New chemical tools to illuminate N-acylphosphatidylethanolamine biosynthesis

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Optimization of WEN091 towards selective PLA2G4E inhibitors

Phospholipase A2ε (PLA2G4E) is a serine hydrolase that belongs to the Group IV or cytosolic phospholipases A_2 (cPLA$_2$).[1,2] This family is involved in phospholipid metabolism, with each of the six members (PLA2G4A–F) having specific PLA$_1$, PLA$_2$, lyso-PLA or acyltransferase activities, calcium-dependency and expression pattern.[1,3] Recently, PLA2G4E was identified as a calcium-dependent *N*-acyltransferase (Ca-NAT) capable of producing *N*-acylphosphatidylethanolamines (NAPEs).[4,5]

In humans, two isoforms of PLA2G4E have been identified. The canonical isoform (868 amino acids, 100 kDa) has an N-terminal C2 domain, which is truncated in the alternative splicing product (834 amino acids, 97 kDa).[5] This C2 domain is characteristic of the PLA2G4 family and functions as a calcium-dependent lipid-binding domain.[6,7] In contrast to other PLA2G4 enzymes, PLA2G4E has an additional C-terminal polybasic (PB) domain which is also involved in lipid-binding.[5,8] These positively charged amino acids (KKKRLK) are able to bind phosphatidylinositide phosphates (PIPs), which localizes the enzymes to the membranes of the clathrin-independent endocytic machinery. Here, PLA2G4E is involved in tubule formation required for major histocompatibility complex (MHC) class I protein recycling.[8] Both the C2 and PB domain are needed for catalytic activity and correct cellular distribution.[5] In the catalytic domain, a Ser-Asp catalytic dyad, characteristic of the PLA2G4 family, is responsible for the *N*-acyltransferase activity.[2,4] The nucleophilic serine of PLA2G4E preferentially cleaves the *sn*-1 ester of phosphatidylcholine (PC), which leads to the release of lyso-PC. The acyl chain is subsequently transferred to the amine of phosphatidylethanolamine (PE), thereby producing NAPEs.

NAPEs are a low-abundant class of phospholipids that have both structural and signaling functions.[9] They control membrane dynamics by supporting their structural integrity and inducing membrane fusion[10–12], and regulate the localization of intracellular membrane-interacting proteins.[13] They have inhibitory effects on food-intake[14,15] and inflammation[16] and are highly elevated during ischemia and stress, which is suggested to be a cytoprotective mechanism.[17–19] Furthermore, NAPEs are the precursors to *N*-acylethanolamines (NAEs) through the hydrolytic activity of NAPE-specific phospholipase D (NAPE-PLD).[20] NAEs are a class of lipids with highly diverse signaling functions, including satiety, nociception and anxiety.[21–24] Activity of NAEs is terminated by fatty acid amide hydrolase (FAAH) (Figure 4.1).[25]

Figure 4.1. Schematic overview of biosynthesis and degradation of NAPEs and NAEs. Alternative pathways are not depicted. PE: phosphatidylethanolamine, PC: phosphatidylcholine, NAPE: *N*-acylphosphatidylethanolamine, NAE: *N*-acylethanolamine, FFA: free fatty acid, PLA2G4E: phospholipase A_2 Group IV E, PLAATs: phospholipase A/acyltransferases, NAPE-PLD: NAPE-specific phospholipase D, FAAH: fatty acid amide hydrolase.

The calcium-dependent activity of PLA2G4E is currently hypothesized to be the major pathway of NAPE biosynthesis.[26,27] Additionally, NAPEs are also synthesized calcium-independently by *N*-acyltransferase activity of phospholipases/acyltransferases (PLAATs) 1–5.[28,29] Acute, selective inhibition of PLA2G4E would help elucidate the physiological roles of these two pathways. In Chapter 3, **WEN091** was identified as a potent inhibitor of PLA2G4E that was able to reduce production of NAPEs in cells overexpressing PLA2G4E. However, biological profiling revealed off-target activity on NAE-degrading enzyme FAAH, which may complicate the interpretation of downstream signaling events. In this chapter, the synthesis and biochemical profiling of novel analogues of **WEN091** are described with a focus on their selectivity profile.

Results

Design and synthesis of PLA2G4E inhibitors 1–38

WEN091 is an irreversible, covalent PLA2G4E inhibitor that belongs to the class of triazole ureas. The electrophilic carbonyl of the urea acts as a warhead by carbamoylating the catalytic serine of PLA2G4E (Chapter 2). The triazole functions as a leaving group, while the amine group that is covalently bound to the enzyme is referred to as the staying group. In this chapter, 38 new analogues of **WEN091** were synthesized to further explore the structure-activity relationships on PLA2G4E and FAAH (Figure 4.2). Previously, it was shown that irreversible inhibitors with high intrinsic reactivity may bind in a non-specific manner to other members of the same enzyme family (e.g. FAAH).[30,31] The reactivity of the triazole urea was, therefore, investigated in compounds **1**–**3** (Table 4.1) and the steric and electronic properties of the leaving group were modulated in compounds **4**–**31** (Table 4.1–Table 4.3). In addition, the role of the staying group was further explored in compounds **32**–**38** (Table 4.4) (Figure 4.2).

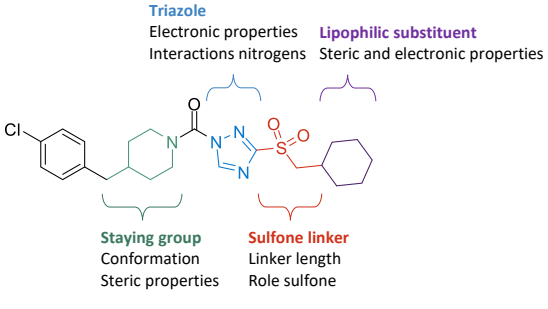


Figure 4.2. Optimization strategy for improving WEN091's selectivity profile. The structure of **WEN091** is depicted with parts of the molecule that were investigated in this chapter highlighted.

