Universiteit Leiden
The Netherlands

Inhibitor selectivity: profiling and prediction

Janssen, A.P.A.

Citation

Janssen, A. P. A. (2019, May 1). *Inhibitor selectivity: profiling and prediction*. Retrieved from https://hdl.handle.net/1887/71808

Version:	Not Applicable (or Unknown)
License:	Leiden University Non-exclusive license
Downloaded from:	https://hdl.handle.net/1887/71808

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

Leiden University Repository

The following handle holds various files of this Leiden University dissertation:
http://hdl.handle.net/1887/71808

Author: Janssen, A.P.A.
Title: Inhibitor selectivity: profiling and prediction
Issue Date: 2019-05-01

I'd far rather be happy than right any day.
Douglas Adams

3 Towards drug-like peripherally restricted DAGL-inhibitors

Introduction

The signalling of 2-arachidonoylglycerol (2-AG) via the endocannabinoid system is implicated in a wide range of physiological effects, such as neurotransmission, inflammation and energy metabolism.[1,2,3] The diacylglycerol lipase (DAGL) inhibitors, introduced in Chapter 2, have reasonable physicochemical properties and showed promising activity to study the role of DAGL in 2-AG signalling *in vivo*. They lacked, however, subtype selectivity over DAGL-α and DAGL-β, and also inhibited α/β-hydrolase domain containing protein 6 (ABHD6).[4] Despite studies using genetic knock-out mice, the role and importance of the two distinct DAGL isoforms is still poorly understood, and should ideally

be studied with isoform-selective inhibitors.[1,5,6] Since there is no structural data of these enzymes available, the search for such selective inhibitors is challenging. The spatial distribution of the isoforms in the body does point towards an alternative way to obtain largely selective modulation of DAGL activity: DAGL-α is predominantly expressed in the central nervous system (CNS), whereas DAGL-β is more highly expressed in the periphery.[7] By employing the drug design principles that do not allow drugs to pass the blood-brain barrier, compounds could in principle be restricted to the periphery, making them spatially selective towards DAGL-β. By limiting the brain penetration, unwanted CNS-side effects such as those observed for the retracted drug Rimonabant could be reduced or even prevented completely.[8]

It is generally accepted that the topological polar surface area (TPSA), together with the lipophilicity, expressed as (C)LogP, are the best predictors of brain penetration.[9] Hitchcock and Pennington described an optimal range for the lipophilicity where the LogP is between 2 and 4 and the limit for the polar surface area is below 90 Å^2. Further requirements include a maximum of three hydrogen bond donors. Compounds that do not meet these criteria are less likely to penetrate the brain.

The work presented here builds on the findings of Chapter 2 to work towards more drug-like and peripherally restricted compounds. Ultimately, these inhibitors could aid in the elucidation of the different physiological roles the DAG lipases play in human health and disease.

Structure activity relationship study on 4-substituted piperazine derivatives

During the hit optimization, which led to the discovery of *in vivo* active compound **1** (Figure 3.1, Chapter 2), a similarity in the structure activity relationships (SAR) with other triazole urea inhibitors was observed. The activity of DAGL inhibitor DO34 (Figure 3.1) demonstrated that there is ample room for variation in the 2-benzylpiperidine moiety[10], therefore it was envisioned that optimization of this part of **1** could be exploited to increase the TPSA. First, the substituted piperazine featured in DO34 was resynthesized in a 7-step procedure from commercially available *N*-Boc-phenylalanine and *N*-benzylglycine (Scheme S3.1), and was subsequently coupled to the two most promising scaffolds of Chapter 2, i.e. the 1,2,4-triazoles substituted with either an *N*-methylaniline-sulfonamide (**2**) or a benzyl-sulfone (**3**) (Table 3.1).

The sulfone analogue **3** showed comparable activity to **2** for DAGL-α and -β in the previously published surrogate substrate assay employing para-nitrophenol butyrate (PNPB) (Table 3.1).[11] In view of its synthetic accessibility, the sulfone scaffold was selected

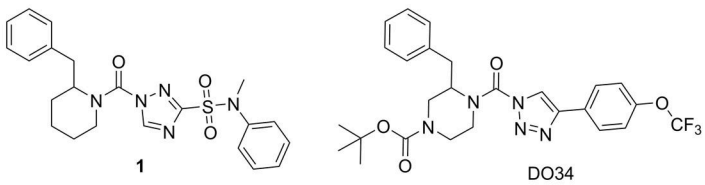
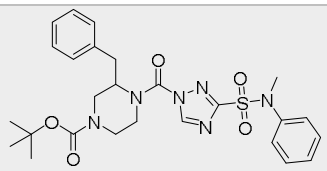
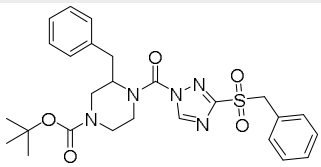


Figure 3.1 | Triazole urea compound **1**, presented in Chapter 2, and DO34, a published DAGL inhibitor featuring a 4-substituted piperazine.

Table 3.1 | Matched molecular pair analysis to validate the sulfone as synthon for a DAGL inhibitor library.

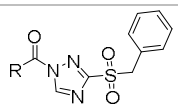
#	Structure	PNPB pIC_{50} DAGL-α	ABPP pIC_{50} DAGL-α	PNPB pIC_{50} DAGL-β	CLogP	TPSA (Å^2)	LipE DAGL-α
2		8.97 ± 0.08	8.62 ± 0.13	8.25 ± 0.07	4.63	115.2	4.34
3		8.71 ± 0.06	7.88 ± 0.14	8.40 ± 0.07	4.97	112.0	3.74

to quickly generate SAR by varying the 4-piperazine substitutions in a matched molecular pair analysis. A small library was synthesized (Scheme S3.1) starting from the two 1-protected piperazine intermediates, which were synthesized according to Scheme S3.2. The 4-position was modified by 1-step reactions to generate the desired substitution. The resulting piperazines were deprotected and coupled to 3-(benzylsulfonyl)-1*H*-1,2,4-triazole, which is available in two high-yielding steps from commercial building blocks. The resulting library was tested in both the PNPB-assay for DAGL-α and DAGL-β activity and in a competitive activity-based protein profiling (ABPP) assay using MB064 as the activity-based probe (Table 3.2).

First, several amide variations were made with substituents increasing in size and lipophilicity from an acetyl (**4**), via cyclic and bulky alkyl substituents (**5** and **6**) to a benzoyl substituent (**7**). The potencies of the amides were all lower than *tert*-butyl carbamate containing compound **3**. The largest potency reduction (200-fold) was observed for the acetyl substitution in **4**. The more lipophilic substituents (**5**-**7**) retained most of their activity, with about a 10-fold reduced potency. The keto-piperazine analogue **8** showed a 10-fold drop as compared to the highly similar benzoyl substituted compound **7**. Three related urea moieties were introduced: unsubstituted (**9**), ethyl substituted (**10**) and a Boc-like *tert*-butyl urea (**11**). These followed the same trend in activity with the more substituted and lipophilic substituents showing higher inhibitory activity.

The introduction of a small alkyl substituent, generating a basic tertiary amine, did not dramatically reduce potency for the DAGLs (**12**-**13**). The calculated LipE for **13** (3.97) was the highest observed thus far, and was slightly better when using the (calculated) LogD for lipophilicity. As with the amide and urea substitutions, increasing the bulk and lipophilicity of the substituent increased the potency, reaching single digit nanomolar IC_{50}s for all variations (**14**-**18**). However, the TPSA of the most potent compounds (**13**-**18**) was actually reduced compared to the Boc-substituted **3**, and was below the 90 Å^2. Therefore, sulfonamide substitutions **19**-**22** were designed to incorporate another highly polar functionality in the scaffold. Again, a small substituent showed a large decrease in activity (**19**), but potency could be recovered by the introduction of an isopropyl (**20**) or phenyl substituent (**21**, **22**). The sulfonamide substitution increased the TPSA to 120 Å^2, making it unlikely these compounds will be centrally active. The low CLogP combined with rather high potency for DAGL-α resulted in a LipE for **20** of 4.13, slightly higher than amine **13**.

Table 3.2 | Piperazine variations of the 1,2,4-triazole-sulfone scaffold.



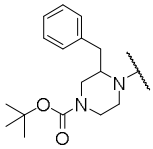
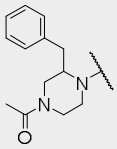
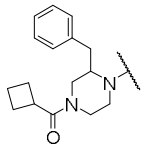
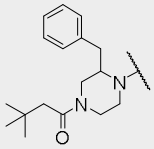
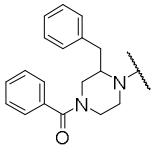
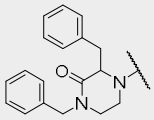
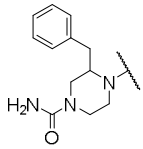
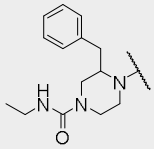
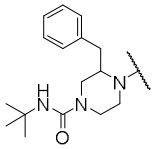
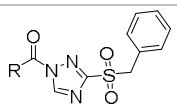
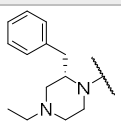
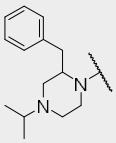
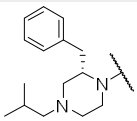
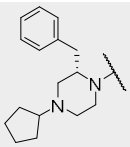
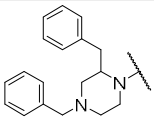
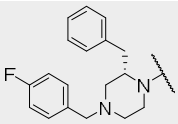
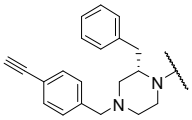
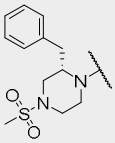
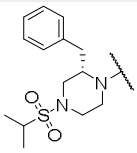
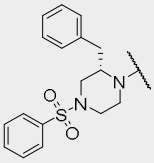
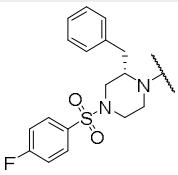
#	R	PNPB pIC_{50} DAGL-α	ABPP pIC_{50} DAGL-α	PNPB pIC_{50} DAGL-β	CLogP	TPSA (Å^2)	LipE DAGL-α
3		8.71 ± 0.06	7.88 ± 0.14	8.40 ± 0.07	4.97	112.0	3.74
4		6.25 ± 0.08	5.61 ± 0.22	5.74 ± 0.22	2.58	102.7	3.67
5		7.41 ± 0.07	7.57 ± 0.45	7.25 ± 0.09	3.49	102.7	3.92
6		7.74 ± 0.07	7.91 ± 0.15	7.24 ± 0.07	4.44	102.7	3.30
7		7.71 ± 0.06	6.87 ± 0.25	7.24 ± 0.06	4.16	102.7	3.55
8		6.66 ± 0.20	6.14 ± 0.30	5.69 ± 0.14	4.58	102.7	2.08
9		< 5	< 5	< 5	1.77	128.7	-
10		6.52 ± 0.19	5.95 ± 0.19	6.27 ± 0.18	3.59	114.8	2.93
11		6.77 ± 0.05	6.54 ± 0.13	6.68 ± 0.07	4.14	114.8	2.63

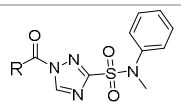
Table 3.2 (continued) | Piperazine variations of the 1,2,4-triazole-sulfone scaffold.

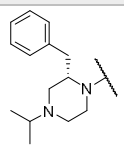
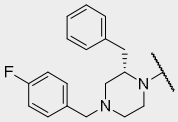
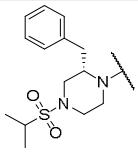
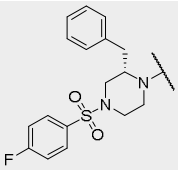


#	R	PNPB pIC_{50} DAGL-α	ABPP pIC_{50} DAGL-α	PNPB pIC_{50} DAGL-β	CLogP	TPSA (Å²)	LipE DAGL-α
12		7.46 ± 0.05	N.D.	7.54 ± 0.23	4.09	85.7	3.37
13		8.37 ± 0.08	7.95 ± 0.25	7.99 ± 0.08	4.40	85.7	3.97
14		8.92 ± 0.03	8.67 ± 0.09	8.35 ± 0.11	5.02	85.7	3.90
15		8.56 ± 0.10	N.D.	8.81 ± 0.18	4.82	85.7	3.74
16		9.05 ± 0.06	8.53 ± 0.15	8.98 ± 0.10	5.28	85.7	3.77
17		8.12 ± 0.11	8.26 ± 0.14	N.D.	5.42	85.7	2.70
18		8.58 ± 0.11	7.83 ± 0.15	9.28 ± 0.12	5.55	85.7	3.03
19		6.75 ± 0.07	N.D.	6.73 ± 0.20	3.00	119.8	3.75
20		7.97 ± 0.12	7.72 ± 0.20	6.50 ± 0.31	3.84	119.8	4.13
21		8.50 ± 0.06	8.45 ± 0.10	7.88 ± 0.08	4.68	119.8	3.82
22		7.83 ± 0.11	N.D.	6.39 ± 0.30	4.82	119.8	3.01

Four piperazine substituents of the compounds with highest potency, LipE and drug-like character (Table 3.2) were combined with the *N*-methylaniline substituted 1,2,4-triazole sulfonamide scaffold of **1** to form compounds **23**-**26** (Table 2.2). The potency of the sulfonamide derivatives increased 5-10 fold compared to their sulfone analogues. Both amine compounds **23** and **24** showed sub-nanomolar potencies in the surrogate substrate assay and maintained high potency in the gel-based ABPP assays. Compounds **25** and **26** were slightly less active, but still showed IC_{50}s in the single nanomolar range. All four compounds were slightly less active against DAGL-β, consistent with the observations for compounds **3**-**22**. The lipophilic efficiency was further increased. The TPSA of the amines is just below 90 Å^2, which could allow these compounds to be centrally active. **25** and **26** have a TPSA > 120 Å^2, making it highly unlikely that they are able to cross the blood-brain barrier. Together, they could be employed to elucidate peripheral effects using **25** or **26**, or central effects, by comparing the effects of **23** or **24** with **25** or **26**.

Table 3.3 | *In vitro* profiling of the four most promising piperazine groups combined with the sulfonamide leaving group. Activity-based protein profiling of DAGL-α is performed using mouse brain membrane proteome. For DAGL-β membrane fractions of HEK293T cells overexpressing mDAGL-β were used.

