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Discovery of glycine sulfonamides as dual inhibitors of *sn*-1 diacylglycerol lipase α and α/β hydrolase domain 6^{*}

Introduction

Diacylglycerol lipases (DAGL) are responsible for the synthesis of the endogenous signaling lipid 2-arachidonoylglycerol (2-AG) in the brain and immune system.¹ 2-AG activates the cannabinoid type 1 receptor (CB1R), which is also the target of Δ^9 -tetrahydrocannabinol, the main psychoactive compound in marijuana. 2-AG functions as a retrograde messenger and modulates synaptic plasticity at both GABAergic and glutamatergic synapses by inhibiting neurotransmitter release in various regions of the central nervous system. It is involved in a broad array of neurophysiological functions, such as adult neurogenesis, modulation of reward-related behavior, memory formation, pain sensation and appetite.^{2,3} Activation of CB1R via 2-AG has been implicated in various disorders, including obesity and nicotine addiction.⁴ In addition, 2-AG is a precursor for pro-inflammatory prostaglandins in animal models of neuroinflammation.⁴⁻⁷ As a consequence, inhibitors of 2-AG biosynthesis may become useful for therapeutic intervention in various human diseases.^{8,9} DAGLs are intracellular, multi-domain integral membrane proteins that produce 2-AG. Two homologous isoforms are known: *sn*-1 diacylglycerol lipase- α (DAGL α) and *sn*-1 diacylglycerol lipase- β (DAGL β). The DAG lipases share extensive homology, but differ in size: ~120 and ~70 kD for DAGL α and DAGL β respectively. DAG lipases employ the typical Ser-His-Asp catalytic triad to specifically hydrolyze the ester bond of *sn*-1 acyl chains from arachidonate-containing diacylglycerols. Studies with DAGL-KO mice have shown that DAGL α controls to a large extent the formation of 2-AG in the central nervous system whereas DAGL β partakes in 2-AG production in the periphery during inflammation.¹⁰

^{*} Janssen, F.J., Deng, H., Baggelaar, M.P., Allarà, M., van der Wel, T., den Dulk, H., Ligresti, A., van Esbroeck, A.C.M., McGuire, R., Di Marzo, V., Overkleeft, H.S., van der Stelt, M. 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).

Selective chemical tools (*i.e.* inhibitors and activity-based probes) for DAGL α and DAGL β can be used to obtain a better understanding of the physiological role of 2-AG. Such chemical tools provide a critical counterpart of the DAGL-KO mice and allow the examination of acute versus congenital inhibition. Currently, several different classes of DAGL-inhibitors have been reported. These are a) α -ketoheterocycles,¹¹ b) fluorophosphonates,¹²⁻¹⁴ c) beta-lactones,^{10,15} d) bis-oximino-carbamates,¹⁰ and e) 1,2,3-triazole ureas. The latter have recently been described as non-brain-penetrable subtype selective DAGL β inhibitors, which cross-react with α/β hydrolase domain 6 (ABHD6).¹⁶ This is an enzyme responsible for 2-AG hydrolysis and is involved in obesity-related metabolic disorders.¹⁷ Most of the reported inhibitors are lipid-based molecules with chemically reactive warheads that appear not to be sufficiently efficacious and selective over other lipases and/or lack *in vivo* activity on DAGL. Thus, there is a need for new, subtype selective DAGL-inhibitors, which can be used to study the function of DAGL α and DAGL β in animal models of disease.

DAGLs belong to the class of serine hydrolases, which contains more than 200 members with various physiological functions.¹⁸ In order to gain insight in the biological role of a specific serine hydrolase, it is important to know the selectivity of the chemical tool that is used to study the role of a particular enzyme. Activity-based protein profiling (ABPP) is often used to characterize the selectivity and activity of serine hydrolase inhibitors in proteome wide screens using tagged fluorophosphonates (FP) as broad-spectrum activity-based probes.¹⁹ However, DAGL α does not react with such FP-probes. To overcome this shortcoming, a bodipy tagged tetrahydrolipstatin based β -lactone probe MB064 was recently developed,¹¹ which is able to detect endogenous DAGL α expressed in brain. Probe MB064 was used in conjunction with a commercially available fluorophosphonate TAMRA-FP, to assess the activity and selectivity of novel DAGL α inhibitors. For example, α -ketoheterocycles were identified as a potent class of DAGL α inhibitors with fatty acid amide hydrolase (FAAH) as the only off-target of LEI104 in mouse brain, as assessed by competitive ABPP. This demonstrated the power of comparative ABPP to rapidly identify and characterize novel hits.¹¹

Recently, researchers from Bristol-Myers Squibb disclosed a new reversible class of DAGL α inhibitors.^{20,21} They performed a high throughput screening campaign and prioritized the glycine sulfonamide series as an initial lead for chemical optimization (see also Chapter 6). Compounds **2a-c** (Figure 1) were shown to be active on recombinant human DAGL and were selective over pancreatic lipase and monoacylglycerol (MAG) lipase, the main enzyme in the central nervous system that hydrolyzes 2-AG. Due to the lack of an obvious serine hydrolase warhead, the structure-activity relationships of this novel chemotype were investigated, using **2c** as a starting point. In addition, a broader selectivity profile over the serine hydrolase family in native brain proteome was not available.

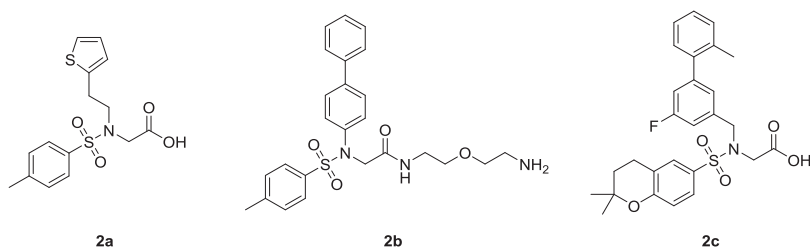
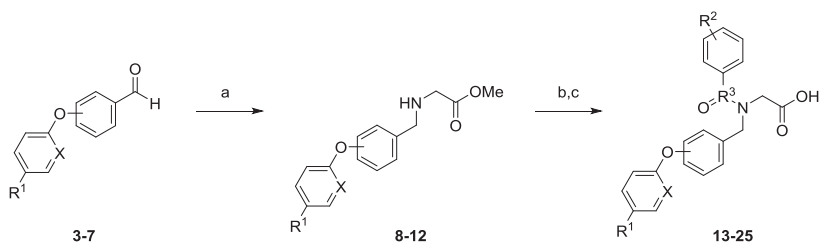


Figure 1. Glycine sulfonamides as reversible class of DAGL α inhibitors.^{20, 21} Compounds **2a** (IC_{50} = 0.5 μ M), **2b** (IC_{50} = 0.05 μ M) and **2c** (IC_{50} = 0.02 μ M) were initial leads for chemical optimization.^{20, 21}

Here, the first study of structure-activity relationships of glycine sulfonamide DAGL α inhibitors is reported, together with the assessment of the selectivity profile over brain membrane proteome using comparative ABPP with MB064 (**1**) and TAMRA-FP. Chemical optimization of the sulfonamide series led to the discovery of **15**, which is a potent DAGL α inhibitor. This study confirmed that glycine sulfonamides are selective over MAGL, but ABPP assays revealed that **15** is also a potent ABHD6 inhibitor. Since ABHD6, a general lysophospholipid hydrolase, is strongly linked to high-fat-diet induced obesity, hepatic steatosis and insulin resistance,¹⁷ this dual inhibition profile may be beneficial for the treatment of obesity-related disorders.

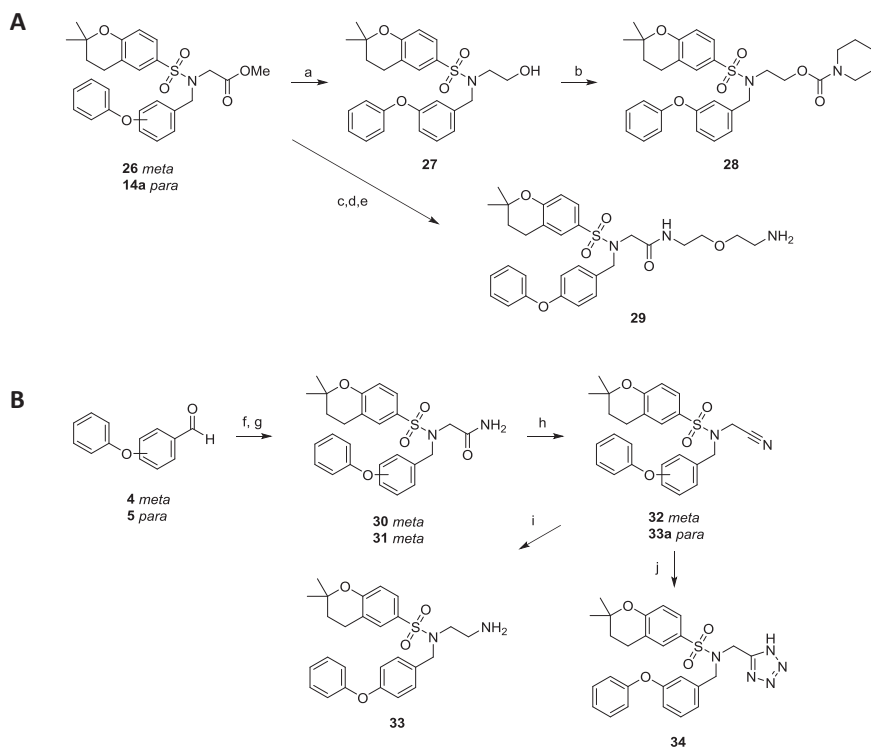
Results

To systematically investigate the structure-activity relationships of glycine sulfonamide series, a general synthetic scheme was developed that allowed for rapid variation of different parts of the molecule. Compound **2c**, with a 2,2-dimethylchroman substituent was used as a starting point, because this was one of the most potent inhibitors reported by BMS.²¹ First, the biphenyl substituent was replaced by biarylethers (**13–17**), which are other well-known fatty acid tail bio-isosters used in clinical candidates (e.g. PF-04457845).²² To probe the size of the binding site, analogs were synthesized with the ether substituent at *ortho* (**13**), *meta* (**14**, **16**) and *para* (**15**, **17**) position of the first phenyl ring. Second, to reduce the polar surface area of the molecules, the sulfonamide was replaced by an amide (**19** vs **23**; **20** vs **22** and **24** vs **25**). Of note, chlorophenyl substituents at R^B were used, because they are metabolically stable analogs of the tolyl substituent in compound **2b** and are commercially available as both a sulfonyl chlorides and acyl chlorides. Finally, since there is no obvious serine hydrolase warhead present in the starting molecule **2c**, the contribution of the carboxylic acid to its activity was investigated by replacing it by ester (**26**), alcohol (**27**), carbamate (**28**) amide (**29**, **30**), nitrile (**32**), amine (**33**) and tetrazole (**34**) functionalities. To this end, reductive amination of glycine methyl ester with benzaldehydes **3–7** was performed, followed by a (sulfon)amide coupling using different acyl chlorides (4-chlorobenzoyl- and 2,6-dichlorobenzoyl chloride) and sulfonyl chlorides (4-chlorophenyl-, 2,6-dichlorophenyl- or 2,2-dimethylchroman-6-sulfonyl chloride). Hydrolysis of the glycine ester yielded final compounds **13–25**.



Scheme 1. Synthesis of glycine sulfonamide derivatives **13-25**. a) Methyl glycinate, AcOH, NaCNBH₃, MeOH, 3Å molecular sieves. rt, 27-80%, **8**: *ortho*, X = CH, R₁ = H, **9**: *meta* X = CH, R₁ = H, **10**: *para*, X = CH, R₁ = H, **11**: *meta* X = N, R₁ = CF₃, **12**: *para* X = N, R₁ = CF₃; b) acylchloride or sulfonylchloride, Et₃N, DMAP or pyridine (cat.), CH₂Cl₂, 0°C to rt. 25-94%; c) NaOH, THF, H₂O, rt, 33-92%, for the structures of final compounds **13-25** see Table 1.