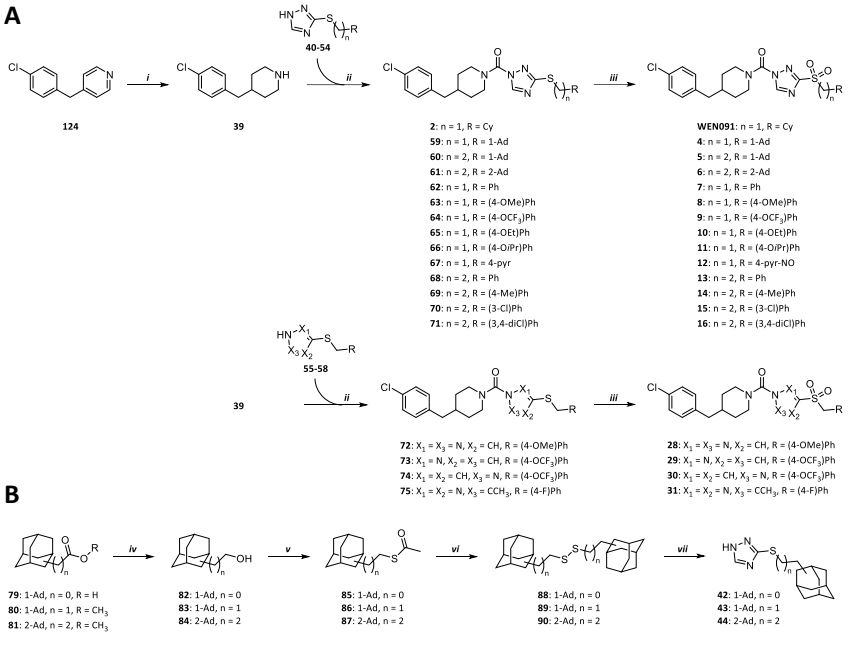
Based on **WEN091**, a similar synthesis plan was deployed for compounds **1**–**16** and **28**–**31** (Scheme 4.1A). Briefly, optimized staying group 4-(4-chlorobenzyl)piperidine (**39**, see Chapter 2) was coupled to different substituted triazole or pyrazole leaving groups (**40**–

58) using triphosgene, yielding triazole or pyrazole ureas **2**, **3**, **59**–**75**. Subsequent oxidation of the thioether in compound **2** to sulfoxide afforded compound **1**. Oxidation of the thioether in **59**–**75** to sulfone afforded inhibitors **4**–**16** and **28**–**31** (Scheme 4.1A).

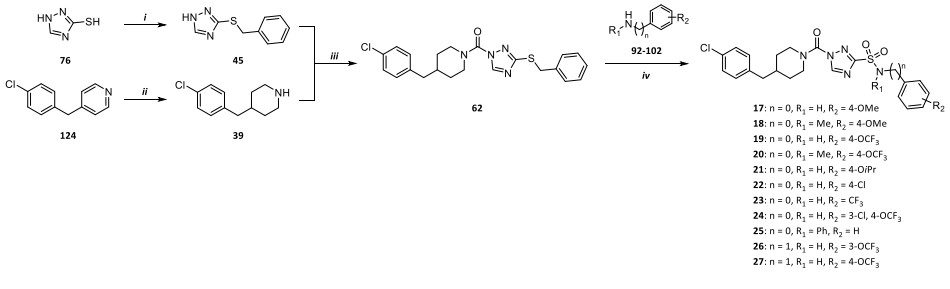
Variations in the triazole leaving group of compounds **1**, **2** and **7**–**16** were introduced by substitution of 1,2,4-triazole-3-thiol (**76**) with various commercial and tailor-made organohalides, leading to compounds **40** and **45**–**54** (Supplementary Scheme S4.1). The triazole amide leaving group **41** of inhibitor **3** was synthesized by reacting 1,2,4-triazole-3-carboxylic acid (**77**) with thionyl chloride, followed by substitution with *N*-methyl-cyclohexylamine (**78**, Supplementary Scheme S4.2). Incorporation of caged hydrocarbons in compounds **4**–**6** requested a different approach (Scheme 4.1B, see also Chapter 6). Adamantanecarboxylic acid (**79**) and the methyl esters **80** and **81** were reduced with $LiAlH_4$. Triflation of the resulting alcohols (**82**–**84**) and subsequent substitution with potassium thioacetate afforded thioacetates **85**–**87**. These were reduced using $LiAlH_4$ and oxidized with molecular bromine, forming disulfides **88**–**90**. Lithiation of 1-(pyrrolidin-1-ylmethyl)-1*H*-1,2,4-triazole (synthesized according to literature)[32] using *n*-BuLi followed by careful addition of disulfides **88**–**90** led to the formation of the desired thioethers, while the pyrrolidin-1-ylmethyl protecting groups were removed immediately by the liberated thiols, providing triazole thioether building blocks **42**–**44**. The byproduct formed in this reaction was easily removed after treatment with $NaBH_4$ in ethanol. For pyrazole building blocks **56** and **57**, the respective aminopyrazole was diazotized using *in situ*-generated nitrous acid, followed by immediate substitution with 4-(trifluoromethoxy)benzyl mercaptan (**91**, Supplementary Scheme S4.3). Each of these azole building blocks was reacted with staying group **39**, forming **2**, **3** and **59**–**75**, and oxidized as depicted in Scheme 4.1A.

Sulfonamide variants **17**–**27** were obtained by a one-pot oxidative chlorination–substitution reaction between triazole urea thioether **62** and desired aniline analogues **92**–**102** (Scheme 4.2, Supplementary Scheme S4.4).

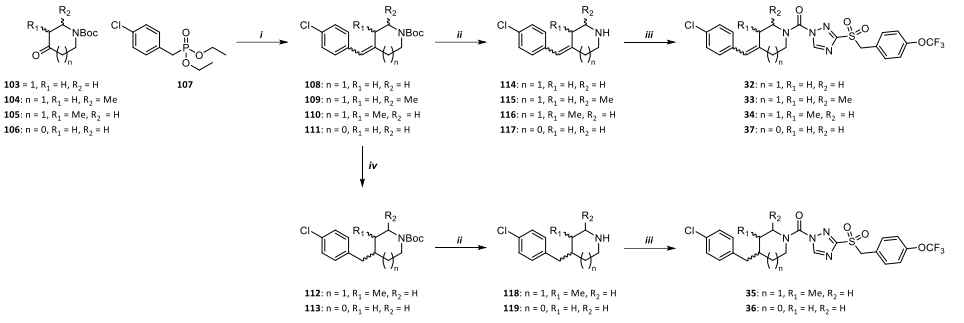
Variations of the 4-(4-chlorobenzyl)piperidine staying group were introduced in inhibitors **32**–**38**. The staying groups in compounds **32**–**37** were synthesized by a Horner-Wadsworth-Emmons olefination of piperidinones **103**–**105** or pyrrolidinone **106** using diethyl (4-chlorobenzyl)phosphonate (**107**), providing **108**–**111** (Scheme 4.3). Hydrogenation of **110** and **111** over palladium on carbon yielded **112** and **113**. **108**–**113** were deprotected to yield the amines **114**–**119**. For compound **38**, Grignard reaction of 4-chlorophenylmagnesium bromide (**120**) with isonipecotic acid ethyl ester **121** and subsequent acidic dehydration afforded amine **122** (Supplementary Scheme S4.5). All amine building blocks were coupled to triazole sulfone leaving group **123** in the triphosgene-mediated urea formation, yielding **32**–**38**.