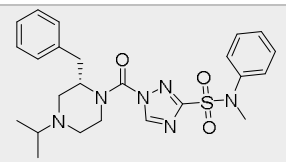
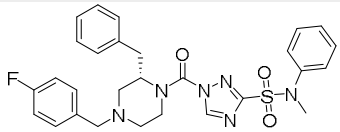
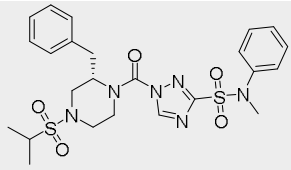
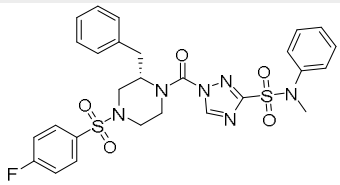


#	R	PNPB pIC_{50} DAGL-α	ABPP pIC_{50} DAGL-α	PNPB pIC_{50} DAGL-β	ABPP pIC_{50} DAGL-β	CLogP	TPSA (Å^2)	LipE DAGL-α
23		9.06 ± 0.07	8.93 ± 0.08	7.84 ± 0.25	8.54 ± 0.06	4.06	88.9	5.00
24		9.91 ± 0.11	9.45 ± 0.04	8.47 ± 0.19	8.44 ± 0.14	5.08	88.9	4.83
25		8.55 ± 0.10	7.97 ± 0.10	8.52 ± 0.11	7.70 ± 0.09	3.50	123.0	5.05
26		8.72 ± 0.12	9.44 ± 0.12	8.12 ± 0.08	8.26 ± 0.25	4.48	123.0	4.24

Cellular activity and selectivity of **23**-**26**

Cellular DAGL-β activity of compounds **23**-**26** was assessed by the treatment of murine neuroblastoma (Neuro-2a) cells for 1 hour with medium containing inhibitor or vehicle (DMSO) followed by gel-based ABPP. Post-lysis treatment with activity-based probes MB064, to visualize DAGL-β and other lipases such as Phospholipase DDHD2 (DDHD2) and ABHD6, showed clear inhibition of DAGL-β (Figure 3.2, Table 3.4). FP-BODIPY (Chapter 6) was used to label the endocannabinoid related hydrolase Fatty Acid Amide Hydrolase (FAAH) and to further assess general selectivity. Overall potency for DAGL-β of the inhibitors was reduced 10-50 fold in this setting. Quite surprisingly, the selectivity observed in the gel-based assays *in vitro* was attenuated *in situ*. The potency of the inhibitors against ABHD6 was equal or greater than that for DAGL-β, and further off-target activity was observed for DDHD2 and FAAH.

Table 3.4 | *In situ* potency of the optimized piperazine derivatives **23**-**26** tested in Neuro-2a cells determined by post-lysis ABPP and SDS-PAGE analysis.

#	Structure	pIC_{50} DAGL-β	pIC_{50} DDHD2	pIC_{50} ABHD6	pIC_{50} FAAH
23		6.68 ± 0.13	5.57 ± 0.07	8.00 ± 0.05	6.16 ± 0.08
24		7.58 ± 0.10	6.76 ± 0.11	7.84 ± 0.05	6.01 ± 0.22
25		6.09 ± 0.12	5.53 ± 0.21	7.06 ± 0.12	5.48 ± 0.13
26		6.70 ± 0.08	6.31 ± 0.11	7.38 ± 0.07	5.35 ± 0.41

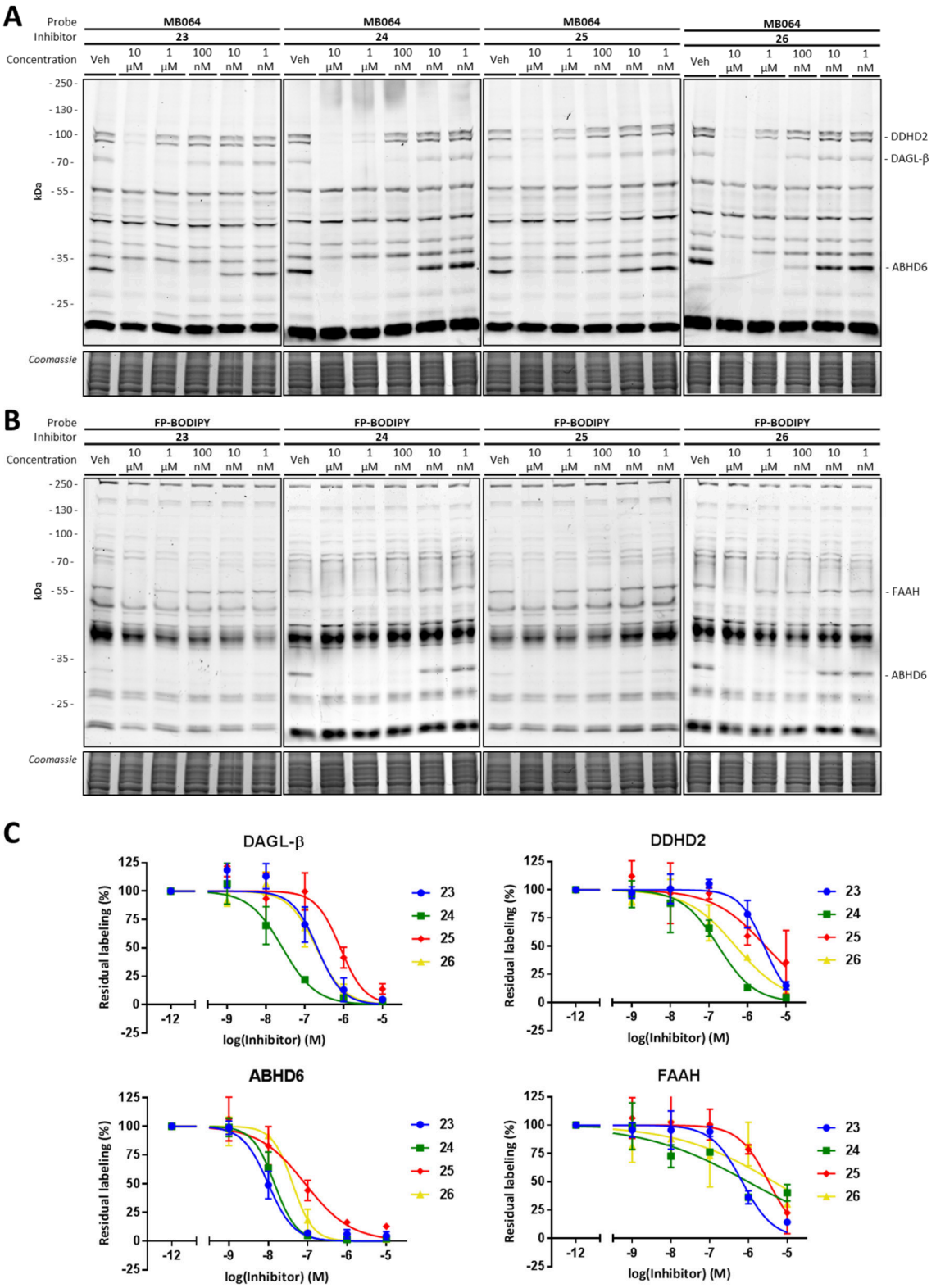


Figure 3.2 | Dose response *in situ* treatment of Neuro-2a cells with piperazine inhibitors **23**-**26**. A) Representative gels of lysates labeled with MB064 (250 nM). B) Representative gels of lysates labeled with FP-BODIPY (500 nM). C) Dose response curves for DAGL-β, DDHD2, ABHD6 and FAAH as based on A and B (N=3).

Discussion and conclusion

The optimization of the physicochemical properties of the DAG lipase inhibitors introduced in Chapter 2 was initiated by incorporating a previously published piperazine as the amine for the triazole urea scaffold.[10] Successful implementation of a matched molecular pair strategy significantly sped up the generation of a library of 2-benzyl-4-substituted piperazine triazole ureas. Similar trends were observed for acyl, alkyl, urea and sulfonyl substituents, with a strong preference for lipophilic and bulky sidechains. Intriguingly, the Boc-group (**3**), 3,3-dimethylbutyric acid (**6**) and *tert*-butylamine urea (**11**) variants show a nearly perfect trend, each decreasing a factor 10 in potency. The CLogP (ChemDraw 16.0) also follows a nearly perfect linear decrease, albeit with a slightly smaller slope: from 4.97 for **3**, 4.44 for **6** to 4.14 for **11**. This likely explains the observed drop in affinity.

The optimized inhibitors **23**-**26** showed (sub)nanomolar potency and good selectivity *in vitro*, but both were attenuated *in situ*. For compounds **25** and **26** the TPSA is probably too high for efficient cell penetration. The decrease in selectivity has not been investigated in detail, but might be attributed to different physical conditions, such as local pH, and differences in enzyme activity *in situ* as compared to *in vitro*. Especially the small selectivity window with respect to FAAH should be noted, as this is the main metabolizing enzyme for anandamide, the other major endocannabinoid.[12] Further profiling of the generated library *in situ* is warranted to possibly find more selective members.

Overall potency on both DAGL isoforms was slightly improved, but more importantly the lipophilic efficiency was increased from 4.34 for **2** to 5.05 for **25**, the highest LipE reported to date for a DAGL inhibitor. The topological polar surface area of **25** and **26** is > 120 $Å^2$, which should be sufficient to restrict these compounds to the periphery. However, their high TPSA likely hampers their cell penetration and may affect their oral bioavailability. Future studies should test whether these compounds can be used in animal models to inhibit DAGL-β in the periphery without inhibiting DAGL-α in the brain.

Acknowledgements

For the optimization of the synthetic route towards 1,3-dibenzylpiperazine Jacob van Hengst is kindly acknowledged. Esmeralda Hemme and Esmee Schoof are acknowledged for the synthetic work towards **40** and **49**, respectively. Annelot van Esbroeck and Floor Stevens are kindly acknowledged for their advice and cooperation in the *in situ* testing of the inhibitors.

Methods

Chemical Biology Methods

Cell Culture

HEK293T (human embryonic kidney) and Neuro-2a (mouse neuroblastoma) cells were cultured at 37 °C under 7% CO_2 in DMEM containing phenol red, stable glutamine, 10% (v/v) New Born Calf Serum (Thermo Fisher), and penicillin and streptomycin (200 µg/mL each; Duchefa). Medium was refreshed every 2-3 days and cells were passaged twice a week at 80-90% confluence by resuspension in fresh medium.
Cells lines were purchased from ATCC and were regularly tested for mycoplasma contamination. Cultures were discarded after 2-3 months of use.

Transient transfection

One day prior to transfection HEK293T cells were seeded to 15-cm dishes (~62.500 cells/cm^2). Prior to transfection, culture medium was aspirated and a minimal amount of medium was added. A 3:1 (m/m) mixture of polyethyleneimine (PEI) (60 µg/dish) and plasmid DNA (20 µg/dish, either mDAGL-α-FLAG or mDAGL-β-FLAG) was prepared in serum-free culture medium and incubated for 15 min at RT. Transfection was performed by dropwise addition of the PEI/DNA mixture to the cells. Transfection with the empty pcDNA3.1 vector was used to generate control samples. After 24 h, medium was refreshed. Medium was aspirated 48 h post-transfection and cells were harvested by resuspension in PBS. Cells were pelleted by centrifugation (5 min, 1,000 *g*) and the pellet was washed with PBS. Supernatant was discarded and cell pellets were frozen in liquid nitrogen and stored at -80 °C until sample preparation.

In situ treatment of Neuro-2a cells

Cells were seeded in 12-well plates. *In situ* treatment was initiated 24 h later. Medium was aspirated and medium (with serum) containing inhibitor or DMSO as vehicle was added (0.1% v/v DMSO). After 1 h exposure to the treatment medium, the medium was aspirated and cells were harvested and stored as described above until sample preparation.

Whole cell lysate

Cell pellets were thawed on ice, resuspended in cold lysis buffer (20 mM HEPES pH 7.2, 2 mM DTT, 250 mM sucrose, 1 mM $MgCl_2$, 2.5 U/mL benzonase) and incubated on ice (15-30 min). The cell lysate was used for membrane preparation (below) or was diluted to 2.0 mg/mL concentration in cold storage buffer (20 mM Hepes, pH 7.2, 2 mM DTT) for use as whole lysate. Protein concentrations were determined by a Quick Start™ Bradford Protein Assay and diluted samples were flash frozen in liquid nitrogen and stored at -80 °C until further use.

Membrane preparation from overexpression lysate

The membrane and cytosolic fractions of cell lysates were separated by ultracentrifugation (93,000 *g*, 45 min, 4 °C). The supernatant was collected (cytosolic fraction) and the membrane pellet was resuspended in cold storage buffer (20 mM HEPES, pH 7.2, 2 mM DTT) by thorough pipetting and passage through an insulin needle. Protein concentrations were determined by a Quick Start™ Bradford Protein Assay and samples were diluted to 2.0 mg/mL with cold storage buffer, flash frozen in liquid nitrogen and stored at -80 °C until further use.

Tissue preparation

Organs were isolated from C57BL/6 mice following standard guidelines as approved by the ethical committee of Leiden University (DEC#13191). Isolated organs were frozen in liquid nitrogen and stored at -80 °C. Organs were thawed on ice and homogenized by a glass stick douncing homogenizer in cold lysis buffer (20 mM HEPES, pH 7.2, 2 mM DTT, 1 mM $MgCl_2$, 2.5 U/mL benzonase). The resulting suspension was centrifuged at 1000 *g* for 5 minutes at 4 °C to get rid of residual solid tissue. The supernatant was centrifuged 45 minutes at 93,000 *g* at 4 °C to separate soluble (cytosol) and insoluble (membrane) fractions. The pellet (membrane) was resuspended in storage buffer (20 mM HEPES, pH 7.2, 2 mM DTT) using an insulin syringe. The protein concentration was measured using a Qubit™ protein assay and was adjusted to 2 mg/mL using storage buffer. The resulting lysates were frozen in liquid nitrogen and stored at -80 °C for later use.