To derivatize the carboxylic acid moiety, methyl esters **14a** and **26** were reduced and subsequent CDI coupling was performed to yield carbamate **28** (see Scheme 2A). Hydrolysis of methyl ester **20** followed by EDC coupling and deprotection provided amide **29**. In addition, reductive aminations using 2-aminoacetamide and aldehydes **4-5** were performed, followed by sulfonylchloride coupling to **30** and **31** (see Scheme 2B). Dehydration to nitriles **32** and **33a** followed by reduction or cyclization yielded amine **33** and tetrazole **34** respectively. All compounds were tested in a colorimetric, biochemical assay using *para*-nitrophenylbutyrate as substrate and HEK293T cell membranes overexpressing human DAGLα, as previously described.^{11, 23}



Scheme 2. Synthetic approach towards glycine sulfonamide derivatives **26–34**. **A** a) LiBH₄, THF, -5°C, 65%; b) CDI, DIPEA, then piperidine, CH₂Cl₂, 0°C to rt, quant; c) NaOH, THF, H₂O, rt, 90%; d) EDC, DIPEA, MeCN, then tert-butyl (2-(2-aminoethoxy)ethyl)carbamate, rt, 21%; e) TFA, CH₂Cl₂, rt, 96%. **B** f) 2-aminoacetamide, AcOH, NaCNBH₃, MeOH, 3 Å molecular sieves, rt, 50% (*meta*, **30a**), 68% (*para*, **31a**); g) 2,2-dimethylchroman-6-sulfonyl chloride, Et₃N, pyridine (cat.), CH₂Cl₂, 0°C to rt, 42% (*meta*, **30**), 63% (*para*, **31**); h) Pyridine, TFAA, THF, 0°C to rt, 69% (*meta*, **32**), 62% (*para*, **33a**); i) borane THF complex, THF, 0°C, 54%; j) Et₃N.HCl, NaN₃, toluene, reflux, 16%;

DAGL α seems to have a large binding cavity, because *ortho* (**13**), *meta* (**14**, **16**) and *para* (**15**, **17**) substitution of the benzylamine moiety with aryloxyethers was allowed at R^A (see Table 1). *Para* substitution was, however, preferred over *ortho* and *meta* substitution. The sulfonamides **13–21** and **24** were well tolerated, whereas amide derivatives **22**, **23** and **25** proved inactive. Thus, a specific orientation of the R^B substituent induced by the sulfonamide is required. The weak activity of compound **19** indicated that the steric tolerance around the sulfonamide functionality is limited, whereas a *para*-chlorophenyl group is allowed (compounds **18** and **22**). This indicated that both electron withdrawing and donating substituents (**2b**) are tolerated at R^B. Thus, the activity in this subpocket seems to be more governed by lipophilic and steric interactions than electronic interactions. In contrast, from the activity of compounds **26–34** it was clear that the carboxylic acid is a crucial pharmacophoric feature, because methylester **26**, alcohol **27**, primary amide **33** and tetrazole **34**, a carboxylic acid bioisoster, were all inactive. Replacement of the acid

functionality by carbamate **28**, which is a well-known warhead for serine hydrolases,²⁴ resulted in loss of activity. It could be that the steric hinder posed by the piperidine moiety caused the drop in activity, however, the primary amide (**30**) was also inactive. Moreover, conversion of the acid moiety into substituted amide **29**, as observed in the original hit **2b**, was detrimental for activity on DAGL α ($IC_{50} > 10 \mu M$). Altogether, this suggests that the carboxylate of the glycine forms an important ionic interaction with a basic amino acid in the enzyme. Surprisingly, replacement of the carboxylic acid with a primary amine yielded compound **33** with an IC_{50} of 512 ± 147 nM. Compound **15** proved the most potent glycine sulfonamide derivative in the biochemical assay with an IC_{50} of 18 ± 6 nM and inhibited the hydrolysis of [^{14}C]-*sn*-1-oleoyl-2-arachidonoyl-glycerol, the natural substrate of DAGL α , with a K_i of $0.7 \pm 0.08 \mu M$.¹⁰

To explain the observed structure-activity-relationships, **15** was docked in a previously reported homology model of DAGL α (built from the crystal structure of the S146A mutant of *Thermomyces (Humicola) lanuginosa* lipase in complex with oleic acid, PDB code 1GT6).¹¹ Docking was performed using the Yasara²⁵ implementation of AutodockVina,²⁶ generating 25 high scoring poses within $1.3 \text{ kcal.mol}^{-1}$ of the calculated binding energy minimum. Two high ranking binding poses (Figure 2) have been observed that place the carboxylic acid in very close proximity to the catalytic serine (Ser472) or histidine (His650) residues. While in both poses pi-cation interactions with the benzylphenoxy pharmacophore are observed, the interactions of the key carboxylic acid moiety differ (Figure 2A,B). In pose 1 a hydrogen bond between the carboxylic acid with the catalytic serine (Ser472) is found, while in pose 2 the carboxylic acid is in close proximity of the catalytic histidine (His650) and a neighboring His471. This may also explain the weak activity of the amine (compound **33**), because a histidine has both an acid and base functionality. The orientations of the two hydrophobic substituents differ as well in the different binding poses. In pose 1 the 2,2-dimethylchroman substituent is in a hydrophobic pocket lined by residues Leu410, Val526, Ile529 and Leu531 and the phenoxybenzyl group is directed outside of the catalytic site into a broader cavity (Figure 2C). In pose 2 the 2,2-dimethylchroman substituent is in another hydrophobic pocket that is only accessible through its perpendicular angle induced by the sulfonamide (Figure 2D). In this pose the phenoxybenzyl group is positioned in the pocket aligned by Leu410, Val526, Ile529 and Leu531. While these binding modes were among the highest ranking docking poses and are in agreement with the observed structure-activity relationships, a co-crystal structure of the glycine sulfonamide inhibitors with DAGL α is required to prove the proposed binding modes.

Table 1. Structure-activity relationship overview.

$$\begin{array}{c} \text{R}^{\text{B}} \quad \text{R}^{\text{C}} \\ | \quad / \\ \text{N} \\ | \\ \text{R}^{\text{A}} \end{array}$$

Entry	R ^A	R ^B	R ^C	DAGL α IC ₅₀ $\pm$ SEM (nM)
13				185 $\pm$ 74
14				56 $\pm$ 11
15 (LEI106)				18 $\pm$ 6
16				143 $\pm$ 60
17				65 $\pm$ 21
18				91 $\pm$ 20
19				1600 $\pm$ 500
20				66 $\pm$ 27
21				695 $\pm$ 335
22				> 10 ⁴
23				> 10 ⁴
24				302 $\pm$ 135
25				> 10 ⁴
26				> 10 ⁴
27				> 10 ⁴
28				> 10 ⁴
29				> 10 ⁴
30				> 10 ⁴

Entry	R ^A	R ^B	R ^C	DAGL α IC ₅₀ $\pm$ SEM (nM)
32				> 10 ⁴
33				512 $\pm$ 147
34				> 10 ⁴

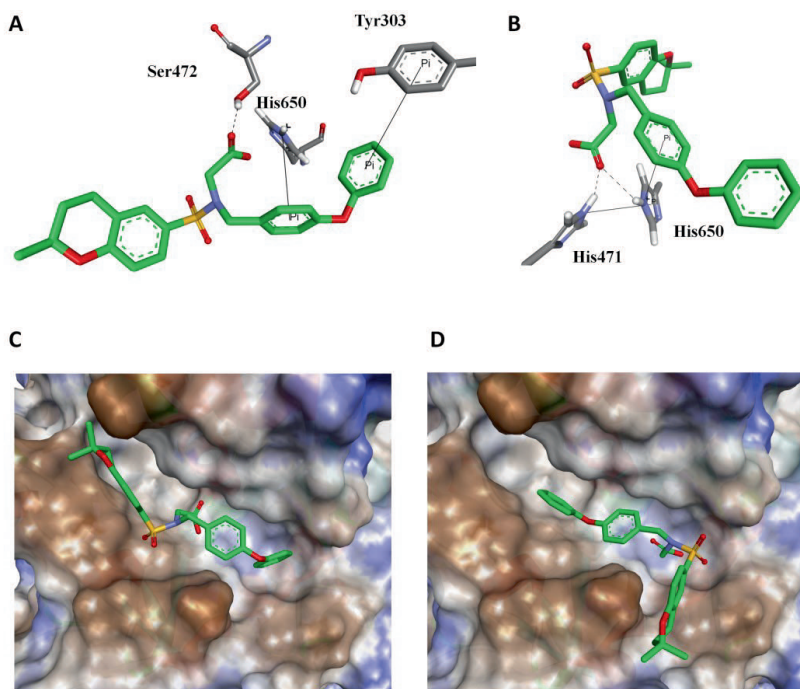


Figure 2. Two highly ranked binding poses of compound **15** in the DAGL α homology model. **A)** Binding mode 1 displaying key interactions with catalytic serine (Ser472) and histidine (His650) and a neighboring Tyr303. **B)** Binding mode 2 displaying key interactions with the catalytic histidine (His650) and a neighboring His471 **C)** Binding mode 1 in the catalytic binding site cavity. **D)** Binding mode 2 in the catalytic binding site cavity.

To determine the activity and selectivity of the glycine sulfonamide series on endogenously expressed DAGL α and other serine hydrolases in brain membrane proteome, the three most potent inhibitors **14**, **15** and **17** were incubated for 20 min with mouse brain membrane homogenates, followed by labeling of the serine hydrolases by MB064 (**1**) or FP-probe (Figure 3C,D). The non-selective lipase inhibitor, tetrahydrolipstatin (THL) was used as a positive control. All compounds were able to block the labeling of DAGL α in the mouse brain proteome in a concentration dependent manner. Complete blockade was observed at 20 μ M of inhibitors **14**, **15** and **17** (Figure 3D). The order of potency was similar to that found in

the biochemical assay. Compound **15** inhibited DAGL α labeling with an IC₅₀ of 124 ± 13 nM (N = 3), which is ~2.5 times more potent than compound **14** and ~6 times more potent than compound **17** (Figure 3A,B). No reduction of MAGL (protein bands 11 and 12, Figure 3C) labeling at 20 μ M was observed for any of the three compounds, which is in line with the reported lack of activity of this chemical series in a biochemical assay. However, compounds **14** and **15** partially prevented labeling of an unknown off-target protein by TAMRA-FP (protein 4 in Figure 3C) and all three compounds reduced labeling of two other proteins (labeled 8 and 9 in Figure 3D) by MB064 (**1**). Protein 9 was identified as ABHD6. Hence, three off-targets for **14** and **15** and two off-targets for **17** were identified in the current setting.