Scheme 4.1. Synthetic routes towards PLA2G4E inhibitors 2, 4–16 and 28–31. A) General synthetic scheme of **2**–**16** and **28**–**31**. Reagents and conditions: *i*) 4 bar H_2, PtO_2, HCl, EtOH, o/n RT; *ii*) 1. **39**, triphosgene, Et_3N or DIPEA, 3 h 0°C → RT, then 2. **40**–**58**, K_2CO_3, DMF, o/n RT; *iii*) AcOOH, DCM, 6 h 0°C → RT. B) Synthesis of adamantane-containing triazole building blocks for **4**–**6**. Reagents and conditions: *iv*) $LiAlH_4$, THF, 0.5 h reflux; *v*) 1. Tf_2O, pyr, DCM, 45 min −15°C → RT, then 2. AcSK, 18-Crown-6, CH_3CN, 72 h RT; *vi*) 1. $LiAlH_4$, THF, 1.5 h RT → 50°C, then 2. Br_2, DCM/H_2O, RT; *vii*) 1. 1-(pyrrolydin-1-ylmethyl)-1*H*-1,2,4-triazole, *n*-BuLi, THF, 21 h −80°C → RT, then 2. $NaBH_4$, EtOH, 5 min RT. **42**–**44** were treated as described in reaction *ii* and *iii* to generate the final compounds. 1-Ad: adamant-1-yl, 2-Ad: adamant-2-yl.



Scheme 4.2. Synthetic route towards PLA2G4E inhibitors 17–27. Reagents and conditions: *i*) BnBr, DMF, 6 h RT; *ii*) 4 bar H_2, PtO_2, HCl, EtOH, o/n RT; *iii*) 1. **39**, triphosgene, DIPEA, THF, 3 h 0°C → RT, then 2. **45**, K_2CO_3, DMF, o/n RT; *iv*) 1. **62**, HCl, NaOCl, 1,4-dioxane/H_2O, 2 h −10°C, then 2. **92**–**100**, or DIPEA and **101**–**102**, o/n −10°C → RT.



Scheme 4.3. Synthetic routes towards PLA2G4E inhibitors 32–37. Reagents and conditions: *i*) KO*t*Bu or NaH, THF, o/n −10°C → RT; *ii*) TFA, DCM, o/n RT; *iii*) 1. **114**–**119**, triphosgene, DIPEA, THF, 3 h 0°C → RT, then 2. **123**, K_2CO_3, DMF, o/n RT; *iv*) 1 atm H_2, Pd/C, EtOAc, 5 h RT.

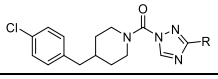
Biochemical evaluation and structure-activity relationships of 1–38

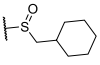
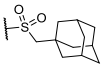
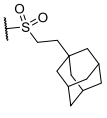
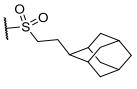
The activity of **1**–**38** was tested in a gel-based competitive activity-based protein profiling (cABPP) assay. This technique assesses the activity and selectivity of a small molecule on multiple enzymes in a biologically relevant context in one experiment. Fluorophosphonate-tetramethylrhodamine (FP-TAMRA) was used as an activity-based probe to measure PLA2G4E and FAAH activity.[33,34] Briefly, lysate of human embryonic kidney (HEK293T) cells overexpressing recombinant hPLA2G4E was treated with inhibitor at different concentrations or vehicle (30 min) and the remaining enzyme activity was labeled with FP-TAMRA (50 nM, 5 min). The proteins were resolved on molecular weight by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and in-gel fluorescence scanning allowed the quantification of residual enzyme activity and determination of the apparent half-maximal inhibitory concentration (IC_{50}). The inhibitory potency of the compounds on FAAH was determined in a similar manner by using mouse brain proteome.[35]

Sulfone linker and small lipophilic substituents are preferred structural elements for PLA2G4E inhibition

Optimization of the triazole leaving group of **WEN091** was started by investigating the role of the sulfone linker between the triazole and the aliphatic substituent (Table 4.1). The decreased potency of sulfoxide, thioether and amide variants **1**–**3** demonstrated the important role of the sulfone. In contrast, activity on FAAH was less strongly affected and **1** and **2** showed a slightly increased activity on FAAH. The apparent selectivity (app. sel.) of **1** for PLA2G4E over FAAH, defined as the ratio of the respective apparent IC_{50} values, was calculated to be 5-fold, whereas that of **WEN091** was 32-fold (Table 4.1). This suggested that the sulfone improves binding to PLA2G4E either through direct interactions with the active site or through its inductive effect on the triazole. Inhibitors with bulky adamantyl substituents (**4**–**6**) showed markedly reduced activity on FAAH, but the activity on PLA2G4E was also lower, indicating smaller lipophilic groups were favored in both enzymes.

Table 4.1. Structure-activity relationships of PLA2G4E inhibitors 1–6. Activity determined with gel-based cABPP on PLA2G4E-overexpressing HEK293T membranes or mouse brain proteome (N ≥ 2). Apparent selectivity (app. sel.) = IC_{50} (PLA2G4E)/IC_{50} (FAAH) = $10^{pIC_{50}(PLA2G4E)-pIC_{50}(FAAH)}$.



		pIC_{50} ± SEM		
ID	**R**	**PLA2G4E**	**FAAH**	**App. sel.**
WEN091		8.01 ± 0.02	6.52 ± 0.04	32
1		7.69 ± 0.01	6.98 ± 0.08	5
2		< 5.0	6.93 ± 0.09	< 1
3		5.15 ± 0.39	6.42 ± 0.04	< 1
4		7.12 ± 0.01	5.26 ± 0.08	72
5		7.40 ± 0.19	< 5.0	> 251
6		7.25 ± 0.20	< 5.0	> 177

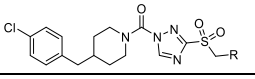
***para*-Substituted benzyl groups provided highest potency and selectivity**

Next, the electronic properties of the leaving group were investigated by substituting the cyclohexylmethyl of **WEN091** with various aromatic groups (Table 4.2). **7**, which contains a benzyl group, was a slightly less potent inhibitor than **WEN091**. Compounds with ether substituents on the *para* position of the benzyl group (**8**–**11**) were tolerated and showed both increasing potency on PLA2G4E and selectivity over FAAH with increasing size and lipophilicity. **12**, which contains a polar 4-pyridine-*N*-oxide, showed decreased activity on PLA2G4E. This suggested that a lipophilic pocket is available in the active site of PLA2G4E, but not in FAAH. Trifluoromethoxy-substituted compound **9** demonstrated over 800-fold higher activity on PLA2G4E (pIC_{50} = 8.1) than on FAAH (pIC_{50} = 5.2), which makes it the most potent and selective PLA2G4E inhibitor identified in this study. Compound **13**, with an ethylene linker between the sulfone and the phenyl, had slightly lower potency on PLA2G4E, which was similar with *para* (**14**, **16**) and further decreased with *meta* (**15**) substitutions on the phenyl ring. These compounds, however, did not show increased selectivity over FAAH (e.g. **16**: app. sel. 79-fold), indicating they did not have similar PLA2G4E-specific interactions as compounds **9** and **11**.

