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Author: Janssen, Freek J.
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CHAPTER 5

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

Introduction

Diacylglycerol lipases (DAGL) are responsible for the synthesis of the endogenous signaling lipid 2-arachidonoylglycerol (2-AG) in the brain and immune system. 2-AG activates the cannabinoid type 1 receptor (CB1R), which is also the target of Δ⁹-tetrahydrocannabinol, the main psychoactive compound in marijuana. 2-AG functions as a retrograde messenger and modulates synaptic plasticity at both GABAergic and glutamatergic synapses by inhibiting neurotransmitter release in various regions of the central nervous system. It is involved in a broad array of neurophysiological functions, such as adult neurogenesis, modulation of reward-related behavior, memory formation, pain sensation and appetite. Activation of CB1R via 2-AG has been implicated in various disorders, including obesity and nicotine addiction. In addition, 2-AG is a precursor for pro-inflammatory prostaglandins in animal models of neuroinflammation. As a consequence, inhibitors of 2-AG biosynthesis may become useful for therapeutic intervention in various human diseases. DAGLs are intracellular, multi-domain integral membrane proteins that produce 2-AG. Two homologous isoforms are known: sn-1 diacylglycerol lipase-α (DAGLα) and sn-1 diacylglycerol lipase-β (DAGLβ). The DAG lipases share extensive homology, but differ in size: ~120 and ~70 kD for DAGLα and DAGLβ respectively. DAG lipases employ the typical Ser-His-Asp catalytic triad to specifically hydrolyze the ester bond of sn-1 acyl chains from arachidonate-containing diacylglycerols. Studies with DAGL-KO mice have shown that DAGLα controls to a large extent the formation of 2-AG in the central nervous system whereas DAGLβ partakes in 2-AG production in the periphery during inflammation.

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

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

Recently, researchers from Bristol-Myers Squibb disclosed a new reversible class of DAGLα inhibitors. They performed a high throughput screening campaign and prioritized the glycine sulfonamide series as an initial lead for chemical optimization (see also Chapter 6). Compounds 2a-c (Figure 1) were shown to be active on recombinant human DAGL and were selective over pancreatic lipase and monoacylglycerol (MAG) lipase, the main enzyme in the central nervous system that hydrolyzes 2-AG. Due to the lack of an obvious serine hydrolase warhead, the structure-activity relationships of this novel chemotype were investigated, using 2c as a starting point. In addition, a broader selectivity profile over the serine hydrolase family in native brain proteome was not available.
Here, the first study of structure-activity relationships of glycine sulfonamide DAGLα inhibitors is reported, together with the assessment of the selectivity profile over brain membrane proteome using comparative ABPP with MB064 (1) and TAMRA-FP. Chemical optimization of the sulfonamide series led to the discovery of 15, which is a potent DAGLα inhibitor. This study confirmed that glycine sulfonamides are selective over MAGL, but ABPP assays revealed that 15 is also a potent ABHD6 inhibitor. Since ABHD6, a general lysophospholipid hydrolase, is strongly linked to high-fat-diet induced obesity, hepatic steatosis and insulin resistance,17 this dual inhibition profile may be beneficial for the treatment of obesity-related disorders.

Results

To systematically investigate the structure-activity relationships of glycine sulfonamide series, a general synthetic scheme was developed that allowed for rapid variation of different parts of the molecule. Compound 2c, with a 2,2-dimethylchroman substituent was used as a starting point, because this was one of the most potent inhibitors reported by BMS.21 First, the biphenyl substituent was replaced by biarylethers (13-17), which are other well-known fatty acid tail bio-isosters used in clinical candidates (e.g. PF-04457845).22 To probe the size of the binding site, analogs were synthesized with the ether substituent at ortho (13), meta (14, 16) and para (15, 17) position of the first phenyl ring. Second, to reduce the polar surface area of the molecules, the sulfonamide was replaced by an amide (19 vs 23; 20 vs 22 and 24 vs 25). Of note, chlorophenyl substituents at R8 were used, because they are metabolically stable analogs of the tolyl substituent in compound 2b and are commercially available as both a sulfonoyl chlorides and acyl chlorides. Finally, since there is no obvious serine hydrolase warhead present in the starting molecule 2c, the contribution of the carboxylic acid to its activity was investigated by replacing it by ester (26), alcohol (27), carbamate (28) amide (29, 30), nitrile (32), amine (33) and tetrazole (34) functionalities. To this end, reductive amination of glycine methyl ester with benzaldehydes 3-7 was performed, followed by a (sulfon)amide coupling using different acyl chlorides (4-chlorobenzoyl- and 2,6-dichlorobenzoyl chloride) and sulfonyl chlorides (4-chlorophenyl-, 2,6-dichlorophenyl- or 2,2-dimethylchroman-6-sulfonyl chloride). Hydrolysis of the glycine ester yielded final compounds 13-25.
Scheme 1. Synthesis of glycine sulfonamide derivatives 13-25. a) Methyl glycinate, AcOH, NaCNBH$_3$, MeOH, 3Å molecular sieves. rt, 27-80%; b) acylchloride or sulfonylchloride, Et$_3$N, DMAP or pyridine (cat.), CH$_2$Cl$_2$, 0°C to rt. 25-94%; c) NaOH, THF, H$_2$O, rt, 33-92%, for the structures of final compounds 13-25 see Table 1.

To derivatize the carboxylic acid moiety, methyl esters 14a and 26 were reduced and subsequent CDI coupling was performed to yield carbamate 28 (see Scheme 2A). Hydrolysis of methyl ester 20 followed by EDC coupling and deprotection provided amide 29. In addition, reductive aminations using 2-aminoacetamide and aldehydes 4-5 were performed, followed by sulfonylchloride coupling to 30 and 31 (see Scheme 2B). Dehydration to nitriles 32 and 33a followed by reduction or cyclization yielded amine 33 and tetrazole 34 respectively. All compounds were tested in a colorimetric, biochemical assay using para-nitrophenylbutyrate as substrate and HEK293T cell membranes overexpressing human DAGL$\alpha$, as previously described.$^{11,23}$
Discovery of glycine sulfonamides as dual inhibitors of DAGLα and ABHD6

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

DAGLα seems to have a large binding cavity, because ortho (13), meta (14, 16) and para (15, 17) substitution of the benzylamine moiety with arylethers was allowed at R² (see Table 1). Para substitution was, however, preferred over ortho and meta substitution. The sulfonamides 13-21 and 24 were well tolerated, whereas amide derivatives 22, 23 and 25 proved inactive. Thus, a specific orientation of the R² substituent induced by the sulfonamide is required. The weak activity of compound 19 indicated that the steric tolerance around the sulfonamide functionality is limited, whereas a para-chlorophenyl group is allowed (compounds 18 and 22). This indicated that both electron withdrawing and donating substituents (2b) are tolerated at R². Thus, the activity in this subpocket seems to be more governed by lipophilic and steric interactions than electronic interactions. In contrast, from the activity of compounds 26-34 it was clear that the carboxylic acid is a crucial pharmacophoric feature, because methylester 26, alcohol 27, primary amide 33 and tetrazole 34, a carboxylic acid bioisoster, were all inactive. Replacement of the acid
functionality by carbamate 28, which is a well-known warhead for serine hydrolases, resulted in loss of activity. It could be that the steric hinder posed by the piperidine moiety caused the drop in activity, however, the primary amide (30) was also inactive. Moreover, conversion of the acid moiety into substituted amide 29, as observed in the original hit 2b, was detrimental for activity on DAGLα (IC₅₀ > 10 μM). Altogether, this suggests that the carboxylate of the glycine forms an important ionic interaction with a basic amino acid in the enzyme. Surprisingly, replacement of the carboxylic acid with a primary amine yielded compound 33 with an IC₅₀ of 512 ± 147 nM. Compound 15 proved the most potent glycine sulfonamide derivative in the biochemical assay with an IC₅₀ of 18 ± 6 nM and inhibited the hydrolysis of [¹⁴C]-sn-1-oleoyl-2-arachidonoyl-glycerol, the natural substrate of DAGLα, with a Kᵢ of 0.7 ± 0.08 μM.¹⁰

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

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<th>Entry</th>
<th>$R^A$</th>
<th>$R^B$</th>
<th>$R^C$</th>
<th>DAGL$\alpha$ IC$_{50}$ ± SEM (nM)</th>
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Figure 2. Two highly ranked binding poses of compound 15 in the DAGLα homology model. A) Binding mode 1 displaying key interactions with catalytic serine (Ser472) and histidine (His650) and a neighboring Tyr303. B) Binding mode 2 displaying key interactions with the catalytic histidine (His650) and a neighboring His471 C) Binding mode 1 in the catalytic binding site cavity. D) Binding mode 2 in the catalytic binding site cavity.