Surrogate substrate assay

The biochemical DAGL-α or -β activity assay is based on the method previously described.[11] 200 µL reactions were performed in flat bottom Greiner 96-wells plates in a 50 mM pH 7.2 HEPES buffer, for DAGL-β this buffer was supplemented with 5 mM $CaCl_2$. Membrane protein fractions from HEK293T cells transiently transfected with mDAGL-α or -β (0.05 µg/µL final concentration) were used as mDAGL source. Inhibitors were introduced in 5.0 µL DMSO. The mixtures were incubated for 20 minutes before 10.0 µL 6 mM (DAGL-α) or 12 mM (DAGL-β) PNP-butyrate (final concentration 0.3 mM) in 50% DMSO was added (final DMSO concentration 5.0%). Reactions were allowed to progress for 30 minutes at 20 °C before OD (420 nm) was measured using a TECAN GENios plate reader. All experiments were performed at N=2, n=2 for experimental measurements and N=2, n=4 for controls.

Z'-factor of each plate was determined for the validation of each experiment, using the following formula: $Z' = 1\text{-}3(\sigma_{pc}+ \sigma_{nc})/(\mu_{pc} - \mu_{nc})$. The OD from the positive control (pc: DAGL DMSO), and the negative control (nc: 10 µM THL) were used. Plates were accepted for further analysis when Z' > 0.6. Measurements were corrected for the average absorption 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 7.0 for the calculation of the pIC_{50} using a non-linear dose-response analysis with variable slope.

Activity-based protein profiling

For *in vitro* inhibition, lysates (19 µL per sample, 2 µg/µL) were thawed on ice. 0.5 µL of the inhibitor (40x stock in DMSO) or pure DMSO (as vehicle) was added to the sample, vortexed briefly and incubated for 20 minutes at RT. Subsequently, 0.5 µL probe (40x stock in DMSO, final concentration 250 nM for MB064[11], 500 nM for FP-TAMRA[13], 1 µM for DH379[10]) was added to the proteome sample, vortexed briefly and incubated for 15 minutes at RT. For *in situ* inhibition, the *in situ*-treated cells (19.5 µL whole lysate) were directly incubated with the activity based probe (40x stock in DMSO, final concentration 2 µM MB064 or 500 nM FP-BODIPY, 20 min, RT). Final volume in all cases was 20 µL (max. 5% DMSO). The reaction was quenched by the addition of 7.5 µL of 4*Laemmli-buffer (final concentrations: 60 mM Tris (pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol, 1.25% (v/v) β-mercaptoethanol, 0.01% (v/v) bromophenol blue). 10 µL (14 µg protein) of quenched reaction mixture was resolved on 10% acrylamide SDS-PAGE (180 V, 75 min). Fluorescence was measured using a Biorad ChemiDoc MP system (fluorescence channels Cy2 (460-490 nm), Cy3 (520-545 nm), Cy5 (625-650 nm) filters). Gels were then stained using coomassie staining and imaged for protein loading control.

Labeling quantification

Fluorescence quantification was performed using Imagelab 6.0 (Biorad). Intensities were normalized to the DMSO control and corrected for protein loading by coomassie staining. pIC_{50} values were calculated with GraphPad Prism 7.0 using a non-linear dose-response analysis with variable slope. For all pIC_{50} determinations three replicates of each condition were used.

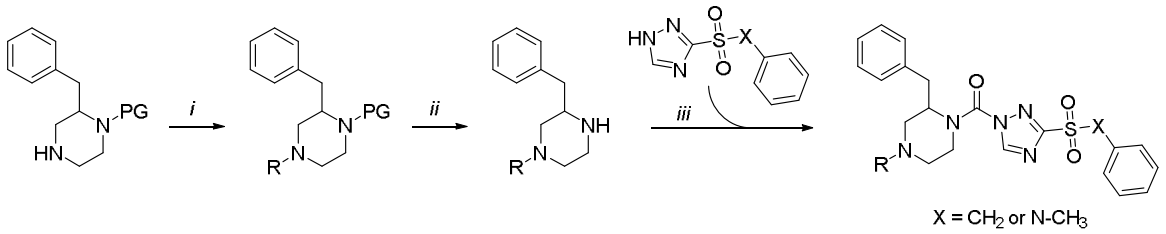
Synthetic Methods

General remarks

All reactions were performed using oven- or flame-dried glassware and dry (molecular sieves) solvents. Reagents were purchased from Alfa Aesar, Sigma-Aldrich, Acros, and Merck and used without further purification unless noted otherwise. All moisture sensitive reactions were performed under an argon or nitrogen atmosphere. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 (300 MHz), AV-400 (400 MHz) or DRX-500 (500 MHz). Used software for interpretation of NMR-data was Bruker TopSpin 1.3 and MestreNova 11.0. 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; ACN-*d*3: δ 1.94 for ^1H, δ 1.32 for ^{13}C; MeOD: δ 3.31 for ^1H, δ 49.00 for ^{13}C).[14] Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, td = triple doublet, t = triplet, q = quartet, bs = broad singlet, m = multiplet), coupling constants *J* (Hz), and integration.

Liquid chromatography analysis was performed on a Finnigan Surveyor LC/MS system, equipped with a C18 column. 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 $KMnO_4$ stain (K_2CO_3 (40 g), $KMnO_4$ (6 g), and water (600 mL)) or CAM stain ($Ce(NH_4)_4(SO_4)_4{\cdot}2H_2O$ (ceric ammonium sulfate: 10 g); ammonium molybdate (25 g); conc. H_2SO_4 (100 mL); H_2O (900 mL)). Preparative HPLC (Waters, 515 HPLC pump M; Waters, 515 HPLC pump L; Waters, 2767 sample manager; Waters SFO

System Fluidics Organizer; Waters Acquity Ultra Performance LC, SQ Detector; Waters Binary Gradient Module) was performed on a Waters XBridgeTM column (5 µM C18, 150 x 19 mm). Diode detection was done between 210 and 600 nm. Gradient: ACN in (H_2O + 0.2% TFA). High resolution mass spectra (HRMS) were recorded by direct injection on a q-TOF mass spectrometer (Synapt G2-Si) equipped with an electrospray ion source in positive mode with Leu-enkephalin (m/z = 556.2771) as an internal lock mass. The instrument was calibrated prior to measurement using the MS/MS spectrum of Glu-1-fibrinopeptide B.



Scheme S3.1 | General scheme for the synthesis of the triazole urea inhibitors. *i*) introduction or substituent R; *ii*) deprotection of amine, General procedure 1 or 2; *iii*) triphosgene coupling to triazoles, General procedure 3.

General Procedure 1: Debocylation of piperazine intermediate

To a 1 M HCl solution in MeOH *tert*-butyl 4-substituted-2-benzylpiperazine-1-carboxylate was added (± 0.1 M). The mixture was stirred for 4-18 h until LC/MS analysis showed full conversion of starting material. Volatiles were removed under reduced pressure to yield the HCl salt of the deprotected piperazine.

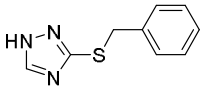
General Procedure 2: Removal of trifluoroacetyl protecting group from piperazine intermediate

1-(2-Benzyl-4-substituted-piperazin-1-yl)-2,2,2-trifluoroethan-1-one was dissolved in 20% H_2O in MeOH (± 0.1 M). 3 Equivalents of K_2CO_3 were added. The mixture was stirred for 20-40 h until LC/MS analysis showed full conversion of starting material. The solids were filtered off and the filtrate concentrated *in vacuo* to obtain the deprotected piperazine.

General Procedure 3: Triphosgene coupling of piperazine derivative to 3-substituted-1*H*-1,2,4-triazoles

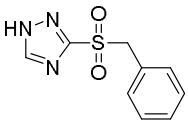
1-Substituted-3-benzylpiperazine was dissolved in dry DCM (± 0.1 M) and 3 equivalents of Na_2CO_3 were added. The mixture was cooled to 0 °C and 0.75 equivalent of triphosgene was added. The mixture was stirred at 0 °C for 1.5 h. The solids were filtered off and the solvent was evaporated under reduced pressure. The residue was redissolved in dry THF (± 0.1 M), brought under N_2-atmosphere and 0.95 equivalent of 3-substituted-1*H*-1,2,4-triazole, 0.1 equivalent of DMAP and 2 equivalents of DIPEA were added. The mixture was refluxed for 6-18 h. The reaction mixture was concentrated *in vacuo*.

3-(Benzylthio)-1*H*-1,2,4-triazole (**27**)

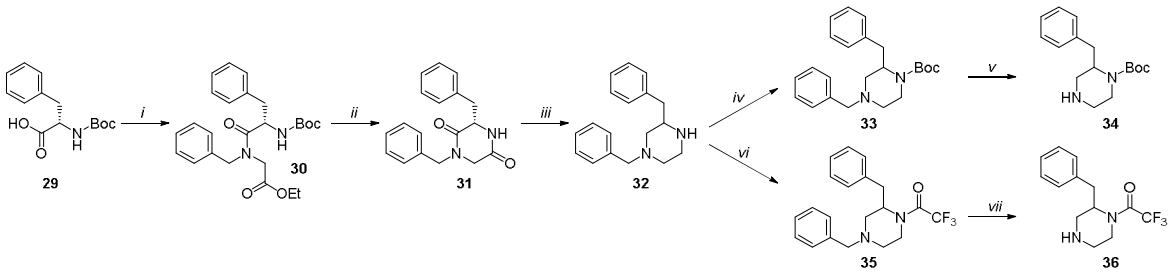


1*H*-1,2,4-triazole-3-thiol (1.01 g, 10.0 mmol) and benzylbromide (1.09 mL, 10.0 mmol) were dissolved in DMF (10 mL) and stirred for 18 h. The reaction mixture was then diluted with EtOAc (50 mL) and washed with aqueous saturated $NaHCO_3$ (50 mL). The organic phase was separated and the aqueous phase was extracted with EtOAc (50 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL). The volatiles are removed *in vacuo* and the product was obtained as white solid (1.86 g, 9.75 mmol, 98%). ^{1}H NMR (400 MHz, MeOD) δ 8.30 (s, 1H), 7.35 – 7.14 (m, 5H), 4.33 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 147.99, 138.64, 129.91, 129.52, 128.48, 37.80.

3-(Benzylsulfonyl)-1*H*-1,2,4-triazole (**28**)

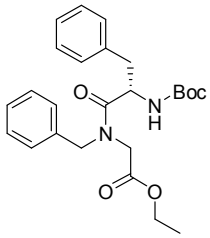


27 (2.91 g, 15.2 mmol) was dissolved in DCM (40 mL) and peracetic acid (8.9 mL, 47 mmol) was slowly added. The reaction was stirred for 48 h at RT during which a white precipitate formed. The precipitate was filtered off and taken up in EtOAc (50 mL). This was washed with 1:1 saturated aqueous $Na_2S_2O_3$ and Na_2CO_3 (50 mL). The aqueous phase was extracted with EtOAc (2x 50 mL). Combined organic layers were dried ($MgSO_4$), filtered and concentrated to afford the title compound (2.60 g, 11.6 mmol, 76%).^{1}H NMR (400 MHz, MeOD) δ 8.67 (s, 1H), 7.48 – 7.11 (m, 5H), 4.70 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 161.51, 146.94, 132.20, 129.90, 129.58, 128.47, 61.59.



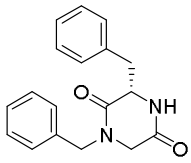
Scheme S3.2 | Reagents and conditions: *i*) HBTU, DIPEA, ethyl benzylglycinate, DCM, RT, 99%; *ii*) a: TFA, DCM, b: DIPEA, MeOH, RT, 82%; *iii*) $LiAlH_4$, THF, 65 °C, 90%; *iv*) Boc_2O, DCM, RT, 95%; *v*) ammonium formate, Pd/C, EtOH, 75 °C, 100%; *vi*) $(CF_3CO)_2O$, NEt_3, Et_2O, 100%; *vii*) ammonium formate, Pd/C, EtOH, 75 °C, 95%.

Ethyl *N*-benzyl-*N*-((*tert*-butoxycarbonyl)-L-phenylalanyl)glycinate (**30**)



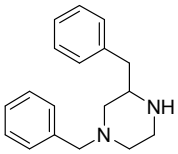
(*tert*-Butoxycarbonyl)-L-phenylalanine **29** (4.38 g, 16.5 mmol) and DIPEA (3.41 mL, 19.5 mmol) were dissolved in DCM (200 mL) on ice. HBTU (6.26 g, 16.5 mmol) was added and the mixture was stirred for 10 minutes. Ethyl benzylglycinate (2.76 mL, 15.0 mmol) was added and the mixture was stirred for 18 h at RT. The reaction was quenched with sat. aq. NH_4Cl (100 mL) and the organic phase was separated. The aqueous layer was extracted with EtOAc (100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound as a yellow oil (6.61 g, 15.0 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 6.97 (m, 10H), 5.37 (dd, *J* = 22.3, 8.9 Hz, 1H), 5.03 – 4.90 (m, 1H), 4.78 – 4.43 (m, 2H), 4.23 – 4.07 (m, 2H), 4.02 (dd, *J* = 20.2, 17.8 Hz, 1H), 3.78 (dd, *J* = 40.3, 17.8 Hz, 1H), 3.19 – 3.02 (m, 1H), 3.02 – 2.87 (m, 1H), 1.39 (s, 9H), 1.30 – 1.20 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.89, 168.83, 155.03, 136.65, 136.46, 135.98, 135.41, 129.73, 129.58, 129.54, 128.95, 128.66, 128.52, 128.42, 128.01, 127.72, 127.35, 126.89, 126.86, 79.83, 61.73, 61.24, 51.98, 51.63, 50.11, 48.32, 47.12, 39.61, 28.34, 14.20 (carbon spectrum shows a mixture of rotamers).

(S)-1,3-Dibenzylpiperazine-2,5-dione (**31**)



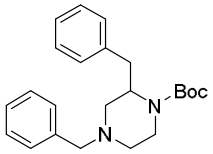
30 (6.61 g, 15.0 mmol) was dissolved in DCM (100 mL) and TFA (20 mL) was added. The mixture was stirred for 3 h at RT. Volatiles were removed under reduced pressure and the residue was taken up in methanol (50 mL). DIPEA (26.2 mL, 150 mmol) was added and the mixture stirred for 18 h. The mixture was concentrated *in vacuo* and purified by column chromatography. Product was isolated as a white gum (3.62 g, 12.3 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 3H), 7.26 – 7.18 (m, 1H), 7.18 – 7.06 (m, 7H), 4.41 (t, *J* = 3.7 Hz, 1H), 4.36 (t, *J* = 4.3 Hz, 1H), 4.22 (s, 1H), 3.38 (dd, *J* = 17.6, 2.8 Hz, 1H), 3.28 (dt, *J* = 13.7, 3.6 Hz, 1H), 3.02 (dt, *J* = 13.7, 3.7 Hz, 1H), 2.61 (dd, *J* = 17.6, 2.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.56, 169.05, 137.52, 137.50, 133.19, 131.83, 131.70, 131.58, 131.17, 130.47, 59.39, 52.73, 51.19, 43.44.