Compounds **17–27** (Table 4.3), which contain a sulfonamide instead of a sulfone, were synthesized, based on the structures of **8, 9** and **11.** It was hypothesized that a weaker electron-withdrawing effect of the sulfonamide could decrease the reactivity of the urea,

thereby reducing inhibitory potency on FAAH and improving the selectivity. Sulfonamides **17** and **18** were equally potent on PLA2G4E to **8** (pIC_{50} = 7.0). Sulfonamides **19** and **21**, however, showed a 10-fold decrease in potency on PLA2G4E compared to sulfones **9** and **11**, respectively, while methylated sulfonamide **20** showed an even larger reduction (pIC_{50} = 6.4). **17**, **19** and **21** had a lower activity on FAAH than their respective sulfone counterparts, but the apparent selectivity window of **19** and **21** was not improved (**19**: app. sel. 148-fold, **21**: 100-fold). Compounds with lipophilic but electron-withdrawing substituents on the *para* position of the phenyl ring (**22**, **23**) had lower potency on PLA2G4E compared to the ones with electron-donating ether groups (**17**, **21**). Inhibitors **24** and **25**, with bulkier substituents, also showed a decreased potency (pIC_{50} < 7.0), while **26** and **27**, with a methylene linker between the sulfonamide and the phenyl, were tolerated but did not have an improved selectivity over FAAH (**26**: app. sel. 123-fold, **27**: 60-fold).

Table 4.2. Structure-activity relationships of PLA2G4E inhibitors 7–16. Activity determined with gel-based cABPP on PLA2G4E-overexpressing HEK293T membranes or mouse brain proteome (N ≥ 2). Apparent selectivity (app. sel.) = IC_{50} (PLA2G4E)/IC_{50} (FAAH) = $10^{pIC_{50}(PLA2G4E)-pIC_{50}(FAAH)}$.



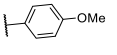
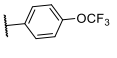
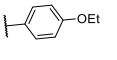
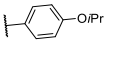
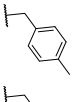
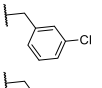
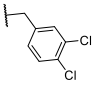
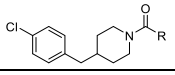
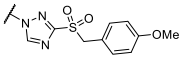
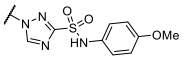
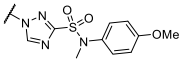
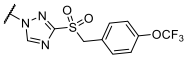
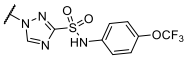
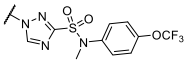
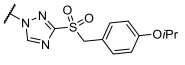
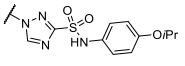
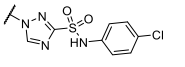
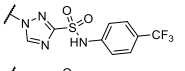
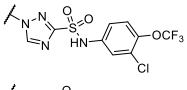
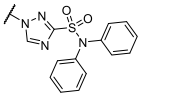
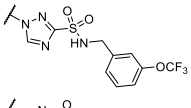
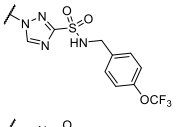
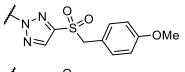
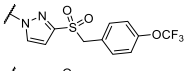
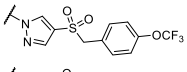
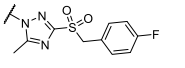
		pIC_{50} ± SEM		
ID	**R**	**PLA2G4E**	**FAAH**	**App. sel.**
WEN091		8.01 ± 0.02	6.52 ± 0.04	32
7		7.23 ± 0.04	7.39 ± 0.06	< 1
8		7.06 ± 0.05	6.43 ± 0.05	4
9 (IK015)		8.10 ± 0.02	5.18 ± 0.18	832
10		7.67 ± 0.03	6.49 ± 0.14	15
11		8.03 ± 0.04	5.35 ± 0.05	479
12		5.44 ± 0.07	5.46 ± 0.03	1
13		7.73 ± 0.05	7.06 ± 0.08	5
14		7.76 ± 0.19	5.99 ± 0.04	59
15		7.18 ± 0.05	6.65 ± 0.10	3
16		7.84 ± 0.15	5.94 ± 0.12	79

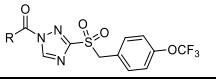
Table 4.3. Structure-activity relationships of PLA2G4E inhibitors 17–31. Activity determined with gel-based cABPP on PLA2G4E-overexpressing HEK293T membranes or mouse brain proteome (N ≥ 2). Apparent selectivity (app. sel.) = IC_{50} (PLA2G4E)/IC_{50} (FAAH) = $10^{pIC_{50}(PLA2G4E)-pIC_{50}(FAAH)}$.

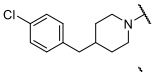
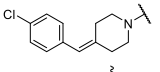
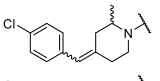
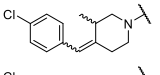
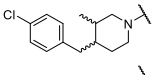
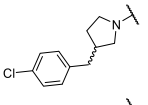
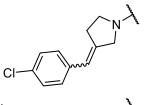
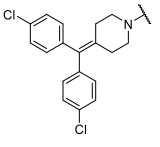


		pIC$_{50}$ ± SEM		
ID	**R**	**PLA2G4E**	**FAAH**	**App. sel.**
8		7.06 ± 0.05	6.43 ± 0.05	4
17		7.01 ± 0.07	5.70 ± 0.15	20
18		7.04 ± 0.07	6.17 ± 0.07	7
9		8.10 ± 0.02	5.18 ± 0.18	832
19 (WEN222)		7.04 ± 0.05	4.87 ± 0.10	148
20		6.38 ± 0.03	6.02 ± 0.07	2
11		8.03 ± 0.04	5.35 ± 0.05	479
21		7.08 ± 0.04	5.08 ± 0.10	100
22		6.58 ± 0.04	4.98 ± 0.36	40
23		6.66 ± 0.05	4.94 ± 0.12	52
24		6.74 ± 0.04	< 5.0	> 54
25		6.21 ± 0.04	< 5.0	> 16
26		7.13 ± 0.03	5.04 ± 0.13	123
27		6.76 ± 0.03	4.98 ± 0.17	60
28		8.07 ± 0.02	6.42 ± 0.09	45
29		5.24 ± 0.06	< 5.0	> 1
30 (WEN258)		< 5.0	< 5.0	NA
31		< 5.0	< 5.0	NA