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

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

Since ABHD6 is a hydrolase that is capable of metabolizing 2-AG, the natural product of DAGLα, a natural substrate-based assay for ABHD6 was set up. This assay employs ABHD6 to convert 2-AG into glycerol, which is coupled to the oxidation of commercially available fluorogenic Amplifu™Red via a multi-enzyme cascade (Figure 4A). It was found that membrane preparations of HEK293T cells overexpressing human ABHD6 are able to hydrolyze 2-AG with an apparent Kₘ of 25.4 ± 3.3 μM and a Vₘₐₓ of 5.94 ± 0.28 nmol/min/mg protein. Pre-incubation of the three inhibitors dose-dependently inhibited ABHD6 with a Kᵢ of 0.8, 2.7 and 18.3 μM for compound 15, 17 and 14, respectively (Figure 4C). This indicates that the glycine sulfonamides are a class of dual inhibitors for DAGLα and ABHD6.
Figure 4. Biochemical hABHD6 activity assay and dose response analysis. A) Enzymatic activity of hABHD6 was measured in a natural substrate based activity assay as previously described.\textsuperscript{27} B) Normalized and corrected (for control) fluorescence measurements over time. C) Concentration response analysis for ABHD6 inhibition by 14 (black, ---, $K_i = 18.3 \pm 1.8 \, \mu M$ ), 15 (grey ---, $K_i = 0.8 \pm 0.1 \, \mu M$ ) and 17 (black, ---, $K_i = 2.7 \pm 0.4 \, \mu M$ ) as measured by resorufin fluorescence (SEM, N = 2, n = 2).
Discussion & Conclusion

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

In contrast to a previous report, which claimed that glycine sulfonamide were selective inhibitors for DAGLα, proteome-wide ABPP assay indicated that the glycine sulfonamides do interact with other proteins. Compound 15 prevented labeling of at least three other unknown proteins targeted by MB064 and TAMRA-FP. One off-target was identified as ABHD6. Using a recently developed activity assay for ABHD6 that employs 2-AG as natural substrate, it was discovered that 15 prevented the hydrolysis of 2-AG with a Ki of 0.8 ± 0.1 μM, which is very similar as for DAGLα (Ki = 0.7 ± 0.08 μM). Thus, compound 15 is a dual inhibitor of DAGLα and ABHD6. Of note, it seems that ABHD6 is a common off-target for DAGLα inhibitors in general, because also the triazole ureas,16 fluorophosphonates12-14 and beta-lactones10,15 cross-react with this enzyme. To investigate DAGL biology it is necessary to identify compounds that do not cross-react with ABHD6 and the two other unknown off-targets.

Recently it has been reported that ABHD6 is linked to metabolic disorders and is a regulator of de novo lipogenesis in the liver by acting as a general lysophospholipid hydrolase using lyso-phosphatidylglycerol, lyso-phosphatidylethanolamine, lyso-phosphatic acid and lyso-phosphatidyl serine as substrates. Peripheral knock-down of ABHD6 protected mice from high-fat-diet induced obesity, hepatic steatosis and insulin resistance. In vivo inhibition of ABHD6 with a small molecule resulted in reduced glucose intolerance and induced weight loss, thereby providing rationale for the development of small molecule inhibitors of ABHD6 for the treatment of obesity-induced metabolic disorders.17 Since inhibition of DAGLα is also strongly linked with reduced body weight and resistance against high-fat diet induced obesity,14 via reduced cannabinoid CB1R activation,4,28,29 it can be envisioned that dual inhibition of both ABHD6 and DAGLα may provide additional therapeutical benefits. Since both DAGLα and ABHD6 are highly expressed in the brain, it would be of interest to develop compounds with restricted access to the central nervous system to prevent adverse neuropsychiatric side effects as previously observed with the cannabinoid CB1R antagonist Rimonabant. The glycine sulfonamide series, as exemplified by 15, may be an interesting lead series to provide such tool compounds, because their polar surface area is quite large (> 70 Å²).
In summary, this study provides the first structure-activity relationship of the glycine sulfonamide series of DAGLα inhibitors and discovered that this class of compounds is not selective over ABHD6. This provided important insights in the direction for optimization of 15, LEI106, as a selective DAGLα tool compound. In addition, this dual DAGLα/ ABHD6 inhibition profile might provide a new lead for the treatment of diet-induced obesity and metabolic syndrome.
Experimental

Experimental procedures computational chemistry

Docking in DAGLα homology model. Docking was performed as previously reported (Chapter 2).\textsuperscript{11}

Experimental procedures biochemistry

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

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

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

Biochemical DAGLα activity assay. The biochemical hDAGLα activity assay was performed as previously reported.\textsuperscript{11}

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

The assay was performed in HEMNB buffer (50 mM HEPES pH 7.4, 1 mM EDTA, 5 mM MgCl₂, 100 mM NaCl, 0.5% (w/v) BSA) in black, flat bottom Greiner 96-wells plates. Final protein concentration of membrane preparations of HEK293T cells overexpressing hABHD6 was 0.04 µg/µL. Inhibitors were added from 40x concentrated DMSO stocks. After 30 min. incubation, assay mix containing glycerol kinase (GK), glycerol-1-phosphate oxidase (GPO), horse radish peroxidase (HRP), adenosine triphosphate (ATP), Amplifu™Red and 2-
arachidonoyl glycerol (2-AG) was added and fluorescence was measured in 5 min. intervals for 75 min. (λ<sub>ex</sub> = 535 nm, λ<sub>em</sub> = 595 nm) at 21°C on a plate reader (TECAN GENios microplate reader). Final assay concentrations: 0.2 U/mL GK, GPO and HRP, 0.125 mM ATP, 10 µM Amplifu™Red, 25 µM 2-AG, 5% DMSO, 1% ACN in a final volume of 200 µL. Slopes were determined in the linear interval of t = 10 to t = 35 min. All experiments were performed at N = 2, n = 2 for experimental measurements and N = 2, n = 4 for controls.