1,3-Dibenzylpiperazine (**32**)



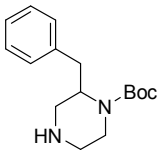
To a 0 °C suspension of **31** (3.61 g, 12.26 mmol) in dry THF (250 mL) was added in batches 2 M $LiAlH_4$ in THF (30.7 mL, 61.3 mmol). The mixture was stirred on ice for 15 min after which the reaction was heated and refluxed for 4 h. The solution was cooled and the excess $LiAlH_4$ was quenched by the careful addition of 2 M NaOH (8 mL). The cloudy mixture was stirred ON. Solids that formed were filtered off and the filtrate concentrated to provide 1,3-dibenzylpiperazine (2.95 g, 11.1 mmol, 90%) as a pale coloured oil. ^{1}H NMR (400 MHz, MeOD) δ 7.41 – 7.09 (m, 10H), 3.56 – 3.38 (m, 2H), 3.02 – 2.90 (m, 1H), 2.86 (dt, *J* = 12.7, 3.0 Hz, 1H), 2.81 – 2.68 (m, 3H), 2.65 – 2.59 (m, 2H), 2.07 (td, *J* = 11.5, 3.2 Hz, 1H), 1.84 (t, *J* = 10.6 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ 139.36, 138.11, 130.70, 130.21, 129.56, 129.26, 128.39, 127.51, 64.22, 59.67, 57.28, 53.79, 45.98, 41.36.

tert-Butyl 2,4-dibenzylpiperazine-1-carboxylate (**33**)



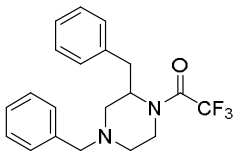
32 (0.70 g, 2.6 mmol) was dissolved in DCM (30 mL) and Boc-anhydride (0.64 mL, 2.8 mmol) was added. The mixture was stirred for 4 h. Volatiles were evaporated under reduced pressure and the resulting residue was purified by column chromatography yielding the title compound as a colourless oil (0.915 g, 2.50 mmol, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 – 6.95 (m, 10H), 4.16 (s, 1H), 3.94 (s, 1H), 3.55 (d, *J* = 12.9 Hz, 1H), 3.38 (d, *J* = 12.9 Hz, 1H), 3.22 (td, *J* = 12.7, 3.4 Hz, 1H), 3.08 (t, *J* = 11.0 Hz, 1H), 2.86 (d, *J* = 11.1 Hz, 2H), 2.65 (dt, *J* = 11.4, 1.9 Hz, 1H), 2.09 (td, *J* = 11.7, 3.5 Hz, 1H), 1.99 (dd, *J* = 11.5, 3.9 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.46, 129.36, 128.40, 127.29, 126.13, 79.63, 63.10, 53.44, 28.49.

tert-Butyl 2-benzylpiperazine-1-carboxylate (**34**)



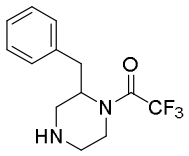
33 (964 mg, 2.63 mmol) was dissolved in absolute EtOH and ammonium formate (829 mg, 13.2 mmol) was added. The mixture was brought under N_2-atmosphere and 10% Pd on carbon (50 mg) was added. The mixture was refluxed for 48 h. The catalyst was filtered off and the volatiles were evaporated. Coevaporation with toluene afforded the pure product in quantitative yield (727 mg, 2.63 mmol, 100%). ^{1}H NMR (300 MHz, MeOD) δ 7.47 – 7.12 (m, 5H), 4.46 – 4.12 (m, 1H), 4.12 – 3.72 (m, 1H), 3.27 – 2.53 (m, 7H), 1.34 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.01, 140.16, 130.37, 129.37, 127.22, 80.68, 46.25, 35.86, 28.82, 28.54.

1-(2,4-Dibenzylpiperazin-1-yl)-2,2,2-trifluoroethan-1-one (**35**)



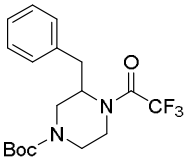
32 (2.01 g, 7.51 mmol) was dissolved in diethyl ether (30 mL) and triethylamine (1.15 mL, 8.26 mmol) was added. The mixture was cooled to 0 °C and 2,2,2-trifluoroacetic anhydride (1.15 mL, 8.26 mmol) was added slowly. The mixture was stirred for 3 h at RT. The reaction was quenched by the addition of saturated aqueous Na_2CO_3 (20 mL). The organic phase was separated and the water layer extracted with Et_2O (2x 25 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to yield **35** (2.72 g, 7.51 mmol, 100%) as a yellow oil, which was used without further purification for the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 6.81 (m, 10H), 4.68 – 4.29 (m, 1H), 4.13 – 3.90 (m, 1H), 3.81 – 3.11 (m, 4H), 3.11 – 2.61 (m, 3H), 2.19 – 1.84 (m, 2H) (proton spectrum shows a mixture of rotamers). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.55 (q, *J* = 35.5 Hz), 155.35 (q, *J* = 35.5 Hz), 137.59, 137.43, 129.42, 129.32, 129.22, 128.55, 128.35, 127.45, 127.42, 126.65, 126.47, 116.69 (q, *J* = 288.1 Hz), 116.45 (q, *J* = 288.0 Hz), 62.51 (q, *J* = 7.0 Hz), 56.08 (q, *J* = 3.1 Hz), 52.74, 45.60, 41.90 (q, *J* = 3.7 Hz), 39.14, 36.67, 35.51, 29.69 (carbon spectrum shows a mixture of rotamers).

1-(2-Benzylpiperazin-1-yl)-2,2,2-trifluoroethan-1-one (**36**)



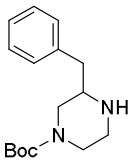
35 (1.09 g, 3.00 mmol) was dissolved in absolute ethanol (30 mL) and ammonium formate (0.95 g, 15 mmol) was added. The mixture was brought under N_2-atmosphere and 10% Pd/C catalyst (50 mg) was added. The mixture was refluxed for 70 h. Catalyst was filtered off and the solvent removed *in vacuo*. The residue was taken up in EtOAc (25 mL) and washed with saturated aqueous Na_2CO_3. The layers were separated and the aqueous phase extracted twice with EtOAc (25 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to yield the title compound (0.773 g, 2.84 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.05 (m, 5H), 4.74 – 3.96 (m, 1H), 3.79 – 3.58 (m, 1H), 3.58 – 3.39 (m, 1H), 3.28 – 2.47 (m, 7H) (proton spectrum shows a mixture of rotamers). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.80 (d, *J* = 35.4 Hz), 155.61 (d, *J* = 35.4 Hz), 137.50, 137.44, 129.31, 129.28, 128.75, 128.52, 126.89, 126.68, 116.70 (q, *J* = 288.1 Hz), 116.45 (q, *J* = 288.2 Hz), 55.92, 52.16, 46.31 (d, *J* = 45.7 Hz), 46.14 (d, *J* = 55.0 Hz), 42.47 (q, *J* = 3.6 Hz), 39.50, 35.66, 34.64 (carbon spectrum shows a mixture of rotamers).

tert-Butyl 3-benzyl-4-(2,2,2-trifluoroacetyl)piperazine-1-carboxylate (**37**)



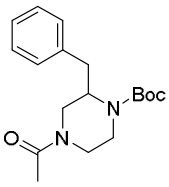
36 (192 mg, 0.705 mmol) was dissolved in DCM (10 mL) and Boc-anhydride (0.180 mL, 0.776 mmol) was added. The mixture was stirred for 18 h at RT. The solvents were removed under reduced pressure. The crude residue (263 mg, 0.706 mmol, quant.) was used without further purification in the next reaction.

tert-Butyl 3-benzylpiperazine-1-carboxylate (**38**)



tert-Butyl 3-benzylpiperazine-1-carboxylate was synthesized from **37** (263 mg, 0.706 mmol) according to General Procedure 2. The crude residue (195 mg, 0.706 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.13 (m, 5H), 4.27 – 3.77 (m, 2H), 3.42 (bs, 1H), 3.04 – 2.73 (m, 4H), 2.71 – 2.47 (m, 3H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.65, 137.49, 129.22, 128.70, 126.73, 79.84, 56.16, 45.36, 39.78, 28.41.

tert-Butyl 4-acetyl-2-benzylpiperazine-1-carboxylate (**39**)

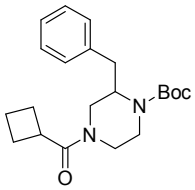


Acetylchloride (0.20 mL, 2.9 mmol), Net_3 (0.5 mL, 3.4 mmol) and **34** (720 mg, 2.6 mmol) were dissolved in DCM (20 mL) and stirred at 0 °C for 2 hours. The solution was washed with water (20 mL) and the water layer was extracted with EtOAc (2x 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was obtained as a yellow oil in quantitative yield (0.83 g, 2.6 mmol, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 – 6.86 (m, 5H), 4.41 – 3.99 (m, 2H), 3.92 – 3.47 (m, 2H), 3.09 – 2.82 (m, 2H), 2.81 – 2.40 (m, 3H), 1.83 (s, 3H), 1.18 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.19, 168.85, 153.78, 137.65, 137.26, 129.04, 128.63, 128.13, 127.86, 126.18, 125.85, 79.41, 59.66, 47.12, 45.49, 42.22, 40.67, 35.48, 34.93, 27.69, 20.85, 20.60 (carbon spectrum shows a mixture of rotamers).

1-(3-Benzylpiperazin-1-yl)ethan-1-one hydrochloride (**40**)

1-(3-Benzylpiperazin-1-yl)ethan-1-one was synthesized from **39** (490 mg, 1.54 mmol) according to General Procedure 1. The crude residue (370 mg, 1.45 mmol, 94%) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

tert-Butyl (S)-2-benzyl-4-(cyclobutanecarbonyl)piperazine-1-carboxylate (**41**)

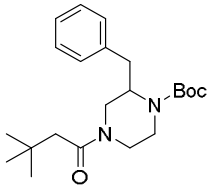


Cyclobutanecarboxylic acid (38.8 mg, 0.388 mmol), DIPEA (77 µL, 0.44 mmol) and HBTU (154 mg, 0.406 mmol) were dissolved in DCM (5 mL) and stirred for 20 minutes at RT. **34** (102 mg, 0.369 mmol) was then added as a solution in DCM (1 mL). The mixture was stirred for 20 h. Volatiles were removed *in vacuo* and the residue was purified by column chromatograpy. The title compound was obtained as a white solid (106 mg, 0.296 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.10 (m, 5H), 4.55 – 4.20 (m, 2H), 3.99 (s, 1H), 3.71 – 3.24 (m, 1H), 3.23 – 2.82 (m, 3H), 2.82 – 2.60 (m, 2H), 2.57 – 2.32 (m, 2H), 2.32 – 2.12 (m, 2H), 2.12 – 1.97 (m, 1H), 1.97 – 1.75 (m, 2H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.26, 173.81, 154.49, 154.33, 138.20, 137.74, 129.60, 129.19, 128.65, 128.50, 126.76, 126.46, 80.23, 44.82, 43.27, 41.65, 37.33, 36.88, 36.04, 29.76, 28.24, 25.61, 24.95, 18.11 (carbon spectrum shows a mixture of rotamers).

(3-Benzylpiperazin-1-yl)(cyclobutyl)methanone hydrochloride (**42**)

(3-Benzylpiperazin-1-yl)(cyclobutyl)methanone was synthesized from **41** (106 mg, 0.30 mmol) according to General Procedure 1. The crude residue (87 mg, 0.30 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

tert-Butyl 2-benzyl-4-(3,3-dimethylbutanoyl)piperazine-1-carboxylate (**43**)

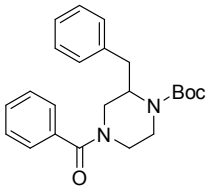


3,3-Dimethylbutanoic acid (45.0 mg, 0.388 mmol), DIPEA (77 µL, 0.44 mmol) and HBTU (154 mg, 0.406 mmol) were dissolved in DCM (5 mL) and stirred for 20 minutes at RT. **34** (102 mg, 0.369 mmol) was then added as a solution in DCM (1 mL). The mixture is stirred for 20 h. Volatiles were removed *in vacuo* and the residue was purified by column chromatograpy. The title compound was obtained as a white solid (67 mg, 0.18 mmol, 49%). ^{1}H NMR (400 MHz, DMSO, 353 K) δ 7.37 – 7.03 (m, 5H), 4.24 (s, 2H), 4.08 – 3.59 (m, 2H), 3.37 – 3.11 (m, 2H), 2.93 – 2.54 (m, 3H), 2.30 (s, 2H), 1.31 (s, 9H), 1.03 (s, 9H). ^{13}C NMR (101 MHz, DMSO, 293 K) δ 169.88, 153.28, 137.94, 128.79, 127.77, 125.74, 78.59, 43.43, 30.44, 29.34, 27.54.

1-(3-Benzylpiperazin-1-yl)-3,3-dimethylbutan-1-one hydrochloride (**44**)

1-(3-Benzylpiperazin-1-yl)-3,3-dimethylbutan-1-one was synthesized from **43** (67 mg, 0.18 mmol) according to General Procedure 1. The crude residue (56 mg, 0.18 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

tert-Butyl 4-benzoyl-2-benzylpiperazine-1-carboxylate (**45**)

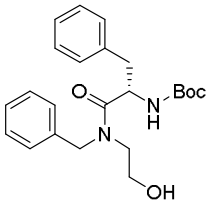


34 (120 mg, 0.434 mmol) was dissolved in DCM (5 mL) to which triethylamine (73 µL, 0.52 mmol) was added. Benzoyl chloride (55 µL, 0.48 mmol) was added and the mixture was stirred for 18 h. The reaction was quenched by the addition of 0.1 M aqueous HCl (5 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound (106 mg, 0.279 mmol, 64%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$, 323 K) δ 7.42 (m, 5H), 7.17 (m, 5H), 4.33 (s, 2H), 4.05 – 3.85 (m, 1H), 3.10 (m, 3H), 2.91 – 2.54 (m, 2H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$, 323 K) δ 171.10, 154.42, 137.91, 135.67, 129.99, 129.40, 128.62, 128.50, 127.33, 126.53, 80.25, 53.48, 39.07, 36.10, 28.33 (carbon spectrum shows a mixture of rotamers).