Next, the role of the triazole in inhibitor binding and reactivity was investigated (Table 4.3). 1,2,3-Triazole urea **28** was a more potent PLA2G4E inhibitor than its 1,2,4-triazole counterpart **8**, but it also had a higher activity on FAAH compared to **9**. Pyrazole ureas **29** and **30** showed greatly diminished activity on both PLA2G4E and FAAH. This might be explained by the higher pK_a of pyrazole (14.2[36], compared to 10.1 for 1,2,4-triazole[37]) and corresponding lower leaving group ability, or by loss of interactions between the third nitrogen and residues in the active sites. Compound **31**, which contains a small methyl substituent on the C5 position of the 1,2,4-triazole, had completely abolished activity on both PLA2G4E and FAAH (pIC_{50} < 5.0), possibly induced by a detrimental steric clash of the methyl group with the enzymes.

Table 4.4. Structure-activity relationships of PLA2G4E inhibitors 32–38. Activity determined with gel-based cABPP on PLA2G4E-overexpressing HEK293T membranes or mouse brain proteome (N ≥ 2). Apparent selectivity (app. sel.) = IC_{50} (PLA2G4E)/IC_{50} (FAAH) = $10^{pIC_{50}(PLA2G4E)-pIC_{50}(FAAH)}$.



		pIC_{50} ± SEM		
ID	**R**	**PLA2G4E**	**FAAH**	**App. sel.**
9		8.10 ± 0.02	5.18 ± 0.18	832
32		7.23 ± 0.02	5.03 ± 0.04	158
33		6.35 ± 0.04	6.19 ± 0.04	1
34		5.95 ± 0.06	< 5.0	> 8
35		6.47 ± 0.10	< 5.0	> 29
36		6.30 ± 0.03	5.63 ± 0.02	5
37		6.69 ± 0.04	4.64 ± 0.06	112
38		< 5.0	< 5.0	NA

Variations of the benzylpiperidine staying group did not improve selectivity

In Chapter 2, a 4-(4-chlorobenzyl)piperidine staying group was found to provide inhibitors with a high potency on PLA2G4E. To improve the selectivity profile of the inhibitors, the conformational and steric properties of this staying group in compound **9** were further explored (Table 4.4). **32**, which has a conformationally restricted staying group, was less potent on PLA2G4E than **9**, while its activity on FAAH was similar. **33**–**35** showed a further decreased activity (pIC_{50} < 6.5), indicating methyl substituents on the 2- or 3-position of the piperidine were not tolerated in PLA2G4E's active site. However, 2-methylpiperidine **33** was a more potent FAAH inhibitor than **32**. Johnson *et al*. have previously described benzylpyrrolidine ureas to be less potent FAAH inhibitors than benzylpiperidine urea analogs.[38] In this study, however, benzylpyrrolidine **36** did not show reduced activity on FAAH compared to benzylpiperidine **9**, while benzylpyrrolidine **37** showed only little reduction. Instead, both **36** and **37** showed a larger decrease in potency on PLA2G4E, leading to an app. sel. of 5-fold and 112-fold, respectively. **38**, which contains an additional phenyl group, was completely inactive on both PLA2G4E and FAAH (pIC_{50} < 5.0), indicating both active sites cannot accommodate this bulky structure.

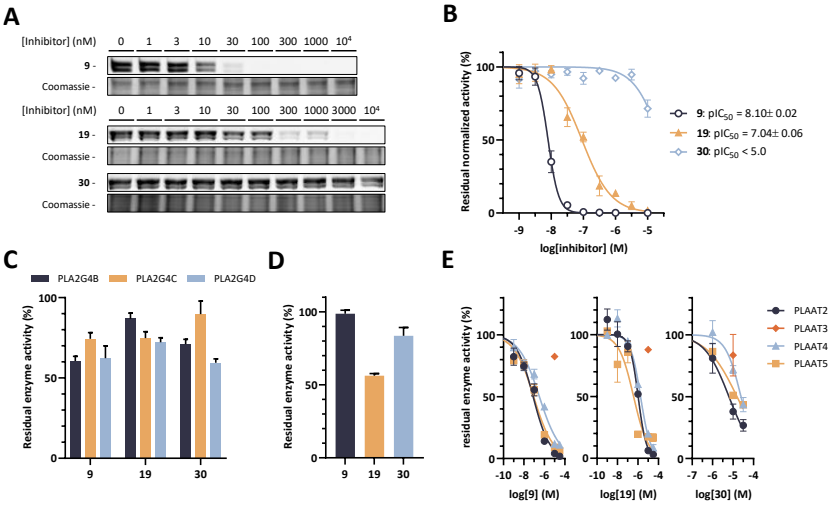


Figure 4.3. Activity of 9, 19 and 30 on PLA2G4B–E and enzymes involved in NAPE metabolism. A) Representative gel excerpts of cABPP experiments with **9**, **19** and **30** on PLA2G4E overexpression lysate. B) Corresponding inhibition curves and pIC_{50} values. Data presented as mean ± SEM (N ≥ 2). C) At 10 µM, **9**, **19** and **30** show less than 50% inhibition (compared to vehicle) of other PLA2G4 family members. Data presented as mean ± SEM of cABPP experiments on overexpression lysate (N = 2). D) At 10 µM, **9**, **19** and **30** show less than 50% inhibition (compared to vehicle) of NAPE-PLD. Data presented as mean ± SEM of fluorigenic substrate (PED6) assays on overexpression lysate (N = 2, n = 2). E) Inhibition curves of **9**, **19** and **30** on PLAAT family members. Data presented as mean ± SEM of cABPP experiments on overexpression lysate (N = 2).

Table 4.5. Inhibition values of 9, 19 and 30 on PLA2G4B–E and enzymes involved in NAPE metabolism. Inhibition values obtained from cABPP experiments (PLA2G4B–E, PLAAT2–5) or PED6 substrate assay (NAPE-PLD) on overexpression lysate, corresponding to Figure 4.3. Data presented as pIC_{50} ± SEM. When pIC_{50} could not be determined within the used concentration range, mean % inhibition ± SEM is reported (N ≥ 2).

