized according to the procedure illustrated in Scheme 4.3, starting from the nitriles. A similar reaction sequence was adopted as shown in Scheme 4.2. Aminium chlorides **103a-k** were condensed with 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**) to form sulfonamides **104a-k**. Subsequent alkylation and saponification yielded the final compounds **33-34**, **36-37** and **39-45**. Glycine sulfonamides **35** and **38** were synthesized from compound **27** via a Suzuki-Miyaura coupling and Pd-catalyzed introduction of the cyano group.



Scheme 4.1 Synthesis of glycine sulfonamides **1-27**. (A) a) corresponding sulfonyl chloride, Et_3N or DIPEA, anhydrous DCM, rt, 20-100%; b) methyl 2-bromoacetate or ethyl 2-bromoacetate, BEMP, anhydrous DMF, 80 °C, 39%-quant.; c) 2 M or 1 M aq. NaOH or 1 M aq. LiOH, MeOH/THF, rt, 8-86%; (B) a) NaH, acetyl chloride or 3-bromoprop-1-yne or CH_3I, anhydrous DMF, rt, 80% for **9**, 19% for **10**, 16% for **23**.



Scheme 4.2 Synthesis of glycine sulfonamides **28-32**. a) 1,4-dibromobutane, TBABr, KOH, toluene, H_2O, reflux, 81%; b) *i.* KOH, ethylene glycol, reflux; *ii.* 6 M aq. HCl in 1,4-dioxane, reflux, 43%; c) DPPA, Et_3N, anhydrous *t*-BuOH, 30 °C-reflux, 54%; d) 3 M aq. HCl in MeOH, rt, 80%; e) corresponding sulfonyl chloride, DIPEA, anhydrous DCM, rt, 30-76%; f) ethyl 2-bromoacetate, BEMP, anhydrous DMF, 80 °C, 25-67%; g) 2 M aq. NaOH or 1 M aq. LiOH, MeOH/THF, rt, 6-59%.



Scheme 4.3 Synthesis of glycine sulfonamides **33-45**. a) 1,3-dibromopropane or 1,4-dibromobutane, TBABr, KOH, toluene, H_2O, reflux, 24-87%; b) KOH, ethylene glycol, reflux, or 9 M aq. H_2SO_4, reflux, 45%-quant.; c)

DPPA, Et_3N, anhydrous *t*-BuOH, 30 °C-reflux, 27-93%; d) 3 M aq. HCl in MeOH, rt, 38-100%; e) 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**), DIPEA, anhydrous DCM, rt, 39-88%; f) ethyl 2-bromoacetate, BEMP, anhydrous DMF, 80 °C, 58-86%; g) 1 M aq. LiOH, MeOH/THF, rt, 29%-99%; h) Methyl boronic acid, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, K_2CO_3, 1,4-dioxane/H_2O, 85 °C, 49% for **35**; $Pd(OAc)_2$, $K_4Fe(CN)_6 \cdot 3H_2O$, Na_2CO_3, *i*-PrOH, H_2O, DMF, 100 °C, 12% for **38**.

4.2.2 Biochemical evaluation and structure-activity-relationship of compounds 1-45

4.2.2.1 Optimization of the sulfonyl substituent

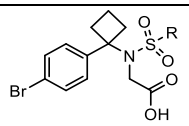
The biochemical activities of compounds **1-27**, with varying sulfonyl substituents, were evaluated using the DAGL EnzChek lipase substrate assay and the corresponding results are presented in Table 4.1. In Chapter 3, three compounds (**49**, **51** and **52** in Chapter 3**)** exhibited around a 3-fold selectivity for DAGLβ over DAGLα. Building upon this, the optimization started by reintroducing the cyclobutyl moiety to the glycine sulfonamide core structure in compounds **1-3**, resulting in an increase in potency and a slight improvement in selectivity compared to their counterparts without the cyclobutyl moiety. In light of this observation, compounds **4-7**, featuring similar sulfonyl substituents, were designed, synthesized, and evaluated. Compounds **4-6** demonstrated, however, a reduction in potency for both DAGL enzymes, likely attributed to the orientation of these sulfonyl substituents. In line, compound **7** was significantly more potent than its counterpart **5**. However, none of these compounds displayed an improved selectivity for DAGLβ compared to compound **3**.

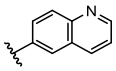
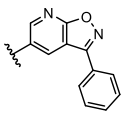
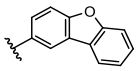
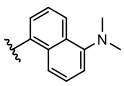
To further extend the SAR study and identify more selective compounds, diverse substituents were systematically investigated. The incorporation of a tetrahydroquinoline onto the sulfonyl led to compound **8**, which showed moderate and comparable activity for DAGLα and DAGLβ. Introducing an acetyl (**9**) or a propargyl (**10**) on the amine resulted in decreased potency. Removing a phenyl ring in compound **3** gave rise to compound **11** with a benzofuran moiety. This caused approximately a 10-fold reduction in potency compared to compound **3**. The selectivity was retained, while the lipophilic efficiency (LipE) was enhanced due to lower lipophilicity. This outcome suggests that the phenyl ring plays an important role for potency. Substituting the furan in compound **11** to a dioxolane resulted in compound **12,** which demonstrated a 3-fold selectivity for DAGLβ. Expanding the five-membered dioxolane to a six-membered dioxane in compound **13** brought a remarkable increase in both potency and selectivity. Compound **13** exhibited a negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) of 7.59 ± 0.06 and a 20-fold selectivity for DAGLβ.

Subsequent modifications were based on compound **13**. Opening the dioxane resulted in compound **14** with 3- and 4-methoxy groups. This modification led to a more than 100-fold decrease in potency and a complete loss of selectivity for DAGLβ. Changing 3-methoxy to a benzyloxy (compound **15**) improved potency, particularly for DAGLα. Meanwhile, changing 4-methoxy or both methoxy groups to a benzyloxy (compounds **16** and **17**) retained most of the activity for both DAGL enzymes. Introducing a double bond (compound **18**) as well as substituting the *para* oxygen with a methylene (compound **19**) decreased potency for DAGLβ,

but increased potency for DAGLα, resulting in lower selectivity. Introducing a dimethyl group near the *para* oxygen in **13** (compound **20**) led to decreased selectivity. Conversely, introducing the dimethyl group near the *meta* oxygen (compound **21)** retained most of potency and selectivity. Substituting the *para* oxygen in **13** to an amine led to compound **22**, which exhibited a reduction in potency and selectivity. Introducing a methyl group to the amine (compound **23**) regained some potency. Introduction of a chlorine on the *meta* position of the phenyl ring (compound **24**) further restored potency as well as selectivity. Changing the methylmorpholine in **23** to a methylmorpholinone (compound **25)** reduced the activity likely due to increased hydrophilicity. Expanding the six-membered dioxane to an eight-membered dioxocane (compound **26**) increased potency for DAGLα while retaining potency for DAGLβ, resulting in a decrease in selectivity. However, expanding dioxane to a seven-membered dioxepane obtained compound **27**, which displayed a notable decrease in the potency for DAGLα but not for DAGLβ. Compound **27** exhibited a pIC_{50} of 7.48 ± 0.09 and a selectivity of 36-fold for DAGLβ, representing the compound with the highest selectivity thus far. Additionally, this compound demonstrated high lipophilic efficiency (a LipE of 6.1), indicating favourable druglikeness.

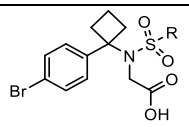
Table 4.1 Biochemical results and physicochemical properties of glycine sulfonamides **1-27**.[a]

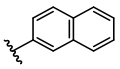
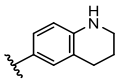
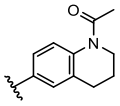
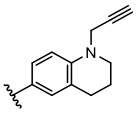
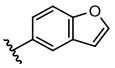
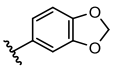
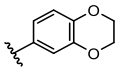
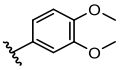
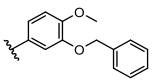
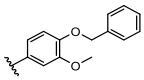
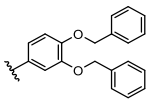
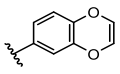


ID	R	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
1		6.08 ± 0.09	5.33 ± 0.13	5.6	1.4	99	4.7
2		6.47 ± 0.13	5.72 ± 0.04	5.6	1.9	125	4.6
3		7.85 ± 0.14	7.03 ± 0.03	6.6	2.9	99	5.0
4		5.27 ± 0.07	5.20 ± 0.10	1.3	1.3	99	4.0
5		6.01 ± 0.08	5.49 ± 0.07	3.3	2.3	86	3.7
6		6.39 ± 0.08	5.82 ± 0.08	3.7	2.2	89	4.2

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

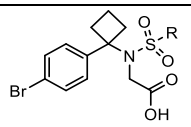
Table 4.1 (continued) Biochemical results and physicochemical properties of glycine sulfonamides **1-27**.[a]

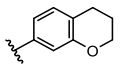
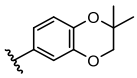
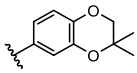
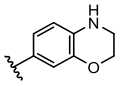
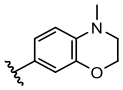
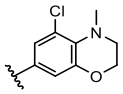
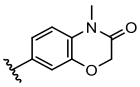
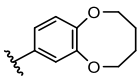
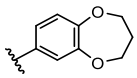


ID	R	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
7		7.89 ± 0.12	7.22 ± 0.04	4.7	2.3	86	5.6
8		6.46 ± 0.09	6.32 ± 0.05	1.4	1.1	98	5.4
9		5.52 ± 0.12	5.48 ± 0.08	1.1	1.6	106	3.9
10		6.16 ± 0.10	6.31 ± 0.08	0.7	1.5	89	4.7
11		6.84 ± 0.12	6.15 ± 0.10	4.9	1.6	99	5.2
12		6.16 ± 0.24	5.72 ± 0.16	2.8	1.2	104	5.0
13		7.59 ± 0.06	6.29 ± 0.05	20	1.1	104	6.5
14		5.29 ± 0.21	5.49 ± 0.10	0.6	1.0	104	4.3
15		5.61 ± 0.14	6.62 ± 0.06	0.1	2.4	104	3.2
16		5.37 ± 0.18	5.44 ± 0.07	0.9	2.4	104	3.0
17		5.43 ± 0.14	5.64 ± 0.11	0.6	3.8	104	1.6
18		7.32 ± 0.11	6.66 ± 0.06	4.6	0.8	104	6.5

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

Table 4.1 (continued) Biochemical results and physicochemical properties of glycine sulfonamides **1-27**.[a]



ID	R	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
19		6.85 ± 0.10	6.43 ± 0.06	2.6	1.7	95	5.2
20		6.75 ± 0.21	6.70 ± 0.07	1.1	1.7	104	5.0
21		7.28 ± 0.23	6.06 ± 0.11	17	1.7	104	5.6
22		5.92 ± 0.10	5.82 ± 0.21	1.3	0.5	107	5.4
23		6.40 ± 0.10	6.16 ± 0.18	1.7	0.9	98	5.5
24		7.04 ± 0.13	5.94 ± 0.13	13	1.5	98	5.5
25		5.47 ± 0.30	5.37 ± 0.21	1.3	0.3	115	5.2
26		7.56 ± 0.16	6.73 ± 0.08	6.8	1.7	104	5.9
27		7.48 ± 0.09	5.93 ± 0.16	35	1.4	104	6.1

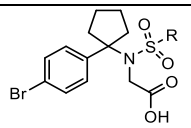
[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

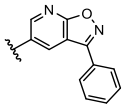
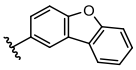
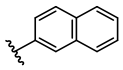
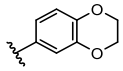
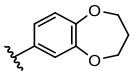
4.2.2.2 Combination of the optimal sulfonyl substituents and modifications on the amine moiety

Based on a previously published SAR study with glycine sulfonamides for DAGLα[19], a (4-bromophenyl)cyclopentane on the amine was combined with the optimal sulfonyl substituents, leading to compounds **28-32** (Table 4.2). Remarkably, the potencies of compounds **28-32** were found to be comparable to their cyclobutyl counterparts. Specifically, compound **31** was 2-fold less potent than its counterpart **13**, while compound **32** was 4-fold more potent than its counterpart **27**. Furthermore, in comparison with the cyclobutyl analogs, the cyclopentyl

slightly decreased the selectivity of compounds, except for compound **32**. In conclusion, compound **32**, featuring a 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine on the sulfonyl and a (4-bromophenyl)cyclopentane on the amine, was the most potent and selective inhibitor so far, exhibiting a pIC_{50} of 8.08 ± 0.13 and a selectivity of 37-fold for DAGLβ.

Table 4.2 Biochemical results and physicochemical properties of glycine sulfonamides **28-32**.[a]



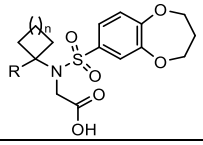
ID	R	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
28		6.38 ± 0.11	5.88 ± 0.10	3.2	2.2	125	4.2
29		7.71 ± 0.14	7.07 ± 0.08	4.4	3.2	99	4.5
30		8.00 ± 0.06	7.40 ± 0.06	4.0	2.7	86	5.3
31		7.19 ± 0.15	5.98 ± 0.18	16	1.4	104	5.8
32		8.08 ± 0.13	6.51 ± 0.06	37	1.8	104	6.3

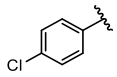
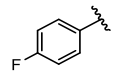
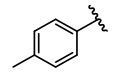
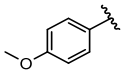
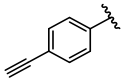
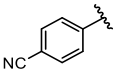
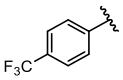
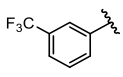
[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

Finally, the substitution pattern of the benzyl group was investigated (compounds **33-45**, Table 4.3). Changing the bromine in compound **27** to chlorine or fluorine yielded compounds **33** and **34**, respectively, which displayed a significant reduction in potency and selectivity. This reduction may be caused by a decreased size of these substituents. Substituting the bromine to a methyl (**35**) or a methoxy (**36**) also significantly decreased the potency and selectivity, which may be due to the electron-donating property of these two groups. Therefore, larger electron-withdrawing groups were investigated. Altering the bromine to an alkyne (**37**) reduced both potency and selectivity, while replacing it with a more electron-withdrawing cyano group (**38**) decreased potency, but it retained most of the selectivity. The reduction in potency may be explained by an increased hydrophilicity of the cyano group. Based on these observations, electron-withdrawing and lipophilic substituents, trifluoromethyl and trifluoromethoxy, were introduced, resulting in compounds **39-41**. Compound **39**, with a *para* trifluoromethyl group, displayed slightly higher potency, but lower selectivity than compound **27**. Moving the trifluoromethyl group from the *para* position to the *meta* position resulted in compound **40**,

which had a slightly decreased potency and selectivity. Compound **41**, with a *para* trifluoromethoxy moiety, displayed a significant increase in potency but a slight decrease in selectivity compared with compound **27**. As both *para* and *meta* substitutions were allowed, electron withdrawing and lipophilic groups were subsequently introduced at these two positions. Compound **42** with 3- and 4-dichloride exhibited a significant increase in potency compared with compound **27**. Notably, its selectivity was also slightly increased. Changing the *para* chloride to a larger trifluoromethyl group (**43**) further improved potency and retained selectivity. Based on compound **32**, the cyclopentyl group was introduced, resulting in compounds **44** and **45**. Compound **44** exhibited higher potency and selectivity than compound **42**, while compound **45** showed similar potency and selectivity to its counterpart **43**. To conclude, compound **42-45** were the most optimal compounds with high potency and selectivity for DAGLβ.

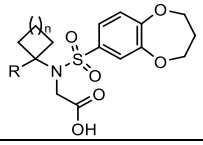
Table 4.3 Biochemical results and physicochemical properties of glycine sulfonamides **33-45**.[a]

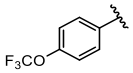
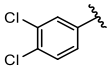
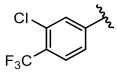
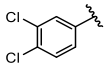
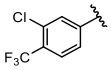


ID	n	R	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
33	1		7.30 ± 0.17	6.02 ± 0.13	19	1.3	104	6.0
34	1		6.84 ± 0.16	5.58 ± 0.09	18	0.8	104	6.0
35	1		7.02 ± 0.13	6.07 ± 0.09	8.9	1.1	104	5.9
36	1		6.41 ± 0.20	5.14 ± 0.14	19	0.6	114	5.8
37	1		6.94 ± 0.10	5.73 ± 0.07	16	0.8	104	6.1
38	1		6.74 ± 0.07	5.22 ± 0.06	33	0.5	128	6.2
39	1		7.55 ± 0.16	6.08 ± 0.06	30	1.6	104	6.0
40	1		7.44 ± 0.06	5.99 ± 0.05	28	1.6	104	5.8

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

Table 4.3 (continued) Biochemical results and physicochemical properties of glycine sulfonamides **33-45**.[a]



ID	n	R	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	LipE DAGLβ
41	1		7.78 ± 0.10	6.29 ± 0.07	31	1.8	114	6.0
42	1		7.88 ± 0.09	6.27 ± 0.07	41	1.9	104	6.0
43	1		7.94 ± 0.08	6.34 ± 0.09	40	2.2	104	5.7
44	2		8.07 ± 0.09	6.36 ± 0.05	51	2.3	104	5.8
45	2		7.96 ± 0.06	6.37 ± 0.05	39	2.5	104	5.5

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using the EnzChek lipase substrate assay. The apparent selectivity regards the selectivity for DAGLβ over DAGLα. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA), were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

4.3 Conclusion

In this Chapter, a comprehensive structure-activity relationship (SAR) study was conducted, focusing on the modification of the sulfonyl group of the glycine sulfonamide chemotype. The exploration led to the discovery of 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine moiety as the optimal substituent on the sulfonyl group. To further optimize the potency and selectivity, investigations were extended to other components of this chemotype while maintaining the 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine moiety on the sulfonyl group. Within this study, compound **44** emerged as the most potent and selective compound, displaying a pIC_{50} of 8.07 ± 0.09 and a selectivity of 51-fold for DAGLβ over DAGLα. Importantly, compound **44** also exhibited desirable druglike properties, including a cLogD of 2.3 and a LipE of 5.8. Alongside compound **44**, compounds **27**, **32**, **42**, **43**, and **45** also showed promising potency, selectivity and physiochemical characteristics. These findings position these compounds as the first-in-class DAGLβ selective inhibitors deserving further profiling and exploration.

4.4 Acknowledgements

Danique van Workum and Brian Herry are acknowledged for their contribution to the synthesis and biochemical evaluation. Hans van den Elst is kindly acknowledged for preparative HPLC purification and HRMS measurements.

4.5 Experimental methods

Biology

EnzChek lipase substrate assay for DAGLα and DAGLβ in 384-well plate

The DAGL EnzChek lipase substrate assay was performed as described in Chapter 3.

Chemistry

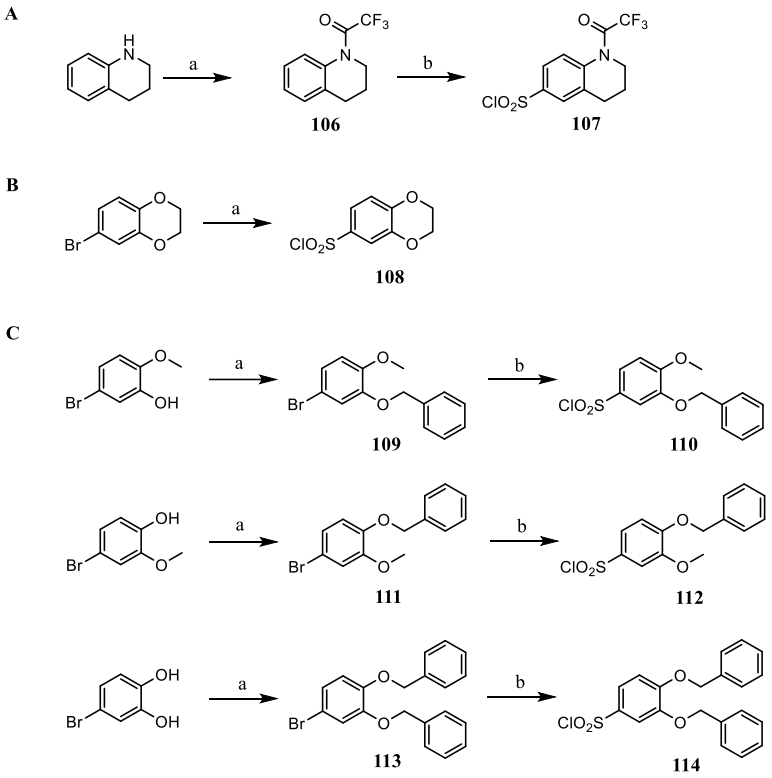
General remarks

All purchased chemicals were used without purification unless stated otherwise. All reactions were performed in oven-dried or flame-dried glassware. Anhydrous solvents were dried by activated 3 Å or 4 Å molecular sieves. Thin layer chromatography (TLC) analysis was performed on Merck silica gel 60 F_{254} aluminium sheets and the compounds were visualized by using UV absorption at 254 nm and/or $KMnO_4$ staining (5 g/L $KMnO_4$ and 25 g/L K_2CO_3 in water). TLC plates were analysed with the Advion CMS Plate Express® connected to the Advion Expression® L-MS using 90% MeOH in H_2O with 0.1% formic acid as the solvent. Liquid chromatography-mass spectrometry (LC-MS) analysis was performed on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI^+) coupled to a Surveyor HPLC system equipped with a C18 column (50 × 4.6 mm, 3 µm particle size, Macherey-Nagel) or a Thermo Finnigan LCQ Fleet ion-trap mass spectrometer (ESI^+) coupled to a Vanquish UHPLC system using H_2O, CH_3CN and 0.1% aq. TFA as eluents. Purification was performed on manual silica gel column chromatography (40-63 µm, 60 Å silica gel, Macherey-Nagel) or automated silica gel column chromatography (40-63 µm, 60 Å pre-packed silica gel, Screening Devices) on a Biotage Isolera™ Four 3.0 system. Alternatively, purification was performed using preparative HPLC on a Waters Acquity Ultra performance LC equipped with a C18 column (21 × 150 mm, 5 µm particle size, Phenomenex). ^1H and ^{13}C spectra were recorded on a Bruker AV 400 MHz (400 MHz for ^1H and 101 MHz for ^{13}C) or AV 500 MHz (500 MHz for ^1H and 126 MHz for ^{13}C) or AV 850 MHz spectrometer (850 MHz for ^1H and 214 MHz for ^{13}C) in deuterated solvents. Chemical shifts are reported in ppm with tetramethylsilane (TMS) or solvent resonance as the internal standard ($CDCl_3$: δ 7.26 for ^1H, δ 77.16 for ^{13}C; CD_3OD: δ 3.31 for ^1H, 49.00 for ^{13}C; DMSO-*d6*: δ 2.50 for ^1H, δ 39.52 for ^{13}C). Data is reported as follows: chemical shifts δ (ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, t = triplet, td = triplet of doublets, tt = triplet of triplets, q = quartet, quintet = p, bs = broad singlet, m = multiplet), coupling constants *J* (Hz) and integration. High resolution mass spectrometry (HRMS) analysis was performed on

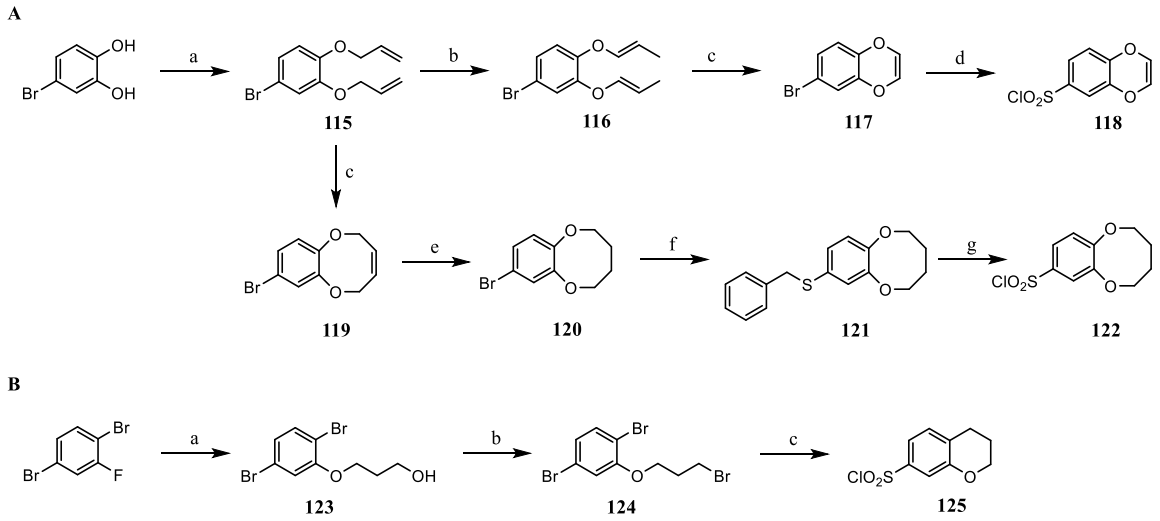
a Thermo Finnigin LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctyl phthalate (m/z = 391.28428) as a lock mass.

Synthesis of sulfonyl chlorides

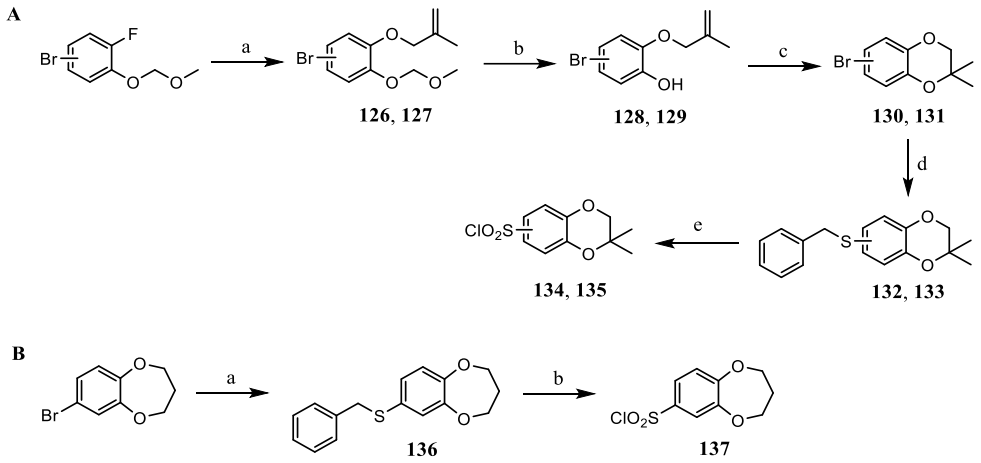
The sulfonyl chlorides used in the synthesis of the final compounds outlined in Table 4.1 were prepared following the procedures depicted in Schemes 4.4-4.7. Sulfonyl chloride **107** was synthesized by trifluoroacetylation of tetrahydroisoquinoline, followed by chlorosulfonation using chlorosulfonic acid (Scheme 4.4A). Sulfonyl chloride **108** was obtained from the bromide-containing starting material via a two-step chlorosulfonation (Scheme 4.4B).[20] This process involved a bromide-lithium exchange and nucleophilic addition to sulfur dioxide, followed by oxidative chlorination using *N*-chlorosuccinimide. Similarly, sulfonyl chlorides **110**, **112** and **114** were made in a comparable fashion (Scheme 4.4C). Sulfonyl chlorides **118** and **122** (Scheme 4.5A) were obtained from 4-bromobenzene-1,2-diol through a series of synthetic steps. The process involved initial allylation (**115**), followed by Ruthenium-catalyzed isomerization[21], resulting in enol-ether **116**. Subsequent ring-closing metathesis and a two-step chlorosulfonation yielded sulfonyl chloride **118**. For the synthesis of **122**, ring-closing metathesis of **119**, catalytic hydrogenation (**120**), and Pd-catalyzed aromatic substitution (**121**) were performed, followed by chlorosulfonation.[22] Nucleophilic aromatic substitution at 1,4-dibromo-2-fluorobenzene with propane-1,3-diol[23] followed by phosphorus tribromide treatment afforded tribromide **124** (Scheme 4.5B). The subsequent two-step chlorosulfonation yielded sulfonyl chloride **125**. Sulfonyl chlorides **134** and **135** (Scheme 4.6A) were synthesized starting from 4-bromo-2-fluoro-1-(methoxymethoxy)benzene and 4-bromo-1-fluoro-2-(methoxymethoxy)benzene, respectively. The synthesis involved nucleophilic aromatic substitution, MEM group cleavage and ring-closure, resulting in compounds **130** and **131**. A second nucleophilic aromatic substitution followed by chlorosulfonation led to the formation of sulfonyl chlorides **134** and **135**. Compound **137** was synthesized (Scheme 4.6B) through nucleophilic aromatic substitution and chlorosulfonation. Sulfonyl chlorides **142**, **145** and **148** (Scheme 4.7) were synthesized from intermediate **138**, derived from 2-amino-5-bromophenol and 2-chloroacetyl chloride. Then, amide reduction (**139**) followed by sequential trifluoro acetylation, nucleophilic aromatic substitution and chlorosulfonation formed sulfonyl chloride **142**. Methylation of **138** and **139** obtained compounds **143** and **146**, respectively. These were then transformed to sulfonyl chlorides **145** and **148** via nucleophilic aromatic substitution and chlorosulfonation, and nucleophilic aromatic substitution and chlorosulfonation/chlorination processes, respectively.



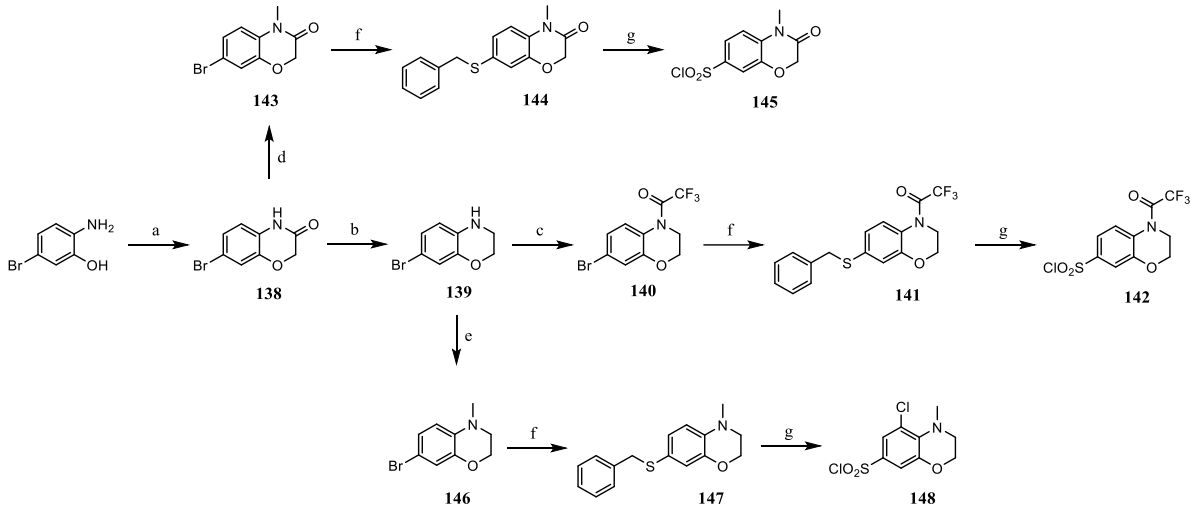
Scheme 4.4 Synthesis of sulfonyl chlorides **107**, **108**, **110**, **112** and **114**. (A) a) TFAA, Et_3N, Et_2O, rt, 87%; b) HSO_3Cl, anhydrous DCM, 0 °C-rt, 52%; (B) a) *i.* *n*-BuLi, anhydrous THF, -78 °C; *ii.* SO_2 in hexane, anhydrous THF, -78 to -40 °C; *iii.* *N*-chlorosuccinimide, DCM, 0 °C, 27%; (C) a) benzyl bromide, K_2CO_3, anhydrous DMF, rt or 90-106 °C, 67% for **109**, 92% for **111**, 75% for **113**; b) *i.* *n*-BuLi, anhydrous THF, -78 °C; *ii.* SO_2, anhydrous THF, -78 to -40 °C; *iii.* *N*-chlorosuccinimide, DCM, 0 °C, 39% for **110**, 71% for **112**, 38% for **114**.



Scheme 4.5 Synthesis of sulfonyl chlorides **118**, **122**, **125**. (A) a) 3-bromoprop-1-ene, K_2CO_3, anhydrous DMF, 60 °C, 25%; b) $RuClH(CO)(PPh_3)_3$, anhydrous benzene, 80 °C, 46%; c) Grubbs catalyst (II), anhydrous DCM, reflux, 88% for **117**, 22% for **119**; d) *i.* *n*-BuLi, anhydrous THF, -78 °C; *ii.* SO_2, anhydrous THF, -78 to -40 °C; *iii.* *N*-chlorosuccinimide, DCM, 0 °C, 49%; e) $RhCl(PPh_3)_3$, H_2, EtOH, 50 °C, 63%; f) benzyl mercaptan, DIPEA, xantphos, $Pd_2(dba)_3$, 1,4-dioxane, 100 °C, 75%; g) *N*-chlorosuccinimide, AcOH, H_2O, rt, 84%; (B) a) propane-1,3-diol, *t*-BuOK, 1-methylpyrrolidin-2-one, 100 °C, 80%; b) PBr_3, toluene, 0 °C-rt, 71%; c) *i.* *n*-BuLi, anhydrous THF, -78 °C; *ii.* SO_2, anhydrous THF, -78 to -40 °C; *iii.* *N*-chlorosuccinimide, DCM, 0 °C, 50%.

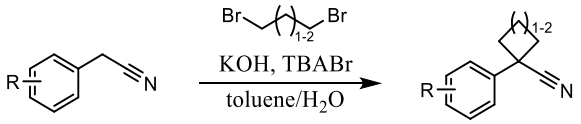


Scheme 4.6 Synthesis of sulfonyl chloride **134**, **135**, and **137**. (A) a) NaH, 2-methylprop-2-en-1-ol, anhydrous DMF, 0 °C-rt, 61% for **126**, 51% for **127**; b) TFA, DCM, 0 °C, 80%; c) HCOOH, reflux, 35% for **130**, 41% for **131**; d) benzyl mercaptan, DIPEA, xantphos, $Pd_2(dba)_3$, 1,4-dioxane, 100 °C, 74% for **132**, 87% for **133**; e) *N*-chlorosuccinimide, AcOH, H_2O, rt, 96% for **134**, quant. for **135**; (B) a) benzyl mercaptan, DIPEA, xantphos, $Pd_2(dba)_3$, 1,4-dioxane, 100 °C, 74%; b) *N*-chlorosuccinimide, AcOH, H_2O, rt, 96%.



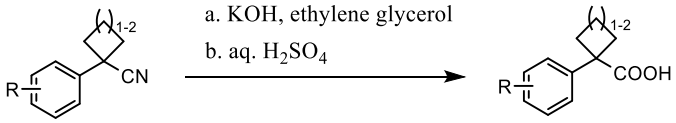
Scheme 4.7 Synthesis of sulfonyl chlorides **143**, **145** and **148**. a) 2-chloroacetyl chloride, K_2CO_3, anhydrous DMF, 80 °C, 81%; b) borane-THF complex, anhydrous THF, reflux, 88%; c) TFAA, Et_3N, Et_2O, 0 °C-rt, 92%; d) *t*-BuOK, CH_3I, anhydrous DMF, rt, 57%; e) NaH, CH_3I, anhydrous DMF, 0 °C-rt, 76%; f) benzyl mercaptan, DIPEA, xantphos, $Pd_2(dba)_3$, 1,4-dioxane, 100 °C, 88% for **141**, 61% for **144**, 56% for **147**; g) *N*-chlorosuccinimide, AcOH, H_2O, rt, 74% for **142**, quant. for **145**, 69% for **148**.

General procedure A



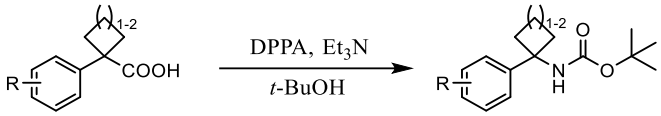
A mixture of corresponding phenyl acetonitrile (1 eq), 1,3-dibromopropane or 1,4-dibromobutane (1 eq) and TBABr (0.1 eq) in toluene (0.35 M) and solid KOH (8 eq) in H_2O (75% w/w) was heated to 100 °C with occasionally slow stirring to facilitate liquification of the inorganic phase. The reaction was then refluxed with continuous vigorous stirring for 1-2 h. The mixture was diluted in water and extracted 3× with EtOAc. Combined organic layers were washed with sat. NH_4Cl, brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product**.**

General procedure B



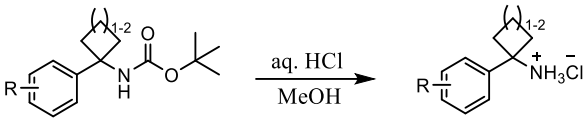
The mixture of corresponding nitrile (1 eq) and KOH (6 eq) in ethylene glycol (0.4-0.8 M) was refluxed until completion. The mixture was diluted in water and washed 1× with Et_2O. The pH of water layer was adjusted by 2 M aq. HCl solution to 2 and extracted 3× with EtOAc. The combined organic layers were dried by anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product. Alternatively, the mixture of corresponding nitrile (1 eq) in 9 M aq. H_2SO_4 (0.4 M) was refluxed until the reaction finished. The mixture was diluted in EtOAc and extracted 3× with 3 M aq. NaOH. The aqueous layer was adjusted to pH 2 by using 2 M aq. HCl and the fluffy precipitate was extracted 3× with EtOAc. The combined organic layers were washed with brine, dried by anhydrous Na_2SO_4, filtered and concentrated to obtain the product.

General procedure C



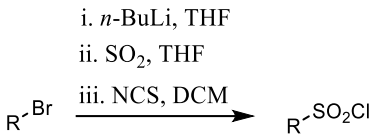
To a solution of corresponding carboxylic acid (1 eq) in anhydrous *t*-BuOH (0.08-0.2 M) with 4 Å molecular sieves was added diphenylphosphoryl azide (1 eq) and Et_3N (1.1 eq). The reaction was stirred at 30 °C for 1 h and then reflux until completion. The mixture was filtered and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography to afford the product.

General procedure D



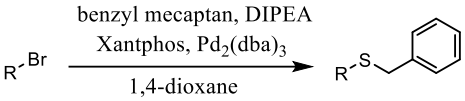
Boc-protected amine (1 eq) was dissolved in 3 M aq. HCl in MeOH and the reaction was stirred at rt until completion. The solvent was removed and the residue was washed 2× with Et_2O and filtered to afford the product.

General procedure E



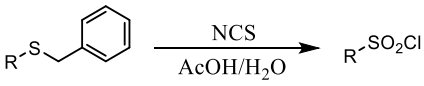
To a solution of corresponding aromatic bromide in anhydrous THF (0.15 M) at -78 °C was added *n*-BuLi (1-2 eq) dropwise and the mixture was stirred at -78 °C for 1-2 h. SO_2 (1.2 M in THF, 1.5 eq) was added and the mixture was stirred between -78 °C to -40 °C for 1 h, and at rt for 1 h. The mixture was concentrated and diluted in anhydrous DCM (0.15 M). *N*-chlorosuccinimide (1.2-1.5 eq) was added portion-wise at 0 °C and the mixture was stirred at 0 °C for 1 h. The mixture was diluted in DCM and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure F



To a stirred solution of the corresponding aromatic bromide in degassed 1,4-dioxane (0.24 M) was added DIPEA (2 eq) and the mixture was purged with N_2 for 30 min. Subsequently, xantphos (0.1 eq), $Pd_2(dba)_3$ (0.05 eq) and benzyl mercaptan (1 eq) were added and the reaction mixture was heated at 100 °C for 4 h. The reaction mixture was filtered through Celite. The filtrate was poured into water and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

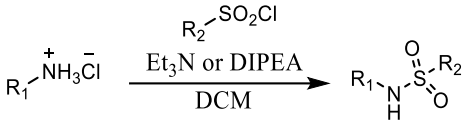
General procedure G



To a stirred solution of the corresponding benzyl sulfane (1 eq) in AcOH/H_2O (7.5:1, 0.23 M) was added *N*-chlorosuccinimide (4 eq) portion-wise and the mixture was stirred at rt for 2-6 h. The reaction mixture was diluted in water and extracted 3× with EtOAc. The combined organic

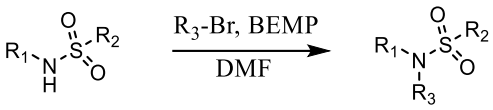
layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure H



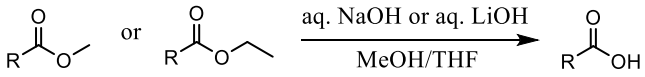
To a mixture of corresponding aminium chloride (1 eq) and Et_3N or DIPEA (3-12 eq) in anhydrous DCM at 0 °C was added corresponding sulfonyl chloride (1-2 eq). The reaction was stirred at rt until completion. The mixture was diluted in water or 0.2 M aq. HCl and extracted 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure I



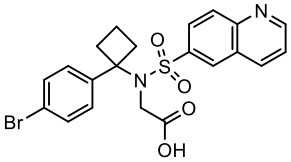
To a solution of corresponding sulfonamide (1 eq) in anhydrous DMF was added methyl 2-bromoacetate or ethyl 2-bromoacetate (1.5-3 eq) and 2-(tert-butylimino)-*N*,*N*-diethyl-1,3-dimethyl-1,3,2λ^5-diazaphosphinan-2-amine (1 M BEMP in hexane, 1.5-3 eq) and the reaction was heated to 80 °C. After completion, the mixture was diluted in EtOAc and washed with water or 0.2 M aq. HCl and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford the product.

General procedure J



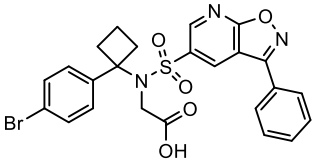
To a solution of corresponding methyl or ethyl esters (1 eq) in MeOH/THF (1:1, 0.1 M) was added 1 M or 2 M aq. NaOH or 1 M aq. LiOH (2-6 eq) and the reaction was stirred at rt. After completion, the mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography or preparative HPLC to afford the product.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-(quinolin-6-ylsulfonyl)glycine (1)



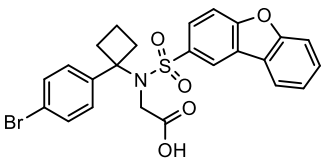
The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-(quinolin-6-ylsulfonyl)glycinate (**70**, 30 mg, 0.060 mmol, 1 eq) and 1 M aq. LiOH (361 µL, 0.361 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5-10% MeOH in DCM) afforded the product (12 mg, 0.025 mmol, 41%). ^{1}H NMR (400 MHz, DMSO) δ 9.01 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.46 – 8.40 (m, 1H), 7.97 (d, *J* = 8.7 Hz, 1H), 7.90 (d, *J* = 1.9 Hz, 1H), 7.66 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.60 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 2H), 7.14 (d, *J* = 8.6 Hz, 2H), 4.25 (s, 2H), 2.80 – 2.67 (m, 2H), 2.49 – 2.40 (m, 2H), 1.71 – 1.61 (m, 1H), 1.44 – 1.35 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.66, 152.27, 146.18, 141.62, 141.30, 135.96, 130.56, 129.33, 129.28, 129.27, 128.21, 123.57, 122.76, 120.65, 64.73, 48.65, 34.42, 14.14. HRMS $[C_{21}H_{19}BrN_2O_4+H]^+$: 475.03217/477.03005 calculated, 475.03195/477.02957 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((3-phenylisoxazolo[5,4-*b*]pyridin-5-yl)sulfonyl)glycine (2)



The title compound was synthesized according to general procedure **J** using methyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((3-phenylisoxazolo[5,4-*b*]pyridin-5-yl)sulfonyl)glycinate (**71**, 29 mg, 0.052 mmol, 1 eq) and 1 M aq. NaOH (187 µL, 0.187 mmol, 3.6 eq). Total time: overnight at rt. Silica gel column chromatography (10-30% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product as a white solid (13.6 mg, 25.0 µmol, 48%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 8.82 (d, *J* = 2.2 Hz, 1H), 8.36 (d, *J* = 2.2 Hz, 1H), 7.95 – 7.90 (m, 2H), 7.64 – 7.59 (m, 3H), 7.33 – 7.28 (m, 2H), 7.12 – 7.08 (m, 2H), 4.36 (s, 2H), 2.84 – 2.74 (m, 2H), 2.56 – 2.47 (m, 2H), 1.80 – 1.72 (m, 1H), 1.59 – 1.49 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 172.65, 170.86, 158.46, 149.90, 141.07, 136.55, 133.38, 132.01, 131.62, 130.09, 130.00, 128.31, 127.84, 122.33, 112.00, 66.16, 48.61, 35.37, 14.87. HRMS $[C_{24}H_{20}BrN_3O_5S+H]^+$: 542.03798/544.03589 calculated, 542.03778/544.03560 found.

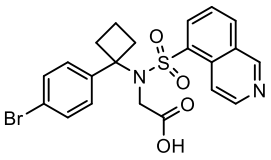
_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-(dibenzo[*b*,*d*]furan-2-ylsulfonyl)glycine (3)



The title compound was synthesized according to general procedure **J** using methyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-(dibenzo[*b*,*d*]furan-2-ylsulfonyl)glycinate (**72**, 39 mg, 0.074 mmol, 1 eq) and 1 M aq. NaOH (0.35 mL, 0.35 mmol, 4.7 eq). Total time: 3 h at rt. Silica gel column chromatography (10-30% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product as a white solid (24 mg, 0.047 mmol, 63%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.90 (ddd, *J* = 7.7, 1.4, 0.7 Hz, 1H), 7.79 (dd, *J* = 2.0, 0.6 Hz, 1H), 7.62 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.57 (dt, *J* = 8.3, 1.0 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.40 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.26 (m, 2H), 7.22 – 7.18 (m, 2H), 4.15 (s, 2H), 2.94 – 2.80 (m, 2H), 2.57 – 2.45 (m, 2H), 1.83 – 1.71 (m, 1H), 1.63 – 1.49 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 172.98, 158.39, 157.71, 142.07, 136.24, 131.66, 129.75, 128.99, 126.69, 125.05, 124.27,

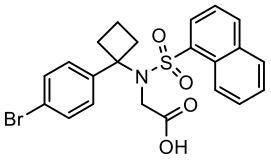
123.65, 122.09, 121.94, 121.19, 112.41, 112.11, 66.00, 48.62, 35.56, 15.03. HRMS $[C_{24}H_{20}BrNO_5S+Na]^+$: 536.01378/538.01168 calculated, 536.01367/538.01160 found.

N-(1-(4-Bromophenyl)cyclobutyl)-*N*-(isoquinolin-5-ylsulfonyl)glycine (4)



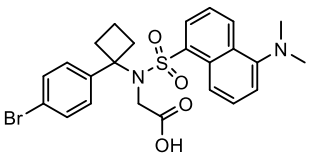
The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(isoquinolin-5-ylsulfonyl)glycinate (**73**, 13.5 mg, 28.0 µmol, 1 eq) and 2 M aq. NaOH (56.0 µL, 0.112 mmol, 4 eq). Preparative HPLC afforded the product as a white powder (1.0 mg, 2.1 µmol, 8%). ^{1}H NMR (400 MHz, DMSO) δ 9.37 (d, *J* = 0.9 Hz, 1H), 8.58 (d, *J* = 6.2 Hz, 1H), 8.32 (d, *J* = 8.1 Hz, 1H), 8.13 (dt, *J* = 6.1, 1.1 Hz, 1H), 8.09 – 8.03 (m, 1H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.32 – 7.25 (m, 2H), 7.17 – 7.10 (m, 2H), 4.29 (s, 2H), 2.83 – 2.76 (m, 2H), 2.67 (p, *J* = 1.8 Hz, 1H), 2.47 – 2.37 (m, 2H), 2.33 (p, *J* = 1.8 Hz, 1H). HRMS $[C_{21}H_{19}BrN_2O_4S+H]^+$: 475.03217/477.03005 calculated, 475.03215/477.02999 found.

N-(1-(4-Bromophenyl)cyclobutyl)-*N*-(naphthalen-1-ylsulfonyl)glycine (5)



The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(naphthalen-1-ylsulfonyl)glycinate (**74**, 37 mg, 0.077 mmol, 1 eq) and 2 M aq. NaOH (154 µL, 0.308 mmol, 4 eq). Silica gel column chromatography (30-70% EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product as a white powder (13 mg, 0.027 mmol, 35%). ^{1}H NMR (400 MHz, DMSO) δ 12.78 (bs, 1H), 8.40 – 8.31 (m, 1H), 8.13 (dt, *J* = 8.4, 1.0 Hz, 1H), 8.05 – 7.98 (m, 1H), 7.87 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.68 – 7.57 (m, 2H), 7.45 (dd, *J* = 8.2, 7.4 Hz, 1H), 7.35 – 7.27 (m, 2H), 7.22 – 7.14 (m, 2H), 4.24 (s, 2H), 2.86 – 2.73 (m, 2H), 2.48 – 2.38 (m, 2H), 1.75 – 1.63 (m, 1H), 1.44 – 1.28 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.11, 141.50, 136.57, 133.70, 133.67, 130.35, 129.22, 128.93, 128.78, 127.82, 127.61, 126.68, 124.38, 124.28, 120.45, 64.86, 47.87, 34.03, 14.17. HRMS $[C_{22}H_{20}BrNO_4S+Na]^+$: 496.01886/498.01675 calculated, 496.01875/498.01659 found.

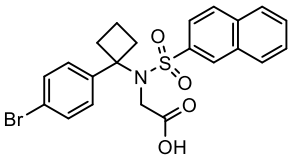
N-(1-(4-Bromophenyl)cyclobutyl)-*N*-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)glycine (6)



The title compound was synthesized according to general procedure **J** using methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((5-(dimethylamino)naphthalen-1-yl)-sulfonyl)glycinate (**75**, 49 mg, 0.093 mmol, 1 eq) and 2 M aq. NaOH (186 µL, 0.372 mmol, 4 eq). Preparative HPLC afforded the product as a white powder (5.0 mg, 9.6 µmol, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (dt, *J* = 8.6, 1.1 Hz, 1H), 8.24 (d, *J* = 8.7 Hz, 1H), 8.02 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.51 (dd, *J* = 8.7, 7.6 Hz, 1H), 7.42 (dd, *J* = 8.6, 7.4 Hz, 1H), 7.28 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.24 – 7.15 (m, 4H), 4.23 (s, 2H), 3.01 (s, 6H), 2.95 – 2.82 (m, 2H), 2.52 – 2.43 (m, 2H), 1.84 – 1.73 (m, 1H), 1.56 – 1.47 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.07, 149.19, 140.99, 137.11, 131.15, 129.93, 129.70, 129.41, 129.11, 129.06, 127.94, 124.00, 121.74,

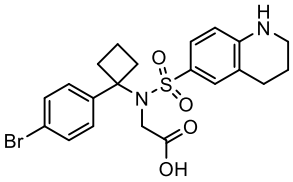
121.13, 115.93, 65.84, 47.97, 45.87, 34.56, 14.78. HRMS $[C_{24}H_{25}BrN_2O_4S+H]^+$: 517.07912/519.07701 calculated, 517.07893/519.07678 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-(naphthalen-2-ylsulfonyl)glycine (7)



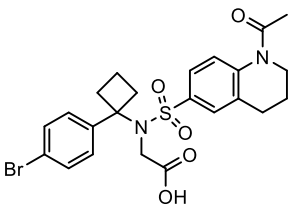
The title compound was synthesized according to general procedure **J** using methyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-(naphthalen-2-ylsulfonyl)glycinate (**76**, 43 mg, 0.089 mmol, 1 eq) and 2 M aq. NaOH (187 µL, 0.356 mmol, 4 eq). Silica gel column chromatography (30-70% EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product as a white powder (25 mg, 0.052 mmol, 59%). ^{1}H NMR (400 MHz, DMSO) δ 8.00 – 7.85 (m, 4H), 7.71 – 7.57 (m, 2H), 7.48 (dd, _J_ = 8.7, 2.0 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.28 – 7.20 (m, 2H), 4.17 (s, 2H), 2.79 – 2.67 (m, 2H), 2.49 – 2.41 (m, 2H), 1.73-1.61 (m, 1H), 1.48 – 1.32 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.46, 141.55, 137.98, 133.86, 131.42, 130.66, 129.34, 129.22, 128.69, 128.67, 127.65, 127.39, 122.19, 120.62, 64.70, 48.00, 34.39, 14.14. HRMS$[C_{22}H_{20}BrNO_4S+Na]^+$: 496.01886/498.01675 calculated, 496.01877/498.01660 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((1,2,3,4-tetrahydroquinolin-6-yl)sulfonyl)glycine (8)



The title compound was synthesized according to general procedure **J** using methyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((1-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroquinolin-6-yl)sulfonyl)glycinate (**77**, 23 mg, 0.039 mmol, 1 eq) and 2 M aq. NaOH (78.0 µL, 0.156 mmol, 4 eq). Total time: overnight at rt. Preparative HPLC afforded the product as an off-white solid (7.0 mg, 0.015 mmol, 37%). ^{1}H NMR (500 MHz, DMSO) δ 7.47 – 7.36 (m, 4H), 7.00 (dd, _J_ = 8.6, 2.3 Hz, 1H), 6.44 (s, 2H), 6.28 (d, _J_ = 8.6 Hz, 1H), 3.73 (s, 2H), 3.20 – 3.14 (m, 2H), 2.89 – 2.81 (m, 2H), 2.47 – 2.42 (m, 2H), 2.37 – 2.29 (m, 2H), 1.78 – 1.71 (m, 2H), 1.70 – 1.60 (m, 1H), 1.48-1.37 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 157.89, 157.65, 148.22, 130.44, 129.42, 127.83, 126.20, 119.89, 118.28, 111.37, 64.19, 49.55, 40.37, 34.33, 26.51, 20.66, 14.45. HRMS $[C_{21}H_{23}BrN_2O_4S+H]^+$: 479.06347/481.06135 calculated, 479.06355/481.06145 found.

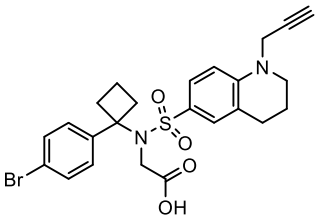
_N_-((1-Acetyl-1,2,3,4-tetrahydroquinolin-6-yl)sulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycine (9)



To a solution of _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((1,2,3,4-tetrahydroquinolin-6-yl)sulfonyl)glycine (**8**, 30 mg, 0.063 mmol, 1 eq) in anhydrous THF (0.6 mL, 0.1 M) was added NaH (60% w/w in mineral oil, 10 mg, 0.025 mmol, 4 eq). The mixture was stirred at rt for 1 h. Acetyl chloride (26.8 µL, 0.375 mmol, 6 eq) was added and the mixture was stirred at rt for overnight. The mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by preparative HPLC to afford the product as a white solid (26 mg, 0.050 mmol, 80%). ^{1}H NMR (500 MHz, DMSO) δ 12.81 (bs, 1H), 7.52 (d, _J_ = 9.0 Hz, 1H), 7.34 (s, 4H), 7.26 (dd, _J_ = 8.7, 2.4 Hz, 1H), 6.77 (d,

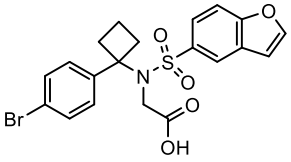
J = 2.3 Hz, 1H), 4.13 (s, 2H), 3.67 (t, *J* = 6.2 Hz, 2H), 2.78 – 2.68 (m, 2H), 2.55 (t, *J* = 6.6 Hz, 2H), 2.48 – 2.40 (m, 2H), 2.20 (s, 3H), 1.85 (p, *J* = 6.5 Hz, 2H), 1.75 – 1.65 (m, 1H), 1.47 – 1.34 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 169.68, 141.73, 141.56, 135.69, 131.15, 130.55, 129.36, 126.79, 124.05, 120.41, 64.40, 48.19, 44.12, 34.58, 26.41, 23.44, 23.03, 14.10. HRMS $[C_{23}H_{25}BrN_2O_5S+H]^+$: 521.07403/523.07193 calculated, 521.07417/523.07207 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((1-(prop-2-yn-1-yl)-1,2,3,4-tetrahydroquinolin-6-yl)sulfonyl)glycine (10)



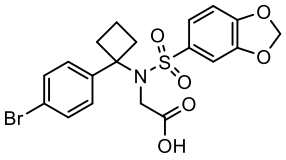
To a solution of *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((1,2,3,4-tetrahydroquinolin-6-yl)sulfonyl)glycine (**8**, 30 mg, 0.063 mmol, 1 eq) in anhydrous THF (0.6 mL, 0.1 M) was added NaH (60% w/w in mineral oil, 10 mg, 0.025 mmol, 4 eq). The mixture was stirred at rt for 1 h. 3-bromoprop-1-yne (23.7 µL, 0.313 mmol, 5 eq) was added and the mixture was stirred at rt for overnight. The mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by preparative HPLC to afford the product as a yellow solid (6.0 mg, 0.012 mmol, 19%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.20 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.60 (s, 1H), 6.52 (d, *J* = 8.8 Hz, 1H), 4.01 (d, *J* = 2.4 Hz, 2H), 3.96 (s, 2H), 3.37 (t, *J* = 5.8 Hz, 2H), 2.83 – 2.75 (m, 2H), 2.59 – 2.52 (m, 4H), 2.22 (t, *J* = 2.4 Hz, 1H), 1.95 (p, *J* = 6.1 Hz, 2H), 1.86 – 1.80 (m, 1H), 1.61 – 1.53 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.08, 141.60, 131.45, 129.12, 128.03, 127.53, 123.30, 121.64, 110.33, 78.58, 71.82, 65.51, 49.36, 40.72, 35.32, 27.65, 21.74, 14.88. HRMS $[C_{24}H_{25}BrN_2O_4S+Na]^+$: 539.06106/541.05896 calculated, 539.06081/541.05867 found.

_N_-(Benzofuran-5-ylsulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycine (11)



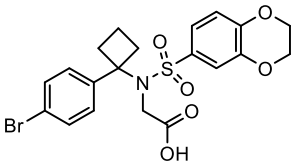
The title compound was synthesized according to general procedure **J** using ethyl *N*-(benzofuran-5-ylsulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (**78**, 7.7 mg, 0.016 mmol, 1 eq) and 1 M aq. LiOH (48 µL, 0.048 mmol, 3 eq). Total time: 4.5 h at rt. Silica gel column chromatography (3-10% MeOH in DCM) afforded the product (4.6 mg, 0.010 mmol, 64%). ^{1}H NMR (400 MHz, DMSO) δ 8.12 (d, *J* = 2.2 Hz, 1H), 7.71 (t, *J* = 1.3 Hz, 1H), 7.57 – 7.53 (m, 2H), 7.46 – 7.41 (m, 2H), 7.32 – 7.27 (m, 2H), 6.96 (d, *J* = 2.2 Hz, 1H), 3.86 (s, 2H), 2.90 – 2.78 (m, 2H), 2.39 – 2.29 (m, 2H), 1.69 – 1.57 (m, 1H), 1.47 – 1.34 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 155.39, 147.90, 142.80, 137.18, 130.52, 129.44, 126.92, 123.19, 120.90, 120.12, 111.17, 107.31, 64.60, 49.82, 34.16, 14.37. HRMS $[C_{20}H_{18}BrNO_5S+Na]^+$: 485.99813/487.99601 calculated, 485.99846/487.99614 found.

_N_-(Benzo[_d_][1,3]dioxol-5-ylsulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycine (12)



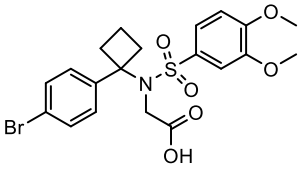
The title compound was synthesized according to the general procedure **J** using ethyl _N_-(benzo[_d_][1,3]dioxol-5-ylsulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycinate (**79**, 49 mg, 0.099 mmol, 1 eq) and 1 M aq. LiOH (591 µL, 0.591 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (10% MeOH in DCM) afforded the product (31 mg, 0.066 mmol, 67%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.35 – 7.26 (m, 4H), 7.05 (dd, _J_ = 8.2, 1.9 Hz, 1H), 6.70 (d, _J_ = 1.8 Hz, 1H), 6.69 (d, _J_ = 8.3 Hz, 1H), 6.03 (s, 2H), 4.06 (s, 2H), 2.87 – 2.76 (m, 2H), 2.50 – 2.42 (m, 2H), 1.81 – 1.72 (m, 1H), 1.61 – 1.48 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 173.01, 151.67, 148.32, 142.21, 135.12, 131.66, 129.65, 123.13, 121.90, 108.20, 107.90, 103.00, 65.81, 48.64, 35.27, 14.95. HRMS $[C_{19}H_{18}BrNO_6S+Na]^+$: 489.99304/491.99093 calculated, 489.99298/491.99076 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((2,3-dihydrobenzo[_b_][1,4]dioxin-6-yl)sulfonyl)glycine (13)



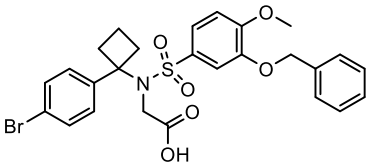
The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,3-dihydrobenzo[_b_][1,4]dioxin-6-yl)sulfonyl)glycinate (**80**, 22 mg, 0.043 9 mmol, 1 eq) and 2 M aq. NaOH (86 µL, 0.086 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (10-30% EtOAc in dis. _n_-pentane with a drop of con. HCl) afforded the product as a white solid (15 mg, 0.031 mmol, 72%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.33 – 7.28 (m, 2H), 7.28 – 7.23 (m, 2H), 6.97 (dd, _J_ = 8.6, 2.3 Hz, 1H), 6.74 (d, _J_ = 8.6 Hz, 1H), 6.71 (d, _J_ = 2.3 Hz, 1H), 4.31 – 4.20 (m, 4H), 4.05 (s, 2H), 2.86 – 2.75 (m, 2H), 2.50 – 2.41 (m, 2H), 1.80 – 1.71 (m, 1H), 1.59 – 1.48 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 172.75, 147.64, 143.49, 141.93, 133.91, 131.54, 129.48, 121.83, 120.87, 117.61, 117.19, 65.63, 65.07, 64.72, 48.47, 35.24, 14.86. HRMS $[C_{20}H_{20}BrNO_6S+Na]^+$: 504.00869/506.00659 calculated, 504.00884/506.00676 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((3,4-dimethoxyphenyl)sulfonyl)glycine (14)



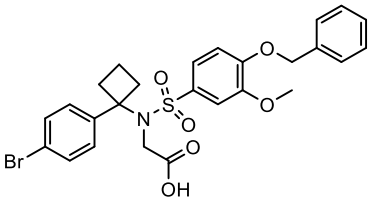
The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((3,4-dimethoxyphenyl)sulfonyl)glycinate (**81**, 30 mg, 0.059 mmol, 1 eq) and 1 M aq. LiOH (234 µL, 0.234 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-50% EtOAc in dis. _n_-pentane with a drop of conc. HCl) afforded the product as a white solid (21 mg, 0.043 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.35 (s, 4H), 7.15 (dd, _J_ = 8.4, 2.2 Hz, 1H), 7.11 (d, _J_ = 2.2 Hz, 1H), 6.77 (d, _J_ = 8.5 Hz, 1H), 4.08 (s, 2H), 3.93 (s, 3H), 3.84 (s, 3H), 2.88 – 2.74 (m, 2H), 2.52 – 2.41 (m, 2H), 1.84 – 1.71 (m, 1H), 1.63 – 1.51 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 172.13, 152.12, 148.51, 141.56, 133.10, 131.01, 129.09, 121.29, 121.10, 109.95, 109.68, 65.25, 56.00, 55.89, 47.58, 34.44, 14.46. HRMS $[C_{20}H_{22}BrNO_6S+NH_4]^+$: 501.06895/503.06685 calculated, 501.06877/503.06651 found.

_N_-((3-(Benzyloxy)-4-methoxyphenyl)sulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycine (15)



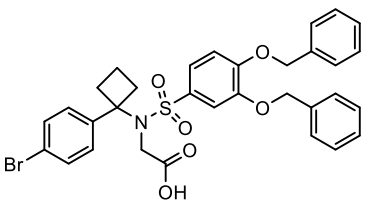
The title compound was synthesized according to the general procedure **J** using ethyl _N_-((3-(benzyloxy)-4-methoxyphenyl)sulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycinate (**82**, 20 mg, 0.034 mmol, 1 eq) and 1 M aq. LiOH (136 µL, 0.136 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in dis. *n*-pentane with a drop of conc. HCl) afforded the product as a white powder (9.0 mg, 0.016 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.44 (m, 2H), 7.43 – 7.37 (m, 2H), 7.35 – 7.28 (m, 3H), 7.19 – 7.14 (m, 2H), 7.12 (d, *J* = 2.2 Hz, 1H), 7.02 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.72 (d, *J* = 8.6 Hz, 1H), 5.13 (s, 2H), 3.94 (s, 3H), 3.91 (s, 2H), 2.73 – 2.62 (m, 2H), 2.44 – 2.36 (m, 2H), 1.75 – 1.66 (m, 1H), 1.55 – 1.46 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.52, 153.13, 147.58, 141.26, 136.42, 132.51, 131.35, 129.05, 128.89, 128.35, 127.59, 121.75, 121.65, 112.09, 110.57, 70.95, 65.38, 56.36, 47.96, 34.67, 14.64. HRMS $[C_{26}H_{26}BrNO_6S+NH_4]^+$: 577.10025/579.09818 calculated, 577.10021/579.09804 found.