(3-Benzylpiperazin-1-yl)(phenyl)methanone hydrochloride (**46**)

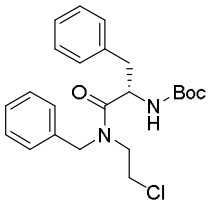
(3-Benzylpiperazin-1-yl)(phenyl)methanone was synthesized from **45** (106 mg, 0.279 mmol) according to General Procedure 1. The crude residue (88 mg, 0.28 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

tert-Butyl (*S*)-(1-(benzyl(2-hydroxyethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**47**)



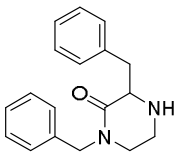
DIPEA (1.55 mL, 8.9 mmol) was added to a solution of Boc-L-phenylalanine (1.99 g, 7.5 mmol) in DCM (100 mL) on ice. HBTU (2.85 g, 7.5 mmol) was added and the mixture was stirred for 10 minutes. 2-Benzylaminoethanol (0.97 mL, 6.8 mmol) was added to the reaction mixture. The reaction mixture was stirred at RT for 24 h. The mixture was washed with 0.1 M HCl (aq., 100 mL) The water layer was extracted with EtOAc (3x 50 mL). The organic layers were combined and washed with 1 M NaOH (aq., 50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by column chromatography affording the title compound as a yellow oil (1.61 g, 4.0 mmol, 59%).^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 6.89 (m, 11H), 5.36 (dd, *J* = 40.4, 8.1 Hz, 1H), 5.07 – 4.74 (m, 1H), 4.34 (dd, *J* = 48.6, 16.0 Hz, 1H), 3.73 – 3.23 (m, 2H), 3.19 – 2.70 (m, 4H), 1.64 (s, 1H), 1.41 (s, 9H) (proton spectrum shows a mixture of rotamers).

tert-Butyl (*S*)-(1-(benzyl(2-chloroethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**48**)



Triethylamine (0.63 mL, 5.1 mmol) was added to a solution of **47** (0.88 g, 2.2 mmol) in DCM (20 mL) on ice. Methanesulfonylchloride (0.18 mL, 2.4 mmol) was added and the mixture was refluxed for 5 h. The mixture was washed with saturated aqueous $NaHCO_3$ (20 mL). The water layer was extracted with EtOAc (20 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified by column chromatography yielding the title compound (0.64 g, 1.5 mmol, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 6.93 (m, 10H), 5.62 (t, *J* = 9.9 Hz, 1H), 4.99 – 4.74 (m, 1H), 4.74 – 4.29 (m, 2H), 3.79 – 3.23 (m, 4H), 3.21 – 2.86 (m, 2H), 1.46 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.75, 172.64, 155.36, 155.07, 136.72, 136.58, 136.11, 129.65, 129.60, 128.99, 128.70, 128.64, 128.02, 127.93, 127.63, 127.07, 127.00, 79.91, 79.75, 52.44, 51.82, 49.44, 48.39, 48.26, 40.89, 39.97, 28.40 (carbon spectrum shows a mixture of rotamers).

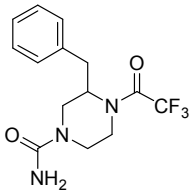
1,3-Dibenzylpiperazin-2-one (**49**)



48 (640 mg, 1.53 mmol) was dissolved in 1 M HCl in MeOH (20 mL) and stirred for 19 h. The volatiles were removed *in vacuo* and the residue was taken up in dry DMF (20 mL) and brought under argon atmosphere. K_2CO_3 (300 mg, 2.17 mmol) and a catalytic amount of tetrabutylammonium iodide were added and the temperature was raised to 80 °C. The mixture was stirred for 24 h. The mixture was diluted with water (20 mL). The aqueous phase was extracted with EtOAc (3x 40 mL), the combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography

affording the title compound as a white solid (150 mg, 0.54 mmol, 35%). ^{1}H NMR (300 MHz, MeOD) δ 7.67 – 7.02 (m, 10H), 4.62 (dt, *J* = 31.0, 7.0 Hz, 1H), 4.37 – 4.16 (m, 1H), 3.99 – 3.75 (m, 1H), 3.75 – 3.49 (m, 1H), 3.49 – 3.27 (m, 3H), 3.24 – 3.03 (m, 2H).

3-Benzyl-4-(2,2,2-trifluoroacetyl)piperazine-1-carboxamide (**50**)

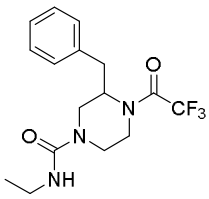


36 (156 mg, 0.573 mmol) and potassium cyanate (93 mg, 1.15 mmol) were dissolved in H_2O/AcOH (1:1, 6 mL) and stirred for 18 h at RT. The reaction was quenched by the addition of sat. aq. Na_2CO_3. The mixture was extracted with EtOAc (3x 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound as a white solid (91 mg, 0.29 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.13 (m, 5H), 5.24 (s, 2H), 4.81 – 4.66 (m, 1H), 4.51 – 4.01 (m, 2H), 3.80 (t, *J* = 13.4 Hz, 1H), 3.66 (dt, *J* = 13.6, 2.1 Hz, 1H), 3.50 (ddd, *J* = 14.3, 11.8, 3.5 Hz, 1H), 3.27 – 3.13 (m, 1H), 3.10 (dd, *J* = 13.7, 4.0 Hz, 1H), 3.04 – 2.80 (m, 4H) (proton spectrum shows a mixture of rotamers). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.67, 156.00 (q, *J* = 35.8 Hz), 155.71 (q, *J* = 36.0 Hz), 136.46 (d, *J* = 3.2 Hz), 129.39, 129.07, 128.86, 127.42, 127.20, 116.52 (q, *J* = 287.9 Hz), 116.25 (q, *J* = 288.0 Hz), 55.68 (d, *J* = 3.5 Hz), 53.53, 52.52, 44.66, 44.43, 43.16 (d, *J* = 4.1 Hz), 41.23 (q, *J* = 3.3 Hz), 38.37, 36.23, 35.20 (carbon spectrum shows a mixture of rotamers).

3-Benzylpiperazine-1-carboxamide (**51**)

3-Benzylpiperazine-1-carboxamide was synthesized from **50** (91 mg, 0.29 mmol) according to General Procedure 2. The crude residue (63 mg, 0.29 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

3-Benzyl-*N*-ethyl-4-(2,2,2-trifluoroacetyl)piperazine-1-carboxamide (**52**)

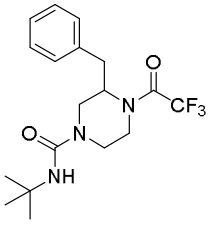


36 (112 mg, 0.411 mmol) was dissolved in DCM (5 mL) and isocyanatoethane (39 µL, 0.49 mmol) was added. The mixture was stirred for 18 h at RT. The reaction was quenched by the addition of 0.1 M HCl (aq., 10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to yield the title compound (141 mg, 0.411 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.15 (m, 5H), 4.81 – 4.40 (m, 2H), 4.24 – 4.07 (m, 1H), 3.98 – 3.66 (m, 1H), 3.64 – 3.42 (m, 1H), 3.42 – 3.12 (m, 3H), 3.08 – 2.78 (m, 4H), 1.16 (td, *J* = 7.2, 3.1 Hz, 3H) (proton spectrum shows a mixture of rotamers). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.47, 156.00 (q, *J* = 35.8 Hz), 136.70, 129.40, 129.06, 128.86, 127.41, 127.20, 116.57 (q, *J* = 288.1 Hz), 116.30 (q, *J* = 288.0 Hz), 55.70 (q, *J* = 3.0 Hz), 53.53, 52.54, 44.56, 44.19, 43.11, 43.00, 41.42 (q, *J* = 3.3 Hz), 38.54, 36.21, 35.91, 35.88, 35.09, 30.34, 29.74, 15.48 (carbon spectrum shows a mixture of rotamers).

3-Benzyl-*N*-ethylpiperazine-1-carboxamide (**53**)

3-Benzyl-*N*-ethylpiperazine-1-carboxamide was synthesized from **52** (141 mg, 0.41 mmol) according to General Procedure 2. The crude residue (102 mg, 0.41 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

3-Benzyl-*N*-(*tert*-butyl)-4-(2,2,2-trifluoroacetyl)piperazine-1-carboxamide (**54**)

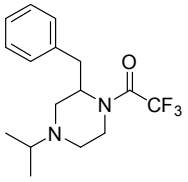


36 (114 mg, 0.419 mmol) was dissolved in DCM (6 mL) and isocyanatoethane (70 µL, 0.61 mmol) was added. The mixture was stirred for 18 h at RT. The reaction was quenched by the addition of 0.1 M HCl (aq., 10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to yield the title compound (156 mg, 0.419 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.12 (m, 5H), 4.75 – 4.64 (m, 1H), 4.47 – 4.09 (m, 2H), 3.82 (d, *J* = 14.0 Hz, 1H), 3.61 – 3.37 (m, 2H), 3.26 – 3.15 (m, 1H), 3.08 – 2.94 (m, 1H), 2.92 – 2.70 (m, 2H), 1.35 (s, 9H) (proton spectrum shows a mixture of rotamers). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.52, 156.48, 155.91 (q, *J* = 35.9 Hz), 136.75, 136.68, 129.32, 129.26, 129.07, 128.85, 127.38, 127.14, 116.53 (q, *J* = 287.8 Hz), 116.25 (q, *J* = 288.0 Hz), 55.50 (q, *J* = 2.6 Hz), 52.52, 51.02, 44.88, 44.36, 42.74, 42.60, 41.41 (q, *J* = 3.0 Hz), 38.56, 36.06, 34.91, 31.83, 30.26, 29.28 (carbon spectrum shows a mixture of rotamers).

3-Benzyl-*N*-(*tert*-butyl)piperazine-1-carboxamide (**55**)

3-Benzyl-*N*-(*tert*-butyl)piperazine-1-carboxamide was synthesized from **54** (156 mg, 0.419 mmol) according to General Procedure 2. The crude residue (119 mg, 0.419 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

1-(2-Benzyl-4-isopropylpiperazin-1-yl)-2,2,2-trifluoroethan-1-one (**56**)

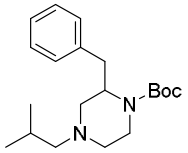


36 (194 mg, 0.71 mmol) and acetone (78 µL, 1.1 mmol) were dissolved in DCM (5 mL). The mixture was cooled to 0 °C and sodium triacetoxyborohydride (166 mg, 0.78 mmol) and acetic acid (49 µL, 0.86 mmol) were added. The mixture was stirred for 48 h at RT. The reaction was quenched by the addition of sat. aq. Na_2CO_3 (10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound (120 mg, 0.382 mmol, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.12 (m, 5H), 4.74 – 4.28 (m, 1H), 4.14 – 3.60 (m, 1H), 3.59 – 3.40 (m, 1H), 3.31 – 3.13 (m, 1H), 2.94 – 2.57 (m, 4H), 2.38 – 2.22 (m, 1H), 2.22 – 2.05 (m, 1H), 1.06 – 0.91 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.66 (q, *J* = 35.4 Hz), 155.45 (q, *J* = 35.4 Hz), 138.06, 137.92, 129.55, 129.44, 128.74, 128.54, 126.89, 126.65, 116.84 (q, *J* = 288.1 Hz), 116.58 (q, *J* = 288.2 Hz), 56.20 (d, *J* = 3.0 Hz), 54.54, 54.49, 52.83, 49.20, 48.77, 48.56, 48.32, 42.46 (d, *J* = 3.8 Hz), 39.62, 36.54, 35.40, 18.76, 18.70, 17.67, 17.58 (carbon spectrum shows a mixture of rotamers).

3-Benzyl-1-isopropylpiperazine (**57**)

3-Benzyl-1-isopropylpiperazine was synthesized from **56** (120 mg, 0.38 mmol) according to General Procedure 2. The crude residue (83 mg, 0.38 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

tert-Butyl 2-benzyl-4-isobutylpiperazine-1-carboxylate (**58**)

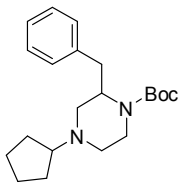


34 (53 mg, 0.19 mmol) and isobutyraldehyde (53 µL, 0.58 mmol) were dissolved in DCM (5 mL). The mixture was cooled to 0 °C and sodium triacetoxyborohydride (81 mg, 0.38 mmol) and acetic acid (24 µL, 0.42 mmol) were added. The mixture was stirred for 18 h at RT. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound (58 mg, 0.17 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.10 (m, 5H), 4.17 (s, 1H), 3.91 (s, 1H), 3.18 (td, *J* = 12.7, 3.5 Hz, 1H), 3.07 (d, *J* = 11.4 Hz, 1H), 2.97 – 2.69 (m, 2H), 2.60 (dt, *J* = 11.6, 1.9 Hz, 1H), 2.04 (dd, *J* = 12.0, 7.6 Hz, 1H), 2.00 – 1.87 (m, 3H), 1.72 (m, 1H), 1.39 (s, 9H), 0.94 (m, 6H) (proton spectrum shows a mixture of rotamers). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.83, 139.66, 129.52, 128.44, 126.18, 79.50, 66.99, 55.11, 53.74, 36.42, 28.46, 25.58, 21.01.

3-Benzyl-1-isobutylpiperazine hydrochloride (**59**)

3-Benzyl-1-isobutylpiperazine was synthesized from **58** (58 mg, 0.17 mmol) according to General Procedure 1. The crude residue (47 mg, 0.17 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

tert-Butyl 2-benzyl-4-cyclopentylpiperazine-1-carboxylate (**60**)

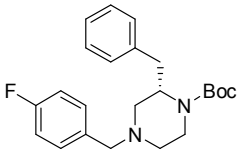


34 (50 mg, 0.18 mmol) and cyclopentanone (80 µL, 0.91 mmol) were dissolved in DCM (5 mL). The mixture was cooled to 0 °C and sodium triacetoxyborohydride (77 mg, 0.36 mmol) and acetic acid (23 µL, 0.40 mmol) were added. The mixture was stirred for 18 h at RT. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound (54 mg, 0.16 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.12 (m, 5H), 4.16 (s, 1H), 3.91 (s, 1H), 3.24 – 3.01 (m, 2H), 3.00 – 2.80 (m, 2H), 2.74 (d, *J* = 11.5 Hz, 1H), 2.52 – 2.33 (m, 1H), 1.95 (qd, *J* = 12.8, 12.2, 3.6 Hz, 2H), 1.83 (dtd, *J* = 11.5, 7.2, 2.8 Hz, 1H), 1.77 – 1.60 (m, 2H), 1.61 – 1.46 (m, 2H), 1.39 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.78, 139.65, 129.57, 128.45, 126.22, 79.55, 67.36, 53.18, 52.38, 36.42, 30.54, 30.29, 28.48, 24.15.