	PLA2G4B	PLA2G4C	PLA2G4D	PLAAT2	PLAAT3	PLAAT4	PLAAT5	NAPE-PLD
9	39 ± 5%	26 ± 5%	38 ± 11%	7.10 ± 0.11	18 ± 2%	6.49 ± 0.11	7.05 ± 0.10	1 ± 5%
19	13 ± 5%	25 ± 6%	28 ± 4%	5.99 ± 0.08	12 ± 3%	5.75 ± 0.08	6.43 ± 0.21	44 ± 3%
30	29 ± 4%	10 ± 12%	41 ± 3%	5.20 ± 0.11	17 ± 24%	28 ± 5%	49 ± 3%	17 ± 11%

Biological profiling of 9, 19 and 30

In vitro selectivity over related enzymes

Compounds **9** and **19** were selected for further biological characterization, because **9** was the most potent PLA2G4E inhibitor with the highest selectivity over FAAH and **19** is a close structural analog of **9** with lower lipophilicity (Supplementary Table S4.1). Analog **30** was selected as a potential control compound that would inhibit the off-targets of compound **9**, but not PLA2G4E (Figure 4.3). **9**, **19** and **30** did not bind the CB_1 and CB_2 receptors (Supplementary Table S4.2) and did not inhibit NAPE-PLD, PLA2G4B, PLA2G4C or PLA2G4D (Figure 4.3C-D, Table 4.5). Of note, the activity on PLA2G4A and PLA2G4F could not be determined due to a lack of expression of the proteins in HEK293T cells. Surprisingly, **9** and **19** did inhibit the *N*-acyltransferases PLAAT2, PLAAT4 and PLAAT5 with submicromolar activity, but not phospholipase PLAAT3 (Figure 4.3E, Table 4.5).[29,39,40] **30** only inhibited PLAAT2 with IC_{50} < 10 µM. PLAATs are structurally distinct from PLA2G4E, but their active sites may share common structural features that not only recognize similar endogenous substrates, but also the same synthetic inhibitors.

The selectivity of **9** and **19** and control compound **30** over other serine hydrolases was assessed with cABPP on mouse brain lysate (Figure 4.4). **9** and **19** displayed in general good selectivity (>30-fold) over the other identified hydrolases. Both inhibited one unidentified serine hydrolase (~70–80 kDa) with a similar IC_{50} value as for PLA2G4E and α/β hydrolase domain-containing protein 6 (ABHD6) at approximately four times higher concentration (**9**: pIC_{50} (ABHD6) = 7.4, **19**: pIC_{50} (ABHD6) = 6.5, Table 4.6). ABHD12, monoacylglycerol lipase (MAGL), diacylglycerol lipase α (DAGLα) and acyl protein thioesterases 1 and 2 (LyPLA1 and 2) were inhibited at concentrations ≥ 1 µM. **30** had a similar off-target profile as **9**, albeit slightly less potent on most enzymes (Table 4.6). In conclusion, **9** and **19** are selective inhibitors of NAPE-producing enzymes regardless whether these proteins can be activated by calcium ions or not.

Cellular activity on Neuro-2a serine hydrolases

Finally, compounds **9**, **19** and **30** were also assessed on their cellular activity. Briefly, Neuro-2a cells were treated with increasing concentrations of the inhibitors (30 min), harvested and lysed, and the remaining enzyme activity was determined by gel-based cABPP (Figure 4.5). In general, the inhibitors maintained their overall selectivity profile, albeit that ABHD6

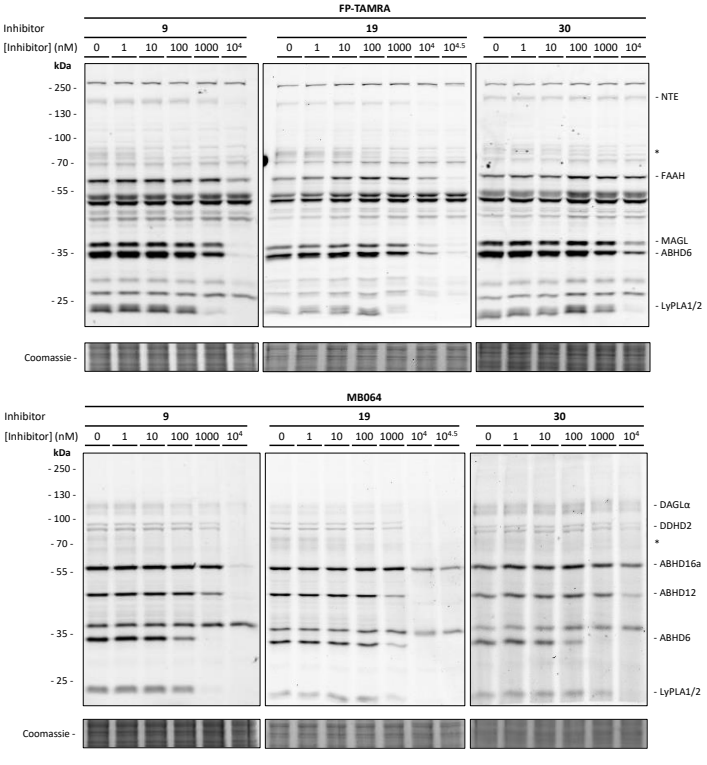


Figure 4.4. *In vitro* activity of 9, 19 and 30 on mouse brain serine hydrolases. Representative gel images of cABPP experiments on mouse brain membrane proteome. Names of identified off-targets of these inhibitors are indicated. Unidentified off-target indicated with *.

Table 4.6. *In vitro* pIC_{50} values of 9, 19 and 30 on mouse brain serine hydrolases. Inhibition values obtained from cABPP experiments on mouse brain membrane proteome using FP-TAMRA and MB064, corresponding to Figure 4.4. When pIC_{50} could not be determined within the used concentration range, % inhibition at 10 μM is reported. Data reported as mean ± SEM (N ≥ 2). Inhibition of unidentified ~70-80 kDa protein could not reliably be quantified.

	ABHD6	ABHD12	ABHD16a	DAGLα	DDHD2	FAAH	LyPLA1/2	MAGL	NTE
9	7.43 ± 0.02	6.37 ± 0.06	5.96 ± 0.02	5.98 ± 0.15	6.14 ± 0.03	5.18 ± 0.18	6.73 ± 0.05	5.95 ± 0.04	5.04 ± 0.11
19	6.48 ± 0.05	6.34 ± 0.07	4.65 ± 0.18	5.48 ± 0.10	5.83 ± 0.06	4.87 ± 0.10	5.82 ± 0.21	5.13 ± 0.09	5.30 ± 0.08
30	7.10 ± 0.04	5.41 ± 0.05	4.90 ± 0.23	4.89 ± 0.20	4.98 ± 0.24	0 ± 5%	5.71 ± 0.16	5.24 ± 0.10	9 ± 4%

and FAAH inhibition by **9** and **30** was 10- to 100-fold increased compared to the mouse brain lysate (Table 4.6 and Table 4.7). Interestingly, **19** also showed increased activity on FAAH, but not on ABHD6.