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

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

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

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

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

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

General remarks

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

$^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker AV 400 MHz spectrometer at 400.2 ($^1$H) and 100.6 ($^{13}$C) MHz using CDCl$_3$ or CD$_2$OD as solvent, unless stated otherwise. Chemical shift values are reported in ppm with tetramethylsilane or solvent resonance as the internal standard (CDCl$_3$: $\delta$ 7.26 for $^1$H, $\delta$ 77.16 for $^{13}$C, CD$_2$OD: $\delta$ 3.31 for $^1$H, $\delta$ 49.00 for $^{13}$C). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, td = triple doublet, t = triplet, q = quartet, quintet = quint, br = broad, m = multiplet), coupling constants $J$ (Hz), and integration. HPLC purification was performed on a preparative LC-MS system (Agilent 1200 series) with an Agilent 6130 Quadruple MS detector. High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific LTQ Orbitrap XL. Liquid chromatography was performed on a Finnigan Surveyor LC-MS system, equipped with a C18 column. Melting points were measured on a Stuart SMP30 melting point apparatus. Flash chromatography was performed using SiliCycle silica gel type SiliaFlash P60 (230 – 400 mesh). TLC analysis was performed on Merck silica gel 60/Kieselguhr F254, 0.25 mm. Compounds were visualized using either Seebach’s reagent (a mixture of phosphomolybdic acid (25 g), cerium (IV) sulfate (7.5 g), H$_2$O (500 mL) and H$_2$SO$_4$ (25 mL)) or a KMnO$_4$ stain (K$_2$CO$_3$ (40 g), KMnO$_4$ (6 g) and H$_2$O (600 mL)).

Methyl (2-phenoxybenzyl)glycinate (8)

To a stirred solution of 2-phenoxybenzaldehyde (3, 150 µL, 0.87 mmol) in MeOH (50 mL) with 3 Å MS was added methyl glycinate (377.6 mg, 3.01 mmol) and AcOH (260 µL, 4.54 mmol) at rt, the reaction mixture was stirred for 15 minutes. Lastly was added NaCNBH$_3$ (151.4 mg, 2.41 mmol) and the reaction mixture was stirred for 2 hours at rt. After completion the reaction was diluted with EtOAc and washed with NaHCO$_3$, water and brine, dried, concentrated in vacuo and purified by flash chromatography to yield methyl (2-phenoxybenzyl)glycinate (141.9 mg, 0.52 mmol, 60 %) as a white solid.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.41 (d, $J$ = 7.3 Hz, 1H), 7.32 (t, $J$ = 7.6 Hz, 2H), 7.22 (t, $J$ = 7.7 Hz, 1H), 7.16 – 7.05 (m, 2H), 6.96 (d, $J$ = 8.0 Hz, 2H), 6.88 (d, $J$ = 8.0 Hz, 1H), 3.88 (s, 2H), 3.68 (s, 3H), 3.45 (s, 2H), 1.92 (bs, 1H).

$^{13}$C NMR (CDCl$_3$, 101 MHz): $\delta$ 170.51, 157.54, 155.07, 130.54, 129.89(2C), 128.76, 125.85, 123.91, 123.12, 119.17, 118.24(2C), 54.19, 51.97, 49.87.

Methyl (3-phenoxybenzyl)glycinate (9)

The title compound was synthesized from 3-phenoxybenzaldehyde (4, 100 µL, 0.58 mmol) according to the procedure described for compound 8. This yielded methyl (3-phenoxybenzyl)glycinate (125.7 mg, 0.46 mmol, 80 %) as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.39 – 7.22 (m, 3H), 7.13 – 7.04 (m, 2H), 7.04 – 6.97 (m, 3H), 6.90 (ddd, $J$ = 8.2, 2.5, 1.0 Hz, 1H), 3.79 (s, 2H), 3.73 (s, 3H), 3.42 (s, 2H).

$^{13}$C NMR (CDCl$_3$, 101 MHz): $\delta$ 170.73, 157.37, 156.38, 134.38, 129.89(2C), 129.84, 123.36, 123.14, 119.04(2C), 118.73, 117.71, 99.91, 53.09, 51.74, 50.02. Spectroscopic data are in agreement with those reported in literature.

Methyl (4-phenoxybenzyl)glycinate (10)

The title compound was synthesized from 4-phenoxybenzaldehyde (5, 100 mg, 0.50 mmol) according to the procedure described for compound 8. This yielded methyl (4-phenoxybenzyl)glycinate (240 mg, 0.88 mmol, 44 %) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.38 – 7.22 (m, 4H), 7.08 (t, $J$ = 7.4 Hz, 1H), 6.98 (ddd, $J$ = 10.1, 7.2, 1.5 Hz, 4H), 3.77 (s, 2H), 3.72 (s, 3H), 3.42 (s, 2H), 2.18 (s, 1H); $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 172.87, 157.37, 156.38, 134.38, 129.75(2C), 129.72(2C), 123.18, 118.97(2C), 118.76(2C), 52.68, 51.82, 49.85.
3-((5-(Trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (11a)

The title compound was synthesized from 3-hydroxybenzaldehyde (366 mg, 3 mmol) and 2-chloro-5-(trifluoromethyl)pyridine (363 mg, 2 mmol) according to the procedure described for compound 12a. This yielded 3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (135 mg, 0.5 mmol, 30%) after flash chromatography as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz): δ 10.02 (s, 1H), 8.45 – 8.40 (m, 1H), 7.96 (dd, J = 8.6, 2.3 Hz, 2H), 7.77 – 7.79 (m, 1H), 7.71 – 7.67 (m, 1H), 7.62 (t, J = 7.8 Hz, 1H), 7.43-7.46 (m, 1H), 7.10 (d, 1H, J = 8.8 Hz). Spectroscopic data are in agreement with those reported in literature.$^{31}$

Methyl [3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl]glycinate (11)

The title compound was synthesized from 3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (6a, 183 mg, 0.68 mmol) according to the procedure described for compound 8. This yielded methyl [3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl]glycinate (84 mg, 0.25 mmol, 37%) as a colorless oil. $^1$H NMR (CDCl$_3$, 400 MHz): δ 8.43 (s, 1H), 7.86 – 7.89 (m, 1H), 7.36 – 7.39 (m, 1H), 7.14 – 7.23 (m, 2H), 6.98 – 7.01 (m, 2H), 3.86 (s, 2H), 3.72 (s, 3H), 3.45 (s, 2H).

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

To a stirred solution of 4-hydroxybenzaldehyde (366 mg, 3 mmol) in DMSO (30 mL) was added K$_2$CO$_3$ (360 mg, 6 mmol) at room temperature. After 30 minutes of stirring was added dropwise a solution of 2-fluoro-5-(trifluoromethyl)pyridine (0.241 mL, 2 mmol) in DMSO (2 mL). After full completion (24 h) HCl (1 M) was added. The mixture was poured into water, and the product was extracted with EtOAc, washed with water and brine, dried, concentrated in vacuo and purified by flash chromatography to yield 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzaldehyde (366 mg, 1.37 mmol, 70%) as a white solid. $^1$H NMR (CDCl$_3$, 400 MHz): δ 10.01 (s, 1H), 8.45 (s, 1H), 7.95 – 7.99 (m, 3H), 7.31 – 7.35 (m, 2H), 7.11 – 7.13 (m, 1H).

$^{13}$C NMR (101 MHz, CDCl$_3$): δ 190.94, 164.87, 158.25, 145.49 (q, J = 4.3 Hz), 137.24 (q, J = 3.2 Hz), 133.58, 131.69 (2C), 123.68 (q, J = 272.6 Hz), 122.50 (q, J = 33.5 Hz), 121.92 (2C), 112.24.