3-Benzyl-1-cyclopentylpiperazine hydrochloride (**61**)

3-Benzyl-1-cyclopentylpiperazine was synthesized from **60** (54 mg, 0.16 mmol) according to General Procedure 1. The crude residue (44 mg, 0.16 mmol, quant.) was of sufficient purity to be used without further purification in the next reaction, as judged by LC/MS analysis.

tert-Butyl (*S*)-2-benzyl-4-(4-fluorobenzyl)piperazine-1-carboxylate (**62**)

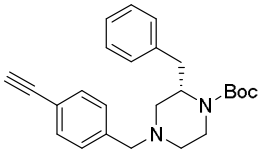


Commercial *tert*-butyl (S)-3-benzylpiperazine-1-carboxylate (50 mg, 0.18 mmol) and 4-fluorobenzaldehyde (21.4 µL, 0.199 mmol) were dissolved in DCM (5 mL). The mixture was cooled to 0 °C and sodium triacetoxyborohydride (46 mg, 0.22 mmol) and acetic acid (16 µL, 0.22 mmol) were added. The mixture was stirred for 18 h at RT. The reaction was quenched by the addition of sat. aq. Na_2CO_3 (10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound (30 mg, 0.08 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 6.91 (m, 9H), 4.13 (bs, 1H), 3.82 (bs, 1H), 3.51 (d, *J* = 13.0 Hz, 1H), 3.31 (d, *J* = 12.9 Hz, 1H), 3.20 (td, *J* = 12.8, 3.4 Hz, 1H), 3.12 – 2.94 (m, 1H), 2.93 – 2.74 (m, 2H), 2.60 (dt, *J* = 11.4, 1.9 Hz, 1H), 2.12 – 2.01 (m, 1H), 1.95 (dd, *J* = 11.5, 3.9 Hz, 1H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.13 (d, *J* = 245.0 Hz), 154.72, 139.20, 133.91, 130.76 (d, *J* = 7.9 Hz), 129.37, 128.31, 126.11, 115.06 (d, *J* = 21.3 Hz), 79.62, 62.13, 53.27, 28.35.

(*S*)-3-Benzyl-1-(4-fluorobenzyl)piperazine 2,2,2-trifluoroacetate (**63**)

62 (30 mg, 0.08 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C. 2,2,2-trifluoroacetic acid (1 mL, 13 mmol) was added and the mixture was stirred for 18 h at RT. The mixture was concentrated *in vacuo*. The residue was coevaporated with toluene (2x 5 mL). The residue was used without further purification in the next reactions.

tert-Butyl (*S*)-2-benzyl-4-(4-ethynylbenzyl)piperazine-1-carboxylate (**64**)

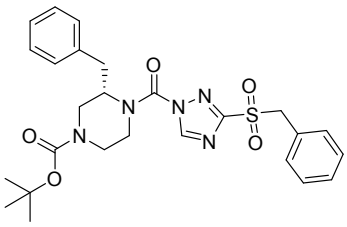


Commercial *tert*-butyl (S)-3-benzylpiperazine-1-carboxylate (50 mg, 0.18 mmol) and 4-ethynylbenzaldehyde (25.9 mg, 0.199 mmol) were dissolved in DCM (5 mL). The mixture was cooled to 0 °C and sodium triacetoxyborohydride (46 mg, 0.22 mmol) and acetic acid (16 µL, 0.22 mmol) were added. The mixture was stirred for 18 h at RT. The reaction was quenched by the addition of sat. aq. Na_2CO_3 (10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound (43 mg, 0.11 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.42 (m, 2H), 7.31 (d, *J* = 7.8 Hz, 2H), 7.24 – 6.92 (m, 5H), 4.37 – 3.67 (m, 2H), 3.52 (d, *J* = 13.1 Hz, 1H), 3.34 (d, *J* = 13.1 Hz, 1H), 3.20 (td, *J* = 12.5, 3.4 Hz, 1H), 3.08 (s, 1H), 3.01 (d, *J* = 12.4 Hz, 1H), 2.94 – 2.69 (m, 2H), 2.63 – 2.54 (m, 1H), 2.15 – 2.02 (m, 1H), 1.97 (dd, *J* = 11.6, 3.8 Hz, 1H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.42, 132.18, 129.47, 129.27, 128.43, 126.21, 121.03, 83.72, 79.67, 62.73, 53.47, 29.83, 28.45.

(*S*)-3-Benzyl-1-(4-ethynylbenzyl)piperazine 2,2,2-trifluoroacetate (**65**)

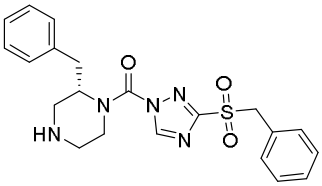
64 (43 mg, 0.11 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C. 2,2,2-trifluoroacetic acid (1 mL, 13 mmol) was added and the mixture was stirred for 18 h at RT. The mixture was concentrated *in vacuo*. The residue was coevaporated with toluene (2x 5 mL). The residue was used without further purification in the next reactions.

tert-Butyl (*S*)-3-benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazine-1-carboxylate (**66**)



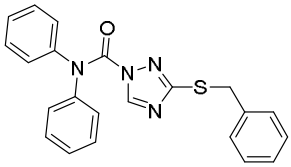
28 (178 mg, 0.796 mmol) was reacted with commercial *tert*-butyl (S)-3-benzylpiperazine-1-carboxylate (200 mg, 0.724 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (363 mg, 0.691 mmol, 95%). Spectral data in accordance with **3** (*vide infra*).

(*S*)-(2-Benzylpiperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone hydrochloride (**67**)



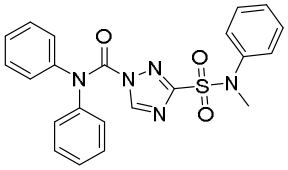
66 (363 mg, 0.691 mmol) was dissolved in diethylether (10 mL) and cooled to 0 °C. Hydrochloric acid (2 M in Et_2O, 1.7 mL, 3.4 mmol) was added and the mixture was stirred for 24 h at RT. The mixture was concentrated *in vacuo*. The residue was used without further purification in the next reactions.

3-(Benzylthio)-*N,N*-diphenyl-1*H*-1,2,4-triazole-1-carboxamide (**68**)



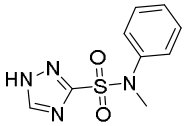
27 (2.137 g, 11.17 mmol) was dissolved in dry THF (40 mL) and diphenylcarbamic chloride (2.85 g, 12.3 mmol), DIPEA (2.34 mL, 13.4 mmol) and a catalytic amount of DMAP were added. The mixture was refluxed for 5 h and stirred 18 h at RT. The reaction was quenched by the addition of sat. aq. Na_2CO_3 (40 mL). The organic layer was separated and the aqueous phase extracted with EtOAc (2x 40 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated. Column chromatography afforded the title compound as a white solid (3.81 g, 9.86 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.46 – 7.10 (m, 15H), 3.86 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.81, 148.53, 147.18, 142.64, 136.61, 129.58, 129.04, 128.65, 127.60, 127.42, 126.80, 35.89.

3-(*N*-Methyl-*N*-phenylsulfamoyl)-*N,N*-diphenyl-1*H*-1,2,4-triazole-1-carboxamide (**69**)



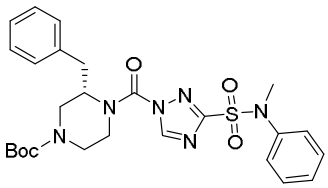
4 N HCl in dioxane (2 mL) was added to DCM (5 mL) and cooled to -10 °C. 15‰$_{wt}$ NaOCl (aq., 2 mL) was added slowly, forming a yellow-green Cl_2 solution. After 30 min **68** (100 mg, 0.259 mmol) was added dropwise as a solution in DCM (1 mL). After 20 min *N*-methylaniline (1 mL, 9 mmol) was added. The mixture was stirred for 2 h at RT. The reaction was quenched by the addition of 0.1 M HCl (10 mL). The organic layer was separated and the water layer was extracted with EtOAc (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound as a brown solid (78 mg, 0.18 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.47 – 6.99 (m, 15H), 3.07 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.95, 147.73, 147.28, 141.75, 140.35, 129.45, 128.87, 127.58, 127.47, 126.76, 126.40, 39.02.

N-Methyl-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (**70**)



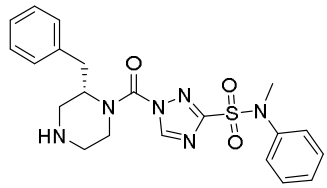
69 (1.05 g, 2.42 mmol) was dissolved in 2 N KOH in a 1:1 (v/v) H_2O/THF mixture (50 mL). The mixture was stirred for 18 h at RT. The mixture was concentrated and the residue was purified by column chromatography to yield the title compound (308 mg, 1.29 mmol, 53%). ^{1}H NMR (400 MHz, MeOD) δ 8.63 (s, 1H), 7.46 – 7.09 (m, 5H), 3.42 (s, 3H).

tert-Butyl (*S*)-3-benzyl-4-(3-(*N*-methyl-*N*-phenylsulfamoyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazine-1-carboxylate (**71**)



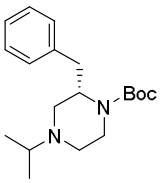
70 (30 mg, 0.13 mmol) was reacted with commercial *tert*-butyl (*S*)-3-benzylpiperazine-1-carboxylate (38.3 mg, 0.139 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (54 mg, 0.10 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (bs, 1H), 7.38 – 7.08 (m, 9H), 6.98 (d, *J* = 8.0 Hz, 1H), 4.97 – 4.47 (m, 1H), 4.44 – 3.83 (m, 3H), 3.49 (s, 3H), 3.42 (td, *J* = 13.1, 3.6 Hz, 1H), 3.28 – 2.59 (m, 4H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.79, 147.38, 140.53, 136.59, 129.31, 129.21, 128.91, 127.96, 127.22, 126.95, 80.87, 56.58, 40.60, 39.69, 35.88, 28.45.

(*S*)-1-(2-Benzylpiperazine-1-carbonyl)-*N*-methyl-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide 2,2,2-trifluoroacetate (**72**)



71 (54 mg, 0.10 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C. 2,2,2-trifluoroacetic acid (1 mL, 13 mmol) was added and the mixture was stirred for 5 h at RT. The mixture was concentrated *in vacuo*. The residue was coevaporated with toluene (2x 5 mL). The residue was used without further purification in the next reactions.

tert-Butyl (*S*)-2-benzyl-4-isopropylpiperazine-1-carboxylate (**73**)

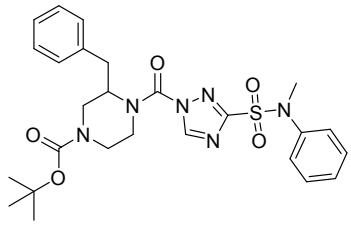


Commercial *tert*-butyl (*S*)-3-benzylpiperazine-1-carboxylate (100 mg, 0.362 mmol) and acetone (268 µL, 3.62 mmol) were dissolved in DCM (10 mL). The mixture was cooled to 0 °C and sodium triacetoxyborohydride (153 mg, 0.724 mmol) and acetic acid (46 µL, 0.80 mmol) were added. The mixture was stirred for 18 h at RT. The reaction was quenched by the addition of sat. aq. Na_2CO_3 (10 mL). The organic layer was separated and the aqueous phase was extracted with DCM (2x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography to yield the title compound as a white solid (88 mg, 0.28 mmol, 76%). HPLC/MS: Calculated for $[C_{19}H_{30}N_2O_2 + H]^+$ = 319.24, found = 319.07.

(*S*)-3-Benzyl-1-isopropylpiperazine 2,2,2-trifluoroacetate (**74**)

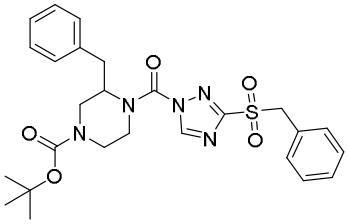
73 was dissolved in DCM (5 mL) and TFA (1 mL) was added. The mixture was stirred for 6 h at RT, when TLC showed full conversion of the starting material. The reaction mixture was concentrated *in vacuo* and used without further purification for the next reaction.

tert-Butyl 3-benzyl-4-(3-(*N*-methyl-*N*-phenylsulfamoyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazine-1-carboxylate (**2**)



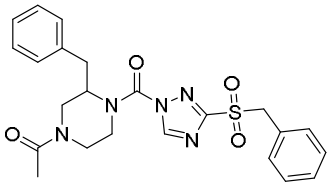
70 (50 mg, 0.21 mmol) was reacted with **38** (124 mg, 0.449 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (41 mg, 0.076 mmol, 36%). ^{1}H NMR (400 MHz, $CDCl_3$, 328K) δ 8.19 (bs, 1H), 7.49 – 6.81 (m, 10H), 4.74 (s, 1H), 4.09 (m, 3H), 3.48 (m, 3H), 3.46 – 3.34 (m, 1H), 3.19 – 2.68 (m, 4H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$, 333K) δ 161.53, 154.89, 147.62, 140.92, 136.80, 129.40, 129.34, 128.93, 127.89, 127.24, 127.01, 80.88, 56.52, 53.46, 43.13, 39.69, 36.06, 28.54. HRMS: Calculated for $[C_{26}H_{32}N_6O_5S + Na]^+$ = 563.2047, found = 563.2053.

tert-Butyl 3-benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazine-1-carboxylate (**3**)



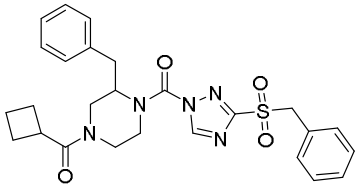
28 (150 mg, 0.67 mmol) was reacted with **38** (195 mg, 0.706 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (254 mg, 0.483 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.45 – 7.13 (m, 9H), 7.00 (s, 1H), 4.77 – 4.46 (m, 3H), 4.39 – 3.73 (m, 3H), 3.48 – 3.28 (m, 1H), 3.18 – 2.60 (m, 4H), 1.51 (s, 9H). HRMS: Calculated for $[C_{26}H_{31}N_5O_5S + Na]^+$ = 548.1938, found = 548.1945.