_N_-((4-(Benzyloxy)-3-methoxyphenyl)sulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycine (16)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-((4-(benzyloxy)-3-methoxyphenyl)sulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycinate (**83**, 19 mg, 0.032 mmol, 1 eq) and 1 M aq. LiOH (130 µL, 0.130 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in dis. *n*-pentane with a drop of conc. HCl) afforded the product as a white powder (15 mg, 0.027 mmol, 83%). ^{1}H NMR (500 MHz, MeOD+$CDCl_3$) δ 7.46 – 7.41 (m, 2H), 7.39 – 7.35 (m, 2H), 7.33 – 7.27 (m, 5H), 7.04 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.02 (d, *J* = 2.2 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 5.16 (s, 2H), 4.11 (s, 2H), 3.79 (s, 3H), 2.87 – 2.78 (m, 2H), 2.49 – 2.40 (m, 2H), 1.79 – 1.71 (m, 1H), 1.58 – 1.50 (m, 1H). ^{13}C NMR (126 MHz, MeOD+$CDCl_3$) δ 173.19, 152.35, 150.00, 142.62, 137.17, 134.38, 131.82, 130.03, 129.34, 128.89, 128.25, 122.02, 121.66, 113.21, 111.12, 71.69, 66.14, 56.47, 48.56, 35.41, 15.11. HRMS $[C_{26}H_{26}BrNO_6S+NH_4]^+$: 577.10025/579.09818 calculated, 577.09999/579.09781 found.

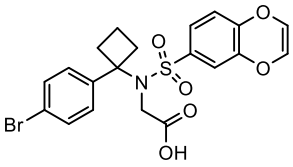
_N_-((3,4-bis(Benzyloxy)phenyl)sulfonyl)-_N_-(1-(4- bromophenyl)cyclobutyl)glycine (17)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-((3,4-bis(benzyloxy)phenyl)sulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycinate (**84**, 20 mg, 0.029 mmol, 1 eq) and 1 M aq. LiOH (177 µL, 0.177 mmol, 6 eq). Total time: 4 h at rt. Silica gel column chromatography (20-40% EtOAc in dis. *n*-pentane with a drop of

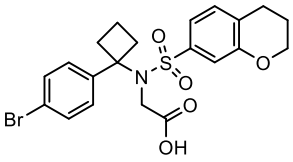
conc. HCl) afforded the product (4.9 mg, 7.7 µmol, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.43 (m, 4H), 7.42 – 7.28 (m, 8H), 7.21 – 7.14 (m, 2H), 7.14 (d, *J* = 2.2 Hz, 1H), 6.99 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 5.21 (s, 2H), 5.12 (s, 2H), 3.93 (s, 2H), 2.73 – 2.63 (m, 2H), 2.44 – 2.36 (m, 2H), 1.76 – 1.66 (m, 1H), 1.56 – 1.43 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.53, 152.38, 148.15, 141.30, 136.61, 136.31, 133.04, 131.35, 129.07, 128.82, 128.79, 128.31, 128.24, 127.52, 127.35, 121.66, 121.63, 113.03, 113.00, 71.10, 65.40, 47.94, 34.67, 34.26, 14.64. HRMS $[C_{32}H_{30}BrNO_6+Na]^+$: 658.08694/660.08494 calculated, 658.08667/660.08451 found.

_N_-(Benzo[_b_][1,4]dioxin-6-ylsulfonyl)-_N_-(1-(4-bromophenyl)cyclobutyl)glycine (18)



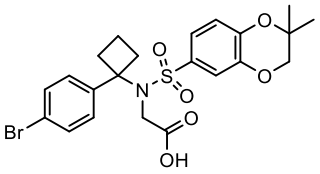
The title compound was synthesized according to the general procedure **J** using ethyl *N*-(benzo[*b*][1,4]dioxin-6-ylsulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (**85**, 15 mg, 0.030 mmol, 1 eq) and 1 M aq. LiOH (118 µL, 0.118 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-30% EtOAc in dis. *n*-pentane with a drop of conc. HCl) afforded the product as a white solid (6.5 mg, 0.014 mmol, 46%). ^{1}H NMR (500 MHz, MeOD+$CDCl_3$) δ 7.41 – 7.34 (m, 2H), 7.32 – 7.25 (m, 2H), 6.95 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 5.91 – 5.85 (m, 2H), 4.02 (s, 2H), 2.84 – 2.71 (m, 2H), 2.50 – 2.43 (m, 2H), 1.81 – 1.72 (m, 1H), 1.60 – 1.50 (m, 1H). ^{13}C NMR (126 MHz, MeOD+$CDCl_3$) δ 172.50, 146.68, 142.85, 141.84, 137.01, 131.62, 129.48, 127.56, 127.00, 124.12, 122.07, 116.36, 115.68, 65.72, 48.30, 35.13, 14.84. HRMS $[C_{20}H_{18}BrNO_6S+NH_4]^+$: 497.03765/499.03560 calculated, 497.03741/499.03515 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-(chroman-7-ylsulfonyl)glycine (19)



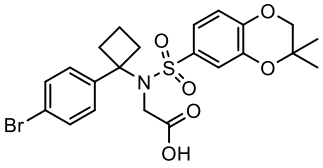
The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(chroman-7-ylsulfonyl)glycinate (**86**, 24 mg, 0.047 mmol, 1 eq) and 1 M aq. LiOH (189 µL, 0.189 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-30% EtOAc in dis. *n*-pentane with a drop of conc. HCl) afforded the product as a white solid (16 mg, 0.033 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.35 – 7.24 (m, 4H), 7.01 – 6.96 (m, 1H), 6.92 (dd, *J* = 8.0, 1.9 Hz, 1H), 6.67 (d, *J* = 1.9 Hz, 1H), 4.25 – 4.17 (m, 2H), 4.11 (s, 2H), 2.91 – 2.77 (m, 4H), 2.54 – 2.44 (m, 2H), 2.07 – 1.98 (m, 2H), 1.84 – 1.73 (m, 1H), 1.64 – 1.50 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 171.86, 154.22, 140.85, 139.29, 130.60, 129.55, 128.57, 126.85, 120.98, 117.63, 114.97, 66.28, 64.73, 47.68, 34.31, 24.39, 21.27, 13.93. HRMS $[C_{21}H_{22}BrNO_5S+NH_4]^+$: 497.07403/499.07192 calculated, 497.07396/499.07175 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((2,2-dimethyl-2,3-dihydrobenzo[_b_][1,4]dioxin-6-yl)sulfonyl)glycine (20)



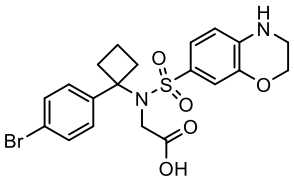
The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)sulfonyl)glycinate (**87**, 31.5 mg, 59.0 µmol, 1 eq) and 1 M aq. LiOH (234 µL, 0.234 mmol, 4 eq). Total time: 4 h at rt. Silica gel column chromatography (20-30% EtOAc in dis. *n*-pentane with a drop of conc. HCl) afforded the product as a white solid (25 mg, 49 µmol, 84%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.35 – 7.24 (m, 4H), 6.98 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 6.69 (d, *J* = 8.6 Hz, 1H), 4.04 (s, 2H), 3.89 (s, 2H), 2.89 – 2.76 (m, 2H), 2.52 – 2.42 (m, 2H), 1.82 – 1.69 (m, 1H), 1.59 – 1.46 (m, 1H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 172.92, 146.99, 142.39, 142.16, 133.37, 131.67, 129.64, 121.99, 121.63, 117.89, 116.77, 74.12, 72.31, 65.80, 48.55, 35.40, 23.43, 14.97. HRMS $[C_{22}H_{24}BrNO_6S+NH_4]^+$: 527.08460/529.08251 calculated, 527.08469/529.08251 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((3,3-dimethyl-2,3-dihydrobenzo[_b_][1,4]dioxin-6-yl)sulfonyl)glycine (21)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,3-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)sulfonyl)glycinate (**88**, 42 mg, 0.078 mmol, 1 eq) and 1 M aq. LiOH (313 µL, 0.313 mmol, 4 eq). Total time: 4 h at rt. Silica gel column chromatography (20-30% EtOAc in dis. *n*-pentane with a drop of conc. HCl) afforded the product as a white solid (27 mg, 0.053 mmol, 68%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.34 – 7.25 (m, 4H), 6.97 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.60 (d, *J* = 2.3 Hz, 1H), 4.05 (s, 2H), 3.92 (s, 2H), 2.88 – 2.77 (m, 2H), 2.52 – 2.42 (m, 2H), 1.82 – 1.71 (m, 1H), 1.60 – 1.47 (m, 1H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 172.98, 146.57, 142.79, 142.21, 134.27, 131.70, 129.67, 121.89, 120.72, 117.47, 117.18, 73.41, 72.53, 65.79, 48.59, 35.44, 23.35, 15.00. HRMS $[C_{22}H_{24}BrNO_6S+NH_4]^+$: 527.08460/529.08251 calculated, 527.08486/529.08261 found.

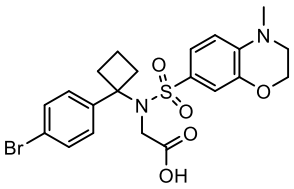
_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]oxazin-7-yl)sulfonyl)glycine (22)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-7-yl)sulfonyl)glycinate (**89**, 30 mg, 0.059 mmol, 1 eq) and 1 M aq. LiOH (356 µL, 0.356 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5-10% MeOH in DCM) afforded the product as a white solid (11 mg, 0.023 mmol, 39%). ^{1}H NMR (400 MHz, DMSO+MeOD) δ 7.34 – 7.27 (m, 4H), 6.76 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 1H), 6.39 (d, *J* = 8.4 Hz, 1H), 4.07 – 4.03 (m, 2H), 3.91 (s, 2H), 3.33 – 3.26 (m, 2H), 2.76 – 2.65 (m, 2H), 2.40 – 2.32 (m, 2H), 1.71 – 1.60 (m, 1H), 1.47 – 1.36 (m, 1H). ^{13}C NMR (101 MHz, DMSO+MeOD)

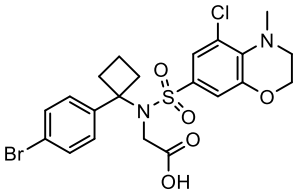
δ 172.73, 143.19, 142.41, 139.80, 131.53, 129.96, 127.98, 121.91, 121.39, 115.78, 113.42, 65.53, 65.11, 48.02, 40.39, 35.20, 14.89. HRMS $[C_{20}H_{21}BrN_2O_5S+Na]^+$: 503.02468/505.02257 calculated, 503.02460/505.02245 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((4-methyl-3,4-dihydro-2_H_-benzo[_b_][1,4]oxazin-7-yl)sulfonyl)glycine (23)



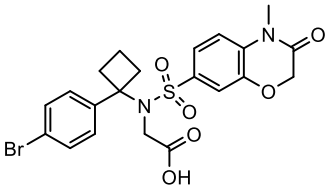
To a solution of *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-7-yl)sulfonyl)glycine (**22**, 12 mg, 26 µmol, 1 eq) in anhydrous DMF (111 µL, 0.23 M) was added NaH (60% w/w in mineral oil, 2.0 mg, 0.051 mmol, 2 eq) portion-wise at 0 °C under N_2. The mixture was stirred at 0 °C for 20 min. CH_3I (3.5 µL, 0.056 mmol, 2.2 eq) was following added and the mixture was stirred at 0 °C for 30 min and at rt for 48 h. The reaction was quenched by water and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by HPLC to afford the product (2.0 mg, 4.0 µmol, 16%). ^{1}H NMR (500 MHz, DMSO) δ 7.38 (s, 4H), 6.90 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.53 (d, *J* = 8.6 Hz, 1H), 6.48 (d, *J* = 2.2 Hz, 1H), 4.19 (t, *J* = 4.4 Hz, 2H), 3.94 (s, 2H), 3.45-3.24 (m, 2H), 2.90 (s, 3H), 2.76 – 2.66 (m, 2H), 2.41 – 2.35 (m, 2H), 1.73 – 1.64 (m, 1H), 1.48 – 1.37 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 169.66, 142.17, 139.68, 130.55, 129.21, 121.09, 120.28, 113.50, 110.16, 64.37, 63.99, 48.03, 47.75, 37.79, 34.20, 14.17. HRMS $[C_{21}H_{23}BrN_2O_5S+Na]^+$: 517.04033/519.03822 calculated, 517.04044/519.03818 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((5-chloro-4-methyl-3,4-dihydro-2_H_-benzo[_b_][1,4]oxazin-7- yl)sulfonyl)glycine (24)



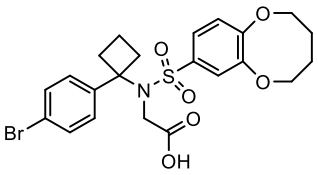
The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((5-chloro-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-7-yl)sulfonyl)glycinate (**90**, 33 mg, 0.060 mmol, 1 eq) and 1 M aq. LiOH (358 µL, 0.358 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5-10% MeOH in DCM) afforded the product as a white solid (19 mg, 0.036 mmol, 61%). ^{1}H NMR (400 MHz, DMSO) δ 7.36 – 7.29 (m, 4H), 6.68 (d, *J* = 2.2 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 4.18 – 4.10 (m, 4H), 3.08 (t, *J* = 4.3 Hz, 2H), 2.85 (s, 3H), 2.75 – 2.65 (m, 2H), 2.46 – 2.38 (m, 2H), 1.75 – 1.65 (m, 1H), 1.45 – 1.36 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.65, 147.76, 141.28, 136.93, 134.14, 130.54, 129.26, 126.24, 120.71, 119.90, 114.07, 64.43, 60.34, 48.72, 48.30, 42.67, 34.62, 14.11. HRMS $[C_{21}H_{22}BrClN_2O_5S+H]^+$: 529.01941/531.01711 calculated, 529.01912/531.01659 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((4-methyl-3-oxo-3,4-dihydro-2_H_-benzo[_b_][1,4]oxazin-7- yl)sulfonyl)glycine (25)



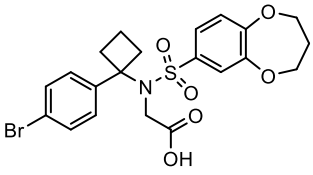
The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((4-methyl-3-oxo-3,4-dihydro-2_H_- benzo[_b_][1,4]oxazin-7-yl)sulfonyl)glycinate (**91**, 13 mg, 0.024 mmol, 1 eq) and 1 M aq. LiOH (145 µL, 0.145 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5-10% MeOH in DCM) to afford the product (6.2 mg, 0.012 mmol, 50%). ^{1}H NMR (500 MHz, DMSO) δ 7.41 – 7.34 (m, 4H), 7.20 (dd, _J_ = 8.5, 2.1 Hz, 1H), 7.07 (d, _J_ = 8.6 Hz, 1H), 6.83 (d, _J_ = 2.0 Hz, 1H), 4.69 (s, 2H), 3.97 (s, 2H), 3.28 (s, 3H), 2.82 – 2.72 (m, 2H), 2.41 – 2.34 (m, 2H), 1.71 – 1.63 (m, 1H), 1.45 – 1.38 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 172.10, 163.90, 143.74, 142.14, 136.28, 132.28, 130.55, 129.34, 121.43, 120.30, 114.82, 114.37, 66.89, 64.49, 49.18, 34.20, 27.85, 14.18. HRMS $[C_{21}H_{21}BrN_2O_6S+Na]^+$: 531.01959/533.01750 calculated, 531.01937/533.01713 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((2,3,4,5-tetrahydrobenzo[_b_][1,4]dioxocin-8-yl)sulfonyl)glycine (26)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((2,3,4,5-tetrahydrobenzo[_b_][1,4]dioxocin-8-yl)sulfonyl)glycinate (**92**, 33 mg, 0.061 mmol, 1 eq) and 1 M aq. LiOH (363 µL, 0.363 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5% MeOH in DCM) afforded the product (27 mg, 0.052 mmol, 86%). ^{1}H NMR (500 MHz, MeOD) δ 7.38 – 7.32 (m, 4H), 7.06 (dd, _J_ = 8.5, 2.4 Hz, 1H), 6.84 (d, _J_ = 2.4 Hz, 1H), 6.82 (d, _J_ = 8.5 Hz, 1H), 4.44 (t, _J_ = 5.5 Hz, 2H), 4.22 (t, _J_ = 5.4 Hz, 2H), 4.14 (s, 2H), 2.91 – 2.82 (m, 2H), 2.54 – 2.46 (m, 2H), 1.94 (p, _J_ = 5.8 Hz, 2H), 1.84 (p, _J_ = 5.6 Hz, 2H), 1.81 – 1.72 (m, 1H), 1.58 – 1.50 (m, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.78, 155.38, 148.71, 143.15, 136.11, 132.16, 130.41, 124.10, 123.78, 122.39, 122.31, 74.95, 72.84, 66.40, 49.13, 35.99, 28.70, 26.56, 15.30. HRMS $[C_{22}H_{24}BrNO_6S+Na]^+$: 532.03999/534.03790 calculated, 532.03995/534.03765 found.

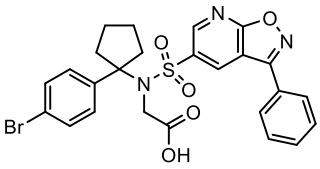
_N_-(1-(4-Bromophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (27)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (**93**, 20.5 mg, 39.1 µmol, 1 eq) and 1 M aq. LiOH (235 µL, 0.235 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5% MeOH in DCM) afforded the product (16.5 mg, 33.2 µmol, 85%). ^{1}H NMR (400 MHz, MeOD) δ 7.38 – 7.31 (m, 4H), 7.03 (dd, _J_ = 8.5, 2.3 Hz, 1H), 6.83 (d, _J_ = 8.5 Hz, 1H), 6.74 (d, _J_ = 2.3 Hz, 1H), 4.25 (t, _J_ = 5.6 Hz, 2H), 4.20 (t, _J_ = 5.7 Hz, 2H), 4.14 (s, 2H), 2.93 – 2.80 (m, 2H), 2.55 – 2.45 (m, 2H), 2.23 – 2.16 (m, 2H), 1.83 – 1.72 (m, 1H), 1.60 – 1.49 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ

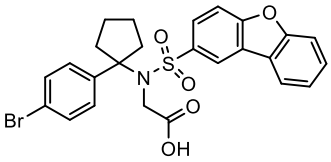
155.93, 151.71, 143.09, 136.67, 132.16, 130.45, 123.47, 122.34, 122.30, 121.86, 71.82, 71.80, 66.36, 49.21, 36.04, 32.23, 15.30. HRMS $[C_{21}H_{22}BrNO_6S+Na]^+$: 518.02434/520.02224 calculated, 518.02422/520.02196 found.

N-(1-(4-Bromophenyl)cyclopentyl)-*N*-((3-phenylisoxazolo[5,4-*b*]pyridin-5-yl)sulfonyl)glycine (28)



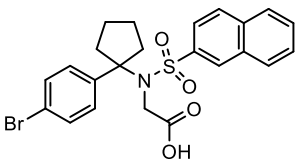
The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclopentyl)-*N*-((3-phenylisoxazolo[5,4-*b*]pyridin-5-yl)sulfonyl)glycinate (**99a**, 20 mg, 0.034 mmol, 1 eq) and 2 M aq. NaOH (51 µL, 0.102 mmol, 3 eq). Total time: 3 h at rt. Silica gel column chromatography (6-13% MeOH in DCM) afforded the product (1.1 mg, 2.0 µmol, 6%). LC-MS $[C_{25}H_{22}BrN_3O_5S+H]^+$: 556.05/558.05 calculated, 555.93/557.80 found. HRMS $[C_{25}H_{22}BrN_3O_5S+H]^+$: 556.05363/558.05154 calculated, 556.05374/558.05152 found.

N-(1-(4-Bromophenyl)cyclopentyl)-*N*-(dibenzo[*b*,*d*]furan-2-ylsulfonyl)glycine (29)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclopentyl)-*N*-(dibenzo[*b*,*d*]furan-2-ylsulfonyl)glycinate (**99b**, 19 mg, 0.035 mmol, 1 eq) and 2 M aq. NaOH (52.5 µL, 0.105 mmol, 3 eq). Total time: 4 h at rt. Silica gel column chromatography (30% EtOAc in dis. *n*-pentane with a drop of conc. HCl) afforded the product (6.5 mg, 0.012 mmol, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 1.9 Hz, 1H), 7.94 (dt, *J* = 7.5, 1.1 Hz, 1H), 7.84 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.62 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.43 (td, *J* = 7.4, 1.0 Hz, 1H), 7.30 – 7.26 (m, 2H), 7.25 – 7.21 (m, 2H), 4.26 (s, 2H), 2.45 – 2.36 (m, 2H), 2.34 – 2.24 (m, 2H), 1.74 – 1.63 (m, 2H), 1.41 – 1.22 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.62, 158.03, 157.16, 141.25, 136.25, 131.27, 129.19, 128.59, 126.68, 124.68, 123.84, 123.26, 121.83, 121.48, 121.26, 112.14, 111.91, 73.74, 48.92, 38.10, 21.58. HRMS $[C_{25}H_{22}BrNO_5S+Na]^+$: 550.02943/552.02734 calculated, 550.02954/552.02728 found.

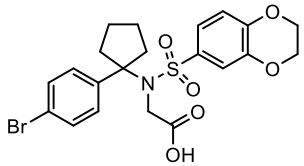
N-(1-(4-Bromophenyl)cyclopentyl)-*N*-(naphthalen-2-ylsulfonyl)glycine (30)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-bromophenyl)cyclopentyl)-*N*-(naphthalen-2-ylsulfonyl)glycinate (**99c**, 21 mg, 0.040 mmol, 1 eq) and 2 M aq. NaOH (60 µL, 0.12 mmol, 3 eq). Total time: 3.5 h at rt. Silica gel column chromatography (2-6% MeOH in DCM) afforded the product (6.8 mg, 0.014 mmol, 35%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.85 – 7.79 (m, 2H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.64 – 7.56 (m, 2H), 7.19 (s, 4H), 4.25 (s, 2H), 2.45 – 2.21 (m, 4H), 1.73 – 1.59 (m, 2H), 1.39 – 1.28 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.82, 141.16, 138.57, 134.68, 132.05, 131.24, 129.61, 129.19, 128.98, 128.92, 127.88, 127.57,

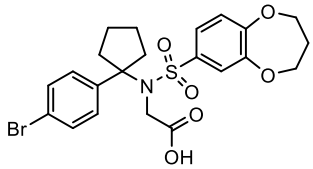
122.83, 121.80, 73.67, 49.26, 38.05, 21.54. HRMS $[C_{23}H_{22}BrNO_4S+Na]^+$: 510.03451/512.03240 calculated, 510.03497/512.03274 found.

_N_-(1-(4-Bromophenyl)cyclopentyl)-_N_-((2,3-dihydrobenzo[_b_][1,4]dioxin-6-yl)sulfonyl)glycine (31)



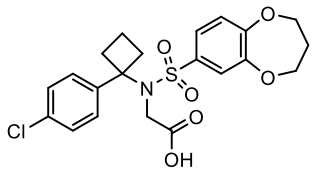
The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclopentyl)-_N_-((2,3-dihydrobenzo[_b_][1,4]dioxin-6-yl)sulfonyl)glycinate (**99d**, 16.5 mg, 31.5 µmol, 1 eq) and 1 M aq. LiOH (94 µL, 0.094 mmol, 3 eq). Silica gel column chromatography (25-30% EtOAc in dis. _n_-pentane with a drop of conc. HCl) afforded the product (1.0 mg, 2.0 µmol, 7%). LC-MS $[C_{21}H_{22}BrNO_6S+NH_4]^+$: 513.07/515.07 calculated, 512.58/514.58 found. HRMS $[C_{21}H_{22}BrNO_6S+Na]^+$: 518.02434/520.02224 calculated, 518.02369/520.02154 found.

_N_-(1-(4-Bromophenyl)cyclopentyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (32)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-bromophenyl)cyclopentyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (**99e**, 48 mg, 0.089 mmol, 1 eq) and 1 M aq. LiOH (267 µL, 0.267 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product as a white solid (26 mg, 0.051 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.31 (m, 2H), 7.26 – 7.20 (m, 3H), 7.14 (d, _J_ = 2.3 Hz, 1H), 6.91 (d, _J_ = 8.5 Hz, 1H), 4.32 (t, _J_ = 5.8 Hz, 2H), 4.26 (t, _J_ = 5.8 Hz, 2H), 4.18 (s, 2H), 2.41 – 2.32 (m, 2H), 2.31 – 2.20 (m, 4H), 1.75 – 1.65 (m, 2H), 1.39 – 1.30 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.80, 154.65, 150.41, 141.32, 135.84, 131.22, 129.14, 122.89, 121.71, 121.53, 121.41, 73.53, 70.61, 70.53, 49.07, 37.94, 30.95, 21.58. HRMS $[C_{22}H_{24}BrNO_6S+Na]^+$: 532.03999/534.03790 calculated, 532.04016/534.03809 found.

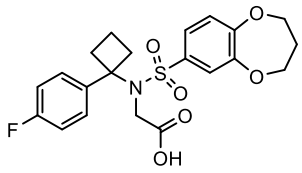
_N_-(1-(4-Chlorophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (33)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-chlorophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105a**, 33 mg, 0.069 mmol, 1 eq) and 1 M aq. LiOH (414 µL, 0.414 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5% MeOH in DCM) afforded the product as a white powder (20 mg, 0.044 mmol, 65%). ^{1}H NMR (400 MHz, MeOD) δ 7.43 – 7.37 (m, 2H), 7.23 – 7.18 (m, 2H), 7.04 (dd, _J_ = 8.5, 2.4 Hz, 1H), 6.83 (d, _J_ = 8.5 Hz, 1H), 6.74 (d, _J_ = 2.4 Hz, 1H), 4.24 (t, _J_ = 5.8 Hz, 2H), 4.19 (t, _J_ = 5.8 Hz, 2H), 4.14 (s, 2H), 2.92 – 2.82 (m, 2H), 2.55 – 2.47 (m, 2H), 2.18 (p, _J_ = 5.8 Hz, 2H), 1.82 – 1.72 (m, 1H), 1.59 – 1.49 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 173.96, 155.93, 151.71, 142.58, 136.66, 134.18,

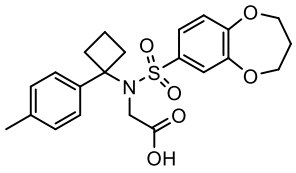
130.11, 129.15, 123.46, 122.34, 121.88, 71.76, 66.32, 49.21, 36.06, 32.22, 15.30. HRMS $[C_{21}H_{22}ClNO_6S+Na]^+$: 474.07486 calculated, 474.07469 found.

_N_-(1-(4-Fluorophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (34)



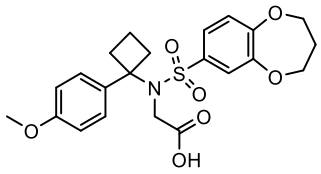
The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-fluorophenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105b**, 18 mg, 0.034 mmol, 1 eq) and 1 M aq. LiOH (207 µL, 0.207 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5% MeOH in DCM) afforded the product as a white powder (24 mg, 0.055 mmol, 86%). ^{1}H NMR (400 MHz, MeOD) δ 7.48 – 7.42 (m, 2H), 7.06 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.97 – 6.90 (m, 2H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.74 (d, *J* = 2.3 Hz, 1H), 4.23 (t, *J* = 5.8 Hz, 2H), 4.17 (t, *J* = 5.8 Hz, 2H), 4.11 (s, 2H), 2.92 – 2.81 (m, 2H), 2.56 – 2.47 (m, 2H), 2.18 (p, *J* = 5.7 Hz, 2H), 1.81 – 1.71 (m, 1H), 1.58 – 1.48 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 174.00, 163.34 (d, J_{C-F} = 245.1 Hz), 155.86, 151.71, 139.76 (d, J_{C-F} = 3.3 Hz), 136.78, 130.45 (d, J_{C-F} = 8.1 Hz), 123.48, 122.31, 121.95, 115.69 (d, J_{C-F} = 21.5 Hz), 71.72, 71.68, 66.35, 49.28, 36.13, 32.22, 15.32. HRMS $[C_{21}H_{22}FNO_6S+Na]^+$: 458.10441 calculated, 458.10419 found.

_N_-((3,4-Dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)-_N_-(1-(_p_-tolyl)cyclobutyl)glycine (35)



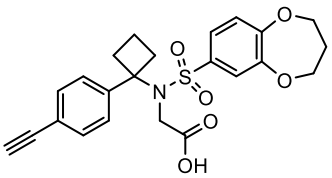
To a mixture of *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycine (**27**, 35 mg, 0.071 mmol, 1 eq), K_2CO_3 (29.2 mg, 0.212 mmol, 3 eq) and methylboronic acid (8.4 mg, 0.14 mmol, 2 eq) in degassed 1,4-dioxane/H_2O (0.5 mL/0.5 mL, 0.07 M) under N_2 was added $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (5.8 mg, 7.1 µmol, 0.1 eq). The mixture was heated to 85 °C and stirred overnight. The reaction mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered through Celite and concentrated. The residue was purified by silica gel column chromatography (20-40% EtOAc in *n*-heptane with a drop of conc. HCl) to afford the product as a white solid (15 mg, 0.035 mmol, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 2H), 7.12 – 7.06 (m, 3H), 7.02 (d, *J* = 2.3 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.27 (t, *J* = 5.7 Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 2H), 4.01 (s, 2H), 2.85 – 2.74 (m, 2H), 2.60 – 2.51 (m, 2H), 2.34 (s, 3H), 2.22 (p, *J* = 5.7 Hz, 2H), 1.84 – 1.74 (m, 1H), 1.64 – 1.52 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.95, 154.56, 150.42, 139.04, 137.54, 135.03, 129.09, 127.12, 122.93, 121.38, 70.54, 70.44, 65.83, 47.98, 34.87, 31.03, 21.19, 14.84. HRMS $[C_{22}H_{25}NO_6S+Na]^+$: 454.12948 calculated, 454.12958 found.

_N_-(1-(4-Methoxyphenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (36)



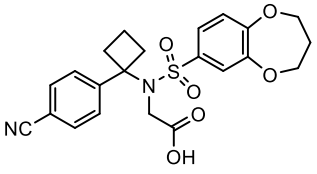
The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-methoxyphenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105c**, 17 mg, 0.036 mmol, 1 eq) and 1 M aq. LiOH (214 µL, 0.214 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5% MeOH in DCM) afforded the product as a white powder (14 mg, 0.032 mmol, 89%). ^{1}H NMR (400 MHz, MeOD) δ 7.34 – 7.28 (m, 2H), 7.04 (dd, _J_ = 8.5, 2.4 Hz, 1H), 6.81 (d, _J_ = 8.5 Hz, 1H), 6.76 – 6.72 (m, 2H), 6.70 (d, _J_ = 2.4 Hz, 1H), 4.22 (t, _J_ = 5.7 Hz, 2H), 4.15 (t, _J_ = 5.7 Hz, 2H), 4.08 (s, 2H), 3.79 (s, 3H), 2.90 – 2.78 (m, 2H), 2.55 – 2.47 (m, 2H), 2.17 (p, _J_ = 5.7 Hz, 2H), 1.79 – 1.70 (m, 1H), 1.57 – 1.48 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 174.12, 160.33, 155.76, 151.68, 136.73, 135.38, 129.56, 123.53, 122.16, 122.05, 114.39, 71.65, 66.42, 55.67, 49.00, 36.17, 32.28, 15.42. HRMS $[C_{22}H_{25}NO_7S+Na]^+$: 470.12439 calculated, 470.12427 found.

_N_-(1-(4-Ethynylphenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (37)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(4-ethynylphenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105d**, 30 mg, 0.064 mmol, 1 eq) and 1 M aq. LiOH (385 µL, 0.385 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5% MeOH in DCM) afforded the product as a white powder (28 mg, 0.063 mmol, 99%). ^{1}H NMR (400 MHz, DMSO+MeOD) δ 7.36 – 7.28 (m, 2H), 7.27 – 7.18 (m, 2H), 6.98 (dd, _J_ = 8.4, 2.3 Hz, 1H), 6.77 (d, _J_ = 8.5 Hz, 1H), 6.62 (d, _J_ = 2.3 Hz, 1H), 4.15 (t, _J_ = 5.5 Hz, 2H), 4.09 (t, _J_ = 5.6 Hz, 2H), 4.03 (s, 2H), 3.70 (s, 1H), 2.82 – 2.69 (m, 2H), 2.45 – 2.34 (m, 2H), 2.08 (p, _J_ = 5.6 Hz, 2H), 1.72 – 1.61 (m, 1H), 1.48 – 1.37 (m, 1H). ^{13}C NMR (101 MHz, DMSO+MeOD) δ 173.08, 155.21, 151.04, 144.25, 136.45, 132.22, 128.10, 123.03, 122.04, 121.85, 121.37, 84.17, 79.94, 71.37, 71.30, 65.85, 49.18, 35.49, 31.74, 14.97. HRMS $[C_{23}H_{23}NO_6S+Na]^+$: 464.11383 calculated, 464.11366 found.

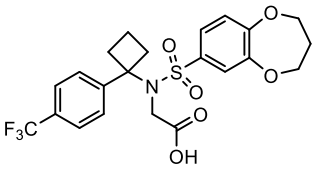
_N_-(1-(4-Cyanophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (38)



_i_-PrOH (70 µL), H_2O (175 µL), Na_2CO_3 (27 mg, 0.25 mmol, 2.5 eq), $Pd(OAc)_2$ (2.3 mg, 0.010 mmol, 0.1 eq), potassium hexacyanoferrate(II) trihydrate (12.5 mg, 0.300 mmol, 3 eq) and _N_-(1-(4-bromophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (**27**, 50 mg, 0.10 mmol, 1 eq) were ordinally added into a microtube equipped with DMF (1.2 mL, 0.08 M). The mixture was degassed under argon and heated to 100 °C and stirred overnight. The mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. Combined organic layers were washed with brine, dried over

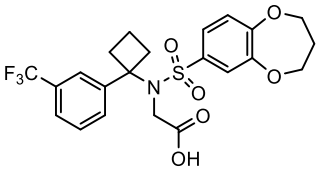
anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by preparative HPLC to afford the product as a white solid (5.2 mg, 0.012 mmol, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 4H), 7.11 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.83 (d, *J* = 2.4 Hz, 1H), 4.30 (t, *J* = 5.8 Hz, 2H), 4.25 (t, *J* = 5.9 Hz, 2H), 4.13 (s, 2H), 2.89 – 2.78 (m, 2H), 2.59 – 2.50 (m, 2H), 2.24 (p, *J* = 5.8 Hz, 2H), 1.89 – 1.79 (m, 1H), 1.64 – 1.53 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.66, 154.73, 150.36, 147.85, 134.72, 132.13, 128.10, 122.39, 121.58, 120.91, 118.76, 111.56, 70.57, 70.52, 65.58, 48.04, 34.77, 30.78, 14.63. HRMS $[C_{22}H_{22}N_2O_6S+NH_4]^+$: 460.15368 calculated, 460.15377 found.

_N_-(1-(4-(Trifluoromethyl)phenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (39)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(4-(trifluoromethyl)phenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105e**, 18 mg, 0.034 mmol, 1 eq) and 1 M aq. LiOH (207 µL, 0.207 mmol, 6 eq). Total time: 2 h at rt. Silica gel column chromatography (5% MeOH in DCM) afforded the product as a white powder (15 mg, 0.032 mmol, 92%). ^{1}H NMR (400 MHz, MeOD) δ 7.66 – 7.61 (m, 2H), 7.55 – 7.50 (m, 2H), 6.96 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 4.21 (t, *J* = 5.8 Hz, 2H), 4.17 (s, 2H), 4.15 (t, *J* = 5.8 Hz, 2H), 2.98 – 2.86 (m, 2H), 2.60 – 2.51 (m, 2H), 2.17 (p, *J* = 5.7 Hz, 2H), 1.85 – 1.75 (m, 1H), 1.62 – 1.53 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 173.90, 155.94, 151.79, 148.44 (q, $J_{C\text{-}F}$ = 1.3 Hz), 136.59, 130.45 (q, $J_{C\text{-}F}$ = 32.1 Hz), 129.03, 126.00 (q, $J_{C\text{-}F}$ = 4.0 Hz), 125.67 (q, $J_{C\text{-}F}$ = 272.7 Hz), 123.52, 122.34, 121.76, 71.73, 71.64, 66.57, 49.28, 35.99, 32.18, 15.29. HRMS $[C_{22}H_{22}F_3NO_6S+Na]^+$: 508.10121 calculated, 508.10112 found.

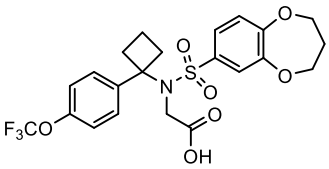
_N_-((3,4-Dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)-_N_-(1-(3-(trifluoromethyl)phenyl)cyclobutyl)glycine (40)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)-*N*-(1-(3-(trifluoromethyl)phenyl)cyclobutyl)glycinate (**105f**, 19 mg, 0.037 mmol, 1 eq) and 1 M aq. LiOH (111 µL, 0.111 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (15 mg, 0.031 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.68 (m, 1H), 7.58 (t, *J* = 2.0 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.03 – 6.96 (m, 2H), 6.83 – 6.77 (m, 1H), 4.27 (t, *J* = 5.7 Hz, 2H), 4.21 (t, *J* = 5.9 Hz, 2H), 4.14 (s, 2H), 2.93 – 2.81 (m, 2H), 2.61 – 2.52 (m, 2H), 2.21 (p, *J* = 5.7 Hz, 2H), 1.90 – 1.79 (m, 1H), 1.65 – 1.54 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.07, 154.60, 150.43, 143.47, 134.94, 130.76 (q, $J_{C\text{-}F}$ = 32.4 Hz), 130.56, 128.90, 124.51 (q, $J_{C\text{-}F}$ = 3.9 Hz), 124.17 (q, $J_{C\text{-}F}$ = 3.9 Hz), 124.11 (q, $J_{C\text{-}F}$ = 273.5

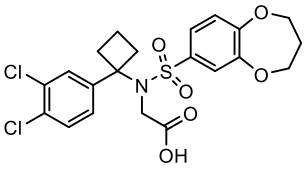
Hz), 122.47, 121.50, 120.90, 70.48, 70.30, 65.53, 48.09, 34.88, 30.86, 14.63. HRMS $[C_{22}H_{22}F_3NO_6S+NH_4]^+$: 503.14582 calculated, 503.14599 found.

_N_-((3,4-Dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)-_N_-(1-(4-(trifluoromethoxy)phenyl)cyclobutyl)glycine (41)



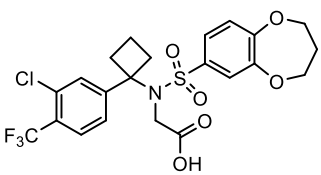
The title compound was synthesized according to the general procedure **J** using ethyl _N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)-_N_-(1-(4-(trifluoromethoxy)phenyl)cyclobutyl)glycinate (**105g**, 40 mg, 0.076 mmol, 1 eq) and 1 M aq. LiOH (227 µL, 0.227 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product (28 mg, 0.056 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.46 (m, 2H), 7.15 – 7.09 (m, 2H), 7.06 (d, J = 2.3 Hz, 1H), 7.03 (dd, J = 8.4, 2.4 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 4.28 (t, 2H), 4.22 (t, J = 5.8 Hz, 2H), 4.08 (s, 2H), 2.88 – 2.77 (m, 2H), 2.59 – 2.50 (m, 2H), 2.22 (p, J = 5.8 Hz, 2H), 1.87 – 1.76 (m, 1H), 1.65 – 1.51 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.29, 154.64, 150.43, 148.58 (q, J_{C-F} = 1.9 Hz), 140.95, 135.03, 128.90, 122.57, 121.50, 121.10, 120.57, 120.54 (q, J_{C-F} = 257.7 Hz), 70.53, 70.42, 65.41, 47.91, 34.90, 30.89, 14.68. HRMS $[C_{22}H_{22}F_3NO_7S+NH_4]$: 519.14073 found, 519.14031 found.

_N_-(1-(3,4-Dichlorophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (42)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(3,4-dichlorophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105h**, 9.0 mg, 0.018 mmol, 1 eq) and 1 M aq. LiOH (53 µL, 0.053 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in _n_-heptane with a drop of conc. HCl) afforded the product (2.5 mg, 5.1 µmol, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 1.9 Hz, 1H), 7.37 – 7.30 (m, 2H), 7.08 (dd, J = 8.5, 2.4 Hz, 1H), 6.91 (d, J = 2.3 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 4.31 (t, J = 5.8 Hz, 2H), 4.24 (t, J = 5.9 Hz, 2H), 4.14 (s, 2H), 2.86 – 2.76 (m, 2H), 2.55 – 2.45 (m, 2H), 2.23 (p, J = 5.8 Hz, 2H), 1.87 – 1.77 (m, 1H), 1.64 – 1.54 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.51, 154.74, 150.45, 142.59, 134.66, 132.51, 131.81, 130.20, 129.76, 126.73, 122.46, 121.50, 120.85, 70.57, 70.43, 65.16, 48.08, 35.02, 30.84, 14.67. HRMS $[C_{21}H_{21}Cl_2NO_6S+NH_4]^+$: 503.08049/505.07749 calculated, 503.08045/505.07739 found.

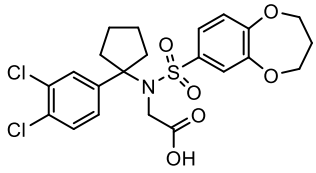
_N_-(1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycine (43)



The title compound was synthesized according to the general procedure **J** using ethyl _N_-(1-(3-chrolo-4-(trifluoromethyl)phenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105i**, 36 mg, 0.066

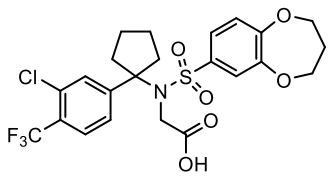
mmol, 1 eq) and 1 M aq. LiOH (197 µL, 0.197 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (32 mg, 0.062 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.59 (d, *J* = 8.0 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.00 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 4.28 (t, *J* = 5.8 Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 2H), 4.14 (s, 2H), 2.93 – 2.81 (m, 2H), 2.55 – 2.45 (m, 2H), 2.21 (p, *J* = 5.8 Hz, 2H), 1.88 – 1.78 (m, 1H), 1.66 – 1.54 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 172.09, 154.46, 150.35, 148.40, 135.06, 132.07 (q, J_{C-F} = 2.1 Hz), 130.64, 127.26 (q, J_{C-F} = 5.4 Hz), 127.21 (q, J_{C-F} = 31.6 Hz), 125.34, 122.80 (q J_{C-F} = 273.3 Hz), 122.28, 121.29, 120.48, 70.41, 70.25, 65.01, 48.08, 34.76, 30.75, 14.48. HRMS $[C_{22}H_{21}ClF_3NO_6S+NH_4]^+$: 537.10685 calculated, 537.10718 found.

N-(1-(3,4-Dichlorophenyl)cyclopentyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycine (44)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(3,4-dichlorophenyl)cyclopentyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105j**, 44 mg, 0.083 mmol, 1 eq) and 1 M aq. LiOH (333 µL, 0.333 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (33.5 mg, 66.9 µmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.29 (m, 2H), 7.23 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.19 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.08 (d, *J* = 2.3 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 4.36 – 4.30 (m, 2H), 4.27 (t, *J* = 5.9 Hz, 2H), 4.24 (s, 2H), 2.41 – 2.33 (m, 2H), 2.32 – 2.20 (m, 4H), 1.79 – 1.68 (m, 2H), 1.41 – 1.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.83, 154.68, 150.41, 142.75, 135.57, 132.28, 131.65, 129.90, 129.76, 126.81, 122.66, 121.54, 121.11, 73.12, 70.58, 70.42, 48.99, 38.12, 30.87, 21.56. HRMS $[C_{22}H_{23}Cl_2NO_6S+Na]^+$: 522.05153 calculated, 522.05107 found.

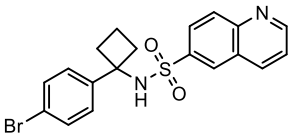
N-(1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclopentyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycine (45)



The title compound was synthesized according to the general procedure **J** using ethyl *N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (**105k**, 26 mg, 0.046 mmol, 1 eq) and 1 M aq. LiOH (184 µL, 0.184 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-40% EtOAc in *n*-heptane with a drop of conc. HCl) afforded the product (20 mg, 0.037 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.0 Hz, 1H), 7.42 – 7.35 (m, 2H), 7.13 – 7.06 (m, 2H), 6.87 (dt, *J* = 8.4, 1.0 Hz, 1H), 4.32 (t, *J* = 5.8 Hz, 2H), 4.25 (s, 2H), 4.25 (t, *J* = 5.8 Hz, 2H), 2.45 – 2.36 (m, 2H), 2.35 – 2.27 (m, 2H), 2.23 (p, *J* = 5.8 Hz, 2H), 1.83 – 1.70 (m, 2H), 1.45 – 1.34 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.53, 154.80, 150.54, 148.47, 135.25, 132.16 (q, J_{C-F} = 1.7 Hz), 130.62, 127.45 (q, J_{C-F} = 31.9 Hz), 127.26 (q, J_{C-F} = 5.3 Hz), 125.51, 122.85 (q, J_{C-F} = 273.7 Hz), 122.71, 121.57, 121.02, 73.27,

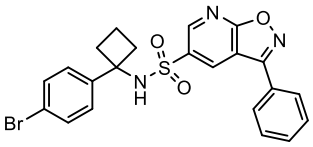
70.55, 70.37, 48.97, 38.24, 30.84, 21.68. HRMS $[C_{23}H_{23}ClF_3NO_6S+Na]^+$: 556.07789 calculated, 556.07726 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)quinoline-6-sulfonamide (46)



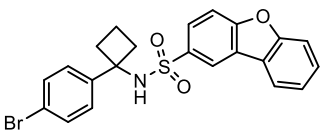
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (53.0 mg, 0.193 mmol, 1.1 eq), quinoline-6-sulfonyl chloride (41.6 mg, 0.176 mmol, 1 eq) and DIPEA (127 µL, 0.703 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (1% MeOH in DCM) afforded the product (34 mg, 0.081 mmol, 44%). ^{1}H NMR (400 MHz, DMSO) δ 8.99 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.70 (s, 1H), 8.45 – 8.32 (m, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.78 (d, *J* = 1.9 Hz, 1H), 7.63 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.55 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.02 – 6.96 (m, 2H), 6.95 – 6.89 (m, 2H), 2.54 – 2.48 (m, 2H), 2.39 – 2.31 (m, 2H), 2.02 – 1.89 (m, 1H), 1.66 – 1.52 (m, 1H). ^{13}CNMR (101 MHz, DMSO) δ 152.08, 146.14, 142.35, 141.72, 135.90, 130.21, 129.07, 128.93, 128.45, 127.62, 123.29, 122.55, 119.89, 60.40, 34.06, 15.17. LC-MS $[C_{19}H_{17}BrN_2O_2S+H]^+$: 417.03/419.02 calculated, 417.17/419.00 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-3-phenylisoxazolo[5,4-*b*]pyridine-5-sulfonamide (47)



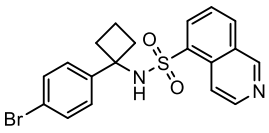
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (42.8 mg, 0.163 mmol, 1 eq), 3-phenylisoxazolo[5,4-*b*]pyridine-5-sulfonyl chloride (50 mg, 0.17 mmol, 1.04 eq) and DIPEA (123 µL, 0.707 mmol, 4.6 eq). Total time: overnight at rt. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product as a white solid (62.2 mg, 0.128 mmol, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 – 8.74 (m, 1H), 8.05 – 7.96 (m, 1H), 7.89 – 7.74 (m, 2H), 7.59 – 7.47 (m, 3H), 6.98 – 6.84 (m, 4H), 6.35 (s, 1H), 2.63 – 2.42 (m, 4H), 2.13 – 1.96 (m, 1H), 1.76 – 1.60 (m, 1H).

_N_-(1-(4-Bromophenyl)cyclobutyl)dibenzo[*b*,*d*]furan-2-sulfonamide (48)



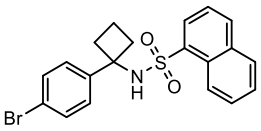
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (79.1 mg, 0.301 mmol, 1 eq), dibenzo[*b*,*d*]furan-2-sulfonyl chloride (83.6 mg, 0.314 mmol, 1.04 eq) and DIPEA (241 µL, 1.39 mmol, 4.6 eq). Total time: overnight at rt. Silica gel column chromatography (10-40% EtOAc in *n*-pentane) afforded the product (129 mg, 0.283 mmol, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 – 7.84 (m, 1H), 7.82 (d, *J* = 1.9 Hz, 1H), 7.64 – 7.49 (m, 3H), 7.47 – 7.38 (m, 2H), 7.09 – 6.97 (m, 4H), 5.40 (s, 1H), 2.67 – 2.46 (m, 4H), 2.18 – 1.95 (m, 1H), 1.82 – 1.64 (m, 1H).

***N*-(1-(4-Bromophenyl)cyclobutyl)isoquinoline-5-sulfonamide (49)**



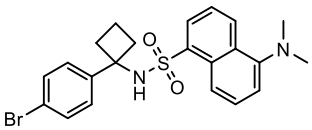
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)-cyclobutan-1-aminium chloride (30.0 mg, 0.114 mmol, 1 eq), isoquinoline-5-sulfonyl chloride (31.2 mg, 0.137 mmol, 1.2 eq) and Et_3N (48 µL, 0.34 mmol, 3 eq). Silica gel column chromatography (30-70% EtOAc in *n*-pentane) afforded the product (9.5 mg, 0.023 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (d, *J* = 1.0 Hz, 1H), 8.67 (d, *J* = 6.1 Hz, 1H), 8.25 (dt, *J* = 6.1, 1.0 Hz, 1H), 8.05 (dt, *J* = 8.2, 1.1 Hz, 1H), 7.69 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.33 (dd, *J* = 8.2, 7.4 Hz, 1H), 6.87 – 6.79 (m, 2H), 6.78 – 6.69 (m, 2H), 5.80 (s, 1H), 2.66 – 2.48 (m, 4H), 2.13 – 1.96 (m, 1H), 1.78 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.33, 145.02, 140.00, 135.23, 133.45, 132.76, 130.75, 130.57, 128.67, 128.61, 125.88, 121.40, 117.15, 61.45, 35.91, 15.21. LC-MS $[C_{19}H_{17}BrN_2O_2S+H]^+$: 417.03/419.03 calculated, 417.08/419.00 found.

***N*-(1-(4-Bromophenyl)cyclobutyl)naphthalene-1-sulfonamide (50)**



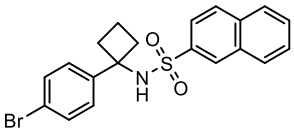
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)-cyclobutan-1-aminium chloride (30.0 mg, 0.114 mmol, 1 eq), naphthalene-1-sulfonyl chloride (31.1 mg, 0.137 mmol, 1.2 eq) and Et_3N (48 µL, 0.34 mmol, 3 eq). Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (37 mg, 0.088 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (dq, *J* = 8.6, 0.9 Hz, 1H), 7.91 (dt, *J* = 8.3, 1.2 Hz, 1H), 7.89 – 7.85 (m, 1H), 7.67 – 7.54 (m, 3H), 7.20 (dd, *J* = 8.2, 7.4 Hz, 1H), 6.82 – 6.76 (m, 2H), 6.73 – 6.66 (m, 2H), 5.76 (s, 1H), 2.64 – 2.53 (m, 2H), 2.52 – 2.43 (m, 2H), 2.09 – 1.96 (m, 1H), 1.72 – 1.59 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.32, 135.71, 133.91, 133.53, 130.35, 129.79, 129.14, 128.31, 128.13, 127.70, 126.59, 124.29, 124.14, 121.12, 61.39, 35.63, 15.26.

***N*-(1-(4-Bromophenyl)cyclobutyl)-5-(dimethylamino)naphthalene-1-sulfonamide (51)**



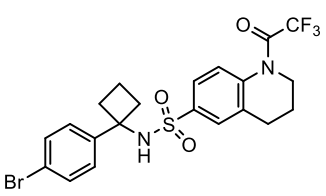
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)-cyclobutan-1-aminium chloride (40.0 mg, 0.152 mmol, 1 eq), 5-(dimethylamino)naphthalene-1-sulfonyl chloride (49.3 mg, 0.183 mmol, 1.2 eq) and Et_3N (64 µL, 0.46 mmol, 3 eq). Silica gel column chromatography (5-15% EtOAc in *n*-pentane) afforded the product (60.5 mg, 0.132 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (dt, *J* = 8.5, 1.2 Hz, 1H), 8.14 (dt, *J* = 8.8, 1.0 Hz, 1H), 7.57 (dd, *J* = 7.4, 1.3 Hz, 1H), 7.52 (dd, *J* = 8.6, 7.6 Hz, 1H), 7.17 (dd, *J* = 8.5, 7.4 Hz, 1H), 7.14 (dd, *J* = 7.6, 0.9 Hz, 1H), 6.85 – 6.81 (m, 2H), 6.75 – 6.71 (m, 2H), 5.66 (s, 1H), 2.89 (s, 6H), 2.63 – 2.53 (m, 2H), 2.52 – 2.42 (m, 2H), 2.08 – 1.96 (m, 1H), 1.72 – 1.61 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.99, 140.49, 135.92, 130.39, 129.77, 129.66, 129.44, 129.16, 128.49, 128.18, 123.11, 121.07, 118.68, 114.77, 61.36, 45.58, 35.73, 15.26. LC-MS $[C_{22}H_{23}BrN_2O_2S+H]^+$: 459.07/461.07 calculated, 459.00/461.00 found.

N-(1-(4-Bromophenyl)cyclobutyl)naphthalene-2-sulfonamide (52)



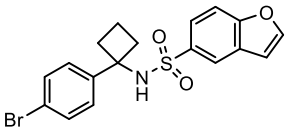
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)-cyclobutan-1-aminium chloride (30.0 mg, 0.114 mmol, 1 eq), naphthalene-2-sulfonyl chloride (31.1 mg, 0.137 mmol, 1.2 eq) and Et_3N (48 µL, 0.34 mmol, 3 eq). Silica gel column chromatography (5-15% EtOAc in *n*-pentane) afforded the product (36 mg, 0.086 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.82 (m, 1H), 7.78 – 7.76 (m, 1H), 7.75 – 7.70 (m, 2H), 7.63 – 7.55 (m, 2H), 7.52 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.02 – 6.92 (m, 4H), 5.93 (s, 1H), 2.65 – 2.46 (m, 4H), 2.11 – 2.00 (m, 1H), 1.75 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.81, 138.18, 134.29, 131.81, 130.85, 129.25, 128.93, 128.71, 128.35, 127.79, 127.57, 122.00, 121.36, 61.39, 35.55, 15.34.

N-(1-(4-Bromophenyl)cyclobutyl)-1-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroquinoline-6-sulfonamide (53)



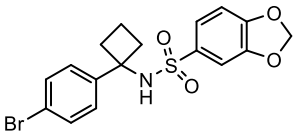
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (100 mg, 0.381 mmol, 1 eq), 1-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroquinoline-6-sulfonyl chloride (**107**, 137 mg, 0.419 mmol, 1.1 eq) and DIPEA (265 µL, 1.52 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (10-30% EtOAc in *n*-pentane) afforded the product as a white solid (196 mg, 0.379 mmol, 99%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.58 (bs, 1H), 7.38 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.19 – 7.10 (m, 2H), 7.03 – 6.96 (m, 2H), 6.81 (s, 1H), 3.85 (t, *J* = 5.9 Hz, 2H), 2.69 (t, *J* = 7.0 Hz, 2H), 2.65 – 2.55 (m, 2H), 2.54 – 2.43 (m, 2H), 2.18 – 2.06 (m, 3H), 1.81 – 1.67 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 156.40, 141.45, 139.60, 139.27, 130.84, 128.99, 128.32, 124.89, 124.22, 120.82, 116.73 (q, $J_{C\text{-}F}$ = 288.2 Hz), 60.91, 45.47 (q, $J_{C\text{-}F}$ = 3.6 Hz), 35.20, 25.93, 23.29, 15.56.

N-(1-(4-Bromophenyl)cyclobutyl)benzofuran-5-sulfonamide (54)



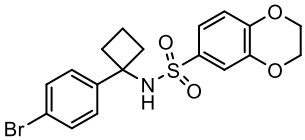
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (50 mg, 0.19 mmol, 1 eq), benzofuran-5-sulfonyl chloride (43.2 mg, 0.199 mmol, 1.05 eq) and DIPEA (133 µL, 0.760 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-30% EtOAc in *n*-pentane) afforded the product (22 mg, 0.054 mmol, 27%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 2.2 Hz, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 7.45 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 1H), 7.11 – 7.05 (m, 2H), 7.03 – 6.95 (m, 2H), 6.73 (dd, *J* = 2.2, 0.9 Hz, 1H), 5.67 (s, 1H), 2.60 – 2.47 (m, 4H), 2.09 – 1.99 (m, 1H), 1.76 – 1.66 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.26, 147.10, 141.10, 136.46, 130.91, 128.83, 127.25, 123.00, 121.38, 121.19, 111.59, 107.11, 61.36, 35.62, 15.30.

_N_-(1-(4-Bromophenyl)cyclobutyl)benzo[_d_][1,3]dioxole-5-sulfonamide (55)



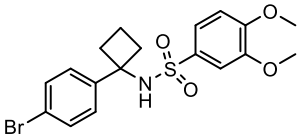
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (287 mg, 1.09 mmol, 1.1 eq), benzo[*d*][1,3]dioxole-5-sulfonyl chloride (219 mg, 0.993 mmol, 1 eq) and DIPEA (0.69 mL, 4.0 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20-25% EtOAc in *n*-pentane) afforded the product as an off-white solid (409 mg, 0.996 mmol, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 – 7.20 (m, 2H), 7.05 – 6.99 (m, 3H), 6.68 (d, *J* = 1.8 Hz, 1H), 6.64 (d, *J* = 8.2 Hz, 1H), 6.04 (s, 2H), 5.60 (s, 1H), 2.63 – 2.44 (m, 4H), 2.15 – 1.99 (m, 1H), 1.80 – 1.64 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.76, 147.45, 141.34, 134.94, 130.98, 128.82, 122.51, 121.13, 107.79, 107.49, 102.34, 61.23, 35.40, 15.35.

_N_-(1-(4-Bromophenyl)cyclobutyl)-2,3-dihydrobenzo[_b_][1,4]dioxine-6-sulfonamide (56)



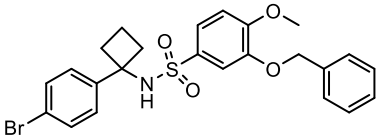
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)-cyclobutan-1-aminium chloride (49.2 mg, 0.188 mmol, 1 eq), 2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride (**108**, 44.0 mg, 0.188 mmol, 1 eq) and DIPEA (0.13 mL, 0.75 mmol, 4 eq). Silica gel column chromatography (20-50% EtOAc in *n*-pentane) afforded the product as a white solid (56.6 mg, 0.133 mmol, 71%). ^{1}H NMR (400 MHz, DMSO) δ 8.31 (s, 1H), 7.26 – 7.21 (m, 2H), 7.07 – 7.01 (m, 2H), 6.88 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.60 (d, *J* = 2.2 Hz, 1H), 4.31 – 4.20 (m, 4H), 2.50 – 2.42 (m, 2H), 2.35 – 2.26 (m, 2H), 1.99 – 1.89 (m, 1H), 1.66 – 1.53 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 145.99, 142.41, 142.30, 134.70, 130.29, 128.55, 119.67, 119.57, 116.86, 115.45, 64.29, 64.03, 60.16, 34.00, 15.13.

_N_-(1-(4-Bromophenyl)cyclobutyl)-3,4-dimethoxybenzenesulfonamide (57)



The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (40.0 mg, 0.152 mmol, 1 eq), 3,4-dimethoxybenzenesulfonyl chloride (43.3 mg, 0.183 mmol, 1.2 eq) and DIPEA (106 µL, 0.609 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (15-35% EtOAc in *n*-pentane) afforded the product as a colorless oil (56.7 mg, 0.133 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 – 7.13 (m, 2H), 7.06 – 6.99 (m, 2H), 6.98 – 6.91 (m, 2H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.00 (s, 1H), 3.92 (s, 3H), 3.80 (s, 3H), 2.64 – 2.42 (m, 4H), 2.14 – 1.99 (m, 1H), 1.76 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.09, 148.59, 141.32, 133.25, 130.85, 128.76, 121.02, 120.93, 109.97, 109.17, 61.21, 56.28, 56.12, 35.36, 15.33.

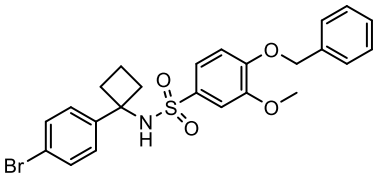
3-(Benzyloxy)-_N_-(1-(4-bromophenyl)cyclobutyl)-4-methoxybenzenesulfonamide (58)



The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (92.3 mg, 0.352 mmol, 1 eq), 3-(benzyloxy)-4-methoxybenzenesulfonyl chloride (**110**, 100 mg, 0.320 mmol, 0.9 eq) and DIPEA (245 µL, 1.41

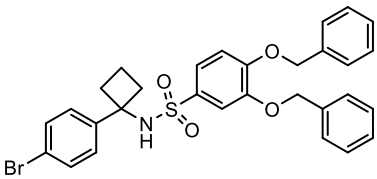
mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product (83.2 mg, 0.166 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.45 (m, 2H), 7.43 – 7.36 (m, 2H), 7.34 – 7.28 (m, 1H), 7.10 (d, *J* = 2.2 Hz, 1H), 7.08 – 7.03 (m, 2H), 6.84 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.80 – 6.74 (m, 2H), 6.56 (d, *J* = 8.5 Hz, 1H), 6.06 (s, 1H), 5.07 (s, 2H), 3.90 (s, 3H), 2.53 – 2.33 (m, 4H), 2.08 – 1.92 (m, 1H), 1.72 – 1.57 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.67, 147.40, 141.16, 136.42, 132.98, 130.69, 128.71, 128.24, 127.76, 121.21, 120.82, 111.50, 110.27, 70.70, 61.05, 56.27, 35.19, 15.31.

4-(Benzyloxy)-*N*-(1-(4-bromophenyl)cyclobutyl)-3-methoxybenzenesulfonamide (59)



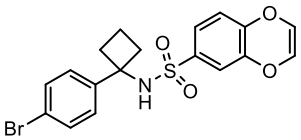
The title compound was synthesized according to general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (200 mg, 0.762 mmol, 1 eq), 4-(benzyloxy)-3-methoxybenzenesulfonyl chloride (**112**, 246 mg, 0.838 mmol, 1.1 eq) and DIPEA (531 µL, 3.05 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product as a white solid (274 mg, 0.545 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.48 – 7.38 (m, 4H), 7.37 – 7.31 (m, 1H), 7.18 – 7.11 (m, 2H), 6.99 (dd, *J* = 8.7, 2.2 Hz, 2H), 6.88 – 6.82 (m, 2H), 6.66 (d, *J* = 8.1 Hz, 1H), 5.18 (s, 2H), 3.79 (s, 3H), 2.60 – 2.42 (m, 4H), 2.09 – 2.00 (m, 1H), 1.77 – 1.67 (m, 1H). ^{13}C NMR (101 MHz, , $CDCl_3$+MeOD) δ 150.89, 148.72, 141.42, 136.10, 133.69, 130.65, 128.63, 128.12, 127.28, 120.72, 120.33, 112.08, 109.52, 70.87, 60.69, 55.83, 35.06, 15.14.

3,4-Bis(benzyloxy)-*N*-(1-(4-bromophenyl)cyclobutyl)benzenesulfonamide (60)



The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (228 mg, 0.869 mmol, 1.1 eq), 3,4-bis(benzyloxy)benzenesulfonyl chloride (**114**, 300 mg, 0.771 mmol, 1 eq) and DIPEA (540 µL, 3.09 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (278 mg, 0.581 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.44 (m, 4H), 7.43 – 7.29 (m, 6H), 7.10 – 7.05 (m, 2H), 7.03 (d, *J* = 2.2 Hz, 1H), 6.86 – 6.76 (m, 3H), 6.64 (d, *J* = 8.5 Hz, 1H), 5.60 (s, 1H), 5.18 (s, 2H), 5.07 (s, 2H), 2.51 – 2.37 (m, 4H), 2.05 – 1.92 (m, 1H), 1.72 – 1.62 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.02, 147.99, 141.12, 136.67, 136.46, 133.49, 130.82, 128.82, 128.78, 128.72, 128.23, 127.67, 127.34, 121.22, 121.00, 112.78, 112.52, 71.11, 70.96, 61.12, 35.46, 15.28.

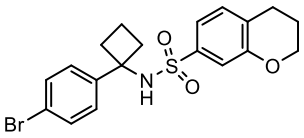
***N*-(1-(4-Bromophenyl)cyclobutyl)benzo[*b*][1,4]dioxine-6-sulfonamide (61)**



The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (34.0 mg, 0.129 mmol, 1.2 eq), benzo[*b*][1,4]dioxine-6-sulfonyl chloride (**118**, 25.0 mg, 0.107 mmol, 1 eq) and DIPEA (75 µL, 0.43 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (100% DCM) afforded the product as

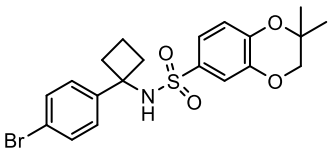
a white powder (40 mg, 0.095 mmol, 88%). ^{1}H NMR (400 MHz, DMSO) δ 8.42 (s, 1H), 7.39 – 7.26 (m, 2H), 7.13 – 7.00 (m, 2H), 6.86 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.59 (d, *J* = 8.5 Hz, 1H), 6.35 (d, *J* = 2.2 Hz, 1H), 6.21 (q, *J* = 3.6 Hz, 2H), 2.50 – 2.40 (m, 2H), 2.39 – 2.28 (m, 2H), 2.04 – 1.90 (m, 1H), 1.71 – 1.54 (m, 1H).