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

To a stirred solution of methyl (2-phenoxybenzyl)glycinate (8, 63.9 mg, 0.24 mmol) in CH$_2$Cl$_2$ (50 mL) was added 2,2-dimethylchromene-6-sulfonyl chloride (96 mg, 0.368 mmol), triethylamine (0.1 mL, 0.717 mmol) and 1 drop of pyridine (or 4-dimethylaminopyridine) at rt. After completion (2 h), the reaction mixture was diluted with EtOAc, washed with sat. NaHCO$_3$, brine and purified using column chromatography to yield methyl [N-((2,2-dimethylchroman-6-yl)sulfonyl)-N-(2-phenoxybenzyl)glycinate (96.0 mg, 0.19 mmol, 82%) as an oil. $^1$H NMR (CDCl$_3$, 400 MHz): δ 7.57 – 7.46 (m, 3H), 7.33 – 7.27 (m, 2H), 7.25 – 7.19 (m, 1H), 7.14 – 7.04 (m, 2H), 6.87 – 6.74 (m, 4H), 4.55 (s, 2H), 4.02 (s, 2H), 3.52 (s, 3H), 2.74 (t, J = 6.7 Hz, 2H), 1.80 (t, J = 6.7 Hz, 2H), 1.34 (s, 6H).$^{13}$C NMR (CDCl$_3$, 101 MHz): δ 169.68, 157.96, 156.85, 155.46, 131.49, 130.43 (2C), 129.95, 129.57, 129.51, 127.15, 126.53, 123.89, 123.52, 121.31, 118.57 (2C), 118.26, 117.79, 75.73, 52.06, 48.00, 46.26, 32.38, 27.01 (2C), 22.45.
**N-((2,2-Dimethylchroman-6-yl)sulfonyl)-N-(2-phenoxybenzyl)glycine (13)**

To a stirred solution of methyl N-((2,2-dimethylchroman-6-yl)sulfonyl)-N-(2-phenoxybenzyl)glycinate (13a, 60 mg, 0.121 mmol) in THF (1 mL): methanol (1 mL) was added aqueous NaOH (2 mL, 4.00 mmol) at rt. After completion (30 mins), to the reaction was added Amberlight 150 H resin until neutral, the mixture was filtered, concentrated in vacuo, coevaporated with toluene and purified by flash chromatography to yield N-((2,2-dimethylchroman-6-yl)sulfonyl)-N-(2-phenoxybenzyl)glycine (25.3 mg, 0.053 mmol, 43% yield) as a white solid.

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

The title compound was synthesized from methyl (3-phenoxybenzyl)glycinic acid (9, 37.5 mg, 0.14 mmol) according to the procedure described for compound 13a. This yielded methyl N-((2,2-dimethylchroman-6-yl)sulfonyl)-N-(3-phenoxybenzyl)glycinate (64.5 mg, 0.13 mmol, 94 %) as an oil. 1H NMR (CDCl₃, 400 MHz) δ 7.62 – 7.51 (m, 2H), 7.33 (t, J = 8.4, 7.5 Hz, 2H), 7.29 – 7.23 (m, 1H), 7.11 (t, J = 7.4 Hz, 1H), 7.03 – 6.94 (m, 3H), 6.94 – 6.80 (m, 3H), 4.44 (s, 2H), 3.92 (s, 2H), 3.57 (s, 3H), 2.80 (t, J = 6.7 Hz, 2H), 1.83 (t, J = 6.7 Hz, 2H), 1.35 (s, 6H). 13C NMR (CDCl₃, 101 MHz): δ 173.82, 158.16, 156.62, 155.63, 131.64, 130.46, 130.02(2C), 129.76, 129.62, 127.20, 126.06, 123.88, 123.71, 121.49, 119.71(2C), 118.18, 118.85, 75.83, 48.15, 46.95, 32.31, 27.00(2C), 22.42. Purity >95% as determined by LC-MS.

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

The title compound was synthesized from methyl (3-phenoxybenzyl)glycinic acid (9, 37.5 mg, 0.14 mmol) according to the procedure described for compound 13. This yielded N-((2,2-dimethylchroman-6-yl)sulfonyl)-N-(3-phenoxybenzyl)glycine (27.9 mg, 0.06 mmol, 55 %) as a white solid after lyophilization. mp 171-173 °C. HRMS (ESI+) m/z: calculated for C₂₅H₂₃NO₅S ([M + H]⁺), 482.1632, found: 482.1630. 1H NMR (CDCl₃, 400 MHz): δ 7.70 – 7.52 (m, 2H), 7.33 (t, J = 7.9 Hz, 2H), 7.25 – 7.29 (m, 1H), 7.15 – 7.06 (m, 1H), 7.05 – 6.87 (m, 4H), 6.87 – 6.77 (m, 2H), 4.42 (s, 2H), 3.94 (s, 2H), 2.78 (t, J = 6.6 Hz, 2H), 1.82 (t, J = 6.7 Hz, 2H), 1.34 (s, 6H). 13C NMR (CDCl₃, 101 MHz): δ 174.65, 158.14, 157.67, 150.99, 149.11, 148.01, 139.35, 131.83, 129.66, 127.15, 127.15, 123.48, 123.44, 121.45, 119.06, 119.02(2C), 118.39, 117.95, 75.82, 52.13, 51.25, 46.95, 32.36, 26.99(2C), 22.48.

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

See compound 26.

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

The title compound was synthesized from methyl N-((2,2-dimethylchroman-6-yl)sulfonyl)-N-(4-phenoxybenzyl)glycinic acid (9, 37.5 mg, 0.14 mmol) according to the procedure described for compound 13. This yielded N-((2,2-dimethylchroman-6-yl)sulfonyl)-N-(4-phenoxybenzyl)glycine (54 mg, 0.11 mmol, 90%) as a white solid.

**Discovery of glycine sulfonamides as dual inhibitors of DAGLα and ABHD6**
The title compound was synthesized from methyl (3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (11, 34 mg, 0.016 mmol) according to the procedure described for compound 13a. This yielded methyl N-(2,2-dimethylchroman-6-yl)sulfonyl)-N-(3-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (16a, 34 mg, 0.016 mmol) as a white solid. 

$^1$H NMR (CDCl$_3$, 400 MHz): δ 8.45 (s, 1H), 7.92 – 7.95 (m, 1H), 7.62 – 7.65 (m, 2H), 7.34 – 7.36 (m, 2H), 7.11 – 7.14 (m, 2H), 7.02 – 7.03 (m, 2H), 6.90 (d, 1H, J = 8.4 Hz), 4.52 (s, 2H), 3.98 (s, 2H), 3.61 (s, 3H), 2.28 (t, 2H, J = 6.4 Hz, 2H), 1.87 (t, 2H, J = 6.8 Hz), 1.30 (s, 6H).

$^{13}$C NMR (CDCl$_3$, 101 MHz): δ 171.96, 165.66, 158.33, 153.54, 154.43 (q, J = 4.1 Hz), 137.51, 137.11 (q, J = 3.2 Hz), 130.29, 129.72, 129.60, 127.22, 125.87, 123.72 (q, J = 8.4 Hz) 123.73 (q, J = 271.5 Hz), 121.83, 121.65 (q, J = 33.4 Hz), 121.60, 121.46, 118.01, 111.83, 75.91, 51.27, 48.33, 32.32, 26.99(2C), 22.47. Purity >95% as determined by LC-MS.

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

The title compound was synthesized from methyl (4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (12, 34 mg, 0.016 mmol) and 2,2-dimethylchroman-6-sulfonyl chloride (65 mg, 0.25 mmol) according to the procedure described for compound 13a. This yielded methyl N-(2,2-dimethylchroman-6-yl)sulfonyl)-N-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (17a, 42 mg, 0.075 mmol) as a white solid after lyophilization.

$^1$H NMR (CDCl$_3$, 400 MHz): δ 8.45 (s, 1H), 7.93 – 7.95 (m, 1H), 7.62 – 7.65 (m, 2H), 7.34 – 7.36 (m, 2H), 7.11 – 7.14 (m, 2H), 7.02 (d, J = 8.8 Hz), 6.90 (d, 1H, J = 8.4 Hz), 4.52 (s, 2H), 3.98 (s, 2H), 3.61 (s, 3H), 2.28 (t, 2H, J = 6.4 Hz, 2H), 1.87 (t, 2H, J = 6.8 Hz), 1.30 (s, 6H).