1-(3-Benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazin-1-yl)ethan-1-one (**4**)



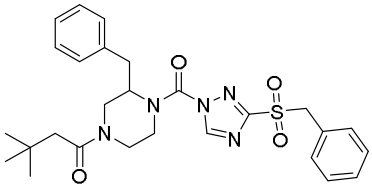
28 (49 mg, 0.22 mmol) was reacted with **40** (51 mg, 0.20 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (67 mg, 0.143 mmol, 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 – 8.47 (m, 1H), 7.51 – 6.90 (m, 10H), 4.91 (s, 2H), 4.69 – 4.21 (m, 2H), 4.03 (q, *J* = 7.1 Hz, 1H), 3.74 (d, *J* = 13.9 Hz, 1H), 3.60 – 3.36 (m, 1H), 3.24 (t, *J* = 12.6 Hz, 1H), 2.98 – 2.66 (m, 3H), 2.14 (s, 1H), 1.99 (s, 2H) (spectrum shows a mixture of rotamers). HRMS: Calculated for $[C_{23}H_{25}N_5O_4S + H]^+$ = 468.1699, found = 468.1699.

(3-Benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazin-1-yl)(cyclobutyl)methanone (**5**)



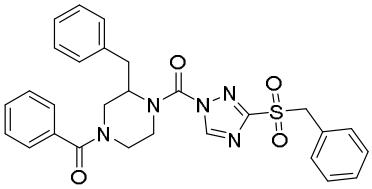
28 (62 mg, 0.28 mmol) was reacted with **42** (76 mg, 0.29 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (46 mg, 0.091 mmol, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 – 7.79 (m, 1H), 7.53 – 6.72 (m, 10H), 4.97 – 4.68 (m, 1H), 4.70 – 4.51 (m, 3H), 4.22 – 4.04 (m, 1H), 3.94 – 3.53 (m, 1H), 3.54 – 3.09 (m, 3H), 3.07 – 2.51 (m, 3H), 2.51 – 1.79 (m, 6H) (spectrum shows a mixture of rotamers). HRMS: Calculated for $[C_{27}H_{33}N_5O_4S + H]^+$ = 524.232, found = 524.2328.

1-(3-Benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazin-1-yl)-3,3-dimethylbutan-1-one (**6**)



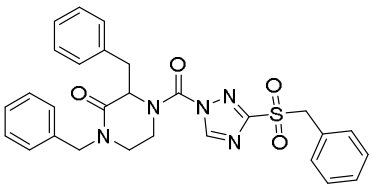
28 (60 mg, 0.27 mmol) was reacted with **44** (78 mg, 0.28 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (88 mg, 0.17 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.45 – 6.84 (m, 10H), 5.04 – 4.74 (m, 1H), 4.73 – 4.53 (m, 3H), 4.24 – 3.99 (m, 1H), 3.99 – 3.72 (m, 1H), 3.59 – 3.18 (m, 2H), 3.17 – 2.98 (m, 1H), 2.98 – 2.60 (m, 2H), 2.44 – 2.07 (m, 2H), 1.18 – 0.96 (m, 9H) (spectrum shows a mixture of rotamers). HRMS: Calculated for $[C_{27}H_{33}N_5O_4S + H]^+$ = 524.2325, found = 524.2328.

(4-Benzoyl-2-benzylpiperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**7**)



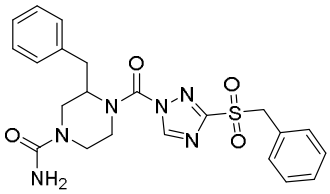
28 (53 mg, 0.24 mmol) was reacted with **46** (70 mg, 0.25 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (26.2 mg, 0.049 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.61 – 6.57 (m, 15H), 4.71 (bs, 1H), 4.65 – 4.51 (m, 2H), 4.31 – 4.03 (m, 1H), 4.01 – 3.66 (m, 1H), 3.62 – 3.29 (m, 2H), 3.29 – 2.84 (m, 3H) (spectrum shows a mixture of rotamers). HRMS: Calculated for $[C_{28}H_{27}N_5O_4S + H]^+$ = 530.1856, found = 530.1857.

1,3-Dibenzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazin-2-one (**8**)



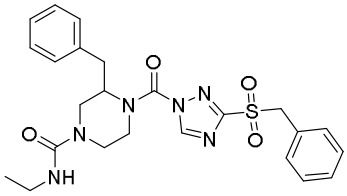
28 (53 mg, 0.24 mmol) was reacted with **49** (70 mg, 0.25 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (118 mg, 0.223 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.52 – 7.02 (m, 14H), 6.92 (s, 1H), 5.61 (s, 1H), 5.10 (s, 1H), 4.81 – 4.51 (m, 2H), 4.45 – 4.04 (m, 2H), 3.41 (m, 2H), 3.31 – 3.04 (m, 2H), 2.92 – 2.66 (m, 1H). HRMS: Calculated for $[C_{28}H_{27}N_5O_4S + H]^+$ = 530.1856, found = 530.1859.

3-Benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)piperazine-1-carboxamide (**9**)



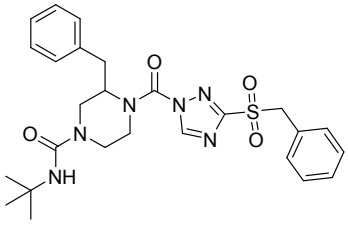
28 (61 mg, 0.27 mmol) was reacted with **51** (63 mg, 0.29 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (81.3 mg, 0.174 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.43 – 7.11 (m, 9H), 6.99 (s, 1H), 5.01 (s, 2H), 4.78 – 4.48 (m, 3H), 4.26 – 4.05 (m, 1H), 3.91 (m, 1H), 3.75 (d, *J* = 13.7 Hz, 1H), 3.43 (t, *J* = 12.5 Hz, 1H), 3.15 (dd, *J* = 13.7, 3.8 Hz, 1H), 3.09 – 2.89 (m, 2H), 2.86 – 2.71 (m, 1H). HRMS: Calculated for $[C_{22}H_{24}N_6O_4S + H]^+$ = 469.1652, found = 469.1658.

3-Benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)-*N*-ethylpiperazine-1-carboxamide (**10**)



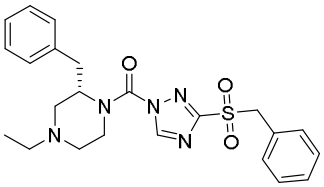
28 (87 mg, 0.39 mmol) was reacted with **53** (102 mg, 0.41 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (140 mg, 0.283 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.42 – 7.11 (m, 9H), 7.00 (s, 1H), 4.84 – 4.52 (m, 4H), 4.32 – 4.04 (m, 1H), 3.93 (d, *J* = 18.4 Hz, 1H), 3.72 (d, *J* = 13.6 Hz, 1H), 3.51 – 3.36 (m, 1H), 3.34 – 3.16 (m, 2H), 3.17 – 2.87 (m, 3H), 2.87 – 2.75 (m, 1H), 1.15 (t, *J* = 7.2 Hz, 3H). HRMS: Calculated for $[C_{24}H_{28}N_6O_4S + H]^+$ = 497.1965, found = 497.1969.

3-Benzyl-4-(3-(benzylsulfonyl)-1*H*-1,2,4-triazole-1-carbonyl)-*N*-(*tert*-butyl)piperazine-1-carboxamide (**11**)



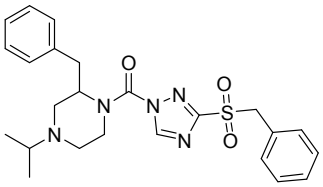
28 (92 mg, 0.41 mmol) was reacted with **55** (119 mg, 0.43 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (184 mg, 0.351 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.44 – 7.15 (m, 9H), 7.05 (s, 1H), 4.73 – 4.49 (m, 3H), 4.40 – 4.01 (m, 2H), 3.97 – 3.80 (m, 1H), 3.56 (d, *J* = 13.2 Hz, 1H), 3.41 (d, *J* = 12.3 Hz, 1H), 3.14 – 2.95 (m, 2H), 2.95 – 2.77 (m, 2H), 1.35 (s, 9H). HRMS: Calculated for $[C_{26}H_{32}N_6O_4S + H]^+$ = 525.2278, found = 525.2286.

(2-Benzyl-4-ethylpiperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**12**)



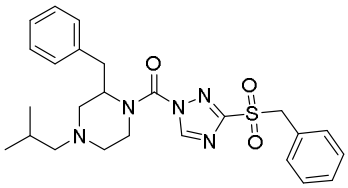
67 (60 mg, 0.13 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C. Acetaldehyde (8.0 µL, 0.14 mmol), acetic acid (9.1 µL, 0.16 mmol) and sodium triacetoxyhydroborate (33.0 mg, 0.156 mmol) were added and the mixture was stirred for 18 h warming up to RT. The reaction was quenched with saturated aqueous Na_2CO_3 and the organic layer was separated. The water layer was extracted twice with DCM. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Column chromatography yielded the title compound as a white solid (20 mg, 44 µmol, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.54 – 6.82 (m, 10H), 4.73 – 4.49 (m, 3H), 4.21 – 3.82 (m, 1H), 3.52 (t, *J* = 12.9 Hz, 1H), 3.38 – 3.16 (m, 1H), 3.03 (s, 1H), 2.93 – 2.68 (m, 2H), 2.60 – 2.00 (m, 4H), 1.10 (t, *J* = 7.3 Hz, 3H). HRMS: Calculated for $[C_{23}H_{27}N_5O_3S + H]^+$ = 454.1907, found = 454.1909.

(2-Benzyl-4-isopropylpiperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**13**)



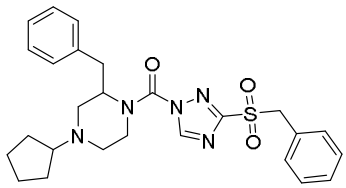
28 (81 mg, 0.36 mmol) was reacted with **57** (83 mg, 0.38 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (66.9 mg, 0.143 mmol, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.25 (m, 9H), 7.07 – 6.91 (m, 1H), 4.66 – 4.49 (m, 3H), 4.19 – 3.82 (m, 1H), 3.47 (t, *J* = 12.5 Hz, 1H), 3.28 (t, *J* = 11.4 Hz, 1H), 3.02 – 2.61 (m, 4H), 2.53 – 2.18 (m, 2H), 1.08 – 0.92 (m, 6H). HRMS: Calculated for $[C_{24}H_{29}N_5O_3S + H]^+$ = 468.2063, found = 468.2066.

(2-Benzyl-4-isobutylpiperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**14**)



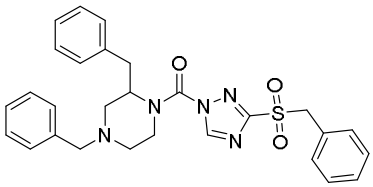
28 (43 mg, 0.19 mmol) was reacted with **59** (40 mg, 0.17 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (63.6 mg, 0.132 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.44 – 6.83 (m, 10H), 4.71 – 4.41 (m, 3H), 4.21 – 3.69 (m, 1H), 3.51 (td, *J* = 12.9, 3.5 Hz, 1H), 3.36 – 3.20 (m, 1H), 3.08 – 2.66 (m, 3H), 2.33 – 1.85 (m, 4H), 1.85 – 1.54 (m, 1H), 0.95 (d, *J* = 6.5 Hz, 6H). HRMS: Calculated for $[C_{25}H_{31}N_5O_3S + H]^+$ = 482.2220, found = 482.2225.

(2-Benzyl-4-cyclopentylpiperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**15**)



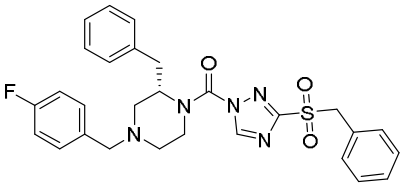
28 (39 mg, 0.17 mmol) was reacted with **61** (38 mg, 0.16 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (20.5 mg, 0.042 mmol, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.43 – 6.90 (m, 10H), 4.69 – 4.47 (m, 3H), 4.34 – 3.84 (m, 1H), 3.71 – 2.65 (m, 6H), 2.62 – 1.28 (m, 10H) (spectrum shows a mixture of rotamers). HRMS: Calculated for $[C_{26}H_{31}N_5O_3S + H]^+$ = 494.2220, found = 494.2223.

(3-(Benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)(2,4-dibenzylpiperazin-1-yl)methanone (**16**)



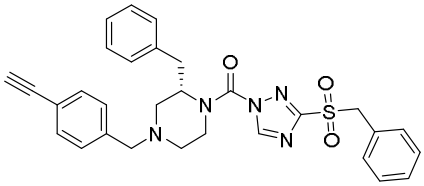
28 (46.1 mg, 0.206 mmol) was reacted with **32** (50 mg, 0.19 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (85 mg, 0.17 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.45 – 7.19 (m, 11H), 7.19 – 6.73 (m, 5H), 4.60 (m, 3H), 4.12 (q, *J* = 7.0 Hz, 1H), 3.64 – 3.37 (m, 3H), 3.29 (t, *J* = 11.3 Hz, 1H), 3.01 (d, *J* = 11.9 Hz, 1H), 2.79 (dd, *J* = 28.0, 9.1 Hz, 2H), 2.34 – 2.07 (m, 2H). HRMS: Calculated for $[C_{28}H_{29}N_5O_3S + H]^+$ = 516.2063, found = 516.2070.

(*S*)-(2-Benzyl-4-(4-fluorobenzyl)piperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**17**)



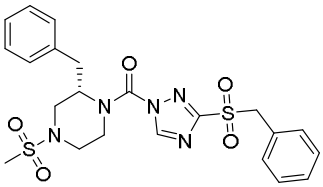
28 (19 mg, 85 µmol) was reacted with **63** (22 mg, 77 µmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (33 mg, 62 µmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.42 – 6.81 (m, 14H), 4.60 (m, 3H), 4.23 – 3.83 (m, 1H), 3.62 – 3.12 (m, 4H), 2.99 (d, *J* = 11.8 Hz, 1H), 2.76 (dd, *J* = 26.6, 9.8 Hz, 2H), 2.37 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.33 (d, *J* = 245.7 Hz), 160.66, 147.88, 147.61, 137.47, 133.35 (d, *J* = 3.2 Hz), 131.14, 130.74, 128.97, 128.87, 127.00, 126.37, 115.37 (d, *J* = 21.3 Hz), 77.36, 61.88, 60.65, 56.96, 55.45, 55.06, 53.02, 52.52, 43.66, 41.47, 36.74, 35.95. HRMS: Calculated for $[C_{28}H_{28}FN_5O_3S + H]^+$ = 534.1969, found = 534.1975.