_N_-(1-(4-Bromophenyl)cyclobutyl)chromane-7-sulfonamide (62)



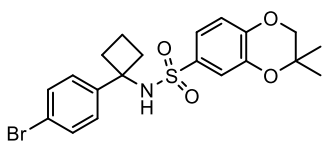
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (40.6 mg, 0.155 mmol, 1.2 eq), chromane-7-sulfonyl chloride (**125**, 30.0 mg, 0.129 mmol, 1 eq) and DIPEA (90 µL, 0.52 mmol, 4 eq). Total time: overnight at rt. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product (42 mg, 0.099 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.19 – 7.13 (m, 2H), 7.04 – 6.98 (m, 2H), 6.91 – 6.86 (m, 1H), 6.86 – 6.82 (m, 1H), 6.63 (d, *J* = 1.8 Hz, 1H), 4.23 – 4.16 (m, 2H), 2.78 (t, *J* = 6.4 Hz, 2H), 2.63 – 2.54 (m, 2H), 2.50 – 2.40 (m, 2H), 2.13 – 1.98 (m, 3H), 1.78 – 1.66 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 153.90, 141.21, 139.93, 130.28, 129.52, 128.19, 126.25, 120.30, 117.51, 114.90, 66.33, 60.32, 34.34, 24.45, 21.43, 14.95.

_N_-(1-(4-Bromophenyl)cyclobutyl)-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (63)



The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (25 mg, 0.095 mmol, 1 eq), 2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride (**134**, 35.0 mg, 0.133 mmol, 1.4 eq) and DIPEA (196 µL, 1.13 mmol, 12 eq). Total time: overnight at rt. Silica gel column chromatography (10-15% EtOAc in *n*-pentane) afforded the product as a white solid (37 mg, 0.081 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.19 (m, 2H), 7.10 – 7.03 (m, 2H), 6.98 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.83 (d, *J* = 2.2 Hz, 1H), 6.66 (d, *J* = 8.5 Hz, 1H), 5.71 (s, 1H), 3.88 (s, 2H), 2.62 – 2.43 (m, 4H), 2.12 – 2.00 (m, 1H), 1.76 – 1.64 (m, 1H), 1.35 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.05, 141.51, 141.39, 133.44, 130.91, 128.87, 121.04, 120.96, 117.51, 116.32, 73.44, 71.89, 61.15, 35.51, 23.39, 15.35. LC-MS $[C_{20}H_{22}BrNO_4S+H]^+$: 452.05/454.05 calculated, 451.92/453.83 found.

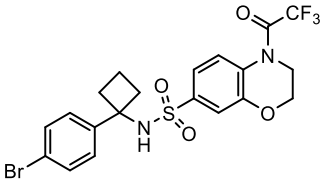
_N_-(1-(4-Bromophenyl)cyclobutyl)-3,3-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (64)



The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (25 mg, 0.095 mmol, 1 eq), 3,3-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride (**135**, 50 mg, 0.19 mmol, 2 eq) and DIPEA (196 µL, 1.13 mmol, 12 eq). Total time: overnight at rt. Silica gel column chromatography (10-15% EtOAc in *n*-pentane) afforded the product (41 mg, 0.091 mmol, 94%). ^{1}H NMR (400 MHz,

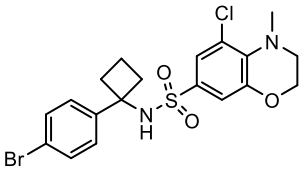
$CDCl_3$) δ 7.22 – 7.16 (m, 2H), 7.07 – 7.01 (m, 2H), 6.97 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.76 (d, *J* = 2.2 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 5.74 (s, 1H), 3.93 (s, 2H), 2.64 – 2.43 (m, 4H), 2.11 – 2.01 (m, 1H), 1.79 – 1.64 (m, 1H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.53, 141.90, 141.44, 134.30, 130.88, 128.81, 120.94, 119.92, 117.05, 116.76, 72.72, 72.06, 61.14, 35.44, 23.35, 15.37. LC-MS $[C_{20}H_{22}BrNO_4S+H]^+$: 452.05/454.05 calculated, 451.75/454.00 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-4-(2,2,2-trifluoroacetyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonamide (65)



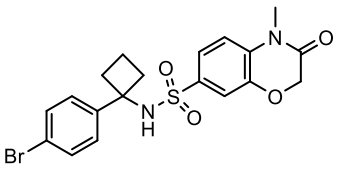
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (35 mg, 0.13 mmol, 1 eq), 4-(2,2,2-trifluoroacetyl)-3,4-dihydro-*2H*-benzo[*b*][1,4]oxazine-7-sulfonyl chloride (**142**, 53 mg, 0.16 mmol, 1.2 eq) and DIPEA (232 µL, 1.33 mmol, 10 eq). Total time: overnight at rt. Silica gel column chromatography (25-30% EtOAc in *n*-pentane) afforded the product (58.9 mg, 0.113 mmol, 85%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.76 (bs, 1H), 7.14 – 7.08 (m, 2H), 7.02 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.96 – 6.91 (m, 2H), 6.58 (d, *J* = 2.1 Hz, 1H), 4.42 – 4.36 (m, 2H), 3.97 – 3.93 (m, 2H), 2.61 – 2.49 (m, 2H), 2.49 – 2.38 (m, 2H), 2.11 – 2.01 (m, 1H), 1.75 – 1.61 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 146.22, 141.29, 140.18, 130.84, 128.77, 126.32, 124.08, 120.76, 118.30, 116.94, 116.24 (q, $J_{C\text{-}F}$ =289.9 Hz), 65.89, 60.82, 43.37, 34.94, 15.45.

_N_-(1-(4-Bromophenyl)cyclobutyl)-5-chloro-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonamide (66)



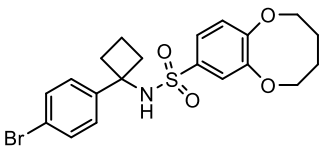
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (68 mg, 0.26 mmol, 1 eq), 5-chloro-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonyl chloride (**148**, 66.4 mg, 0.235 mmol, 1 eq) and DIPEA (410 µL, 2.35 mmol, 10 eq). Total time: overnight at rt. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (38 mg, 0.081 mmol, 34%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.22 – 7.14 (m, 2H), 7.05 – 6.98 (m, 2H), 6.96 – 6.92 (m, 1H), 6.65 – 6.59 (m, 1H), 4.18 – 4.10 (m, 2H), 3.17 – 3.10 (m, 2H), 2.92 (s, 3H), 2.64 – 2.53 (m, 2H), 2.53 – 2.44 (m, 2H), 2.16 – 2.07 (m, 1H), 1.80 – 1.66 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 147.83, 141.14, 136.50, 135.09, 130.58, 128.67, 127.29, 120.74, 120.60, 114.68, 60.68, 60.23, 49.46, 43.21, 35.04, 15.30. LC-MS $[C_{19}H_{20}BrClN_2O_3S+H]^+$: 471.01/473.01 calculated, 471.25/473.08 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-4-methyl-3-oxo-3,4-dihydro-2_H_-benzo[_b_][1,4]oxazine-7-sulfonamide (67)



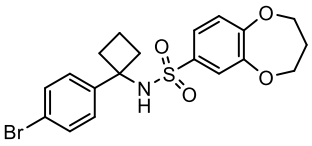
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (43.0 mg, 0.164 mmol, 1 eq), 4-methyl-3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonyl chloride (**145**, 51.0 mg, 0.197 mmol, 1.2 eq) and DIPEA (285 µL, 1.64 mmol, 10 eq). Total time: overnight at rt. Silica gel column chromatography (30-40% EtOAc in *n*-pentane) afforded the product (19 mg, 0.042 mmol, 25%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.18 – 7.12 (m, 2H), 7.03 – 6.98 (m, 3H), 6.81 (d, *J* = 2.0 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 4.64 (s, 2H), 3.37 (s, 3H), 2.61 – 2.52 (m, 2H), 2.51 – 2.45 (m, 2H), 2.15 – 2.05 (m, 1H), 1.79 – 1.66 (m, 1H). LC-MS $[C_{19}H_{19}BrN_2O_4S+H]^+$: 451.03/453.03 calculated, 451.00/453.00 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-2,3,4,5-tetrahydrobenzo[_b_][1,4]dioxocine-8-sulfonamide (68)



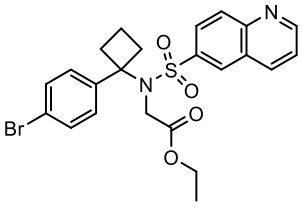
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (101 mg, 0.184 mmol, 1.1 eq), 2,3,4,5-tetrahydrobenzo[*b*][1,4]dioxocine-8-sulfonyl chloride (**122**, 92 mg, 0.35 mmol, 1 eq) and DIPEA (0.61 mL, 3.5 mmol, 10 eq). Total time: over-weekend at rt. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product as an orange solid (38 mg, 0.084 mmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.19 (m, 2H), 7.08 – 7.01 (m, 3H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 5.79 (s, 1H), 4.45 (t, *J* = 5.5 Hz, 2H), 4.21 (t, *J* = 5.5 Hz, 2H), 2.61 – 2.45 (m, 4H), 2.09 – 2.01 (m, 1H), 1.95 (p, *J* = 5.9 Hz, 2H), 1.85 (p, *J* = 5.6 Hz, 2H), 1.77 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.86, 147.25, 141.39, 135.05, 130.94, 128.84, 122.79, 122.74, 121.49, 121.07, 73.87, 71.81, 61.14, 35.49, 27.69, 25.58, 15.35. LC-MS $[C_{20}H_{22}BrNO_4S+H]^+$: 452.05/454.05 calculated, 452.00/453.75 found.

_N_-(1-(4-Bromophenyl)cyclobutyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (69)



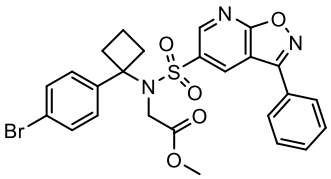
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclobutan-1-aminium chloride (43.0 mg, 0.168 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 49.0 mg, 0.197 mmol, 1.2 eq) and DIPEA (285 µL, 1.68 mmol, 10 eq). Total time: over-weekend at rt. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (73.5 mg, 0.168 mmol, quant). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.19 (m, 2H), 7.08 – 7.02 (m, 2H), 7.00 (dt, *J* = 8.5, 1.8 Hz, 1H), 6.88 (t, *J* = 1.8 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 5.54 (s, 1H), 4.28 (t, *J* = 5.8 Hz, 2H), 4.21 (t, *J* = 6.2 Hz, 2H), 2.62 – 2.47 (m, 4H), 2.22 (p, *J* = 5.9 Hz, 2H), 2.12 – 2.00 (m, 1H), 1.78 – 1.69 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.26, 150.09, 141.29, 135.60, 130.98, 128.90, 122.17, 121.36, 121.12, 120.86, 70.55, 70.47, 61.18, 35.59, 30.97, 15.35.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(quinolin-6-ylsulfonyl)glycinate (70)



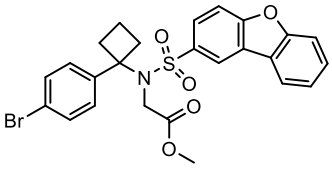
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)quinoline-6-sulfonamide (**46**, 34 mg, 0.081 mmol, 1 eq), ethyl 2-bromoacetate (18 µL, 0.16 mmol, 2 eq) and BEMP (1 M in hexane, 162 µL, 0.162 mmol, 2 eq). Total time: 12 h at 80 °C. Silica gel column chromatography (1% MeOH in DCM) afforded the product (30 mg, 0.060 mmol, 75%). ^{1}H NMR (400 MHz, MeOD) δ 8.97 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.40 (ddd, *J* = 8.4, 1.7, 0.8 Hz, 1H), 7.98 – 7.94 (m, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.65 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.60 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.11 – 7.04 (m, 2H), 4.39 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 2.90 – 2.79 (m, 2H), 2.57 – 2.49 (m, 2H), 1.80 – 1.70 (m, 1H), 1.58 – 1.47 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.94, 153.21, 147.28, 143.60, 142.24, 138.05, 132.06, 131.20, 131.00, 130.41, 129.25, 124.76, 124.36, 122.49, 66.57, 62.64, 49.72, 36.03, 15.20, 14.46. LC-MS $[C_{23}H_{23}BrN_2O_4S+H]^+$: 503.06/505.06 calculated, 503.17/505.00 found.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3-phenylisoxazolo[5,4-*b*]pyridin-5-yl)sulfonyl)glycinate (71)



The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-3-phenylisoxazolo[5,4-*b*]pyridine-5-sulfonamide (**47**, 48 mg, 0.098 mmol, 1 eq), methyl 2-bromoacetate (19.0 µL, 0.196 mmol, 2 eq) and BEMP (1 M in hexane, 196 µL, 0.196 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product (29 mg, 0.052 mmol, 53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (d, *J* = 2.2 Hz, 1H), 8.50 (d, *J* = 2.2 Hz, 1H), 8.00 – 7.93 (m, 2H), 7.66 – 7.57 (m, 3H), 7.38 – 7.30 (m, 2H), 7.24 – 7.17 (m, 2H), 4.30 (s, 2H), 3.80 (s, 3H), 2.83 – 2.65 (m, 2H), 2.59 – 2.42 (m, 2H), 1.84 – 1.70 (m, 1H), 1.62 – 1.49 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.77, 157.86, 149.67, 140.45, 136.00, 132.83, 131.50, 131.43, 129.56, 129.48, 127.94, 127.62, 122.22, 111.63, 65.92, 52.81, 48.00, 34.78, 14.67.

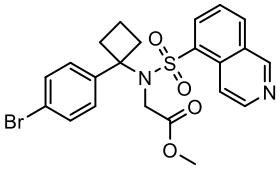
Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(dibenzo[*b*,*d*]furan-2-ylsulfonyl)glycinate (72)



The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)dibenzo[*b*,*d*]furan-2-sulfonamide (**48**, 61.6 mg, 0.135 mmol, 1 eq), methyl 2-bromoacetate (26 µL, 0.27 mmol, 2 eq) and BEMP (1 M in hexane, 0.27 mL, 0.27 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product (39 mg, 0.074 mmol, 55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, *J* = 2.0, 0.5 Hz, 1H), 7.92 (ddd, *J* = 7.6, 1.4, 0.7 Hz, 1H), 7.75 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.63 – 7.41 (m, 4H), 7.37 – 7.28 (m, 4H), 4.13 (s, 2H), 3.74 (s, 3H), 2.89 – 2.73 (m, 2H), 2.74 – 2.56 (m, 2H), 1.93 – 1.76 (m, 1H), 1.64 – 1.49 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.74, 157.89, 157.13, 141.51, 136.17,

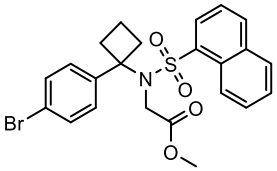
131.86, 131.31, 128.47, 126.41, 124.56, 123.77, 123.26, 121.76, 121.40, 120.94, 112.08, 111.78, 65.63, 52.58, 47.91, 34.86, 14.72.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(isoquinolin-5-ylsulfonyl)glycinate (73)



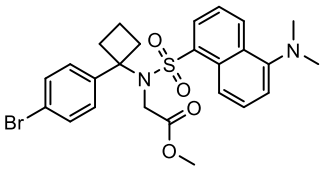
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-isoquinoline-5-sulfonamide (**49**, 9.5 mg, 0.023 mmol, 1 eq), methyl 2-bromoacetate (4.3 µL, 0.046 mmol, 2 eq) and BEMP (1 M in hexane, 46 µL, 0.046 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded and used in the next step without purification (13.5 mg, 27.6 µmol, quant.). LC-MS $[C_{22}H_{21}BrN_2O_4S+H]^+$: 489.04/491.05 calculated, 489.00/491.00 found.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(naphthalen-1-ylsulfonyl)glycinate (74)



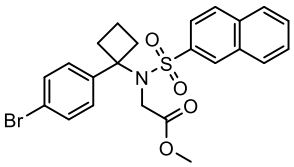
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)-cyclobutyl)naphthalene-1-sulfonamide (**50**, 37 mg, 0.088 mmol, 1 eq), methyl 2-bromoacetate (17.0 µL, 0.176 mmol, 2 eq) and BEMP (1 M in hexane, 176 µL, 0.176 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded and used in the next step without purification (37 mg, 0.077 mmol, 87%). LC-MS $[C_{23}H_{22}BrNO_4S+Na]^+$: 510.03/512.03 calculated, 510.00/512.00 found.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)glycinate (75)



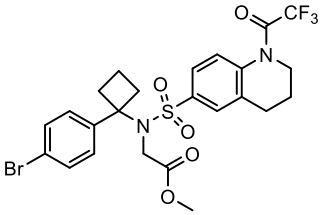
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-Bromophenyl)cyclobutyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**51**, 60.5 mg, 0.132 mmol, 1 eq), methyl 2-bromoacetate (25 µL, 0.263 mmol, 2 eq) and BEMP (1 M in hexane, 263 µL, 0.263 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (10-30% dist. EtOAc in *n*-heptane) afforded the product (49 mg, 0.093 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (dt, *J* = 8.5, 1.1 Hz, 1H), 8.12 (dt, *J* = 8.7, 0.9 Hz, 1H), 8.04 (dd, *J* = 7.4, 1.3 Hz, 1H), 7.47 (dd, *J* = 8.7, 7.5 Hz, 1H), 7.37 (dd, *J* = 8.5, 7.4 Hz, 1H), 7.26 – 7.21 (m, 2H), 7.21 – 7.13 (m, 3H), 4.24 (s, 2H), 3.61 (s, 3H), 2.93 – 2.82 (m, 8H), 2.50 – 2.38 (m, 2H), 1.82 – 1.69 (m, 1H), 1.59 – 1.40 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.48, 151.74, 141.13, 137.32, 131.00, 130.07, 129.84, 129.81, 129.25, 127.97, 123.14, 121.58, 119.44, 115.13, 65.73, 52.38, 47.85, 45.55, 34.45, 14.75. LC-MS $[C_{25}H_{27}BrN_2O_4S+H]^+$: 531.09/533.09 calculated , 531.00/533.00 found.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(naphthalen-2-ylsulfonyl)glycinate (76)



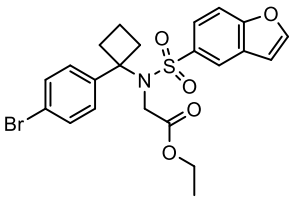
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)-cyclobutyl)naphthalene-2-sulfonamide (**52**, 36 mg, 0.086 mmol, 1 eq), methyl 2-bromoacetate (16 µL, 0.17 mmol, 2 eq) and BEMP (1 M in hexane, 173 µL, 0.173 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded and used in the next step without purification (43 mg, 0.089 mmol, quant.). LC-MS $[C_{23}H_{22}BrNO_4S+NH_4]^+$: 505.08/507.08 calculated , 504.92/506.83 found.

Methyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((1-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroquinolin-6-yl)sulfonyl)glycinate (77)



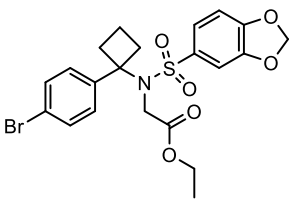
The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-1-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroquinoline-6-sulfonamide (**53**, 20 mg, 0.039 mmol, 1 eq), methyl 2-bromoacetate (7.4 µL, 0.077 mmol, 2 eq) and BEMP (1 M in hexane, 77 µL, 0.077 mmol, 2 eq). Total time: overnight at 80 °C. The product was afforded and used in the next step without purification (23 mg, 0.039 mmol, quant.).

Ethyl *N*-(benzofuran-5-ylsulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (78)



The title compound was synthesized according to general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)benzofuran-5-sulfonamide (**54**, 22 mg, 0.054 mmol, 1 eq), ethyl 2-bromoacetate (11.9 µL, 0.108 mmol, 2 eq) and BEMP (1 M in hexane, 108 µL, 0.108 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (5-10% EtOAc in dis. *n*-pentane) afforded the product (10 mg, 0.021 mmol, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 2.2 Hz, 1H), 7.71 (dd, *J* = 2.0, 0.6 Hz, 1H), 7.60 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.47 (dt, *J* = 8.7, 0.8 Hz, 1H), 7.32 (s, 4H), 6.80 (dd, *J* = 2.2, 1.0 Hz, 1H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.07 (s, 2H), 2.86 – 2.74 (m, 2H), 2.53 – 2.44 (m, 2H), 1.81 – 1.71 (m, 1H), 1.64 – 1.48 (m, 1H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.28, 156.47, 147.09, 141.63, 136.42, 131.33, 129.30, 127.46, 123.64, 122.09, 121.59, 111.61, 107.32, 65.56, 61.66, 48.03, 34.72, 14.72, 14.23. HRMS $[C_{22}H_{22}BrNO_5S+Na]^+$: 514.02943/516.02732 calculated, 514.02945/516.02724 found.

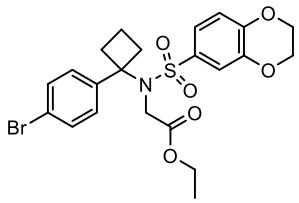
Ethyl *N*-(benzo[*d*][1,3]dioxol-5-ylsulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (79)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)benzo[*d*][1,3]dioxole-5-sulfonamide (**55**, 40 mg, 0.097 mmol, 1 eq), ethyl 2-bromoacetate (22.0 µL, 0.195 mmol, 2 eq) and BEMP (1 M in hexane, 195 µL, 0.195 mmol, 2 eq). Total

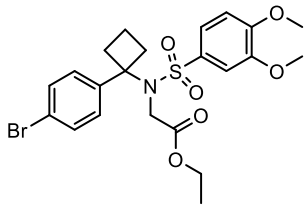
time: 12 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (49 mg, 0.099 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.31 (m, 4H), 7.19 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.93 (d, *J* = 1.9 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.06 (s, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.06 (s, 2H), 2.84 – 2.71 (m, 2H), 2.51 – 2.41 (m, 2H), 1.83 – 1.72 (m, 1H), 1.61 – 1.51 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.27, 150.97, 147.72, 141.64, 135.26, 131.29, 129.26, 122.87, 121.60, 107.86, 107.83, 102.33, 65.45, 61.68, 48.09, 34.64, 14.68, 14.24.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)sulfonyl)glycinate (80)



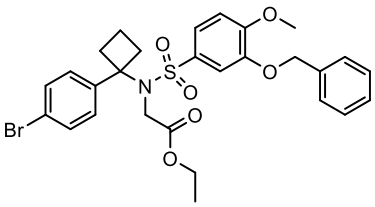
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (**56**, 56.6 mg, 0.133 mmol, 1 eq), ethyl 2-bromoacetate (30.0 µL, 0.267 mmol, 2 eq) and BEMP (1 M in hexane, 267 µL, 0.267 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20% EtOAc in dis. *n*-pentane) afforded the product as a white powder (41 mg, 0.080 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.30 (m, 4H), 7.12 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.96 (d, *J* = 2.2 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 1H), 4.32 – 4.25 (m, 4H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.06 (s, 2H), 2.83 – 2.72 (m, 2H), 2.50 – 2.42 (m, 2H), 1.81 – 1.72 (m, 1H), 1.60 – 1.50 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.28, 147.04, 143.03, 141.60, 134.27, 131.21, 129.22, 121.54, 120.77, 117.29, 117.10, 65.34, 64.66, 64.28, 61.62, 48.11, 34.67, 14.65, 14.22.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,4-dimethoxyphenyl)sulfonyl)glycinate (81)



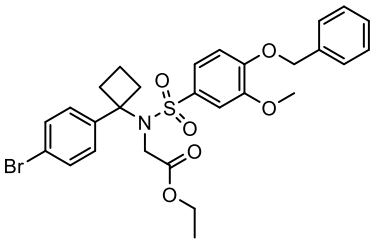
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-3,4-dimethoxybenzenesulfonamide (**57**, 56.7 mg, 0.133 mmol. 1 eq), ethyl 2-bromoacetate (29.5 µL, 0.266 mmol, 2 eq) and BEMP (1 M in hexane, 266 µL, 0.266 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20-30% EtOAc in dis. *n*-pentane) afforded the product (53.8 mg, 0.105 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (s, 4H), 7.27 – 7.20 (m, 2H), 6.78 (d, *J* = 8.4 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.07 (s, 2H), 3.93 (s, 3H), 3.87 (s, 3H), 2.82 – 2.72 (m, 2H), 2.48 – 2.40 (m, 2H), 1.81 – 1.71 (m, 1H), 1.59 – 1.51 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.37, 152.28, 148.74, 141.71, 133.69, 131.23, 129.32, 121.54, 121.30, 110.05, 110.02, 65.46, 61.55, 56.24, 56.22, 47.79, 34.44, 14.68, 14.20. LC-MS $[C_{22}H_{26}BrNO_6S+NH_4]^+$: 529.10/531.10 calculated, 528.75/530.67 found.

Ethyl *N*-((3-(benzyloxy)-4-methoxyphenyl)sulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (82)



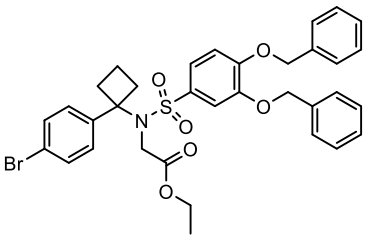
The title compound was synthesized according to the general procedure **I** using 3-(benzyloxy)-*N*-(1-(4-bromophenyl)cyclobutyl)-4-methoxybenzenesulfonamide (**58**, 85 mg, 0.17 mmol, 1 eq), ethyl 2-bromoacetate (36 µL, 0.33 mmol, 2 eq) and BEMP (1 M in hexane, 248 µL, 0.248 mmol , 1.5 eq). Total time: overnight at 80 °C. Silica gel column chromatography (15-20% EtOAc in dis. *n*-pentane) to afford the product as a light yellow oil (97 mg, 0.16 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.45 (m, 2H), 7.42 – 7.36 (m, 2H), 7.34 – 7.30 (m, 3H), 7.28 – 7.21 (m, 3H), 7.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 5.16 (s, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 3.94 (s, 2H), 3.93 (s, 3H), 2.72 – 2.60 (m, 2H), 2.40 – 2.31 (m, 2H), 1.71 – 1.62 (m, 1H), 1.55 – 1.44 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.29, 152.84, 147.55, 141.61, 136.47, 133.54, 131.17, 129.15, 128.74, 128.20, 127.60, 121.59, 121.41, 112.18, 110.47, 70.91, 65.27, 61.53, 56.26, 48.02, 34.38, 14.59, 14.21.

Ethyl *N*-((4-(benzyloxy)-3-methoxyphenyl)sulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (83)



The title compound was synthesized according to the general procedure **I** using 4-(benzyloxy)-*N*-(1-(4-bromophenyl)cyclobutyl)-3-methoxybenzenesulfonamide (**59**, 274 mg, 0.545 mmol, 1 eq), ethyl 2-bromoacetate (121 µL, 1.09 mmol, 2 eq) and BEMP (1 M in hexane, 1.1 mL, 1.1 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (15-20% EtOAc in dis. *n*-pentane) afforded the product as an oil (288 mg, 0.489 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.31 (m, 9H), 7.21 (d, *J* = 2.2 Hz, 1H), 7.17 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.80 (d, *J* = 8.5 Hz, 1H), 5.21 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.05 (s, 2H), 3.87 (s, 3H), 2.84 – 2.71 (m, 2H), 2.49 – 2.39 (m, 2H), 1.80 – 1.70 (m, 1H), 1.63 – 1.49 (m, 1H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.41, 151.42, 149.27, 141.75, 136.24, 134.04, 131.28, 129.35, 128.87, 128.34, 127.40, 121.59, 121.05, 112.30, 110.51, 71.03, 65.50, 61.60, 56.30, 47.87, 34.51, 14.72, 14.24. LC-MS $[C_{28}H_{30}BrNO_6S+NH_4]^+$: 605.13/607.13 calculated, 604.67/606.50 found.

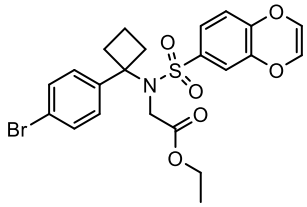
Ethyl *N*-((3,4-bis(benzyloxy)phenyl)sulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (84)



The title compound was synthesized according to the general procedure **I** using 3,4-bis(benzyloxy)-*N*-(1-(4-bromophenyl)cyclobutyl)benzenesulfonamide (**60**, 278 mg, 0.481 mmol, 1 eq), ethyl 2-bromoacetate (107 µL, 0.962 mmol, 2 eq) and BEMP (1 M in hexane, 962 µL, 0.962 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column

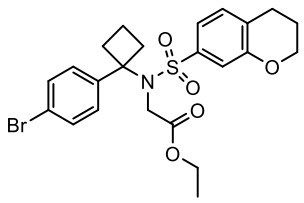
chromatography (15-20% EtOAc in *n*-pentane) afforded the product (199 mg, 0.299 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.42 (m, 4H), 7.41 – 7.36 (m, 4H), 7.36 – 7.26 (m, 5H), 7.25 – 7.21 (m, 2H), 7.11 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.79 (d, *J* = 8.6 Hz, 1H), 5.21 (s, 2H), 5.15 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.95 (s, 2H), 2.73 – 2.62 (m, 2H), 2.40 – 2.31 (m, 2H), 1.73 – 1.58 (m, 1H), 1.55 – 1.45 (m, 1H), 1.26 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.30, 152.11, 148.16, 141.65, 136.70, 136.42, 134.05, 131.22, 129.21, 128.78, 128.71, 128.24, 128.15, 127.54, 127.30, 121.53, 121.47, 113.14, 113.01, 71.10, 71.06, 65.33, 61.58, 48.04, 34.44, 14.63, 14.23. LC-MS $[C_{34}H_{34}BrNO_6S+NH_4]^+$: 681.16/683.16 calculated, 680.67/682.50 found.

Ethyl *N*-(benzo[*b*][1,4]dioxin-6-ylsulfonyl)-*N*-(1-(4-bromophenyl)cyclobutyl)glycinate (85)



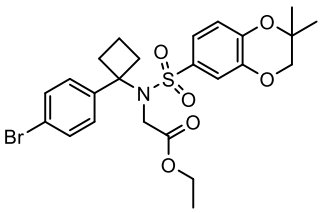
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)benzo[*b*][1,4]dioxine-6-sulfonamide (**61**, 36 mg, 0.086 mmol, 1 eq), ethyl 2-bromoacetate (19 µL, 0.17 mmol, 2 eq) and BEMP (1 M in hexane, 171 µL, 0.171 mmol, 2 eq). Total time: 6 h at 80 °C. Silica gel column chromatography (15% EtOAc in dis. *n*-pentane) afforded the product as a colorless oil (23 mg, 0.045 mmol, 53%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.40 (m, 2H), 7.37 – 7.32 (m, 2H), 7.08 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.70 (d, *J* = 2.3 Hz, 1H), 6.54 (d, *J* = 8.5 Hz, 1H), 5.89 – 5.86 (m, 2H), 4.19 (q, *J* = 7.1 Hz, 2H), 4.03 (s, 2H), 2.80 – 2.71 (m, 2H), 2.50 – 2.43 (m, 2H), 1.82 – 1.74 (m, 1H), 1.61 – 1.52 (m, 1H), 1.29 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.16, 146.26, 142.53, 141.49, 137.28, 131.38, 129.26, 127.26, 126.69, 124.00, 121.85, 116.12, 115.67, 65.50, 61.72, 48.02, 34.66, 14.70, 14.23.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-(chroman-7-ylsulfonyl)glycinate (86)



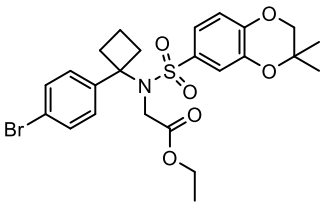
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)chromane-7-sulfonamide (**62**, 42 mg, 0.099 mmol, 1 eq), ethyl 2-bromoacetate (22 µL, 0.19 mmol, 2 eq) and BEMP (1 M in hexane, 189 µL, 0.189 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (10-20% EtOAc in dis. *n*-pentane) afforded the product as a colorless oil (34 mg, 0.067 mmol, 67%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.31 (m, 4H), 7.04 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.01 – 6.97 (m, 1H), 6.92 (d, *J* = 1.9 Hz, 1H), 4.22 – 4.19 (m, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.06 (s, 2H), 2.83 – 2.73 (m, 4H), 2.50 – 2.41 (m, 2H), 2.05 – 1.98 (m, 2H), 1.79 – 1.71 (m, 1H), 1.59 – 1.50 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.20, 154.78, 141.53, 140.53, 131.21, 130.04, 129.21, 127.05, 121.59, 118.41, 115.84, 66.79, 65.38, 61.59, 48.18, 34.62, 25.05, 21.89, 14.65, 14.19.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)sulfonyl)glycinate (87)



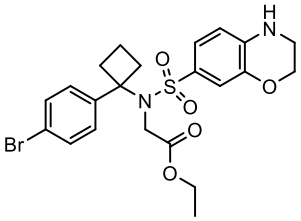
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (**63**, 37 mg, 0.081 mmol, 1 eq), ethyl 2-bromoacetate (17.9 µL, 0.162 mmol, 2 eq) and BEMP (162 µL, 0.162 mmol, 2 eq). Total time: 4 h at 80 °C. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product as a pale-yellow oil (31.5 mg, 58.5 µmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.30 (m, 4H), 7.14 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.00 (d, *J* = 2.3 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.04 (s, 2H), 3.90 (s, 2H), 2.86 – 2.73 (m, 2H), 2.53 – 2.41 (m, 2H), 1.81 – 1.73 (m, 1H), 1.59 – 1.49 (m, 1H), 1.36 (s, 6H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.27, 146.32, 141.78, 141.70, 133.56, 131.25, 129.22, 121.59, 121.36, 117.50, 116.62, 73.53, 71.90, 65.39, 61.59, 48.08, 34.68, 23.41, 14.68, 14.21. LC-MS $[C_{24}H_{28}BrNO_6S+Na]^+$: 560.07/562.07 calculated, 560.08/562.08 found.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,3-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)sulfonyl)glycinate (88)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-3,3-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (**64**, 41 mg, 0.090 mmol, 1 eq), ethyl 2-bromoacetate (19.8 µL, 0.180 mmol, 2 eq) and BEMP (180 µL, 0.180 mmol, 2 eq). Total time: 4 h at 80 °C. Silica gel column chromatography (10-20% EtOAc in *n*-pentane) afforded the product as a pale-yellow oil (42 mg, 0.078 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.29 (m, 4H), 7.11 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.87 (d, *J* = 2.3 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.04 (s, 2H), 3.92 (s, 2H), 2.86 – 2.74 (m, 2H), 2.52 – 2.43 (m, 2H), 1.82 – 1.72 (m, 1H), 1.61 – 1.48 (m, 1H), 1.34 (s, 6H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.25, 145.77, 142.18, 141.71, 134.42, 131.24, 129.19, 121.47, 120.38, 117.28, 116.76, 72.77, 72.12, 65.33, 61.59, 48.12, 34.71, 23.32, 14.67, 14.21. LC-MS $[C_{24}H_{28}BrNO_6S+Na]^+$: 560.07/562.07 calculated, 560.08/562.00 found.

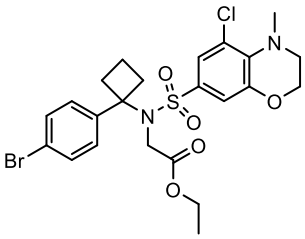
Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,4-dihydro-*2H*-benzo[*b*][1,4]oxazin-7-yl)sulfonyl)glycinate (89)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-4-(2,2,2-trifluoroacetyl)-3,4-dihydro-*2H*-benzo[*b*][1,4]oxazine-7-sulfonamide (**65**, 58.9 mg, 0.113 mmol, 1 eq), ethyl 2-bromoacetate (25.0 µL, 0.227 mmol, 2 eq) and BEMP (1 M in hexane, 227 µL, 0.227 mmol, 2 eq). Total time: 4 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (30 mg, 0.059 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40

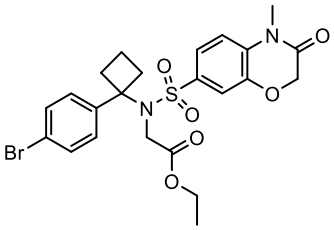
– 7.32 (m, 4H), 7.01 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.91 (d, *J* = 2.1 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 4.28 (bs, 1H), 4.23 – 4.20 (m, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 3.99 (s, 2H), 3.48 – 3.44 (m, 2H), 2.85 – 2.74 (m, 2H), 2.48 – 2.40 (m, 2H), 1.81 – 1.70 (m, 1H), 1.61 – 1.47 (m, 1H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.38, 142.48, 141.99, 137.85, 131.21, 129.73, 129.15, 121.48, 121.40, 116.14, 113.64, 65.28, 64.72, 61.49, 48.04, 40.53, 34.54, 14.66, 14.19. LC-MS $[C_{22}H_{25}BrN_2O_5S+H]^+$: 509.07/511.07 calculated, 509.08/511.00 found.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((5-chloro-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-7-yl)sulfonyl)glycinate (90)



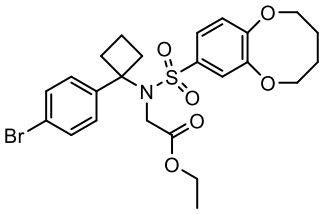
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-5-chloro-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonamide (**66**, 38 mg, 0.080 mmol, 1 eq), ethyl 2-bromoacetate (18 µL, 0.16 mmol, 2 eq) and BEMP (1 M in hexane, 161 µL, 0.161 mmol, 2 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (33 mg, 0.059 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.28 (m, 4H), 7.01 (d, *J* = 2.2 Hz, 1H), 6.84 (d, *J* = 2.2 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 4.18 – 4.14 (m, 2H), 4.11 (s, 2H), 3.17 – 3.12 (m, 2H), 2.93 (s, 3H), 2.84 – 2.74 (m, 2H), 2.52 – 2.44 (m, 2H), 1.83 – 1.74 (m, 1H), 1.60 – 1.51 (m, 1H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.22, 148.17, 141.27, 137.08, 135.06, 131.17, 129.24, 127.45, 121.66, 121.06, 115.07, 65.30, 61.75, 60.44, 49.69, 48.33, 43.28, 34.90, 14.65, 14.24. LC-MS $[C_{23}H_{26}BrClN_2O_5S+H]^+$: 557.05/559.05 calculated, 557.25/559.17 found.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((4-methyl-3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-7-yl)sulfonyl)glycinate (91)



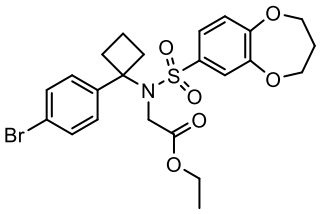
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-4-methyl-3-oxo-3,4-dihydro-*2H*-benzo[*b*][1,4]oxazine-7-sulfonamide (**67**, 19 mg, 0.042 mmol, 1 eq), ethyl 2-bromoacetate (14.0 µL, 0.125 mmol, 3 eq) and BEMP (125 µL, 0.125 mmol, 3 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (30-35% EtOAc in *n*-pentane) afforded the product (13 mg, 0.024 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.31 (m, 4H), 7.24 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.08 (d, *J* = 2.1 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 4.67 (s, 2H), 4.22 (q, *J* = 7.2 Hz , 2H), 4.11 (s, 2H), 3.38 (s, 3H), 2.82 – 2.71 (m, 2H), 2.53 – 2.44 (m, 2H), 1.83 – 1.73 (m, 1H), 1.59 – 1.51 (m, 1H), 1.30 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.27, 164.28, 144.47, 141.38, 132.77, 131.27, 129.89, 129.40, 122.20, 121.64, 115.84, 114.30, 67.54, 65.32, 61.79, 48.17, 34.79, 28.38, 14.68, 14.26.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((2,3,4,5-tetrahydrobenzo[*b*][1,4]dioxocin-8-yl)sulfonyl)glycinate (92)



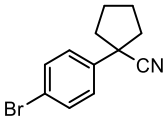
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-2,3,4,5-tetrahydrobenzo[*b*][1,4]dioxocine-8-sulfonamide (**68**, 38 mg, 0.084 mmol, 1 eq), ethyl 2-bromoacetate (19 µL, 0.17 mmol, 2 eq) and BEMP (1 M in hexane, 169 µL, 0.169 mmol, 2 eq). Total time: 3 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (33 mg, 0.061 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.32 (m, 4H), 7.21 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.14 (d, *J* = 2.4 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 4.48 (t, *J* = 5.5 Hz, 2H), 4.24 (t, *J* = 5.5 Hz, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.04 (s, 2H), 2.84 – 2.73 (m, 2H), 2.51 – 2.41 (m, 2H), 1.96 (p, *J* = 5.9 Hz, 2H), 1.86 (p, *J* = 5.9 Hz, 2H), 1.82 – 1.72 (m, 1H), 1.59 – 1.50 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.20, 154.26, 147.32, 141.67, 135.17, 131.28, 129.21, 123.32, 123.25, 121.58, 121.43, 74.04, 71.66, 65.40, 61.60, 48.06, 34.63, 27.80, 25.38, 14.67, 14.21. LC-MS $[C_{24}H_{28}BrNO_6S+Na]^+$: 560.07/562.07 calculated, 560.17/562.08 found.

Ethyl *N*-(1-(4-bromophenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (93)



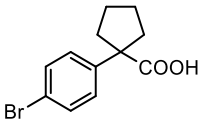
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclobutyl)-3,4-dihydro-*2H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**69**, 73.5 mg, 0.168 mmol, 1 eq), ethyl 2-bromoacetate (37.0 µL, 0.335 mmol, 2 eq) and BEMP (1 M in hexane, 335 µL, 0.335 mmol, 2 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (60.0 mg, 0.114 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 4H), 7.17 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.06 (d, *J* = 2.4 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 4.30 (t, *J* = 5.8 Hz, 2H), 4.24 (t, *J* = 5.9 Hz, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 4.05 (s, 2H), 2.83 – 2.72 (m, 2H), 2.50 – 2.43 (m, 2H), 2.23 (p, *J* = 5.8 Hz, 2H), 1.81 – 1.72 (m, 1H), 1.59 – 1.50 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.22, 154.44, 150.33, 141.61, 135.85, 131.28, 129.27, 122.58, 121.59, 121.40, 121.14, 70.54, 70.49, 65.39, 61.65, 48.12, 34.69, 30.97, 14.68, 14.22.

1-(4-Bromophenyl)cyclopentane-1-carbonitrile (94)



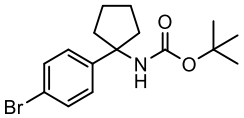
The title compound was synthesized according to the general procedure **A** using 2-(4-bromophenyl)acetonitrile (2.00 g, 10.2 mmol, 1 eq), 1,4-dibromobutane (1.2 mL 10.2 mmol, 1 eq), TBABr (33.0 mg, 0.102 mmol, 0.01 eq) and KOH (4.58 g, 81.6 mmol, 8 eq). Total time: 1 h at reflux. Silica gel column chromatography (5% Et_2O in *n*-pentane) afforded the product (2.12 g, 8.48 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.37 (m, 2H), 7.35 – 7.21 (m, 2H), 2.52 – 2.35 (m, 2H), 2.13 – 1.80 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.96, 132.00, 127.84, 123.93, 121.82, 47.42, 40.49, 24.25.

1-(4-Bromophenyl)cyclopentane-1-carboxylic acid (95)



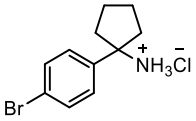
The title compound was synthesized according to the general procedure **B** using 1-(4-bromophenyl)cyclopentane-1-carbonitrile (**94**, 2.12 g, 8.48 mmol, 1 eq) and KOH (2.85 g, 50.9 mmol, 6 eq) at reflux for 3 h and then 6 M aq. HCl in 1,4-dioxane (6 mL) at reflux for 2 days. The reaction was cooled down to rt and 1 M aq. NaOH was added to pH >12. The aqueous layer was washed 2× with Et_2O. Afterwards, 3 M aq. HCl was added to pH < 2 and the aqueous layer was extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the product (0.99 g, 3.7 mmol, 43%). ^{1}H NMR (400 MHz, MeOD) δ 7.40 (d, *J* = 7.7 Hz, 2H), 7.33 – 7.23 (m, 2H), 2.71 – 2.52 (m, 2H), 1.92 – 1.78 (m, 2H), 1.78 – 1.67 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 178.82, 143.60, 131.87, 129.49, 121.18, 59.34, 36.73, 24.13.

***tert*-Butyl (1-(4-bromophenyl)cyclopentyl)carbamate (96)**



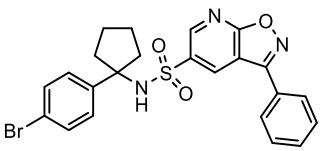
The title compound was synthesized according to the general procedure **C** using 1-(4-bromophenyl)cyclopentane-1-carboxylic acid (**95**, 989 mg, 3.68 mmol, 1 eq), diphenylphosphoryl azide (531 µL, 3.68 mmol, 1 eq) and Et_3N (567 µL, 4.04 mmol, 1.1 eq) in anhydrous *t*-BuOH (48 mL, 0.08 M). Total time: 1 h at 30 °C and overnight at reflux. Silica gel column chromatography (5% EtOAc in *n*-pentane) afforded the product (675 mg, 1.98 mmol, 54%). 1H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.36 (m, 2H), 7.31 – 7.23 (m, 2H), 4.96 (s, 1H), 2.33 – 1.91 (m, 4H), 1.90 – 1.71 (m, 4H), 1.37 (s, 9H).

1-(4-Bromophenyl)cyclopentan-1-aminium chloride (97)



The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(4-bromophenyl)cyclopentyl)carbamate (**96**, 612 mg, 1.98 mmol) in 3 M aq. HCl in MeOH (25 mL, 0.08 M). Total time: 36 h at rt. The mixture was concentrated, washed with Et_2O and filtered to afford the product (399 mg, 1.44 mmol, 80%). ^{1}H NMR (400 MHz, MeOD) δ 7.69 – 7.57 (m, 2H), 7.52 – 7.43 (m, 2H), 2.37 – 2.21 (m, 4H), 2.08 – 1.84 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 140.84, 133.23, 129.41, 123.82, 67.13, 38.59, 23.83. LC-MS $[C_{11}H_{14}BrN–NH_2]^+$: 223.01/225.01 calculated, 222.93/224.93 found.

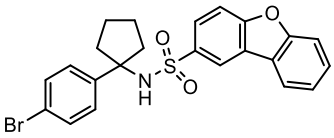
***N*-(1-(4-Bromophenyl)cyclopentyl)-3-phenylisoxazolo[5,4-*b*]pyridine-5-sulfonamide (98a)**



The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclopentan-1-aminium chloride (**97**, 80.0 mg, 0.289 mmol, 1 eq), 3-phenylisocazolo[5,4-*b*]pyridine-5-sulfonyl chloride (89.6 mg, 0.303 mmol, 1.05 eq) and DIPEA (202 µL, 1.16 mmol, 4 eq). Total time: 3 days at rt. Silica gel column chromatography (10-40% EtOAc in *n*-pentane) afforded the product (90.6 mg, 0.182 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (d, *J* = 2.1 Hz, 1H), 8.09 (d, *J* = 2.1 Hz, 1H), 7.94 – 7.87 (m, 2H), 7.64 –

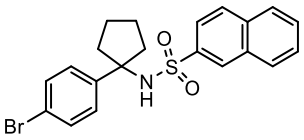
7.56 (m, 3H), 7.00 – 6.94 (m, 2H), 6.92 – 6.85 (m, 2H), 6.57 (s, 1H), 2.54 – 2.45 (m, 2H), 2.01 – 1.87 (m, 4H), 1.82 – 1.70 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.33, 157.53, 149.50, 139.72, 135.50, 131.76, 131.41, 130.86, 129.47, 129.24, 127.81, 127.52, 121.58, 111.22, 68.87, 38.91, 22.06. LC-MS $[C_{23}H_{20}BrN_3O_3S+H]^+$: 498.05/500.05 calculated, 497.92/499.83 found.

_N_-(1-(4-Bromophenyl)cyclopentyl)dibenzo[_b,d_]furan-2-sulfonamide (98b)



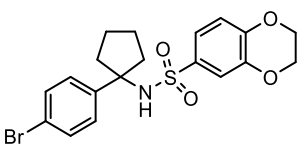
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclopentan-1-aminium chloride (**97**, 40.0 mg, 0.145 mmol, 1 eq), dibenzo[*b*,*d*]furan-2-sulfonyl chloride (40.5 mg, 0.152 mmol, 1.05 eq) and DIPEA (101 µL, 0.578 mmol, 4 eq). Total time: 4 days at rt. Silica gel column chromatography (5-60% EtOAc in *n*-pentane) afforded the product (45 mg, 0.095 mmol, 66%). ^{1}H NMR (400 MHz, DMSO) δ 8.11 (d, *J* = 7.8, 1.3 Hz, 1H), 8.05 (s, 1H), 7.89 (t, *J* = 1.3 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.64 – 7.61 (m, 2H), 7.60 – 7.54 (m, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.02 – 6.97 (m, 2H), 6.94 – 6.89 (m, 2H), 2.47 – 2.36 (m, 2H), 1.81 – 1.51 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 156.42, 156.33, 141.59, 137.18, 129.90, 128.79, 128.36, 125.62, 123.66, 123.21, 122.73, 121.64, 120.03, 119.72, 111.80, 111.58, 67.64, 38.08, 21.81.

_N_-(1-(4-Bromophenyl)cyclopentyl)naphthalene-2-sulfonamide (98c)



The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclopentan-1-aminium chloride (**97**, 80.0 mg, 0.289 mmol, 1 eq), naphtalene-2-sulfonyl chloride (68.7 mg, 0.303 mmol, 1.05 eq) and DIPEA (202 µL, 1.16 mmol, 4 eq). Total time: 2 days at rt. Silica gel column chromatography (10-40% EtOAc in *n*-pentane) afforded the product (59.4 mg, 0.138 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.82 (m, 1H), 7.79 (d, *J* = 1.9 Hz, 1H), 7.76 – 7.70 (m, 2H), 7.65 – 7.56 (m, 2H), 7.53 (dd, *J* = 8.7, 1.9 Hz, 1H), 6.99 – 6.91 (m, 2H), 6.89 – 6.82 (m, 2H), 5.76 (s, 1H), 2.50 – 2.38 (m, 2H), 2.01 – 1.63 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.49, 138.30, 134.24, 131.85, 131.34, 130.63, 129.26, 128.95, 128.65, 128.17, 127.79, 127.58, 121.99, 121.30, 68.75, 39.15, 22.17.

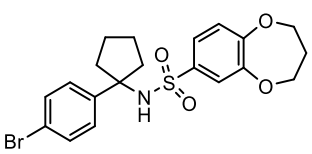
_N_-(1-(4-Bromophenyl)cyclopentyl)-2,3-dihydrobenzo[_b_][1,4]dioxine-6-sulfonamide (98d)



The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclopentan-1-aminium chloride (**97**, 61.7 mg, 0.223 mmol, 1 eq), 2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride (**108**, 54.9 mg, 0.234 mmol, 1.05 eq) and DIPEA (156 µL, 0.892 mmol, 4 eq). Total time: 3 days at rt. Silica gel column chromatography (100% EtOAc) afforded the product (29.5 mg, 0.290 mmol, 30%). ^{1}H NMR (400 MHz, DMSO) δ 7.88 (s, 1H), 7.21 – 7.16 (m, 2H), 7.06 – 7.01 (m, 2H), 6.91 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 4.33 – 4.19 (m, 4H), 2.41 – 2.29 (m, 2H), 1.79 – 1.66 (m, 4H), 1.65 – 1.53 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ

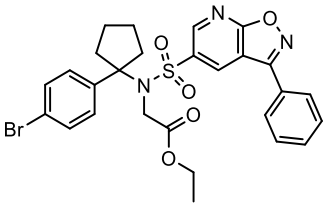
145.96, 142.33, 142.12, 134.79, 130.11, 128.85, 119.65, 119,64, 116.82, 115.42, 67.46, 64.30, 64.05, 38.11, 21.86.

_N_-(1-(4-Bromophenyl)cyclopentyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (98e)



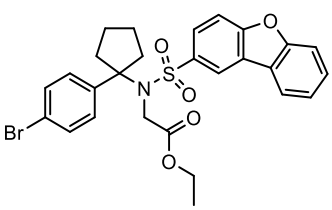
The title compound was synthesized according to the general procedure **H** using 1-(4-bromophenyl)cyclopentan-1-aminium chloride (**97**, 32.0 mg, 0.116 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 43.2 mg, 0.174 mmol, 1.5 eq) and DIPEA (61.0 µL, 0.347 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (15-25% EtOAc in *n*-pentane) afforded the product (40 mg, 0.088 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 – 7.13 (m, 2H), 7.05 – 6.99 (m, 3H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 5.44 (s, 1H), 4.30 (t, *J* = 5.8 Hz, 2H), 4.22 (t, *J* = 5.8 Hz, 2H), 2.46 – 2.32 (m, 2H), 2.22 (p, *J* = 5.8 Hz, 2H), 1.98 – 1.86 (m, 4H), 1.78 – 1.65 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.18, 150.13, 141.08, 135.75, 130.77, 129.09, 122.15, 121.34, 121.04, 120.74, 70.57, 70.46, 68.49, 39.16, 30.99, 22.16.

Ethyl _N_-(1-(4-bromophenyl)cyclopentyl)-_N_-((3-phenylisoxazolo[5,4-_b_]pyridin-5-yl)sulfonyl)glycinate (99a)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclopentyl)-3-phenylisoxazolo[5,4-*b*]pyridine-5-sulfonamide (**98a**, 90.6 mg, 0.182 mmol, 1 eq), ethyl 2-bromoacetate (40.2 µL, 0.364 mmol, 2 eq) and BEMP (1 M in hexane, 364 µL, 0.364 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (5-10% EtOAc in dis. *n*-pentane) afforded the product (26.5 mg, 45.3 µmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (d, *J* = 2.2 Hz, 1H), 8.68 (d, *J* = 2.2 Hz, 1H), 8.03 – 7.97 (m, 2H), 7.65 – 7.57 (m, 3H), 7.31 – 7.27 (m, 2H), 7.21 – 7.16 (m, 2H), 4.34 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 2.44 – 2.36 (m, 2H), 2.22 – 2.10 (m, 2H), 1.73 – 1.60 (m, 2H), 1.38 – 1.34 (m, 2H), 1.32 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.96, 170.77, 157.90, 150.05, 140.27, 136.62, 133.20, 131.49, 131.16, 129.60, 129.54, 127.95, 127.66, 122.20, 111.66, 73.89, 61.99, 48.71, 37.77, 21.54, 14.28. HRMS $[C_{27}H_{26}BrN_3O_5S+H]^+$: 584.08493/586.08286 calculated, 584.08474/586.08264 found.

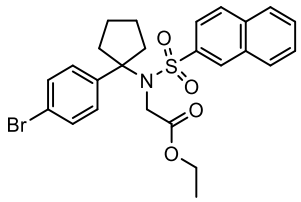
Ethyl _N_-(1-(4-bromophenyl)cyclopentyl)-_N_-(dibenzo[_b_,_d_]furan-2-ylsulfonyl)glycinate (99b)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclopentyl)dibenzo[*b*,*d*]furan-2-sulfonamide (**98b**, 41 mg, 0.087 mmol, 1 eq), ethyl 2-bromoacetate (19.3 µL, 0.174 mmol, 2 eq) and BEMP (1 M in hexane, 174 µL, 0.174 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (5-10% EtOAc in dis. *n*-

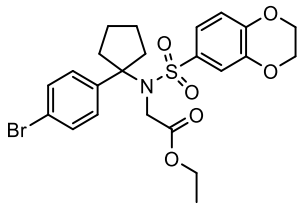
pentane) afforded the product (26 mg, 0.046 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 1.7 Hz, 1H), 7.98 – 7.94 (m, 2H), 7.62 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.58 (dd, *J* = 8.7, 0.5 Hz, 1H), 7.56 – 7.51 (m, 1H), 7.43 (td, *J* = 7.5, 1.1 Hz, 1H), 7.27 (s, 4H), 4.21 (s, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.38 – 2.22 (m, 4H), 1.70 – 1.60 (m, 2H), 1.40 – 1.30 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.82, 157.94, 157.14, 141.58, 137.00, 131.11, 129.33, 128.45, 126.82, 124.50, 123.74, 123.40, 121.63, 121.47, 121.30, 112.10, 111.85, 73.69, 61.60, 48.94, 37.81, 21.66, 14.28. HRMS $[C_{27}H_{26}BrNO_5S+Na]^+$: 578.06073/580.05865 calculated, 578.06057/580.05840 found.

Ethyl *N*-(1-(4-bromophenyl)cyclopentyl)-*N*-(naphthalen-2-ylsulfonyl)glycinate (99c)



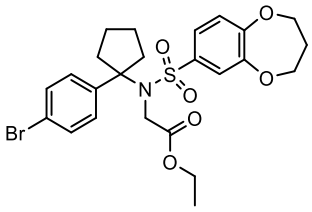
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclopentyl)naphthalene-2-sulfonamide (**98c**, 54.9 mg, 0.128 mmol, 1 eq), ethyl 2-bromoacetate (28.4 µL, 0.256 mmol, 2 eq) and BEMP (1 M in hexane, 256 µL, 0.256 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (5-10% EtOAc in dis. *n*-pentane) afforded the product (39 mg, 0.075 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 1.3 Hz, 1H), 7.91 – 7.82 (m, 4H), 7.67 – 7.56 (m, 2H), 7.30 – 7.19 (m, 4H), 4.22 (s, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 2.37 – 2.21 (m, 4H), 1.69 – 1.59 (m, 2H), 1.38 – 1.29 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.73, 141.41, 139.18, 134.65, 132.05, 131.12, 129.60, 129.37, 129.32, 128.90, 128.79, 127.87, 127.45, 123.04, 121.68, 73.65, 61.57, 48.95, 37.71, 21.61, 14.24. HRMS $[C_{25}H_{26}BrNO_4S+Na]^+$: 538.06581/540.06371 calculated, 538.06572/540.06351 found.

Ethyl *N*-(1-(4-bromophenyl)cyclopentyl)-*N*-((2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)sulfonyl)glycinate (99d)



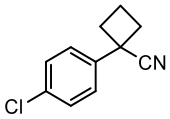
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclopentyl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (**98d**, 30 mg, 0.067 mmol, 1 eq), ethyl 2-bromoacetate (15.0 µL, 0.135 mmol, 2 eq) and BEMP (1 M in hexane, 135 µL, 0.135 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (10-20% EtOAc in dis. *n*-pentane) afforded the product (18.5 mg, 35.3 µmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.29 (m, 3H), 7.29 – 7.24 (m, 2H), 7.19 (d, *J* = 2.3 Hz, 1H), 6.86 (d, *J* = 8.6 Hz, 1H), 4.34 – 4.26 (m, 4H), 4.18 (q, *J* = 7.2 Hz, 2H), 4.12 (s, 2H), 2.36 – 2.18 (m, 4H), 1.73 – 1.63 (m, 2H), 1.42 – 1.30 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.77, 147.10, 143.08, 141.67, 135.05, 131.05, 129.26, 121.51, 121.21, 117.44, 117.36, 73.45, 64.69, 64.29, 61.49, 48.97, 37.64, 21.66, 14.24. HRMS $[C_{23}H_{26}BrNO_6S+Na]^+$: 546.05564/548.05355 calculated, 546.05521/548.05318 found.

Ethyl *N*-(1-(4-bromophenyl)cyclopentyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (99e)



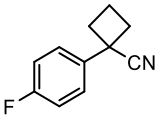
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-bromophenyl)cyclopentyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**98e**, 60.0 mg, 0.133 mmol, 1 eq), ethyl 2-bromoacetate (44 µL, 0.40 mmol, 3 eq) and BEMP (1 M in hexane, 398 µL, 0.398 mmol, 3 eq). Total time: overnight at 80 °C. Silica gel column chromatography (15-20% EtOAc in dis. *n*-pentane) afforded the product (48 mg, 0.089 mmol, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 6H), 6.93 (d, *J* = 8.5 Hz, 1H), 4.31 (t, *J* = 5.7 Hz, 2H), 4.26 (t, *J* = 5.8 Hz, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.12 (s, 2H), 2.37 – 2.15 (m, 6H), 1.74 – 1.59 (m, 2H), 1.40 – 1.31 (m, 2H), 1.27 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.65, 154.47, 150.34, 141.65, 136.53, 131.07, 129.23, 122.95, 121.50, 121.47, 121.45, 73.48, 70.55, 70.49, 61.48, 48.97, 37.63, 30.98, 21.64, 14.20.

1-(4-Chlorophenyl)cyclobutane-1-carbonitrile (100a)



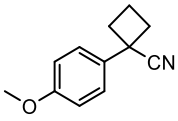
The title compound was synthesized according to the general procedure **A** using 2-(4-chlorophenyl)acetonitrile (2.00 g, 13.2 mmol, 1 eq), 1,3-dibromopropane (1.35 mL, 13.2 mmol, 1 eq), TBABr (420 mg, 1.32 mmol, 0.1 eq) and KOH (5.92 g, 106 mmol, 8 eq). Total time: 2 h at reflux. Silica gel column chromatography (4-5% Et_2O in *n*-pentane) afforded the product (790 mg, 4.13 mmol, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.21 (m, 4H), 2.86 – 2.77 (m, 2H), 2.63 – 2.53 (m, 2H), 2.49 – 2.38 (m, 1H), 2.12 – 2.01 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.34, 133.83, 129.12, 127.10, 124.00, 39.74, 34.69, 17.06.

1-(4-Fluorophenyl)cyclobutane-1-carbonitrile (100b)



The title compound was synthesized according to the general procedure **A** using 2-(4-fluorophenyl)acetonitrile (2.00 g, 14.8 mmol, 1 eq), 1,3-dibromopropane (1.51 mL, 14.8 mmol, 1 eq) and TBABr (480 mg, 1.48 mmol, 0.1 eq) and KOH (6.64 g, 118 mmol, 8 eq). Total time: 2 h at reflux. Silica gel column chromatography (4-5% Et_2O in *n*-pentane) afforded the product (1.40 g, 7.99 mmol, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.34 (m, 2H), 7.12 – 7.03 (m, 2H), 2.86 – 2.77 (m, 2H), 2.64 – 2.54 (m, 2H), 2.49 – 2.35 (m, 1H), 2.12 – 2.01 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.18 (d, $J_{C\text{-}F}$ = 247.3 Hz), 135.70 (d, $J_{C\text{-}F}$ = 3.3 Hz), 127.44 (d, $J_{C\text{-}F}$ = 8.2 Hz), 124.27, 115.86 (d, $J_{C\text{-}F}$ = 21.7 Hz), 39.67, 34.78, 17.03.

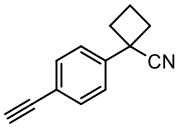
1-(4-Methoxyphenyl)cyclobutane-1-carbonitrile (100c)



The title compound was synthesized according to the general procedure **A** using 2-(4-methoxyphenyl)acetonitrile (2.00 g, 13.6 mmol, 1 eq), 1,3-dibromopropane (1.39 mL, 13.6 mmol, 1 eq), TBABr (440 mg, 1.36 mmol, 0.1 eq) and KOH (6.10 g, 109 mmol, 8 eq) and. Total time: 2 h at reflux. Silica gel column

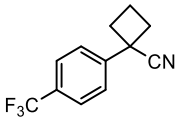
chromatography (5-10% Et_2O in *n*-pentane) afforded the product (1.27 g, 6.76 mmol, 50%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.30 (m, 2H), 6.94 – 6.89 (m, 2H), 3.81 (s, 3H), 2.84 – 2.76 (m, 2H), 2.63 – 2.54 (m, 2H), 2.44 – 2.36 (m, 1H), 2.10 – 2.00 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.18, 131.95, 126.87, 124.75, 114.31, 55.44, 39.71, 34.89, 17.10.

1-(4-Ethynylphenyl)cyclobutane-1-carbonitrile (100d)



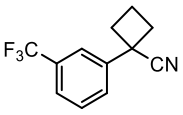
The title compound was synthesized according to the general procedure **A** using 2-(4-ethynylphenyl)acetonitrile (2.00 g, 14.2 mmol, 1 eq), 1,3-dibromopropane (1.45 mL, 14.17 mmol, 1 eq), TBABr (0.46 g, 1.42 mmol, 0.1 eq) and KOH (6.36 g, 113 mmol, 8 eq). Total time: 2 h at reflux. Silica gel column chromatography (5% Et_2O in *n*-pentane) afforded the product (1.16 g, 6.40 mmol, 45%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.49 (m, 2H), 7.41 – 7.35 (m, 2H), 3.11 (s, 1H), 2.88 – 2.78 (m, 2H), 2.66 – 2.55 (m, 2H), 2.50 – 2.37 (m, 1H), 2.14 – 2.02 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.41, 132.74, 125.73, 124.03, 121.89, 82.95, 78.11, 40.15, 34.70, 17.12.

1-(4-(Trifluoromethyl)phenyl)cyclobutane-1-carbonitrile (100e)



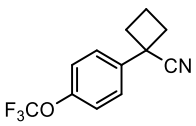
The title compound was synthesized according to the general procedure **A** using 2-(4-(trifluoromethyl)phenyl)acetonitrile (2.00 g, 10.8 mmol, 1 eq), 1,3-dibromopropane (1.10 mL, 10.8 mmol, 1 eq), TBABr (350 mg, 1.08 mmol, 0.1 eq) and KOH (4.85 g, 86.4 mmol, 8 eq). Total time: 2 h at reflux. Silica gel column chromatography (4-5% Et_2O in *n*-pentane) afforded the product (1.35 g, 5.99 mmol, 55%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 8.2 Hz, 2H), 2.92 – 2.79 (m, 2H), 2.69 – 2.57 (m, 2H), 2.54 – 2.40 (m, 1H), 2.18 – 2.06 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.75 (q, $J_{C\text{-}F}$ = 1.1 Hz), 130.30 (q, $J_{C\text{-}F}$ = 33.0 Hz), 126.23, 126.08 (q, $J_{C\text{-}F}$ = 3.7 Hz), 123.94 (q, $J_{C\text{-}F}$ = 273.1 Hz), 123.73, 40.11, 34.69, 17.17.