$^{13}$C NMR (CDCl$_3$, 101 MHz): δ 171.93, 165.65, 158.35, 153.54, 154.43 (q, J = 4.1 Hz), 137.08 (q, J = 3.1 Hz), 132.38, 130.32(2C), 129.72, 127.21, 123.72 (q, J = 272.5 Hz), 121.90 (q, J = 33.3 Hz), 121.89(2C), 121.62, 118.03, 111.81, 75.94, 51.06, 46.76, 32.34, 27.00(2C), 22.50. One quaternary carbon signal overlaps and is not reported. Purity >95% as determined by LC-MS.
Methyl \( N\)-((4-chlorophenyl)sulfonyl)-\( N\)-(3-phenoxybenzyl)glycinate (18a)

The title compound was synthesized from \(3\)-\(N\)-(3-phenoxybenzyl)glycinate (9, 116.5 mg, 0.429 mmol) according to the procedure described for compound 13a. This yielded methyl \( N\)-((4-chlorophenyl)sulfonyl)-\( N\)-(3-phenoxybenzyl)glycinate (83.0 mg, 0.186 mmol, 43 % yield) as an oil. \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.84 – 7.76 (m, 2H), 7.51 – 7.43 (m, 2H), 7.38 – 7.24 (m, 3H), 7.13 (tt, \(J = 7.6, 1.2\) Hz, 1H), 7.02 – 6.95 (m, 3H), 6.93 (dd, \(J = 8.2, 2.5, 1.0\) Hz, 1H), 6.85 (t, \(J = 2.0\) Hz, 1H), 4.46 (s, 2H), 3.96 (s, 2H), 3.57 (s, 3H). \(^{13}\)C NMR (CDCl\(_3\), 101 MHz): \(\delta\) 169.08, 157.86, 156.83, 139.41, 138.37, 136.76, 130.33, 129.96(2C), 129.36(2C), 128.98(2C), 123.71, 123.27, 119.12(2C), 118.81, 118.56, 52.28, 51.14, 46.68.

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

The title compound was synthesized from methyl \( N\)-((4-chlorophenyl)sulfonyl)-\( N\)-(3-phenoxybenzyl)glycinate (18a, 32.2 mg, 0.07 mmol) according to the procedure described for compound 13. This yielded \( N\)-((4-chlorophenyl)sulfonyl)-\( N\)-(3-phenoxybenzyl)glycine (11.7 mg, 0.03 mmol, 38 %) after crystallization from EtoAc/pentane as a white solid. mp 147-149 °C. HRMS (ESI+) m/z: calculated for C\(_{21}\)H\(_{19}\)ClNO\(_2\)S (\([\text{M + H]}\)), 432.0667, found: 432.0669. \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.82 – 7.74 (m, 2H), 7.48 – 7.41 (m, 2H), 7.39 – 7.31 (m, 2H), 7.30 – 7.27 (m, 1H), 7.13 (tt, \(J = 7.2, 1.2\) Hz, 1H), 7.01 – 6.94 (m, 6H), 6.92 (dd, \(J = 8.3, 2.5, 1.0\) Hz, 6.81 (t, \(J = 2.0\) Hz, 1H), 5.44 (bs, 1H), 4.45 (s, 2H), 3.98 (s, 2H). \(^{13}\)C NMR (CDCl\(_3\), 101 MHz): \(\delta\) 173.48, 157.97, 156.76, 139.57, 138.17, 136.55, 130.43, 130.00(2C), 129.45(2C), 128.95(2C), 128.80, 123.24, 119.18(2C), 118.70, 118.65, 51.27, 46.59. Purity >95% as determined by LC-MS.

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

The title compound was synthesized from methyl \(3\)-\(N\)-(3-phenoxybenzyl)glycinate (9, 126.1 mg, 0.47 mmol) and 2,6-dichlorobenzenesulfonyl chloride (202.8 mg, 0.83 mmol) according to the procedure described for compound 13a. This yielded \( N\)-((2,6-dichlorophenyl)sulfonyl)-\( N\)-(3-phenoxybenzyl)glycinate (57.7 mg, 0.12 mmol, 26 %) as an oil. \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.36 – 7.30 (m, 4H), 7.29 – 7.26 (m, 2H), 7.17 – 7.12 (m, 1H), 6.97 (d, \(J = 11.2\) Hz, 4H), 6.88 (t, \(J = 2.0\) Hz, 1H), 4.45 (s, 2H), 4.18 (s, 2H), 3.74 (s, 3H).

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

The title compound was synthesized from methyl \( N\)-((2,6-dichlorophenyl)sulfonyl)-\( N\)-(3-phenoxybenzyl)glycinate (19a, 23.9 mg, 0.05 mmol) according to the procedure described for compound 13. This yielded \( N\)-((2,6-dichlorophenyl)sulfonyl)-\( N\)-(3-phenoxybenzyl)glycine (14.2 mg, 0.03 mmol, 61 %) as an oil. HRMS (ESI+) m/z: calculated for C\(_{25}\)H\(_{19}\)Cl\(_2\)NO\(_2\)S\(_2\)Na (\([\text{M + Na]}\)), 488.0097, found: 488.0095. \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.43 – 7.37 (m, 2H), 7.37 – 7.31 (m, 2H), 7.30 – 7.22 (m, 2H), 7.18 (tt, \(J = 7.6, 1.6\) Hz, 1H), 6.99 – 6.86 (m, 4H), 6.70 (t, \(J = 2.0\) Hz, 1H), 5.07 (bs, 1H), 4.62 (s, 2H), 4.15 (s, 2H). \(^{13}\)C NMR (CDCl\(_3\), 101 MHz): \(\delta\) 173.49, 157.91, 156.83, 140.60, 136.56, 135.90(2C), 135.52, 132.79, 131.75(2C), 130.36, 129.99(2C), 123.70, 123.35, 119.16(2C), 118.62, 51.41, 47.47. Purity >95% as determined by LC-MS.

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

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (10, 41 mg, 0.15 mmol) and 4-chlorobenzene-1-sulfonic chloride (80 mg, 0.38 mmol) according to the procedure described for compound 13a. This yielded methyl \( N\)-((4-chlorophenyl)sulfonyl)-\( N\)-(4-phenoxybenzyl)glycinate (62 mg, 0.14 mmol, 93 %) as a white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.83 (d, \(J = 8.7\) Hz, 2H), 7.50 (d, \(J = 8.7\) Hz, 2H), 7.38 – 7.31 (m, 2H), 7.21 (d, \(J = 8.6\) Hz, 2H), 7.17 – 7.08 (m, 1H), 7.03 – 6.91 (m, 4H), 4.45 (s, 2H), 3.96 (s, 2H), 3.58 (s, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 169.16, 157.64, 156.80, 139.37, 138.48, 130.30(2C), 129.96(2C), 129.35(2C), 129.13, 129.01(2C), 123.77, 119.27(2C), 118.94(2C), 52.26, 50.87, 46.52.