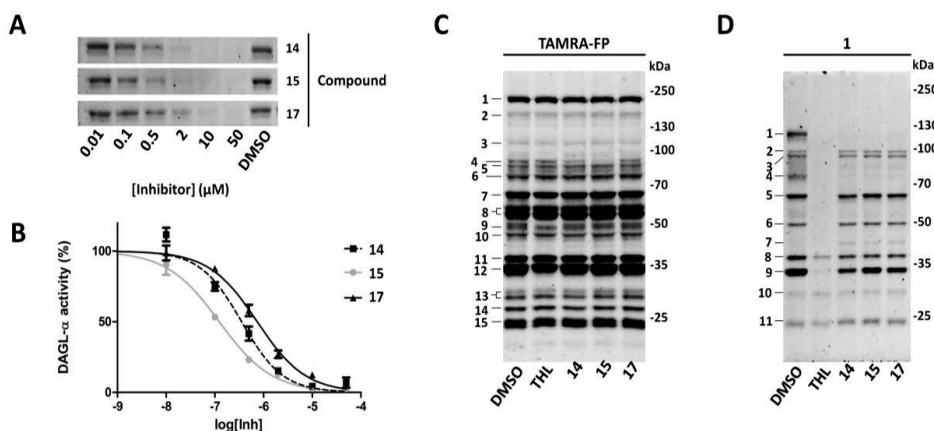
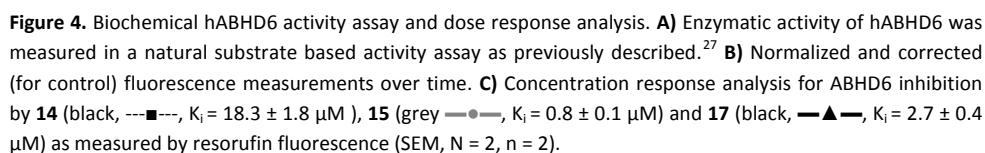


Figure 3: Activity-based protein profiling assay with TAMRA-FP and MB064 (**1**). **A**) Concentration dependent inhibition of DAGL α in the mouse brain membrane proteome by **14**, **15** and **17**. **B**) Dose–response curves of DAGL α inhibition by **14** (black, ---■---, IC₅₀ = 355 ± 54 nM), **15** (grey —●—, IC₅₀ = 124 ± 13 nM) and **17** (black, —▲—, IC₅₀ = 743 ± 72 nM) as measured by competitive ABPP with MB064 (**1**, SEM, N = 3). **C**) and **D**) Competitive ABPP with the DAGL α inhibitors **14**, **15** and **17** (20 μ M) using ABPs carboxytetramethylrhodamine fluorophosphonate (TAMRA-FP) and MB064 (**1**) in mouse brain membrane proteome. The quantified data of the selectivity screening can be found in the supplementary information.

Since ABHD6 is a hydrolase that is capable of metabolizing 2-AG, the natural product of DAGL α , a natural substrate-based assay for ABHD6 was set up.²⁷ This assay employs ABHD6 to convert 2-AG into glycerol, which is coupled to the oxidation of commercially available fluorogenic Amplifu™Red via a multi-enzyme cascade (Figure 4A). It was found that membrane preparations of HEK293T cells overexpressing human ABHD6 are able to hydrolyze 2-AG with an apparent K_m of 25.4 ± 3.3 μ M and a V_{max} of 5.94 ± 0.28 nmol/min/mg protein. Pre-incubation of the three inhibitors dose-dependently inhibited ABHD6 with a K_i of 0.8, 2.7 and 18.3 μ M for compound **15**, **17** and **14**, respectively (Figure 4C). This indicates that the glycine sulfonamides are a class of dual inhibitors for DAGL α and ABHD6.



Discussion & Conclusion

This study reports on the first structure-activity-relationship study of non-covalent small molecule inhibitors of DAGL α based on the glycine sulfonamide scaffold. It was found that *i*) the enzyme tolerates a variety of biaryl substituents, *ii*) the sulfonamide is required for inducing a specific orientation of a 2,2-dimethylchroman substituent and *iii*) a carboxylic acid is essential for its activity. Analysis of potential binding modes of the most potent inhibitor **15** in a homology model of DAGL α indicated that the carboxylic acid may interact with the catalytic Ser472 or His650 and that the two hydrophobic substituents may address different hydrophobic binding pockets.

In contrast to a previous report, which claimed that glycine sulfonamide were selective inhibitors for DAGL α , proteome-wide ABPP assay indicated that the glycine sulfonamides do interact with other proteins. Compound **15** prevented labeling of at least three other unknown proteins targeted by MB064 and TAMRA-FP. One off-target was identified as ABHD6. Using a recently developed activity assay for ABHD6 that employs 2-AG as natural substrate, it was discovered that **15** prevented the hydrolysis of 2-AG with a K_i of 0.8 ± 0.1 μ M, which is very similar as for DAGL α ($K_i = 0.7 \pm 0.08$ μ M). Thus, compound **15** is a dual inhibitor of DAGL α and ABHD6. Of note, it seems that ABHD6 is a common off-target for DAGL α inhibitors in general, because also the triazole ureas,¹⁶ fluorophosphonates¹²⁻¹⁴ and beta-lactones^{10,15} cross-react with this enzyme. To investigate DAGL biology it is necessary to identify compounds that do not cross-react with ABHD6 and the two other unknown off-targets.

Recently it has been reported that ABHD6 is linked to metabolic disorders and is a regulator of *de novo* lipogenesis in the liver by acting as a general lysophospholipid hydrolase using *lyso*-phosphatidylglycerol, *lyso*-phosphatidylethanolamine, *lyso*-phosphatic acid and *lyso*-phosphatidyl serine as substrates. Peripheral knock-down of ABHD6 protected mice from high-fat-diet induced obesity, hepatic steatosis and insulin resistance. *In vivo* inhibition of ABHD6 with a small molecule resulted in reduced glucose intolerance and induced weight loss, thereby providing rationale for the development of small molecule inhibitors of ABHD6 for the treatment of obesity-induced metabolic disorders.¹⁷ Since inhibition of DAGL α is also strongly linked with reduced body weight and resistance against high-fat diet induced obesity,¹⁴ via reduced cannabinoid CB1R activation,^{4,28,29} it can be envisioned that dual inhibition of both ABHD6 and DAGL α may provide additional therapeutical benefits. Since both DAGL α and ABHD6 are highly expressed in the brain, it would be of interest to develop compounds with restricted access to the central nervous system to prevent adverse neuropsychiatric side effects as previously observed with the cannabinoid CB1R antagonist Rimobabant. The glycine sulfonamide series, as exemplified by **15**, may be an interesting lead series to provide such tool compounds, because their polar surface area is quite large (> 70 Å²).

In summary, this study provides the first structure-activity relationship of the glycine sulfonamide series of DAGL α inhibitors and discovered that this class of compounds is not selective over ABHD6. This provided important insights in the direction for optimization of **15**, LEI106, as a selective DAGL α tool compound. In addition, this dual DAGL α / ABHD6 inhibition profile might provide a new lead for the treatment of diet-induced obesity and metabolic syndrome.

Experimental

Experimental procedures computational chemistry

Docking in DAGL α homology model. Docking was performed as previously reported (Chapter 2).¹¹

Experimental procedures biochemistry

Cloning procedures. Full length human hDAGL α and hABHD6 cDNA was purchased from Biosource and cloned into mammalian expression vector pcDNA3.1, containing genes for ampicillin and neomycin resistance. The empty vector was used as a negative control (mock). All plasmids were grown in XL-10 Z-competent cells and prepped (Maxi Prep, Qiagen). The sequences were confirmed by sequence analysis at the Leiden Genome Technology Centre.

Cell culture and membrane preparation. HEK293T cells were grown in DMEM with stable glutamine and phenolred (PAA or Sigma) with 10% New Born Calf serum, penicillin and streptomycin. Cells were passaged every 2-3 days by resuspension in medium and seeding to the appropriate confluence. Membranes were prepared from transiently transfected HEK293T cells. One day prior to transfection 10^7 cells were seeded in a 15 cm petri dish. Cells were transfected by the addition of a 3:1 mixture of polyethyleneimine (60 μ g) and plasmid DNA (20 μ g) in 2 mL serum free medium. The medium was refreshed after 24 hours, and after 72 h the cells were harvested by suspending them in 20 mL medium. The suspension was centrifuged for 10 min at 1000 rpm, and the supernatant was removed. The cell pellet was stored at -80 °C until use.

Cell pellets were thawed on ice and suspended in lysis buffer A (20 mM HEPES, pH 7.2, 2 mM DTT, 0.25 M sucrose, 1 mM MgCl₂, 1x protease inhibitor cocktail (Roche cOmplete EDTA free), 25U/mL Benzonase). For hABHD6 membrane preparations, the protease inhibitor cocktail was not added. The suspension was homogenized by polytrone (3 $\times$ 7 sec) and incubated for 30 min on ice. The suspension was subjected to ultracentrifugation (93.000 $\times$ g, 30 min, 4 °C, Beckman Coulter, Type Ti70 rotor) to yield the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was resuspended in lysis buffer B (20 mM HEPES, pH 7.2, 2 mM DTT, 1x protease inhibitor cocktail). The protein concentration was determined with Quick Start Bradford assay (Biorad). The protein fractions were diluted to a total protein concentration of 1 mg/mL and stored in small aliquots at -80 °C until use.

Biochemical DAGL α activity assay. The biochemical hDAGL α activity assay was performed as previously reported.¹¹

Biochemical ABHD6 activity assay. The biochemical hABHD6 activity assay is based on the production of glycerol by 2-arachidonoyl glycerol (2-AG) hydrolysis by membrane preparations from HEK293T cells transiently transfected with hABHD6, as previously reported.²⁷ The produced glycerol is coupled to the oxidation of commercially available Ampliflu™Red via a multi-enzyme cascade, resulting in a fluorescent signal from the dye resorufin. Assay reagents and enzymes (glycerol kinase from *Cellulomonas sp.*, glycerol-1-phosphate oxidase from *Streptococcus thermophilus*, horse radish peroxidase from *Horseradish*) were purchased from Sigma Aldrich.

The assay was performed in HEMNB buffer (50 mM HEPES pH 7.4, 1 mM EDTA, 5 mM MgCl₂, 100 mM NaCl, 0.5% (w/v) BSA) in black, flat bottom Greiner 96-wells plates. Final protein concentration of membrane preparations of HEK293T cells overexpressing hABHD6 was 0.04 μ g/ μ L. Inhibitors were added from 40x concentrated DMSO stocks. After 30 min. incubation, assay mix containing glycerol kinase (GK), glycerol-1-phosphate oxidase (GPO), horse radish peroxidase (HRP), adenosine triphosphate (ATP), Ampliflu™Red and 2-

arachidonoyl glycerol (2-AG) was added and fluorescence was measured in 5 min. intervals for 75 min. ($\lambda_{\text{ex}} = 535 \text{ nm}$, $\lambda_{\text{em}} = 595 \text{ nm}$) at 21°C on a plate reader (TECAN GENios microplate reader). Final assay concentrations: 0.2 U/mL GK, GPO and HRP, 0.125 mM ATP, 10 μM Amplifu™Red, 25 μM 2-AG, 5% DMSO, 1% ACN in a final volume of 200 μL . Slopes were determined in the linear interval of $t = 10$ to $t = 35 \text{ min}$. All experiments were performed at $N = 2$, $n = 2$ for experimental measurements and $N = 2$, $n = 4$ for controls.

The Z'-factor for each measurement was calculated as previously reported,¹¹ but with ABHD6 and DMSO as a positive control and with slope values determined in the interval of $t = 10$ to $t = 35 \text{ min}$. Plates with $Z' \geq 0.6$ were accepted for further analysis. Fluorescence values were corrected for the average fluorescence of the negative control. The average, standard deviation (SD) and standard error of mean (SEM) were calculated and normalized to the corrected positive control. Data was exported to Graphpad Prism 5.0 for the calculation of the IC_{50} using a non-linear dose-response analysis.

For K_M and V_{max} determination, the concentration of 2-AG was varied and slopes of corrected fluorescence in time were exported to GraphPad Prism 5.0 (Michaelis-Menten analysis). The calculated V_{max} value in RFU/min was then converted to a value in nmol converted glycerol/min/mg protein according to a standard curve with the rate of increase in fluorescence as a function of converted glycerol.

Preparation of mouse brain membrane proteome. Mouse brains were isolated according to guidelines approved by the ethical committee of Leiden University (DEC#13191). Mouse brains were thawed on ice and homogenized by polytrone (3 $\times$ 7 sec.) in pH 7.2 lysis buffer A (20 mM HEPES, 2 mM DTT, 1 mM MgCl_2 , 25 U/mL Benzonase) and incubated for 15 minutes on ice, followed by low speed spin (2500 $\times$ g, 3 min. at 4 °C) to remove debris. The supernatant was subjected to ultracentrifugation (100.000 $\times$ g, 45 min. 4 °C, Beckman Coulter, Type Ti70 rotor) to yield the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was resuspended in lysis buffer B (20 mM HEPES, 2 mM DTT). The total protein concentration was determined with Quick Start Bradford assay (Biorad). Membranes were stored in small aliquots at -80 °C until use.