1-(3-(Trifluoromethyl)phenyl)cyclobutane-1-carbonitrile (100f)



The title compound was synthesized according to the general procedure **A** using 2-(3-(trifluoromethyl)phenyl)acetonitrile (2.00 g, 10.8 mmol, 1 eq), 1,3-dibromopropane (1.10 mL, 10.8 mmol, 1 eq), TBABr (350 mg, 1.08 mmol, 0.1 eq) and KOH (4.85 g, 86.4 mmol, 8 eq). Total time: 2 h at reflux. Silica gel column chromatography (2-5% EtOAc in *n*-pentane) afforded the product (1.52 g, 6.74 mmol, 62%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.49 (m, 4H), 2.95 – 2.81 (m, 2H), 2.72 – 2.58 (m, 2H), 2.57 – 2.39 (m, 1H), 2.22 – 2.04 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.93, 131.55 (q, $J_{C\text{-}F}$ = 32.6 Hz), 129.72, 129.30 (q, $J_{C\text{-}F}$ = 1.5 Hz), 124.96 (q, $J_{C\text{-}F}$ = 3.8 Hz), 123.93 (q, $J_{C\text{-}F}$ = 273.1 Hz), 123.80, 122.55 (q, $J_{C\text{-}F}$ = 3.9 Hz), 40.12, 34.73, 17.20.

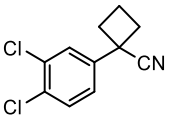
1-(4-(Trifluoromethoxy)phenyl)cyclobutane-1-carbonitrile (100g)



The title compound was synthesized according to the general procedure **A** using 2-(4-(trifluoromethoxy)phenyl)acetonitrile (2.00 g, 9.95 mmol, 1 eq), 1,3-dibromopropane(1.01 mL, 9.95 mmol , 1 eq), TBABr (350 mg, 1.08

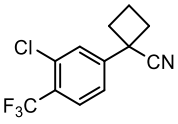
mmol, 0.1 eq) and KOH (4.85 g, 86.4 mmol, 8 eq). Total time: 3.5 h at reflux. Silica gel column chromatography (0-1% EtOAc in *n*-pentane) afforded the product as a colourless liquid (620 mg, 2.57 mmol, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ7.50 – 7.40 (m, 2H), 7.29 – 7.19 (m, 2H), 2.90 – 2.79 (m, 2H), 2.68 – 2.55 (m, 2H), 2.53 – 2.36 (m, 1H), 2.15 – 2.02 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.98 (q, J_{C-F} = 2.0 Hz), 138.78, 127.53, 124.24, 121.71, 120.71 (q, J_{C-F} = 258.6 Hz), 40.01, 35.00, 17.31.

1-(3,4-Dichlorophenyl)cyclobutane-1-carbonitrile (100h)



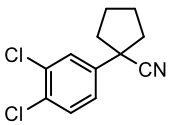
The title compound was synthesized according to the general procedure **A** using 2-(3,4-dichlorophenyl)acetonitrile (2.00 g, 10.7 mmol, 1 eq), 1,3-dibromopropane (1.09 mL, 10.7 mmol, 1 eq), TBABr (350 mg, 1.07 mmol, 0.1 eq) and KOH (4.83 g, 86.1 mmol, 8 eq). Total time: 3 h at reflux. Silica gel column chromatography (0-2% EtOAc in *n*-pentane) afforded the product as a colourless liquid (1.11 g, 4.90 mmol, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 2.3 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.26 (dd, J = 8.4, 2.3 Hz, 1H), 2.89 – 2.77 (m, 2H), 2.66 – 2.52 (m, 2H), 2.52 – 2.37 (m, 1H), 2.16 – 2.02 (m, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 139.99, 133.28, 132.31, 131.03, 127.95, 125.22, 123.53, 39.60, 34.71, 17.10.

1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclobutane-1-carbonitrile (100i)



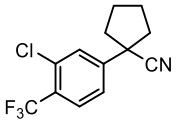
The title compound was synthesized according to the general procedure **A** using 2-(3-chloro-4-(trifluoromethyl)phenyl)acetonitrile (2.00 g, 9.11 mmol, 1 eq), 1,3-dibromopropane (930 µL, 9.11 mmol, 1 eq), TBABr (0.29 g, 0.91 mmol, 0.1 eq) and KOH (4.0 g, 73 mmol, 8 eq). Total time: 1 h at reflux. Silica gel column chromatography (2-4% EtOAc in *n*-pentane) afforded the product (1.02 g, 3.93 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.73 (d, *J* = 8.2 Hz, 1H), 7.57 (d, *J* = 1.9 Hz, 1H), 7.43 (dd, *J* = 8.3, 1.8 Hz, 1H), 2.94 – 2.80 (m, 2H), 2.69 – 2.57 (m, 2H), 2.56 – 2.42 (m, 1H), 2.20 – 2.07 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 145.25 (q, J_{C-F} = 1.0 Hz), 133.35 (q, J_{C-F} = 2.0 Hz), 128.97, 128.40 (q, J_{C-F} = 5.1 Hz), 128.21 (q, J_{C-F} = 32.3 Hz), 124.21, 123.15, 122.69 (q, J_{C-F} = 273.7 Hz), 39.82, 34.67, 17.19.

1-(3,4-Dichlorophenyl)cyclopentane-1-carbonitrile (100j)



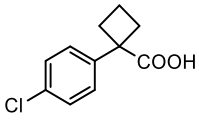
The title compound was synthesized according to the general procedure **A** using 2-(3,4-dichlorophenyl)acetonitrile (2.00 g, 10.7 mmol, 1 eq), 1,4-dibromobutane (1.28 mL, 10.7 mmol, 1 eq), TBABr (350 mg, 1.08 mmol, 0.1 eq) and KOH (4.83 g, 86.1 mmol, 8 eq). Total time: 2 h at reflux. Silica gel column chromatography (4-5% Et_2O in *n*-pentane) afforded the product (2.22 g, 9.25 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 2.3 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.30 (dd, *J* = 8.4, 2.3 Hz, 1H), 2.53 – 2.42 (m, 2H), 2.11 – 1.90 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.15, 133.15, 132.18, 130.89, 128.28, 125.65, 123.54, 47.27, 40.58, 24.29.

1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclopentane-1-carbonitrile (100k)



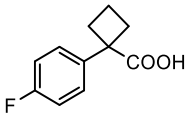
The title compound was synthesized according to the general procedure **A** using 2-(3-chloro-4-(trifluoromethyl)phenyl)acetonitrile (1.00 g, 4.55 mmol, 1 eq), 1,4-dibromobutane (0.60 mL, 5.0 mmol, 1.1 eq), TBABr (0.15 g, 0.46 mmol, 0.1 eq) and KOH (2.0 g, 36 mmol, 8 eq). Total time: 2 h at reflux. Silica gel column chromatography (4-5% Et_2O in *n*-pentane) afforded the product (1.08 g, 3.95 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.3 Hz, 1H), 7.60 (d, *J* = 1.9 Hz, 1H), 7.48 (ddd, *J* = 8.3, 2.1, 0.9 Hz, 1H), 2.57 – 2.45 (m, 2H), 2.14 – 1.94 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.59, 133.16 (q, J_{C-F} = 1.9 Hz), 129.27, 128.24 (q, J_{C-F} = 5.3 Hz), 128.10 (q, J_{C-F} = 32.3 Hz), 124.62, 123.19, 122.70 (q, J_{C-F} = 273.7 Hz), 47.62, 40.74, 24.46.

1-(4-Chlorophenyl)cyclobutane-1-carboxylic acid (101a)



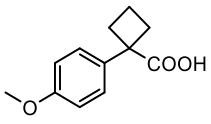
The title compound was synthesized according to the general procedure **B** using 1-(4-chlorophenyl)cyclobutane-1-carbonitrile (**100a**, 790 mg, 4.13 mmol, 1 eq) and KOH (1.39 g, 24.8 mmol, 6 eq). Total time: 16 h at reflux. Silica gel column chromatography (20% EtOAc in *n*-pentane with 0.1% TFA) afforded the product as a solid (830 mg, 3.93 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.29 (bs, 1H), 7.33 – 7.20 (m, 4H), 2.88 – 2.79 (m, 2H), 2.53-2.43 (m, 2H), 2.13 – 2.04 (m, 1H), 1.92-1.81 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.35, 141.69, 132.93, 128.59, 128.04, 51.86, 32.37, 16.69.

1-(4-Fluorophenyl)cyclobutane-1-carboxylic acid (101b)



The title compound was synthesized according to the general procedure **B** using 1-(4-fluorophenyl)cyclobutane-1-carbonitrile (**100b**, 1.40 g, 7.99 mmol, 1 eq) and KOH (2.69 g, 47.9 mmol, 6 eq) at reflux for overnight, and 6 M aq. HCl (3.5 mL) at reflux for 6 days. The pH of the reaction mixture was adjusted by 1 M aq. NaOH to pH > 12 and the aqueous layer was extracted 2× with Et_2O. The pH of the aqueous layer was adjusted by 3 M aq. HCl to pH < 2 and extracted 3× with EtOAc. The combined EtOAc layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the product (830 mg, 4.25 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.92 (bs, 1H), 7.30 – 7.23 (m, 2H), 7.05 – 6.97 (m, 2H), 2.89 – 2.79 (m, 2H), 2.55 – 2.44 (m, 2H), 2.14 – 2.01 (m, 1H), 1.93 – 1.81 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.53, 161.86 (d, J_{C-F} = 245.6 Hz), 138.94 (d, J_{C-F} = 3.1 Hz), 128.25 (d, J_{C-F} = 8.1 Hz), 115.29 (d, J_{C-F} = 21.5 Hz), 51.76, 32.45, 16.65.

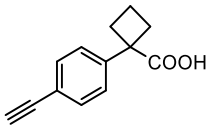
1-(4-Methoxyphenyl)cyclobutane-1-carboxylic acid (101c)



The title compound was synthesized according to the general procedure **B** using 1-(4-methoxyphenyl)cyclobutane-1-carbonitrile (**100c**, 1.26 g, 6.76 mmol, 1 eq) and KOH (2.28 g, 40.5 mmol, 6 eq). Total time: 16 h at reflux. Silica gel column chromatography (20% EtOAc in *n*-pentane with 0.1% TFA) afforded the

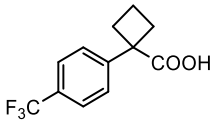
product (1.50 g, 7.27 mmol, quant.). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 2H), 6.89 – 6.84 (m, 2H), 3.79 (s, 3H), 2.85 – 2.77 (m, 2H), 2.54 – 2.43 (m, 2H), 2.10 – 1.99 (m, 1H), 1.91 – 1.80 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 182.80, 158.58, 135.27, 127.68, 113.86, 55.41, 51.62, 32.35, 16.62.

1-(4-Ethynylphenyl)cyclobutane-1-carboxylic acid (101d)



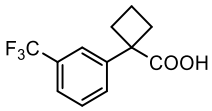
The title compound was synthesized according to the general procedure **B** using 1-(4-ethynylphenyl)cyclobutane-1-carbonitrile (**100d**, 1.16 g, 6.40 mmol, 1 eq) and KOH (2.16 g, 38.4 mmol, 6 eq). Total time: 16 h at reflux. Silica gel column chromatography (20% EtOAc in *n*-pentane with 0.1% TFA) afforded the product (730 mg, 3.63 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.85 (bs, 1H), 7.48 – 7.42 (m, 2H), 7.29 – 7.23 (m, 2H), 3.05 (s, 1H), 2.89 – 2.79 (m, 2H), 2.56 – 2.45 (m, 2H), 2.15 – 2.02 (m, 1H), 1.93 – 1.80 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.11, 143.97, 132.23, 126.61, 120.82, 83.53, 77.38, 52.28, 32.36, 16.73.

1-(4-(Trifluoromethyl)phenyl)cyclobutane-1-carboxylic acid (101e)



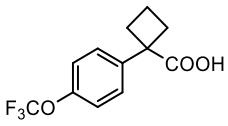
The title compound was synthesized according to the general procedure **B** using 1-(4-(trifluoromethyl)phenyl)cyclobutane-1-carbonitrile (**100e**, 1.35 g, 5.99 mmol, 1 eq) and KOH (2.0 g, 36 mmol, 6 eq). Total time: 16 h at reflux. Silica gel column chromatography (20% EtOAc in *n*-pentane with 0.1% TFA) afforded the product (1.37 g, 5.61 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.55 (m, 2H), 7.45 – 7.37 (m, 2H), 2.94 – 2.83 (m, 2H), 2.59 – 2.48 (m, 2H), 2.20 – 2.06 (m, 1H), 1.95 – 1.83 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.77, 147.18 (q, $J_{C\text{-}F}$ = 1.6 Hz), 129.32 (q, $J_{C\text{-}F}$ = 32.5 Hz), 127.03, 125.45 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.25 (q, $J_{C\text{-}F}$ = 272.7 Hz), 52.28, 32.46, 16.82.

1-(3-(Trifluoromethyl)phenyl)cyclobutane-1-carboxylic acid (101f)



The title compound was synthesized according to the general procedure **B** using 1-(3-(trifluoromethyl)phenyl)cyclobutane-1-carbonitrile (**100f**, 1.49 g, 6.61 mmol, 1 eq) and KOH (2.22 g, 39.6 mmol, 6 eq). Total time: 2 h at reflux. The product was afforded without purification (1.69 g, 6.93 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.40 (m, 4H), 2.99 – 2.82 (m, 2H), 2.62 – 2.49 (m, 2H), 2.22 – 2.05 (m, 1H), 1.99 – 1.83 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.41, 144.53, 130.73 (q, $J_{C\text{-}F}$ = 32.1 Hz), 130.05, 128.88, 124.21 (q, $J_{C\text{-}F}$ = 272.7 Hz), 123.75 (q, $J_{C\text{-}F}$ = 3.8 Hz), 123.31 (q, $J_{C\text{-}F}$ = 3.8 Hz), 52.14, 32.39, 16.72.

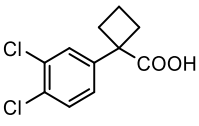
1-(4-(Trifluoromethoxy)phenyl)cyclobutane-1-carboxylic acid (101g)



The title compound was synthesized according to the general procedure **B** using 1-(4-(trifluoromethoxy)phenyl)cyclobutane-1-carbonitrile (**100g**, 620 mg, 2.57 mmol, 1 eq) and KOH (0.95 g, 17 mmol, 6.6 eq). Total time: 3.5 h at reflux. Silica gel column chromatography (20-40% EtOAc in *n*-pentane with a drop of

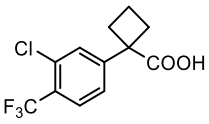
conc. HCl) afforded the product (590 mg, 2.26 mmol, 88%). ^{1}H NMR (850 MHz, $CDCl_3$) δ 7.38 – 7.28 (m, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 2.90 – 2.83 (m, 2H), 2.59 – 2.47 (m, 2H), 2.15 – 2.04 (m, 1H), 1.93 – 1.82 (m, 1H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 181.97, 148.15 (q, J_{C-F} = 1.8 Hz), 141.96, 128.07, 120.90, 120.59 (q, J_{C-F} = 256.8 Hz), 51.88, 32.44, 16.64.

1-(3,4-Dichlorophenyl)cyclobutane-1-carboxylic acid (101h)



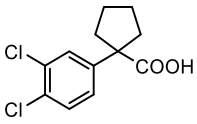
The title compound was synthesized according to the general procedure **B** using 1-(3,4-dichlorophenyl)cyclobutane-1-carbonitrile (**100h**, 1.11 g, 4.90 mmol, 1 eq) and KOH (1.65 g, 29.4 mmol, 6 eq). Total time: 4 h at reflux. Silica gel column chromatography (40% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (1.10 g, 4.49 mmol, 91%). ^{1}H NMR (850 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.6 Hz, 1H), 7.36 (d, *J* = 2.5 Hz, 1H), 7.12 (dd, *J* = 8.3, 2.3 Hz, 1H), 2.85 – 2.79 (m, 2H), 2.49 – 2.42 (m, 2H), 2.12 – 2.05 (m, 1H), 1.90 – 1.83 (m, 1H). ^{13}C NMR (214 MHz, $CDCl_3$) δ 180.75, 143.49, 132.44, 131.04, 130.31, 128.76, 126.08, 51.64, 32.27, 16.59.

1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclobutane-1-carboxylic acid (101i)



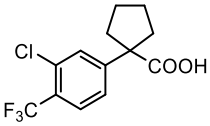
The title compound was synthesized according to the general procedure **B** using 1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutane-1-carbonitrile (**100i**, 1.02 g, 3.93 mmol, 1 eq) and KOH (1.32 g, 23.6 mmol, 6 eq). Total time: 2 h at reflux. The product was afforded without purification (1.06 g, 3.82 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.64 (d, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 1.3 Hz, 1H), 7.28 (dd, *J* = 8.2, 1.1 Hz, 1H), 2.94 – 2.82 (m, 2H), 2.57 – 2.46 (m, 2H), 2.22 – 2.08 (m, 1H), 1.97 – 1.84 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 180.69, 148.92, 132.43 (q, J_{C-F} = 2.0 Hz), 129.77, 127.65 (q, J_{C-F} = 5.1 Hz), 126.91 (q, J_{C-F} = 31.3 Hz), 125.01, 122.95 (q, J_{C-F} = 274.7 Hz), 51.99, 32.34, 16.74.

1-(3,4-Dichlorophenyl)cyclopentane-1-carboxylic acid (101j)



The title compound was synthesized according to the general procedure **B** using 1-(3,4-dichlorophenyl)cyclopentane-1-carbonitrile (**100j**, 2.00 g, 8.33 mmol, 1 eq) in 9 M H_2SO_4 (20 mL, 0.4 M) at reflux until completion. Silica gel chromatography (30-40% EtOAc in *n*-pentane with a drop of conc. HCl) afforded the product (970 mg, 3.74 mmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.48 (d, *J* = 2.3 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.3 Hz, 1H), 2.67 – 2.56 (m, 2H), 1.93 – 1.80 (m, 2H), 1.81 – 1.67 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 177.87, 143.87, 132.12, 130.70, 130.05, 129.15, 126.60, 58.36, 36.09, 23.51.

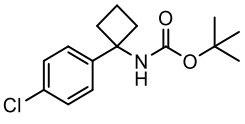
1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclopentane-1-carboxylic acid (101k)



The title compound was synthesized according to the general procedure **B** using 1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentane-1-carbonitrile (**100k**, 1.08 g, 3.95 mmol, 1 eq) in 9 M H_2SO_4 (10 mL, 0.4 M) at reflux until completion. Silica gel chromatography (40-50% EtOAc in *n*-pentane with a drop of conc. HCl)

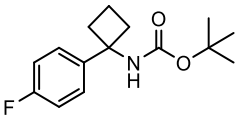
to afford the product (810 mg, 2.78 mmol, 70%). ^{1}H NMR (400 MHz, MeOD) δ 7.70 (d, *J* = 8.3 Hz, 1H), 7.59 (d, *J* = 1.8 Hz, 1H), 7.49 (ddd, *J* = 8.3, 1.9, 0.9 Hz, 1H), 2.70 – 2.61 (m, 2H), 1.95 – 1.84 (m, 2H), 1.82 – 1.72 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 178.01, 151.50, 132.85 (q, J_{C-F} = 2.0 Hz), 131.23, 128.54 (q, J_{C-F} = 5.3 Hz), 127.48 (q, J_{C-F} = 31.5 Hz), 126.96, 124.41 (q, J_{C-F} = 272.0 Hz), 60.16, 37.11, 24.51.

tert-Butyl (1-(4-chlorophenyl)cyclobutyl)carbamate (102a)



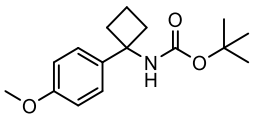
The title compound was synthesized according to the general procedure **C** using 1-(4-chlorophenyl)cyclobutane-1-carboxylic acid (**101a**, 830 mg, 3.93 mmol, 1 eq), diphenylphosphoryl azide (850 µL, 3.93 mmol, 1 eq) and Et_3N (600 µL, 4.32 mmol, 1.1 eq) in anhydrous *t*-BuOH (20 mL, 0.2 M). Total time: 2 h at 30 °C and overnight at reflux. Silica gel column chromatography (5-7% EtOAc in *n*-pentane) afforded the product (820 mg, 2.92 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 2H), 7.32 – 7.27 (m, 2H), 5.17 (s, 1H), 2.60 – 2.35 (m, 4H), 2.13 – 2.05 (m, 1H), 1.88 – 1.80 (m, 1H), 1.37 (s, 9H). LC-MS $[C_{15}H_{20}ClNO_2+H]^+$: 282.13 calculated, 281.83 found.

tert-Butyl (1-(4-fluorophenyl)cyclobutyl)carbamate (102b)



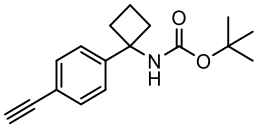
The title compound was synthesized according to the general procedure **C** using 1-(4-fluorophenyl)cyclobutane-1-carboxylic acid (**101b**, 820 mg, 4.25 mmol, 1 eq), diphenylphosphoryl azide (920 µL, 4.25 mmol, 1 eq) and Et_3N (650 µL, 4.68 mmol, 1.1 eq) in anhydrous *t*-BuOH (21 mL, 0.2 M). Total time: 2 h at 30 °C and overnight at reflux. Silica gel column chromatography (5% EtOAc in *n*-pentane) afforded the product (980 mg, 3.68 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.34 (m, 2H), 7.04 – 6.96 (m, 2H), 5.15 (s, 1H), 2.62 – 2.30 (m, 4H), 2.16 – 2.02 (m, 1H), 1.90 – 1.75 (m, 1H), 1.35 (s, 9H).

tert-Butyl (1-(4-methoxyphenyl)cyclobutyl)carbamate (102c)



The title compound was synthesized according to the general procedure **C** using 1-(4-methoxyphenyl)cyclobutane-1-carboxylic acid (**101c**, 1.50 g, 7.27 mmol, 1 eq), diphenylphosphoryl azide (1.57 mL, 7.27 mmol, 1 eq) and Et_3N (1.10 mL, 8.00 mmol, 1.1 eq) in anhydrous *t*-BuOH (36 mL, 0.2 M). Total time: 2 h at 30 °C and overnight at reflux. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (760 mg, 2.74 mmol, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.3 Hz, 2H), 6.90 – 6.84 (m, 2H), 5.05 (s, 1H), 3.80 (s, 3H), 2.65 – 2.44 (m, 4H), 2.11 – 1.99 (m, 1H), 1.86 – 1.73 (m, 1H), 1.37 (s, 9H).

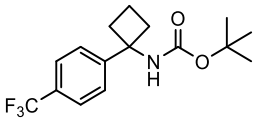
tert-Butyl (1-(4-ethynylphenyl)cyclobutyl)carbamate (102d)



The title compound was synthesized according to the general procedure **C** using 1-(4-ethynylphenyl)cyclobutane-1-carboxylic acid (**101d**, 730 mg, 3.63 mmol, 1 eq), diphenylphosphoryl azide (780 µL, 3.63 mmol, 1 eq) and Et_3N (560 µL, 3.99 mmol, 1.1 eq) in anhydrous *t*-BuOH (18 mL, 0.2 M). Total time: 2

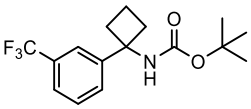
h at 30 °C and overnight at reflux. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (520 mg, 1.90 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.44 (m, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 5.16 (s, 1H), 3.05 (s, 1H), 2.59 – 2.30 (m, 4H), 2.16 – 2.06 (m, 1H), 1.91 – 1.79 (m, 1H), 1.37 (s, 9H).

tert-Butyl (1-(4-(trifluoromethyl)phenyl)cyclobutyl)carbamate (102e)



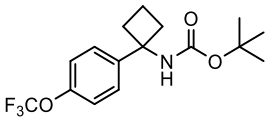
The title compound was synthesized according to the general procedure **C** using 1-(4-(trifluoromethyl)phenyl)cyclobutane-1-carboxylic acid (**101e**, 1.37 g, 5.60 mmol, 1 eq), diphenylphosphoryl azide (1.2 mL, 5.6 mmol, 1 eq) and Et_3N (860 µL, 6.15 mmol, 1.1 eq) in anhydrous *t*-BuOH (28 mL, 0.2 M). Total time: 2 h at 30 °C and overnight at reflux. Silica gel column chromatography (5-7% EtOAc in *n*-pentane) afforded the product (1.20 g, 3.81 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.57 (m, 2H), 7.57 – 7.50 (m, 2H), 5.22 (s, 1H), 2.60 – 2.31 (m, 4H), 2.20 – 2.08 (m, 1H), 1.96 – 1.82 (m, 1H), 1.38 (s, 9H).

tert-Butyl (1-(3-(trifluoromethyl)phenyl)cyclobutyl)carbamate (102f)



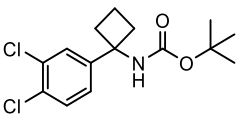
The title compound was synthesized according to the general procedure **C** using 1-(3-(trifluoromethyl)phenyl)cyclobutane-1-carboxylic acid (**101f**, 1.69 g, 6.93 mmol, 1 eq), diphenylphosphoryl azide (1.64 mL, 7.62 mmol, 1.1 eq) and Et_3N (1.93 mL, 13.9 mmol, 2.0 eq) in anhydrous *t*-BuOH (69 mL, 0.1 M). Total time: 2 h at 30 °C and overnight at reflux. Silica gel column chromatography (5-7% EtOAc in *n*-pentane) afforded the product as a white solid (890 mg, 2.82 mmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.18 (m, 4H), 5.19 (s, 1H), 2.68 – 2.36 (m, 4H), 2.22 – 2.10 (m, 1H), 1.97 – 1.81 (m, 1H), 1.32 (s, 9H).

tert-Butyl (1-(4-(trifluoromethoxy)phenyl)cyclobutyl)carbamate (102g)



The title compound was synthesized according to the general procedure **C** using 1-(4-(trifluoromethoxy)phenyl)cyclobutane-1-carboxylic acid (**101g**, 590 mg, 2.27 mmol, 1 eq), diphenyl phosphorazidate (520 µL, 2.38 mmol, 1.05 eq) and Et_3N (950 µL, 6.80 mmol, 3 eq) in anhydrous *t*-BuOH (27 mL, 0.1 M). Total time: 2 h at 30 °C and overnight at reflux. Silica gel column chromatography (3-4% EtOAc in *n*-pentane) afforded the product (0.21 g, 0.60 mmol, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 2H), 5.23 (s, 1H), 2.70 – 2.34 (m, 4H), 2.18 – 2.02 (m, 1H), 1.93 – 1.78 (m, 1H), 1.31 (s, 9H).

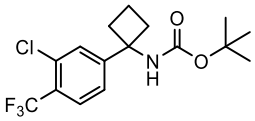
tert-Butyl (1-(3,4-dichlorophenyl)cyclobutyl)carbamate (102h)



The title compound was synthesized according to the general procedure **C** using 1-(3,4-dichlorophenyl)cyclobutane-1-carboxylic acid (**101h**, 1.10 g, 4.50 mmol, 1 eq), diphenylphosphoryl azide (0.97 mL, 4.5 mmol, 1 eq) and Et_3N (1.89 mL, 13.5 mmol, 3 eq) in anhydrous *t*-BuOH (45 mL, 0.1 M). Total time: 1 h at 30 °C and overnight at reflux. Silica gel column chromatography (3-4% EtOAc in *n*-pentane)

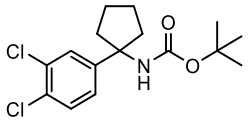
afforded the product (0.80 g, 2.6 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 2.3 Hz, 1H), 7.42 – 7.36 (m, 1H), 7.30 – 7.21 (m, 1H), 5.25 (s, 1H), 2.54 – 2.25 (m, 4H), 2.17 – 2.05 (m, 1H), 1.96 – 1.81 (m, 1H), 1.38 (s, 9H).

tert-Butyl (1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutyl)carbamate (102i)



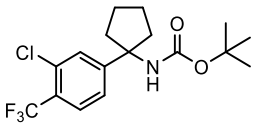
The title compound was synthesized according to the general procedure **C** using 1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutane-1-carboxylic acid (**101i**, 1.06 g, 3.82 mmol, 1 eq), diphenylphosphoryl azide (0.90 mL, 4.2 mmol, 1.1 eq) and Et_3N (1.06 mL, 7.63 mmol, 2 eq) in anhydrous *t*-BuOH (38 mL, 0.1 M). Total time: 1 h at 30 °C and overnight at reflux. Silica gel column chromatography (2-3% EtOAc in *n*-pentane) afforded the product (930 mg, 2.66 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.2 Hz, 1H), 7.54 (d, *J* = 1.9 Hz, 1H), 7.46 – 7.34 (m, 1H), 5.28 (s, 1H), 2.61 – 2.39 (m, 4H), 2.23 – 2.09 (m, 1H), 1.98 – 1.86 (m, 1H), 1.39 (s, 9H).

tert-Butyl (1-(3,4-dichlorophenyl)cyclopentyl)carbamate (102j)



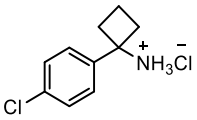
The title compound was synthesized according to the general procedure **C** using 1-(3,4-dichlorophenyl)cyclopentane-1-carboxylic acid (**101j**, 930 mg, 3.59 mmol, 1 eq), diphenylphosphoryl azide (850 µL, 3.95 mmol, 1.1 eq) and Et_3N (1.50 mL, 10.8 mmol, 3 eq) in anhydrous *t*-BuOH (36 mL, 0.1 M). Total time: 2 h at 30 °C and 2 days at reflux. Silica gel column chromatography (2-5% EtOAc in *n*-pentane) afforded the product (770 mg, 2.33 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 2.3 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.91 (s, 1H), 2.28 – 2.08 (m, 2H), 2.03 – 1.91 (m, 2H), 1.91 – 1.69 (m, 4H), 1.33 (s, 9H).

tert-Butyl (1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentyl)carbamate (102k)



The title compound was synthesized according to the general procedure **C** using 1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentane-1-carboxylic acid (**101k**, 810 mg, 2.78 mmol, 1 eq), diphenylphosphoryl azide (660 µL, 3.06 mmol, 1 eq) and Et_3N (1.16 mL, 8.33 mmol, 3 eq) in anhydrous *t*-BuOH (28 mL, 0.1 M). Total time: 2 h at 30 °C and 24 h at reflux. Silica gel column chromatography (5-7% EtOAc in *n*-pentane) afforded the product (940 mg, 2.58 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.3 Hz, 1H), 7.52 (d, *J* = 1.9 Hz, 1H), 7.37 (dd, *J* = 8.2, 1.8 Hz, 1H), 4.97 (s, 1H), 2.33 – 2.08 (m, 2H), 2.06 – 1.93 (m, 2H), 1.93 – 1.75 (m, 4H), 1.30 (s, 9H).

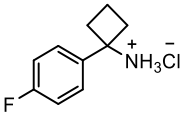
1-(4-Chlorophenyl)cyclobutan-1-aminium chloride (103a)



The title compound was synthesized according to the general procedure **D** using *tert*-butyl 1-(4-chlorophenyl)cyclobutyl)carbamate (**102a**, 820 mg, 2.92 mmol, 1 eq). Total time: over-weekend at rt. The product was afforded as a white solid (560 mg, 2.57 mmol, 88%). ^{1}H NMR (400 MHz, MeOD) δ 7.59 – 7.44 (m, 4H), 2.80 – 2.71 (m, 2H), 2.69 – 2.60 (m, 2H), 2.32 – 2.20 (m, 1H), 2.00 – 1.88 (m, 1H). ^{13}C NMR

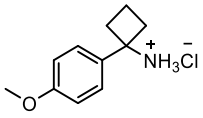
(101 MHz, MeOD) δ 140.02, 135.88, 130.24, 129.09, 59.80, 33.27, 14.79. LC-MS [$C_{10}H_{12}ClN$–NH_2]$^+$: 165.05 calculated, 165.00 found.

1-(4-Fluorophenyl)cyclobutan-1-aminium chloride (103b)



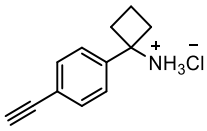
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(4-fluorophenyl)cyclobutyl)carbamate (**102b**, 980 mg, 3.68 mmol, 1 eq). Total time: 16 h at rt. The product was afforded as a white solid (670 mg, 3.31 mmol, 90%). ^{1}H NMR (400 MHz, MeOD) δ 7.60 – 7.53 (m, 2H), 7.26 – 7.18 (m, 2H), 2.81 – 2.72 (m, 2H), 2.70 – 2.59 (m, 2H), 2.31 – 2.19 (m, 1H), 2.01 – 1.87 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 164.19 (d, $J_{C\text{-}F}$ = 247.1 Hz), 137.35 (d, $J_{C\text{-}F}$ = 3.2 Hz), 129.65 (d, $J_{C\text{-}F}$ = 8.5 Hz), 116.88 (d, $J_{C\text{-}F}$ = 22.0 Hz), 59.83, 33.34, 14.77. LC-MS [$C_{10}H_{12}FN$+H]$^+$: 166.10 calculated, 165.83 found.

1-(4-Methoxyphenyl)cyclobutan-1-aminium chloride (103c)



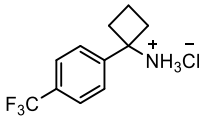
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(4-methoxyphenyl)cyclobutyl)carbamate (**102c**, 760 mg, 2.74 mmol, 1 eq). Total time: 16 h at rt. The product was afforded as a white solid (350 mg, 1.64 mmol, 60%). ^{1}H NMR (400 MHz, MeOD) δ 7.50 – 7.42 (m, 2H), 7.06 – 6.99 (m, 2H), 3.82 (s, 3H), 2.81 – 2.67 (m, 2H), 2.68 – 2.55 (m, 2H), 2.30 – 2.15 (m, 1H), 1.99 – 1.81 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 161.42, 133.00, 128.65, 115.36, 59.91, 55.87, 33.34, 14.82. LC-MS [$C_{11}H_{15}NO$–NH_2]$^+$: 161.10 calculated, 161.00 found.

1-(4-Ethynylphenyl)cyclobutan-1-aminium chloride (103d)



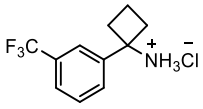
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(4-ethynylphenyl)cyclobutyl)carbamate (**102d**, 520 mg, 1.90 mmol, 1 eq). Total time: 16 h at rt. The product was afforded as a white solid (350 mg, 1.68 mmol, 88%). ^{1}H NMR (400 MHz, MeOD) δ 7.60 – 7.56 (m, 2H), 7.54 – 7.50 (m, 2H), 3.61 (s, 1H), 2.82 – 2.72 (m, 2H), 2.70 – 2.59 (m, 2H), 2.32 – 2.20 (m, 1H), 2.01 – 1.89 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 141.69, 133.66, 127.39, 124.41, 83.52, 79.99, 60.02, 33.23, 14.82. LC-MS [$C_{12}H_{13}N$+H]$^+$: 172.11 calculated, 171.83 found.

1-(4-(Trifluoromethyl)phenyl)cyclobutan-1-aminium chloride (103e)



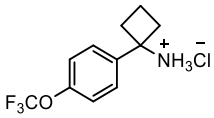
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(4-(trifluoromethyl)phenyl)cyclobutyl)carbamate (**102e**, 1.20 g, 3.81 mmol, 1 eq). Total time: 16 h at rt. The product was afforded as a white solid (870 mg, 3.46 mmol, 91%). ^{1}H NMR (400 MHz, MeOD) δ 7.85 – 7.71 (m, 4H), 2.88 – 2.75 (m, 2H), 2.75 – 2.63 (m, 2H), 2.37 – 2.23 (m, 1H), 2.07 – 1.91 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 145.57 (q, $J_{C\text{-}F}$ = 1.3 Hz), 131.95 (q, $J_{C\text{-}F}$ = 33.3 Hz), 128.16, 127.09 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.4 (q, $J_{C\text{-}F}$ = 271.7 Hz), 59.96, 33.30, 14.84. LC-MS [$C_{11}H_{12}F_3N$+H]$^+$: 216.10 calculated, 215.83 found.

1-(3-(Trifluoromethyl)phenyl)cyclobutan-1-aminium chloride (103f)



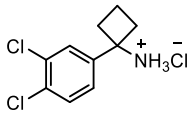
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(3-(trifluoromethyl)phenyl)cyclobutyl)carbamate (**102f**, 860 mg, 2.74 mmol, 1 eq). Total time: 3 days at rt. The product was afforded as a white solid (330 mg, 1.31 mmol, 48%). ^{1}H NMR (400 MHz, MeOD) δ 7.86 – 7.69 (m, 4H), 2.86 – 2.76 (m, 2H), 2.73 – 2.63 (m, 2H), 2.36 – 2.24 (m, 1H), 2.05 – 1.92 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 142.61, 132.36 (q, $J_{C\text{-}F}$ = 32.4 Hz), 131.31 (q, $J_{C\text{-}F}$ = 1.4 Hz), 131.25, 126.82 (q, $J_{C\text{-}F}$ = 3.7 Hz), 125.40 (q, $J_{C\text{-}F}$ = 273.7 Hz), 124.10 (q, $J_{C\text{-}F}$ = 3.7 Hz), 59.95, 33.23, 14.85.

1-(4-(Trifluoromethoxy)phenyl)cyclobutan-1-aminium chloride (103g)



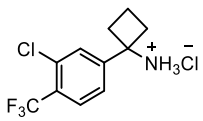
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(4-(trifluoromethoxy)phenyl)cyclobutyl)carbamate (**102g**, 0.20 g, 0.60 mmol, 1 eq). Total time: 24 h at rt. The product was afforded as a white solid (0.16 g, 0.60 mmol, quant.) ^{1}H NMR (400 MHz, MeOD) δ 7.68 – 7.60 (m, 2H), 7.44 – 7.36 (m, 2H), 2.85 – 2.74 (m, 2H), 2.71 – 2.59 (m, 2H), 2.33 – 2.20 (m, 1H), 2.04 – 1.90 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 151.65 (q, $J_{C\text{-}F}$ = 2.0 Hz), 140.44, 129.44, 122.60, 121.87(q, $J_{C\text{-}F}$ = 257.6 Hz), 59.77, 33.34, 14.78.

1-(3,4-Dichlorophenyl)cyclobutan-1-aminium chloride (103h)



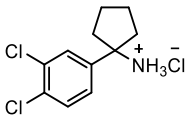
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(3,4-dichlorophenyl)cyclobutyl)carbamate (**102h**, 0.80 g, 2.6 mmol, 1 eq). Total time: 24 h at rt. The product was afforded as a white solid (470 mg, 1.86 mmol, 72%). ^{1}H NMR (400 MHz, MeOD) δ 7.70 (d, *J* = 2.3 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.47 (dd, *J* = 8.4, 2.3 Hz, 1H), 2.81 – 2.71 (m, 2H), 2.68 – 2.57 (m, 2H), 2.32 – 2.20 (m, 1H), 2.02 – 1.90 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ 141.91, 134.07, 134.06, 132.32, 129.81, 127.39, 59.52, 33.20, 14.75.

1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclobutan-1-aminium chloride (103i)



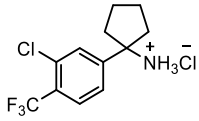
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutyl)carbamate (**102i**, 930 mg, 2.66 mmol, 1 eq). Total time: over-weekend at rt. The product was afforded as a white solid (0.28 g, 0.99 mmol, 38%). ^{1}H NMR (400 MHz, MeOD) δ 7.90 (d, *J* = 8.3 Hz, 1H), 7.78 (d, *J* = 1.9 Hz, 1H), 7.66 (dd, *J* = 8.2, 1.9 Hz, 1H), 2.85 – 2.74 (m, 2H), 2.73 – 2.62 (m, 2H), 2.36 – 2.24 (m, 1H), 2.07 – 1.94 (m, 1H). ^{13}C NMR (101 MHz, MeOD) *δ* 147.21, 133.87 (q, $J_{C\text{-}F}$ = 2.0 Hz), 130.67, 129.53 (q, $J_{C\text{-}F}$ = 5.2 Hz), 129.51 (q, $J_{C\text{-}F}$ = 32.0 Hz), 126.34, 124.02 (q, $J_{C\text{-}F}$ = 272.3 Hz), 59.48, 33.15, 14.77.

1-(3,4-Dichlorophenyl)cyclopentan-1-aminium chloride (103j)



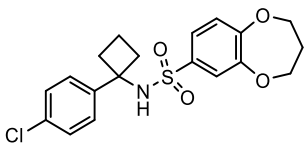
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(3,4-dichlorophenyl)cyclopentyl)carbamate (**102j**, 770 mg, 2.33 mmol, 1 eq). Total time: 16 h at rt. The product was afforded as a white solid (520 mg, 1.95 mmol, 84%). ^{1}H NMR (400 MHz, MeOD) δ 7.72 (d, *J* = 2.3 Hz, 1H), 7.64 (d, *J* = 8.5 Hz, 1H), 7.48 (dd, *J* = 8.5, 2.3 Hz, 1H), 2.36 – 2.22 (m, 4H), 2.04 – 1.88 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 142.22, 134.02, 133.95, 132.24, 129.84, 127.49, 66.85, 38.57, 23.81.

1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclopentan-1-aminium chloride (103k)



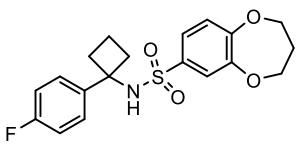
The title compound was synthesized according to the general procedure **D** using *tert*-butyl (1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentyl)carbamate (**102k**, 940 mg, 2.58 mmol, 1 eq). Total time: 2 days at rt. The product was afforded as a white solid (720 mg, 2.39 mmol, 93%). ^{1}H NMR (400 MHz, MeOD) δ 7.89 (d, *J* = 8.3 Hz, 1H), 7.82 (d, *J* = 1.6 Hz, 1H), 7.68 (ddd, *J* = 8.3, 1.9, 0.9 Hz, 1H), 2.38 – 2.27 (m, 4H), 2.07 – 1.90 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 147.83, 133.81 (q, J_{C-F} = 2.0 Hz), 130.76, 129.51 (q, J_{C-F} = 5.3 Hz), 129.40 (q, J_{C-F} = 31.3 Hz), 126.52, 124.11 (q, J_{C-F} = 273.7 Hz), 66.95, 38.84, 24.01.

***N*-(1-(4-Chlorophenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (104a)**



The title compound was synthesized according to the general procedure **H** using 1-(4-chlorophenyl)cyclobutan-1-aminium chloride (**103a**, 35 mg, 0.16 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 48 mg, 0.19 mmol, 1.2 eq) and DIPEA (0.28 mL, 1.6 mmol, 10 eq). Total time: overnight at rt. Silica gel column chromatography (20-25% EtOAc in *n*-pentane) afforded the product (37 mg, 0.093 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 7.13 – 7.02 (m, 4H), 6.98 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.82 (d, *J* = 2.4 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 4.27 (t, *J* = 5.7 Hz, 2H), 4.19 (t, *J* = 5.8 Hz, 2H), 2.66 – 2.53 (m, 2H), 2.53 – 2.42 (m, 2H), 2.21 (p, *J* = 5.7 Hz, 2H), 2.14 – 2.02 (m, 1H), 1.79 – 1.65 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 153.79, 149.72, 140.99, 135.77, 132.25, 128.15, 127.58, 121.70, 121.02, 120.34, 70.31, 70.19, 60.41, 34.79, 30.75, 15.09.

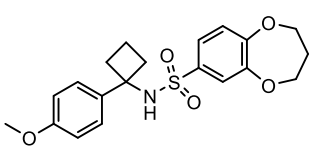
***N*-(1-(4-Fluorophenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (104b)**



The title compound was synthesized according to the general procedure **H** using 1-(4-fluorophenyl)cyclobutan-1-aminium chloride (**103b**, 34 mg, 0.17 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 50 mg, 0.20 mmol, 1.2 eq) and DIPEA (290 µL, 1.69 mmol, 10 eq). Total time: overnight at rt. Silica gel column

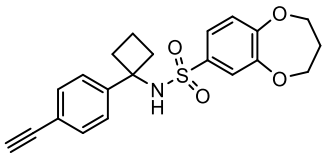
chromatography (20-25% EtOAc in *n*-pentane) afforded the product (36 mg, 0.097 mmol, 57%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.15 – 7.04 (m, 2H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.79 – 6.67 (m, 4H), 4.20 (t, *J* = 5.7 Hz, 2H), 4.14 (t, *J* = 5.9 Hz, 2H), 2.61 – 2.36 (m, 4H), 2.16 (p, *J* = 5.8 Hz, 2H), 2.09 – 1.96 (m, 1H), 1.75 – 1.61 (m, 1H).

_N_-(1-(4-Methoxyphenyl)cyclobutyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (104c)



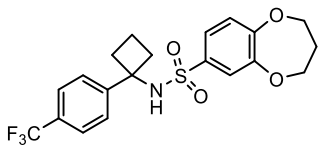
The title compound was synthesized according to the general procedure **H** using 1-(4-methoxyphenyl)cyclobutan-1-aminium chloride (**103c**, 36 mg, 0.17 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 50 mg, 0.20 mmol, 1.2 eq) and DIPEA (290 µL, 1.68 mmol, 10 eq). Total time: overnight at rt. Silica gel column chromatography (20-25% EtOAc in *n*-pentane) afforded the product (42.0 mg, 0.108 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 – 7.06 (m, 2H), 7.02 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.88 (d, *J* = 2.3 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.65 – 6.59 (m, 2H), 5.61 (s, 1H), 4.24 (t, 2H), 4.17 (t, *J* = 5.8 Hz, 2H), 3.76 (s, 3H), 2.59 – 2.49 (m, 4H), 2.18 (p, *J* = 5.7 Hz, 2H), 2.06 – 1.98 (m, 1H), 1.73 – 1.64 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.48, 153.94, 150.00, 136.02, 134.24, 128.23, 122.19, 121.17, 120.98, 113.18, 70.33, 70.25, 61.22, 55.25, 35.62, 31.07, 15.31.

_N_-(1-(4-Ethynylphenyl)cyclobutyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (104d)



The title compound was synthesized according to the general procedure **H** using 1-(4-ethynylphenyl)cyclobutan-1-aminium chloride (**103d**, 35 mg, 0.17 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 50 mg, 0.20 mmol, 1.2 eq) and DIPEA (290 µL, 1.68 mmol, 10 eq). Total time: overnight at rt. Silica gel column chromatography (20-25% EtOAc in *n*-pentane) afforded the product (37 mg, 0.097 mmol, 58%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.23 – 7.18 (m, 2H), 7.14 – 7.09 (m, 2H), 6.99 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.80 (d, *J* = 2.3 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 4.26 (t, *J* = 5.8 Hz, 2H), 4.19 (t, *J* = 5.8 Hz, 2H), 3.12 (s, 1H), 2.64 – 2.55 (m, 2H), 2.54 – 2.45 (m, 2H), 2.20 (p, *J* = 5.8 Hz, 2H), 2.12 – 2.04 (m, 1H), 1.77 – 1.68 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 154.14, 150.00, 143.44, 135.95, 131.62, 126.94, 122.03, 121.37, 120.76, 120.67, 83.61, 77.47, 70.64, 70.47, 61.02, 35.05, 31.10, 15.47.

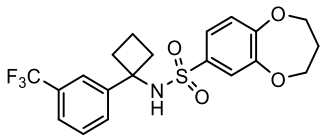
_N_-(1-(4-(Trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (104e)



The title compound was synthesized according to the general procedure **H** using 1-(4-(trifluoromethyl)phenyl)cyclobutan-1-aminium chloride (**103e**, 42.0 mg, 0.167 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 50 mg, 0.20 mmol, 1.2 eq) and DIPEA (290 µL, 1.67 mmol, 10 eq). Total time: overnight at rt. Silica gel

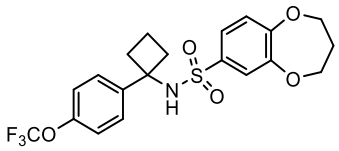
column chromatography (20-25% EtOAc in *n*-pentane) afforded the product (52.1 mg, 0.122 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 4H), 6.96 – 6.89 (m, 2H), 6.70 (d, *J* = 8.3 Hz, 1H), 5.50 (s, 1H), 4.23 (t, *J* = 5.8 Hz, 2H), 4.16 (t, *J* = 5.8 Hz, 2H), 2.65 – 2.51 (m, 4H), 2.19 (p, *J* = 5.8 Hz, 2H), 2.13 – 2.05 (m, 1H), 1.78 – 1.70 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.27, 150.20, 146.22 (q, J_{C-F} = 1.1 Hz), 135.53, 129.34 (q, J_{C-F} = 32.4 Hz), 127.43, 124.93 (q, J_{C-F} = 3.8 Hz), 122.18, 121.34, 120.62, 70.41, 70.32, 61.33, 35.62, 30.89, 15.39 (missing ^{13}C peak of the CF_3 group).

_N_-(1-(3-(Trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (104f)



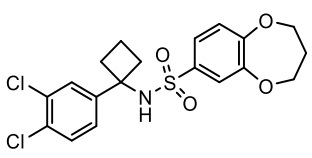
The title compound was synthesized according to the general procedure **H** using 1-(3-(trifluoromethyl)phenyl)cyclobutan-1-aminium chloride (**103f**, 40 mg, 0.16 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 43.5 mg, 0.175 mmol, 1.1 eq) and DIPEA (140 µL, 0.795 mmol, 5 eq). Total time: overnight at rt. Silica gel column chromatography (20-25 % EtOAc in *n*-pentane) afforded the product (31 mg, 0.073 mmol, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dt, *J* = 7.7, 1.6 Hz, 1H), 7.33 – 7.26 (m, 2H), 7.26 – 7.18 (m, 1H), 6.89 – 6.80 (m, 2H), 6.62 (d, *J* = 8.4 Hz, 1H), 5.76 (s, 1H), 4.15 (t, *J* = 5.8 Hz, 2H), 4.09 (t, *J* = 5.9 Hz, 2H), 2.63 – 2.41 (m, 4H), 2.11 (p, *J* = 5.9 Hz, 2H), 2.07 – 1.92 (m, 1H), 1.77 – 1.62 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.11, 150.16, 143.26, 135.54, 130.46 (q, J_{C-F} = 1.1 Hz), 130.35 (q, J_{C-F} = 32.3 Hz), 128.48, 124.07 (q, J_{C-F} = 273.7 Hz), 123.99 (q, J_{C-F} = 3.9 Hz), 123.89 (q, J_{C-F} = 3.7 Hz), 122.02, 121.28, 120.52, 70.31, 70.16, 61.27, 35.64, 30.88, 15.35.

_N_-(1-(4-(Trifluoromethoxy)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (104g)



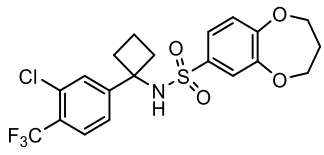
The title compound was synthesized according to the general procedure **H** using 1-(4-(trifluoromethoxy)phenyl)cyclobutan-1-aminium chloride (**103g**, 40 mg, 0.15 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 40.8 mg, 0.164 mmol, 1.1eq) and DIPEA (78 µL, 0.45 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (15-20% EtOAc in *n*-pentane) afforded the product (58 mg, 0.13 mmol, 88%). ^{1}H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.22 – 7.16 (m, 2H), 6.95 – 6.86 (m, 4H), 6.71 – 6.67 (m, 1H), 4.19 (t, *J* = 5.7 Hz, 2H), 4.14 (t, *J* = 5.8 Hz, 2H), 2.62 – 2.52 (m, 2H), 2.50 – 2.40 (m, 2H), 2.16 (p, *J* = 5.8 Hz, 2H), 2.10 – 1.98 (m, 1H), 1.76 – 1.63 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 154.51, 150.61, 148.31 (q, J_{C-F} = 1.8 Hz), 142.24, 136.71, 128.88, 122.30, 121.64, 120.99 (q, J_{C-F} = 257.6 Hz), 120.81, 120.48, 70.89, 70.84, 61.12, 35.45, 31.42, 15.76.

_N_-(1-(3,4-Dichlorophenyl)cyclobutyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (104h)



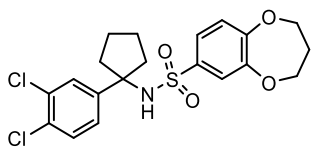
The title compound was synthesized according to the general procedure **H** using 1-(3,4-dichlorophenyl)cyclobutan-1-aminium chloride (**103h**, 60 mg, 0.24 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 65 mg, 0.26 mmol, 1.1 eq) and DIPEA (124 µL, 0.714 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (15% EtOAc in *n*-pentane) afforded the product (40 mg, 0.093 mmol, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.3 Hz, 1H), 7.14 (d, *J* = 2.2 Hz, 1H), 7.10 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.99 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.88 (d, *J* = 2.3 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 5.34 (s, 1H), 4.29 (t, *J* = 5.9 Hz, 2H), 4.21 (t, *J* = 5.9 Hz, 2H), 2.60 – 2.44 (m, 4H), 2.21 (p, *J* = 5.8 Hz, 2H), 2.14 – 2.01 (m, 1H), 1.80 – 1.67 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.35, 150.18, 142.35, 135.29, 132.12, 131.14, 129.87, 129.61, 126.54, 122.15, 121.33, 120.66, 70.48, 70.41, 60.84, 35.75, 30.91, 15.29.

_N_-(1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (104i)



The title compound was synthesized according to the general procedure **H** using 1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutan-1-aminium chloride (**103i**, 40 mg, 0.14 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 38.2 mg, 0.154 mmol, 1.1 eq) and DIPEA (70 µL, 0.42 mmol, 3 eq). Total time: overnight at rt. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (46 mg, 0.096 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.1 Hz, 1H), 7.22 – 7.17 (m, 1H), 7.15 (d, *J* = 1.8 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.86 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.66 (d, *J* = 8.5 Hz, 1H), 5.91 (s, 1H), 4.19 (t, *J* = 5.8 Hz, 2H), 4.12 (t, *J* = 5.9 Hz, 2H), 2.60 – 2.40 (m, 4H), 2.12 (p, *J* = 5.8 Hz, 2H), 2.08 – 1.99 (m, 1H), 1.74 – 1.62 (m, 1H). ^{13}C NMR (101MHz, $CDCl_3$) *δ* 154.40, 150.31, 147.83, 135.21, 131.96 (q, $J_{C\text{-}F}$ = 2.0 Hz), 130.38, 127.19 (q, $J_{C\text{-}F}$ = 5.1 Hz), 126.96 (q, $J_{C\text{-}F}$ = 31.3 Hz), 125.23, 122.83 (q, $J_{C\text{-}F}$ = 273.7 Hz), 122.12, 121.30, 120.37, 70.45, 70.22, 60.87, 35.42, 30.85, 15.36.

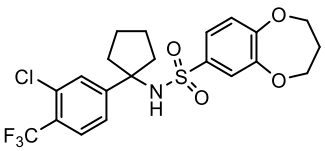
_N_-(1-(3,4-Dichlorophenyl)cyclopentyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (104j)



The title compound was synthesized according to the general procedure **H** using 1-(3,4-dichlorophenyl)cyclopentan-1-aminium chloride (**103j**, 40 mg, 0.15 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 45 mg, 0.18 mmol, 1.2 eq) and DIPEA (105 µL, 0.600 mmol, 4 eq). Total time: 3 days at rt. Silica gel column chromatography (20-25% EtOAc in *n*-pentane) afforded the product (54 mg, 0.12 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.3 Hz, 1H), 7.13 (d, *J* = 2.1 Hz, 1H), 7.07 (td, *J* = 8.1, 2.3 Hz, 2H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 5.85 (s, 1H), 4.30 (t, *J* = 5.7

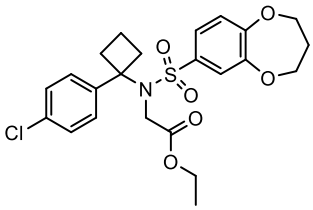
Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 2H), 2.49 – 2.35 (m, 2H), 2.21 (p, *J* = 5.8 Hz, 2H), 2.01 – 1.82 (m, 4H), 1.79 – 1.65 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.21, 150.20, 142.25, 135.49, 131.71, 130.88, 129.73, 129.66, 126.83, 122.04, 121.32, 120.48, 70.44, 70.40, 68.06, 39.05, 30.93, 22.09.

_N_-(1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclopentyl)-3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepine-7-sulfonamide (104k)



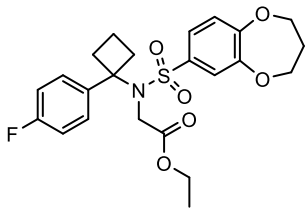
The title compound was synthesized according to the general procedure **H** using 1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentan-1-ammonium chloride (**103k**, 40 mg, 0.13 mmol, 1 eq), 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (**137**, 39.8 mg, 0.160 mmol, 1.2 eq) and DIPEA (93 µL, 0.53 mmol, 4 eq). Total time: 2 days at rt. Silica gel column chromatography (20-25% EtOAc in *n*-pentane) afforded the product (42 mg, 0.089 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.2 Hz, 1H), 7.29 – 7.20 (m, 2H), 7.07 (d, *J* = 2.4 Hz, 1H), 6.97 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 5.89 (s, 1H), 4.28 (t, *J* = 5.7 Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 2H), 2.49 – 2.38 (m, 2H), 2.20 (p, *J* = 5.8 Hz, 2H), 1.97 – 1.85 (m, 4H), 1.80 – 1.69 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.31, 150.44, 147.64, 135.42, 131.69 (q, J_{C-F} = 2.0 Hz), 130.57, 127.03 (q, J_{C-F} = 5.1 Hz), 126.91 (q, J_{C-F} = 30.3 Hz), 125.54, 122.83 (q, J_{C-F} = 273.7 Hz), 122.02, 121.34, 120.30, 70.47, 70.22, 68.19, 39.07, 30.88, 22.11.

Ethyl _N_-(1-(4-chlorophenyl)cyclobutyl)-_N_-((3,4-dihydro-2_H_-benzo[_b_][1,4]dioxepin-7-yl)sulfonyl)glycinate (105a)



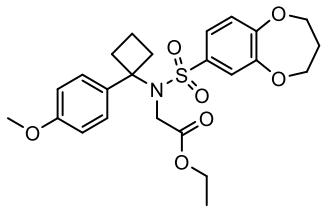
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-chlorophenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104a**, 37 mg, 0.093 mmol, 1 eq), ethyl 2-bromoacetate (21.0 µL, 0.187 mmol, 2 eq) and BEMP (1 M in hexane, 187 µL, 0.187 mmol, 2 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (33 mg, 0.069 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.37 (m, 2H), 7.24 – 7.19 (m, 2H), 7.17 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.06 (d, *J* = 2.4 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 4.29 (t, *J* = 5.8 Hz, 2H), 4.24 (t, *J* = 5.9 Hz, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 4.05 (s, 2H), 2.84 – 2.73 (m, 2H), 2.51 – 2.42 (m, 2H), 2.22 (p, *J* = 5.8 Hz, 2H), 1.81 – 1.72 (m, 1H), 1.59 – 1.50 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.20, 154.41, 150.31, 141.05, 135.85, 133.34, 128.91, 128.29, 122.55, 121.37, 121.14, 70.49, 70.45, 65.33, 61.62, 48.09, 34.71, 30.95, 14.66, 14.21.

Ethyl *N*-(1-(4-fluorophenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (105b)



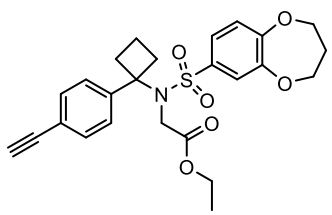
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-fluorophenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104b**, 36 mg, 0.097 mmol, 1 eq), ethyl 2-bromoacetate (21 µL, 0.18 mmol, 2 eq) and BEMP (1 M in hexane, 184 µL, 0.184 mmol, 2 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (31 mg, 0.067 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.41 (m, 2H), 7.19 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.08 (d, *J* = 2.3 Hz, 1H), 6.97 – 6.90 (m, 2H), 6.88 (d, *J* = 8.5 Hz, 1H), 4.28 (t, *J* = 5.8 Hz, 2H), 4.23 (t, *J* = 5.8 Hz, 2H), 4.17 (q, *J* = 7.2 Hz, 2H), 4.04 (s, 2H), 2.83 – 2.73 (m, 2H), 2.52 – 2.44 (m, 2H), 2.22 (p, *J* = 5.8 Hz, 2H), 1.81 – 1.71 (m, 1H), 1.59 – 1.50 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.22, 162.05 (d, $J_{C\text{-}F}$ = 246.6 Hz), 154.35, 150.31, 138.21 (d, $J_{C\text{-}F}$ = 3.3 Hz), 135.99, 129.27 (d, $J_{C\text{-}F}$ = 8.1 Hz), 122.56, 121.36, 121.20, 114.94 (d, $J_{C\text{-}F}$ = 21.3 Hz), 70.46, 70.40, 65.34, 61.58, 47.99, 34.79, 30.96, 14.68, 14.21.

Ethyl *N*-(1-(4-methoxyphenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (105c)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-methoxyphenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104c**, 42.0 mg, 0.108 mmol, 1 eq), ethyl 2-bromoacetate (24 µL, 0.22 mmol, 2 eq) and BEMP (1 M in hexane, 216 µL, 0.216 mmol, 2 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (44 mg, 0.093 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.34 (m, 2H), 7.21 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.11 (d, *J* = 2.3 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.81 – 6.75 (m, 2H), 4.27 (t, *J* = 5.8 Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.01 (s, 2H), 3.81 (s, 3H), 2.82 – 2.71 (m, 2H), 2.52 – 2.44 (m, 2H), 2.21 (p, *J* = 5.8 Hz, 2H), 1.79 – 1.69 (m, 1H), 1.59 – 1.49 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.37, 158.91, 154.27, 150.32, 136.20, 134.29, 128.67, 122.73, 121.33, 121.29, 113.48, 70.43, 70.40, 65.46, 61.51, 55.34, 47.98, 34.75, 31.09, 14.79, 14.23.

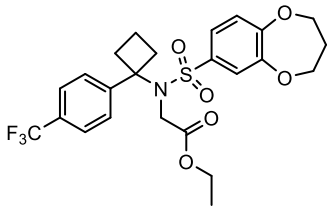
Ethyl *N*-(1-(4-ethynylphenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate(105d)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-ethynylphenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104d**, 37 mg, 0.097 mmol, 1 eq), ethyl 2-bromoacetate (22 µL, 0.19 mmol, 2 eq) and BEMP (1 M in hexane, 194 µL, 0.194 mmol, 2 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (30 mg, 0.064 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.33 (m, 4H), 7.16 (dd, *J* =

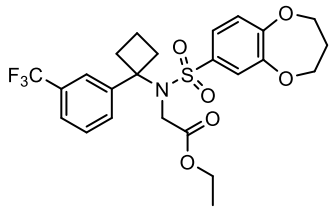
8.5, 2.3 Hz, 1H), 7.04 (d, *J* = 2.3 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 4.29 (t, *J* = 5.4 Hz, 2H), 4.24 (t, *J* = 5.8 Hz, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.05 (s, 2H), 3.08 (s, 1H), 2.87 – 2.73 (m, 2H), 2.54 – 2.43 (m, 2H), 2.21 (p, *J* = 5.8 Hz, 2H), 1.83 – 1.70 (m, 1H), 1.62 – 1.49 (m, 1H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.22, 154.40, 150.27, 143.31, 135.78, 131.95, 127.33, 122.53, 121.36, 121.26, 121.19, 83.49, 77.60, 70.46, 70.44, 65.57, 61.61, 48.21, 34.68, 30.96, 14.69, 14.21.

Ethyl *N*-(1-(4-(trifluoromethyl)phenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7- yl)sulfonyl)glycinate (105e)



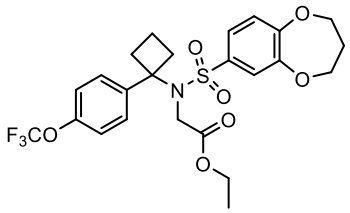
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104e**, 52.1 mg, 0.122 mmol, 1 eq), ethyl 2-bromoacetate (27 µL, 0.24 mmol, 2 eq) and BEMP (1 M in hexane, 244 µL, 0.244 mmol, 2 eq). Total time: 16 h at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (47 mg, 0.092 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.50 (m, 4H), 7.11 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.08 (d, *J* = 2.3 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.27 (t, *J* = 5.8 Hz, 2H), 4.21 (t, *J* = 5.8 Hz, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.07 (s, 2H), 2.89 – 2.78 (m, 2H), 2.55 – 2.47 (m, 2H), 2.25 – 2.17 (m, 2H), 1.85 – 1.76 (m, 1H), 1.62 – 1.53 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.11, 154.48, 150.39, 146.74 (q, $J_{C\text{-}F}$ = 1.3 Hz), 135.69, 129.69 (q, $J_{C\text{-}F}$ = 32.3 Hz), 127.74, 125.20 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.19 (q, $J_{C\text{-}F}$ = 273.7 Hz), 122.61, 121.42, 121.04, 70.48, 70.40, 65.56, 61.69, 48.18, 34.69, 30.93, 14.67, 14.20.

Ethyl *N*-(1-(3-(trifluoromethyl)phenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7- yl)sulfonyl)glycinate (105f)



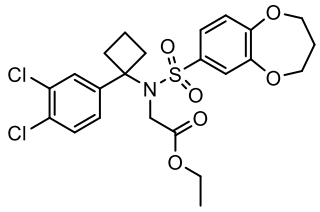
The title compound was synthesized according to the general procedure **I** using *N*-(1-(3-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104f**, 25 mg, 0.056 mmol, 1 eq), ethyl 2-bromoacetate (13 µL, 0.12 mmol, 2 eq) and BEMP (1 M in hexane, 117 µL, 0.117 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (19 mg, 0.037 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dt, *J* = 8.0, 1.6 Hz, 1H), 7.61 (t, *J* = 2.0 Hz, 1H), 7.51 – 7.47 (m, 1H), 7.45 – 7.38 (m, 1H), 7.11 – 7.04 (m, 2H), 6.82 (d, *J* = 8.3 Hz, 1H), 4.27 (t, *J* = 5.8 Hz, 2H), 4.21 (t, *J* = 5.9 Hz, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.10 (s, 2H), 2.90 – 2.78 (m, 2H), 2.56 – 2.45 (m, 2H), 2.21 (p, *J* = 5.8 Hz, 2H), 1.85 – 1.74 (m, 1H), 1.65 – 1.51 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.09, 154.41, 150.37, 143.76, 135.72, 130.76, 130.60 (q, $J_{C\text{-}F}$ = 32.2 Hz), 128.78, 124.34 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.18 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.15 (q, $J_{C\text{-}F}$ = 273.7 Hz), 122.48, 121.41, 120.94, 70.45, 70.28, 65.48, 61.69, 48.21, 34.70, 30.91, 14.62, 14.19.