Discovery of glycine sulfonamides as dual inhibitors of DAGL\(\alpha\) and ABHD6
**N-((4-Chlorophenyl)sulfonyl)-N-(4-phenoxybenzyl)glycine (20)**

The title compound was synthesized from methyl N-((4-chlorophenyl)sulfonyl)-N-(4-phenoxybenzyl)glycinate (20a, 62.1 mg, 0.13 mmol) according to the procedure described for compound 13. This yielded N-((4-chlorophenyl)sulfonyl)-N-(4-phenoxybenzyl)glycine (38 mg, 0.09 mmol, 70 %) as a white solid after lyophilization. mp 188-190 °C. HRMS (ESI+) calculated for C_{37}H_{33}ClNO_S ([M + H]). 432.0667, found: 432.0662. ^1H NMR (CDCl₃, 400 MHz): δ 7.75 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.28 – 7.30 (m, 2H), 7.12 (d, J = 8.4 Hz, 1H), 7.04 – 7.08 (m, 2H), 6.93 (d, J = 7.6 Hz, 2H), 6.88 (d, J = 8.0 Hz, 2H), 4.38 (s, 2H), 3.93 (s, 2H). ^13C NMR (CDCl₃, 101 MHz): δ 174.93, 169.19, 157.57, 157.32, 156.76, 136.72, 133.16, 131.71, 130.35(2C), 129.83(2C), 129.19(2C), 128.88(2C), 123.62, 119.11, 118.83(2C), 118.79(2C), 50.60, 46.28. Purity >95% as determined by LC-MS.

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

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

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

The title compound was synthesized from methyl N-((2,6-dichlorophenyl)sulfonyl)-N-(4-phenoxybenzyl)glycinate (21a, 17.1 mg, 0.036 mmol) according to the procedure described for compound 13. This yielded N-((2,6-dichlorophenyl)sulfonyl)-N-(4-phenoxybenzyl)glycine (15 mg, 0.033 mmol, 92 %) as a white solid after lyophilization. mp 140-142 °C. HRMS (ESI+) calculated for C_{37}H_{33}ClNO_S ([M + H]). 466.0274, found: 466.0277. ^1H NMR (CDCl₃, 400 MHz): δ 7.50 (d, J = 8.0 Hz, 2H), 7.35 – 7.39 (m, 3H), 7.15 – 7.17 (m, 3H), 7.02 (d, J = 7.6 Hz, 2H), 6.94 (d, J = 8.4 Hz, 2H), 4.67 (s, 2H), 4.20 (s, 2H). ^13C NMR (CDCl₃, 101 MHz): δ 175.08, 157.61, 156.64, 135.81, 135.61, 132.71, 131.69(2C), 130.21(2C), 129.87(2C), 128.76(2C), 123.72, 119.23(2C), 118.84(2C), 51.12, 46.20. Purity >95% as determined by LC-MS.

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

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

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

The title compound was synthesized from methyl N-((4-chlorobenzoyl)-N-(4-phenoxybenzyl)glycinate (22a, 15.8 mg, 0.039 mmol) according to the procedure described for compound 13. This yielded N-((4-chlorobenzoyl)-N-(4-phenoxybenzyl)glycine (12 mg, 0.031 mmol, 80 % yield) as a white solid after lyophilization. mp 225-227 °C. HRMS (ESI+) calculated for C_{37}H_{33}ClNO_S ([M + H]). 396.0997, found: 396.0991. ^1H NMR (CDCl₃, 400 MHz): δ 7.28 – 7.33 (m, 7H), 7.06 – 7.08 (m, 2H), 6.92 – 6.95 (m, 4H), 4.69 (s, 0.6H), 4.52 (s, 1.4H), 4.12 (s, 1.4H), 3.83 (s, 0.6H). ^13C NMR (CDCl₃, 101 MHz): δ 172.11, 157.59, 157.32, 156.76, 136.72, 133.16, 131.71, 130.35(2C), 129.87(2C), 128.86(2C), 128.30(2C), 124.93(2C), 119.35, 116.03(2C), 53.63, 46.64. Signals split (7:3) due to rotamers. Purity >95% as determined by LC-MS.
Methyl N-(2,6-dichlorobenzoyl)-N-(4-phenoxybenzyl)glycinate (23a)

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (10, 33 mg, 0.12 mmol) and 2,6-dichlorobenzoyl chloride (63 mg, 0.30 mmol) according to the procedure described for compound 13a. This yielded methyl N-(2,6-dichlorobenzoyl)-N-(4-phenoxybenzyl)glycinate (50 mg, 0.11 mmol, 92%) as a white solid, which was directly converted to the corresponding acid 17. 1H NMR (CDCl3, 400 MHz): δ 7.13-7.25 (m, 7H), 7.05-7.07 (m, 1H), 6.86-6.95 (m, 4H), 4.97 (s, 0.5H), 4.46 (s 1.5H), 4.19 (s, 1.5H), 3.76 (s, 0.5H), 3.75 (s, 2.1H), 3.68 (s, 0.9H). Signals split (3:1) due to rotamers.

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

The title compound was synthesized from methyl N-(2,6-dichlorobenzoyl)-N-(4-phenoxybenzyl)glycinate (23a, 57.4 mg, 0.129 mmol) according to the procedure described for compound 13. This yielded N-(2,6-dichlorobenzoyl)-N-(4-phenoxybenzyl)glycine (25 mg, 0.058 mmol, 45%) as an oily white solid after lyophilization. mp not determined. HRMS (ESI+) calculated for C22H19Cl2NO4 (M + H)+: 501.0493, found: 501.0491.

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

The title compound was synthesized from methyl 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (12, 41 mg, 0.12 mmol) and 4-chlorobenzene-1-sulfonyl chloride (53 mg, 0.3 mmol) according to the procedure described for compound 13a. This yielded methyl N-(4-chlorophenyl)sulfonyl)-N-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (22 mg, 0.04 mmol, 36%) as a white solid. 1H NMR (CDCl3, 400 MHz): δ 8.41-8.44 (m, 1H), 7.90-7.93 (m, 1H), 7.82-7.85 (m, 2H), 7.50-7.52 (m, 2H), 7.33 (d, 2H, J = 8.4 Hz), 7.11-7.14 (m, 2H), 7.03 (d, 1H, J = 8.4 Hz), 4.53 (s, 2H), 4.14(s, 2H), 3.62 (s, 3H).

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

The title compound was synthesized from methyl N-(4-chlorophenyl)sulfonyl)-N-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (24a, 16 mg, 0.031 mmol) according to the procedure described for compound 13. This yielded N-(4-chlorophenyl)sulfonyl)-N-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (14 mg, 0.027 mmol, 88%) as a white solid after lyophilization. mp 150-152 °C. HRMS (ESI+) calculated for C22H18ClF2NS (+[M + H]). 501.0493, found: 501.0491.

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

The title compound was synthesized from methyl 4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (12, 41 mg, 0.12 mmol) and 4-chlorobenzoyl chloride (53 mg, 0.3 mmol) according to the procedure described for compound 13a. This yielded methyl N-(4-chlorobenzoyl)-N-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (48 mg, 0.10 mmol, 83%) as a white solid. 1H NMR (CDCl3, 400 MHz): δ 8.36 (s, 1H), 7.84-7.86 (m, 1H), 7.38-7.46 (m, 5H), 7.20 (d, 2H, J = 8.4 Hz), 7.10 (d, 2H, J = 8.0 Hz), 6.98 (d, 1H, J = 8.8 Hz), 4.73 (s, 0.7H), 4.57 (s, 1.3H), 4.12 (s, 1.3H), 3.84 (s, 0.7H), 3.71 (s, 2.1H), 3.65 (s, 0.9H). Signals split (7:3) due to rotamers.
Chapter 5