Activity-based protein profiling activity assay. The IC_{50} of inhibitors was determined against endogenously expressed DAGL α in the mouse brain membrane proteome. Inhibitors were incubated at the indicated concentrations (total volume 20 μL) for 30 min at rt, prior to incubation with MB064 for 10 min at rt. The reaction was quenched with 10 μL standard 3 $\times$ SDS page sample buffer, and resolved on 10 % SDS-page. The gels were scanned with a ChemiDoc™ MP sytem (Cy3 settings; 605/50 filter). The percentage activity remaining was determined by measuring the integrated optical intensity of the bands using Image Lab 4.1. software. IC_{50} values were determined from a dose-response curve generated using Prism software (GraphPad).

Activity-based protein profiling selectivity assay. For gel based ABPP experiments mouse brain proteome (2.0 mg/mL, 20 μL) was preincubated for 30 min with vehicle (0.5 μL DMSO) or inhibitor in 0.5 μL DMSO at rt and subsequently treated with 250 nM (final concentration) ABP 1 or 500 nM (final concentration) TAMRA-FP (from Thermo Fischer Scientific) for 15 minutes at rt. The reactions were quenched with 10 μL standard 3 $\times$ SDS page sample buffer. The samples were directly loaded and resolved on SDS page gel (10 % acrylamide). The gels were scanned with a ChemiDoc™ MP sytem (Cy 3 settings; 605/50 filter) and analyzed using Image Lab 4.1.

Quantification of protein bands for the determination of selectivity of 14, 15 and 17 The percentage activity remaining was determined by measuring the integrated optical intensity of the bands using Image Lab 4.1 software. This activity was corrected for the total protein loading per lane as determined by Coomassie stain and imaging with a ChemiDoc™ MP sytem, followed by determination of the integrated optical intensity per lane by using Image Lab 4.1. The intensity of the protein bands from the protein samples treated with vehicle was set to 100%.

Experimental procedures chemistry

General remarks

All reactions were performed using oven or flame-dried glassware and dry solvents. Reagents were purchased from Sigma Aldrich, Acros and Merck and used without further purification unless noted otherwise. All moisture sensitive reactions were performed under an argon atmosphere. Traces of water were removed from starting compounds by co-evaporation with toluene.

^1H - and ^{13}C -NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 (^1H) and 100.6 (^{13}C) MHz using CDCl_3 or CD_3OD as solvent, unless stated otherwise. Chemical shift values are reported in ppm with tetramethylsilane 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 , δ 49.00 for ^{13}C). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, td = triple doublet, t = triplet, q = quartet, quintet = quint, br = broad, m = multiplet), coupling constants J (Hz), and integration. HPLC purification was performed on a preparative LC-MS system (Agilent 1200 series) with an Agilent 6130 Quadrupole MS detector. High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific LTQ Orbitrap XL. Liquid chromatography was performed on a Finnigan Surveyor LC-MS system, equipped with a C18 column. Melting points were measured on a Stuart SMP30 melting point apparatus. Flash chromatography was performed using SiliCycle silica gel type SiliaFlash P60 (230 – 400 mesh). TLC analysis was performed on Merck silica gel 60/Kieselguhr F254, 0.25 mm. Compounds were visualized using either Seebach's reagent (a mixture of phosphomolybdic acid (25 g), cerium (IV) sulfate (7.5 g), H_2O (500 mL) and H_2SO_4 (25 mL)) or a KMnO_4 stain (K_2CO_3 (40 g), KMnO_4 (6 g) and H_2O (600 mL)).

Methyl (2-phenoxybenzyl)glycinate (8)

To a stirred solution of 2-phenoxybenzaldehyde (**3**, 150 μL , 0.87 mmol) in MeOH (50 mL) with 3 Å MS was added methyl glycinate (377.6 mg, 3.01 mmol) and AcOH (260 μL , 4.54 mmol) at rt, the reaction mixture was stirred for 15 minutes. Lastly was added NaCNBH_3 (151.4 mg, 2.41 mmol) and the reaction mixture was stirred for 2 hours at rt. After completion the reaction was diluted with EtOAc and washed with NaHCO_3 , water and brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield methyl (2-phenoxybenzyl)glycinate (141.9 mg, 0.52 mmol, 60 %) as a white solid. ^1H NMR (CDCl_3 , 400 MHz): δ 7.41 (d, J = 7.3 Hz, 1H), 7.32 (t, J = 7.6 Hz, 2H), 7.22 (t, J = 7.7 Hz, 1H), 7.16 – 7.05 (m, 2H), 6.96 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 8.0 Hz, 1H), 3.88 (s, 2H), 3.68 (s, 3H), 3.45 (s, 2H), 1.92 (bs, 1H). ^{13}C NMR (CDCl_3 , 101 MHz): δ 170.51, 157.54, 155.07, 130.54, 129.89(2C), 128.76, 125.85, 123.91, 123.12, 119.17, 118.24(2C), 54.19, 51.97, 49.87.

Methyl (3-phenoxybenzyl)glycinate (9)

The title compound was synthesized from 3-phenoxybenzaldehyde (**4**, 100 μL , 0.58 mmol) according to the procedure described for compound **8**. This yielded methyl (3-phenoxybenzyl)glycinate (125.7 mg, 0.46 mmol, 80 %) as a white solid. ^1H NMR (CDCl_3 , 400 MHz): δ 7.39 – 7.27 (m, 3H), 7.13 – 7.04 (m, 2H), 7.04 – 6.97 (m, 3H), 6.90 (ddd, J = 8.2, 2.5, 1.0 Hz, 1H), 3.79 (s, 2H), 3.73 (s, 3H), 3.42 (s, 2H). ^{13}C NMR (CDCl_3 , 101 MHz): δ 170.73, 157.76, 157.16, 133.93, 129.88(2C), 129.84, 123.36, 123.14, 119.04(2C), 118.73, 117.71, 99.91, 53.09, 51.74, 50.02. Spectroscopic data are in agreement with those reported in literature.³⁰

Methyl (4-phenoxybenzyl)glycinate (10)

The title compound was synthesized from 4-phenoxybenzaldehyde (**5**, 100 mg, 0.50 mmol) according to the procedure described for compound **8**. This yielded methyl (4-phenoxybenzyl)glycinate (240 mg, 0.88 mmol, 44 %) as a white solid. ^1H NMR (400 MHz, CDCl_3): δ 7.38 – 7.22 (m, 4H), 7.08 (t, J = 7.4 Hz, 1H), 6.98 (ddd, J = 10.1, 7.2, 1.5 Hz, 4H), 3.77 (s, 2H), 3.72 (s, 3H), 3.42 (s, 2H), 2.18 (s, 1H); ^{13}C NMR (101 MHz, CDCl_3): δ 172.87, 157.37, 156.38, 134.38, 129.75(2C), 129.72(2C), 123.18, 118.97(2C), 118.76(2C), 52.68, 51.82, 49.85.

3-((5-(Trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (11a)

The title compound was synthesized from 3-hydroxybenzaldehyde (366 mg, 3 mmol) and 2-chloro-5-(trifluoromethyl)pyridine (363 mg, 2 mmol) according to the procedure described for compound **12a**. This yielded 3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (135 mg, 0.5 mmol, 30%) after flash chromatography as a white solid. ¹H NMR (CDCl₃, 400 MHz): δ 10.02 (s, 1H), 8.45 – 8.40 (m, 1H), 7.96 (dd, *J* = 8.6, 2.3 Hz, 2H), 7.77–7.79 (m, 1H), 7.71 – 7.67 (m, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.43–7.46 (m, 1H), 7.10 (d, 1H, *J* = 8.8 Hz). Spectroscopic data are in agreement with those reported in literature.³¹

Methyl 3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzylglycinate (11)

The title compound was synthesized from 3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (**6**, 183mg, 0.68mmol) according to the procedure described for compound **8**. This yielded methyl 3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzylglycinate (84 mg, 0.25 mmol, 37 %) as a colorless oil. ¹H NMR (CDCl₃, 400 MHz): δ 8.43 (s, 1H), 7.86 – 7.89 (m, 1H), 7.36 – 7.39 (m, 1H), 7.14 – 7.23 (m, 2H), 6.98 – 7.01 (m, 2H), 3.86 (s, 2H), 3.72 (s, 3H), 3.45 (s, 2H).

4-((5-(Trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (12a)

To a stirred solution of 4-hydroxybenzaldehyde (366 mg, 3 mmol) in DMSO (30 mL) was added K₂CO₃ (360 mg, 6 mmol) at room temperature. After 30 minutes of stirring was added dropwise a solution of 2-fluoro-5-(trifluoromethyl)pyridine (0.241 mL, 2 mmol) in DMSO (2 mL). After full completion (24 h) HCl (1 M) was added. The mixture was poured into water, and the product was extracted with EtOAc, washed with water and brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (366 mg, 1.37 mmol, 70 %) as a white solid. ¹H NMR (CDCl₃, 400 MHz): δ 10.01 (s, 1H), 8.45 (s, 1H), 7.95 – 7.99 (m, 3H), 7.31 – 7.35 (m, 2H), 7.11 – 7.13 (m, 1H). ¹³C NMR (101 MHz, CDCl₃): δ 190.94, 164.87, 158.25, 145.49 (q, *J* = 4.3 Hz), 137.24 (q, *J* = 3.2 Hz), 133.58, 131.69(2C), 123.68 (q, *J* = 272.6 Hz), 122.50 (q, *J* = 33.5 Hz), 121.92(2C), 112.24.

Methyl 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzylglycinate (12)

The title compound was synthesized from 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (**7**, 267 mg, 1 mmol) according to the procedure described for compound **8**. This yielded methyl 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzylglycinate (215 mg, 0.63 mmol, 63 %) as a colorless oil. ¹H NMR (CDCl₃, 400 MHz): δ 8.44 (s, 1H), 7.88–7.91 (m, 1H), 7.40 (d, 2H, *J* = 8.4 Hz), 7.10–7.13(m, 2H), 7.00 (d, 1H, *J* = 8.8 Hz), 3.83 (s, 2H), 3.75 (s, 3H), 3.46 (s, 2H).

Methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(2-phenoxybenzyl)glycinate (13a)

To a stirred solution of methyl (2-phenoxybenzyl)glycinate (**8**, 63.9 mg, 0.24 mmol) in CH₂Cl₂ (50 mL) was added 2,2-dimethylchroman-6-sulfonyl chloride (96 mg, 0.368 mmol), triethylamine (0.1 mL, 0.717 mmol) and 1 drop of pyridine (or 4-dimethylaminopyridine) at rt. After completion (2 h), the reaction mixture was diluted with EtOAc, washed with sat. NaHCO₃, brine and purified using column chromatography to yield methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(2-phenoxybenzyl)glycinate (96.0 mg, 0.19 mmol, 82 %) as an oil. ¹H NMR (CDCl₃, 400 MHz): δ 7.57 – 7.46 (m, 3H), 7.33 – 7.27 (m, 2H), 7.25 – 7.19 (m, 1H), 7.14 – 7.04 (m, 2H), 6.87 – 6.74 (m, 4H), 4.55 (s, 2H), 4.02 (s, 2H), 3.52 (s, 3H), 2.74 (t, *J* = 6.7 Hz, 2H), 1.80 (t, *J* = 6.7 Hz, 2H), 1.34 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 169.68, 157.96, 156.85, 155.46, 131.49, 130.43(2C), 129.95, 129.57, 129.51, 127.15, 126.53, 123.89, 123.52, 121.31, 118.57(2C), 118.26, 117.79, 75.73, 52.06, 48.00, 46.26, 32.38, 27.01(2C), 22.45.