Ethyl *N*-(1-(4-(trifluoromethoxy)phenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7- yl)sulfonyl)glycinate (105g)



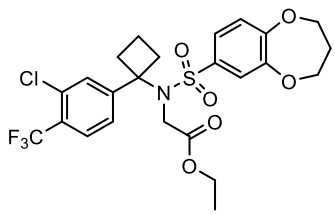
The title compound was synthesized according to the general procedure **I** using *N*-(1-(4-(trifluoromethoxy)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104g**, 58 mg, 0.13 mmol, 1 eq), ethyl 2-bromoacetate (29 µL, 0.26 mmol, 2 eq) and BEMP (1 M in hexane, 262 µL, 0.262 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (40 mg, 0.076 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.41 (m, 2H), 7.10 (d, *J* = 2.3 Hz, 1H), 7.07 – 7.00 (m, 3H), 6.78 (d, *J* = 8.5 Hz, 1H), 4.20 (t, 2H), 4.15 (t, *J* = 5.8 Hz, 2H), 4.08 (q, *J* = 7.1 Hz, 2H), 3.97 (s, 2H), 2.79 – 2.66 (m, 2H), 2.47 – 2.37 (m, 2H), 2.14 (p, *J* = 5.8 Hz, 2H), 1.76 – 1.64 (m, 1H), 1.55 – 1.41 (m, 1H), 1.19 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.13, 154.44, 150.37, 148.44 (q, J_{C-F} = 1.9 Hz), 141.25, 135.89, 128.99, 122.57, 121.41, 121.12, 120.54 (q, J_{C-F} = 258.6 Hz), 120.44, 70.49, 70.39, 65.36, 61.61, 48.02, 34.69, 30.94, 14.64, 14.17.

Ethyl *N*-(1-(3,4-dichlorophenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (105h)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(3,4-dichlorophenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104h**, 29 mg, 0.068 mmol, 1 eq), ethyl 2-bromoacetate (15 µL, 0.14 mmol, 2 eq) and BEMP (1 M in hexane, 136 µL, 0.136 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (28 mg, 0.054 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 2.0 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.13 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.01 (d, *J* = 2.4 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 4.30 (t, *J* = 5.8 Hz, 2H), 4.24 (t, *J* = 5.8 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.12 (s, 2H), 2.85 – 2.73 (m, 2H), 2.50 – 2.39 (m, 2H), 2.22 (p, *J* = 5.8 Hz, 2H), 1.84 – 1.73 (m, 1H), 1.61 – 1.51 (m, 1H), 1.30 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.16, 154.46, 150.32, 142.92, 135.61, 132.28, 131.51, 130.01, 129.93, 126.87, 122.40, 121.38, 120.84, 70.51, 70.39, 65.02, 61.75, 48.19, 34.79, 30.89, 14.61, 14.23. HRMS $[C_{23}H_{25}Cl_2NO_6S+Na]^+$: 536.06718/538.06419 calculated, 536.06655/538.06343 found.

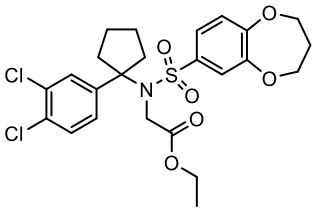
Ethyl *N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (105i)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104i**, 46 mg, 0.10 mmol, 1 eq), ethyl 2-bromoacetate (22 µL, 0.20 mmol, 2 eq) and BEMP (1 M in hexane, 0.20 mL, 0.20 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column

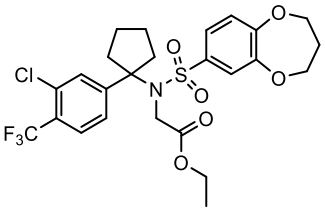
chromatography (20% EtOAc in *n*-pentane) afforded the product (36 mg, 0.066 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 8.7 Hz, 1H), 7.54 – 7.49 (m, 2H), 7.09 – 7.03 (m, 2H), 6.86 – 6.81 (m, 1H), 4.29 (t, *J* = 5.8 Hz, 2H), 4.23 (t, *J* = 5.8 Hz, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 4.15 (s, 2H), 2.88 – 2.76 (m, 2H), 2.54 – 2.43 (m, 2H), 2.21 (p, *J* = 5.8 Hz, 2H), 1.87 – 1.77 (m, 1H), 1.66 – 1.53 (m, 1H), 1.30 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.05, 154.56, 150.47, 148.52, 135.40, 132.20 (q, J_{C-F} = 2.0 Hz), 130.74, 127.37 (q, J_{C-F} = 5.2 Hz), 127.35 (q, J_{C-F} = 32.3 Hz), 125.50, 122.87 (q, J_{C-F} = 274.7 Hz), 122.42, 121.39, 120.72, 70.47, 70.30, 65.16, 61.81, 48.26, 34.74, 30.84, 14.58, 14.21. HRMS $[C_{24}H_{25}ClF_3NO_6S+NH_4]^+$: 565.13815 calculated, 565.13779 found.

Ethyl *N*-(1-(3,4-dichlorophenyl)cyclopentyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (105j)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(3,4-dichlorophenyl)cyclopentyl)-3,4-dihydro-*2H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104j**, 54 mg, 0.12 mmol, 1 eq), ethyl 2-bromoacetate (27 µL, 0.24 mmol, 2 eq) and BEMP (1 M in hexane, 244 µL, 0.244 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (50 mg, 0.095 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 2.1 Hz, 1H), 7.31 – 7.25 (m, 3H), 7.18 (d, *J* = 2.4 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 4.32 (t, *J* = 5.8 Hz, 2H), 4.26 (t, *J* = 5.8 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.18 (s, 2H), 2.36 – 2.28 (m, 2H), 2.27 – 2.19 (m, 4H), 1.76 – 1.63 (m, 2H), 1.42 – 1.32 (m, 2H), 1.29 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.60, 154.51, 150.33, 143.06, 136.19, 132.11, 131.43, 129.90, 129.77, 126.93, 122.71, 121.45, 121.18, 73.09, 70.52, 70.38, 61.63, 48.90, 37.83, 30.91, 21.62, 14.23. HRMS $[C_{24}H_{27}Cl_2NO_6S+Na]^+$: 550.08283/552.07984 calculated, 550.08267/552.07949 found.

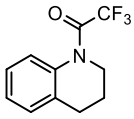
Ethyl *N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentyl)-*N*-((3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepin-7-yl)sulfonyl)glycinate (105k)



The title compound was synthesized according to the general procedure **I** using *N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclopentyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (**104k**, 42 mg, 0.089 mmol, 1 eq), ethyl 2-bromoacetate (19.7 µL, 0.178 mmol, 2 eq) and BEMP (1 M in hexane, 178 µL, 0.178 mmol, 2 eq). Total time: overnight at 80 °C. Silica gel column chromatography (20% EtOAc in *n*-pentane) afforded the product (36 mg, 0.064 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.2 Hz, 1H), 7.48 (d, *J* = 1.8 Hz, 1H), 7.43 (ddd, *J* = 8.1, 1.8, 0.9 Hz, 1H), 7.22 – 7.15 (m, 2H), 6.88 (d, *J* = 8.4 Hz, 1H), 4.31 (t, *J* = 5.9 Hz, 2H), 4.25 (t, *J* = 5.9 Hz, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 4.19 (s, 2H), 2.40 – 2.18 (m, 6H), 1.78 – 1.67 (m, 2H), 1.44 – 1.33 (m, 2H), 1.29 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.48, 154.62, 150.48, 148.86, 135.88, 132.00 (q, J_{C-F} = 1.9 Hz), 130.74, 127.25 (q, J_{C-F} = 32.3 Hz), 127.12 (q, J_{C-F} = 5.2 Hz), 125.60, 122.88 (q, J_{C-F} = 273.7 Hz), 122.75, 121.47, 121.11, 73.27,

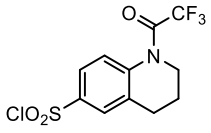
70.50, 70.33, 61.71, 48.93, 37.96, 30.89, 21.76, 14.22. HRMS $[C_{25}H_{27}ClF_3NO_6S+NH_4]^+$: 579.15380 calculated, 579.15333 found.

1-(3,4-Dihydroquinolin-1(2*H*)-yl)-2,2,2-trifluoroethan-1-one (106)



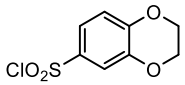
To a solution of 1,2,3,4-tetrahydroquinoline (1.00 g, 7.51 mmol, 1 eq) and Et_3N (3.14 mL, 22.5 mmol, 3 eq) in Et_2O (10 mL, 0.75 M) at 0 °C was added 2,2,2-trifluoroacetic anhydride (2.1 mL, 15 mmol, 2 eq) in Et_2O (3 mL) dropwise. The reaction was warmed to rt and stirred for overnight. The reaction mixture was diluted in water and extracted 3× with Et_2O. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (5-20% Et_2O in *n*-pentane) to afford the product (1.50 g, 6.54 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (bs, 1H), 7.25 – 7.04 (m, 3H), 3.84 (t, *J* = 6.2 Hz, 2H), 2.88 (bs, 2H), 2.08 (p, *J* = 6.7 Hz, 2H).

1-(2,2,2-Trifluoroacetyl)-1,2,3,4-tetrahydroquinoline-6-sulfonyl chloride (107)



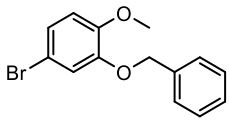
1-(3,4-Dihydroquinolin-1(2*H*)-yl)-2,,2,2-trifluoroethan-1-one (**106**, 0.50 g, 2.2 mmol, 1 eq) was dissolved in anhydrous DCM (10 mL, 0.2 M) and chlorosulfonic acid (150 µL, 2.18 mmol, 1 eq) was added dropwise at 0 °C. The mixture was stirred for 2 h and chlorosulfonic acid (150 µL, 2.18 mmol, 1 eq) was added dropwise at 0 °C. The reaction mixture was slowly warmed to rt and stirred for another 2 h. The reaction was quenched with cool water and extracted 3× with DCM. Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to afford the product (370 mg, 1.13 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 7.97 (m, 1H), 7.94 – 7.87 (m, 2H), 3.97 – 3.90 (m, 2H), 3.05 (t, *J* = 6.9 Hz, 2H), 2.25 – 2.13 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.35 (q, $J_{C\text{-}F}$ = 37.0 Hz), 142.95, 141.15, 132.70, 128.31, 125.79, 125.07, 116.39 (q, $J_{C\text{-}F}$ = 288.3 Hz), 45.26 (q, $J_{C\text{-}F}$ = 3.6 Hz), 26.48, 22.92.

2,3-Dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride (108)



The title compound was synthesized according to general procedure **E** using 6-bromo-2,3-dihydrobenzo[*b*][1,4]dioxine (150 mg, 0.698 mmol, 1 eq) and *n*-BuLi (1.6 M in hexane, 910 µL, 1.46 mmol, 2.1 eq) at -78 °C for 1.5 h, and subsequently SO_2 (0.5 M in hexane, 2.8 mL, 1.4 mmol, 2 eq) at -78 °C to -40 °C for 1 h and *N*-chlorosuccinimide (112 mg, 0.837 mmol, 1.2 eq) at 0 °C for 1 h. Silica gel column chromatography (0-15% EtOAc in *n*-pentane) afforded the product (44.0 mg, 0.188 mmol, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.50 (m, 2H), 7.03 (d, *J* = 8.5 Hz, 1H), 4.40 – 4.36 (m, 2H), 4.36 – 4.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.85, 143.80, 136.56, 121.14, 118.26, 116.90, 64.85, 64.23.

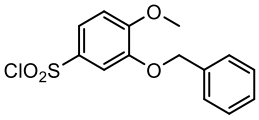
2-(Benzyloxy)-4-bromo-1-methoxybenzene (109)



To a solution of 5-bromo-2-methoxyphenol (2.00 g, 9.80 mmol, 1 eq) in anhydrous DMF (35 mL, 0.28 M) was added K_2CO_3 (2.70 g, 19.6 mmol, 2

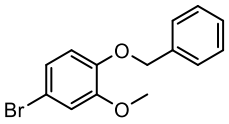
eq) and benzyl bromide (1.40 mL, 11.8 mmol, 1.2 eq). The mixture was heated to 106 °C and stirred overnight. The mixture was cooled down and diluted in EtOAc and water and the aqueous layer was extracted 2× with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (5% EtOAc in *n*-pentane) to afford the product as a white solid (1.9 g, 6.6 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.45 (m, 2H), 7.44 – 7.39 (m, 2H), 7.38 – 7.33 (m, 1H), 7.10 – 7.05 (m, 2H), 6.81 – 6.77 (m, 1H), 5.14 (s, 2H), 3.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.08, 149.03, 136.50, 128.72, 128.17, 127.50, 124.05, 117.29, 113.13, 112.61, 71.26, 56.22.

3-(Benzyloxy)-4-methoxybenzenesulfonyl chloride (110)



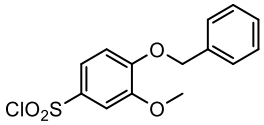
The title compound was synthesized according to general procedure **E** using 2-(benzyloxy)-4-bromo-1-methoxybenzene (**109**, 960 mg, 3.29 mmol, 1 eq) and *n*-BuLi (2.5 M in hexane, 1.58 mL, 3.95 mmol, 1.2 eq) at -78 °C for 1 h, SO_2 (1.2 M in THF, 5.48 mL, 6.58 mmol, 2 eq) at -78 °C to -40 °C for 1 h and then at rt for 1 h, and subsequently *N*-chlorosuccinimide (660 mg, 4.94 mmol. 1.5 eq) at 0 °C for 1 h and at rt for 1 h. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product (390 mg, 1.26 mmol, 39%). ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.66 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.50 (d, *J* = 2.3 Hz, 1H), 7.48 – 7.42 (m, 2H), 7.41 – 7.29 (m, 3H), 6.98 (d, *J* = 8.7 Hz, 1H), 5.16 (s, 2H), 3.95 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.42, 148.40, 135.72, 135.48, 128.80, 128.52, 127.78, 122.03, 111.26, 110.92, 71.40, 56.50.

1-(Benzyloxy)-4-bromo-2-methoxybenzene (111)



To a solution of 4-bromo-2-methoxyphenol (1.45 g, 7.14 mmol, 1 eq) in anhydrous DMF (20 mL, 0.36 M) was added K_2CO_3 (2.80 g, 20.3 mmol, 2.84 eq) and the mixture was stirred at rt for 10 min. Benzyl bromide (1.02 mL, 8.57 mmol, 1.2 eq) was added and the mixture was then stirred at rt for overnight. The mixture was diluted in EtOAc and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (3-10% EtOAc in *n*-pentane) to afforded the product as a white solid (1.93 g, 6.58 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.26 (m, 5H), 6.98 (d, *J* = 2.3 Hz, 1H), 6.93 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 5.09 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.51, 147.41, 136.75, 128.65, 128.04, 127.34, 123.37, 115.36, 115.27, 113.39, 71.19, 56.18.

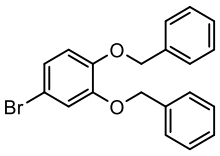
4-(Benzyloxy)-3-methoxybenzenesulfonyl chloride (112)



The title compound was synthesized according to general procedure **E** using 1-(benzyloxy)-4-bromo-2-methoxybenzene (**111**, 800 mg, 2.73 mmol, 1 eq) and *n*-BuLi (2.5 M in hexane, 1.10 mL, 2.73 mmol, 1 eq) at -78 °C for 1 h, and SO_2 (1.2 M in THF, 3.40 mL, 4.09 mmol, 1.5 eq) at -78 °C to -40 °C for 1 h and then at rt for 1 h, and subsequently *N*-chlorosuccinimide (440 mg, 3.27 mmol, 1.2 eq) at

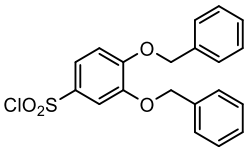
0 °C for 1 h and at rt for 1 h. Silica gel column chromatography (5-20% EtOAc in *n*-pentane) afforded the product as a light yellow solid (610 mg, 1.93 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.48 – 7.31 (m, 6H), 7.00 (d, *J* = 8.7 Hz, 1H), 5.24 (s, 2H), 3.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.96, 149.85, 136.18, 135.44, 128.95, 128.59, 127.38, 121.59, 112.41, 109.44, 71.25, 56.53.

(((4-Bromo-1,2-phenylene)bis(oxy))bis(methylene))dibenzene (113)



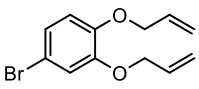
To a solution of 2-bromobenzene-1,2-diol (1.03 g, 5.43 mmol, 1 eq) in anhydrous DMF (22 mL, 0.25 M) was added K_2CO_3 (1.88 g, 13.6 mmol, 2.5 eq) and the mixture stirred at rt for 30 min. Benzyl bromide (1.60 mL, 13.6 mmol, 2.5 eq) was then added dropwise at rt. The reaction mixture was stirred at 90 °C for overnight. The reaction was quenched with sat. NH_4Cl and extracted 3× with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (2-5% EtOAc in *n*-pentane) to afford the product as a white crystalline solid (1.49 g, 4.05 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.27 (m, 10H), 7.06 (d, *J* = 2.3 Hz, 1H), 6.98 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 5.11 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.93, 148.24, 136.99, 136.70, 128.70, 128.65, 128.15, 128.05, 127.47, 127.40, 124.28, 118.20, 116.54, 113.54, 71.56, 71.47.

3,4-Bis(benzyloxy)benzenesulfonyl chloride (114)



The title compound was synthesized according to general procedure **E** using (((4-bromo-1,2-phenylene)bis(oxy))bis(methylene))dibenzene (**113**, 760 mg, 2.05 mmol, 1 eq) and *n*-BuLi (2.5 M in hexane, 0.82 mL, 2.05 mmol, 1 eq) at -78 °C for 1 h, SO_2 (1.2 M in THF, 2.60 mL, 3.12 mmol, 1.5 eq) at -78 °C to -40 °C for 1 h and at rt for 2 h, and subsequently *N*-chlorosuccinimide (330 mg, 2.46 mmol, 1.2 eq) at 0 °C for 1 h and at rt for 1 h. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product as a white crystalline solid (300 mg, 0.783 mmol, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.53 (d, *J* = 2.3 Hz, 1H), 7.48 – 7.31 (m, 10H), 7.01 (d, *J* = 8.7 Hz, 1H), 5.25 (s, 2H), 5.20 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.66, 148.89, 136.14, 135.77, 135.67, 128.91, 128.83, 128.50, 128.48, 127.63, 127.20, 122.00, 113.01, 112.19, 71.57, 71.19.

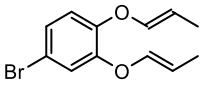
1,2-Bis(allyloxy)-4-bromobenzene (115)



To a mixture of 4-bromobenzene-1,2-diol (1.00 g, 5.29 mmol, 1 eq) in anhydrous DMF (10.6 mL, 0.5 M) was added K_2CO_3 (2.20 g, 15.9 mmol, 3 eq) and allyl bromide (1.37 mL, 15.9 mmol, 3 eq). The mixture was heated to 60 °C and stirred overnight. The mixture was diluted in water and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (2-10% EtOAc in *n*-pentane) to afford the product (362 mg, 1.35 mmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 – 6.93 (m,

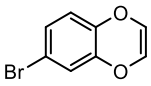
2H), 6.79 – 6.71 (m, 1H), 6.13 – 5.95 (m, 2H), 5.43 (dq, *J* = 8.5, 1.6 Hz, 1H), 5.38 (dq, *J* = 8.6, 1.6 Hz, 1H), 5.30 (dq, *J* = 7.7, 1.3 Hz, 1H), 5.27 (dq, *J* = 7.7, 1.3 Hz, 1H), 4.62 – 4.49 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.39, 147.82, 133.17, 132.87, 123.85, 118.12, 117.95, 117.35, 115.47, 113.12, 70.16, 70.10.

4-Bromo-1,2-bis((prop-1-en-1-yl)oxy)benzene (116)



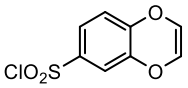
To a solution of 1,2-bis(allyloxy)-4-bromobenzene (**115**, 1.00 g, 3.72 mmol, 1 eq) in anhydrous benzene (12.4 mL, 0.3 M) was added $RuClH(CO)(PPh_3)_3$ (35 mg, 0.037 µmol, 1%) under argon and the mixture was heated to 80 °C for 24 h. The reaction mixture was purified by silica gel column chromatography (1% EtOAc in *n*-pentane) to afford the product (460 mg, 1.71 mmol, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, *J* = 2.3 Hz, 1H), 7.09 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 1H), 6.36 – 6.21 (m, 2H), 5.00 – 4.85 (m, 2H), 1.73 (d, *J* = 1.8 Hz, 3H), 1.72 (d, *J* = 1.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.29, 146.83, 141.43, 140.98, 125.68, 120.15, 118.42, 114.87, 109.13, 108.28, 9.56, 9.54.

6-Bromobenzo[*b*][1,4]dioxine (117)



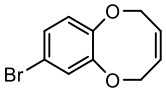
A solution of 4-bromo-1,2-bis((prop-1-en-1-yl)oxy)benzene (**116**, 460 mg, 1.71 mmol, 1 eq) in anhydrous DCM (17 mL, 0.1 M) was degassed under argon. Grubbs catalyst (II) (73 mg, 0.085 mmol, 5%) was added and the mixture was degassed again under argon. The mixture was heated to reflux for overnight. The mixture was diluted in DCM and concentrated. The residue was purified by silica gel column chromatography (100% *n*-pentane) to afford the product (320 mg, 1.50 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.48 (d, *J* = 8.5 Hz, 1H), 5.86 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.55, 142.14, 127.01, 126.77, 119.57, 117.62, 115.57.

Benzo[*b*][1,4]dioxine-6-sulfonyl chloride (118)



The title compound was synthesized according to general procedure **E** using 6-bromobenzo[*b*][1,4]dioxine (**117**, 0.15 g, 0.70 mmol, 1 eq) and *n*-BuLi (2.5 M in hexane, 0.28 mL, 0.70 mmol, 1 eq) at -78 °C for 1 h, SO_2 (1.2 M in THF, 880 µL, 1.06 mmol, 1.5 eq) at -78 °C to -40 °C for 1 h and at rt for 1 h, and subsequently *N*-chlorosuccinimide (113 mg, 0.850 mmol, 1.2 eq) at 0 °C for 1 h and at rt for 1 h. Silica gel column chromatography (6% EtOAc in *n*-pentane) afforded the product (80 mg, 0.34 mmol, 49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.24 (d, *J* = 2.3 Hz, 1H), 6.76 (d, *J* = 8.6 Hz, 1H), 5.93 (q, *J* = 3.6 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.03, 143.49, 139.52, 127.45, 126.87, 124.52, 117.09, 115.38.

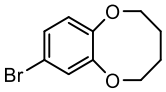
8-Bromo-2,5-dihydrobenzo[*b*][1,4]dioxocine (119)



A solution of 1,2-bis(allyloxy)-4-bromobenzene (**115**, 730 mg, 2.71 mmol, 1 eq) in anhydrous DCM (27 mL, 0.1 M) was degassed with argon. Grubbs catalyst (II) (115 mg, 0.140 mmol, 5%) was added. The mixture was degassed again with argon and refluxed for overnight. The reaction mixture was diluted in DCM and concentrated. The residue

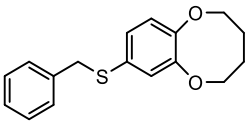
was purified by silica gel column chromatography(0-15% EtOAc in *n*-pentane) to afford the product (0.11 g, 0.46 mmol, 17%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, *J* = 2.4 Hz, 1H), 7.04 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 1H), 5.92 – 5.83 (m, 2H), 4.93 – 4.89 (m, 2H), 4.88 – 4.85 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.14, 147.48, 129.97, 129.00, 126.64, 125.46, 124.24, 115.41, 70.81, 69.97.

8-Bromo-2,3,4,5-tetrahydrobenzo[*b*][1,4]dioxocine (120)



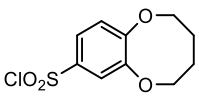
To a solution of 8-bromo-2,5-dihydrobenzo[*b*][1,4]dioxocine (**119**, 54.9 mg, 0.228 mmol, 1 eq) in degassed EtOH (1.9 mL, 0.12 M) was added $RhCl(PPh_3)_3$ (6.1 mg, 6.6 µmol, 0.03 eq). The mixture was degassed with N_2. Subsequently, the N_2 was changed to H_2 and the reaction was bubbled for 2 h at 50 °C. The mixture was filtered through Celite and washed with methanol. The filtrate was concentrated and the residue was purified by silica gel column chromatography (100% *n*-pentane) to afford the product (35.0 mg, 0.144 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 2.5 Hz, 1H), 7.03 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 1H), 4.34 (t, *J* = 5.2 Hz, 2H), 4.29 (t, *J* = 5.2 Hz, 2H), 1.94 – 1.84 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.54, 148.71, 126.35, 125.40, 123.96, 115.03, 72.99, 72.79, 27.00, 26.66.

8-(Benzylthio)-2,3,4,5-tetrahydrobenzo[*b*][1,4]dioxocine (121)



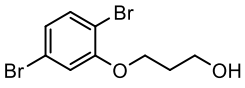
The title compound was synthesized according to the general procedure **F** using 8-bromo-2,3,4,5-tetrahydrobenzo[*b*][1,4]dioxocine (**120**, 63.2 mg, 0.260 mmol, 1 eq), benzyl mercaptan (31 µL, 0.26 mmol, 1 eq), DIPEA (91 µL, 0.52 mmol, 2 eq), xantphos (15 mg, 0.026 mmol, 0.1 eq) and $Pd_2(dba)_3$ (12 mg, 0.013 mmol, 0.05 eq). Total time: 4 h at 100 °C. Silica gel column chromatography (100% *n*-pentane) afforded the product (55.8 mg, 0.195 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.21 (m, 5H), 6.93 (d, *J* = 2.2 Hz, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.86 (s, 1H), 4.34 – 4.25 (m, 4H), 4.03 (s, 2H), 1.91 – 1.84 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.32, 149.04, 137.80, 129.70, 129.00, 128.53, 127.20, 126.67, 125.49, 122.67, 73.02, 72.66, 40.31, 27.06, 26.70.

2,3,4,5-Tetrahydrobenzo[*b*][1,4]dioxocine-8-sulfonyl chloride (122)



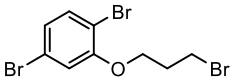
The title compound was synthesized according to the general procedure **G** using 8-(benzylthio)-2,3,4,5-tetrahydrobenzo[*b*][1,4]dioxocine (**121**, 55.8 mg, 0.195 mmol, 1 eq) and *N*-chlorosuccinimide (104 mg, 0.779 mmol, 4 eq). Total time: 2 h at rt. The product was afforded without purification (91.7 mg, 0.164 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 2.5 Hz, 1H), 7.64 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 4.62 (t, *J* = 5.7 Hz, 2H), 4.30 (t, *J* = 5.8 Hz, 2H), 2.07 – 2.00 (m, 2H), 1.90 – 1.84 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.74, 147.07, 136.98, 124.04, 123.81, 121.94, 75.12, 71.29, 28.19, 24.49.

3-(2,5-Dibromophenoxy)propan-1-ol (123)



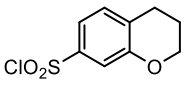
To a mixture of 1,4-dibromo-2-fluorobenzene (520 mg, 2.05 mmol, 1 eq) in propane-1,3-diol (2.7 mL) and 1-methylpyrrolidin-2-one (0.26 mL) was added *t*-BuOK (800 mg, 7.17 mmol, 3.5 eq) portion-wise under N_2 at rt. The resulting dark mixture was stirred at 100 °C for overnight. The mixture was poured into water and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (5-20% EtOAc in *n*-pentane) to afford the product (510 mg, 1.64 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 2.1 Hz, 1H), 6.97 (dd, *J* = 8.3, 2.1 Hz, 1H), 4.16 (t, *J* = 5.8 Hz, 2H), 3.90 (t, *J* = 5.7 Hz, 2H), 2.33 (s, 1H), 2.10 (p, *J* = 5.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.76, 134.10, 125.03, 121.67, 116.41, 110.93, 67.59, 60.44, 31.72.

1,4-Dibromo-2-(3-bromopropoxy)benzene (124)



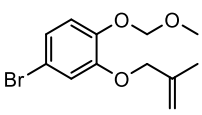
To a solution of 3-(2,5-dibromophenoxy)propan-1-ol (**123**, 500 mg, 1.61 mmol, 1 eq) in toluene (6.5 mL, 0.25 M) was added PBr_3 (0.22 g, 0.81 mmol, 0.5 eq) at 0 °C. The mixture was heated to 100 °C for 2 h. The mixture was poured into ice-water and extracted 3× with EtOAc. Combined organic layers were washed with sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-1% EtOAc in *n*-pentane) to afford the product (430 mg, 1.14 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.3 Hz, 1H), 7.03 (d, *J* = 2.1 Hz, 1H), 6.98 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.14 (t, *J* = 5.7 Hz, 2H), 3.67 (t, *J* = 6.3 Hz, 2H), 2.36 (p, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.72, 134.24, 125.17, 121.65, 116.72, 111.19, 66.73, 32.16, 29.93.

Chromane-7-sulfonyl chloride (125)



The title compound was synthesized according to general procedure **E** using 1,4-dibromo-2-(3-bromopropoxy)benzene (**124**, 0.20 g, 0.54 mmol, 1 eq) and *n*-BuLi (2.5 M in hexane, 430 µL, 1.07 mmol, 1 eq) at -78 °C for 2 h, SO_2 (1.2 M in THF, 670 µL, 0.805 mmol, 1.5 eq) at -78 °C to -40 °C for 1 h and at rt for 1 h, and subsequently *N*-chlorosuccinimide (86 mg, 0.64 mmol, 1.2 eq) at 0 °C for 1 h and at rt for 1 h. Silica gel column chromatography (6% EtOAc in *n*-pentane) afforded the product (62.0 mg, 0.266 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.38 (m, 2H), 7.28 – 7.21 (m, 1H), 4.30 – 4.24 (m, 2H), 2.89 (t, *J* = 6.5 Hz, 2H), 2.10 – 2.02 (m, 2H).

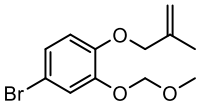
4-Bromo-1-(methoxymethoxy)-2-((2-methylallyl)oxy)benzene (126)



To a stirred solution of 2-methylprop-2-en-1-ol (2.06 g, 28.5 mmol, 2 eq) in anhydrous DMF (27 mL, 0.5 M) was added NaH (60% w/w in mineral oil, 1.14 g, 15.3 mmol, 2 eq) portion-wise under N_2 at 0 °C. The mixture was stirred at 0 °C for 30 min. To the mixture was added 4-bromo-2-fluoro-1-

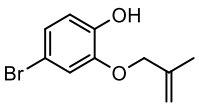
(methoxymethoxy)benzene (3.35 g, 14.3 mmol, 1 eq) in anhydrous DMF (2 mL) and the mixture was stirred at rt for 24 h. The reaction was quenched with ice cold water and extracted 3× with EtOAc. The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (1-3% EtOAc in *n*-pentane) to afford the product (2.48 g, 8.64 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (s, 3H), 5.18 (s, 2H), 5.09 (p, *J* = 1.3 Hz, 1H), 5.00 (p, *J* = 1.3 Hz, 1H), 4.48 (s, 2H), 3.51 (s, 3H), 1.82 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.04, 146.08, 140.22, 123.99, 118.80, 117.38, 114.72, 113.22, 95.83, 72.87, 56.38, 19.42.

4-Bromo-2-(methoxymethoxy)-1-((2-methylallyl)oxy)benzene (127)



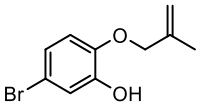
To a stirred solution of 2-methylprop-2-en-1-ol (1.10 g, 15.3 mmol, 2 eq) in anhydrous DMF (15 mL, 0.5 M) was added NaH (60% w/w in mineral oil, 610 mg, 15.3 mmol, 2 eq) portion-wise under N_2 at 0 °C. The mixture was stirred at 0 °C for 30 min. To the mixture was added 4-bromo-1-fluoro-2-(methoxymethoxy)benzene (1.80 g, 7.66 mmol, 1 eq) in anhydrous DMF (0.4 mL) and the mixture was stirred at rt for 3 days. The reaction was quenched with water and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (2-5% EtOAc in *n*-pentane) to afford the product (1.12 g, 3.90 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 2.4 Hz, 1H), 7.05 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 5.20 (s, 2H), 5.08 – 5.04 (m, 1H), 5.00 – 4.95 (m, 1H), 4.47 (s, 2H), 3.51 (s, 3H), 1.81 (dd, *J* = 1.5, 0.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.41, 147.64, 140.46, 125.23, 120.48, 115.34, 113.00, 112.94, 95.72, 72.83, 56.36, 19.33.

4-Bromo-2-((2-methylallyl)oxy)phenol (128)



To a solution of 4-bromo-1-(methoxymethoxy)-2-((2-methylallyl)oxy)benzene (**126**, 2.44 g, 8.50 mmol, 1 eq) in anhydrous DCM (28.3 mL, 0.25 M) was added TFA (5.66 mL, 8.7 eq) at 0 °C. The reaction was stirred at 0 °C until completion. The mixture was diluted in DCM and washed with cold water, cold sat. $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (1-5% EtOAc in *n*-pentane) to afford the product (1.88 g, 7.72 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 – 6.95 (m, 2H), 6.81 (d, *J* = 8.3 Hz, 1H), 5.61 (s, 1H), 5.10 – 5.06 (m, 1H), 5.06 – 5.02 (m, 1H), 4.48 (t, *J* = 1.2 Hz, 2H), 1.83 (dd, *J* = 1.5, 0.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.40, 145.15, 139.86, 124.52, 116.00, 115.61, 113.99, 111.58, 73.06, 19.49.

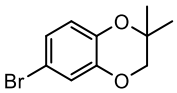
5-Bromo-2-((2-methylallyl)oxy)phenol (129)



To a solution of 4-bromo-2-(methoxymethoxy)-1-((2-methylallyl)oxy)benzene (**127**, 1.00 g, 3.48 mmol, 1 eq) in anhydrous DCM (17 mL, 0.16 M) was added TFA (5 mL, 18.8 eq) slowly at 0 °C. The reaction was stirred at 0 °C until completion. The mixture was diluted in DCM and washed with cold

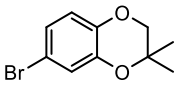
water, cold sat. $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (1-5% EtOAc in *n*-pentane) to afford the product (670 mg, 2.77 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, *J* = 2.4 Hz, 1H), 6.93 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.71 (d, *J* = 8.6 Hz, 1H), 5.71 (s, 1H), 5.08 – 5.05 (m, 1H), 5.04 – 5.01 (m, 1H), 4.48 (s, 2H), 1.82 (dd, *J* = 1.5, 0.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.80, 145.01, 140.09, 122.87, 118.10, 113.84, 113.61, 113.46, 73.00, 19.47.

6-Bromo-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (130)



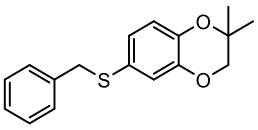
The solution of 4-bromo-2-((2-methylallyl)oxy)phenol (**128**, 1.85 g, 7.61 mmol, 1 eq) in formic acid (15 mL, 0.5 M) was refluxed for 30 min. The reaction mixture was diluted in EtOAc and washed with sat. $NaHCO_3$, water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-4% EtOAc in *n*-pentane) to afford the product (650 mg, 2.68 mmol, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (d, *J* = 2.3 Hz, 1H), 6.94 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.70 (d, *J* = 8.6 Hz, 1H), 3.86 (s, 2H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.14, 142.04, 124.71, 119.98, 118.98, 112.26, 72.60, 71.97, 23.37.

7-Bromo-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (131)



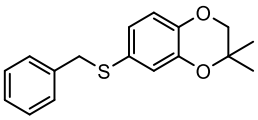
The solution of 5-bromo-2-((2-methylallyl)oxy)phenol (**129**, 674 mg, 2.77 mmol, 1 eq) in formic acid (14 mL, 0.2 M) was refluxed for 30 min. The reaction mixture was diluted in EtOAc and washed with sat. $NaHCO_3$, water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-2% EtOAc in *n*-pentane) to afford the product (276 mg, 1.14 mmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (d, *J* = 2.3 Hz, 1H), 6.92 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 3.86 (s, 2H), 1.33 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.65, 141.64, 123.74, 120.65, 118.27, 113.29, 72.83, 71.93, 23.39.

6-(Benzylthio)-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (132)



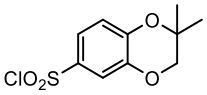
The title compound was synthesized according to the general procedure **F** using 6-bromo-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (**130**, 620 mg, 2.55 mmol, 1 eq), benzyl mercaptan (299 µL, 2.55 mmol, 1 eq), DIPEA (888 µL, 5.10 mmol, 2 eq), xantphos (148 mg, 0.26 mmol, 0.1 eq) and $Pd_2(dba)_3$ (116 mg, 0.130 mmol, 0.05 eq). Total time: 4 h at 100 °C. Silica gel column chromatography (1-5% EtOAc in *n*-pentane) afforded the product (547 mg, 1.91 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.18 (m, 5H), 6.89 (d, *J* = 2.2 Hz, 1H), 6.82 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 4.00 (s, 2H), 3.83 (s, 2H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.29, 142.25, 138.00, 128.97, 128.48, 127.13, 126.77, 125.67, 120.43, 117.96, 72.58, 71.92, 40.90, 23.39.

7-(Benzylthio)-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (133)



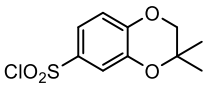
The title compound was synthesized according to the general procedure **F** using 7-bromo-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (**131**, 250 mg, 1.03 mmol, 1 eq), benzyl mercaptan (121 µL, 1.03 mmol, 1 eq), DIPEA (358 µL, 2.03 mmol, 2 eq), xantphos (59.5 mg, 0.100 mmol, 0.1 eq) and $Pd_2(dba)_3$ (47 mg, 0.050 mmol, 0.05 eq). Total time: 4 h at 100 °C. Silica gel column chromatography (1-5% EtOAc in *n*-pentane) afforded the product (257 mg, 0.897 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.17 (m, 5H), 6.86 (d, *J* = 1.7 Hz, 1H), 6.78 – 6.76 (m, 2H), 4.01 (s, 2H), 3.84 (s, 2H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.77, 141.74, 137.92, 128.98, 128.49, 127.92, 127.13, 124.42, 120.80, 117.25, 72.52, 71.99, 40.69, 23.39.

2,2-Dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride (134)



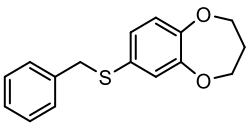
The title compound was synthesized according to the general procedure **G** using 6-(benzylthio)-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (**132**, 537 mg, 1.88 mmol, 1 eq) and *N*-chlorosuccinimide (1.00 g, 7.50 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (1-5% EtOAc in *n*-pentane) afforded the product (472 mg, 1.80 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.51 (m, 2H), 6.98 (d, *J* = 8.5 Hz, 1H), 3.96 (s, 2H), 1.40 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.24, 142.47, 136.07, 121.61, 118.44, 116.59, 74.48, 71.87, 23.45.

3,3-Dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonyl chloride (135)



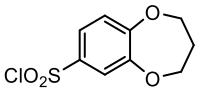
The title compound was synthesized according to the general procedure **G** using 7-(benzylthio)-2,2-dimethyl-2,3-dihydrobenzo[*b*][1,4]dioxine (**133**, 257 mg, 0.900 mmol, 1 eq) and *N*-chlorosuccinimide (479 mg, 3.58 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (1-5% EtOAc in *n*-pentane) afforded the product (244 mg, 0.929 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.47 (m, 2H), 7.09 – 6.99 (m, 1H), 3.99 (s, 2H), 1.38 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.55, 143.00, 137.05, 120.60, 117.78, 117.15, 73.36, 72.29, 23.35.

7-(Benzylthio)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine (136)



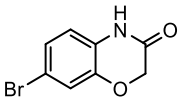
The title compound was synthesized according to the general procedure **F** using 7-bromo-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine (250 mg, 1.09 mmol, 1 eq), benzyl mercaptan (128 µL, 1.09 mmol, 1 eq), DIPEA (380 µL, 2.18 mmol, 2 eq), xantphos (63 mg, 0.11 mmol, 0.1 eq) and $Pd_2(dba)_3$ (50 mg, 0.060 mmol, 0.05 eq). Total time: 4 h at 100 °C. Silica gel column chromatography (1-5% EtOAc in *n*-pentane) afforded the product (219 mg, 0.804 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.19 (m, 5H), 6.96 (d, *J* = 2.2 Hz, 1H), 6.89 – 6.83 (m, 2H), 4.19 – 4.15 (m, 4H), 4.03 (s, 2H), 2.16 (p, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.30, 150.48, 137.69, 129.97, 128.95, 128.54, 127.22, 126.17, 124.19, 122.00, 70.67, 70.64, 40.14, 31.83.

3,4-Dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonyl chloride (137)



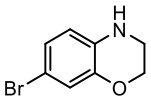
The title compound was synthesized according to the general procedure **G** using 7-(benzylthio)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine (**136**, 219 mg, 0.810 mmol, 1 eq) and *N*-chlorosuccinimide (430 mg, 3.22 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (1-5% EtOAc in *n*-pentane) afforded the product as a white solid (181 mg, 0.728 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 2.4 Hz, 1H), 7.58 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.09 (d, *J* = 8.6 Hz, 1H), 4.41 (t, *J* = 6.0 Hz, 2H), 4.35 (t, *J* = 6.0 Hz, 2H), 2.29 (p, *J* = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.01, 150.77, 137.86, 122.65, 122.25, 121.12, 70.72, 70.42, 30.36.

7-Bromo-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (138)



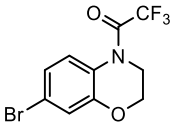
To a solution of 2-amino-5-bromophenol (300 mg, 1.60 mmol, 1 eq) in anhydrous DMF (6.4 mL, 0.25 M) was added K_2CO_3 (440 mg, 3.19 mmol, 2 eq) at rt and the mixture was stirred for 30 min. 2-chloroacetyl chloride (127 µL, 1.60 mmol, 1 eq) was then added dropwise and the reaction was heated to 80 °C for 3 h. The reaction mixture was diluted in water and extracted 3× with DCM. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (40% EtOAc in *n*-pentane) to afford the product (290 mg, 1.29 mmol, 81%). ^{1}H NMR (400 MHz, DMSO) δ 10.82 (s, 1H), 7.17 (d, *J* = 2.1 Hz, 1H), 7.13 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.82 (d, *J* = 8.3 Hz, 1H), 4.60 (s, 2H).

7-Bromo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (139)



To a solution of 7-bromo-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (**138**, 270 mg, 1.19 mmol, 1 eq) in anhydrous THF (7.4 mL, 0.16 M) under argon was added borane-THF complex (1 M in THF, 2.38 mL, 2.38 mmol, 2 eq). The mixture was refluxed for 1 h. The reaction was quenched with water and 2 M aq. NaOH and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (20% EtOAc in *n*-pentane) to afford the product (220 mg, 1.04 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (d, *J* = 2.2 Hz, 1H), 6.84 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.44 (d, *J* = 8.4 Hz, 1H), 4.24 – 4.19 (m, 2H), 3.76 (bs, 1H), 3.41 – 3.36 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.84, 132.96, 124.05, 119.72, 116.58, 109.73, 65.26, 40.75.

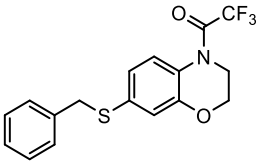
1-(7-Bromo-2,3-dihydro-4*H*-benzo[*b*][1,4]oxazin-4-yl)-2,2,2-trifluoroethan-1-one (140)



To a solution of 7-bromo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**139**, 220 mg, 1.04 mmol, 1 eq) and Et_3N (440 µL, 3.13 mmol, 3 eq) in Et_2O (2.9 mL, 0.25 M) at 0 °C was added 2,2,2-trifluoroacetic anhydride (290 µL, 2.09 mmol, 2 eq) in Et_2O (1.25 mL) dropwise. The reaction was warmed to rt and stirred overnight. The mixture was diluted in water and extracted 3× with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified

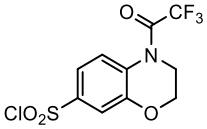
by silica gel column chromatography (10% EtOAc in *n*-pentane) to afford the product (0.30 g, 0.96 mmol, 92%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (bs, 1H), 7.11 (d, *J* = 2.2 Hz, 1H), 7.07 (dd, *J* = 8.9, 2.3 Hz, 1H), 4.42 – 4.35 (m, 2H), 3.98 (t, *J* = 4.7 Hz, 2H).

1-(7-(Benzylthio)-2,3-dihydro-4*H*-benzo[*b*][1,4]oxazin-4-yl)-2,2,2-trifluoroethan-1-one (141)



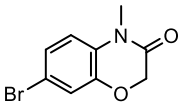
The title compound was synthesized according to the general procedure **F** using 1-(7-bromo-2,3-dihydro-4*H*-benzo[*b*][1,4]oxazin-4-yl)-2,2,2-trifluoroethan-1-one (**140**, 0.27 g, 0.87 mmol, 1 eq), benzyl mercaptan (0.10 mL, 0.87 mmol, 1 eq), DIPEA (300 µL, 1.73 mmol, 2 eq), xantphos (50 mg, 0.087 mmol, 0.1 eq) and $Pd_2(dba)_3$ (40 mg, 0.043 mmol, 0.05 eq). Total time: 4 h at 100°C. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (0.27 g, 0.76 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.35 – 7.19 (m, 5H), 6.90 – 6.84 (m, 2H), 4.37 – 4.32 (m, 2H), 4.11 (s, 2H), 3.95 (t, *J* = 4.6 Hz, 2H).

4-(2,2,2-Trifluoroacetyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonyl chloride (142)



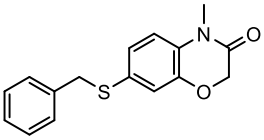
The title compound was synthesized according to the general procedure **G** using 1-(7-(benzylthio)-2,3-dihydro-4*H*-benzo[*b*][1,4]oxazin-4-yl)-2,2,2-trifluoroethan-1-one (**141**, 268 mg, 0.597 mmol, 1 eq) and *N*-chlorosuccinimide (406 mg, 3.04 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (10-15% EtOAc in *n*-pentane) afforded the product (186 mg, 0.565 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 8.8 Hz, 1H), 7.65 – 7.59 (m, 2H), 4.51 – 4.46 (m, 2H), 4.09 – 4.04 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.08 (q, $J_{C\text{-}F}$ = 37.5 Hz), 147.30, 142.02, 129.90, 125.01, 119.13, 116.86, 116.05 (q, $J_{C\text{-}F}$ = 288.9 Hz), 65.92, 43.27 (q, $J_{C\text{-}F}$ = 3.9 Hz).

7-Bromo-4-methyl-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (143)



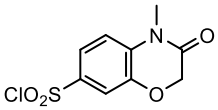
To a solution of 7-bromo-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (**138**, 300 mg, 1.32 mmol, 1 eq) in anhydrous DMF (5.3 mL, 0.25 M) under N_2 was added *t*-BuOK (295 mg, 2.63 mmol, 2 eq) and the mixture was stirred at rt for 30 min. CH_3I (160 µL, 2.63 mmol, 2 eq) was added drop-wise and stirred at rt for overnight. The reaction was quenched with water and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (25% EtOAc in *n*-pentane) to afford the product (183 mg, 0.756 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.62 (s, 2H), 3.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.08, 145.83, 128.98, 125.73, 120.17, 116.08, 116.00, 67.64, 28.21.

7-(Benzylthio)-4-methyl-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (144)



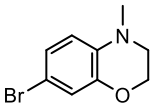
The title compound was synthesized according to the general procedure **F** using 7-bromo-4-methyl-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (**143**, 160 mg, 0.660 mmol, 1 eq), benzyl mercaptan (78 µL, 0.66 mmol, 1 eq), DIPEA (230 µL, 1.62 mmol, 2 eq), xantphos (38 mg, 0.066 mmol, 0.1 eq) and $Pd_2(dba)_3$ (30 mg, 0.033 mmol, 0.05 eq). Total time: 4 h at 100 °C. Silica gel column chromatography (20-25% EtOAc in *n*-pentane) afforded the product (0.11 g, 0.39 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.20 (m, 5H), 6.99 – 6.93 (m, 2H), 6.87 – 6.77 (m, 1H), 4.58 (s, 2H), 4.07 (s, 2H), 3.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.28, 145.22, 137.39, 131.34, 128.91, 128.63, 128.43, 127.38, 125.04, 118.85, 115.08, 67.65, 39.73, 28.15. LC-MS $[C_{16}H_{15}NO_2S+H]^+$: 286.09 calculated, 286.17 found.

4-Methyl-3-oxo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonyl chloride (145)



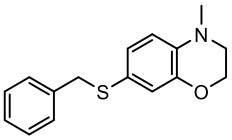
The title compound was synthesized according to the general procedure **G** using 7-(benzylthio)-4-methyl-2*H*-benzo[*b*][1,4]oxazin-3(*4H*)-one (**144**, 115 mg, 0.400 mmol, 1 eq) and *N*-chlorosuccinimide (214 mg, 1.61 mmol, 4 eq). Total time: 2 h at rt. The product was afforded without purification (110 mg, 0.420 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.63 (d, *J* = 2.2 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 4.75 (d, *J* = 0.6 Hz, 2H), 3.43 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.21, 145.02, 138.77, 135.53, 122.29, 115.59, 115.18, 67.29, 28.47.

7-Bromo-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (146)



To a solution of 7-bromo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**139**, 230 mg, 1.07 mmol, 1 eq) in anhydrous DMF (4.7 mL, 0.23 M) under N_2 at 0 °C was added NaH (60% w/w in mineral oil, 86.0 mg, 2.15 mmol, 2 eq). The mixture was stirred at 0 °C for 20 min. Subsequently, CH_3I (74.0 µL, 1.18 mmol, 1.1 eq) was added and the mixture was stirred at 0 °C for 30 min. More CH_3I (74.0 µL, 1.18 mmol, 1.1 eq) was added and the mixture was stirred at rt for another 4 h. The mixture was diluted in water and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-pentane) to afford the product (185 mg, 0.811 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.88 (d, *J* = 2.3 Hz, 1H), , 6.49 (d, *J* = 8.5 Hz, 1H), 4.30 – 4.22 (m, 2H), 3.26 – 3.19 (m, 2H), 2.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.08, 135.89, 124.08, 118.82, 113.56, 109.31, 65.07, 48.90, 38.79. LC-MS $[C_9H_{10}BrNO+H]^+$: 228.00 calculated, 228.08 found.

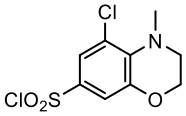
7-(Benzylthio)-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (147)



The title compound was synthesized according to the general procedure **F** using 7-bromo-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**146**, 185 mg, 0.810 mmol, 1 eq), benzyl mercaptan (95 µL, 0.81 mmol, 1 eq), DIPEA

(280 µL, 1.62 mmol, 2 eq), xantphos (47 mg, 0.081 mmol, 0.1 eq) and $Pd_2(dba)_3$ (37 mg, 0.041 mmol, 0.05 eq). Total time: 4 h at 100 °C. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (123 mg, 0.453 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.16 (m, 5H), 6.84 – 6.79 (m, 2H), 6.51 (d, *J* = 8.8 Hz, 1H), 4.27 – 4.20 (m, 2H), 3.97 (s, 2H), 3.26 – 3.19 (m, 2H), 2.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.21, 138.40, 136.29, 134.86, 128.96, 128.50, 126.97, 126.22, 120.04, 112.51, 64.86, 49.01, 41.49, 38.69. LC-MS $[C_{16}H_{17}NOS+H]^+$: 272.11 calculated, 272.17 found.

5-Chloro-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-7-sulfonyl chloride (148)



The title compound was synthesized according to the general procedure **G** using 7-(benzylthio)-4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**147**, 123 mg, 0.450 mmol, 1 eq) and *N*-chlorosuccinimide (242 mg, 1.81 mmol, 4 eq). Total time: 2 h at rt. Silica gel column chromatography (5-10% EtOAc in *n*-pentane) afforded the product (88 mg, 0.31 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.3 Hz, 1H), 7.42 (d, *J* = 2.3 Hz, 1H), 4.23 – 4.18 (m, 2H), 3.27 – 3.23 (m, 2H), 3.07 (s, 3H). LC-MS $[C_9H_9Cl_2NO_3S+H]^+$: 281.98 calculated, 282.00 found.

Reference

1. Baggelaar, M. P., Maccarrone, M. & van der Stelt, M. 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. *Prog. Lipid. Res.* **71**, 1–17 (2018).
2. Zhu, N., Janssen, A. P. A. & van der Stelt, M. Understanding and Targeting the Endocannabinoid System with Activity-Based Protein Profiling. *Isr. J. Chem.* **63**, e202200115 (2023).
3. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163**, 463–468 (2003).
4. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356 (2007).
5. Kozak, K. R., Rowlinson, S. W. & Marnett, L. J. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J. Biol. Chem.* **275**, 33744–33749 (2000).
6. Rouzer, C. A. & Marnett, L. J. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: Cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem. Rev.* **111**, 5899–5921 (2011).
7. Gao, Y. *et al.* Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J. Neurosci.* **30**, 2017–2024 (2010).
8. Hsu, K.-L. *et al.* DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat. Chem. Biol.* **8**, 999–1007 (2012).
9. Baggelaar, M. P. *et al.* Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J. Am. Chem. Soc.* **137**, 8851–8857 (2015).
10. Viader, A. *et al.* A chemical proteomic atlas of brain serine hydrolases identifies cell type-specific pathways regulating neuroinflammation. *Elife* **5**, 1–24 (2016).
11. Shin, M., Buckner, A., Prince, J., Bullock, T. N. J. & Hsu, K. L. Diacylglycerol Lipase-β Is Required for TNF-α Response but Not $CD8^+$ T Cell Priming Capacity of Dendritic Cells. *Cell Chem. Biol.* **26**, 1036–1041 (2019).
12. Jenniches, I. *et al.* Anxiety, Stress, and Fear Response in Mice with Reduced Endocannabinoid Levels. *Biol. Psychiatry* **79**, 858–868 (2016).
13. Cavener, V. S. *et al.* Inhibition of Diacylglycerol Lipase Impairs Fear Extinction in Mice. *Front. Neurosci.* **12**, 1–10 (2018).
14. Beins, E. C. *et al.* Cannabinoid receptor 1 signalling modulates stress susceptibility and microglial responses to chronic social defeat stress. *Transl. Psychiatry* **11**, (2021).

15. Zhong, P. *et al.* Monoacylglycerol lipase inhibition blocks chronic stress-induced depressive-like behaviors via activation of mTOR signaling. *Neuropsychopharmacology* **39**, 1763–1776 (2014).

16. Zhang, Z. *et al.* Blockade of 2-arachidonoylglycerol hydrolysis produces antidepressant-like effects and enhances adult hippocampal neurogenesis and synaptic plasticity. *Hippocampus* **25**, 16–26 (2015).

17. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 26–33 (2016).

18. Wilkerson, J. L. *et al.* Diacylglycerol lipase β inhibition reverses nociceptive behaviour in mouse models of inflammatory and neuropathic pain. *Br. J. Pharmacol.* **173**, 1678–1692 (2016).

19. Chupak, L. S. *et al.* Structure activity relationship studies on chemically non-reactive glycine sulfonamide inhibitors of diacylglycerol lipase. *Bioorg. Med. Chem.* **24**, 1455–1468 (2016).

20. Lavey, B. J. *et al.* Optimization of triaryl bis-sulfones as cannabinoid-2 receptor ligands. *Bioorg Med. Chem. Lett.* **17**, 3760–3764 (2007).

21. Morgans, G. L. *et al.* Synthesis of unsaturated 1,4-heteroatom-containing benzo-fused heterocycles using a sequential isomerization – ring-closing metathesis strategy. *Tetrahedron* **65**, 10650–10659 (2009).

22. Martín-Gago, P. *et al.* A PDE6δ-KRas Inhibitor Chemotype with up to Seven H-Bonds and Picomolar Affinity that Prevents Efficient Inhibitor Release by Arl2. *Angew. Chem. Int. Ed.* **56**, 2423–2428 (2017).

23. Yu, W. *et al.* Discovery of Chromane Containing Hepatitis C Virus (HCV) NS5A Inhibitors with Improved Potency against Resistance-Associated Variants. *J. Med. Chem.* **59**, 10228–10243 (2016).

Chapter 5

Profiling DAGL inhibitors in the $GRAB_{eCB2.0}$ assay reveals the primary role of DAGLα in ATP-stimulated 2-AG production in Neuro2A

5.1 Introduction

The endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) are widely distributed throughout central and peripheral nervous systems, where they play physiological roles primarily by activating cannabinoid receptors type 1 and 2 (CB_1R and CB_2R).[1] Endocannabinoids, cannabinoid receptors and enzymes responsible for the biosynthesis and degradation of endocannabinoids constitute the endocannabinoid system (ECS). In the brain, endocannabinoids are produced and released from the postsynaptic terminal upon demand[2] to activate CB_1R on the presynaptic terminal. This process is distinct from classical neurotransmitters that are stored in synaptic vesicles and released from presynaptic compartments.[3,4] In addition, glial cells[5] and intracellular organelles such as mitochondria[6,7] also contain components of the endocannabinoid system.

The biosynthesis of 2-AG is primarily mediated by *sn*1-specific diacylglycerol lipases (DAGLs), after which 2-AG is degraded by monoacylglycerol lipase (MAGL) and α/β-hydrolase domain-containing 6 and 12 (ABHD6/12) to form arachidonic acid and glycerol.[8] The production and degradation of 2-AG are tightly regulated to maintain 2-AG levels. To qualitatively and quantitatively measure 2-AG levels, liquid chromatography coupled to mass spectrometry (LC/MS) is commonly employed.[9,10] Despite being highly sensitive, this method requires sample preparation and lipid extraction, limiting its application in living cells and organisms and resulting in poor temporal and spatial resolution. In recent years, various genetically encoded fluorescent sensors[11–13] based on G protein-coupled receptors (GPCRs) and circular-permutated fluorescent proteins (cpFPs) have been developed. These sensors enable the real-time detection of neurotransmitters and neuromodulators.[14] This technology relies on the conformational change[15] of the specific receptors induced by the binding of the ligands to elicit fluorescence of cpFP. To detect the dynamics of endocannabinoids, Yulong Li's lab recently developed a GPCR activation-based endocannabinoid sensor called $GRAB_{eCB2.0}$ (Figure 5.1) based on human CB_1R and circular permutated enhanced green fluorescent protein (cpEGFP).[16] The $GRAB_{eCB2.0}$ sensor exhibits high specificity and rapid kinetics for 2-AG and AEA, and it has been successfully applied in cultured neurons, brain slices and living animals.[16–19]

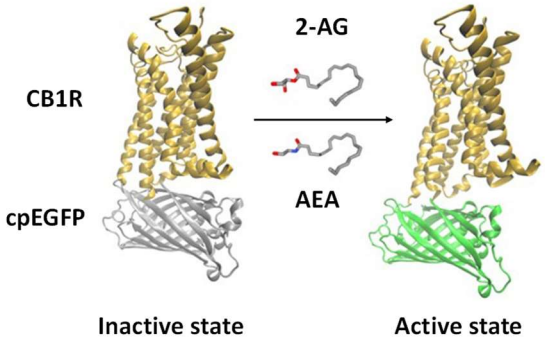


Figure 5.1 Schematic illustration of $GRAB_{eCB2.0}$ activation upon the binding of 2-AG and AEA.[16] The figure was adapted from Dong, A. *et al. Nat. Biotechnol.* **40**, 787-798 (2021).

In view of the successful applications of GRAB$_{eCB2.0}$ in monitoring the 2-AG dynamics, we aimed to develop a fluorescence-based plate reader assay by using this sensor to evaluate the cellular activity of DAGL inhibitors. To achieve this, GRAB$_{eCB2.0}$ was initially transiently transfected into mouse neuroblastoma cells (Neuro2A). The success of transient transfection and expression was confirmed by EVOS fluorescent imaging and kinetic fluorescent measurement in the plate reader. The sensor demonstrated direct activation upon the addition of 2-AG and AEA, as well as indirect activation by adding adenosine triphosphate (ATP) to the medium. Subsequently, a total of 23 DAGL inhibitors were profiled in the GRAB$_{eCB2.0}$ assay using Neuro2A cells stably expressing the sensor. This profiling revealed the primary role of DAGLα in ATP-stimulated 2-AG production.

5.2 Results and discussion

5.2.1 Transient expression and activation of the GRAB$_{eCB2.0}$ in Neuro2A

To express the GRAB$_{eCB2.0}$ sensor, Neuro2A cells were transfected with eCB2.0 plasmid. At the same time, inactive eCBmut, green fluorescent protein (GFP) and an empty vector were transfected as well to serve as controls. Strong green fluorescence was observed for Neuro2A cells transfected with GFP (Figure 5.2), indicating the success of transfection and protein expression. Green fluorescence was also detectable in cells transfected with eCB2.0, which proved that the sensor was successfully expressed in the cells and the basal level of 2-AG was able to partially activate the sensor. In contrast, the basal fluorescent signal in cells expressing inactive eCBmut was low, and no fluorescence was detected in mock transfected and untransfected Neuro2A cells. Addition of a synthetic agonist of the CB_1R, CP55,940 (1 μM), increased fluorescence in the eCB2.0 transfected but not in the eCBmut transfected cells (Figure 5.2, bottom row). The fluorescent signal was different in each Neuro2A cell because of the heterogeneity of transient expression.

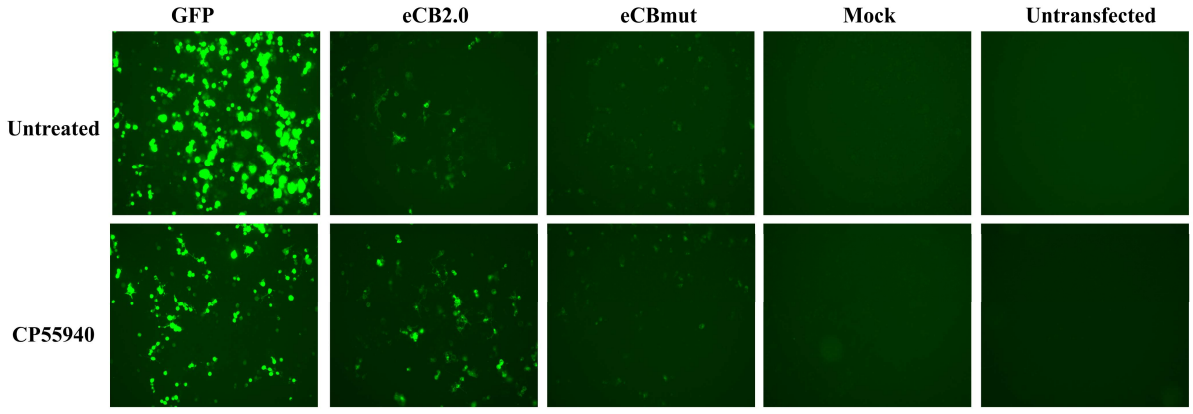


Figure 5.2 EVOS microscopy images of Neuro2A cells with different transient transfections before and after adding CB_1R agonist CP55,940.

To increase the throughput, the Neuro2A GRAB$_{eCB2.0}$ assay was adapted for a 96-well plate format. The procedure involved measuring the baseline fluorescent signal of transiently expressed GRAB$_{eCB2.0}$ in Neuro2A cells. Following this, agents were added and kinetic fluorescence measurement started immediately. Endocannabinoids 2-AG and AEA induced a concentration-dependent activation of GRAB$_{eCB2.0}$ as shown in Figure 5.3A and B. The fluorescent signal peaked within 2 min, followed by a decline and stabilization at a plateau response. 2-AG, a full agonist of CB$_1$R, increased the fluorescence by a maximum of 3-fold, which was significantly higher than the partial agonist AEA (1.5-fold). The area under the curves (AUC, Figure 5.3C, D) indicated concentration-dependent activation of GRAB$_{eCB2.0}$ in Neuro2A, with negative logarithms of half-maximal effective concentrations (pEC$_{50}$) of 6.37 ± 0.29 and 6.16 ± 0.19 for 2-AG and AEA, respectively (Figure 5.3E). Stimulation of calcium-permeable ionotropic receptors by ATP (1 mM) resulted in a pronounced increase in the fluorescence by 0.9-fold at maximum within 10 min, followed by a continuous decline (Figure 5.3F). This effect was completely blocked by CB$_1$R antagonist rimonabant (1 µM). Together, these results demonstrate that changes in GRAB$_{eCB2.0}$ fluorescence could be reliably measured in a 96-well plate reader to detect stimulus-induced 2-AG production.