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

The title compound was synthesized from methyl N-(4-chlorobenzoyl)-N’-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycinate (25a, 47.8 mg, 0.1 mmol) according to the procedure described for compound 13. This yielded N-(4-chlorobenzoyl)-N’-(4-((5-(trifluoromethyl)pyridin-2-yl)oxy)benzyl)glycine (13 mg, 0.03 mmol, 33%) as a white solid after lyophilization. mp 202–204 °C. HRMS (ESI+) calculated for C_{27}H_{23}ClF_{3}N_{2}O_{5} ([M + H]), 465.0821. 1H NMR (MeOD, 500 MHz) δ 8.42 (s, 1H), 8.13 – 8.05 (m, 1H), 7.56 – 7.42 (m, 5H), 7.32 (d, J = 8.3 Hz, 1H), 7.20 – 7.16 (m, 2H), 7.14 (t, J = 8.5 Hz, 1H), 4.82 (s, 1H), 4.64 (s, 1H), 4.16 (s, 1H), 3.86 (s, 1H). 13C NMR (MeOD, 125 MHz) δ 173.62 (0.5C), 173.56 (0.5C), 167.33 (0.5C), 156.22 (0.5C), 154.17 (0.5C), 146.28 – 146.05 (m, 1C), 138.40 – 138.64 (m, 1C), 137.25 (0.5C), 137.07 (0.5C), 135.68, 135.28 (0.5C), 135.18 (0.5C), 134.63, 131.00 (2C), 129.95, 129.92, 129.59, 129.46, 125.19 (q, 0.5C, 1H). Signals split (1:1) due to rotamers. Purity >95% as determined by LC-MS.

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

The title compound was synthesized from methyl (4-phenoxybenzyl)glycinate (84 mg, 17 mmol, 92%) as a white solid. mp 86–88 °C. HRMS (ESI+) calculated for C_{27}H_{23}ClF_{3}N_{2}O_{5} ([M + H]), 496.1788, found: 496.1788.

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

To a stirred solution of methyl N-(2,2-dimethylchroman-6-yl)sulfonyl)-N’-(3-phenoxybenzyl)glycinate (26, 34.4 mg, 0.07 mmol) in MeOD (0.1 mL, 2M in THF). After completion (4 h), saturated KHCO_{3} was carefully added at -5 °C to quench the reaction. The mixture was slowly warmed up to rt, diluted with EtOAc, washed with brine, dried, concentrated in vacuo and purified by flash chromatography to yield N-(2-hydroxyethyl)-2,2-dimethyl-N’-(3-phenoxybenzyl)chromane-6-sulfonamide (21.1 mg, 0.05 mmol, 65%) as a white solid. mp 148–150 °C. HRMS (ESI+) m/z: calculated for C_{27}H_{23}O_{5}N_{2}S ([M + H]), 468.1839, found: 468.1839.

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

To a stirred solution of N-(2-hydroxyethyl)-2,2-dimethyl-N’-(3-phenoxybenzyl)chromane-6-sulfonamide (27, 8.1 mg, 0.02 mmol) in CH_{2}Cl_{2} (2 mL) at 0 °C was added DIPEA (3 µL, 0.017 mmol) and di(1H-imidazol-1-yl)methanone (6.6 mg, 0.04 mmol). After 10 minutes was added piperidine (6 µL, 0.06 mmol) and the mixture was stirred for 30 mins and allowed to warm up to rt. After completion (1 h), the mixture was diluted with EtOAc, washed with brine, dried, concentrated in vacuo to yield 2-((2,2-dimethyl-N’-(3-phenoxybenzyl)chromane)-6-sulfonamido)ethyl piperidine-1-carboxylate (10.0 mg, 0.02 mmol, 100%) as a white solid after lyophilization. mp not determined. HRMS (ESI+) m/z: calculated for C_{27}H_{23}O_{5}N_{2}S ([M + H]), 465.0824, found: 465.0821.
The title compound was synthesized from 2-((3-phenoxybenzyl)amino)acetamide (29a, 184 mg, 0.72 mmol) according to the procedure described for compound 29a. This yielded 2-((3-phenoxybenzyl)amino)acetamide (30a, 184 mg, 0.72 mmol) as a white solid. mp 122-124 °C. HRMS (ESI+) m/z: calculated for C_{29}H_{32}N_{2}O_{4}S ([M + H]), 568.2475, found: 568.2476. 1H NMR (MeOD, 400 MHz): δ 7.66 – 7.54 (m, 2H), 7.40 – 7.31 (m, 2H), 7.29 – 7.21 (m, 2H), 7.16 – 7.09 (m, 1H), 7.01 – 6.93 (m, 2H), 6.93 – 6.82 (m, 3H), 4.35 (s, 2H), 3.73 (s, 2H), 3.66 (t, J = 4.8 Hz, 2H), 3.48 (t, J = 5.4 Hz, 2H), 3.29 (t, J = 5.6, 2H), 3.12 (t, J = 4.8 Hz, 2H), 2.87 (t, J = 6.9 Hz, 2H), 1.87 (t, J = 6.7 Hz, 2H), 1.36 (s, 6H). 13C NMR (MeOD, 101 MHz): δ 174.67, 159.10, 158.50, 140.69, 131.14, 130.93(2C), 124.68, 124.54, 120.02, 119.97(2C), 119.11, 53.29, 50.65. Signals split (7:3) due to rotamers.
NMR (CDCl₃, 101 MHz): δ 171.07, 158.72, 157.83, 156.91, 136.92, 130.37, 129.93(2C), 129.72, 127.58, 127.17, 123.82, 123.67, 122.01, 119.30, 119.09(2C), 118.85, 118.35, 76.10, 54.04, 51.43, 32.19, 27.00(2C), 22.46. Purity >95% as determined by LC-MS.

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

The title compound was synthesized from 4-phenoxbenzaldehyde (5, 300 µL, 1.71 mmol) and 2-aminoacetamide (226 mg, 2.06 mmol) according to the procedure described for compound 8. This yielded 2-((4-phenoxbenzyl)amino)acetamide (300 mg, 1.17 mmol, 68 %) as a white solid. ¹H NMR (MeOD, 400 MHz): δ 7.35 – 7.24 (m, 4H), 7.04 (t, J = 7.4 Hz, 1H), 6.94 – 6.85 (m, 4H), 3.68 (s, 1.2H), 3.58 (0.8H), 3.20 (s, 2H).

¹³C NMR (MeOD, 101 MHz): δ 176.70, 158.70, 158.08(0.6C), 157.92(0.4C), 135.41(0.6C), 134.27(0.4C), 131.72, 131.09(2C), 130.88(0.6C), 130.86(0.4C), 131.09(0.6C), 119.86, 119.80, 119.75, 119.69, 59.22(0.6C), 57.57(0.4C), 53.47(0.6C), 51.39(0.4C). Signals split (3:2) due to rotamers.

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

The title compound was synthesized from 2-((4-phenoxybenzyl)amino)acetamide (31a, 200 mg, 0.780 mmol) according to the procedure described for compound 13a. This yielded 2-((2,2-dimethyl-N-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (237.0 mg, 0.493 mmol, 63 % yield) as a white solid.

¹H NMR (CDCl₃, 400 MHz): δ 7.59 (d, J = 1.6 Hz, 1H), 7.52 (dd, J = 8.6, 2.1 Hz, 1H), 7.45 – 7.29 (m, 3H), 7.25 – 7.22 (m, 1H), 7.20 (s, 1H), 7.17 – 7.09 (m, 1H), 7.05 (s, 1H), 7.02 – 6.96 (m, 2H), 6.95 – 6.90 (m, 2H), 6.84 (d, J = 8.6 Hz, 1H), 4.34 (s, 2H), 3.66 (s, 2H), 2.78 (t, J = 6.7 Hz, 2H), 1.79 (t, J = 6.7 Hz, 2H), 1.30 (s, 6H).