***N*-((2,2-Dimethylchroman-6-yl)sulfonyl)-*N*-(2-phenoxybenzyl)glycine (13)**

To a stirred solution of methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(2-phenoxybenzyl)glycinate (**13a**, 60 mg, 0.121 mmol) in THF (1 mL): methanol (1 mL) was added aqueous NaOH (2 mL, 4.00 mmol) at rt. After completion (30 mins), to the reaction was added Amberlight 150 H resin until neutral, the mixture was filtered, concentrated *in vacuo*, coevaporated with toluene and purified by flash chromatography to yield *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(2-phenoxybenzyl)glycine (25.3 mg, 0.053 mmol, 43% yield) as a white solid. mp 155-157 °C. HRMS (ESI+) *m/z*: calculated for C₂₆H₂₈NO₆S ([M + H]), 482.1632, found: 482.1631. ¹H NMR (CDCl₃, 400 MHz): δ 7.72 – 7.35 (m, 3H), 7.33 – 7.27 (m, 2H), 7.23 (t, *J* = 7.5 Hz, 1H), 7.17 – 6.98 (m, 2H), 6.94 – 6.65 (m, 4H), 4.54 (s, 2H), 4.08 (s, 2H), 2.71 (t, *J* = 6.6 Hz, 2H), 1.78 (t, *J* = 6.4 Hz, 2H), 1.33 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 173.82, 158.16, 156.62, 155.63, 131.64, 130.46, 130.02(2C), 129.76, 129.62, 127.20, 126.06, 123.88, 123.71, 121.49, 118.71(2C), 118.18, 117.85, 75.83, 48.15, 46.90, 32.31, 27.00(2C), 22.42. Purity >95% as determined by LC-MS.

Methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (14a)

The title compound was synthesized from methyl (3-phenoxybenzyl)glycinate (**9**, 37.5 mg, 0.14 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (64.5 mg, 0.13 mmol, 94 %) as an oil. ¹H NMR (CDCl₃, 400 MHz) δ 7.62 – 7.51 (m, 2H), 7.33 (t, *J* = 8.4, 7.5 Hz, 2H), 7.29 – 7.23 (m, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 7.03 – 6.94 (m, 3H), 6.94 – 6.80 (m, 3H), 4.44 (s, 2H), 3.92 (s, 2H), 3.57 (s, 3H), 2.80 (t, *J* = 6.7 Hz, 2H), 1.83 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz) δ 169.45, 158.14, 157.67, 156.99, 137.43, 130.15, 129.96, 129.92(2C), 129.64, 127.15, 123.58, 123.44, 121.45, 119.06, 119.02(2C), 118.39, 117.95, 75.82, 52.13, 51.25, 46.95, 32.36, 26.99(2C), 22.48.

***N*-((2,2-Dimethylchroman-6-yl)sulfonyl)-*N*-(3-phenoxybenzyl)glycine (14)**

The title compound was synthesized from methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (**14a**, 52.4 mg, 0.11 mmol) according to the procedure described for compound **13**. This yielded *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-phenoxybenzyl)glycine (27.9 mg, 0.06 mmol, 55 %) as a white solid after lyophilization. mp 171-173 °C. HRMS (ESI+) *m/z*: calculated for C₂₆H₂₈NO₆S ([M + H]), 482.1632, found: 482.1630. ¹H NMR (CDCl₃, 400 MHz): δ 7.70 – 7.52 (m, 2H), 7.33 (t, *J* = 7.9 Hz, 2H), 7.25 – 7.29 (m, 1H), 7.15 – 7.06 (m, 1H), 7.05 – 6.87 (m, 4H), 6.87 – 6.77 (m, 2H), 4.42 (s, 2H), 3.94 (s, 2H), 2.78 (t, *J* = 6.6 Hz, 2H), 1.82 (t, *J* = 6.7 Hz, 2H), 1.34 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 173.84, 158.34, 157.82, 156.91, 137.14, 130.28, 129.95(2C), 129.67, 129.56, 127.18, 123.66, 123.39, 121.63, 119.08(2C), 118.97, 118.51, 118.02, 75.93, 51.40, 46.87, 32.32, 27.00(2C), 22.47. Purity >95% as determined by LC-MS.

Methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (15a)

See compound **26**.

***N*-((2,2-Dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (15)**

The title compound was synthesized from methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (**15a**, 62 mg, 0.125 mmol) according to the procedure described for compound **13**. This yielded *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (54 mg, 0.11 mmol, 90%) as a white solid. mp 192-194 °C. HRMS (ESI+) *m/z*: calculated for C₂₆H₂₈NO₆S ([M + H]) 482.1632, found 482.1631. ¹H NMR (400 MHz, DMSO): δ 7.58 (d, *J* = 2.2 Hz, 1H), 7.52 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.40 (dd, *J* = 8.5, 7.5 Hz, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 7.15 (t, *J* = 7.4 Hz, 1H), 7.04 – 6.97 (m, 2H), 6.93 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 1H), 4.37 (s, 2H), 3.84 (s, 2H), 2.80 (t, *J* = 6.6 Hz, 2H), 1.81 (t, *J* = 6.7 Hz, 2H), 1.31 (s, 6H); ¹³C NMR (101 MHz, DMSO): δ 170.47, 157.70, 157.04, 156.54, 131.36, 130.66, 130.61(2C), 130.51(2C), 129.63, 127.05, 123.94,

122.03, 119.02(2C), 118.89(2C), 117.66, 76.13, 51.14, 48.15, 32.00, 27.02(2C), 22.11. Purity >95% as determined by LC-MS.

Methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (16a)

The title compound was synthesized from methyl 3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzylglycinate (**11**, 34 mg, 0.1 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (19 mg, 0.03 mmol, 35 %) as a white solid. ¹H NMR (CDCl₃, 400 MHz): δ 8.34 (s, 1H), 7.82-7.85 (m, 1H), 7.50-7.52 (m, 2H), 7.28-7.32 (m, 1H), 7.07-7.09 (m, 1H), 7.00 (d, 2H, *J* = 8.0 Hz), 6.94 (d, 1H, *J* = 8.8 Hz), 6.76 (d, 1H, *J* = 8.4 Hz), 4.43 (s, 2H), 3.89 (s, 2H), 3.62 (s, 3H), 2.73 (t, 2H, *J* = 6.4 Hz), 1.75 (t, 2H, *J* = 6.8 Hz), 1.26 (s, 6H).

***N*-((2,2-Dimethylchroman-6-yl)sulfonyl)-*N*-(3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (16)**

The title compound was synthesized from methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (**16a**, 9.0 mg, 0.016 mmol) according to the procedure described for compound **13**. This yielded *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (5.6 mg, 0.010 mmol, 63% yield) as a white solid after lyophilization. mp 149-151 °C. HRMS (ESI+) calculated for C₂₆H₂₅F₃N₂O₆S ([M + H]). 551.1458, found: 551.1456. ¹H NMR (CDCl₃, 400 MHz): δ 8.47 (s, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.58 – 7.62 (m, 2H), 7.30 – 7.32 (m, 1H), 7.03 – 7.11 (m, 4H), 6.85 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 3.96 (s, 2H), 2.82 (t, *J* = 6.4 Hz, 2H), 1.85 (t, *J* = 6.8 Hz, 2H), 1.28 (s, 6H). ¹³C NMR (151 MHz, CDCl₃): δ 171.96, 165.66, 158.33, 153.54, 145.43 (q, *J* = 4.1 Hz), 137.51, 137.11 (q, *J* = 3.2 Hz), 130.29, 129.72, 129.60, 127.22, 125.87, 123.73 (q, *J* = 271.5 Hz), 121.83, 121.65 (q, *J* = 33.4 Hz), 121.60, 121.46, 118.01, 111.83, 75.91, 51.27, 46.83, 32.32, 26.99(2C), 22.47. Purity >95% as determined by LC-MS.

Methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (17a)

The title compound was synthesized from methyl 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzylglycinate (**12**, 34 mg, 0.1 mmol) and 2,2-dimethylchroman-6-sulfonyl chloride (65 mg, 0.25 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (45 mg, 0.080 mmol, 80 %) as a white solid. ¹H NMR (CDCl₃, 400 MHz): δ 8.45 (s, 1H), 7.92-7.95 (m, 1H), 7.62-7.65 (m, 2H), 7.34-7.36 (m, 2H), 7.11-7.14 (m, 2H), 7.02 (d, 1H, *J* = 8.8 Hz), 6.90 (d, 1H, *J* = 8.4 Hz), 4.52 (s, 2H), 3.98 (s, 2H), 3.61 (s, 3H), 2.28 (t, 2H, *J* = 6.4 Hz), 1.87 (t, 2H, *J* = 6.8 Hz), 1.30 (s, 6H).

***N*-((2,2-Dimethylchroman-6-yl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (17)**

The title compound was synthesized from methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (**17a**, 42 mg, 0.075 mmol) according to the procedure described for compound **13**. This yielded *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (35 mg, 0.064 mmol, 85 %) as a white solid after lyophilization. mp 193-195 °C. HRMS (ESI+) calculated for C₂₆H₂₆F₃N₂O₆S ([M + H]). 551.1458, found: 551.1456. ¹H NMR (CDCl₃, 400 MHz): δ 8.44 (s, 1H), 7.91 – 7.94 (m, 1H), 7.59 – 7.62 (m, 2H), 7.30 – 7.33 (m, 2H), 7.04 – 7.11 (m, 2H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 4.47 (s, 2H), 3.97 (s, 2H), 2.82 (t, *J* = 6.4 Hz, 2H), 1.85 (t, *J* = 6.8 Hz, 2H), 1.36 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 173.12, 165.66, 158.34, 153.08, 145.49 (q, *J* = 4.3 Hz), 137.08 (q, *J* = 3.1 Hz), 132.38, 130.32(2C), 129.72, 127.21, 123.72 (q, *J* = 272.5 Hz), 121.90 (q, *J* = 33.3 Hz), 121.89(2C), 121.62, 118.03, 111.81, 75.94, 51.06, 46.76, 32.34, 27.00(2C), 22.50. One quaternary carbon signal overlaps and is not reported. Purity >95% as determined by LC-MS.

Methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (18a)

The title compound was synthesized from (3-phenoxybenzyl)glycinate (**9**, 116.5 mg, 0.429 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (83.0 mg, 0.186 mmol, 43 % yield) as an oil. ^1H NMR (CDCl_3 , 400 MHz): δ 7.84 – 7.76 (m, 2H), 7.51 – 7.43 (m, 2H), 7.38 – 7.24 (m, 3H), 7.13 (tt, J = 7.6, 1.2 Hz, 1H), 7.02 – 6.95 (m, 3H), 6.93 (ddd, J = 8.2, 2.5, 1.0 Hz, 1H), 6.85 (t, J = 2.0 Hz, 1H), 4.46 (s, 2H), 3.96 (s, 2H), 3.57 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz): δ 169.08, 157.86, 156.83, 139.41, 138.37, 136.76, 130.33, 129.96(2C), 129.36(2C), 128.98(2C), 123.71, 123.27, 119.12(2C), 118.81, 118.56, 52.28, 51.14, 46.68.

***N*-((4-Chlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycine (18)**

The title compound was synthesized from methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (**18a**, 32.2 mg, 0.07 mmol) according to the procedure described for compound **13**. This yielded *N*-((4-chlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycine (11.7 mg, 0.03 mmol, 38 %) after crystallization from EtOAc/pentane as a white solid. mp 147–149 °C. HRMS (ESI+) m/z : calculated for $\text{C}_{21}\text{H}_{19}\text{ClNO}_5\text{S}$ ($[\text{M} + \text{H}]$), 432.0667, found: 432.0669. ^1H NMR (CDCl_3 , 400 MHz): δ 7.82 – 7.74 (m, 2H), 7.48 – 7.41 (m, 2H), 7.39 – 7.31 (m, 2H), 7.30 – 7.27 (m, 1H), 7.13 (tt, J = 7.2, 1.2 Hz, 1H), 7.01 – 6.94 (m, 3H), 6.92 (dd, J = 8.3, 2.5, 1H), 6.81 (t, J = 2.0 Hz, 1H), 5.44 (bs, 1H), 4.45 (s, 2H), 3.98 (s, 2H). ^{13}C NMR (CDCl_3 , 101 MHz): δ 173.48, 157.97, 156.76, 139.57, 138.17, 136.55, 130.43, 130.00(2C), 129.45(2C), 128.95(2C), 123.80, 123.24, 119.18(2C), 118.70, 118.65, 51.27, 46.59. Purity >95% as determined by LC-MS.