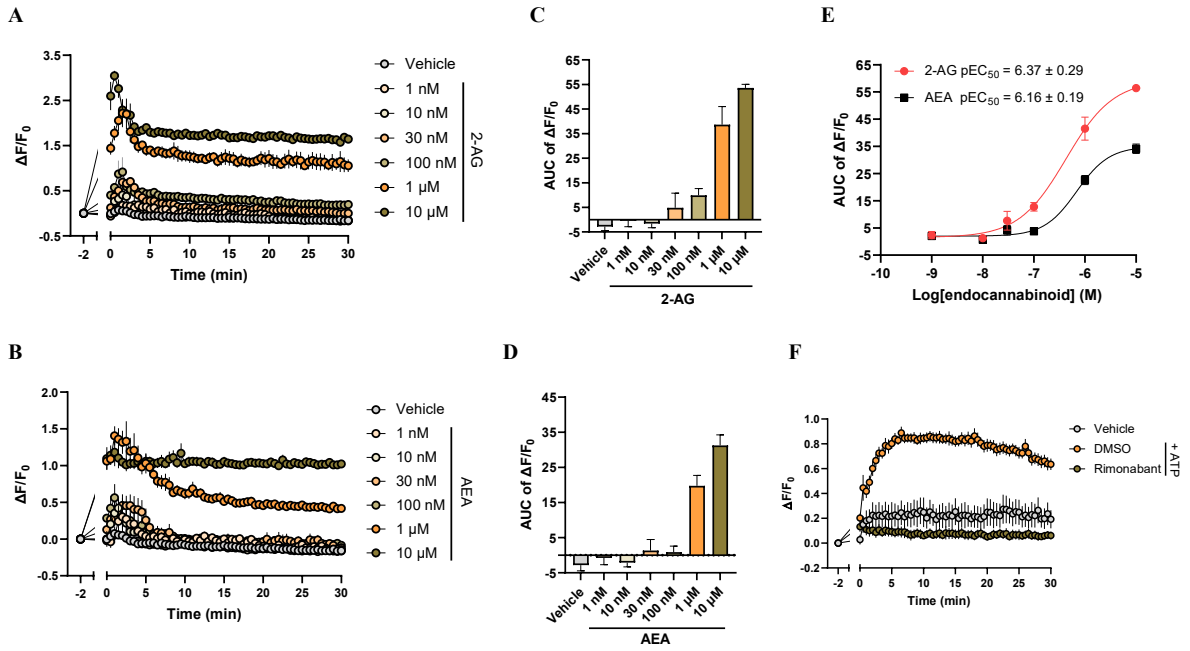


Figure 5.3 The fluorescent detection of GRAB$_{eCB2.0}$ transiently expressed in Neuro2A by a 96-well plate reader upon different stimuli. (A, B) Time course of $\Delta F/F_0$ for the vehicle and different concentrations of 2-AG (A) and AEA (B). (C, D) Area under the curve (AUC) of $\Delta F/F_0$. (E) Concentration-response curves of 2-AG and AEA. (F) Time course of $\Delta F/F_0$ for the vehicle and ATP stimulation with and without CB$_1$R antagonist rimonabant. Data shown are mean ± SEM (n = 3-6).

5.2.2 Profiling DAGL inhibitors in the Neuro2A GRAB$_{eCB2.0}$ assay

To evaluate the potential of the developed GRAB$_{eCB2.0}$ assay for assessing the cellular activity of DAGL inhibitors, Neuro2A cells stably expressing GRAB$_{eCB2.0}$ (Neuro2A GRAB$_{eCB2.0}$) were established and the inhibitory effect of DAGL inhibitors DH376 and LEI-106 on ATP-

stimulated 2-AG production was investigated. To this end, Neuro2A GRAB$_{eCB2.0}$ were seeded in a clear-bottom 96-well plate a day before the treatment. The cells were then treated with different concentrations of inhibitors or DMSO for 1 h after which the baseline fluorescence was measured. Subsequently, ATP or MilliQ (vehicle) was added and the kinetic fluorescence measurement started immediately. ATP induced a rapid and transient fluorescent signal, which was blocked by DH376 and LEI-106 in a concentration-dependent manner (Figure 5.4A, B). At the highest tested concentrations, DH376 (1 µM and 100 nM) and LEI-106 (10 µM) showed lower $\Delta F/F_0$ and AUC values than the vehicle control (Figure 5.4C, D), which might be indicative of constitutive 2-AG production. Based on AUC values, the residual activity of DAGL was calculated and plotted to obtain the concentration-response curves (Figure 5.4E, F), which showed that DH376 and LEI-106 inhibited endogenous DAGL in Neuro2A with a pEC$_{50}$ of 7.51 ± 0.20 and 5.82 ± 0.12, respectively. The activity of DH376 and LEI-106 in Neuro2A was significantly lower than their biochemical activity (Table 1), possibly due to the different affinity of these compounds to mouse and human DAGL as well as cellular permeability. Notably, the carboxyl group in LEI-106 may limit cellular permeability at physiological pH.

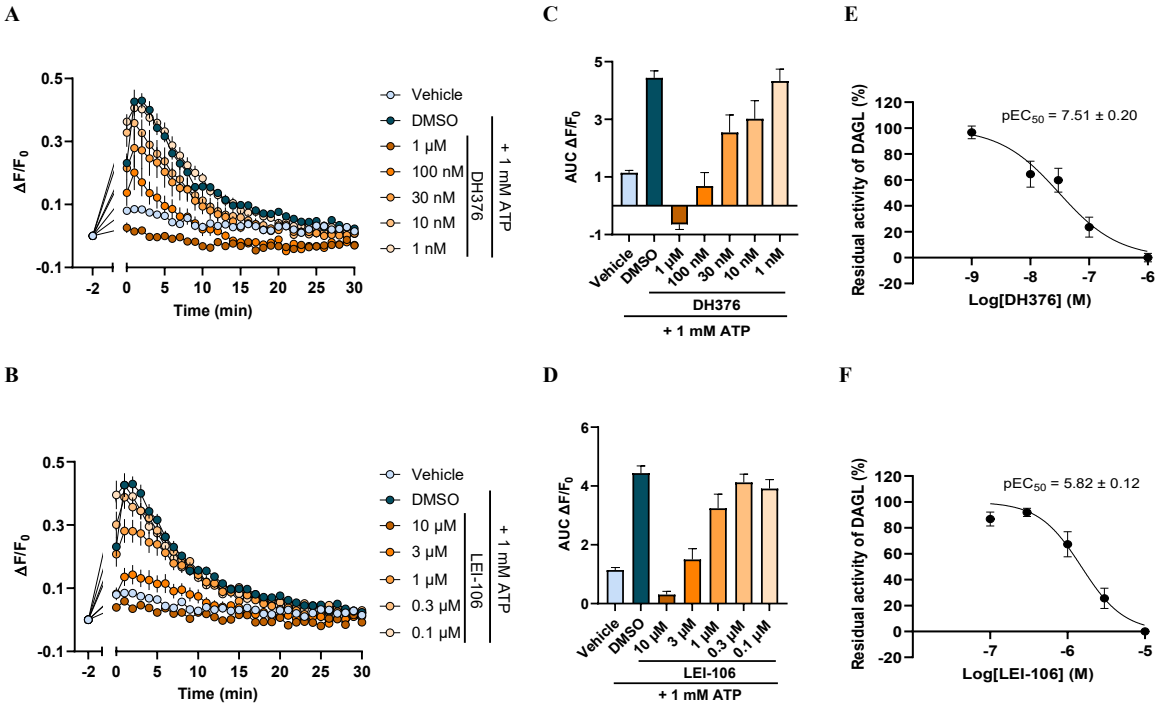


Figure 5.4 The evaluation of DAGL inhibitors DH376 and LEI-106 in the Neuro2A GRAB$_{eCB2.0}$ assay. (A, B) Time course of $\Delta F/F_0$ for the vehicle, DMSO and different concentrations of DH376 (A) and LEI-106 (B) upon ATP stimulation. (C, D) Area under the curve (AUC) of $\Delta F/F_0$. (E, F) Concentration-response curves of DH376 (E) and LEI-106 (F) with their pEC$_{50}$ values. Data shown are mean ± SEM (n = 5, N = 2).

The structure-activity relationship of glycine sulfonamides as inhibitors of DAGLα and DAGLβ was investigated in depth in Chapter 3 and 4, leading to the discovery of numerous inhibitors with varying biochemical activity and DAGL subtype selectivity. To assess their cellular inhibitory activity for DAGLs, a diverse set (chemical structures in Supplementary Figure S5.1) were tested in the Neuro2A GRAB$_{eCB2.0}$ assay within a concentration range from

10 µM to 0.1 µM. The tested inhibitors were categorized into two classes based on selectivity: non-selective DAGL and DAGLβ selective inhibitors. The concentration-response curves of non-selective DAGL inhibitors are shown in Figure 5.5 and a summary of their biochemical and cellular results is presented in Table 5.1. Similar to LEI-106, these inhibitors exhibited lower cellular activity compared to their biochemical activity. Compounds **1-5** demonstrated cellular activity with a pEC_{50} higher than 6. Among them, compound **1** displayed the highest inhibitory activity with a pEC_{50} of 6.48 ± 0.12. Compounds **6-11** were less active than LEI-106 with a pEC_{50} lower than 6. From these results, a general trend was observed that the inhibitor with a higher affinity for DAGLα would show a higher cellular activity. Notably, cellular activity can also be influenced by other factors such as lipophilicity and permeability.

The concentration-response curves of DAGLβ selective inhibitors are depicted in Figure 5.6 and their biochemical and cellular results are summarized in Table 5.2. In general, DAGLβ selective inhibitors showed lower cellular activity compared with the non-selective ones. Compounds **12-15** demonstrated activity in the Neuro2A $GRAB_{eCB2.0}$ assay with cellular activity in the micromolar range. However, the standard deviation of pEC_{50} of compounds **13-15** could not be determined due to the steep slope of the concentration-response curves. Despite being highly active for DAGLβ in the biochemical assay, compounds **16**, **17**, **19** and **20** were inactive (pEC_{50} < 5) in the Neuro2A $GRAB_{eCB2.0}$ assay. In contrast, compounds **18** and **21** showed some cellular activity.

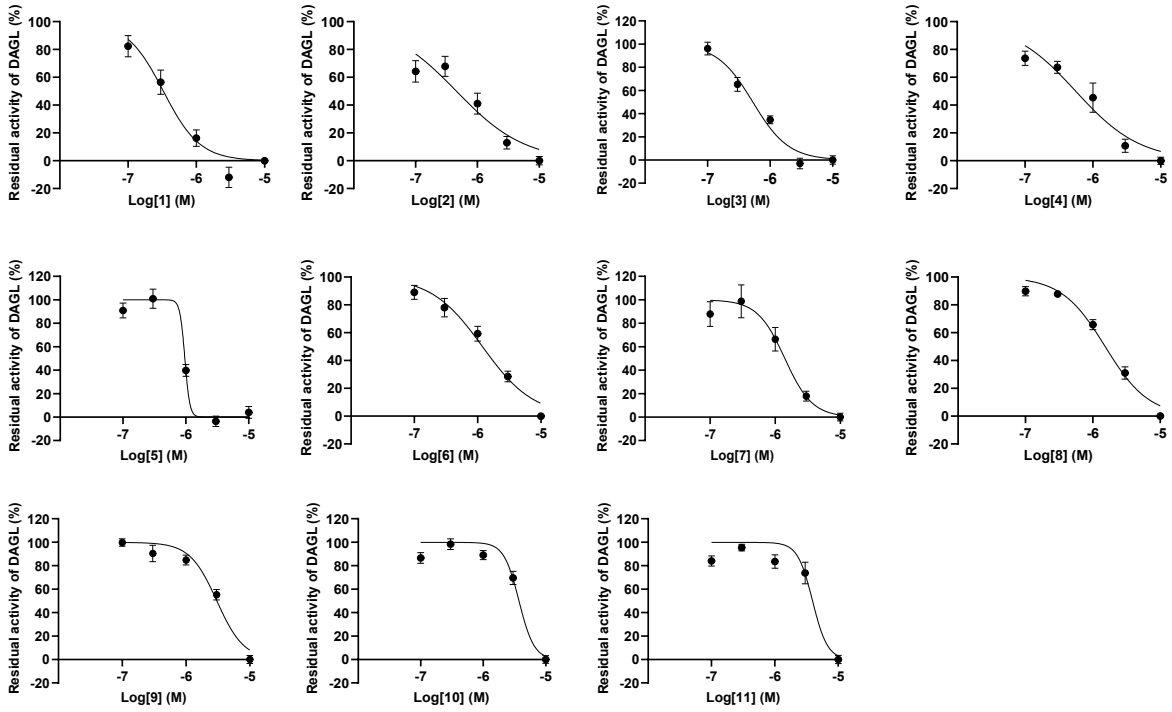


Figure 5.5 Concentration-response curves of non-selective DAGL inhibitors **1-11**. Data shown are mean ± SEM (n = 5, N = 2).

Table 5.1 Biochemical and cellular results of non-selective DAGL inhibitors **1-11**.[a]

Compound	pIC$_{50}$ DAGLβ	pIC$_{50}$ DAGLα	Apparent selectivity	cLogD	tPSA (Å^2)	pEC$_{50}$
DH376	8.79 ± 0.08	8.90 ± 0.04	0.8	4.9	80	7.51 ± 0.20
LEI-106	6.69 ± 0.14	7.35 ± 0.06	0.2	1.9	104	5.82 ± 0.12
1	7.22 ± 0.17	7.60 ± 0.08	0.4	4.5	95	6.48 ± 0.12
2	7.01 ± 0.09	7.41 ± 0.09	0.4	3.8	95	6.34 ± 0.18
3	7.22 ± 0.12	8.17 ± 0.21	0.1	3.8	95	6.28 ± 0.10
4	7.34 ± 0.18	7.89 ± 0.11	0.3	2.3	95	6.25 ± 0.15
5	7.36 ± 0.10	7.75 ± 0.11	0.4	3.2	104	6.02[b]
6	7.73 ± 0.09	7.83 ± 0.09	0.8	4.5	95	5.93 ± 0.11
7	6.96 ± 0.10	7.36 ± 0.14	0.4	4.2	95	5.85 ± 0.13
8	7.17 ± 0.10	7.20 ± 0.03	0.9	2.3	95	5.82 ± 0.07
9	7.07 ± 0.11	7.19 ± 0.04	0.8	3.0	95	5.52 ± 0.08
10	6.66 ± 0.07	6.91 ± 0.05	0.6	2.3	95	5.43 ± 0.08
11	6.66 ± 0.18	6.82 ± 0.11	0.7	4.5	95	5.40 ± 0.10

[a]The negative logarithms of half maximal inhibitory concentration (pIC$_{50}$) and half maximal effective concentration (pEC$_{50}$) were determined in DAGL EnzChek lipase substrate assay and Neuro2A GRAB$_{eCB2.0}$ assay, respectively. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. [b]The pEC$_{50}$ could not be reliably determined due to the steep slope of the concentration-response curves.

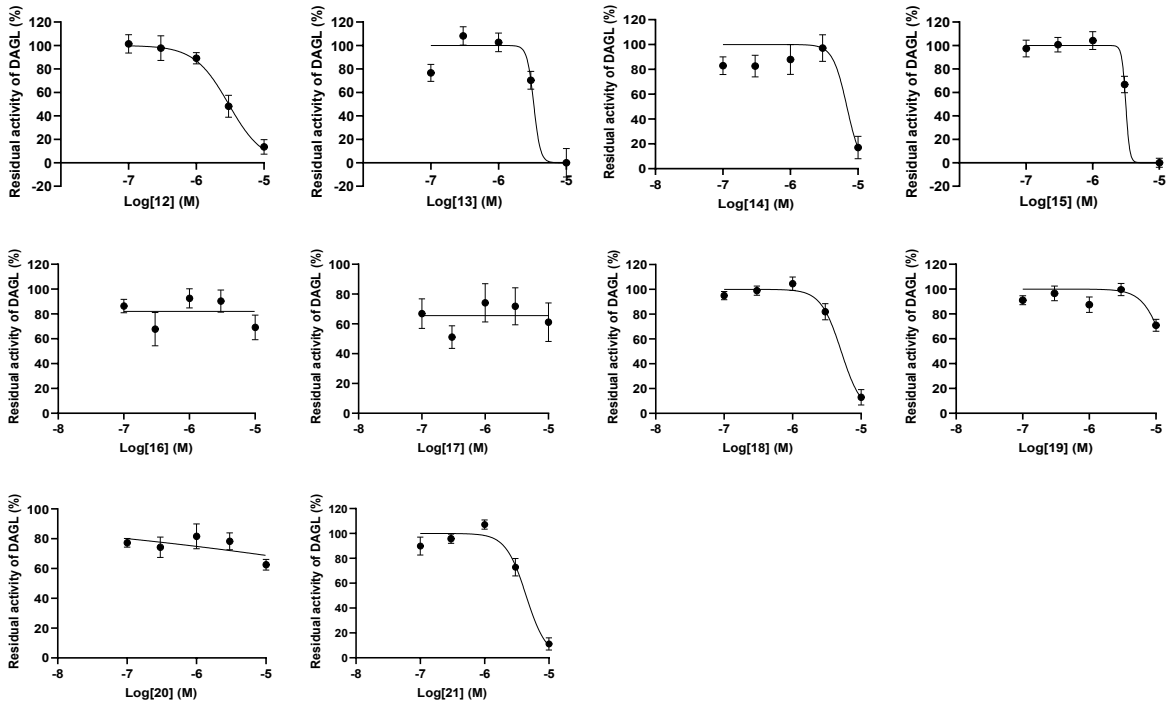


Figure 5.6 Concentration-response curves of DAGLβ selective inhibitors **12-21**. Data shown are mean ± SEM (n = 5, N = 2).

Table 5.2 Biochemical and cellular results of DAGLβ selective inhibitors **12**-**21**.[a]

Compound	pIC_{50} DAGLβ	pIC_{50} DAGLα	Apparent selectivity	cLogD	tPSA (Å²)	pEC_{50}
12	7.89 ± 0.12	7.22 ± 0.04	4.7	2.3	86	5.52 ± 0.14
13	7.85 ± 0.14	7.03 ± 0.03	6.6	2.9	99	5.48[b]
14	7.61 ± 0.12	6.50 ± 0.12	13	2.9	104	5.17[b]
15	8.29 ± 0.08	7.15 ± 0.08	14	3.2	104	5.50[b]
16	7.59 ± 0.06	6.29 ± 0.05	20	1.1	104	< 5[c]
17	7.48 ± 0.09	5.93 ± 0.16	36	1.4	104	< 5[c]
18	7.96 ± 0.06	6.37 ± 0.05	39	2.5	104	5.29 ± 0.09
19	7.94 ± 0.08	6.34 ± 0.09	40	2.2	104	< 5[c]
20	7.88 ± 0.09	6.27 ± 0.07	41	1.9	104	< 5[c]
21	8.07 ± 0.09	6.36 ± 0.05	51	2.3	104	5.35 ± 0.10

[a]The negative logarithms of half maximal inhibitory concentration (pIC_{50}) and half maximal effective concentration (pEC_{50}) were determined in DAGL EnzChek lipase substrate assay and Neuro2A $GRAB_{eCB2.0}$ assay, respectively. The calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. [b]The pEC_{50} could not be reliably determined due to the steep slope of the concentration-response curves. [c]The compound was inactive in Neuro2A $GRAB_{eCB2.0}$ assay .

5.2.3 DAGLα is responsible for ATP-stimulated 2-AG production in Neuro2A

The cellular activity of DAGL inhibitors in the Neuro2A $GRAB_{eB2.0}$ assay enabled a correlation analysis between biochemical and cellular activities to elucidate the isoform of DAGL involved in ATP-stimulated 2-AG production. For this purpose, the pEC_{50} values of the inhibitors obtained in the Neuro2A $GRAB_{eCB2.0}$ assay were plotted against the biochemical pIC_{50} values of the inhibitors for DAGLα or DAGLβ. A significant correlation between the pEC_{50} values and the pIC_{50} values for DAGLα was observed with a Pearson correlation coefficient (Pearson r) of 0.91 ($p < 0.0001$) (Figure 5.7A). Generally, inhibitors with higher affinity for DAGLα exhibited better inhibitory activity in the Neuro2A $GRAB_{eB2.0}$ assay. In contrast, there was a low correlation (Pearson r) of -0.04 ($p = 0.87$) between the pEC_{50} values and the pIC_{50} values for DAGLβ, as indicated by the scattered data points in Figure 5.7B. The non-selective and DAGLβ selective inhibitors were clustered separately, with DAGLβ selective inhibitors tending to have lower pEC_{50} than non-selective ones. Additionally, the correlation between pEC_{50} values and physiochemical properties of inhibitors, such as cLogD (representing lipophilicity) and tPSA (representing topological polar surface area), was analyzed (Figure 5.7C, D). The pEC_{50} exhibited a significant positive correlation with cLogD while displaying a negative correlation with tPSA. In this context, it cannot be ruled out that the reduced cellular activity of DAGLβ selective inhibitors in the Neuro2A $GRAB_{eCB2.0}$ assay could be attributed to generally lower lipophilicity and higher tPSA resulting in reduced cell permeability of these compounds compared to the non-selective inhibitors. Despite LEI-106 and compound **20** sharing the same tPSA and cLogD values, LEI-106 demonstrated activity in the Neuro2A $GRAB_{eCB2.0}$ assay

while compound **20** was inactive. Furthermore, compounds **14**, **15**, **18**, **19**, and **21**, possessing the same tPSA and higher cLogD compared to LEI-106, all exhibited significantly lower cellular activity than LEI-106. The reduced cellular activity in these cases was dominantly attributed to the lower potency of these compounds for DAGLα compared to LEI-106. Consequently, it can be concluded that ATP-stimulated 2-AG production in Neuro2A cells is most likely mediated by DAGLα rather than DAGLβ. Previous immunostaining studies provided valuable insights into the cellular localization of DAGLα and DAGLβ in Neuro2A cells.[20] These studies revealed that DAGLα was located on plasma membranes, whereas DAGLβ was predominantly found in perinuclear lipid droplets. The distinction in localization between DAGLα and DAGLβ may offer a plausible explanation for DAGLα's role in ATP-stimulated 2-AG production.

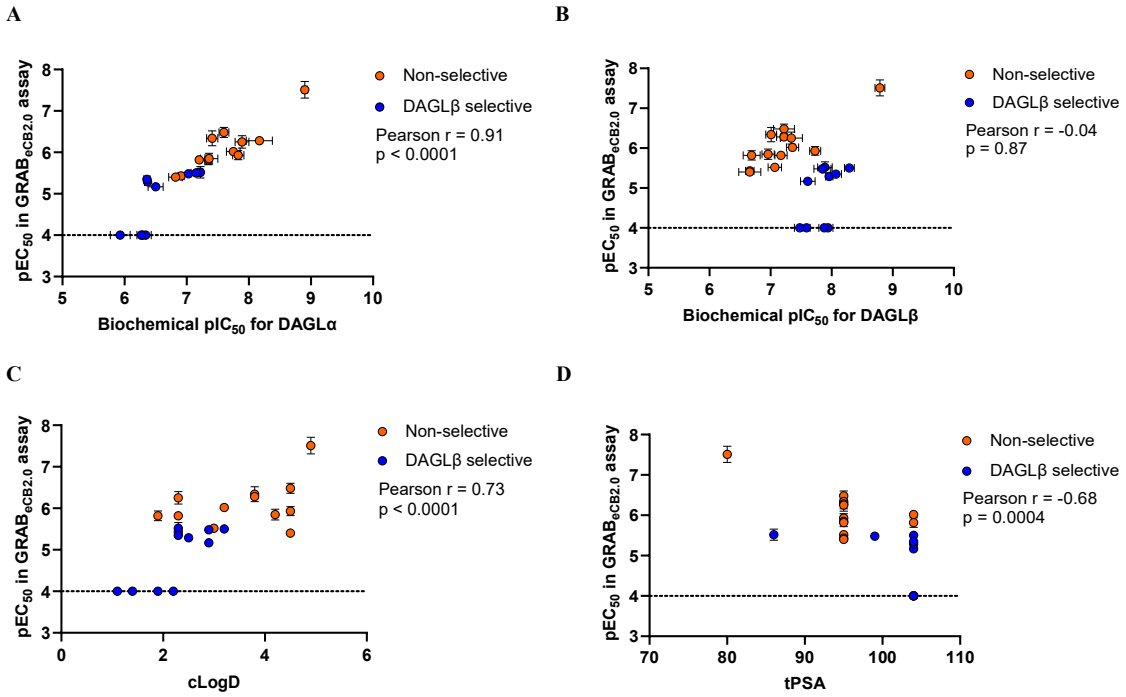


Figure 5.7 Correlation plots. (A) pEC_{50} vs biochemical pIC_{50} for DAGLα. (B) pEC_{50} vs biochemical pIC_{50} for DAGLβ. (C) pEC_{50} vs cLogD. (D) pEC_{50} vs tPSA. The pEC_{50} value for compounds that were inactive ($pEC_{50} < 5$) in the Neuro2A GRAB$_{eCB2.0}$ assay is set at 4.

5.3 Conclusion

In this Chapter, the GRAB$_{eCB2.0}$ sensor, whether transiently or stably expressed in Neuro2A cells, demonstrated exquisite sensitivity to the concentrations of 2-AG. This facilitated the kinetic measurement of ATP-induced 2-AG production in a 96-well plate format and the assessment of DAGL inhibitors in inhibiting 2-AG production. A total of 23 DAGL inhibitors with diverse activity and isoform selectivity were evaluated in the Neuro2A GRAB$_{eCB2.0}$ assay. These inhibitors concentration-dependently blocked the production of 2-AG by inhibiting DAGL with varying potencies. Correlation analysis revealed that the cellular activity of these inhibitors in the Neuro2A GRAB$_{eCB2.0}$ assay was influenced by their lipophilicity, permeability

and potency for DAGLα. Parallel comparison of the cellular activity of DAGLβ selective inhibitors and non-selective DAGL inhibitor LEI-106 with similar tPSA and cLogD values enables the conclusion that DAGLα is the dominant isoform in Neuro2A responsible for 2-AG production upon ATP stimulation. These findings further underscore the importance of isoform-specific DAGL inhibitors in unraveling the intricate mechanisms of DAGL and their roles in biological processes.

5.4 Acknowledgements

Prof. dr. Nephi Stella is gratefully acknowledged for providing plasmids of eCB2.0 and eCBmut. Tom van der Wel and Verena Straub are kindly acknowledged for generating the Neuro2A cell line stably expressing $GRAB_{eCB2.0}$. Matthijs R. van Wijngaarden is kindly acknowledged for his contribution to profiling the DAGL inhibitors in the Neuro2A $GRAB_{eCB2.0}$ assay.

5.5 Experimental methods

Cell culture

Wild type Neuro2A and Neuro2A GRAB$_{eCB2.0}$ cells were cultured at 37 °C under 7% CO_2 in Dulbecco′s modified Eagle′s medium (DMEM, Sigma Aldrich) containing phenol red, GlutaMax (2 mM), penicillin/streptomycin (200 µg/mL each, Duchefa) and 10% newborn calf serum (Thermo Fischer). Cells were passaged every 3 to 4 days and for no longer than 25 generations and the medium was refreshed every 2 to 3 days. Blasticidin S was added to the cultured Neuro2A GRAB$_{eCB2.0}$ cells once a week at a concentration of 5 µg/mL.

EVOS fluorescent microscopic imaging of Neuro2A

One day before transfection, 3×10^4 Neuro2A cells were seeded to each inner well of a clear-bottom black 96-well plate (Greiner-Bio-One, REF 655090). The outer wells were filled with 100 µL PBS (Sigma Aldrich). The cells were incubated at 37 °C under 7% CO_2 for 24 h. After that, 40 µL of medium was removed and cells were transfected with 0.3 µg PEI and 0.1 µg DNA (eCB2.0, eCBmut, GFP or empty vector) in 10 µL of serum-free DMEM. 24 h post-transfection, the medium was replaced with 100 µL PBS containing 1 mM $CaCl_2$ and 0.55 mM $MgCl_2$ and cells were incubated at rt for 20 min. Background fluorescence images were taken on Invitrogen EVOS FL Auto 2 in GFP channel. 1 µL of CB_1R agonist CP55,940 in DMSO (final concentration 1 µM) was added and fluorescence images were taken again.

2-AG, AEA and ATP Activation of GRAB$_{eCB2.0}$ in Neuro2A

Transient transfection was done as previously described. 24 h after transfection, the medium was replaced with 95 µL PBS containing 1 mM $CaCl_2$ and 0.55 mM $MgCl_2$ and cells were incubated at rt for 20 min. After incubation, baseline fluorescence was measured (30 sec/cycle, 5 cycles) in CLARIOstar® (excitation 470-15 nm, emission 515-20 nm, bottom optical, focal height ~4.1 mm). 5 µL of 2-AG or AEA at different concentrations (dissolved in PBS with 2 mg/mL BSA) was then added and kinetic fluorescence measurement (30 sec/cycle, 61 cycles) was started immediately. PBS with 2 mg/mL BSA (final concentration of 0.1 mg/mL) was used as a vehicle control. The assay was performed in n = 3. For ATP activation, 1 µL of DMSO or rimonabant at 100 µM (final concentration of 1 µM) was added followed by the baseline fluorescence measurement. 5 µL of 20 mM ATP (final concentration 1 mM) or MilliQ (vehicle) was added and kinetic fluorescence measurement started immediately. The assay was performed in n = 6. The $\Delta F/F_0$ value was calculated by the formula: $\Delta F/F_0 = (F-F_0)/F_0$, where F and F_0 represent the measured fluorescence at any time point and the baseline fluorescence, respectively. Area under the curve (AUC) of $\Delta F/F_0$ was calculated in GraphPad Prism 9.0.0, which was corrected by the AUC of vehicle to generate the concentration-response curves using GraphPad Prism 9.0.0 (nonlinear regression, log(agonist) vs. response with variable slope).

96-well plate Neuro2A GRAB$_{eCB2.0}$ assay

Neuro2A GRAB$_{eCB2.0}$ cells were harvested in fresh DMEM medium without phenol red (Sigma Aldrich) and 6×10^4 cells in 100 µL DMEM medium were seeded to each inner well of a clear-bottom black 96-well plate (Greiner-Bio-One, REF 655090). The outer wells were filled with 100 µL PBS (Sigma Aldrich). The cells were incubated at 37 °C under 7% CO_2 for 24 h. The medium was removed by aspiration and 100 µL of Hank's balanced salt solution (HBSS) with Ca^{2+} and Mg^{2+} with DMSO or inhibitor (0.1% DMSO) was added. The plate was incubated at 37 °C without CO_2 for 1 h. After incubation, baseline fluorescence was measured (60 sec/cycle, 5 cycles) in CLARIOstar® (excitation 470-15 nm, emission 515-20 nm, bottom optical, focal height ~4.1 mm, full plate gain adjustment at 25%, spiral scan, 3 mm, 25 flashes). 5.2 µL of 20 mM ATP (final concentration of 1 mM) or MilliQ (vehicle) was added and kinetic fluorescence measurement (60 sec/cycle, 31 cycles) started immediately. The $\Delta F/F_0$ value was calculated by the formula: $\Delta F/F_0 = (F-F_0)/F_0$, where F and F_0 represent the measured fluorescence at any time point and the baseline fluorescence, respectively. Area under the curve (AUC) of $\Delta F/F_0$ was calculated in GraphPad Prism 9.0.0, which was converted to the residual activity of DAGL with the formula: residual activity (%) = $(AUC - AUC_{background})/(AUC_{DMSO} - AUC_{background}) \times 100\%$, where $AUC_{background}$ represents the AUC of the vehicle control or the highest inhibitor concentration. Residual activities were used to generate the concentration-response curves using GraphPad Prism 9.0.0 (nonlinear regression, log(inhibitor) vs. normalized response with variable slope). The assay was performed in n = 5, N = 2.

References

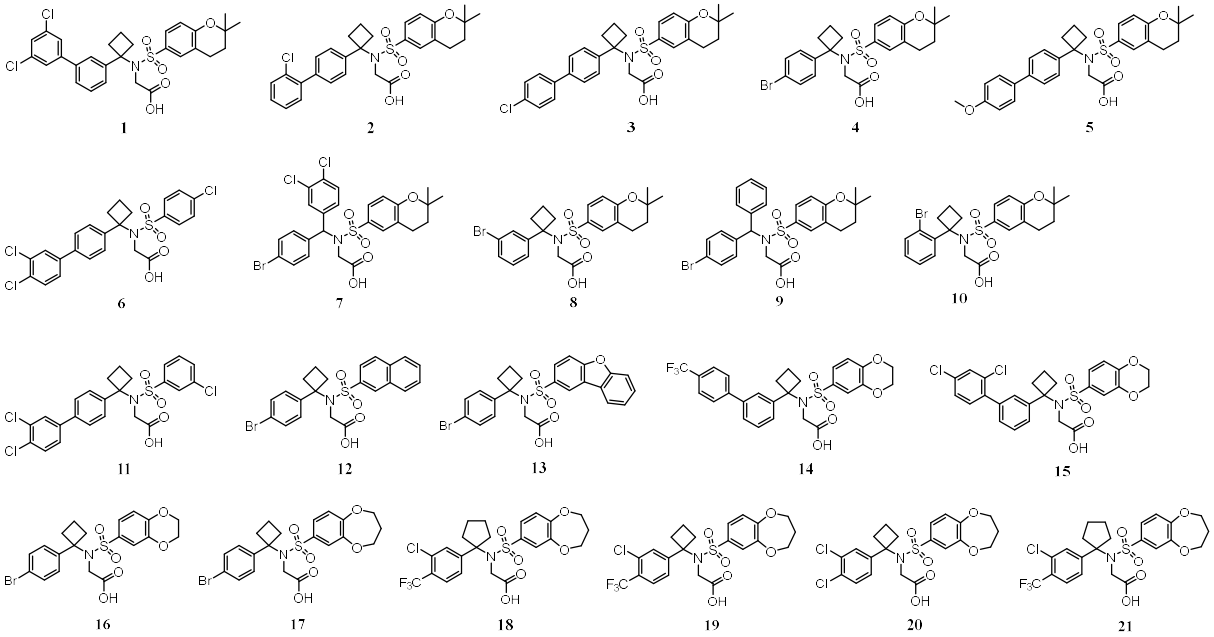
1. Piomelli, D. The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* **4**, 873–884 (2003).
2. Alger, B. E. & Kim, J. Supply and demand for endocannabinoids. *Trends Neurosci.* **34**, 304–315 (2011).
3. Südhof, T. C. Neurotransmitter release: The last millisecond in the life of a synaptic vesicle. *Neuron* **80**, 675–690 (2013).
4. Kavalali, E. T. The mechanisms and functions of spontaneous neurotransmitter release. *Nat. Rev. Neurosci.* **16**, 5–16 (2015).
5. Stella, N. Cannabinoid signaling in glial cells. *Glia* **48**, 267–277 (2004).
6. Hebert-Chatelain, E. *et al.* A cannabinoid link between mitochondria and memory. *Nature* **539**, 555–559 (2016).
7. Jimenez-Blasco, D. *et al.* Glucose metabolism links astroglial mitochondria to cannabinoid effects. *Nature* **583**, 603–608 (2020).
8. Baggelaar, M. P., Maccarrone, M. & van der Stelt, M. 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. *Prog. Lipid Res.* **71**, 1–17 (2018).
9. Buczynski, M. W. & Parsons, L. H. Quantification of brain endocannabinoid levels: Methods, interpretations and pitfalls. *Br. J. Pharmacol.* **160**, 423–442 (2010).
10. Marchioni, C. *et al.* Recent advances in LC-MS/MS methods to determine endocannabinoids in biological samples: Application in neurodegenerative diseases. *Anal. Chim. Acta* **1044**, 12–28 (2018).
11. Sun, F. *et al.* A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of Dopamine in Flies, Fish, and Mice. *Cell* **174**, 481-496 (2018).
12. Jing, M. *et al.* A genetically encoded fluorescent acetylcholine indicator for *in vitro* and *in vivo* studies. *Nat. Biotechnol.* **36**, 726–737 (2018).
13. Wan, J. *et al.* A genetically encoded sensor for measuring serotonin dynamics. *Nat. Neurosci*. **24**, 746–752 (2021).
14. Labouesse, M. A. & Patriarchi, T. A versatile GPCR toolkit to track in vivo neuromodulation: not a one-size-fits-all sensor. *Neuropsychopharmacology* **46**, 2043–2047 (2021).
15. Vardy, E. & Roth, B. L. Conformational ensembles in GPCR activation. *Cell* **152**, 385–386 (2013).
16. Dong, A. *et al.* A fluorescent sensor for spatiotemporally resolved imaging of endocannabinoid dynamics in vivo. *Nat. Biotechnol.* **40**, 787-798 (2021).
17. Yang, N. *et al.* Deficiency in endocannabinoid synthase DAGLB contributes to Parkinson's disease and dopaminergic neuron dysfunction. *Nat. Commun.* **13**, 3490 (2021).

18. Liput, D. J. *et al.* 2-Arachidonoylglycerol mobilization following brief synaptic stimulation in the dorsal lateral striatum requires glutamatergic and cholinergic neurotransmission. *Neuropharmacology* **205**, 108916 (2022).

19. Singh, S. *et al.* Pharmacological characterization of the endocannabinoid sensor $GRAB_{eCB2.0}$. *Cannabis Cannabinoid Res.* (2023).

20. Jung, K. M., Astarita, G., Thongkham, D. & Piomelli, D. Diacylglycerol lipase-α and -β control neurite outgrowth in neuro-2a cells through distinct molecular mechanisms. *Mol. Pharmacol.* **80**, 60–67 (2011).

Supplementary Figures



Supplementary Figure S5.1 Chemical structures of glycine sulfonamide DAGL inhibitors **1-21**.

Chapter 6

Glycine sulfonamides act as selective DAGLβ inhibitors and reduce inflammation

6.1 Introduction

Lipids, such as endocannabinoids[1–4], diacylglycerols[5], eicosanoids[6] and fatty acids[7], have been recognized as signaling molecules that regulate important physiological processes, including neurotransmission, metabolism, cell proliferation and immune response. Distinct from classical chemical messengers stored in vesicles, signaling lipids are produced 'on demand' and rapidly inactivated to terminate their actions.[8] For instance, endocannabinoid 2-arachidonoylglycerol (2-AG) is produced by diacylglycerol lipase α and β (DAGLα and DAGLβ)[9] and degraded by monoacylglycerol lipase (MAGL) and α/β-hydrolase domain-containing 6/12 (ABHD6/12)[10,11] after action. DAGLs regulate diacylglycerols and 2-AG via direct substrate-product relationships. Additionally, they also exert effects indirectly on other bioactive lipids, such as arachidonic acid (AA) and prostaglandins E2 and D2 (PGE_2 and PGD_2), through interconnecting enzymes such as MAGL and cyclooxygenase (COX). These lipids occupy the hubs of distinct but integrated signaling pathways[3,6,12–16] to form a sophisticated lipid crosstalk which affects physiological and pathological processes in a complex and dynamic manner.[14,17]

DAGL enzymes are transmembrane serine hydrolases which employ the classical Ser-His-Asp catalytic triad to specifically hydrolyze the *sn*1 ester bond of diacylglycerols.[9] Genetic studies with constitutive disruption of DAGLα or DAGLβ have confirmed that they contribute differently to the production of 2-AG in various cell and tissue types.[18–20] In general, DAGLα is the dominant regulator of bulk of 2-AG biosynthesis in the brain, whereas DAGLβ is the principal 2-AG biosynthetic enzyme in periphery[18,21] and in immune-related cells.[20,22] Genetic disruption[18] and pharmacological inhibition[23] of DAGLα were found to rewire brain lipid signaling networks, which negatively impacted emotional states such as fear extinction, stress and anxiety.[24,25] By contrast, DAGLβ disruption exerted potentially therapeutic effects related to microglia and macrophage function, by reducing lipopolysaccharide (LPS)-induced inflammation without significant alternations of total brain levels of 2-AG.[20]

In Chapter 4, a series of glycine sulfonamides were discovered as DAGLβ selective inhibitors. Based on their high potency and selectivity over DAGLα, six inhibitors (Figure 6.1) were selected for further profiling in this Chapter. In addition, a negative control compound was generated which is structurally similar to the glycine sulfonamides but inactive against DAGLβ. The results presented in this Chapter reveal that LEI-130 and LEI-131 represent compounds with the most optimal properties. They behave as noncompetitive DAGLβ inhibitors with high selectivity over other serine hydrolases and other proteins in the endocannabinoid system. Moreover, LEI-130 and LEI-131 targeted endogenous DAGLβ and impacted diverse signaling lipid networks depending on the cell type. These findings, taken together, demonstrate that LEI-130 and LEI-131 are potent, selective and cellularly active DAGLβ inhibitors which could lead to potential treatments of inflammation and other disorders.

6.2 Results

6.2.1 LEI-130 and LEI-131 are potent and selective DAGLβ inhibitors

In Chapter 4, six glycine sulfonamides (**1-6**), based on initial screening hit LEI-106, were discovered as selective DAGLβ inhibitors using EnzChek lipase substrate assays (Figure 6.1). The compounds share notable structural similarities, featuring a glycine sulfonamide core structure (red), a substituted benzylamine (black), and a 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine (blue) on the sulfonyl substituent. These inhibitors exhibited high potency (pIC_{50} > 7.4) and good selectivity (> 35-fold) for DAGLβ with low lipophilicity (cLogD < 3) and high lipophilic efficiency (LipE > 5) (Table 6.1 and see also Chapter 4). To assess DAGLβ involvement in biological studies, a negative control compound (**7**, **LEI-132**) lacking DAGL inhibitory activity was generated.

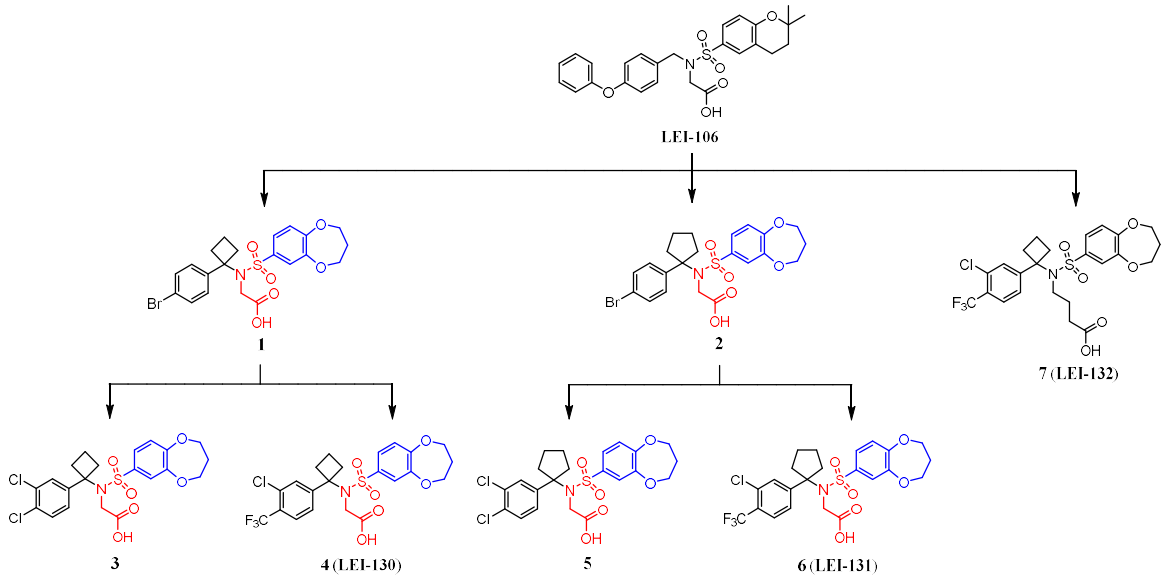


Figure 6.1 Discovery of DAGLβ inhibitors **1-6** and an inactive control **7** starting from hit LEI-106.

Table 6.1 Biochemical results, ABPP results, and physiochemical properties of compounds **1-7**.[a]

ID	Biochemical pIC_{50} DAGLβ	Apparent biochemical selectivity	ABPP pIC_{50} DAGLβ	Apparent ABPP selectivity	MW (Da)	cLogD	tPSA (Å²)	Biochemical LipE DAGLβ	ABPP LipE DAGLβ
1	7.48 ± 0.09	35	7.46 ± 0.09	39	496.37	1.4	104	6.1	6.1
2	8.08 ± 0.13	37	8.19 ± 0.06	37	510.40	1.8	104	6.3	6.4
3	7.88 ± 0.09	41	8.12 ± 0.12	35	486.36	1.9	104	6.0	6.2
4 (LEI-130)	7.94 ± 0.08	40	7.89 ± 0.11	36	519.92	2.2	104	5.7	5.7
5	8.07 ± 0.09	51	8.20 ± 0.08	19	500.38	2.3	104	5.8	5.9
6 (LEI-131)	7.96 ± 0.06	39	8.54 ± 0.11	76	533.94	2.5	104	5.5	6.0
7 (LEI-132)	< 5	-	-	-	547.97	3.1	104	-	-

[a]The negative logarithm of the half-maximal inhibitory concentration (pIC_{50}) was determined using EnzChek lipase substrate assay (biochemical) and activity-based protein profiling (ABPP). Molecular weight (MW), the calculated logarithm of the *n*-octanol-water partition coefficient at pH 7.4 (cLogD) and the topological polar surface area (tPSA) were calculated using DataWarrior 5.0.0. Lipophilic efficiency (LipE) was computed using the formula LipE = pIC_{50} – cLogD.

Here, the DAGL-tailored activity-based probe, DH379 (Figure 6.2B), was employed in activity-based protein profiling (ABPP) as a complementary, orthogonal assay to evaluate the activity and selectivity of inhibitors **1-6** on recombinant human DAGLβ and DAGLα. DH379 effectively labeled DAGL enzymes and pre-incubation with inhibitors **1-6** concentration dependently blocked the labeling of DAGL (Figure 6.2A). The quantification of the fluorescent signals generated concentration-response curves as depicted in Figure 6.2C. These compounds demonstrated high potency and selectivity for DAGLβ over DAGLα in ABPP assays (Table 6.1), aligning with the results observed in DAGL EnzChek lipase substrate assays in Chapter 4. Among these inhibitors, compound **6** exhibited the highest activity and selectivity for DAGLβ with a pIC_{50} of 8.54 ± 0.11 and a 76-fold selectivity. Compound **5** had a pIC_{50} of 8.20 ± 0.08 for DAGLβ but only a 19-fold selectivity in the ABPP assay, significantly lower compared to the biochemical assay. Compound **1** showed the lowest activity (pIC_{50} of 7.46 ± 0.09) for DAGLβ. Compounds **2**, **3** and **4** displayed high potency and moderate selectivity for DAGLβ (~35-fold). Among them, compound **4** belongs the cyclobutyl series and is most structurally similar to compound **6** and the negative control **7**. Taken together, inhibitors **4** (**LEI-130**) and **6** (**LEI-131**) were selected for further biological profiling.

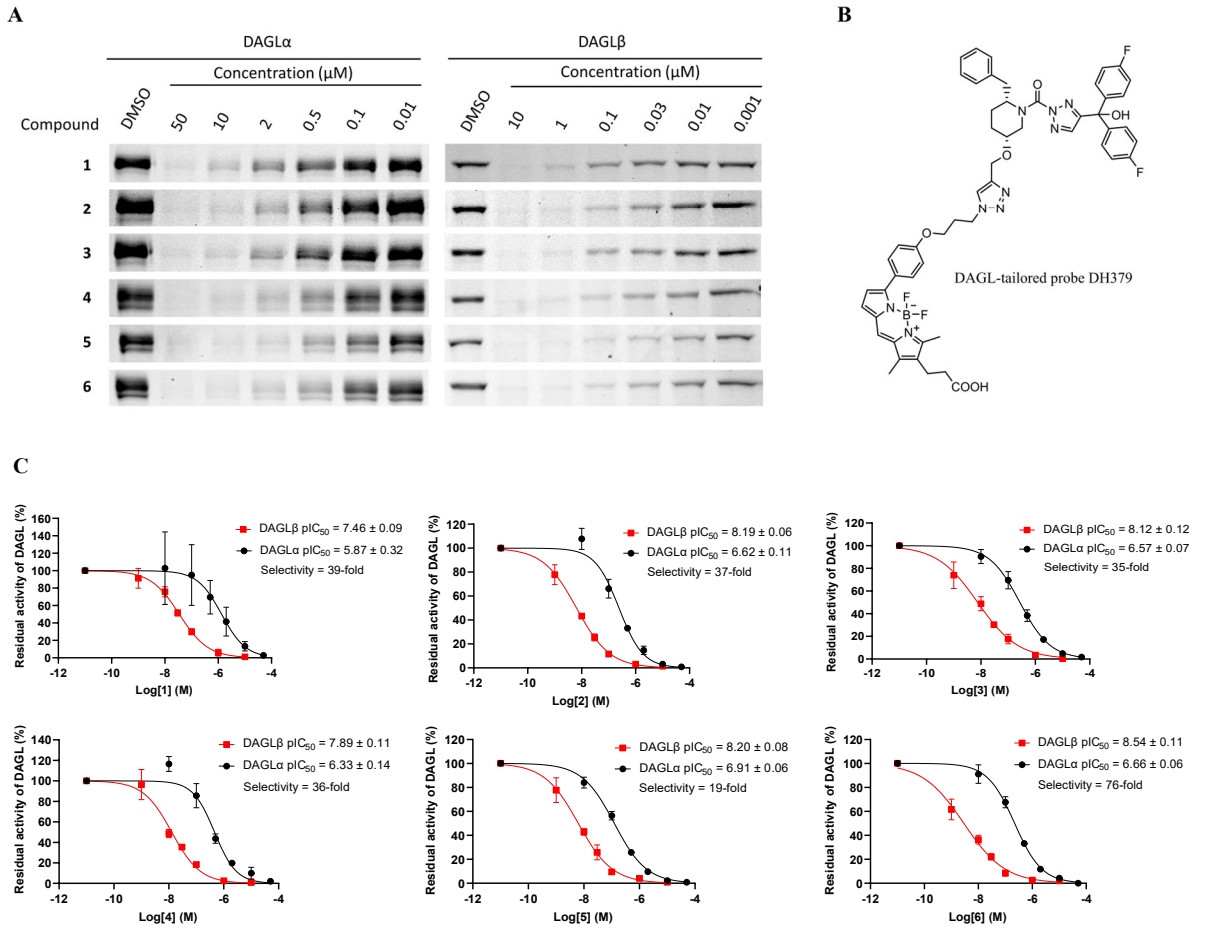


Figure 6.2 *In vitro* activity of compounds **1-6** on recombinant human DAGLα and DAGLβ. (A) Representative gel excerpts of ABPP experiments with compounds **1-6** on recombinant human DAGLα and DAGLβ using probe DH379 (0.5 μM, 10 min). (B) Chemical structure of probe DH379. (C) Concentration-response curves as well as pIC_{50} and selectivity values of inhibitors **1-6**. Data shown are mean ± SD (n = 1, N = 3).

6.2.2 LEI-130 and LEI-131 demonstrate a noncompetitive inhibition mode for DAGLβ

To investigate the inhibition mode of glycine sulfonamides LEI-106, LEI-130 and LEI-131, Michaelis-Menten kinetic assays were performed (Figure 6.3A-C). The kinetic analysis revealed a significant decrease in the maximum rate (V_{max}) with an increase in inhibitor concentration, but the Michaelis constant (K_M) remained relatively stable within the range of 0.24 to 0.31 µM (Supplementary Table S6.1), suggesting these compounds do not inhibit DAGLβ via a competitive mechanism. Lineweaver Burk analysis further demonstrated that LEI-106 inhibited DAGLβ via an uncompetitive inhibition mode (Figure 6.3D), while LEI-130 and LEI-131 exhibited a noncompetitive behaviour (Figure 6.3E, F). Of interest, LEI-106, LEI-130 and LEI-131 displayed an uncompetitive inhibition mode for DAGLα (Supplementary Figure S6.1 and Table S6.2). After determining the inhibition mode, the inhibition constant (K_i) and selectivity could be calculated (Table 6.2). LEI-130 and LEI-131 exhibited a pK_i of 7.94 ± 0.08 and 7.96 ± 0.06 for DAGLβ, respectively, with a 22-fold selectivity.

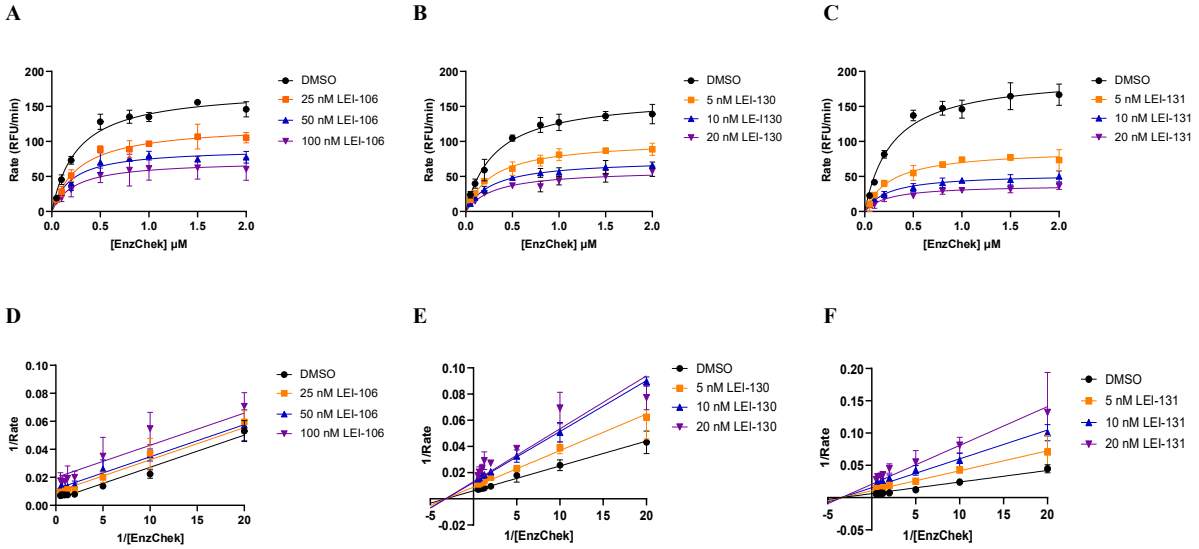


Figure 6.3 Mode-of-inhibition studies of LEI-106, LEI-130 and LEI-131 against DAGLβ. (A-C) Michaelis-Menten kinetic curves. Rate of EnzChek hydrolysis by DAGLβ as a function of substrate concentration in the presence of different concentrations of LEI-106, LEI-130 and LEI-131. (D-F) Lineweaver Burk plots. Data shown are mean ± SD (n = 2, N = 2).

Table 6.2 Biochemical activity, inhibition mode, inhibition constant and selectivity of LEI-106, LEI-130 and LEI131.[a]

Compound	pIC_{50} DAGLα	Inhibition mode DAGLα	pIC_{50} DAGLβ	Inhibition mode DAGLβ	pK_i DAGLα	pK_i DAGLβ	Selectivity DAGLβ
LEI-106	7.35 ± 0.06	uncompetitive	6.69 ± 0.14	uncompetitive	7.60 ± 0.06	6.87 ± 0.14	0.2
LEI-130	6.34 ± 0.09	uncompetitive	7.94 ± 0.08	noncompetitive	6.59 ± 0.09	7.94 ± 0.08	22
LEI-131	6.37 ± 0.05	uncompetitive	7.96 ± 0.06	noncompetitive	6.62 ± 0.05	7.96 ± 0.06	22

[a]The negative logarithm of the inhibition constant (pK_i) was computed using formula $pK_i = pIC_{50} + \log_{10}(1 + K_M/[S])$ and $pK_i = pIC_{50}$ for uncompetitive and noncompetitive inhibitors, respectively. The Michaelis constants (K_M) were 0.39 µM and 0.26 µM for DAGLα and DAGLβ, respectively. Substrate concentration ([S]) was 0.5 µM.

6.2.3 LEI-130 and LEI-131 are selective over other serine hydrolases and proteins in the endocannabinoid system

To assess the selectivity of DAGLβ inhibitors LEI-130 and LEI-131 and the control compound LEI-132 over a panel of serine hydrolases, gel-based competitive ABPP was conducted. This involved pre-incubating the compounds with mouse brain membrane or cytosol proteome, followed by sequential labeling the serine hydrolases using broad-spectrum probes MB064 and FP-BODIPY. As shown in Figure 6.4A, MB064 labeled endogenous DAGLα in mouse brain membrane proteome along with other serine hydrolases such as ABHD6, ABHD12, and ABHD16A. MB064 also labeled a selection of serine hydrolases in the cytosol proteome. LEI-130, LEI-131 and LEI-132 showed no inhibition on these labeled proteins across a broad concentration range from 1 nM to 10 µM. However, LEI-106 at 10 µM completely blocked the labeling of DAGLα and two other bands (band 1 and 2), while enhancing the labeling of a protein (band 3) in both membrane and cytosol proteomes (Supplementary Figure S6.2A). FP-BODIPY labeled different serine hydrolases compared to MB064, such as fatty acid amide hydrolase (FAAH) and MAGL (Figure 6.4B). The labeling of these proteins remained unaffected by LEI-130, LEI-131 and LEI-132. These findings indicate that LEI-130 and LEI-131 are more selective than hit compound LEI-106 and they exhibit selectivity for DAGLβ without interfering with the activity of other serine hydrolases.

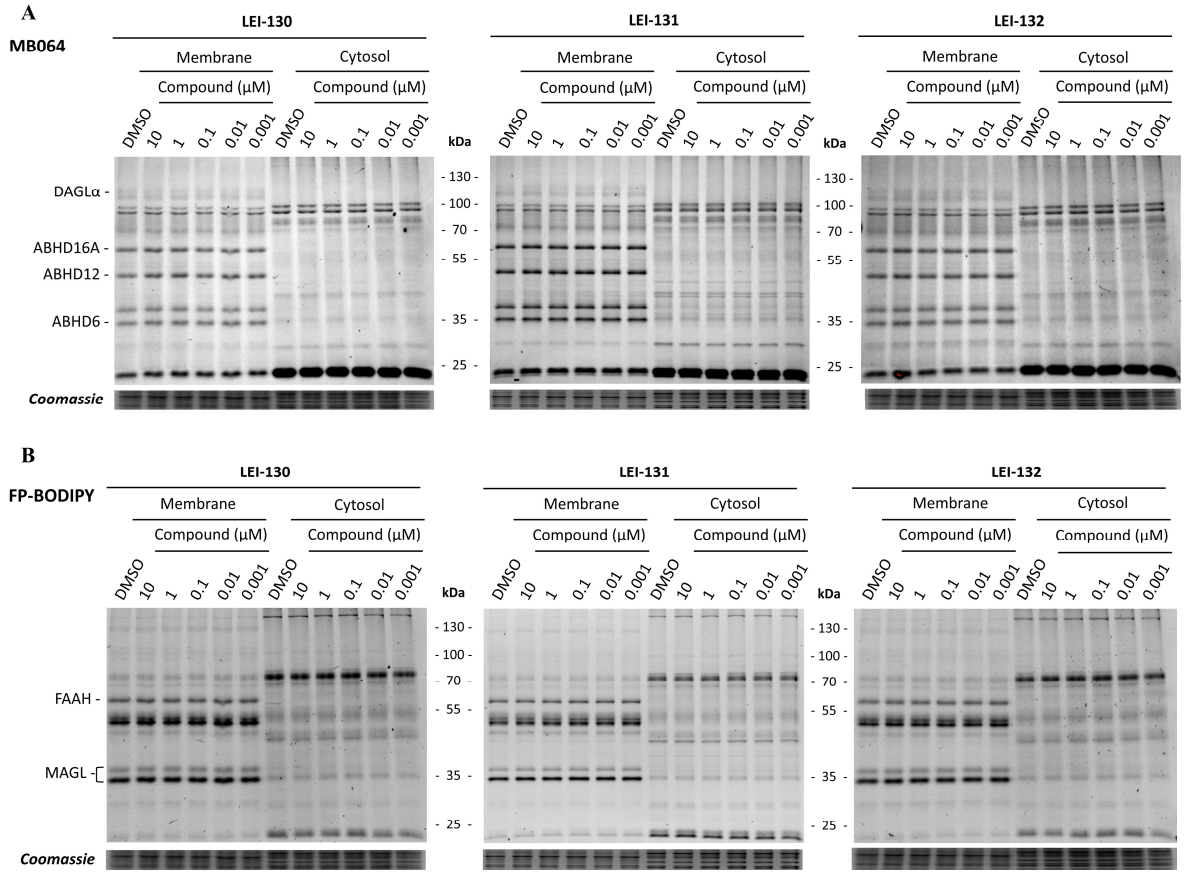


Figure 6.4 *In vitro* selectivity of LEI-130, LEI-131 and LEI-132 on mouse brain proteomes. Representative gels of ABPP experiments using MB064 (A, 250 nM, 10 min) and subsequently FP-BODIPY (B, 100 nM, 10 min).

Next, the selectivity of LEI-130, LEI-131 and LEI-132 over human ABHD6 and MAGL, *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) and cannabinoid CB_1 and CB_2 receptors was determined. These compounds were inactive against ABHD6, MAGL, NAPE-PLD, CB_1R and CB_2R (< 50% inhibition or displacement at 10 µM, Table 6.3). These findings, taken together, demonstrate that LEI-130 and LEI-131 are selective DAGLβ inhibitors and the inactive compound LEI-132 serves as a suitable control compound.

Table 6.3 Biochemical activities of LEI-130, LEI-131 and LEI-132 for representative ECS enzymes and CB receptors. Percentage (%) inhibition or displacement at 10 µM.

Compound	ABHD6	MAGL	NAPE-PLD	CB_1R	CB_2R
LEI-130	20.5 ± 2.3%	26.9 ± 1.7%	4.8 ± 1.1%	-1 ± 8%	-6 ± 16%
LEI-131	10.0 ± 0.4%	35.3 ± 0.7%	3.1 ± 2.2%	-1 ± 9%	-9 ± 9%
LEI-132	4.2 ± 0.8%	24.4 ± 2.1%	6.8 ± 2.2%	-15 ± 11%	1 ± 8%

6.2.4 LEI-130 and LEI-131 play distinct roles in microglia and macrophage

To investigate the cellular target engagement of LEI-130 and LEI-131, a gel-based ABPP experiment was performed. Briefly, N9 microglia or J774A.1 macrophage cells were treated with the inhibitors (10 µM) or vehicle (DMSO) before incubation with probe DH379 (1 µM). Subsequently, the cells were lysed and analyzed using gel-based ABPP. The control compound LEI-132 was included for comparison. Probe DH379 labeled endogenous DAGLβ at the expected molecular weight both in N9 microglia (Figure 6.5A) and J774A.1 macrophage (Figure 6.5B) in the samples treated with the vehicle control. The labeling of DAGLβ, but not other proteins, by DH379 was completely blocked by DAGLβ inhibitors LEI-130 and LEI-131, but not by control compound LEI-132.

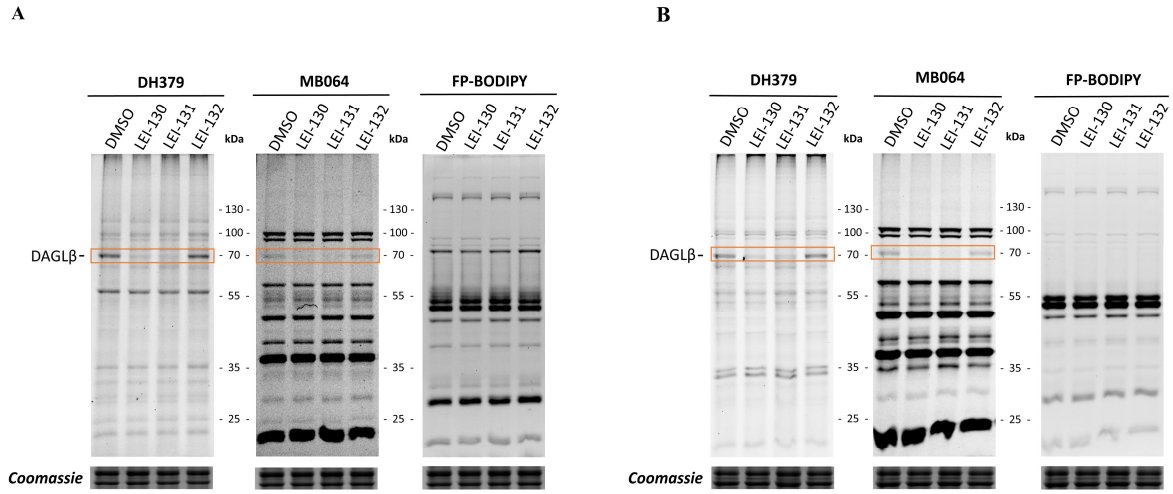


Figure 6.5 *In situ* target engagement and selectivity of LEI-130, LEI-131 and LEI-132. Representative gels of ABPP experiments with LEI-130, LEI-131 and LEI-132 (10 µM, 2 h) in N9 microglia (A) and J774A.1 macrophage (B) using DH379 (*in situ*, 1 µM, 1 h) or the cocktail of MB064 and FP-BODIPY (*in vitro*, 100 nM, 10 min).

Next, the cellular selectivity of LEI-130, LEI-131 and LEI-132 was assessed. N9 and J774A.1 cells were incubated with DMSO, LEI-130, LEI-131 or LEI-132 (10 μM) and subsequently lysed for analysis in gel-based ABPP using a cocktail of probes MB064 and FP-BODIPY (100 nM/each probe). This approach enabled simultaneous labeling of DAGLβ and a number of other serine hydrolases. In line, LEI-130 and LEI-131 efficiently inhibited endogenous DAGLβ without affecting other proteins. In contrast, control compound LEI-132 exhibited no inhibitory activity on any of the detected proteins, including DAGLβ. These findings collectively demonstrate that LEI-130 and LEI-131 are cell-permeable inhibitors capable of selectively targeting endogenous DAGLβ in microglia and macrophage cells.

Having established the cellular target engagement of DAGLβ by LEI-130 and LEI-131 in N9 microglia cells, it was investigated whether the compounds could modulate lipid networks in these cells. LEI-130 and LEI-131 (10 μM, 2 h) exhibited a significant reduction in 2-AG and a noticeable accumulation of 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG) (Figure 6.6A, B). Conversely, a slight increase in 2-AG and no change in SAG were observed in LEI-132-treated cells. These results indicate that DAGLβ is the primary enzyme regulating the SAG and 2-AG levels in N9 microglia. Moreover, LEI-130 and LEI-131 significantly reduced the cellular levels of monoacylglycerols 2-linoleoylglycerol (2-LG) and 2-oleoylglycerol (2-OG), arachidonic acid (AA), PGE_2 and PGD_2, while sparing anandamide (AEA) (Figure 6.7C-H). Notably, these changes were also observed for the control compound LEI-132. These findings suggest the existence of other protein targets modulated by these compounds that regulate the levels of these lipids via DAGLβ-independent mechanisms in N9 microglia.