¹³C NMR (CDCl₃, 101 MHz): δ 159.10, 158.16, 156.69, 135.74, 130.56, 130.02(2C), 129.91, 127.37, 127.02, 123.87, 123.39, 122.22, 119.25(2C), 118.93, 118.85, 118.51, 113.47, 76.17, 50.94, 34.62, 32.23, 27.00(2C), 22.49. Purity >95% as determined by LC-MS.

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

To a stirred solution of 2-((2,2-dimethyl-N-(3-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (30, 46.4 mg, 0.10 mmol) in THF (5 mL) at 0 °C was added pyridine (0.05 mL, 0.62 mmol) and then TFAA (0.04 mL, 0.28 mmol). The reaction was warmed up to rt and stirred for 2h until completion. The mixture was then diluted with EtOAc, washed with water and brine, dried, concentrated in vacuo and purified by flash chromatography to yield N-(cyanomethyl)-2,2-dimethyl-N-(3-phenoxybenzyl)chromane-6-sulfonamide (30.4 mg, 0.07 mmol, 69 %) as a white solid after lyophilization. mp 90-92 °C. HRMS (ESI+) m/z: calculated for C₂₆H₂₆N₂O₄SNa ([M + Na]), 485.1505, found: 485.1504.

¹H NMR (CDCl₃, 400 MHz): δ 7.66 – 7.52 (m, 2H), 7.42 – 7.28 (m, 3H), 7.20 – 7.16 (m, 1H), 7.14 (tt, J = 7.2, 1.2 Hz, 1H), 7.10 – 7.06 (m, 1H), 7.02 – 6.93 (m, 3H), 6.91 (d, J = 8.6 Hz, 1H), 4.28 (s, 2H), 4.06 (s, 2H), 2.85 (t, J = 6.8 Hz 2H), 1.85 (t, J = 6.7 Hz, 2H), 1.36 (s, 6H).

¹³C NMR (CDCl₃, 101 MHz): δ 159.10, 158.16, 156.69, 135.74, 130.56, 130.02(2C), 129.91, 127.37, 127.02, 123.87, 123.39, 122.22, 119.25(2C), 118.93, 118.85, 118.51, 113.47, 76.17, 50.94, 34.62, 32.23, 27.00(2C), 22.49. Purity >95% as determined by LC-MS.

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

The title compound was synthesized from 2-((2,2-dimethyl-N-(4-phenoxybenzyl)chromane)-6-sulfonamido)acetamide (31, 86.2 mg, 0.179 mmol) according to the procedure described for compound 32. This yielded N-(cyanomethyl)-2,2-dimethyl-N-(4-phenoxybenzyl)chromane-6-sulfonamide (51.3 mg, 0.111 mmol, 62 %) as an oil. ¹H NMR (CDCl₃, 400 MHz): δ 7.63 (d, J = 2.3 Hz, 1H), 7.61 (dd, J = 8.6, 2.4 Hz, 1H), 7.40 – 7.32 (m, 2H), 7.32 – 7.27 (m, 2H), 7.18 – 7.10 (m, 1H), 7.06 – 6.95 (m, 4H), 6.93 (d, J = 8.5 Hz, 1H), 4.28 (s, 2H), 4.05 (s, 2H), 2.86 (t, J = 6.7 Hz, 2H), 1.86 (t, J = 6.7 Hz, 2H), 1.37 (s, 6H).¹³C NMR (CDCl₃, 101 MHz): δ 159.08, 158.08, 156.64, 130.52(2C), 130.02(2C), 129.91, 128.02, 127.37, 127.02, 123.94, 122.23, 119.45(2C), 119.04(2C), 118.51, 113.54, 76.17, 50.61, 34.46, 32.27, 27.01(2C), 22.51.
**Discovery of glycine sulfonamides as dual inhibitors of DAGLα and ABHD6**

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

To a stirred solution of N-(cyanomethyl)-2,2-dimethyl-N-(4-phenoxybenzyl)chromane-6-sulfonamide (33a, 21.3 mg, 0.046 mmol) in THF (5 mL) was added borane THF complex (0.092 ml, 0.092 mmol) at 0 °C. The reaction mixture was heated for 10 h. at reflux. After full completion the mixture was quenched with 5 mL MeOH followed by 1M HCl (10 mL) and the reaction was refluxed for 2h, diluted with EtOAc, washed with NaHCO₃, brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield N-(2-aminoethyl)-2,2-dimethyl-N-(4-phenoxybenzyl)chromane-6-sulfonamide (11.6 mg, 0.025 mmol, 54 %) as a white solid after lyophilization. mp not determined. HRMS (ESI+) m/z: calculated for C₂₆H₃₁N₂O₄S ([M + H]), 467.1999, found: 467.1998.

**1H NMR** (CDCl₃, 400 MHz): δ 7.58 (s, 1H), 7.56 – 7.47 (m, 1H), 7.36 – 7.28 (m, 2H), 7.18 (d, J = 8.2 Hz, 2H), 7.09 (t, J = 7.4 Hz, 1H), 7.03 – 6.94 (m, 2H), 6.87 (d, J = 8.1 Hz, 2H), 6.82 (d, J = 8.6 Hz, 1H), 4.27 (s, 2H), 3.41 (m, 2H), 2.96 (m, 2H), 2.79 (t, J = 6.3 Hz, 3H), 1.79 (t, J = 6.6 Hz, 3H), 1.33 (s, 6H).

**13C NMR** (CDCl₃, 101 MHz): δ 159.75, 159.00, 158.17, 132.12, 131.63(2C), 131.00(2C), 130.62, 129.86, 128.06, 124.84, 123.51, 120.17(2C), 119.74(2C), 119.06, 77.07, 54.39, 47.81, 40.11, 33.14, 27.09(2C), 23.28. Purity >95% as determined by LC-MS.

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

To a stirred solution of N-(cyanomethyl)-2,2-dimethyl-N-(3-phenoxybenzyl)chromane-6-sulfonamide (32, 57.4 mg, 0.12 mmol) in toluene (10 mL) was added Et₃N.HCl (51.4 mg, 0.37 mmol) and sodium azide (22.4 mg, 0.35 mmol). The reaction mixture was stirred for 120 h. at reflux. After full completion, the organic layer was washed with brine, dried, concentrated *in vacuo* and purified by flash chromatography to yield N-((1H-tetrazol-5-yl)methyl)-2,2-dimethyl-N-(3-phenoxybenzyl)chromane-6-sulfonamide (10.2 mg, 0.02 mmol, 16 %) as a white solid after lyophilization. mp 53-55 °C. HRMS (ESI+) m/z: calculated for C₂₆H₂₈N₅O₄S ([M + H]), 506.1857, found: 506.1855. ¹H NMR (CDCl₃, 400 MHz): δ 8.01 (bs, 1H), 7.59 – 7.48 (m, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.20 (t, J = 7.9 Hz, 1H), 7.12 (t, J = 7.3 Hz, 1H), 6.97 – 6.82 (m, 5H), 6.82 – 6.74 (m, 1H), 4.52 (s, 2H), 4.35 (s, 2H), 2.80 (t, J = 5.9 Hz, 2H), 1.84 (t, J = 6.0 Hz, 2H), 1.36 (s, 6H). ¹³C NMR (CDCl₃, 101 MHz): δ 159.08, 157.96, 156.72, 152.89, 136.51, 130.41, 130.01(2C), 129.66, 127.34, 127.12, 123.76, 122.29, 119.20(2C), 118.76, 118.66, 118.60, 76.31, 52.79, 40.82, 32.16, 27.03(2C), 22.48. Purity >95% as determined by LC-MS.
References


Discovery of glycine sulfonamides as dual inhibitors of DAGLα and ABHD6


107