Methyl *N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (19a)

The title compound was synthesized from methyl (3-phenoxybenzyl)glycinate (**9**, 126.1 mg, 0.47 mmol) and 2,6-dichlorobenzenesulfonyl chloride (202.8 mg, 0.83 mmol) according to the procedure described for compound **13a**. This yielded *N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (57.7 mg, 0.12 mmol, 26 %) as an oil. ^1H NMR (CDCl_3 , 400 MHz) δ 7.36 – 7.30 (m, 4H), 7.29 – 7.26 (m, 2H), 7.17 – 7.12 (m, 1H), 6.97 (d, J = 11.2 Hz, 4H), 6.88 (t, J = 2.0 Hz, 1H), 4.45 (s, 2H), 4.18 (s, 2H), 3.74 (s, 3H).

***N*-((2,6-Dichlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycine (19)**

The title compound was synthesized from methyl *N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (**19a**, 23.9 mg, 0.05 mmol) according to the procedure described for compound **13**. This yielded *N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(3-phenoxybenzyl)glycine (14.2 mg, 0.03 mmol, 61 %) as an oil. HRMS (ESI+) m/z : calculated for $\text{C}_{22}\text{H}_{17}\text{Cl}_2\text{NO}_5\text{SNa}$ ($[\text{M} + \text{Na}]$), 488.0097, found: 488.0095. ^1H NMR (CDCl_3 , 400 MHz): δ 7.43 – 7.37 (m, 2H), 7.37 – 7.31 (m, 2H), 7.30 – 7.22 (m, 2H), 7.18 (tt, J = 7.6, 1.6 Hz, 1H), 6.99 – 6.86 (m, 4H), 6.70 (t, J = 2.0 Hz, 1H), 5.07 (bs, 1H), 4.62 (s, 2H), 4.15 (s, 2H). ^{13}C NMR (CDCl_3 , 101 MHz): δ 173.49, 157.91, 156.83, 140.60, 136.56, 135.90(2C), 135.52, 132.79, 131.75(2C), 130.36, 129.99(2C), 123.70, 123.35, 119.16(2C), 118.62, 51.41, 47.47. Purity >95% as determined by LC-MS.

Methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (20a)

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (**10**, 41 mg, 0.15 mmol) and 4-chlorobenzene-1-sulfonyl chloride (80 mg, 0.38 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (62 mg, 0.14 mmol, 93 %) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 7.83 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 8.7 Hz, 2H), 7.38 – 7.31 (m, 2H), 7.21 (d, J = 8.6 Hz, 2H), 7.17 – 7.08 (m, 1H), 7.03 – 6.91 (m, 4H), 4.45 (s, 2H), 3.96 (s, 2H), 3.58 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 169.16, 157.64, 156.80, 139.37, 138.48, 130.30(2C), 129.96(2C), 129.35(2C), 129.13, 129.01(2C), 123.77, 119.27(2C), 118.94(2C), 52.26, 50.87, 46.52.

***N*-((4-Chlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (20)**

The title compound was synthesized from methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (**20a**, 62.1 mg, 0.13 mmol) according to the procedure described for compound **13**. This yielded *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (38 mg, 0.09 mmol, 70 %) as a white solid after lyophilization. mp 188–190 °C. HRMS (ESI+) calculated for C₂₁H₁₈ClNO₅S ([M + H]). 432.0667, found: 432.0662. ¹H NMR (CDCl₃, 400 MHz): δ 7.75 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.28 – 7.30 (m, 2H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.04 – 7.08 (m, 2H), 6.93 (d, *J* = 7.6 Hz, 2H), 6.88 (d, *J* = 8.0 Hz, 2H), 4.38 (s, 2H), 3.93 (s, 2H). ¹³C NMR (CDCl₃, 101 MHz): δ 170.52, 157.30, 156.20, 139.20, 139.16, 130.17(2C), 129.83(2C), 129.19(2C), 128.88(2C), 123.62, 119.11, 118.83(2C), 118.79(2C), 50.60, 46.28. Purity >95% as determined by LC-MS.

***Methyl N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (21a)**

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (**10**, 41 mg, 0.15 mmol) and 2,6-dichlorobenzene-1-sulfonyl chloride (93 mg, 0.37 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (17 mg, 0.036 mmol, 25 %) as a white solid. ¹H NMR (CDCl₃, 400 MHz): δ 7.46 (d, *J* = 8.0 Hz, 2H), 7.32 – 7.38 (m, 3H), 7.10 – 7.18 (m, 3H), 6.99 (d, *J* = 7.6 Hz, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 4.67 (s, 2H), 4.12 (s, 2H), 3.63 (s, 3H). ¹³C NMR (CDCl₃, 101 MHz): δ 174.93, 169.19, 157.57, 156.84, 135.96, 135.89, 132.65, 131.72(2C), 130.32(2C), 129.96(2C), 129.25, 123.75, 119.27(2C), 118.95(2C), 52.38, 51.34, 47.17.

***N*-((2,6-Dichlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (21)**

The title compound was synthesized from methyl *N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (**21a**, 17.1 mg, 0.036 mmol) according to the procedure described for compound **13**. This yielded *N*-((2,6-dichlorophenyl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (15 mg, 0.033 mmol, 92 %) as a white solid after lyophilization. mp 140–142 °C. HRMS (ESI+) calculated for C₂₁H₁₈Cl₂NO₅S ([M + H]). 466.0277, found: 466.0274. ¹H NMR (CDCl₃, 400 MHz): δ 7.50 (d, *J* = 8.0 Hz, 2H), 7.35 – 7.39 (m, 3H), 7.15 – 7.17 (m, 3H), 7.02 (d, *J* = 7.6 Hz, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 4.67 (s, 2H), 4.20 (s, 2H). ¹³C NMR (CDCl₃, 101 MHz): δ 175.08, 157.61, 156.64, 135.81, 135.61, 132.71, 131.69(2C), 130.21(2C), 129.87(2C), 128.76(2C), 123.72, 119.23(2C), 118.84(2C), 51.12, 46.20. Purity >95% as determined by LC-MS.

***Methyl N*-(4-chlorobenzoyl)-*N*-(4-phenoxybenzyl)glycinate (22a)**

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (**10**, 33 mg, 0.12 mmol) and 4-chlorobenzoyl chloride (53 mg, 0.30 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-(4-chlorobenzoyl)-*N*-(4-phenoxybenzyl)glycinate (18 mg, 0.04 mmol, 33 %) as a white solid. ¹H NMR (CDCl₃, 400 MHz): δ 7.27–7.48 (m, 7H), 7.12–7.14 (m, 2H), 6.97–7.02 (m, 4H), 4.75 (s, 0.7H), 4.57 (s, 1.3H), 4.15 (s, 1.3H), 3.86 (s, 0.7H), 3.77 (s, 2.1H), 3.71 (s, 0.9H). Signals split (7:3) due to rotamers.

***N*-(4-Chlorobenzoyl)-*N*-(4-phenoxybenzyl)glycine (22)**

The title compound was synthesized from methyl *N*-(4-chlorobenzoyl)-*N*-(4-phenoxybenzyl)glycinate (**22a**, 15.8 mg, 0.039 mmol) according to the procedure described for compound **13**. This yielded *N*-(4-chlorobenzoyl)-*N*-(4-phenoxybenzyl)glycine (12 mg, 0.031 mmol, 80 % yield) as a white solid after lyophilization. mp 225–227 °C. HRMS (ESI+) calculated for C₂₂H₁₉ClNO₄ ([M + H]). 396.0997, found: 396.0991. ¹H NMR (CDCl₃, 400 MHz): δ 7.28 – 7.33 (m, 7H), 7.06 – 7.08 (m, 2H), 6.92 – 6.95 (m, 4H), 4.69 (s, 0.6H), 4.52 (s, 1.4H), 4.12 (s, 1.4H), 3.83 (s, 0.6H). ¹³C NMR (CDCl₃, 101 MHz): δ 172.11, 157.59, 157.32, 156.76, 136.72, 133.16, 131.71, 130.35(2C), 129.87(2C), 128.86(2C), 128.30(2C), 124.93(2C), 119.35, 116.03(2C), 53.63, 46.64. Signals split (7:3) due to rotamers. Purity >95% as determined by LC-MS.

Methyl *N*-(2,6-dichlorobenzoyl)-*N*-(4-phenoxybenzyl)glycinate (23a)

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (**10**, 33 mg, 0.12 mmol) and 2,6-dichlorobenzoyl chloride (63 mg, 0.30 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-(2,6-dichlorobenzoyl)-*N*-(4-phenoxybenzyl)glycinate (50 mg, 0.11 mmol, 92 %) as a white solid, which was directly converted to the corresponding acid **17**. ^1H NMR (CDCl_3 , 400 MHz): δ 7.13-7.25 (m, 7H), 7.05-7.07 (m, 1H), 6.86-6.95 (m, 4H), 4.97 (s, 0.5H), 4.46 (s, 1.5H), 4.19 (s, 1.5H), 3.76 (s, 0.5H), 3.75 (s, 2.1H), 3.68 (s, 0.9H). Signals split (3:1) due to rotamers.

***N*-(2,6-Dichlorobenzoyl)-*N*-(4-phenoxybenzyl)glycine (23)**

The title compound was synthesized from methyl *N*-(2,6-dichlorobenzoyl)-*N*-(4-phenoxybenzyl)glycinate (**23a**, 57.4 mg, 0.129 mmol) according to the procedure described for compound **13**. This yielded *N*-(2,6-dichlorobenzoyl)-*N*-(4-phenoxybenzyl)glycine (25 mg, 0.058 mmol, 45%) as an oily white solid after lyophilization. mp not determined. HRMS (ESI+) calculated for $\text{C}_{22}\text{H}_{18}\text{Cl}_2\text{NO}_4$ ([M + H]). 430.0607, found: 430.0607. ^1H NMR (CDCl_3 , 400 MHz) δ 7.42 – 7.30 (m, 5H), 7.30 – 7.19 (m, 2H), 7.16 – 7.09 (m, 1H), 7.09 – 6.92 (m, 4H), 4.98 (s, 0.5H), 4.46 (s, 1.5H), 4.23 (s, 1.5H), 3.80 (s, 0.5H). ^{13}C NMR (101 MHz, CDCl_3) δ 175.11, 166.03, 157.77, 156.69, 134.28, 132.36(1.5C), 132.19(0.5C), 131.00(0.7C), 130.70(0.3C), 130.22(2C), 130.00(1.5C), 129.96(0.5C), 128.65, 128.54(0.5C), 128.44(1.5C), 123.88(0.7C), 123.67(0.3C), 119.42(1.5C), 119.27(0.5C), 118.99(2C), 52.48, 47.72(0.7C), 44.93(0.3C). Signals split (3:1) due to rotamers. Purity >95% as determined by LC-MS.

Methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (24a)

The title compound was synthesized from methyl (4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (**12**, 41 mg, 0.12 mmol) and 4-chlorobenzene-1-sulfonyl chloride (63 mg, 0.3 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (22 mg, 0.04 mmol, 36 %) as a white solid. ^1H NMR (CDCl_3 , 400 MHz): δ 8.41-8.44 (m, 1H), 7.90-7.93 (m, 1H), 7.82-7.85 (m, 2H), 7.50-7.52 (m, 2H), 7.33 (d, 2H, J = 8.4 Hz), 7.11-7.14 (m, 2H), 7.03 (d, 1H, J = 8.4 Hz), 4.53 (s, 2H), 4.14(s, 2H), 3.62 (s, 3H).