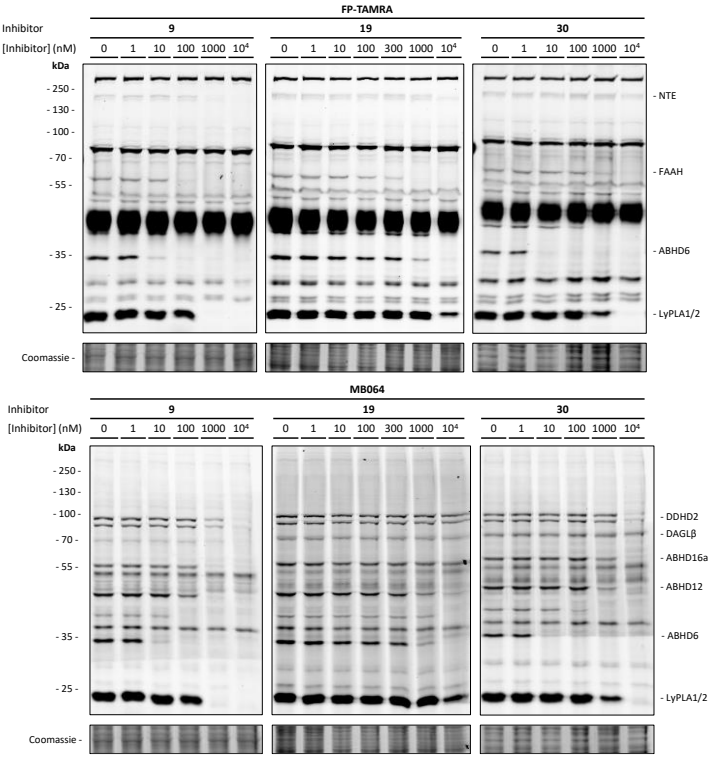


Figure 4.5. Cellular activity of 9, 19 and 30 on Neuro-2a serine hydrolases. Representative gel images of cABPP experiments on Neuro-2a cells. Names of identified off-targets of the inhibitors are indicated.

Table 4.7. Cellular pIC_{50} values of 9, 19 and 30 on Neuro-2a serine hydrolases. Inhibition values obtained from cABPP experiments on Neuro-2a cells using FP-TAMRA and MB064, corresponding to Figure 4.5. When pIC_{50} could not be determined within the used concentration range, % inhibition at 10 µM is reported. Data reported as mean ± SEM (N ≥ 2).

	ABHD6	ABHD12	ABHD16a	DAGLβ	DDHD2	FAAH	LyPLA1/2	NTE
9	8.40 ± 0.03	6.97 ± 0.09	6.43 ± 0.10	5.96 ± 0.19	6.08 ± 0.06	7.29 ± 0.14	6.91 ± 0.06	6.44 ± 0.08
19	6.48 ± 0.07	6.13 ± 0.06	3 ± 14%	8 ± 9%	22 ± 10%	6.74 ± 0.05	5.49 ± 0.08	5.20 ± 0.06
30	8.67 ± 0.05	6.39 ± 0.10	6.04 ± 0.03	7 ± 19%	5.50 ± 0.07	6.50 ± 0.14	6.42 ± 0.07	4.69 ± 0.18

Discussion and conclusions

In this study, **9** (**IK015**) and **19** (**WEN222**) were identified by gel-based cABPP as the most potent and selective PLA2G4E inhibitors identified to date. Interestingly, the NAPE-producing enzymes PLAAT2, PLAAT4 and PLAAT5 were also inhibited by **9** and **19**, which suggests that these compounds are general inhibitors of NAPE-biosynthesis. As such, they could be used to study the inhibition of NAPE biosynthesis on membrane integrity[41], inflammatory responses[16], NAE production[26] or in response to ischemic stress[42] in an acute

setting. Although **9** and **19** do not specifically inhibit the Ca^{2+}-dependent pathway, pan-PLAAT inhibitor **LEI-301** can be used in conjunction to dissect these two pathways.[43] **30** was not active on PLA2G4E *in vitro*, but showed a similar off-target profile to **9** and **19** in both mouse brain proteome and Neuro-2a cells. It is anticipated that **30** (**WEN258**) can therefore be used as a control compound in biological experiments to distinguish off-target activities from PLA2G4E mediated effects (see Chapter 3).

In general, compounds loose activity in cellular systems due to restricted membrane permeability.[44] In contrast, **9** showed increased FAAH activity in an intact cellular system compared to lysates. This was previously also observed with the FAAH inhibitor BIA 10-2474.[31] The increased inhibition was also observed for ABHD6, but not for other enzymes, such as ABHD12 and ABHD16a. ABHD12, ABHD16a and FAAH are all reported to be localized in the endoplasmic reticulum[45,46], suggesting that (subcellular) compound accumulation is not sufficient to explain the increased cellular potency. Further studies are required to explain this phenomenon. While **19** demonstrated activity on several lipases in mouse brain proteome, it was less active on most serine hydrolases in Neuro-2a cells (Table 4.6 and Table 4.7). Its high topological polar surface area (tPSA = 104 Å^2, Supplementary Table S4.1), which impairs cell permeability, may explain this effect.[47,48] The different cellular activity profiles of compounds **9** and **19** indicate that biochemical activity does not always reflect the cellular activity even within one chemical series. To our knowledge, there is currently no cellular system available that exhibits endogenous PLA2G4E activity. This complicates the evaluation of PLA2G4E inhibitors. A cellular target engagement assay using overexpressed PLA2G4E would be a first step to link the biochemical activity of these inhibitors to their cellular effects (see Chapter 5).

Finally, **9** is a useful first chemical tool compound to study the biological role of PLA2G4E *in vitro*, but a better understanding of its molecular interactions with PLA2G4E and PLAAT2–5, obtained e.g. via co-crystallization or cryo-EM experiments, may lead to the discovery of advanced inhibitors that are specific for Ca^{2+}-dependent NAPE biosynthesis. Projecting forward, rapid metabolism of the compound (i.e. hydrolysis of the urea by carboxyl esterases) may pose a problem for *in vivo* studies.[49] Reducing the intrinsic reactivity of the urea or discovery of alternative scaffolds may address this problem. Furthermore, brain-active inhibitors preferentially have tPSA < 90 Å^2 and cLogP 1–3.[48] Therefore, the physico-chemical properties should be taken into careful consideration for the optimization of the next generation of PLA2G4E inhibitors.