DAGLβ deletion protects primary microglia from lipopolysaccharide (LPS)-stimulated inflammation by reducing the production of proinflammatory cytokines.[20] To investigate the influence of acute DAGLβ inhibition on microglia inflammatory processes, N9 microglia cells were pre-treated with LEI-130, LEI-131 and LEI-132 (10 μM, 1 h) and stimulated with the proinflammatory agent LPS (5 ng/mL, 24 h), before analyzing the culture supernatants by the enzyme-linked immunosorbent assay (ELISA). LPS stimulation significantly elevated the levels of secreted interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Figure 6.6I, J). The LPS-induced increase in IL-6 was significantly attenuated by both LEI-130 and LEI-131. However, there was only a modest, but significant, attenuation on LPS-induced secretion of TNF-α by LEI-131. Of note, LEI-132 also significantly reduced the LPS-induced production of IL-6 and TNF-α, suggesting that the cytokine production is not solely modulated by DAGLβ, but also by off-targets of these compounds.

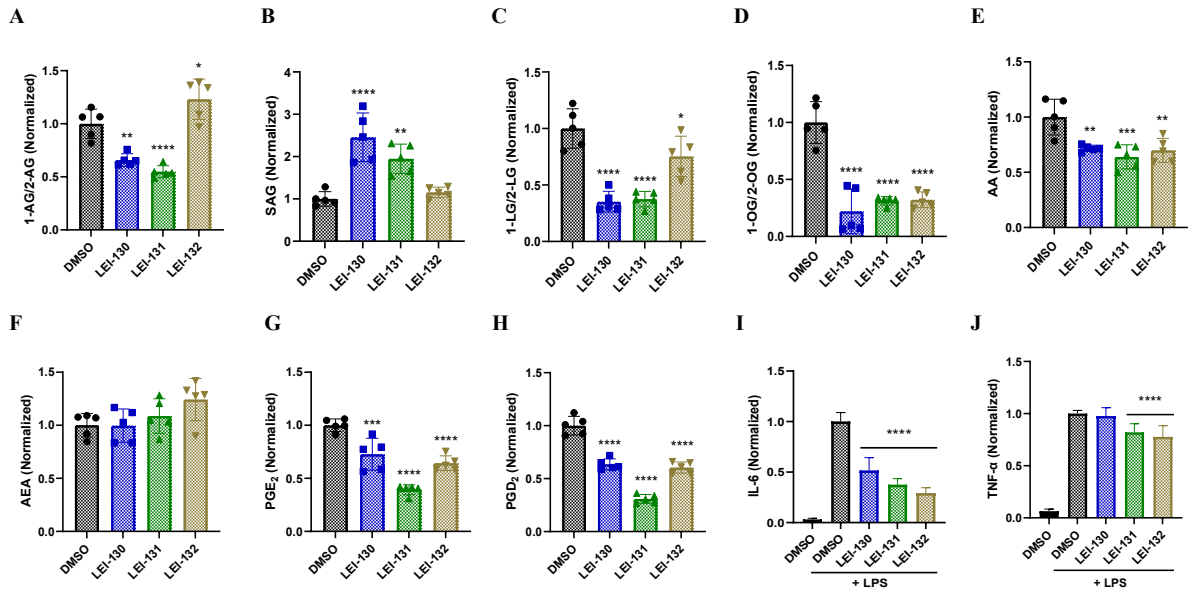


Figure 6.6 Relative levels of lipids and cytokines in N9 microglia. Normalized cellular levels of (A) 2-AG, (B) SAG, (C) 2-LG, (D) 2-OG, (E) AA, (F) AEA, (G) PGE_2, and (H) PGD_2 in N9 microglia cells treated with DMSO, LEI-130, LEI-131 and LEI-132 (10 μM, 2 h). Data shown are mean ± SD (n = 5). Normalized levels of (I) IL-6 and (J) TNF-α secreted by N9 microglia cells pre-treated with DMSO, LEI-130, LEI-131 and LEI-132 (10 μM, 1 h) upon LPS-stimulation (5 ng/mL, 24 h). Data shown are mean ± SD (n = 12-16). Statistical significance was calculated using one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

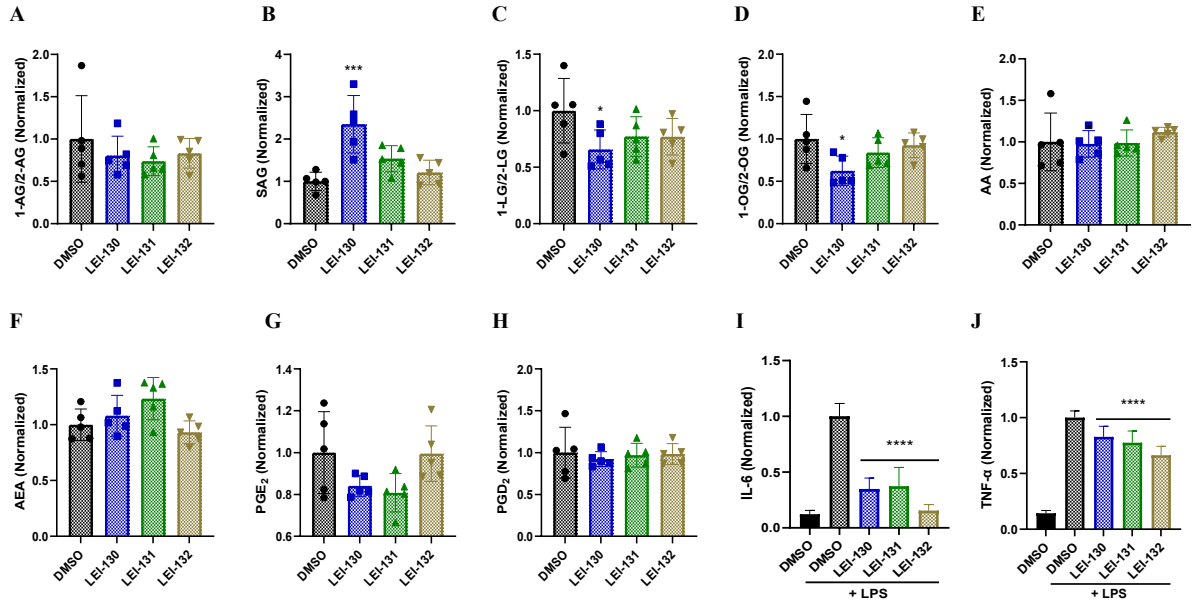


Figure 6.7 Relative levels of lipids and cytokines in J774A.1 macrophages. Normalized cellular levels of (A) 2-AG, (B) SAG, (C) 2-LG, (D) 2-OG, (E) AA, (F) AEA, (G) PGE_2, and (H) PGD_2 in J774A.1 macrophages treated with DMSO, LEI-130, LEI-131 and LEI-132 (10 μM, 2 h). Data shown are mean ± SD (n = 5). Normalized levels of (I) IL-6 and (J) TNF-α secreted by J774A.1 macrophages pre-treated with DMSO, LEI-130, LEI-131 and LEI-132 (10 μM, 1 h) upon LPS-stimulation (5 ng/mL, 24 h). Data shown are mean ± SD (n = 12-16). Statistical significance was calculated using one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Lipidomics analysis and ELISA were also performed for J774A.1 macrophages. In contrast to the results observed in N9 microglia, treatment with LEI-130, LEI-131 and LEI-132 (10 μM, 2 h) in J774A.1 macrophages showed no significant changes in the levels of most bioactive lipids, including 2-AG, AEA, AA, PGE_2, and PGD_2 (Figure 6.7A, E-H). However, a significant accumulation of SAG and a modest reduction in 2-LG and 2-OG were observed in LEI-130-treated cells (Figure 6.7B-D). These results indicate that different metabolic pathways may exist that regulate 2-AG levels in J774A.1 macrophages. Despite their inability to reduce the levels of 2-AG, AA and prostaglandins, LEI-130, LEI-131, and LEI-132 displayed anti-inflammatory effects by significantly suppressing the LPS-stimulated production of proinflammatory cytokines IL-6 and TNF-α.

6.3 Discussion and conclusion

DAGLs play a central role in the lipid networks by integrating signaling pathways of various lipids, including DAG[16], endocannabinoids[3], AA and eicosanoids.[14] Benefitting from the discovery of *in vivo* active inhibitors[22,23], the biological functions of DAGLs have been gradually elucidated. However, understanding the specific roles of individual isoform, either DAGLα or DAGLβ, in biological processes has been challenging due to the lack of isoform-specific inhibitors. In an effort to address this problem, glycine sulfonamide DAGLβ-selective inhibitors LEI-130 and LEI-131 were developed and subjected to cellular studies.

Inhibitors can exert their effects on enzymes through specific ways, such as orthosteric and allosteric binding. A profound understanding of the interactions between inhibitors and enzymes is crucial for elucidating the regulatory mechanisms of enzymatic activity and discovering more specific inhibitors. Due to the absence of crystal structures for DAGL enzymes, our comprehension of these interactions between glycine sulfonamides and DAGL is limited. To address this, a homology model of DAGLα[26] was constructed and employed to understand the structure-activity relationship of glycine sulfonamide DAGL inhibitors.[27] It is important to note that this study initially hypothesized that glycine sulfonamides act as competitive inhibitors, occupying the orthosteric pocket. However, the mode-of-inhibition studies presented here contradict this hypothesis. Specifically, both LEI-130 and LEI-131 were identified as uncompetitive and noncompetitive inhibitors for DAGLα and DAGLβ, respectively, while LEI-106 was shown to be an uncompetitive inhibitor for both DAGL isoforms. This suggests that they occupy an allosteric pocket of DAGLs. During the optimization process, LEI-130 and LEI-131 exhibited increased selectivity and altered their mode of action for DAGLβ, but not for DAGLα. This indicates that the binding site of LEI-130 and LEI-131 in DAGLβ has undergone a slight shift compared to LEI-106, rendering it insensitive to conformational changes induced by substrate binding. Notably, this conformational change could be substrate-dependent, with SAG potentially inducing a different conformational change than EnzChek lipase substrate. The detailed information about the allosteric binding pocket and the conformational changes induced by substrate binding remains unclear. Mutagenesis studies and affinity-based probes could help identify the structural features of DAGL enzymes.

In vitro and *in situ* ABPP studies of LEI-130 and LEI-131 confirmed their activity against DAGLβ with selectivity over a panel of serine hydrolases in mouse brain proteomes and living cells. Acute inhibition of DAGLβ by LEI-130 and LEI-131 resulted in decreased levels of 2-AG, AA, PGE_2 and PGD_2 in N9 microglia cells. However, this effect was not observed in J774A.1 macrophages. Interestingly, an increase of SAG, a substrate of DAGLβ, was observed in both cell lines treated with the inhibitors. These findings indicate that DAGLβ controls SAG levels in both cell lines, whereas the regulation of 2-AG levels is cell line-dependent. Besides being formed from phosphatidylinositol-4,5-bisphosphate (PIP_2) through the sequential actions of phospholipase C (PLC) and DAGL, 2-AG can also be generated from PIP_2 via an alternative pathway involving PIP_2 phosphatase, phospholipase A1 (PLA1) and lysophospholipase C (lysoPLC). This alternative pathway might be the dominant regulatory mechanism for 2-AG levels in J774A.1 macrophages. Although the inactive control LEI-132 showed no effects on the direct flux of 2-AG and SAG, it significantly influenced the production of other bioactive lipids in N9 microglia, likely by targeting DAGLβ-uncoupled pathways. Both DAGLβ active and inactive compounds attenuate LPS-stimulated cytokine productions, indicating that LPS might induce proinflammatory effects through DAGLβ-coupled and -uncoupled mechanisms.

In conclusion, the studies presented in this Chapter demonstrate that glycine sulfonamides LEI-130 and LEI-131 act as selective DAGLβ inhibitors and modulate inflammatory processes in immune cells. These compounds constitute a valuable chemical toolkit along with the control compound LEI-132 for studying the biological functions of DAGLβ. In the future, mass-spectrometry (MS)-based chemical proteomics could help reveal the potential off-targets of LEI-130, LEI-131 and LEI-132 that were not detected in gel-based ABPP.[28] Additionally, the absorption, distribution, metabolism and excretion (ADME) profile of these compounds should be determined prior to progressing to *in vivo* studies.

6.4 Acknowledgements

Wouter Driever is kindly acknowledged for evaluating the biochemical activity of the compounds against NAPE-PLD. Cas van der Horst is kindly acknowledged for evaluating the affinity of the compounds for cannabinoid CB_1 and CB_2 receptors. Mirjam Huizenga and Noëlle van Egmond are kindly acknowledged for their guidance in MAGL biochemical assay and ELISA, respectively. Max Louwerse is acknowledged for providing J774A.1 macrophages. Dr. Xinyu Di is kindly acknowledged for measuring lipid levels.

6.5 Experimental methods

Biology

Cell culture

N9 microglia cells were cultured at 37 °C under 7% CO_2 in Iscove's Modified Dulbecco's Medium (IMDM) containing phenol red, L-glutamine (2 mM), penicillin/streptomycin (200 µg/mL) and 5% sterile-filtered (0.2 µM) fatal calf serum (Thermo Fischer). J774A.1 macrophages were cultured at 37 °C under 5% CO_2 in Dulbecco′s modified Eagle′s medium (DMEM) containing phenol red, Glutamax (2 mM), penicillin/streptomycin (200 µg/mL) and 10% fatal calf serum (FCS, Thermo Fischer). Cells were passaged twice or three times a week by resuspension in fresh medium to appropriate confluence.

Total lysate preparation

Transient transfection was performed as described in Chapter 2. HEK293T cell pellets overexpressing human DAGLα and DAGLβ were thawed on ice and suspended in cold lysis buffer (20 mM HEPES pH 7.2, 250 mM sucrose, 2 mM DTT, 1 mM $MgCl_2$, 2.5 U/mL Benzonase). The suspension was pipetted up and down, incubated on ice for 30 min, and diluted in cold lysis buffer to 1 µg/µL. All samples were flash frozen in liquid N_2 and stored in small aliquots at -80 °C until use.

Preparation of mouse brain membrane and cytosol proteomes

Mouse brains were isolated according to guidelines approved by the ethical committee of Leiden University (AVD1060020171144), immediately flash frozen in liquid N_2 and stored at -80 °C until use. Upon preparation, mouse brains were thawed on ice and homogenized using a Wheaton™ Dounce homogenizer (3×7 sec) in cold lysis buffer (20 mM HEPES pH 7.2, 2 mM DTT, 1 mM $MgCl_2$, 25 U/mL Benzonase) and incubated on ice for 1 h. The lysate was spun at a low speed (2,500 g, 3 min, 4 °C, Eppendorf Centrifuge 5430R) to remove debris. Subsequently, the supernatant was subjected to ultracentrifugation (100,000 g, 45 min, 4 °C, Beckman Coulter, Ti 70.1 rotor) to obtain the membrane fraction as a pellet and the cytosolic fraction in the supernatant. The pellet was resuspended in cold storage buffer (20 mM HEPES pH 7.2, 2 mM DTT) and the total protein concentration was determined using Quick Start™ Bradford assay. The membrane and cytosol proteomes were diluted to 2 µg/µL, flash frozen in liquid N_2 and stored in small aliquots at -80 °C until use.

Activity-based protein profiling using probe DH379

Gel-based ABPP using DAGL-tailored probe DH379 was performed to determine the potency of the inhibitors for recombinant human DAGLβ and DAGLα. The total lysate of HEK293T cells overexpressing human DAGLα or DAGLβ (9.5 µL, 1 µg/µL) was incubated with DMSO or inhibitors at different concentrations (0.5 µL, 20× concentrated DMSO stocks) at rt for 30 min. DH379 (0.34 µL, 15 µM) was added and incubated for 10 min. The final concentration of

DH379 was 0.5 µM. Next, Laemmli blue (3.44 µL, 4× concentrated stock) was added and incubated for 10 min to quench the reaction. 10 µL of the quenched reaction mixture was resolved on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels (180 V, 80 min) and the fluorescence was measured in a Biorad ChemiDoc™ MP system (Cy5: 700/50 filter; Cy3: 602/50 filter). The remaining enzymatic activity was determined based on the integrated fluorescent signal using Image Lab™ 6.0.0, which was corrected using the total protein loading per lane as determined by Coomassie stain (R250) and imaging (Coomassie Blue Gel: 590/110 filter). The pIC_{50} values were determined from the dose-response curves generated using GraphPad Prism 9.0.0 (log(inhibitor) vs. normalized response with variable slope). The assay was performed three times independently (n = 1, N = 3).

Michaelis-Menten kinetic assays

The membrane fraction of HEK293T cells overexpressing human DAGLα or DAGLβ was diluted in assay buffer (50 mM HEPES pH 7.5, 0.0025% Triton X-100) to 2 µg/mL. 1 µL of DMSO or inhibitors at different concentration (100× concentrated DMSO stocks) and 25 µL of protein solution were added to a black 96-well plate (Greiner Bio-One, REF 655076), followed by adding 70 µL assay buffer. After incubating at rt for 30 min, 4 µL of EnzChek at different concentrations (25× concentrated DMSO stocks, 8 concentrations) was added and the measurement was started immediately in CLARIOstar® (excitation 477-14 nm, emission 525-30 nm, gain = 1600, 72 sec/cycle for 61 cycles). The assay was performed in a final volume 100 µL with 0.5 µg/mL protein, different concentrations of inhibitors, different concentrations of EnzChek (0.05, 0.1, 0.2, 0.5, 0.8, 1, 1.5 and 2 µM), 5% DMSO and 0.0025% Triton X-100. The mock membrane fractions subjected to the same treatments were used for background measurements. The enzymatic rate (RFU/min) was determined from the slope between t = 6 min to t = 24 min after background subtraction, which was used to generate the Michaelis-Menten kinetic curves and Lineweaver Burk plots using GraphPad Prism 9.0.0 software. The assay was performed in n = 2, N = 2.

Activity-based protein profiling in mouse brain proteomes

Mouse brain membrane or cytosol proteome (19 µL, 2 µg/µL) was incubated with DMSO or compounds at different concentrations (0.5 µL, 40× concentrated DMSO stocks) at rt for 30 min. MB064 (0.5 µL, 40× concentrated DMSO stock) was added and incubated for 10 min. Subsequently, FP-BODIPY (0.5 µL, 40× concentrated DMSO stock) was added and incubated for another 10 min. Final concentration of probes MB064 and FP-BODIPY were 250 nM and 100 nM, respectively. Laemmli blue (7 µL, 4× concentrated stock) was added and incubated for 10 min to quench the reaction. 10 µL of the quenched reaction mixture was resolved on 10% SDS-PAGE gels (180 V, 80 min). Fluorescence and Coomassie were measured in a Biorad ChemiDocTM MP system (Cy5: 700/50 filter; Cy3: 602/50 filter; Cy2: 532/28 filter; Coomassie Blue Gel: 590/110 filter). The assay was performed twice independently (n = 1, N = 2).

Natural substrate-based fluorescent assay for human ABHD6 and MAGL

The natural substrate assay was performed in HEMNB buffer (50 mM HEPES pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, 100 mM NaCl, 0.5% (w/v) BSA) as previously reported.[27,29] 5 µL of DMSO or compounds at different concentrations (40× concentrated DMSO stocks) was incubated with 95 µL of the membrane fraction of HEK293T cells overexpressing human ABHD6 or MAGL diluted in HEMNB buffer (84 µg/mL for ABHD6, 3.2 µg/mL for MAGL) in a black 96-well plate (Greiner Bio-One, REF 655076) at rt for 30 min. The mock membrane fraction with DMSO was used for background measurement. Next, 100 µL of assay mix containing glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO), horseradish peroxidase (HRP), adenosine triphosphate (ATP), Amplifu™Red and 2-arachidonoylglycerol (2-AG) was added and the measurement was started immediately at rt in CLARIOstar® (λ_{ex} = 535-20 nm, λ_{em} = 595-20 nm, 5 min/cycle for 13 cycles). The assay was performed in a final volume of 200 µL with 40 µg/mL protein for ABHD6, 1.5 µg/mL protein for MAGL, 0.2 U/mL GK, GPO and HRP, 0.125 mM ATP, 10 µM Amplifu™Red, 25 µM 2-AG, 5% DMSO, 1% ACN. The enzymatic rate was determined from the slope between t = 10 min to t = 35 min after background subtraction, which was normalized to generate the dose-dependent response curves using GraphPad Prism 9.0.0 (log(inhibitor) vs. normalized response with variable slope). All measurements were performed in n = 2, N = 2 or n = 4, N = 2 for controls, with Z' ≥ 0.6.

PED6 fluorescence assay for NAPE-PLD

The NAPE-PLD activity assay was performed as previously reported.[30]

Radioligand displacement assay for cannabinoid CB_1 and CB_2 receptors

The [^{3}H]CP55940 displacement assay was performed as previously reported.[31]

In situ target engagement and selectivity profiling

Target engagement 2.5×10^5 N9 microglia cells were seeded one day before the treatment in each well of a 6-well plate (Sarstedt, REF 83.3920). Before treatment, the medium was removed and the cells were washed once with PBS. The cells were treated with DMSO or compounds (1 µL, 10 mM) in 0.9 mL IMDM with 1% sterile-filtered FCS at 37 °C under 7% CO_2 for 1 h. Subsequently, DAGL-tailored probe DH379 (1 µL, 1 mM) in 0.1 mL of IMDM with 1% sterile-filtered FCS was added and the cells were incubated at 37 °C under 7% CO_2 for another 1 h. The final concentrations of compounds and probe DH379 were 10 µM and 1 µM, respectively. After this, the medium was removed and the cells were washed twice with cold PBS. 50 µL cold lysis buffer (20 mM HEPES pH 7.2, 250 mM sucrose, 1 mM $MgCl_2$ and 2.5 U/mL Benzonase) was added and the cells were lysed by scraping. The protein concentration was determined by Quick Start™ Bradford assay and all lysates were diluted to the same concentration (~1 µg/µL). For J774A.1 macrophage, 4×10^5 cells were seeded and incubated at 37 °C under 5% CO_2 and the treatment was done in DMEM with 1% FCS. 21 µL of lysate was incubated with 7 µL Laemmli blue (4× concentrated stock) for 10 min and 20 µL of the mixture

was resolved on 10% SDS-PAGE gels (180 V, 80 min). Fluorescence and Coomassie were measured in a Biorad ChemiDoc™ MP system (Cy5: 700/50 filter; Cy3: 602/50 filter; Coomassie Blue Gel: 590/110 filter). The assay was performed twice independently (n = 1, N = 2).

Selectivity profiling Cells were treated with DMSO or compounds (1 µL, 10 mM) in 1 mL medium with 1% corresponding serum at 37 °C for 2 h, followed by lysing through scraping. All lysates were diluted to the same concentration (~1 µg/µL). Subsequently, 19.5 µL of lysate was incubated with the cocktail of probes MB064 and FP-BODIPY (0.5 µL, 4 µM/each probe) at rt for 10 min. The final concentration of each probe was 100 nM. 6.7 µL Laemmli blue (4× concentrated stock) was then added and incubated for 10 min to quench the reaction. 20 µL of the quenched reaction mixture was resolved on 10% SDS-PAGE gels (180 V, 80 min). Fluorescence and Coomassie were measured in a Biorad ChemiDoc™ MP system (Cy5: 700/50 filter; Cy3: 602/50 filter; Cy2: 532/28 filter; Coomassie Blue Gel: 590/110 filter). The assay was performed twice independently (n = 1, N = 2).

Targeted lipidomics

Sample preparation 5×10^5 N9 microglia cells were seeded one day before the treatment in 6 cm dishes (Sarstedt, REF 83.3901). Before treatment, the medium was removed and the cells were washed once with PBS. The cells were treated with DMSO or compounds (final concentration 10 µM, 0.1% DMSO) in 3 mL IMDM with 1% sterile-filtered FCS at 37 °C under 7% CO_2 for 2 h. The treatment medium was removed and the cells were washed twice with cold PBS. 120 µL cold lysis buffer (20 mM HEPES pH 7.2, 250 mM sucrose, 1 mM $MgCl_2$ and 2.5 U/mL Benzonase) was added and the cells were lysed by scraping. The protein concentration was determined by Quick Start™ Bradford assay and 90 µL of lysate was stored in 1.5 mL Eppendorf safe-lock tubes at -80 °C until lipid extraction. For J774A.1 macrophage, 10^6 cells were seeded and incubated at 37 °C under 5% CO_2 and the treatment was done in DMEM with 1% FCS. The assay was performed in n = 5 for each condition.

Lipid extraction Lipid extraction was performed using liquid-liquid extraction under ice-cool conditions as previously reported.[32]

LC-MS/MS analysis Extracted samples were analyzed using two separate LCMS methods, Method 1 used a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) connected to a SCIEX QTRAP 6500+ mass spectrometer (AB Sciex, Framingham, MA, USA). Separation was performed using a BEH C18 column (50 mm × 2.1 mm, 1.7 µm) from Waters Technologies (Mildford, MA, USA) maintained at 40 °C. The mobile phase was composed of 0.1% acetic acid in water (A), acetonitrile/0.1% acetic acid in methanol (90:10, v/v, B), and 0.1% acetic acid in isopropanol (C). The flow rate was set at 0.7 mL/min and the injection volume was 10 µL preceded by the injection of 20 µL of mobile phase A. Method 2 used a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) connected to a SCIEX QTRAP 7500 mass spectrometer (AB Sciex, Framingham, MA, USA). Separation was performed using a BEH C8 column (50 mm × 2.1 mm, 1.7 µm) from Waters Technologies (Mildford, MA, USA)

maintained at 45 °C. The mobile phase was composed of 10 mM formic acid and 2 mM Ammonium formate in water (A), acetonitrile (B) and isopropanol (C). The flow rate was set at 0.4 mL/min and the injection volume was 5 µL preceded by the injection of 10 µL of mobile phase A. Ionization of the compounds in both methods was performed using electrospray ionization in negative mode. Selected Reaction Mode (SRM) was used for MS/MS acquisition. SRM transitions were individually optimized for targeted analytes and respective internal standards using standard solutions.

Data quality and data pre-processing For each target compound, the ratio between its peak area and the peak area of its respective internal standard was calculated using SCIEX OS-MQ Software and used for further data analysis. The quality of the data was monitored using regular injection of quality control (QC) samples, consisting of blank samples, within the sequence. QC samples were used to correct for inter-batch variations using the in-house developed mzQuality workflow (available at http://www.mzQuality.nl). Relative standard deviations (RSDs) of peak area ratios were calculated for each targeted analyte detected in the QC samples.

Enzyme-linked immunosorbent assay (ELISA)

***In situ* treatment** 2×10^4 N9 cells were seeded one day before the treatment in the wells of a 96-well plate (Sarstedt, REF 83.3924) and incubated at 37 °C under 7% CO_2. Before treatment, the medium in the inner wells was removed and the cells were washed once with PBS. The cells were treated with DMSO or compounds in 70 µL IMDM with 1% sterile-filtered FCS at 37 °C under 7% CO_2 for 1 h. Subsequently, LPS in 30 µL of IMDM with 1% sterile-filtered FCS was added and the cells was incubated at 37°C under 7% CO_2 for 24 h. The final concentrations of compounds and LPS were 10 µM and 5 ng/mL, respectively. The treatment supernatant was collected for ELISA. The cells were incubated with Alamar Blue (3 mM) in IMDM with 5% sterile-filtered FCS for 4 h to check the cell viability. For J774A.1 macrophage, 2×10^4 cells were seeded and incubated at 37 °C under 5% CO_2. The treatment was done in DMEM with 1% FCS and cell viability was assessed by Alamar Blue (3 mM) in DMEM with 10% FCS. The assay was performed in n = 4, N = 4 for each condition.

ELISA Enzyme-linked immunosorbent assay (ELISA) was performed using Invitrogen IL-6 Mouse uncoated ELISA kit (cat 88-7064-88) and TNF alpha Mouse uncoated ELISA kit (cat 88-7324-88) following the protocol from the manufacture with modifications. In brief, half area high binding 96-well plates (Greiner Bio-One, REF 675061) were coated with 25 µL of capture antibody in PBS (1:250) overnight at 4 °C. The plate was washed three times with PBST (1×PBS with 0.05% tween-20) and then blocked with 100 µL of ELISPOT (1×) at rt for 1 h. After another three washes with PBST, 25 µL of treatment supernatant or standard solutions were added and incubated for 2 h. The plate was washed three times and 25 µL of the biotinylated detection antibody (1:250) diluted in ELISPOT was added and incubated for 1 h. Subsequently, the plate was washed three times with PBST and 25 µL of the enzyme Streptavidin-HRP (1:100) diluted in ELISPOT was added and incubated for 30 min. The plate was washed seven times with PBST. Detection was performed by reacting with 25 µL of TMB solution for around 10 min, followed by quenching with 10 µL of 1 M aqueous HCl solution.

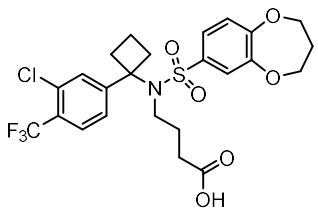
Absorbance was measured at 450 nm and 570 nm in CLARIOstar® and 570 nM values were subtracted from 450 nm values to correct for background. The calibration curve was fitted by a 4P-sigmoidal curve in GraphPad Prism 9.0.0, with logarithmically transformed concentrations plotted against the corrected absorbance values.

Chemistry

General remarks

All purchased chemicals were used without purification unless stated otherwise. All reactions were performed in oven-dried or flame-dried glassware. Anhydrous solvents were dried by activated 3 Å or 4 Å molecular sieves. Thin layer chromatography (TLC) analysis was performed on Merck silica gel 60 F_{254} aluminium sheets and the compounds were visualized by using UV absorption at 254 nm and/or $KMnO_4$ staining (5 g/L $KMnO_4$ and 25 g/L K_2CO_3 in water). TLC plates were analysed with the Advion CMS Plate Express® connected to the Advion Expression® L-MS using 90% MeOH in H_2O with 0.1% formic acid as the solvent. Purification was performed on automated silica gel column chromatography (40-63 µm, 60 Å pre-packed silica gel, Screening Devices) on a Biotage Isolera™ Four 3.0 system. ^1H and ^{13}C spectra were recorded on AV 400 MHz spectrometer (400 MHz for ^1H and 101 MHz for ^{13}C) in deuterated solvents. Chemical shifts are reported in ppm with tetramethylsilane (TMS) or solvent resonance as the internal standard ($CDCl_3$: δ 7.26 for ^1H, δ 77.16 for ^{13}C; CD_3OD: δ 3.31 for ^1H, 49.00 for ^{13}C). Data is reported as follows: chemical shifts δ (ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, quintet = p, m = multiplet), coupling constants *J* (Hz) and integration. High resolution mass spectrometry (HRMS) analysis was performed on a Thermo Finnigin LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctyl phthalate (m/z = 391.28428) as a lock mass.

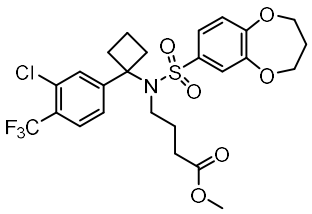
4-((*N*-(1-(3-Chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]diozepine)-7-sulfonamido)butanoic acid (7, LEI-132)



To a solution of methyl 4-((*N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine)-7-sulfonamido)butanoate (**8**, 42 mg, 0.075 mmol, 1 eq) in THF/MeOH (0.75 mL/0.75 mL, 0.05 M) was added 1 M aq. LiOH (0.3 mL, 0.3 mmol, 4 eq). The mixture was stirred at rt for overnight. The reaction mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-3% MeOH in DCM) to afford the product (36 mg, 0.066 mmol, 87%). ^1H NMR (400 MHz, MeOD+$CDCl_3$) δ 7.62 (d, *J* = 8.1 Hz, 1H), 7.56 – 7.47 (m, 2H), 7.00 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 4.26 (t, *J* = 5.7 Hz, 2H), 4.20 (t, *J* = 5.8 Hz,

2H), 3.31 (t, J = 7.2 Hz, 2H), 2.83 – 2.69 (m, 2H), 2.59 – 2.48 (m, 2H), 2.28 (t, J = 6.9 Hz, 2H), 2.19 (p, J = 5.8 Hz, 2H), 1.93 (p, J = 7.1 Hz, 2H), 1.85 – 1.75 (m, 1H), 1.68 – 1.53 (m, 1H). ^{13}C NMR (101 MHz, MeOD+$CDCl_3$) δ 175.79, 155.07, 151.13, 149.91, 135.50, 132.65 (q, $J_{C\text{-}F}$ = 1.9 Hz), 130.75, 127.94 (q, $J_{C\text{-}F}$ = 5.3 Hz), 127.68 (q, $J_{C\text{-}F}$ = 31.9 Hz), 125.80, 123.40 (q, $J_{C\text{-}F}$ = 273.2 Hz), 122.66, 121.99, 120.81, 71.06, 70.88, 65.72, 47.53, 35.75, 31.50, 31.32, 27.22, 14.83. HRMS $[C_{24}H_{25}ClF_3NO_6S+Na]^+$: 570.09354 calculated, 570.09435 found.

Methyl 4-((*N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine)-7-sulfonamido)butanoate (8)



To a solution of *N*-(1-(3-chloro-4-(trifluoromethyl)phenyl)cyclobutyl)-3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine-7-sulfonamide (54.0 mg, 0.117 mmol, 1 eq) in anhydrous DMF (1.2 mL, 0.1 M) was added methyl 4-bromobutanoate (29.6 µL, 0.234 mmol, 2 eq) and BEMP (1 M in hexane, 234 µL, 0.234 mmol, 2 eq). The mixture was heated at 80 °C for overnight. The reaction mixture was diluted in 0.1 M aq. HCl and extracted 3× with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (15-25% EtOAc in *n*-pentane) to afford the product (51 mg, 0.091 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.2 Hz, 1H), 7.56 – 7.51 (m, 1H), 7.50 (d, J = 1.8 Hz, 1H), 7.03 (dd, J = 8.4, 2.4 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 4.29 (t, J = 5.8 Hz, 2H), 4.23 (t, J = 5.9 Hz, 2H), 3.68 (s, 3H), 3.33 – 3.26 (m, 2H), 2.83 – 2.71 (m, 2H), 2.60 – 2.50 (m, 2H), 2.31 (t, J = 6.9 Hz, 2H), 2.22 (p, J = 5.8 Hz, 2H), 2.01 – 1.91 (m, 2H), 1.87 – 1.77 (m, 1H), 1.69 – 1.59 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.44, 154.39, 150.58, 149.44, 135.47, 132.33 (q, $J_{C\text{-}F}$ = 2.0 Hz), 130.21, 127.55 (q, $J_{C\text{-}F}$ = 5.0 Hz), 127.32 (q, $J_{C\text{-}F}$ = 31.5 Hz), 125.35, 122.91 (q, $J_{C\text{-}F}$ = 269.8 Hz), 122.24, 121.51, 120.47, 70.53, 70.37, 65.17, 51.86, 47.06, 35.30, 31.10, 30.90, 26.65, 14.57.

References

1. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50**, 83–90 (1995).
2. Devane, W. A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
3. Busquets-García, A., Bolaños, J. P. & Marsicano, G. Metabolic Messengers: endocannabinoids. *Nat. Metab.* **4**, 848–855 (2022).
4. Castillo, P. E., Younts, T. J., Chávez, A. E. & Hashimotodani, Y. Endocannabinoid Signaling and Synaptic Function. *Neuron* **76**, 70–81 (2012).
5. Mackay, H. J. & Twelves, C. J. Targeting the protein kinase C family: Are we there yet? *Nat. Rev. Cancer.* **7**, 554–562 (2007).
6. Boyce, J. A. Mast cells and eicosanoid mediators: A system of reciprocal paracrine and autocrine regulation. *Immunol. Rev.* **217**, 168–185 (2007).
7. Papackova, Z. & Cahova, M. Fatty acid signaling: The new function of intracellular lipases. *Int. J. Mol. Sci.* **16**, 3831–3855 (2015).
8. Alger, B. E. & Kim, J. Supply and demand for endocannabinoids. *Trends Neurosci.* **34**, 304–315 (2011).
9. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163**, 463–468 (2003).
10. Karlsson, M., Contreras, J. A., Hellman, U., Tornqvist, H. & Holm, C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J. Biol. Chem.* **272**, 27218–27223 (1997).
11. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356 (2007).
12. Griner, E. M. & Kazanietz, M. G. Protein kinase C and other diacylglycerol effectors in cancer. *Nat. Rev. Cancer* **7**, 281–294 (2007).
13. Kolczynska, K., Loza-Valdes, A., Hawro, I. & Sumara, G. Diacylglycerol-evoked activation of PKC and PKD isoforms in regulation of glucose and lipid metabolism: A review. *Lipids Health Dis.* **19**, 1–15 (2020).
14. Rouzer, C. A. & Marnett, L. J. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: Cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem. Rev.* **111**, 5899–5921 (2011).
15. Samovski, D., Jacome-Sosa, M. & Abumrad, N. A. Fatty Acid Transport and Signaling: Mechanisms and Physiological Implications. *Annu. Rev. Physiol.* **85**, 317–337 (2023).
16. Cooke, M. & Kazanietz, M. G. Overarching roles of diacylglycerol signaling in cancer development and antitumor immunity. *Sci. Signal.* **15**, 1–27 (2022).

17. Nomura, D. K. *et al.* Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science* **334**, 809–813 (2011).

18. Gao, Y. *et al.* Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J. Neurosci.* **30**, 2017–2024 (2010).

19. Tanimura, A. *et al.* The Endocannabinoid 2-Arachidonoylglycerol Produced by Diacylglycerol Lipase α Mediates Retrograde Suppression of Synaptic Transmission. *Neuron* **65**, 320–327 (2010).

20. Viader, A. *et al.* A chemical proteomic atlas of brain serine hydrolases identifies cell type-specific pathways regulating neuroinflammation. *Elife* **5**, 1–24 (2016).

21. Berger, N. *et al.* Inhibition of diacylglycerol lipase β modulates lipid and endocannabinoid levels in the ex vivo human placenta. *Front. Endocrinol.* **14**, 1–12 (2023).

22. Hsu, K.-L. *et al.* DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat. Chem. Biol.* **8**, 999–1007 (2012).

23. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 26–33 (2016).

24. Jenniches, I. *et al.* Anxiety, Stress, and Fear Response in Mice with Reduced Endocannabinoid Levels. *Biol. Psychiatry* **79**, 858–868 (2016).

25. Cavener, V. S. *et al.* Inhibition of Diacylglycerol Lipase Impairs Fear Extinction in Mice. *Front. Neurosci.* **12**, 1–10 (2018).

26. Baggelaar, M. P. *et al.* Development of an Activity-Based Probe and In Silico Design Reveal Highly Selective Inhibitors for Diacylglycerol Lipase-α in Brain. *Angew. Chem. Int. Ed.* **52**, 12081–5 (2013).

27. Janssen, F. J. *et al.* Discovery of Glycine Sulfonamides as Dual Inhibitors of sn -1-Diacylglycerol Lipase α and α/β-Hydrolase Domain 6. *J. Med. Chem.* **57**, 6610–6622 (2014).

28. Porta, E. O. J. & Steel, P. G. Activity-based protein profiling: A graphical review. *Curr. Res. Pharmacol. Drug Discov.* **5**, 100164 (2023).

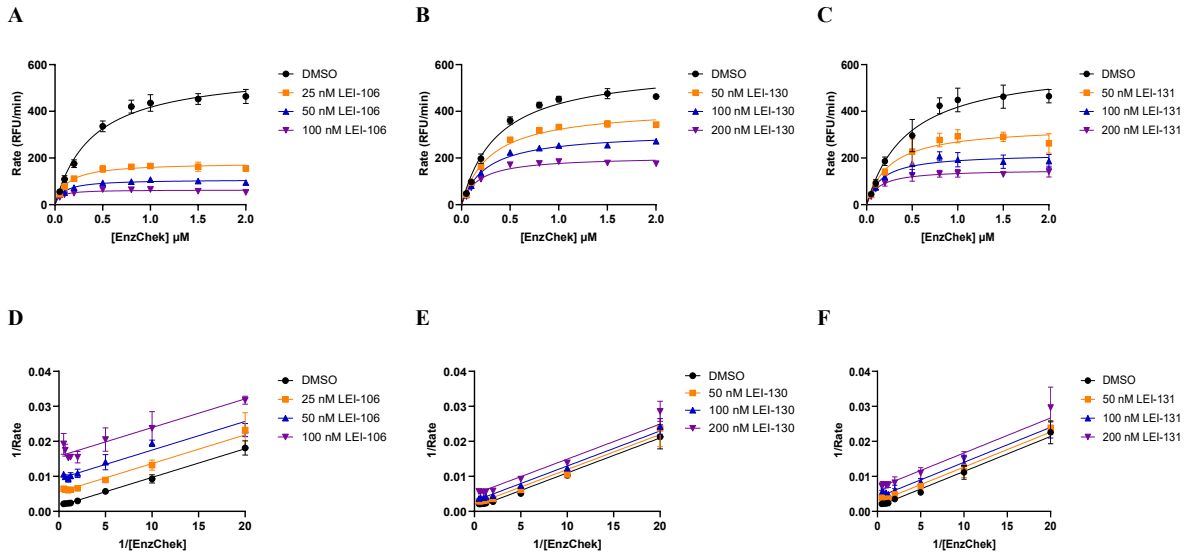
29. Navia-Paldanius, D., Savinainen, J. R. & Laitinen, J. T. Biochemical and pharmacological characterization of human α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). *J. Lipid Res.* **53**, 2413–2424 (2012).

30. Mock, E. D. *et al.* Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. *Nat. Chem. Biol.* **16**, 667–675 (2020).

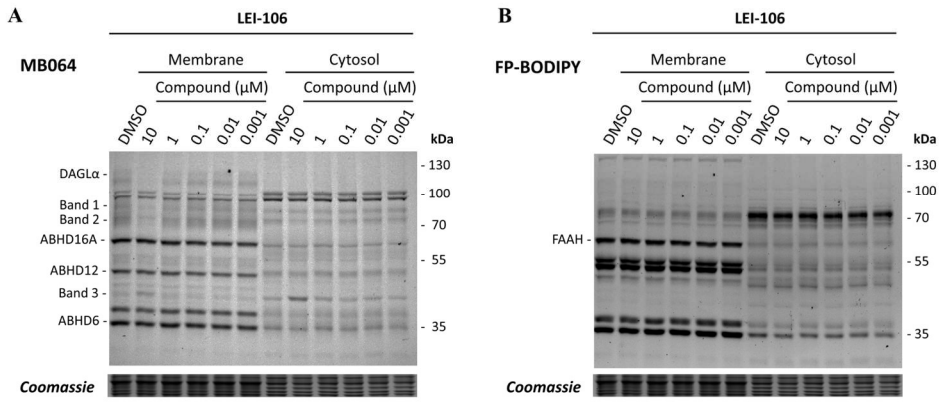
31. Soethoudt, M. *et al.* Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity. *Nat. Commun.* **8**, (2017).

32. Di Zazzo, A. *et al.* Signaling lipids as diagnostic biomarkers for ocular surface cicatrizing conjunctivitis. *J. Mol. Med.* **98**, 751–760 (2020).

Supplementary Figures and Tables



Supplementary Figure S6.1 Mode-of-inhibition studies of LEI-106, LEI-130 and LEI-131 against DAGLα. (A-C) Michaelis-Menten kinetic curves. Rate of EnzChek hydrolysis by DAGLα as a function of substrate concentration in the presence of different concentrations of LEI-106, LEI-130 and LEI-131. (D-F) Lineweaver Burk plots. Data shown are mean ± SD (n = 2, N = 2).



Supplementary Figure S6.2 *In vitro* selectivity of LEI-106 on mouse brain proteomes. Representative gels of ABPP experiments using MB064 (A, 250 nM, 10 min) and subsequently FP-BODIPY (B, 100 nM, 10 min).

Supplementary Table S6.1 Summary of V_{max} and K_M values for DAGLβ in the presence of different concentrations of LEI-106, LEI-130 and LEI-131.

Compound	Concentration (nM)	V_{max} (RFU/min)	K_M (µM)
LEI-106	0	176 ± 11	0.26 ± 0.07
	25	124 ± 11	0.28 ± 0.07
	50	90 ± 7	0.20 ± 0.05
	100	72 ± 10	0.23 ± 0.11
LEI-130	0	165 ± 12	0.31 ± 0.08
	5	102 ± 8	0.29 ± 0.08
	10	74 ± 6	0.28 ± 0.08
	20	60 ± 10	0.31 ± 0.12
LEI-131	0	195 ± 14	0.29 ± 0.07
	5	89 ± 9	0.27 ± 0.11
	10	54 ± 5	0.24 ± 0.10
	20	38 ± 5	0.24 ± 0.09

Supplementary Table S6.2 Summary of V_{max} and K_M values for DAGLα in the presence of different concentrations of LEI-106, LEI-130 and LEI-131.

Compound	Concentration (nM)	V_{max} (RFU/min)	K_M (µM)
LEI-106	0	581 ± 39	0.39 ± 0.07
	25	180 ± 9	0.13 ± 0.03
	50	108 ± 6	0.09 ± 0.02
	100	63 ± 5	0.05 ± 0.02
LEI-130	0	593 ± 35	0.37 ± 0.07
	50	418 ± 19	0.30 ± 0.04
	100	311 ± 11	0.25 ± 0.03
	200	207 ± 9	0.17 ± 0.03
LEI-131	0	607 ± 57	0.45 ± 0.12
	50	337 ± 35	0.25 ± 0.07
	100	219 ± 22	0.16 ± 0.05
	200	151 ± 11	0.13 ± 0.04

Chapter 7

Summary and Future Prospects

7.1 Summary

Endocannabinoids are the endogenous signaling lipids that activate cannabinoid receptors type 1 and 2 (CB_1R and CB_2R) to regulate various biological processes, such as synaptic plasticity, memory formation and learning, mood, energy metabolism, pain sensation and immune response.[1–4] There are two main endocannabinoids: 2-arachidonoylglycerol (2-AG)[5] and *N*-arachidonoylethanolamine (AEA, anandamide).[6] Endocannabinoids are produced 'on demand' by biosynthetic enzymes (*e.g.*, DAGLα/β[7] and NAPE-PLD[8]) and rapidly inactivated by metabolic enzymes (*e.g.*, MAGL[9] and FAAH[10]) to terminate their actions. Endocannabinoids, cannabinoid receptors CB_1R and CB_2R, biosynthetic and metabolic enzymes constitute the endocannabinoid system (ECS). To study biological functions and establish the therapeutic potential of these enzymes, a number of activity-based probes (ABPs) and small molecular inhibitors have been developed, as summarized in **Chapter 1**. Two types of broad-spectrum ABPs have been developed so far: fluorophosphonate-based probes[11,12] and β-lactone-based probes.[13,14] These probes can label numerous serine hydrolases, including most enzymes in the ECS, enabling novel inhibitor discovery and selectivity evaluation. Benefiting from the application of these probes in activity-based protein profiling (ABPP), many potent and selective inhibitors have been discovered for ECS enzymes. Representative inhibitors include, but are not limited to, DH376[15], a triazole urea-based dual DAGLα/β inhibitor; LEI-401[16], a pyrimidine-4-carboxamide-based NAPE-PLD inhibitor; Lu AG06466[17], a carbamate-based MAGL inhibitor; and PF-04457845[18], a pyridazine urea-based FAAH inhibitor.

Additionally, in **Chapter 1**, the biological functions and potential clinical applications of targeting ECS enzymes are discussed. MAGL and FAAH inhibitors have progressed to clinical trials. The MAGL inhibitor, Lu AG06466, demonstrated general safety in clinical trials but did not exhibit efficacy in reducing tics, premonitory urges and comorbidities in patients with Tourette syndrome during a phase 2 clinical trial.[19] The FAAH inhibitor PF-04457845 showed positive effects in a phase 2a study on cannabis use disorder.[20] Another FAAH inhibitor, JNJ-42165279, is currently under clinical investigation for treating post-traumatic stress disorder (PTSD). In contrast, the research progress of DAGL and NAPE-PLD inhibitors has been slower and they are currently being studied in animal models. DAGL inhibitors have showed anti-inflammatory[15,21] effects and analgesic effects.[22] However, inhibiting DAGLα in the brain might potentially lead to psychiatric side effects.[23,24] The first-in-class NAPE-PLD inhibitor, LEI-401, was found to activate the hypothalamus–pituitary–adrenal (HPA) axis and impair fear extinction in mice.[16] In this context, peripherally restricted inhibitors can be essential tools to elucidate the functions of ECS enzymes in peripheral tissues and may have new clinical applications. Moreover, potent and selective inhibitors are still lacking for some ECS enzymes, such as DAGLα and DAGLβ.

The development of selective DAGLβ inhibitors is crucial for understanding the distinct biological functions of this enzyme and discerning the roles of DAGLβ and DAGLα under specific pathophysiological conditions. Moreover, selective DAGLβ inhibitors hold a promise as a valuable approach for treating inflammatory diseases while minimizing the potential for

central nervous system (CNS)-mediated side effects. Therefore, the primary objective of the research outlined in this thesis is to advance the development of selective DAGLβ inhibitors.

In **Chapter 2**, a fluorescence assay using EnzChek lipase substrate was optimized for DAGLα/β and miniaturized to a 384-well plate format. This assay was subsequently applied in a high-throughput screening (HTS) using the purified catalytic domain of DAGLβ to identify new hit compounds. A library of 12,560 serine hydrolase inhibitors from Enamine and an in-house library of 27 glycine sulfonamides[25] were screened at 10 μM. After primary screening, confirmed screening, deselection, and dose-response determination, eight hits classified into four chemotypes were identified, including glycine sulfonamide (hit **1**), ketones (hits **2**, **5**), ureas (hits **3**, **4**) and cyano amides (hits **6-8**). Hit **1**, also known as LEI-106[25] (Figure 7.1A), exhibited the highest potency for DAGLβ with a pIC_{50} of 6.69 ± 0.14 and promising physicochemical properties, but was not selective over DAGLα (pIC_{50} of 7.35 ± 0.06).

In **Chapter 3**, a structure-activity relationship (SAR) study for both DAGLα and DAGLβ was conducted, starting from hit **1**, with the aim of enhancing the potency and selectivity specifically for DAGLβ. A total of 51 analogues were synthesized and biochemically evaluated, resulting in the identification of compound **9** as the most potent and selective DAGLβ inhibitor, albeit a modest selectivity of 2.7-fold (Figure 7.1A). The SAR study revealed that the sulfonyl group was a modification hotspot for improving selectivity, while other components contributed to potency to varying extents.

In **Chapter 4**, an extensive SAR study was conducted, with a specific focus on modifying the sulfonyl group to enhance DAGLβ selectivity. The introduction of a cyclobutyl substituent on the amine moiety and the modification of the dibenzo[*b*,*d*]furan on the sulfonyl group of compound **9** to a 2,3-dihydrobenzo[*b*][1,4]dioxine resulted in compound **10**, which exhibited improved potency (pIC_{50} of 7.59 ± 0.06) and selectivity (20-fold) for DAGLβ (Figure 7.1A). Expanding the dioxane ring in compound **10** to a dioxepane yielded compound **11** with further increased selectivity. However, other modifications on the sulfonyl resulted in less selective compounds. Changing the cyclobutyl in **11** to a cyclopentyl resulted in compound **12**, which displayed improved potency while retaining selectivity for DAGLβ. Replacing the bromide in compounds **11** and **12** with other electron-withdrawing and lipophilic moieties (*e.g.* chloride and trifluoromethyl) resulted in a series of compounds exhibiting high potency ($pIC_{50} > 7.50$), good selectivity (~40-fold), appropriate lipophilicity (cLogD < 3) and lipophilic efficiency (LipE > 5). The comprehensive SAR for glycine sulfonamide DAGL inhibitors is illustrated in Figure 7.1B.

In **Chapter 5**, a fluorescence-based 96-well plate assay was established to assess the cellular activity of DAGL inhibitors using Neuro2A cells expressing the $GRAB_{eCB2.0}$ sensor. This sensor is a circular permuted enhanced green fluorescent protein (cpEGFP)-modified CB_1 receptor[26] that can be activated by the binding of endocannabinoids to elicit fluorescence. The $GRAB_{eCB2.0}$ sensor expressed in Neuro2A cells responds to ATP-induced production of 2-AG. A total of 23 DAGL inhibitors were profiled in this assay, demonstrating a concentration-dependent reduction in the production of 2-AG with varying potencies. Significantly, a strong correlation was observed between the cellular activity in the Neuro2A $GRAB_{eCB2.0}$ assay and

the biochemical activity for DAGLα. However, no such correlation was identified for DAGLβ, indicating that DAGLα is the primary isoform involved in ATP-stimulated 2-AG production in Neuro2A cells.

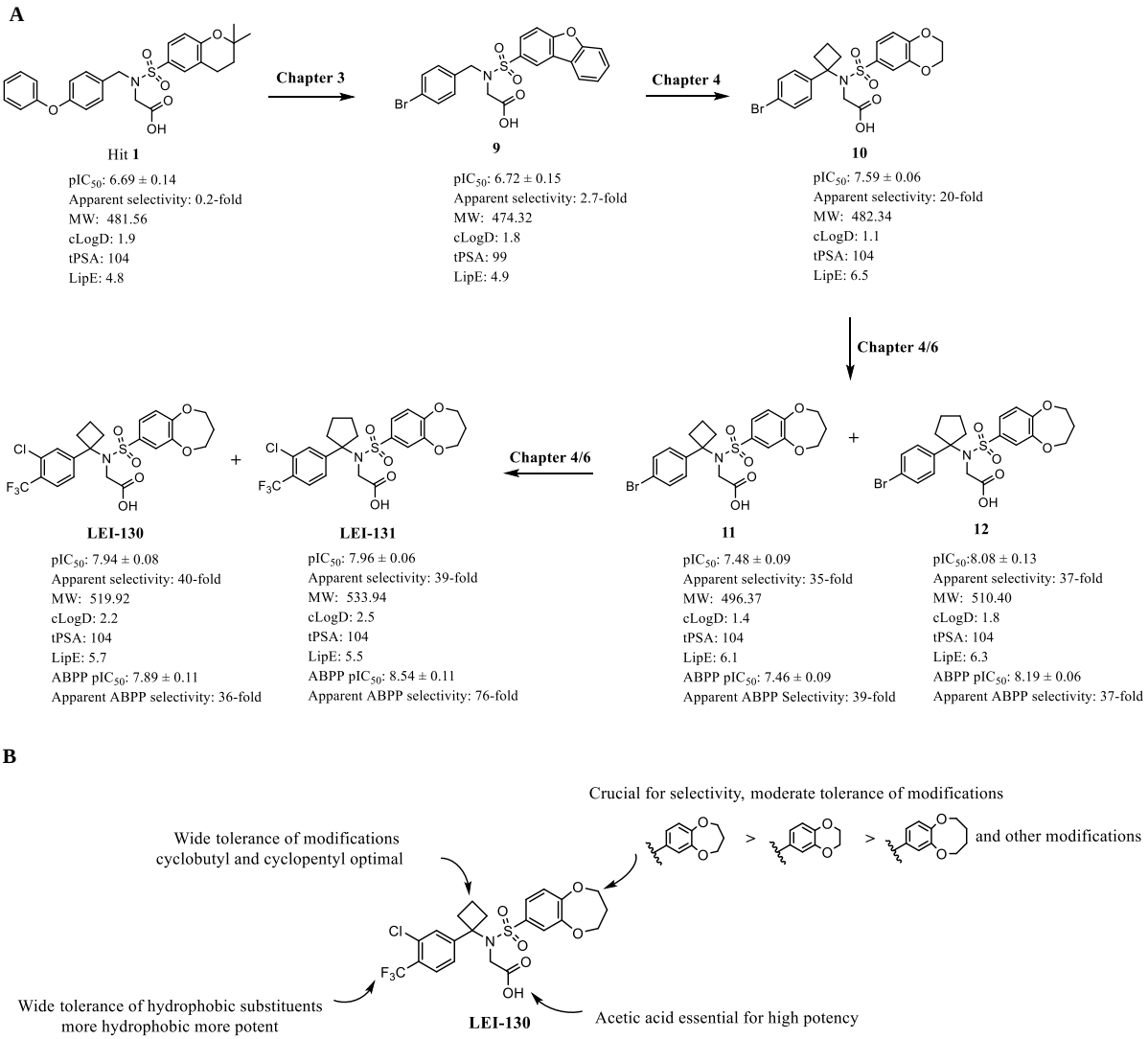


Figure 7.1 Summary of the structure-activity relationship (SAR) study. (A) Chemical structures, biochemical results (pIC_{50}, apparent selectivity and LipE for DAGLβ), physiochemical properties (MW, cLogD and tPSA) and activity-based protein profiling results (ABPP pIC_{50} and apparent ABPP selectivity for DAGLβ) of Hit **1** and representative compounds in SAR. (B) The overall SAR.

In **Chapter 6**, the most promising DAGLβ selective inhibitors developed in Chapter 4 underwent investigation in ABPP assays using DAGL-tailored probe DH379. Considering both potency and selectivity in biochemical and ABPP assays, along with structure similarities, **LEI-130** and **LEI-131** emerged as the most promising candidates for further *in vitro* and *in situ* profiling (Figure 7.1A). Michaelis-Menten kinetic studies using the EnzChek lipase substrate assay demonstrated that both LEI-130 and LEI-131 inhibited DAGLβ through a noncompetitive inhibition mode, suggesting their binding to an allosteric pocket of DAGLβ. *In vitro* selectivity profiling revealed that LEI-130 and LEI-131 did not affect the activity of NAPE-PLD, FAAH,

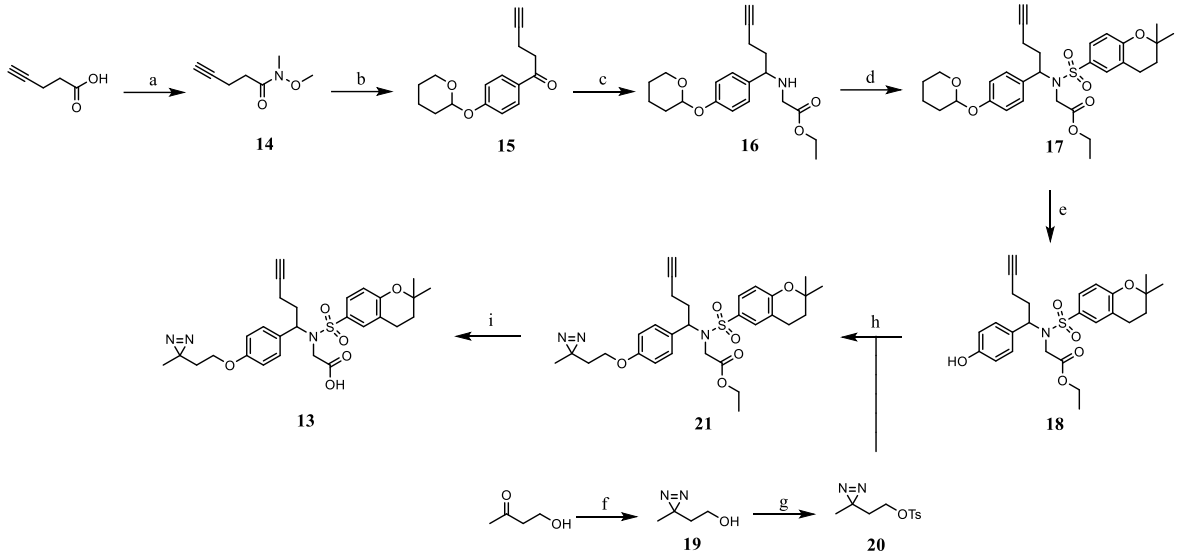
MAGL, ABHD6, ABHD12 and cannabinoid CB_1 and CB_2 receptors. *In situ* target engagement and selectivity profiling in microglia and macrophage cells confirmed cell permeability of LEI-130 and LEI-131, and their capability to inhibit endogenous DAGLβ without interfering with other serine hydrolases. Targeted lipidomics of LEI-130 and LEI-131 demonstrated an increase in SAG levels in N9 microglia cells and J774.1 macrophages. However, a decrease in the levels of 2-AG, arachidonic acid (AA), and prostaglandins PGE_2 and PGD_2 was only observed in N9 cells. LEI-132, a DAGL inactive compound included as a negative control, did not show any effects on 2-AG and SAG levels. This indicates that 2-AG production in J774.1 macrophages, unlike N9 microglia cells, is not dependent on DAGLβ. Interestingly, LEI-130, LEI-131 and LEI-132 were all shown to attenuate LPS-stimulated cytokine production, suggesting that an unknown off-target modulates LPS-stimulated cytokine production.

7.2 Future prospects

7.2.1 Photoaffinity labeling to identify the allosteric binding pocket

LEI-130 and LEI-131 were identified as allosteric inhibitors of DAGLβ utilizing a noncompetitive inhibition mechanism. To unveil the binding pocket of these compounds, an affinity-based photoprobe (**13**) was designed, incorporating a glycine sulfonamide pharmacophore, a diazirine for UV-activated protein crosslinking, and an alkyne handle for reporter ligation. The synthesis of the probe is depicted in Scheme 7.1. Weinreb amide **14** obtained from 4-pentanoic acid was transformed into ketone **15** via Grignard reaction. Subsequently, reductive amination of **15** with ethyl glycinate yielded amine **16**, followed by condensation with 2,2-dimethylchromane-6-sulfonyl chloride, resulting in sulfonamide **17**. THP deprotection afforded key intermediate **18**. Diazirine-linker **20** was obtained from 4-hydroxybutan-2-one by diaziridine introduction under agency of NH_3 and NH_2OSO_3H, followed by oxidation with I_2 to form compound **19**. The hydroxyl group was then transformed to a toysl-ester leaving group (**19** → **20**). Alkyation of **18** with **20** afforded ether **21**, which, after saponification, resulted in the final photoprobe **13**.

The inhibitory activity of photoprobe **13** for DAGL enzymes was assessed in the EnzChek lipase substrate assay, resulting in similar pIC_{50} values for DAGLα and DAGLβ (Figure 7.2).



Scheme 7.1 Synthesis of photoprobe **13**. a) *N*,*O*-dimethylhydroxylammonium chloride, DMAP, EDCI, Et_3N, anhydrous DCM, rt, 42%; b) (4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)magnesium bromide, anhydrous THF, 0 °C-rt, 41%; c) glycine ethyl ester hydrochloride, Et_3N, AcOH, $NaBH_3CN$, anhydrous EtOH, reflux, 49%; d) 2,2-dimethylchromane-6-sulfonyl chloride, DMAP, Et_3N, anhydrous DCM, rt, 75%; e) TsOH, EtOH, rt, 70%; f) *i*. 7 N NH_3 in MeOH, 0 °C; *ii*. NH_2OSO_3H, anhydrous MeOH, rt; *iii*. Et_3N, I_2, anhydrous MeOH, 21%; g) 4-methylbenzenesulfonyl chloride, pyridine, rt, 57%; h) Cs_2CO_3, anhydrous DMF, rt, 25%; i) 1 M aq. LiOH, THF/MeOH, rt, 29%.

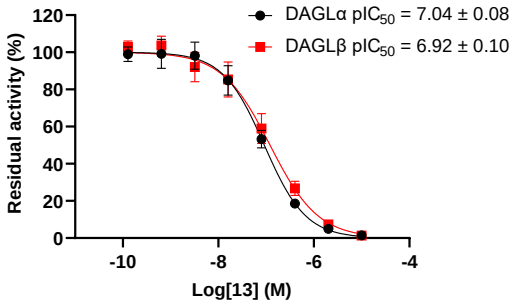


Figure 7.2. Dose-response curves of photoprobe **13** for DAGLα (black) and DAGLβ (red). Data shown are mean ± SD (n = 1, N = 3).

Next, it was tested whether probe **13** could label recombinant human DAGL in HEK293T lysates in a specific manner. DMSO or DAGL inhibitors (DO34, KT109, LEI-105 and LEI-106, Figure 7.3B) were incubated for 30 min before adding probe **13** (100 nM). The mixture was incubated for 10 min, irradiated with 350 nm UV light for 10 min and clicked with a Cy5-N_3 fluorophore (1 μM) for 45 min. The click reaction was quenched by adding Laemmli blue and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Strong fluorescent bands at the expected molecular weights of ~120 kDa and ~70 kDa appeared in the HEK293T cell lysates overexpressing DAGLα and DAGLβ, respectively (lane 2 and 7, Figure 7.3A), but not in the mock lysate (lane 1, Figure 7.3A). Pre-incubation with DAGL inhibitors DO34, KT109 at 1 μM, and LEI-105 and LEI-106 at 10 μM reduced the labeling of these bands. This indicated the successful engagement of DAGLα and DAGLβ by probe **13** and the inhibitors. The activity of DAGLβ selective inhibitors, LEI-130 and LEI-131, was assessed

using photoprobe **13**. The results demonstrated that both LEI-130 and LEI-131 effectively inhibited the labeling of DAGLβ by probe **13** in a concentration-dependent manner (Figure 7.4A), exhibiting pIC_{50} values of 7.12 ± 0.13 and 7.59 ± 0.22 (Figure 7.4B), respectively.

To identify the photoprobe-labeled DAGLβ peptides, chemical proteomics was conducted. However, MS-based proteomics did not enable the identification of these peptides, thereby impeding the elucidation of the allosteric binding pocket of glycine sulfonmaides on DAGLβ.