***N*-((4-Chlorophenyl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (24)**

The title compound was synthesized from methyl *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (**24a**, 16 mg, 0.031 mmol) according to the procedure described for compound **13**. This yielded *N*-((4-chlorophenyl)sulfonyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (14 mg, 0.027 mmol, 88 %) as a white solid after lyophilization. mp 150-152 °C. HRMS (ESI+) calculated for $\text{C}_{21}\text{H}_{17}\text{ClF}_3\text{N}_2\text{O}_5\text{S}$ ([M + H]). 501.0493, found: 501.0491. ^1H NMR (CDCl_3 , 850 MHz) δ 8.46 (s, 1H), 7.93 (dd, J = 8.7, 2.5 Hz, 1H), 7.86 – 7.77 (m, 2H), 7.54 – 7.48 (m, 2H), 7.37 – 7.31 (m, 2H), 7.15 – 7.10 (m, 2H), 7.06 (d, J = 8.7 Hz, 1H), 4.51 (s, 2H), 4.00 (s, 2H). ^{13}C NMR (CDCl_3 , 214 MHz) δ 171.55, 165.60, 153.18, 145.44 (q, J = 4.3 Hz), 139.51, 138.37, 137.16 (q, J = 3.0 Hz), 131.92, 130.31(2C), 129.41(2C), 129.04(2C), 123.71 (q, J = 271.4 Hz), 122.02(2C), 121.97 (q, J = 33.5 Hz), 111.93, 50.84, 46.21. Purity >95% as determined by LC-MS.

Methyl *N*-(4-chlorobenzoyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (25a)

The title compound was synthesized from methyl (4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (**12**, 41 mg, 0.12 mmol) and 4-chlorobenzoyl chloride (53 mg, 0.3 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-(4-chlorobenzoyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (48 mg, 0.10 mmol, 83 %) as a white solid. ^1H NMR (CDCl_3 , 400 MHz): δ 8.36 (s, 1H), 7.84-7.86 (m, 1H), 7.38-7.46 (m, 5H), 7.20 (d, 2H, J = 8.4 Hz), 7.10 (d, 2H, J = 8.0 Hz), 6.98 (d, 1H, J = 8.8 Hz), 4.73 (s, 0.7H), 4.57 (s, 1.3H), 4.12 (s, 1.3H), 3.84 (s, 0.7H), 3.71 (s, 2.1H), 3.65 (s, 0.9H). Signals split (7:3) due to rotamers.

***N*-(4-Chlorobenzoyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (25)**

The title compound was synthesized from methyl *N*-(4-chlorobenzoyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (**25a**, 47.8 mg, 0.1 mmol) according to the procedure described for compound **13**. This yielded *N*-(4-chlorobenzoyl)-*N*-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (13 mg, 0.03 mmol, 33 %) as a white solid after lyophilization. mp 202–204 °C. HRMS (ESI+) calculated for C₂₂H₁₇ClF₃N₂O₄ ([M + H]). 465.0824, found: 465.0821. ¹H NMR (MeOD, 850 MHz) δ 8.42 (s, 1H), 8.13 – 8.05 (m, 1H), 7.56 – 7.42 (m, 5H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.20 – 7.16 (m, 2H), 7.14 (t, *J* = 8.5 Hz, 1H), 4.82 (s, 1H), 4.64 (s, 1H), 4.16 (s, 1H), 3.86 (s, 1H). ¹³C NMR (MeOD, 214 MHz) δ 173.62(0.5C), 173.56(0.5C), 167.33(0.5C), 167.22(0.5C), 154.26(0.5C), 154.17(0.5C), 146.28 – 146.05 (m, 1C), 138.40 – 138.64 (m, 1C), 137.25(0.5C), 137.07(0.5C), 135.68, 135.28(0.5C), 135.18(0.5C), 134.63, 131.00(2C), 129.95, 129.92, 129.59, 129.46, 125.19 (q, 0.5C, *J* = 271.8 Hz), 125.00 (q, 0.5C, *J* = 269.6 Hz), 123.10, 122.84, 122.93 (q, 0.5C, *J* = 34.2 Hz), 122.77 (q, 0.5C, *J* = 32.1 Hz), 112.88(0.5C), 112.77(0.5C), 54.51, 50.10. Signals split (1:1) due to rotamers. Purity >95% as determined by LC-MS.

Methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (26)

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (**10**, 50 mg, 0.18 mmol) according to the procedure described for compound **13a**. This yielded methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycinate (84 mg, 17 mmol, 92%) as a white solid. mp 86–88 °C. HRMS (ESI+) calculated for C₂₇H₃₀NO₆S ([M + H]), 496.1788, found: 496.1788. ¹H NMR (400 MHz, CDCl₃): δ 7.59 (d, *J* = 9.3 Hz, 2H), 7.33 (t, *J* = 7.9 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.11 (t, *J* = 7.4 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 2H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 1H), 4.44 (s, 2H), 3.92 (s, 2H), 3.57 (s, 3H), 2.82 (t, *J* = 6.6 Hz, 2H), 1.84 (t, *J* = 6.7 Hz, 2H), 1.36 (s, 6H); ¹³C NMR (101 MHz, CDCl₃): δ 169.46, 158.08, 157.32, 156.92, 130.28(2C), 130.08, 129.88(2C), 129.84, 129.58, 127.10, 123.60, 121.42, 119.12(2C), 118.84(2C), 117.89, 75.78, 52.04, 50.95, 46.78, 32.33, 26.95(2C), 22.46.

***N*-(2-Hydroxyethyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (27)**

To a stirred solution of methyl *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(3-phenoxybenzyl)glycinate (**14a**, 34.4 mg, 0.07 mmol) in THF (2 mL) at -5 °C was added LiBH₄ (0.1 mL, 2M in THF). After completion (4 h), saturated KHCO₃ was carefully added at -5 °C to quench the reaction. The mixture was slowly warmed up to rt, diluted with EtOAc, washed with brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield *N*-(2-hydroxyethyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (21.1 mg, 0.05 mmol, 65 %) as a white solid. mp 148–150 °C. HRMS (ESI+) *m/z*: calculated for C₂₆H₃₀NO₅S ([M + H]), 468.1839, found: 468.1839. ¹H NMR (CDCl₃, 400 MHz): δ 7.58 – 7.50 (m, 2H), 7.38 – 7.27 (m, 3H), 7.14 – 7.03 (m, 2H), 6.99 – 6.94 (m, 2H), 6.93 – 6.88 (m, 2H), 6.84 (d, *J* = 8.5 Hz, 1H), 4.32 (s, 2H), 3.53 (t, *J* = 5.4 Hz, 2H), 3.23 (t, *J* = 5.4 Hz, 2H), 2.79 (t, *J* = 6.7 Hz, 2H), 2.18 (bs, 1H), 1.83 (t, *J* = 6.7 Hz, 2H), 1.35 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 158.25, 157.75, 157.01, 138.74, 130.25, 129.94(2C), 129.50, 129.12, 126.97, 123.62, 123.15, 121.77, 119.05(2C), 118.72, 118.33, 118.17, 75.93, 61.26, 53.45, 51.07, 32.31, 27.03(2C), 22.50. Purity >95% as determined by LC-MS.

2-((2,2-Dimethyl-*N*-(3-phenoxybenzyl)chromane)-6-sulfonamido)ethyl piperidine-1-carboxylate (28)

To a stirred solution of *N*-(2-hydroxyethyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (**27**, 8.1 mg, 0.02 mmol) in CH₂Cl₂ (2 mL) at 0 °C was added DIPEA (3 μL, 0.017 mmol) and di(1H-imidazol-1-yl)methanone (6.6 mg, 0.04 mmol). After 10 minutes was added piperidine (6 μL, 0.06 mmol) and the mixture was stirred for 30 mins and allowed to warm up to rt. After completion (1 h), the mixture was diluted with EtOAc, washed with brine, dried, concentrated *in vacuo* to yield 2-((2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane)-6-sulfonamido)ethyl piperidine-1-carboxylate (10.0 mg, 0.02 mmol, 100 %) as a white solid after lyophilization. mp not determined. HRMS (ESI+) *m/z*: calculated for C₃₂H₃₉N₂O₆S ([M + H]),

579.2523, found: 579.2523. ^1H NMR (CDCl_3 , 400 MHz): δ 7.56 – 7.49 (m, 2H), 7.37 – 7.30 (m, 2H), 7.25 – 7.15 (m, 1H), 7.10 (tt, J = 7.2, 1.2 Hz, 1H), 7.04 – 6.94 (m, 3H), 6.91 – 6.85 (m, 2H), 6.82 (d, J = 8.5 Hz, 1H), 4.34 (s, 2H), 4.04 (t, J = 5.9 Hz, 2H), 3.41 (t, J = 5.9 Hz, 2H), 3.33 (bs, 4H), 2.79 (t, J = 6.7 Hz, 2H), 1.82 (t, J = 6.7 Hz, 2H), 1.55 – 1.44 (bs, 6H), 1.35 (s, 6H). ^{13}C NMR (CDCl_3 , 101 MHz): δ 157.53, 157.14, 156.52, 154.42, 138.73, 130.19, 130.02, 129.90(2C), 129.33, 126.82, 123.46, 123.02, 121.65, 118.94(2C), 118.71, 118.17, 118.08, 75.83, 62.77, 60.56, 52.35, 47.24, 44.90, 32.35, 27.02(2C), 24.46(2C), 22.49, 21.23. Purity >95% as determined by LC-MS.

Tert-butyl (2-(2-((2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamido)ethoxy)ethyl carbamate (29a)

To a stirred solution of *N*-((2,2-dimethylchroman-6-yl)sulfonyl)-*N*-(4-phenoxybenzyl)glycine (**15**, 27.2 mg, 0.056 mmol) and DIPEA (30 μL , 0.172 mmol) in MeCN (20 mL) was added EDC (12.0 mg, 0.063 mmol) at rt. After 30 min tert-butyl (2-(2-aminoethoxy)ethyl)carbamate (13.6 mg, 0.067 mmol) was added and the reaction mixture was stirred for 10 h. After full completion the mixture was concentrated *in vacuo* and purified by flash chromatography to yield tert-butyl (2-(2-((2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamido)ethoxy)ethyl carbamate (8.0 mg, 0.012 mmol, 21% yield) as an oil. ^1H NMR (CDCl_3 , 400 MHz) δ 7.60 – 7.52 (m, 2H), 7.39 – 7.29 (m, 2H), 7.25 – 7.17 (m, 2H), 7.17 – 7.05 (m, 1H), 7.02 – 6.95 (m, 2H), 6.95 – 6.86 (m, 3H), 6.77 (t, J = 5.7 Hz, 1H), 5.25 (bs, 1H), 4.28 (s, 2H), 3.66 (s, 2H), 3.50 (t, J = 5.1 Hz, 2H), 3.39 (t, J = 5.3 Hz, 2H), 3.36 – 3.25 (m, 4H), 2.82 (t, J = 6.7 Hz, 2H), 1.85 (t, J = 6.7 Hz, 2H), 1.41 (s, 9H), 1.37 (s, 6H).

***N*-(2-(2-Aminoethoxy)ethyl)-2-((2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (29)**

To a stirred solution of tert-butyl (2-(2-((2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamido)ethoxy)ethyl carbamate (**29a**, 6.0 mg, 8.98 μmol) in CH_2Cl_2 (2.0 mL) was added TFA (1 mL) at rt. After full completion (30 min) the mixture was concentrated *in vacuo* and purified by flash chromatography to yield *N*-(2-(2-aminoethoxy)ethyl)-2-((2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (4.9 mg, 8.63 μmol , 96 % yield) as white solid needles. mp 265–267 °C. HRMS (ESI+) m/z : calculated for $\text{C}_{30}\text{H}_{38}\text{N}_3\text{O}_6\text{S}$ ([$\text{M} + \text{H}$]), 568.2476, found: 568.2475. ^1H NMR (MeOD, 400 MHz): δ 7.66 – 7.54 (m, 2H), 7.40 – 7.31 (m, 2H), 7.29 – 7.21 (m, 2H), 7.16 – 7.09 (m, 1H), 7.01 – 6.93 (m, 2H), 6.93 – 6.82 (m, 3H), 4.35 (s, 2H), 3.73 (s, 2H), 3.66 (t, J = 4.8 Hz, 2H), 3.48 (t, J = 5.4 Hz, 2H), 3.29 (t, J = 5.6, 2H), 3.12 (t, J = 4.8 Hz, 2H), 2.87 (t, J = 6.9 Hz, 2H), 1.87 (t, J = 6.7 Hz, 2H), 1.36 (s, 6H). ^{13}C NMR (MeOD, 101 MHz): δ 171.40, 163.07, 159.74, 158.81, 131.96(2C), 131.44, 130.97(2C), 130.76, 130.00, 128.23, 124.72, 123.40, 120.05(2C), 119.66(2C), 118.95, 77.06, 70.71, 67.56, 54.00, 51.43, 40.62, 39.91, 33.16, 27.09(2C), 23.27. Purity 93% as determined by LC-MS.