(*S*)-(2-Benzyl-4-(4-ethynylbenzyl)piperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**18**)



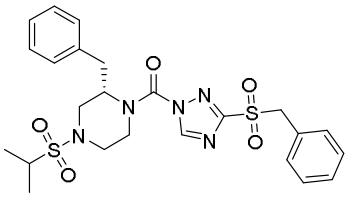
28 (27 mg, 0.12 mmol) was reacted with **65** (32 mg, 0.11 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (37 mg, 69 µmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.56 – 6.83 (m, 14H), 4.60 (m, 3H), 4.05 (m, 1H), 3.71 – 3.14 (m, 4H), 3.10 (s, 1H), 2.99 (s, 1H), 2.76 (dd, *J* = 26.1, 11.7 Hz, 2H), 2.27 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.62, 148.16, 147.86, 147.58, 138.57, 137.39, 132.30, 131.10, 129.22, 129.09, 128.94, 128.85, 126.98, 126.31, 121.38, 83.47, 77.32, 62.27, 60.61, 56.91, 55.14, 53.06, 52.54, 43.63, 41.42, 36.69, 35.78, 30.39. HRMS: Calculated for $[C_{30}H_{29}N_5O_3S + H]^+$ = 540.2063, found = 540.2062.

(*S*)-(2-Benzyl-4-(methylsulfonyl)piperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**19**)



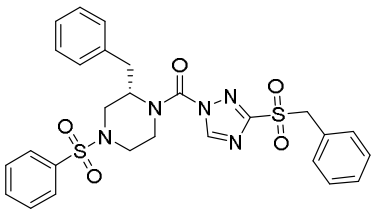
67 (60 mg, 0.13 mmol) was dissolved in DCM (5 mL). Mesyl chloride (12 µL, 0.16 mmol) and Hünig's base (68 µL, 0.39 mmol) were added. The mixture was stirred for 20 h at RT. The reaction mixture was concentrated under reduced pressure and purified by column chromatography to afford the title compound as a white solid (30 mg, 59 µmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.44 – 6.85 (m, 10H), 4.63 (s, 3H), 4.35 – 3.83 (m, 2H), 3.80 – 3.63 (m, 1H), 3.61 – 3.32 (m, 1H), 3.21 (s, 1H), 2.83 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.92, 148.01, 147.84, 136.24, 131.14, 129.34, 129.03, 127.46, 126.34, 77.36, 60.58, 60.51, 56.18, 53.58, 48.15, 45.31, 40.53, 35.80, 34.85, 29.80. HRMS: Calculated for $[C_{22}H_{25}N_5O_5S_2 + Na]^+$ = 526.1189, found = 526.1191.

(*S*)-(2-Benzyl-4-(isopropylsulfonyl)piperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**20**)



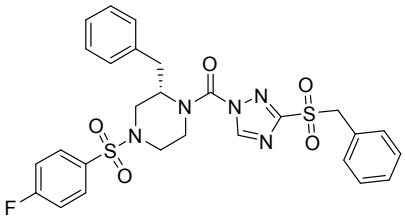
67 (68 mg, 0.15 mmol) was dissolved in DCM (5 mL) to which propane-2-sulfonyl chloride (36 µL, 0.18 mmol) and DIPEA (77 µL, 0.44 mmol) were added. The mixture was stirred for 20 h at RT. The reaction mixture was concentrated under reduced pressure and purified by column chromatography to afford the title compound as a white solid (31.8 mg, 60 µmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.43 – 6.86 (m, 10H), 4.72 – 4.52 (m, 3H), 4.15 (d, *J* = 13.7 Hz, 1H), 4.01 (d, *J* = 13.0 Hz, 1H), 3.79 (d, *J* = 12.9 Hz, 1H), 3.58 – 3.40 (m, 1H), 3.37 – 2.89 (m, 4H), 2.79 (d, *J* = 14.2 Hz, 1H), 1.40 – 1.33 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.88, 148.29, 147.65, 136.45, 131.15, 129.37, 129.28, 129.05, 127.43, 126.44, 77.36, 60.57, 56.54, 53.82, 48.88, 45.68, 41.10, 35.80, 16.95, 16.88. HRMS: Calculated for $[C_{24}H_{29}N_5O_5S_2 + Na]^+$ = 554.1502, found = 554.1511.

(*S*)-(2-Benzyl-4-(phenylsulfonyl)piperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**21**)



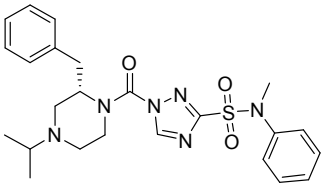
67 (60 mg, 0.13 mmol) was dissolved in DCM (5 mL). Benzenesulfonyl chloride (20 µL, 0.16 mmol) and DIPEA (68 µL, 0.39 mmol) were added. The mixture was stirred for 20 h at RT. The reaction mixture was concentrated under reduced pressure and purified by column chromatography to afford the title compound as a white solid (21.2 mg, 37 µmol, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.86 – 6.87 (m, 15H), 4.80 – 4.51 (m, 3H), 4.26 – 4.08 (m, 1H), 4.08 – 3.86 (m, 1H), 3.86 – 3.40 (m, 2H), 3.38 – 3.15 (m, 1H), 3.05 – 2.79 (m, 1H), 2.60 – 2.24 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.89, 147.91, 147.74, 136.33, 135.02, 133.61, 131.13, 129.81, 129.56, 129.40, 129.33, 129.13, 129.01, 127.83, 127.46, 77.36, 60.54, 56.10, 54.81, 48.59, 46.59, 45.57, 42.58, 40.39, 36.06. HRMS: Calculated for $[C_{27}H_{27}N_5O_5S_2 + Na]^+$ = 588.1346, found = 588.1355.

(*S*)-(2-Benzyl-4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)(3-(benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (**22**)



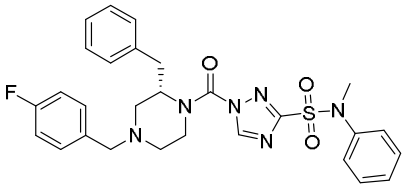
67 (68 mg, 0.15 mmol) was dissolved in DCM (5 mL). 4-fluorobenzenesulfonyl chloride (43 µL, 0.18 mmol) and DIPEA (77 µL, 0.44 mmol) were added. The mixture was stirred for 20 h at RT. The reaction mixture was concentrated under reduced pressure and purified by column chromatography to afford the title compound as a white solid (52 mg, 89 µmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.82 – 6.80 (m, 14H), 4.79 – 4.51 (m, 3H), 4.20 (d, *J* = 13.7 Hz, 1H), 3.94 (d, *J* = 11.7 Hz, 1H), 3.78 – 3.41 (m, 2H), 3.39 – 3.16 (m, 1H), 3.03 – 2.84 (m, 1H), 2.55 – 2.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.58 (d, *J* = 256.4 Hz), 160.86, 148.32, 147.70, 136.15, 131.05, 130.49 (d, *J* = 9.4 Hz), 129.72, 129.29, 129.23, 129.02, 128.92, 127.38, 126.21, 116.80 (d, *J* = 22.6 Hz), 77.27, 60.44, 55.97, 48.42, 46.41, 45.48, 40.22, 35.93. HRMS: Calculated for $[C_{27}H_{26}FN_5O_5S_2 + Na]^+$ = 606.1251, found = 606.1258.

(*S*)-1-(2-Benzyl-4-isopropylpiperazine-1-carbonyl)-*N*-methyl-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (**23**)



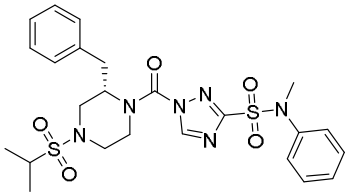
70 (60 mg, 0.25 mmol) was reacted with **72** (92 mg, 0.28 mmol) according to General Procedure 3. Column chromatography afforded the title compound as a white solid (56 mg, 0.12 mmol, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.37 – 6.88 (m, 10H), 4.81 – 4.47 (m, 1H), 4.14 (d, *J* = 13.1 Hz, 1H), 3.56 – 3.44 (m, 4H), 3.34 – 3.18 (m, 1H), 3.07 – 2.87 (m, 1H), 2.87 – 2.64 (m, 3H), 2.55 – 2.14 (m, 2H), 1.13 – 0.87 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.77, 148.18, 147.86, 147.27, 140.64, 137.85, 129.59, 129.32, 128.82, 127.93, 127.02, 126.89, 57.01, 55.42, 54.52, 50.70, 49.20, 48.60, 44.10, 41.84, 39.73, 36.71, 35.74, 18.77, 17.76.

(*S*)-1-(2-Benzyl-4-(4-fluorobenzyl)piperazine-1-carbonyl)-*N*-methyl-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (**24**)



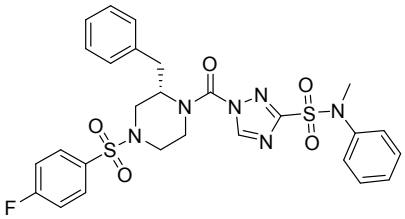
70 (98 mg, 0.18 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C. 4-Fluorobenzaldehyde (27 µL, 0.25 mmol), acetic acid (12 µL, 0.21 mmol) and sodium triacetoxyhydroborate (45 mg, 0.21 mmol) were added and the mixture was stirred for 18 h warming up to RT. The reaction was quenched with saturated aqueous Na_2CO_3 and the organic layer was separated. The water layer was extracted twice with DCM. The combined organic layers were dried ($MgSO_4$) and concentrated. Column chromatography yielded the title compound as a white solid (9.4 mg, 17 µmol, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.47 – 6.77 (m, 14H), 4.83 – 4.48 (m, 1H), 4.32 – 4.06 (m, 1H), 3.64 – 3.12 (m, 6H), 3.12 – 1.86 (m, 6H). HRMS: Calculated for $[C_{28}H_{29}FN_6O_3S + H]^+$ = 549.2078, found = 549.2090.

(*S*)-1-(2-Benzyl-4-(isopropylsulfonyl)piperazine-1-carbonyl)-*N*-methyl-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (**25**)



70 (205 mg, 0.37 mmol) was dissolved in DCM (10 mL). Propane-2-sulfonyl chloride (50 µL, 0.44 mmol) and Hünig's base (194 µL, 1.11 mmol) were added and the reaction mixture was stirred for 18 h at RT. The mixture was concentrated under reduced pressure and purified by column chromatography to yield the title compound as a white solid (127 mg, 232 µmol, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.44 – 6.80 (m, 10H), 4.91 – 4.54 (m, 1H), 4.37 – 4.10 (m, 1H), 4.05 – 3.71 (m, 2H), 3.49 (s, 4H), 3.37 – 2.90 (m, 4H), 2.87 – 2.75 (m, 1H), 1.42 – 1.29 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.93, 148.39, 147.27, 140.41, 136.43, 129.62, 129.27, 129.14, 128.90, 127.95, 127.23, 126.87, 77.33, 61.85, 56.42, 54.95, 53.61, 48.64, 47.00, 45.57, 43.56, 40.93, 39.67, 35.73, 34.96, 16.82, 16.76.

(*S*)-1-(2-Benzyl-4-((4-fluorophenyl)sulfonyl)piperazine-1-carbonyl)-*N*-methyl-*N*-phenyl-1*H*-1,2,4-triazole-3-sulfonamide (**26**)



70 (55 mg, 0.10 mmol) was dissolved in DCM (5 mL). 4-Fluorobenzene-sulfonyl chloride (23 mg, 0.12 mmol) and Hünig's base (52 µL, 0.30 mmol) were added and the reaction mixture was stirred for 18 h at RT. The mixture was concentrated under reduced pressure and purified by column chromatography to yield the title compound as a white solid (44.5 mg, 74 µmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.81 – 6.87 (m, 14H), 5.03 – 4.52 (m, 1H), 4.50 – 4.16 (m, 1H), 3.94 (d, *J* = 11.6 Hz, 1H), 3.82 – 3.53 (m, 2H), 3.48 (s, 3H), 3.40 – 3.16 (m, 1H), 3.08 – 2.80 (m, 1H), 2.63 – 2.27 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.65 (d, *J* = 256.2 Hz), 161.21, 148.18, 147.48, 140.44, 136.29, 131.13, 130.57 (d, *J* = 9.4 Hz), 129.37, 129.07, 128.08, 127.42, 127.07, 116.88 (d, *J* = 22.7 Hz), 56.03, 54.60, 48.54, 46.52, 45.57, 42.53, 40.25, 39.85, 36.07, 35.05. HRMS: Calculated for $[C_{27}H_{27}FN_6O_5S_2 + Na]^+$ = 621.1360, found = 621.1368.

References

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. 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).
3. Hsu, K. *et al.* DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat. Chem. Biol.* **8,** 999–1007 (2012).
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. 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).
6. Janssen, F. J. & van der Stelt, M. Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders. *Bioorg. Med. Chem. Lett.* **26,** 3831–3837 (2016).
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. European Medicines Agency. The European Medicines Agency recommends suspension of the marketing authorisation of Acomplia. https://www.ema.europa.eu/en/news/european-medicines-agency-recommends-suspension-marketing-authorisation-acomplia (2008).
9. Hitchcock, S. A. & Pennington, L. D. Structure−Brain Exposure Relationships. *J. Med. Chem.* **49,** 7559–7583 (2006).
10. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci.* **113,** 26–33 (2016).
11. 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. Chemie Int. Ed.* **52,** 12081–12085 (2013).
12. Patricelli, M. P., Lovato, M. A. & Cravatt, B. F. Chemical and mutagenic investigations of fatty acid amide hydrolase: Evidence for a family of serine hydrolases with distinct catalytic properties. *Biochemistry* **38,** 9804–9812 (1999).
13. 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).
14. Gottlieb, H. E., Kotlyar, V. & Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. *J. Org. Chem.* **62,** 7512–7515 (1997).