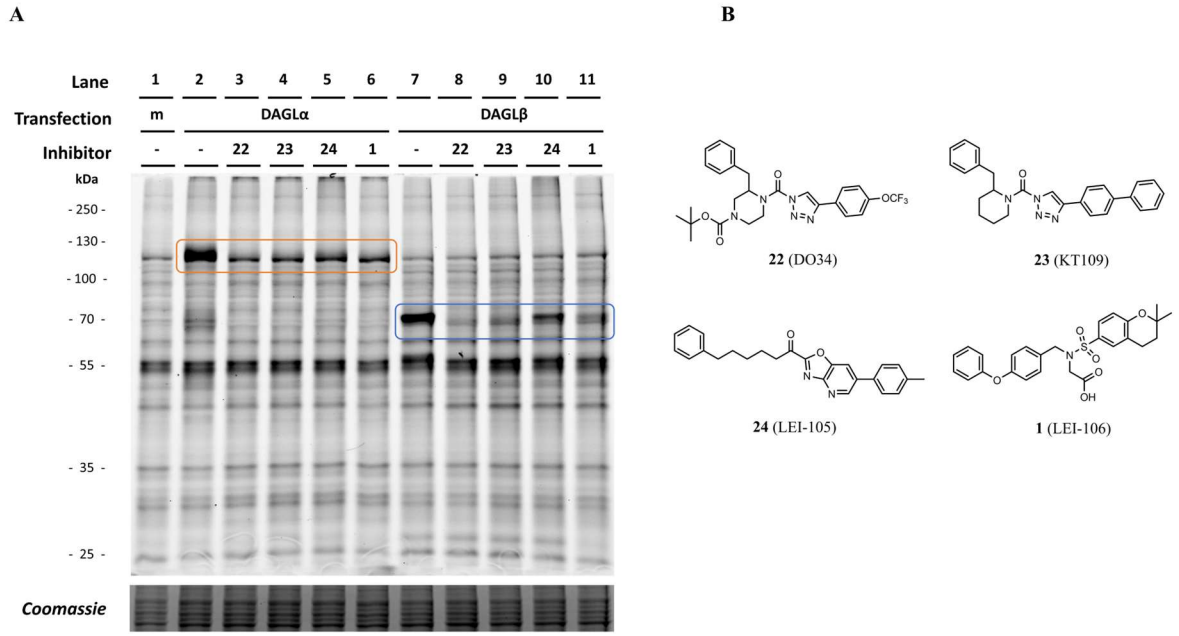


Figure 7.3 Photoprobe **13** enabled *in vitro* labeling and visualization of recombinant human DAGLα and DAGLβ. (A) Representative gel of fluorescent labeling of DAGLα (orange) and DAGLβ (blue) by photoprobe **13** without or with DAGL inhibitors. (B) Chemical structures of DAGL inhibitors DO34, KT109, LEI-105 and LEI-106.

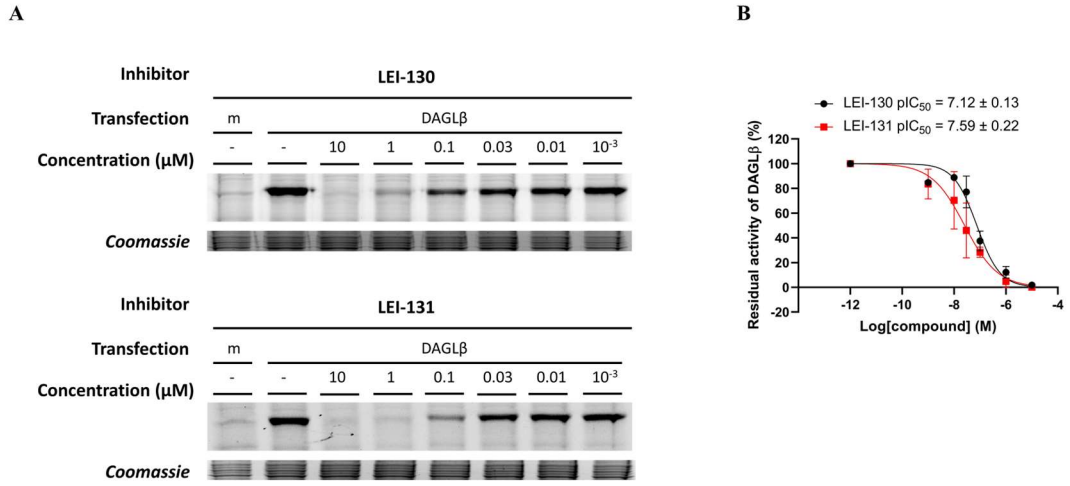


Figure 7.4 LEI-130 and LEI-131 concentration-dependently blocked the labeling of DAGLβ by probe **13**. (A) Representative gel excerpts of fluorescent labeling of DAGLβ by probe **13** without or with LEI-130 and LEI-131 at different concentrations. (B) Dose-response curves of LEI-130 and LEI-131 for DAGLβ. Data shown are mean ± SD (n = 1, N = 3).

7.2.2 Ethyl ester protection is not an effective prodrug strategy for glycine sulfonamide DAGL inhibitors.

In Chapter 5, a set of glycine sulfonamide DAGL inhibitors was assessed in the Neuro2A $GRAB_{eCB2.0}$ assay, revealing a significant decrease in their cellular activity compared to their biochemical activity. The reduction in activity is likely primarily due to the low cell permeability of glycine sulfonamides caused by the carboxylic acid. To enhance cell permeability and cellular activity, the carboxylic acid of LEI-130 and LEI-131 was protected by an ethyl ester in a prodrug approach, resulting in compounds **25** and **26** (Figure 7.5A). When evaluated in the EnzChek lipase substrate assay, compounds **25** and **26** displayed no activity for either DAGLα or DAGLβ (pIC_{50} < 5, Figure 7.5B, C).

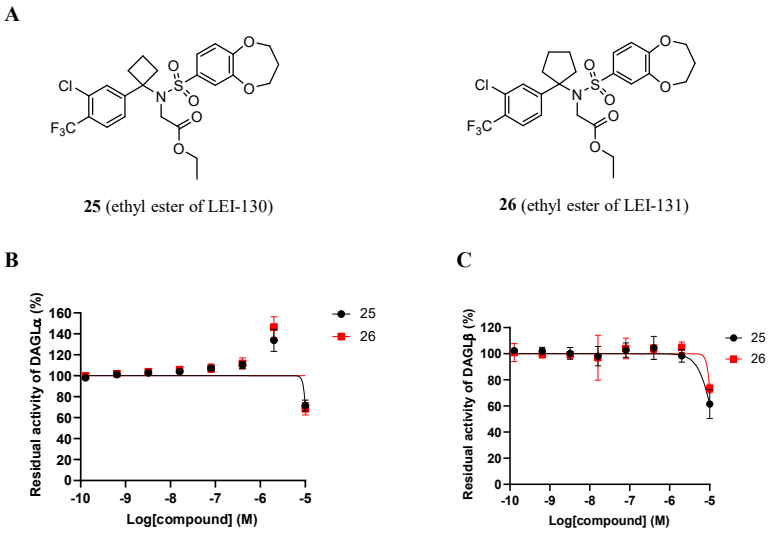


Figure 7.5 Compounds **25** and **26** were not active for DAGL enzymes. (A) Chemical structures of compounds **25** and **26**. (B, C) Dose-response curves of compounds **25** and **26** for DAGLα (B) and DAGLβ (C). Data shown are mean ± SD (n = 1, N = 3).

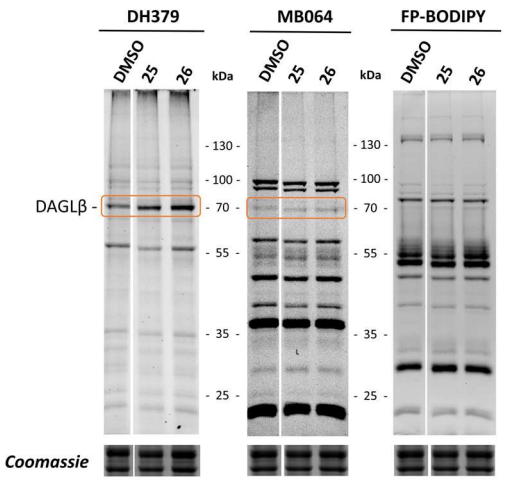


Figure 7.6 *In situ* target engagement and selectivity of compounds **25** and **26**. Representative gels of ABPP experiments with compounds **25** and **26** (10 µM, 2 h) in N9 microglia using DH379 (*in situ*, 1 µM, 1 h) or the cocktail of MB064 and FP-BODIPY (*in vitro*, 100 nM, 10 min).

To investigate whether compounds **25** and **26** could undergo hydrolysis to form active DAGLβ inhibitors in cells, *in situ* gel-based ABPP was conducted in N9 microglia cells, as described in Chapter 6. However, compounds **25** and **26** were not converted to the active parents LEI-130 and LEI-131, as endogenous DAGLβ was not inhibited. Therefore, protecting the carboxyl group with an ethyl ester is not an effective prodrug strategy for glycine sulfonamide DAGL inhibitors.

7.2.3 Targeting DAGLβ-DAG-PKC axis to regulate antitumor immunity

Diacylglycerol (DAG) functions as a physiological activator of protein kinase C (PKC), orchestrating cellular processes.[27] The production of DAG in the plasma membrane from phosphatidylinositol 4,5-bisphosphate (PIP_2) is mediated by phospholipase C (PLC). Subsequently, DAG is metabolized by DAGL and diacylglycerol kinase (DGK) to form monoacylglycerol (MAG) and phosphatidic acid (PA), respectively. DAG interacts with the C1 domain of its effector proteins such as PKC, leading to their recruitment to the membrane.[28,29] The membrane interaction induces a conformational change of PKC, representing a pivotal step to activate PKC. Activated PKC catalyzes the phosphorylation of hydroxyl groups on serine and threonine amino acid residues of its substrates, initiating downstream signaling activation.[27,30] The delicate balance in the biosynthesis and degradation of DAG, as well as the precise regulation of PKC, is crucial for cellular homeostasis. PKC isoenzymes demonstrate a dual role in cancer, acting as both promoters and suppressors, with their specific functions intricately tied to the isoform and cellular context. A distorted pattern of PKC isoenzyme expression has been discovered in most cancer cells, including up-regulation of PKCε, PKCη and PKCθ with oncogenic activities[31–37] and down-regulation of PKCα, PKCβ and PKCδ with tumor-suppressing functions.[38–43] This underscores the complexity of PKC isoenzymes across various cancer types.

Tumor progression is regulated by the interaction between cancer cells and tumor microenvironment (TME).[44] DAG and its effectors exert their influence not only on cancer cells but also extend to noncancerous cells within the TME. DAG plays a pivotal role as a messenger lipid in T cell activation via the T cell receptor (TCR) and tightly regulates the dynamic relationship between cancer cells and T cells.[27,45,46] Within T cells, DAG interacts with the C1 domain to recruit DAG effectors to the immunological synapse. The translocation of DAG effectors triggers the activation of essential transcriptional machinery that governs T cell expansion and activity. PKC signals stimulate the production of interleukin-2 (IL-2), a key cytokine for T cell proliferation and differentiation, as well as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), cytokines contributing to the inhibition of cancer cells.[47] Recently, the production of DAG during T cell receptor signaling was shown to trigger ectocytosis of activated TCRs.[48] The resulting budding ectosomes could be directly endocytosed by target cells, leading to the termination of TCR signaling. Concurrently, the process separates cytotoxic T lymphocytes (CTLs) from target cells, thereby facilitating serial killing. Therefore, maintaining sufficient DAG levels is important for T cell activity and function.

Down-regulated DAG response observed in tumor-infiltrating lymphocytes (TILs) is a hallmark, contributing to hyporesponsive state and reduced lytic activity.[49] Small molecules capable of restoring DAG response could serve as potential treatments to enhance the antitumor potential of cytotoxic T lymphocytes. Diacylglycerol lipase β (DAGLβ) is the predominant enzyme responsible for hydrolyzing DAG in primary macrophage[21] and dendritic cells.[50] DAGLβ disruption in dendritic cells has been discovered to reduce the production of inflammatory cytokine without affecting their capacity for $CD8^+$ T cell priming.[50] However, the involvement of DAGLβ in cancer and antitumor immunity remains understudied. In this research, selective DAGLβ inhibitors, LEI-130 and LEI-131, have been successfully developed. Lipidomics studies demonstrated their ability to significantly elevate the levels of stearoyl-arachidonoyl DAG (SAG) in N9 microglia and J774A.1 macrophages. LEI-130 and LEI-131 hold potential as valuable tool compounds for unraveling the intricate role of DAGLβ in both cancer cells and T cells, with promising applications in cancer therapy.

7.3 Closing remarks

Selective and cellularly active small molecules play a pivotal role in elucidating the physiological and pathological functions of enzymes, as well as in the development of novel therapies. The research described in this thesis presents a reliable biochemical assay for DAGLα and DAGLβ, applicable for high-throughput screening of DAGLβ inhibitors. These methods are anticipated to streamline future drug discovery studies targeting DAGL. Notably, this research details the development of LEI-130 and LEI-131 as pioneering DAGLβ-selective and cellularly active inhibitors, functioning through a noncompetitive inhibition mode. As first-in-class selective DAGLβ inhibitors, LEI-130 and LEI-131 serve as valuable chemical tools to advance our understanding of DAGLβ and unveil its therapeutic potentials.

7.4 Acknowledgements

Matthijs R. van Wijngaarden is acknowledged for evaluating the activity of LEI-130 and LEI-131 in affinity-based protein profiling using the photoprobe. Dr. Stephan M. Hacker is kindly acknowledged for measuring proteomics samples and data analysis. Hans van den Elst is kindly acknowledged for HRMS measurements.

7.5 Experimental methods

Biology

EnzChek lipase substrate assay for DAGLα and DAGLβ in 384-well plate

The DAGL EnzChek lipase substrate assay was performed as described in Chapter 3.

Gel-based photoaffinity labeling

19 µL of HEK293T cell lysate overexpressing DAGLα or DAGLβ was incubated with either 0.5 µL DMSO or inhibitors (40× concentrated stocks in DMSO) for 30 min. As a negative control, mock lysate incubated with DMSO was used. 0.5 µL of the photoprobe (40× concentrated stocks in DMSO, final concentration 100 nM) was added and incubated for 15 min before irradiation under UV (Caprobox, 350 nm) for 10 min at 4 °C. Subsequently, 2.22 µL of click mix (final concentrations: 1 mM $CuSO_4$, 6 mM sodium ascorbate, 1 mM THPTA, and 1 µM Cy5-N_3) was added and incubated for 45 min in the dark. 7.4 µL of Laemmli blue (4×) was added and incubated for 10 min to quench the reaction. 10 µL of the quenched reaction mixture was resolved by 10% SDS-PAGE (180 V, 80 min) and the fluorescence was measured in a Biorad ChemiDoc™ MP system (Cy5: 700/50 filter; Cy3: 602/50 filter). The remaining activity was determined by measuring the integrated fluorescence using Image Lab™ 6.0.0, which was corrected for the total protein loading per lane as determined by Coomassie stain (R250) and imaging (Coomassie Blue Gel: 590/110 filter). The IC_{50} values were determined from a dose-response curve generated using GraphPad Prism 9.0.0 software (log(inhibitor) vs. normalized response with variable slope). The assay was performed three times independently (n = 1, N=3).

MS-based proteomics

1 mL of HEK293T cell lysate overexpressing DAGLβ or mock was incubated with 26 µL of the photoprobe (40× concentrated stocks in DMSO, final concentration 100 nM) for 30 min before irradiated under UV (Caprobox, 350 nm) for 10 min at 4 °C. 114 µL of click mix (final concentrations: 1 mM CuSO4, 6 mM sodium ascorbate, 1 mM THPTA, and 1 µM desthiobiotin-N_3) was added and incubated for 1 h in the dark. The proteins were precipitated in 4 mL cold acetone at -20 °C for overnight. Samples were centrifuged for 10 min at 3,500 g and the supernatants were carefully aspirated. The remaining proteins were washed twice with 1 mL of -80 °C methanol by resuspending using sonication (20% amplitude, 10 s), centrifugation (3,500 g, 3 min), and removing the supernatant. For trypsin digestion, the precipitated proteins were suspended in 300 µL urea buffer (8 M urea in 0.1 M TEAB) by sonication (80% amplitude, 20 s). The samples were spun down (1000 rpm, 1 min) before adding 15 µL of 31 mg/mL DTT and incubation at 37 °C for 45 min with shaking (500 rpm). After this, 15 µL of 74 mg/mL iodoacetamide was added and incubated in the dark for 30 min with rotating. 900 µL of 0.1 M TEAB was added to dilute the sample and 10 µL of 2 mg/mL trypsin was added and incubated at 37 °C for overnight in a shaking incubator (500 rpm). For chymotrypsin digestion, the

precipitated proteins were suspended in 300 µL urea buffer (8 M urea in 0.1 M Tris-HCl at pH 8.0) by sonication (80% amplitude, 20 s). The samples were spun down (1000 rpm, 1 min) before adding 15 µL of 31 mg/mL DTT and incubation at 37 °C for 45 min with shaking (500 rpm). After this, 15 µL of 74 mg/mL iodoacetamide was added and incubated in the dark for 30 min with rotating. 2.1 mL of 0.1 M Tris-HCl at pH 8.0 with 11.43 mM $CaCl_2$ was added to dilute the sample and 20 µL of 1 mg/mL chymotrypsin was added and incubated at 25 °C for overnight in a shaking incubator (500 rpm). 50 µL of Streptavidin high-capacity beads (Thermo Scientific) was added to Falcon tubes and washed three times with PBS by resuspending, centrifugation (1,000 g, 3 min), and removing the supernatant. The washed beads were then suspended in 1.2 mL PBS before adding the digested sample. The mixture of beads and peptides were incubated for 1 h while rotating. The samples were spun down (1,000 g, 3 min) and the supernatants were removed. The remaining beads were suspended in 600 µL PBS and transferred to a Biospin column. The solution was aspirated and the beads were washed twice with 600 µL PBS, three times with 600 µL H_2O, and three time with 600 µL 50% ACN. The columns with beads were then transferred to a 1.5 mL Low-bind Eppendorf tube and the peptides were eluted from the beads using 200 µL of 50% ACN with 0.1% TFA. The elution step was repeated twice using 70 µL of 50% ACN with 0.1% TFA and completed by centrifugation (3,000 g, 3 min). The samples were evaporated at 45 °C to dryness using SpeedVac. The remaining peptides were dissolved in 30 µL of 0.1% TFA in water by pipetting up and down, sonicating (20% amplitude, 10 s), and centrifugation (2,000 g, 1.5 min). Finally, the peptide solutions were filtered through a pre-washed filter (UFC30GVNB) and transferred to MS sample vials.

Fractionated peptide samples were analyzed using a nanoElute 2 LC system (Bruker) coupled to a timsTOF HT mass spectrometer (Bruker). 5 µL of sample was loaded on a trap column (PepMap C18, 5 mm × 0.3 mm, 5 µm, 100 Å, Thermo Scientific) followed by elution and separation on the analytical column (PepSep C18, 25 cm × 75 µm, 1.5 µm, 100 Å, Bruker). A gradient of 2-25% solvent B (0.1% FA in ACN) in 25 min, 25-32% B in 5 min, 32-95% in 5 min and 95% B for 10 min at a flow rate of 300 nL/min (all% values are v/v, water, TFA and ACN solvents were purchased from Bisolve, LC-MS grade). ZDV Sprayer 20 µm (Bruker) installed in the nano-electrospray source (CaptiveSpray source, Bruker) was used with following source parameters: 1600 V of capillary voltage, 3.0 L/min of dry gas, and 180 °C of dry temperature. The MS data was acquired in DDA PASEF mode with an ion mobility window of 0.85 to 1.35 Vs/cm^2 in a mass range from 100 m/z to 1700 m/z with charge states from 0 to 5^+. The dual TIMS analyzer was utilized under a fixed duty cycle, incorporating a 100 ms ramp time, resulting in a total cycle time of 1.17 s. Precursors that reached a target intensity of 20,000 (intensity threshold 2,500) were selected for fragmentation and dynamically excluded for 0.4 min (exclusion window: mass width 0.015 m/z; 1/K0 width 0.015 Vs/cm^2). The collision energy was set to 20 eV at 0.6 Vs/cm^2 and 59 eV at 1.6 Vs/cm^2. The 1/K0 values in between were interpolated linearly and kept constant above or below. The quadrupole isolation width was set to 2 m/z for 700 m/z and to 3 m/z for 800 m/z. Isolation width was constant except for linear interpolation between specified points. For calibration of the TIMS elution voltage, the Agilent

ESI-Low Tuning Mix was used with three selected ions (m/z, 1/K0: 622.0290, 0.9915; 922.0098, 1.1986; 1221.9906, 1.3934). Mass calibration is performed with Na Formate in HPC mode.

In situ target engagement and selectivity in N9 microglia

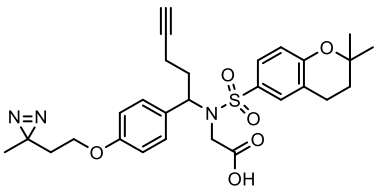
In situ target engagement and selectivity profiling was performed as described in Chapter 6.

Chemistry

General remarks

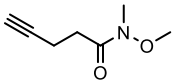
All purchased chemicals were used without purification unless stated otherwise. All reactions were performed in oven-dried or flame-dried glassware. Anhydrous solvents were dried by activated 3 Å or 4 Å molecular sieves. Traces of water in starting materials were removed by co-evaporation with toluene if necessary. Thin layer chromatography (TLC) analysis was performed on Merck silica gel 60 F_{254} aluminium sheets and the compounds were visualized by using UV absorption at 254 nm and/or $KMnO_4$ staining (5 g/L $KMnO_4$ and 25 g/L K_2CO_3 in water). TLC plates were analysed with the Advion CMS Plate Express® connected to the Advion Expression® L-MS using 90% MeOH in H_2O with 0.1% formic acid as the solvent. Liquid chromatography-mass spectrometry (LC-MS) analysis was performed on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI^+) coupled to a Surveyor HPLC system equipped with a C18 column (50×4.6 mm, 3 μm particle size, Macherey-Nagel) or a Thermo Finnigan LCQ Fleet ion-trap mass spectrometer (ESI^+) coupled to a Vanquish UHPLC system using H_2O, CH_3CN and 0.1% aq. TFA as eluents. Purification was performed on manual silica gel column chromatography (40-63 μm, 60 Å silica gel, Macherey-Nagel) or automated silica gel column chromatography (40-63 μm, 60 Å pre-packed silica gel, Screening Devices) on a Biotage Isolera™ Four 3.0 system. ^1H and ^{13}C spectra were recorded on a Bruker AV 400 MHz (400 MHz for ^1H and 101 MHz for ^{13}C) or AV 500 MHz spectrometer (500 MHz for ^1H and 126 MHz for ^{13}C) in deuterated solvents. Chemical shifts are reported in ppm with tetramethylsilane (TMS) or solvent resonance as the internal standard ($CDCl_3$: δ 7.26 for ^1H, δ 77.16 for ^{13}C). Data is reported as follows: chemical shifts δ (ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, t = triplet, td = triplet of doublets, tt = triplet of triplets, q = quartet, quintet = p, bs = broad singlet, m = multiplet), coupling constants *J* (Hz) and integration. High resolution mass spectrometry (HRMS) analysis was performed on a Thermo Finnigin LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctyl phthalate (m/z = 391.28428) as a lock mass.

_N_-((2,2-Dimethylchroman-6-yl)sulfonyl)-_N_-(1-(4-(2-(3-methyl-3_H_-diazirin-3-yl)ethoxy)phenyl)pent-4-yn-1-yl)glycine (13)



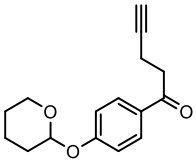
To a solution of ethyl _N_-((2,2-dimethylchroman-6-yl)sulfonyl)-_N_-(1-(4-(2-(3-methyl-3_H_-diazirin-3-yl)ethoxy)phenyl)pent-4-yn-1-yl)glycinate (**21**, 7.3 mg, 0.013 mmol, 1 eq) in THF/MeOH (280 µL/280 µL, 0.02 M) was added 1 M aq. LiOH (77 µL, 0.077 mmol, 6 eq). The mixture was stirred at rt for 20 h. The reaction mixture was diluted in EtOAc and washed with 0.2 M aq. HCl and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (0-5% MeOH in DCM) to obtain the impure product, which was purified again by silica gel column chromatography (10-30% EtOAc in _n_-pentane with a drop of conc. HCl) to obtain the pure product (2.0 mg, 3.7 µmol, 29%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 – 7.58 (m, 2H), 7.08 – 7.03 (m, 2H), 6.87 – 6.83 (m, 1H), 6.81 – 6.76 (m, 2H), 5.06 (t, _J_ = 7.4 Hz, 1H), 3.94 (d, _J_ = 18.3 Hz, 1H), 3.83 (t, _J_ = 6.3 Hz, 2H), 3.68 (d, _J_ = 18.3 Hz, 1H), 2.79 (t, _J_ = 6.7 Hz, 2H), 2.20 – 2.08 (m, 3H), 2.06 – 1.99 (m, 1H), 1.97 (t, _J_ = 2.5 Hz, 1H), 1.85 (t, _J_ = 6.7 Hz, 2H), 1.81 (t, _J_ = 6.2 Hz, 2H), 1.37 (s, 6H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.68, 158.66, 158.44, 130.04, 130.03, 129.65, 129.00, 127.51, 121.68, 118.08, 114.81, 83.18, 75.97, 69.36, 63.05, 59.43, 44.90, 34.39, 32.38, 30.68, 26.96, 24.39, 22.48, 20.39, 15.95. HRMS $[C_{28}H_{33}N_3O_6S+Na]^+$: 562.19823 calculated, 562.19824 found.

_N_-Methoxy-_N_-methylpent-4-ynamide (14)



To a solution of 4-pentynoic acid (1.00 g, 10.2 mmol, 1 eq) in anhydrous DCM (25 mL, 0.4 M) was added DMAP (125 mg, 1.02 mmol, 0.1 eq), EDCI (4.00 g, 20.9 mmol, 2.05 eq), Et_3N (1.56 mL, 11.2 mmol, 1.1 eq) and _N_,_O_-dimethylhydroxylammonium chloride (1.09 g, 11.2 mmol, 1.1 eq). The mixture was stirred at rt for overnight. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was purified by silica gel column chromatography (10-30% EtOAc in _n_-pentane) to afford the product (600 mg, 4.25 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.19 (s, 3H), 2.74 – 2.65 (m, 2H), 2.55 – 2.49 (m, 2H), 2.00 (t, _J_ = 2.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.27, 83.37, 68.65, 61.23, 32.09, 31.03, 13.76.

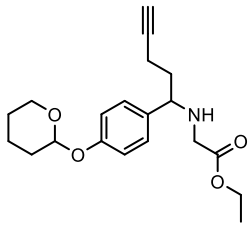
1-(4-((Tetrahydro-2_H_-pyran-2-yl)oxy)phenyl)pent-4-yn-1-one (15)



To a solution of _N_-methoxy-_N_-methylpent-4-ynamide (**14**, 166 mg, 1.18 mmol, 1 eq) in anhydrous THF (6 mL, 0.18 M) at 0 °C under argon was slowly added (4-((tetrahydro-2_H_-pyran-2-yl)oxy)phenyl)magnesium bromide (0.5 M in THF, 2.82 mL, 1.41 mmol, 1.2 eq). The resulting mixture was stirred at 0 °C for 3 h and rt for 3 h. A saturated aq. NH_4Cl solution (6 mL) was added to quench the reaction and the reaction mixture was extracted 3× with DCM. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (2-4% EtOAc in _n_-pentane) to afford the

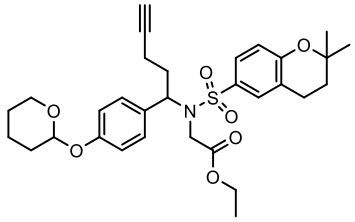
product as a white solid (124 mg, 0.480 mmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 – 7.90 (m, 2H), 7.12 – 7.05 (m, 2H), 5.51 (t, *J* = 3.1 Hz, 1H), 3.84 (ddd, *J* = 11.4, 9.9, 3.1 Hz, 1H), 3.66 – 3.58 (m, 1H), 3.22 – 3.15 (m, 2H), 2.64 – 2.57 (m, 2H), 2.06 – 1.93 (m, 2H), 1.91 – 1.83 (m, 2H), 1.77 – 1.55 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.31, 161.15, 130.14, 116.02, 96.02, 83.57, 68.76, 62.02, 37.20, 30.08, 25.06, 18.48, 13.33.

Ethyl (1-(4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pent-4-yn-1-yl)glycinate (16)



To a stirred solution of 1-(4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pent-4-yn-1-one (**15**, 440 mg, 1.70 mmol, 1 eq) in anhydrous EtOH (15 mL, 0.12 M) with 4 Å molecular sieves was added glycine ethyl ester hydrochloride (713 mg, 5.11 mmol, 3 eq), Et_3N (710 µL, 5.11 mmol, 3 eq), AcOH (150 µL, 2.56 mmol, 1.5 eq) and $NaBH_3CN$ (118 mg, 1.87 mmol, 1.1 eq) at rt. The mixture was refluxed for overnight. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was dissolved in EtOAc and washed 1× with sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (10-15% EtOAc in *n*-pentane) to afford the product as a yellow oil (290 mg, 0.840 mmol, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.13 (m, 2H), 7.06 – 6.97 (m, 2H), 5.40 (t, *J* = 3.5 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.92 (ddd, *J* = 11.8, 9.3, 3.1 Hz, 1H), 3.71 (dd, *J* = 8.0, 5.4 Hz, 1H), 3.66 – 3.55 (m, 1H), 3.31 – 3.13 (m, 2H), 2.22 – 1.56 (m, 12H), 1.24 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.57, 156.56, 134.95, 128.47, 116.51, 96.49, 96.42, 83.99, 68.83, 62.23, 61.16, 61.09, 60.82, 48.76, 36.46, 30.48, 25.29, 18.94, 15.51, 14.26. LC-MS $[C_{20}H_{27}NO_4+H]^+$: 346.21 calculated, 345.73 found.

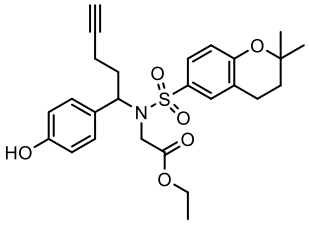
Ethyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(1-(4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pent-4-yn-1-yl)glycinate (17)



To a solution of ethyl (1-(4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pent-4-yn-1-yl)glycinate (**16**, 28 mg, 0.082 mmol, 1 eq) and DMAP (2.0 mg, 16 µmol, 0.2 eq) in anhydrous DCM was added Et_3N (57.3 µL, 0.411 mmol, 5 eq). 2,2-dimethylchromane-6-sulfonyl chloride (64.3 mg, 0.247 mmol, 3 eq) was added at 0 °C and the mixture was warmed to rt and stirred for 5 days. The reaction mixture was concentrated with Celite and purified by silica gel column chromatography (10-15% EtOAc in *n*-pentane) to afford the product (35 mg, 0.061 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.60 (m, 2H), 7.06 – 6.99 (m, 2H), 6.95 – 6.90 (m, 2H), 6.85 (d, *J* = 8.9 Hz, 1H), 5.37 (t, *J* = 3.6 Hz, 1H), 4.98 (t, *J* = 7.5 Hz, 1H), 4.14 – 3.82 (m, 4H), 3.70 – 3.52 (m, 2H), 2.81 (ddd, *J* = 8.0, 6.5, 2.0 Hz, 2H), 2.22 – 1.98 (m, 4H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.88 – 1.80 (m, 4H), 1.72 – 1.55 (m, 4H), 1.37 (s, 6H), 1.19 (td, *J* = 7.2, 0.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.23, 170.21, 158.09, 157.00, 156.95, 130.97, 130.05, 130.02, 129.81, 129.79, 129.54, 129.53, 127.58, 121.34, 117.88, 116.45, 116.39, 96.37, 96.29, 83.56, 75.77, 69.04,

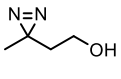
62.21, 61.39, 59.19, 45.09, 45.03, 32.40, 30.82, 30.79, 30.41, 26.93, 25.27, 22.46, 18.86, 16.02, 14.14.

Ethyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(1-(4-hydroxyphenyl)pent-4-yn-1-yl)glycinate (18)



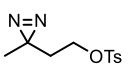
To a solution of ethyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(1-(4-((tetrahydro-2*H*-pyran-2-yl)oxy)phenyl)pent-4-yn-1-yl)glycinate (**17**, 13.5 mg, 24.0 μmol, 1 eq) in ethanol (0.47 mL, 0.05 M) was added TsOH (4.08 mg, 24.0 μmol, 1 eq). The mixture was stirred at rt for overnight. The reaction mixture was diluted in EtOAc and washed 1× with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (20-30% EtOAc in *n*-pentane) to obtain the product as a colorless oil (8.1 mg, 0.017 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.62 (m, 2H), 7.02 – 6.97 (m, 2H), 6.88 – 6.83 (m, 1H), 6.73 – 6.68 (m, 2H), 5.36 (s, 1H), 4.98 (t, *J* = 7.5 Hz, 1H), 4.13 – 3.99 (m, 2H), 3.99 – 3.60 (m, 2H), 2.80 (t, *J* = 6.7 Hz, 2H), 2.20 – 1.97 (m, 4H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.84 (t, *J* = 6.8 Hz, 2H), 1.36 (s, 6H), 1.19 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.32, 158.15, 155.78, 130.86, 130.06, 129.83, 128.92, 127.59, 121.41, 117.91, 115.52, 83.46, 75.83, 69.14, 61.51, 59.16, 45.03, 32.40, 30.71, 26.93, 22.47, 16.00, 14.13.

2-(3-Methyl-3*H*-diazirin-3-yl)ethan-1-ol (19)



A round-bottom flask with 4-hydroxybutan-2-one (2.00 g, 22.7 mmol, 1 eq) under N_2 was cooled to 0 °C and 7 N NH_3 in MeOH (22.6 mL, 158 mmol, 7 eq) was added slowly. After 3 h, an anhydrous methanolic solution of NH_2OSO_3H (2.82 g, 25.0 mmol, 1.1 eq) was added dropwise at 0 °C. The resulting solution was allowed to warm to rt and stirred for overnight. The reaction mixture was evaporated to dryness in the reaction vessel under a stream of dry N_2 and the remaining residue was then resuspended in anhydrous MeOH and filtered. The filtrate was then concentrated under reduced pressure and re-dissolved in anhydrous MeOH (16 mL). The solution was cooled to 0 °C and Et_3N (4.84 mL, 34.7 mmol, 1.5 eq) was added. I_2 (2.36 g, 9.31 mmol, 0.4 eq) was then added in small portions until a dark brown color persisted in the solution for more than 10 min, indicating complete oxidation of the diaziridine intermediate. The solution was then diluted in EtOAc and the organic layer was washed with 1 M aq. HCl and sat. aq. $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to obtain the product as a yellow liquid (0.20 g, 2.0 mmol, 21%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.54 (t, *J* = 6.3 Hz, 2H), 2.02 (bs, 1H), 1.65 (t, *J* = 6.3 Hz, 2H), 1.08 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 57.85, 37.07, 20.35.

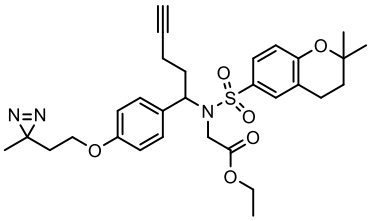
2-(3-Methyl-3*H*-diazirin-3-yl)ethyl 4-methylbenzenesulfonate (20)



To a solution of 2-(3-methyl-3*H*-diazirin-3-yl)ethan-1-ol (**19**, 183 mg, 1.83 mmol, 1 eq) in pyridine (1.5 mL, 1.2 M) was added 4-methylbenzenesulfonyl chloride (523 mg, 2.74 mmol, 1.5 eq) at 0 °C and the mixture was stirred at rt for 1 h. The reaction

mixture was diluted in EtOAc and washed with 1 M aq. HCl, sat. aq. $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (10-15% EtOAc in *n*-pentane) to afford the product as a pale-yellow liquid (264 mg, 1.04 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.78 (m, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 3.96 (t, *J* = 6.4 Hz, 2H), 2.46 (s, 3H), 1.68 (t, *J* = 6.4 Hz, 2H), 1.01 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.18, 132.85, 130.06, 128.13, 65.23, 34.31, 23.53, 21.82, 19.93.

Ethyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(1-(4-(2-(3-methyl-3*H*-diazirin-3-yl)ethoxy)phenyl)pent-4-yn-1-yl)glycinate (21)



To a solution of ethyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(1-(4-hydroxyphenyl)pent-4-yn-1-yl)glycinate (**18**, 30 mg, 0.062 mmol, 1 eq) in anhydrous DMF (618 µL, 0.1 M) under N_2 was added Cs_2CO_3 (60.4 mg, 0.185 mmol, 3 eq). Subsequently, 2-(3-methyl-3*H*-diazirin-3-yl)ethyl 4-methylbenzenesulfonate (**20**, 23.6 mg, 93.0 µmol, 1.5 eq) was added and the reaction was stirred at rt for 5 h. The reaction mixture was diluted in EtOAc and washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography (10-15% EtOAc in *n*-pentane) to afford the product (8.7 mg, 0.015 mmol, 24%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 – 7.61 (m, 2H), 7.07 – 7.01 (m, 2H), 6.87 – 6.82 (m, 1H), 6.79 – 6.74 (m, 2H), 4.99 (t, *J* = 7.5 Hz, 1H), 4.12 – 3.99 (m, 2H), 3.96 (d, *J* = 18.2 Hz, 1H), 3.82 (t, *J* = 6.2 Hz, 2H), 3.63 (d, *J* = 18.2 Hz, 1H), 2.80 (t, *J* = 6.7 Hz, 2H), 2.20 – 2.15 (m, 2H), 2.12 – 1.99 (m, 2H), 1.94 (t, *J* = 2.6 Hz, 1H), 1.84 (t, *J* = 6.8 Hz, 2H), 1.80 (t, *J* = 6.2 Hz, 2H), 1.37 (s, 6H), 1.19 (t, *J* = 7.2 Hz, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.18, 158.45, 158.12, 131.01, 130.06, 129.69, 129.41, 127.61, 121.37, 117.90, 114.59, 83.51, 75.79, 69.09, 63.02, 61.41, 59.20, 45.09, 34.39, 32.44, 30.82, 26.95, 24.37, 22.49, 20.38, 16.03, 14.16. LC-MS $[C_{30}H_{37}N_3O_6S+Na]^+$: 590.23 calculated, 590.07 found.

Reference

1. Baggelaar, M. P., Maccarrone, M. & van der Stelt, M. 2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain. *Prog. Lipid Res.* **71**, 1–17 (2018).
2. Mock, E. D., Gagestein, B. & van der Stelt, M. Anandamide and other *N*-acylethanolamines: A class of signaling lipids with therapeutic opportunities. *Prog. Lipid Res.* **89**, 101194 (2023).
3. Shrestha, N. *et al.* Peripheral modulation of the endocannabinoid system in metabolic disease. *Drug Discov. Today* **23**, 592–604 (2018).
4. Busquets-García, A., Bolaños, J. P. & Marsicano, G. Metabolic Messengers: endocannabinoids. *Nat. Metab.* **4**, 848–855 (2022).
5. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50**, 83–90 (1995).
6. Devane, W. A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
7. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163**, 463–468 (2003).
8. Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T. & Ueda, N. Molecular Characterization of a Phospholipase D Generating Anandamide and Its Congeners. *J. Biol. Chem.* **279**, 5298–5305 (2004).
9. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356 (2007).
10. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
11. Liu, Y., Patricelli, M. P. & Cravatt, B. F. Activity-based protein profiling: The serine hydrolases. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14694–14699 (1999).
12. Patricelli, M. P., Giang, D. K., Stamp, L. M. & Burbaum, J. J. Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *Proteomics* **1**, 1067–1071 (2001).
13. Baggelaar, M. P. *et al.* Development of an Activity-Based Probe and In Silico Design Reveal Highly Selective Inhibitors for Diacylglycerol Lipase-α in Brain. *Angew. Chem. Int. Ed.* **52**, 12081–5 (2013).
14. Baggelaar, M. P. *et al.* Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J. Am. Chem. Soc.* **137**, 8851–8857 (2015).
15. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 26–33 (2016).

16. Mock, E. D. *et al.* Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. *Nat. Chem. Biol.* **16**, 667–675 (2020).

17. Cisar, J. S. *et al.* Identification of ABX-1431, a Selective Inhibitor of Monoacylglycerol Lipase and Clinical Candidate for Treatment of Neurological Disorders. *J. Med. Chem.* **61**, 9062–9084 (2018).

18. Johnson, D. S. *et al.* Discovery of PF-04457845: A highly potent, orally bioavailable, and selective urea FAAH inhibitor. *ACS Med. Chem. Lett.* **2**, 91–96 (2011).

19. Müller-Vahl, K. R. *et al.* Monoacylglycerol Lipase Inhibition in Tourette Syndrome: A 12-Week, Randomized, Controlled Study. *Mov. Disord.* **36**, 2413–2418 (2021).

20. D'Souza, D. C. *et al.* Efficacy and safety of a fatty acid amide hydrolase inhibitor (PF-04457845) in the treatment of cannabis withdrawal and dependence in men: a double-blind, placebo-controlled, parallel group, phase 2a single-site randomised controlled trial. *Lancet Psychiatry* **6**, 35–45 (2019).

21. Hsu, K.-L. *et al.* DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat. Chem. Biol.* **8**, 999–1007 (2012).

22. Khasabova, I. A. *et al.* Inhibition of DAGLβ as a therapeutic target for pain in sickle cell disease. *Haematologica* **108**, 859–869 (2023).

23. Jenniches, I. *et al.* Anxiety, Stress, and Fear Response in Mice with Reduced Endocannabinoid Levels. *Biol. Psychiatry* **79**, 858–868 (2016).

24. Cavener, V. S. *et al.* Inhibition of Diacylglycerol Lipase Impairs Fear Extinction in Mice. *Front. Neurosci.* **12**, 1–10 (2018).

25. Janssen, F. J. *et al.* Discovery of Glycine Sulfonamides as Dual Inhibitors of *sn*-1-Diacylglycerol Lipase α and α/β-Hydrolase Domain 6. *J. Med. Chem.* **57**, 6610–6622 (2014).

26. Dong, A. *et al.* A fluorescent sensor for spatiotemporally resolved imaging of endocannabinoid dynamics in vivo. *Nat. Biotechnol.* (2021) doi:10.1038/s41587-021-01074-4.

27. Cooke, M. & Kazanietz, M. G. Overarching roles of diacylglycerol signaling in cancer development and antitumor immunity. *Sci. Signal.* **15**, 1–27 (2022).

28. Helfand, B. T., Mendez, M. G., Pugh, J., Delsert, C. & Goldman, R. D. Diacylglycerol-dependent Binding Recruits PKCθ and RasGRP1 C1 Domains to Specific Subcellular Localizations in Living T Lymphocytes. *Mol. Biol. Cell.* **15**, 2932–2942 (2004).

29. Katti, S. S. *et al.* Structural anatomy of Protein Kinase C C1 domain interactions with diacylglycerol and other agonists. *Nat. Commun.* **13**, 1–11 (2022).

30. Griner, E. M. & Kazanietz, M. G. Protein kinase C and other diacylglycerol effectors in cancer. *Nat. Rev. Cancer* **7**, 281–294 (2007).

31. Pan, Q. *et al.* Protein kinase Cε is a predictive biomarker of aggressive breast cancer and a validated target for RNA interference anticancer therapy. *Cancer Res.* **65**, 8366–8371 (2005).

32. Bae, K. M. *et al.* Protein kinase Cε is overexpressed in primary human non-small cell lung cancers and functionally required for proliferation of non-small cell lung cancer cells in a p21/Cip1-dependent manner. *Cancer Res.* **67**, 6053–6063 (2007).

33. Aziz, M. H. *et al.* Protein kinase Cε interacts with signal transducers and activators of transcription 3 (Stat3), phosphorylates Stat3Ser727, and regulates its constitutive activation in prostate cancer. *Cancer Res.* **67**, 8828–8838 (2007).

34. Huang, B. *et al.* The expression and role of protein kinase C (PKC) epsilon in clear cell renal cell carcinoma. *J. Exp. Clin. Cancer Res.* **30**, 1–9 (2011).

35. Lau, E. *et al.* PKCε promotes oncogenic functions of ATF2 in the nucleus while blocking its apoptotic function at mitochondria. *Cell* **148**, 543–555 (2012).

36. Porter, S. N. & Magee, J. A. PRKCH regulates hematopoietic stem cell function and predicts poor prognosis in acute myeloid leukemia. *Exp. Hematol.* **53**, 43–47 (2017).

37. Kim, W. K. *et al.* Sustained mutant KIT activation in the Golgi complex is mediated by PKC-θ in gastrointestinal stromal tumors. *Clin. Cancer Res.* **23**, 845–856 (2017).

38. Hill, K. S. *et al.* Protein kinase Cα suppresses Kras-mediated lung tumor formation through activation of a p38 MAPK-TGFβ signaling axis. *Oncogene* **33**, 2134–2144 (2014).

39. Chen, S., Wang, Y., Zhang, Y. & Wan, Y. Low expression of PKCα and high expression of KRAS predict poor prognosis in patients with colorectal cancer. *Oncol. Lett.* **12**, 1655–1660 (2016).

40. Oster, H. & Leitges, M. Protein kinase C α but not PKCζ suppresses intestinal tumor formation in $Apc^{Min/+}$ mice. *Cancer Res.* **66**, 6955–6963 (2006).

41. Hsu, A. H. *et al.* Crosstalk between PKCα and PI3K/AKT Signaling Is Tumor Suppressive in the Endometrium. *Cell Rep.* **24**, 655–669 (2018).

42. Dowling, C. M. *et al.* Protein kinase C beta II suppresses colorectal cancer by regulating IGF-1 mediated cell survival. *Oncotarget* **7**, 20919–20933 (2016).

43. D'Costa, A. M. *et al.* The proapoptotic tumor suppressor protein kinase C-δ is lost in human squamous cell carcinomas. *Oncogene* **25**, 378–386 (2006).

44. Baryła, M. *et al.* Oncometabolites—A Link between Cancer Cells and Tumor Microenvironment. *Biology* **11**, 1–18 (2022).

45. Zhong, X. P. *et al.* Enhanced T cell responses due to diacylglycerol kinase ζ deficiency. *Nat. Immunol.* **4**, 882–890 (2003).

46. Olenchock, B. A. *et al.* Disruption of diacylglycerol metabolism impairs the induction of T cell anergy. *Nat. Immunol.* **7**, 1174–1181 (2006).

47. Salerno, F., Paolini, N. A., Stark, R., Von Lindern, M. & Wolkers, M. C. Distinct PKC-mediated posttranscriptional events set cytokine production kinetics in $CD8^+$ T cells. *Proc. Natl. Acad. Sci. U.S.A* **114**, 9677–9682 (2017).

48. Stinchcombe, J. C. *et al.* Ectocytosis renders T cell receptor signaling self-limiting at the immune synapse. *Science* **380**, 818–823 (2023).

49. Arranz-Nicolas, J. *et al.* Diacylglycerol kinase ζ limits IL-2-dependent control of PD-1 expression in tumor-infiltrating T lymphocytes. *J. Immunother. Cancer* **8**, 1–13 (2020).

50. Shin, M., Buckner, A., Prince, J., Bullock, T. N. J. & Hsu, K. L. Diacylglycerol Lipase-β Is Required for TNF-α Response but Not $CD8^+$ T Cell Priming Capacity of Dendritic Cells. *Cell Chem. Biol.* **26**, 1036-1041 (2019).

Nederlandse Samenvatting

Het onderzoek beschreven in deze scriptie onderzoekt op een systematische methode het ontwerp, de synthese en de biologische evaluatie van remmers voor diacylglycerol lipase β (DAGLβ). Via high-throughput screening en structuur-activiteitsrelatie studies werden selectieve DAGLβ-remmers met cellulaire activiteit ontwikkeld. Als eerste selectieve DAGLβ-remmers bieden deze verbindingen ons de mogelijkheid om de functies van DAGLβ beter te begrijpen en de eventuele therapeutische toepassing te onderzoeken.

Hoofdstuk 1 geeft voornamelijk een overzicht van het endocannabinoïde systeem (ECS) en van de activiteits-gebaseerde probes (ABPs) en kleine moleculaire remmers van enzymen binnen het ECS, evenals de biologische functies en potentiële klinische toepassingen van deze enzymen. Het ECS omvat endocannabinoïden (2-arachidonoylglycerol (2-AG) en *N*-arachidonoylethanolamine (AEA)) en cannabinoïde receptoren type 1 en 2 (CB_1R en CB_2R). Bovendien omvat het enzymen zoals diacylglycerol lipase α en β (DAGLα/β) en *N*-acylfosfatidylethanolaminefosfolipase D (NAPE-PLD), die verantwoordelijk zijn voor de respectieve synthese van 2-AG en AEA, en enzymen zoals monoacylglycerol lipase (MAGL) en vetzuuramidehydrolase (FAAH), die verantwoordelijk zijn voor de respectieve hydrolyse van 2-AG en AEA. Het ECS is betrokken bij het reguleren van verschillende biologische processen, waaronder synaptische plasticiteit, geheugenvorming en cognitie, emoties, energiemetabolisme, pijnperceptie en de immuunrespons. Om de biologische functies van enzymen in het ECS te bestuderen, zijn talloze ABPs en remmers ontwikkeld. Momenteel zijn er twee types breedspectrum ABPs tegen serine hydrolases: op fluorofosfonaat gebaseerde en op β-lacton gebaseerde probes. Deze probes kunnen de meeste enzymen in het ECS labelen, waarmee de ontdekking van nieuwe remmers en de evaluatie van de selectiviteit mogelijk is. Dankzij het gebruik van deze probes in activity-based protein profiling (ABPP) zijn veel potente en selectieve remmers ontdekt voor ECS-enzymen. Voorbeelden van remmers zijn onder andere, maar niet beperkt tot, DH376, een triazol-ureum gebaseerde DAGLα/β-remmer; LEI-401, een pyrimidine-4-carboxamide gebaseerde NAPE-PLD-remmer; Lu AG06466, een carbamaat gebaseerde MAGL-remmer; en PF-04457845, een pyridazine ureum gebaseerde FAAH-remmer.

Waar MAGL- en FAAH-remmers momenteel klinisch getest worden, worden DAGL- en NAPE-PLD-remmers momenteel onderzocht in diermodellen. De MAGL-remmer Lu AG06466, bleek in klinische proeven over het algemeen veilig, maar was niet effectief in het verminderen van tics en bijkomende symptomen bij patiënten met het syndroom van Tourette tijdens een fase 2 klinische proef. De FAAH-remmer PF04457845 vertoonde positieve effecten in een fase 2a studie bij patiënten met een cannabis verslavingsstoornis. Een andere FAAH-remmer, JNJ-42165279, wordt momenteel klinisch onderzocht voor de behandeling van posttraumatische stressstoornis. Bij remming van DAGL zijn ontstekingsremmende en pijnstillende effecten waargenomen. Echter, het remmen van DAGLα in de hersenen kan mogelijk leiden tot psychiatrische bijwerkingen. De NAPE-PLD-remmer LEI-401 bleek de

hypothalamus-hypofyse-bijnier-as te activeren en tot chronische angst te leiden in muizen. In deze context kunnen tot de periferiebeperkte remmers essentiële hulpmiddelen zijn om de functies van ECS-enzymen in perifere weefsels op te helderen en kunnen ze nieuwe klinische toepassingen hebben.

Selectieve DAGLβ-remmers zijn cruciaal om de specifieke biologische functies van dit enzym en het onderscheid tussen DAGLβ en DAGLα onder specifieke pathofysiologische omstandigheden te begrijpen, maar zijn vooralsnog niet beschikbaar. Bovendien kunnen selectieve DAGLβ-remmers een rol spelen in de behandeling van ontstekingsziekten, met mogelijk minimale bijwerkingen in het centrale zenuwstelsel. Daarom is het hoofddoel van het onderzoek dat in deze scriptie wordt beschreven, de ontwikkeling van selectieve DAGLβ-remmers.

In **Hoofdstuk 2** werd een fluorescerentie assay met behulp van het EnzChek-lipasesubstraat geoptimaliseerd voor DAGLα/β en geminiaturiseerd naar een 384-wells plaatformaat. Deze assay werd vervolgens toegepast in een high-throughput screening met gebruik van het gezuiverde katalytische domein van DAGLβ om nieuwe potentiële verbindingen te identificeren. Een bibliotheek van 12.560 serine hydrolaseremmers van Enamine en een in-house bibliotheek van 27 glycinesulfonamiden werden gescreend bij 10 μM. Na primaire en bevestigende screenings, deselectie en dosisresponsbepaling werden acht hits geïdentificeerd, onderverdeeld in vier chemotypen. Hit **1**, gebaseerd op glycinesulfonamide, ook bekend als LEI-106, vertoonde de hoogste activiteit voor DAGLβ met een negatieve logaritme van de halfmaximale remmende concentratie (pIC_{50}) van 6.69 ± 0.14 en veelbelovende fysisch-chemische eigenschappen, maar was niet selectief ten opzichte van DAGLα (pIC_{50} van 7.35 ± 0.06).

In **Hoofdstuk 3** werd een uitgebreide structuur-activiteitsrelatie van glycinesulfonamiden als DAGL-remmers verkend door systematische aanpassingen aan verschillende delen van dit chemotype. In totaal werden 51 analogen gesynthetiseerd en biochemisch geëvalueerd. Waar de meeste verbindingen die in dit hoofdstuk worden besproken, vergelijkbare activiteit vertoonden voor beide DAGL-isoformen of lichte selectiviteit voor DAGLα, vertoonden drie verbindingen met variaties in het sulfonyl-substituent enige selectiviteit voor DAGLβ. Dit suggereert dat het sulfonyl-deel een modificatie-hotspot is om selectiviteit voor DAGLβ te bereiken.

In **Hoofdstuk 4** werd een diepgaande structuur-activiteitsrelatie-studie uitgevoerd om de selectiviteit voor DAGLβ te verbeteren, met de focus op het derivatiseren van het sulfonyl-substituent van het glycinesulfonamide-chemotype. Deze inspanning leidde tot de identificatie van 3,4-dihydro-2*H*-benzo[*b*][1,4]dioxepine als de optimale zijgroep op deze positie. Verdere optimalisatie van de activiteit en selectiviteit omvatte het verkennen van andere componenten van dit chemotype, resulterend in de ontdekking van zes verbindingen met een pIC_{50} tot 8.08 ± 0.13, een selectiviteitsvouw tot 51 ten opzichte van DAGLα en veelbelovende fysiochemische eigenschappen.

In **Hoofdstuk 5** werd een op fluorescentie gebaseerde 96-wells plaatformaat assay opgezet met behulp van een op GPCR-activatie-gebaseerde endocannabinoïde-sensor ($GRAB_{eCB2.0}$) om de cellulaire activiteit van DAGL-remmers te bepalen. Deze sensor bestaat uit een CB_1-receptor gekoppeld aan een circulair gepermuteerd verbeterd groen fluorescerend eiwit (cpEGFP die kan worden geactiveerd door de binding van endocannabinoïden om fluorescentie op te wekken. De $GRAB_{eCB2.0}$-sensor tot expressie gebracht in Neuro2A-cellen fluoresceerde door ATP geïnduceerde productie van 2-AG. In totaal werden 23 DAGL-remmers geprofileerd in deze assay, waarbij een concentratieafhankelijke vermindering van de productie van 2-AG werd aangetoond. Hieruit bleek dat DAGLα de primaire isoform is die betrokken is bij door ATP gestimuleerde 2-AG-productie in Neuro2A-cellen.

In **Hoofdstuk 6** werden representatieve DAGLβ-selectieve remmers **LEI-130** en **LEI-131**, samen met een negatieve controle, **LEI-132**, geprofileerd in *in vitro*- en *in situ*-studies. Onderzoek naar de bindingswijze onthulde een niet-competitief mechanisme voor LEI-130 en LEI-131 tegen DAGLβ, wat wijst op hun binding aan een allosterische pocket. *In vitro*- en *in situ*-ABPP-studies bevestigden de selectiviteit van LEI-130 en LEI-131 tegen DAGLβ ten opzichte van de aanwezige eiwitten in muizenhersenen en levende cellen. Lipidomics-analyse van LEI-130, LEI-131 en LEI-132 in N9 microglia- en J774A.1 macrofaagcellen toonde aan dat DAGLβ de niveaus van 2-AG evenals downstream lipiden reguleerde op een celtype-afhankelijke manier. Ook werd aangetoond dat LEI-130, LEI-131 en LEI-132 allemaal de door LPS gestimuleerde cytokineproductie verminderden, wat wijst op de aanwezigheid van een onbekend off-target dat betrokken is bij het reguleren van dit proces.

In **Hoofdstuk 7** wordt het werk dat in deze scriptie wordt gepresenteerd, samengevat en worden nieuwe richtingen voor toekomstig onderzoek aangegeven.

Summary in Chinese

中文总结

该论文对二酰甘油脂酶β（DAGLβ）抑制剂的设计、合成和生物学评价进行了系统研究。通过高通量筛选以及构效关系研究，发现了具有细胞活性的 DAGLβ 选择性抑制剂。作为突破性的同类第一的 DAGLβ 选择性抑制剂，这些化合物能够推动我们对于 DAGLβ 生物学功能的理解，并揭示其潜在的临床应用价值。

第一章主要概述了内源性大麻素系统（ECS），针对内源性大麻素系统中的酶的活性探针和小分子抑制剂，以及靶向这些酶的生物学功能和其潜在的临床应用价值。ECS 包括内源性大麻素（2-花生四烯酰甘油（2-AG）和 *N*-花生四烯酰乙醇胺（AEA））以及大麻素受体类型 1 和 2（CB_1R 和 CB_2R）。此外，它还包括：内源性大麻素合成酶，如二酰甘油脂酶α/β（DAGLα/β）和 *N*-酰基磷脂醇胺磷脂酶 D（NAPE-PLD），它们分别负责 2-AG 和 AEA 的合成；内源性大麻素降解酶，如单酰甘油脂酶（MAGL）和脂肪酸酰胺水解酶（FAAH），它们分别负责 2-AG 和 AEA 的水解。内源性大麻素系统参与各种生物过程的调节，包括突触可塑性、记忆形成和学习、情绪、能量代谢、疼痛感知和免疫反应。为了研究内源性大麻素系统中的酶的生物学功能，研究人员开发了许多活性探针和小分子抑制剂。到目前为止，广谱活性探针可分为两种：基于氟磷酸酯的探针和基于 β-内酯的探针。这些探针可以标记内源性大麻素系统中的大多数酶以及其他丝氨酸水解酶，从而实现新型抑制剂的发现和选择性评估。得益于这些探针在活性蛋白质分析中的应用，许多针对内源性大麻素系统中的酶的强效和选择性抑制剂已经被开发。代表性的抑制剂包括但不限于 DH376（一种基于三唑脲的 DAGLα/β 抑制剂）、LEI-401（一种基于嘧啶-4-羧酰胺的 NAPE-PLD 抑制剂）、Lu AG06466（一种基于碳酸酯的 MAGL 抑制剂）以及 PF-04457845（一种基于吡嗪脲的 FAAH 抑制剂）。

MAGL 和 FAAH 抑制剂已经进入临床试验，DAGL 和 NAPE-PLD 抑制剂目前则正处于动物模型研究。MAGL 抑制剂 Lu AG06466 在临床试验中表现出普遍的安全性，但在第 2 期临床试验中，对于降低抽动症患者的抽动、先兆冲动和合并症的疗效并不显著。FAAH 抑制剂 FP 04457845 在针对大麻使用障碍的第 2a 期临床研究中显示出积极效果。另一种 FAAH 抑制剂 JNJ-42165279 目前则正处于针对创伤后应激障碍的临床研究。DAGL 抑制剂显示出抗炎和镇痛效果。然而，抑制大脑中的 DAGLα 可能导致精神性的副作用。NAPE-PLD 抑制剂 LEI-401 被发现能激活下丘脑-垂体-肾上腺轴，并损害小鼠的恐惧消退。在这种情况下，外周限制的抑制剂将是阐明这些酶在外周组织中的功能的重要工具，并可能具有新的临床应用价值。

DAGLβ 选择性抑制剂对于理解该酶的独特生物学功能，以及在特定病理生理条件下区分 DAGLβ 和 DAGLα 的作用至关重要，但目前仍然缺乏这样的抑制剂。此外，DAGLβ 选择性抑制剂在治疗炎症性疾病方面具有潜在的应用价值，并同时能够最小化对中枢神经系统的副作用。因此，该论文的主要研究目标是开发 DAGLβ 选择性抑制剂。

第二章介绍了一种基于荧光测定 DAGLα/β 活性的方法。该方法使用 EnzChek 脂肪酶底物，经过优化和小型化后使其适用于 384 孔板。随后，该方法被应用于高通量筛选以鉴定出可以抑制 DAGLβ 的活性化合物。对从 Enamine 购买的 12,560 个丝氨酸水解酶抑制剂和来自内部化合物库的 27 个甘氨酸磺酰胺类似物以 10 微摩尔的浓度进行筛选，经过初步筛选、确认筛选、去除抑制荧光信号的化合物和化合物的剂量-反应关系测定，一共得到了 8 个分属于 4 种化学类型的活性化合物。其中基于甘氨酸磺酰胺的活性化合物 **1**，也被称为 LEI-106，对 DAGLβ 表现出最高的活性。它对 DAGLβ 的半数抑制浓度的负对数（pIC_{50}）为 6.69 ± 0.14，并且具有良好的物理化学性质。但是该化合物对 DAGLβ 不具有选择性，因为它对 DAGLα 也表现出高活性，其对 DAGLα 的 pIC_{50} 值为 7.35 ± 0.06。

第三章通过对活性化合物 **1** 的不同部分进行结构改造，探索了甘氨酸磺酰胺作为 DAGL 抑制剂的构效关系。在此章中，共合成了 51 个甘氨酸磺酰胺类似物并对它们进行了生物化学活性评估。虽然本章讨论的大多数化合物对两个 DAGL 亚型表现出相似的活性或对 DAGLα 表现出轻微的选择性，但是其中三个具有不同磺酰基取代基的化合物表现出对 DAGLβ 的一定选择性。这表明磺酰基部分是实现 DAGLβ 选择性的修饰位点。

第四章对构效关系进行了更深入的研究，旨在提高抑制剂的 DAGLβ 选择性。该研究的重点是对甘氨酸磺酰胺的磺酰基取代基进行优化。这一研究发现了 3,4-二氢-2*H*-苯并[*b*][1,4]二氧杂环庚烷为在这个位置上的最佳取代基。随后，该研究对这一类型的其他结构部分进行探索以进一步优化活性和选择性，最终得到了 6 个具有良好活性、选择性和理化特性的化合物。

第五章介绍了一种基于荧光的 96 孔板细胞活性测定方法。该方法使用基于 G 蛋白偶联受体的内源性大麻素传感器（$GRAB_{eCB2.0}$），对 DAGL 抑制剂的细胞活性进行了研究。该传感器是经环化增强绿色荧光蛋白（cpEGFP）修饰的 CB_1R 受体，其能与内源性大麻素结合并被激活而产生荧光。在小鼠神经母细胞瘤细胞（Neuro2A）中表达的 $GRAB_{eCB2.0}$可以对三磷酸腺苷 （ATP）诱导产生的 2-AG 做出反应。在此实验中，共对 23 个 DAGL 抑制剂进行了活性测试，结果表明这些抑制剂对于抑制 2-AG 的产生具有不同的效力。值得注意的是，相关性分析揭示了在 Neuro2A 细胞中， ATP 诱导的 2-AG 的产生主要依赖于 DAGLα 而非 DAGLβ。

第六章对代表性的 DAGLβ 选择性抑制剂 **LEI-130** 和 **LEI-131**，以及阴性对照化合物 **LEI-132**，进行了体外和原位研究。抑制模式研究揭示了 LEI-130 和 LEI-131 对 DAGL

β 的抑制是非竞争性的 （noncompetititve），表明了它们是结合在 DAGLβ 的异构口袋中。LEI-130 和 LEI-131 的体外和原位活性蛋白质分析研究证实了它们可以有效地抑制 DAGLβ 的活性，并对小鼠脑蛋白质组和活细胞中的一系列丝氨酸水解酶的活性没有影响。N9 小胶质细胞和 J774A.1 巨噬细胞经过化合物 LEI-130、LEI-131 以及 LEI-132 处理后，对其脂质水平和细胞因子水平进行分析。实验结果表明 DAGLβ 以一种依赖于细胞类型的方式调控 2-AG 及其下游脂质的水平。有趣的是，LEI-130、LEI-131 和 LEI-132 都能够减弱脂多糖（LPS）诱导的细胞因子的产生，表明可能除 DAGLβ 外有未知的非靶标蛋白参与了这一过程调控。

第七章对本论文的研究成果进行了归纳和总结，并提供了未来研究的新方向。

List of publications

Discovery of selective DAGLβ inhibitors that reduce inflammation

Zhu, N., Vleig, H. C., Rüegger, J., Straub, V. M., Grether, U., Di, X., van den Berg, R. J. B. H. N., Driever, W. P. F., van Egmond, N., van der Horst, C., Heitman, L. H., Janssen, A. P. A., van der Stelt, M. *Manuscript in preparation*

Structure-activity relationship studies lead to DAGLβ selective inhibitors

Zhu, N., Herry, B. S., de Ruiter, J., van Workum, D., van der Woude, R., van den Berg, R. J. B. H. N., Janssen, A. P. A., van der Stelt, M. *Manuscript in preparation*

Understanding and Targeting the Endocannabinoid System with Activity-Based Protein Profiling

Zhu, N., Janssen, A. P. A., van der Stelt, M. *Isr. J. Chem.* 63, e202200115 (2023).

Dendritic-Polymer-Based Nanomaterials for Cancer Diagnosis and Therapy

Zhu, N., Gong, Q, Gu, Z., Luo, K. *Nanobiomaterials: Classification, Fabrication and Biomedical Applications, Chapter 17* (2017)

Curriculum Vitae

Na Zhu was born on August 25th, 1993, in Ganzhou, Jiangxi province, China. After graduating from Ganxian Middle School, she commenced her bachelor's studies at Sichuan University with a major in pharmacy. She conducted a research internship titled "*Development of linear HPMA-GFLG-Paclitaxel copolymer nanocarriers*" under the supervision of Prof. dr. Kui Luo and Prof. dr. Zhongwei Gu. In 2015, she earned her Bachelor of Science degree.

In 2017, she started her master's studies in Chemistry with a specialization in "Research in Chemistry" at Leiden University. As part of the master's program, she undertook a research internship in the Molecular Physiology group under the supervision of Prof. dr. M. van der Stelt. The research, titled "*Development of Bub1 inhibitors based on OSI-420 analogues*", aimed to develop inhibitors with improved potency against Bub1. In 2019, Na obtained her master's degree.

In the same year, she started her doctoral studies in the same group under the supervision of Prof. dr. M. van der Stelt, Dr. A.P.A. Janssen, and Dr. R.J.B.H.N. van den Berg, which eventually led to the publication of this thesis. Parts of the research described here were orally presented at NWO CHAINS (Veldhoven, 2022) and poster presented at Cannabinoid Function in the CNS Gordon Research Conference (Barcelona, 2023).