2-((3-Phenoxybenzyl)amino)acetamide (30a)

The title compound was synthesized from 3-phenoxybenzaldehyde (**4**, 250 μL , 1.45 mmol) and 2-aminoacetamide (243 mg, 2.20 mmol) according to the procedure described for compound **8**. This yielded 2-((3-phenoxybenzyl)amino)acetamide (184.7 mg, 0.72 mmol, 50 %) as a white solid. ^1H NMR (MeOD, 400 MHz): δ 7.42 – 7.25 (m, 3H), 7.17 – 7.07 (m, 2H), 7.08 – 7.02 (m, 1H), 7.02 – 6.95 (m, 2H), 6.92 (dd, J = 8.2, 1.9 Hz, 1H), 3.85 (s, 2H), 3.37 (s, 1.4H), 3.35 (s, 0.6H). ^{13}C NMR (MeOD, 101 MHz): δ 174.67, 159.10, 158.50, 140.69, 131.14, 130.93(2C), 124.68, 124.54, 120.02, 119.97(2C), 119.11, 53.29, 50.65. Signals split (7:3) due to rotamers.

2-((2,2-Dimethyl-*N*-(3-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (30)

The title compound was synthesized from 2-((3-phenoxybenzyl)amino)acetamide (**30a**, 184 mg, 0.72 mmol) according to the procedure described for compound **13a**. This yielded 2-((2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (144 mg, 0.30 mmol, 42 %) as a white solid. mp 122–124 °C. HRMS (ESI+) m/z : calculated for $\text{C}_{26}\text{H}_{29}\text{N}_2\text{O}_5\text{S}$ ([$\text{M} + \text{H}$]), 481.1792, found: 481.1785. ^1H NMR (CDCl_3 , 400 MHz): δ 7.60 – 7.48 (m, 2H), 7.41 – 7.20 (m, 3H), 7.15 – 7.08 (m, 1H), 7.04 – 6.91 (m, 4H), 6.90 – 6.82 (m, 2H), 6.37 (s, 1H), 5.50 (s, 1H), 4.29 (s, 2H), 3.63 (s, 2H), 2.81 (t, J = 6.7 Hz, 2H), 1.84 (t, J = 6.7 Hz, 2H), 1.36 (s, 6H). ^{13}C

NMR (CDCl₃, 101 MHz): δ 171.07, 158.72, 157.83, 156.91, 136.92, 130.37, 129.93(2C), 129.72, 127.58, 127.17, 123.82, 123.67, 122.01, 119.30, 119.09(2C), 118.85, 118.35, 76.10, 54.04, 51.43, 32.19, 27.00(2C), 22.46. Purity >95% as determined by LC-MS.

2-((4-Phenoxybenzyl)amino)acetamide (**31a**)

The title compound was synthesized from 4-phenoxybenzaldehyde (**5**, 300 μ L, 1.71 mmol) and 2-aminoacetamide (226 mg, 2.06 mmol) according to the procedure described for compound **8**. This yielded 2-((4-phenoxybenzyl)amino)acetamide (300 mg, 1.17 mmol, 68 %) as a white solid. ¹H NMR (MeOD, 400 MHz): δ 7.35 – 7.24 (m, 4H), 7.04 (t, J = 7.4 Hz, 1H), 6.94 – 6.85 (m, 4H), 3.68 (s, 1.2H), 3.58 (0.8H), 3.20 (s, 2H). ¹³C NMR (MeOD, 101 MHz): δ 176.70, 158.70, 158.08(0.6C), 157.92(0.4C), 135.41(0.6C), 134.27(0.4C), 131.72, 131.09(2C), 130.88(0.6C), 130.86(0.4C), 124.42(0.6C), 124.37(0.4C), 119.86, 119.80, 119.75, 119.69, 59.22(0.6C), 57.57(0.4C), 53.47(0.6C), 51.39(0.4C). Signals split (3:2) due to rotamers.

2-((2,2-Dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (**31**)

The title compound was synthesized from 2-((4-phenoxybenzyl)amino)acetamide (**31a**, 200 mg, 0.780 mmol) according to the procedure described for compound **13a**. This yielded 2-((2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (237.0 mg, 0.493 mmol, 63 % yield) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.59 (d, J = 1.6 Hz, 1H), 7.52 (dd, J = 8.6, 2.1 Hz, 1H), 7.45 – 7.29 (m, 3H), 7.25 – 7.22 (m, 1H), 7.20 (s, 1H), 7.17 – 7.09 (m, 1H), 7.05 (s, 1H), 7.02 – 6.96 (m, 2H), 6.95 – 6.90 (m, 2H), 6.84 (d, J = 8.6 Hz, 1H), 4.34 (s, 2H), 3.66 (s, 2H), 2.78 (t, J = 6.7 Hz, 2H), 1.79 (t, J = 6.7 Hz, 2H), 1.30 (s, 6H).

N-(Cyanomethyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (**32**)

To a stirred solution of 2-((2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (**30**, 46.4 mg, 0.10 mmol) in THF (5 mL) at 0 °C was added pyridine (0.05 mL, 0.62 mmol) and then TFAA (0.04 mL, 0.28 mmol). The reaction was warmed up to rt and stirred for 2h until completion. The mixture was then diluted with EtOAc, washed with water and brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield *N*-(cyanomethyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (30.4 mg, 0.07 mmol, 69 %) as a white solid after lyophilization. mp 90-92 °C. HRMS (ESI+) m/z : calculated for C₂₆H₂₆N₂O₄SNa ([M + Na]), 485.1505, found: 485.1504. ¹H NMR (CDCl₃, 400 MHz): δ 7.66 – 7.52 (m, 2H), 7.42 – 7.28 (m, 3H), 7.20 – 7.16 (m, 1H), 7.14 (tt, J = 7.2, 1.2 Hz, 1H), 7.10 – 7.06 (m, 1H), 7.02 – 6.93 (m, 3H), 6.91 (d, J = 8.6 Hz, 1H), 4.28 (s, 2H), 4.06 (s, 2H), 2.85 (t, J = 6.8 Hz, 2H), 1.85 (t, J = 6.7 Hz, 2H), 1.36 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 159.10, 158.16, 156.69, 135.74, 130.56, 130.02(2C), 129.91, 127.37, 127.02, 123.87, 123.39, 122.22, 119.25(2C), 118.93, 118.85, 118.51, 113.47, 76.17, 50.94, 34.62, 32.23, 27.00(2C), 22.49. Purity >95% as determined by LC-MS.

N-(Cyanomethyl)-2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane-6-sulfonamide (**33a**)

The title compound was synthesized from 2-((2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (**31**, 86.2 mg, 0.179 mmol) according to the procedure described for compound **32**. This yielded *N*-(cyanomethyl)-2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane-6-sulfonamide (51.3 mg, 0.111 mmol, 62 %) as an oil. ¹H NMR (CDCl₃, 400 MHz): δ 7.63 (d, J = 2.3 Hz, 1H), 7.61 (dd, J = 8.6, 2.4 Hz, 1H), 7.40 – 7.32 (m, 2H), 7.32 – 7.27 (m, 2H), 7.18 – 7.10 (m, 1H), 7.06 – 6.95 (m, 4H), 6.93 (d, J = 8.5 Hz, 1H), 4.28 (s, 2H), 4.05 (s, 2H), 2.86 (t, J = 6.7 Hz, 2H), 1.86 (t, J = 6.7 Hz, 2H), 1.37 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 159.08, 158.08, 156.64, 130.52(2C), 130.02(2C), 129.91, 128.02, 127.37, 127.15, 123.94, 122.23, 119.45(2C), 119.04(2C), 118.51, 113.54, 76.17, 50.61, 34.46, 32.27, 27.01(2C), 22.51.

***N*-(2-Aminoethyl)-2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane-6-sulfonamide (33)**

To a stirred solution of *N*-(cyanomethyl)-2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane-6-sulfonamide (**33a**, 21.3 mg, 0.046 mmol) in THF (5 mL) was added borane THF complex (0.092 mL, 0.092 mmol) at 0 °C. The reaction mixture was heated for 10 h. at reflux. After full completion the mixture was quenched with 5 mL MeOH followed by 1M HCl (10 mL) and the reaction was refluxed for 2h, diluted with EtOAc, washed with NaHCO₃, brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield *N*-(2-aminoethyl)-2,2-dimethyl-*N*-(4-phenoxybenzyl)chromane-6-sulfonamide (11.6 mg, 0.025 mmol, 54 %) as a white solid after lyophilization. mp not determined. HRMS (ESI+) *m/z*: calculated for C₂₆H₃₁N₂O₄S ([M + H]), 467.1999, found: 467.1998. ¹H NMR (CDCl₃, 400 MHz): δ 7.58 (s, 1H), 7.56 – 7.47 (m, 1H), 7.36 – 7.28 (m, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 7.09 (t, *J* = 7.4 Hz, 1H), 7.03 – 6.94 (m, 2H), 6.87 (d, *J* = 8.1 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 1H), 4.27 (s, 2H), 3.41 (m, 2H), 2.96 (m, 2H), 2.79 (t, *J* = 6.3 Hz, 3H), 1.79 (t, *J* = 6.6 Hz, 3H), 1.33 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 159.75, 159.00, 158.17, 132.12, 131.63(2C), 131.00(2C), 130.62, 129.86, 128.06, 124.84, 123.51, 120.17(2C), 119.74(2C), 119.06, 77.07, 54.39, 47.81, 40.11, 33.14, 27.09(2C), 23.28. Purity >95% as determined by LC-MS.

***N*-((1*H*-Tetrazol-5-yl)methyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (34)**

To a stirred solution of *N*-(cyanomethyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (**32**, 57.4 mg, 0.12 mmol) in toluene (10 mL) was added Et₃N.HCl (51.4 mg, 0.37 mmol) and sodium azide (22.4 mg, 0.35 mmol). The reaction mixture was stirred for 120 h. at reflux. After full completion, the organic layer was washed with brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield *N*-((1*H*-tetrazol-5-yl)methyl)-2,2-dimethyl-*N*-(3-phenoxybenzyl)chromane-6-sulfonamide (10.2 mg, 0.02 mmol, 16 %) as a white solid after lyophilization. mp 53-55 °C. HRMS (ESI+) *m/z*: calculated for C₂₆H₂₈N₅O₄S ([M + H]), 506.1857, found: 506.1855. ¹H NMR (CDCl₃, 400 MHz): δ 8.01 (bs, 1H), 7.59 – 7.48 (m, 2H), 7.34 (t, *J* = 7.8 Hz, 2H), 7.20 (t, *J* = 7.9 Hz, 1H), 7.12 (t, *J* = 7.3 Hz, 1H), 6.97 – 6.82 (m, 5H), 6.82 – 6.74 (m, 1H), 4.52 (s, 2H), 4.35 (s, 2H), 2.80 (t, *J* = 5.9 Hz, 2H), 1.84 (t, *J* = 6.0 Hz, 2H), 1.36 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 159.08, 157.96, 156.72, 152.89, 136.51, 130.41, 130.01(2C), 129.66, 127.34, 127.12, 123.76, 123.29, 122.28, 119.20(2C), 118.76, 118.66, 118.60, 76.31, 52.79, 40.82, 32.16, 27.03(2C), 22.48. Purity >95% as determined by LC-MS.

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