Acknowledgements

Mathijs Wissingh, Ivan Kulyk and Yevhenii Radchenko are kindly acknowledged for performing organic synthesis and cABPP, Wouter Driever and Laura de Paus for selectivity assays, Hans van den Elst for preparative HPLC. Hans den Dulk and Tom van der Wel are acknowledged for plasmid cloning and purification.

Experimental procedures

General remarks

All chemicals and reagents were purchased from Thermo Fisher Scientific or Bio-Rad, unless noted otherwise. Activity-based probes were purchased from Thermo Fisher Scientific (FP-TAMRA) or synthesized in-house (MB064) (chemical structures in Chapter 3 Supplementary Figure S3.8). Inhibitors were synthesized in-house as described below.

Plasmids

The full-length cDNA of wild type human PLA2G4E (GenScript Biotech), murine PLA2G4B, hPLA2G4C, mPLA2G4D (Source BioScience), hPLAAT2, hPLAAT3, hPLAAT4, hPLAAT5 and hNAPE-PLD (kindly provided by Prof. Natsuo Ueda) were cloned into a pcDNA™3.1(+) expression vector in-frame with a C-terminal FLAG tag. Plasmids were isolated from transformed *Escherichia coli* XL-10 using a Qiagen Plasmid Midi kit and stored at 4°C in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). The sequence was determined (Macrogen) and verified using CLC Main Workbench.

Cell culture

HEK293T (human embryonic kidney, ATCC) cells were cultured in DMEM (Sigma-Aldrich, D6546) with additional heat-inactivated new-born calf serum (10% (v/v), Avantor Seradigm), L-Ala-L-Gln (2 mM, Sigma-Aldrich), penicillin and streptomycin (both 200 µg/mL, Duchefa Biochemie) at 37°C, 7% CO_2. Medium was refreshed every 2–3 days and cells were passaged twice a week at 70–80% confluence by aspirating the medium, thorough pipetting in fresh medium and seeding to appropriate density. Cell cultures were regularly tested for mycoplasma and discarded after 2–3 months.

Transient transfection

One day prior to transfection 10^7 HEK293T cells were seeded to a 15 cm dish. Upon transfection medium was aspirated and replaced by 13 mL fresh medium. Plasmid DNA (20 µg per 15 cm dish) and PEI (60 µg per 15 cm dish) were separately dissolved in 1 mL DMEM without serum, mixed, incubated for 15 min and added dropwise to the cells. 24 h p.t. medium was replaced by 25 mL fresh medium. 72 h p.t. medium was aspirated and the cells were washed with RT Dulbecco's PBS, harvested in PBS and centrifuged (3000 × *g*, 15 min, RT). Cell pellets were flash-frozen in liquid N_2 and stored at −80°C until use.

Overexpression lysate preparation

PLA2G4B–E overexpression and lysate preparation was performed as described in Chapter 3. Sample preparation of PLAAT2–5, NAPE-PLD and $CB_{1/2}$ was performed as described before.[43,50]

Mouse brain lysate preparation

Mouse brains were harvested and lysate was prepared as described in Chapter 3.

Activity-based protein profiling

Gel-based ABPP on PLA2G4B–E, PLAAT2–5 and mouse brain proteome was performed as described in Chapter 3.

NAPE-PLD activity assay

The PED6 assay was performed as described in Chapter 3.

CB1/2

The [^{3}H]CP55,940 displacement assay was performed as described before.[50]

Cellular activity assay

Neuro-2a cellular ABPP was performed as described in Chapter 3.

Organic synthesis

General remarks

All reagents were purchased from Sigma-Aldrich, Acros Organics, Merck and Fluorochem and used without further purification. Solvents were purchased from Sigma-Aldrich, VWR Chemicals or Honeywell Riedel-de Haën, common salts from Sigma-Aldrich or Chem-Lab and used without further purification. Moisture-sensitive reactions were carried out in solvents dried over heat-activated molecular sieves (4 Å, Sigma-Aldrich), using flame-dried glassware under an atmosphere of N_2. TLC analysis was performed on Merck silica gel 60 F_{254} aluminum TLC plates, on which compounds were visualized under 254 or 366 nm UV light and using $KMnO_4$ (30 mM $KMnO_4$, 180 mM K_2CO_3 in water) or ninhydrin (7.5 mM ninhydrin, 10% (v/v) AcOH in EtOH) stains. Flash column chromatography was performed using SiO_2 (Macherey-Nagel, 60 M) as stationary phase.

NMR spectra were recorded on a Bruker AV-400 MHz or AV-500 MHz spectrometer at 400 MHz (^{1}H) and 101 MHz (^{13}C) or 500 MHz (^{1}H) and 126 MHz (^{13}C) respectively, using $CDCl_3$ or MeOD (Eurisotop) as solvent. Chemical shifts are reported in ppm with TMS (^{1}H $CHCl_3$, δ 0.00) or solvent resonance (^{1}H MeOD, δ 3.31; ^{13}C MeOD, δ 49.00; ^{13}C $CHCl_3$, δ 77.16) as internal standard. Data are reported as follows: chemical shift δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, p = pentet, dd = doublet of doublets, td = triplet of doublets, qd = quartet of doublets, dt = doublet of triplets, bs = broad singlet (^{1}H), br = broad (^{13}C), m = multiplet), coupling constants *J* (Hz) and integration. HPLC-MS analysis was performed on a Finnigan Surveyor HPLC system equipped with a Macherey-Nagel NUCLEODUR C_{18} Gravity, 5 µm, 50×4.6 mm column followed by a Thermo Scientific LTQ Orbitrap XL spectrometer, using H_2O/CH_3CN + 1% TFA as mobile phase. All compounds used for biological experiments were ≥95% pure based on LC-MS UV absorbance.

General procedure A

Peracetic acid (5–10 eq, 36–40% in AcOH) was added to a solution of triazole urea thioether (1 eq) in DCM (20–30 mL/mmol) and the mixture was stirred for at least 4 h. Upon reaction completion, the mixture was diluted with DCM, washed with water and brine and concentrated *in vacuo*.

General procedure B

4 M HCl in 1,4-dioxane (35 eq) was diluted to 1 M in DCM and cooled to −10°C. To this a 15% NaOCl solution in water (21 eq) was added carefully and the mixture was stirred for 1 h, during which it turned bright yellow. **62** (1 eq) dissolved in DCM (10 mL/mmol) was added dropwise and the mixture was stirred for 1 h. Desired substituted aniline (35 eq) was added dropwise, after which the dark suspension was stirred for 1 h before it was allowed to warm to RT and stirred overnight or until full conversion was confirmed on TLC. The reaction was quenched with 0.1 M aq. HCl, after which the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*.

General procedure C

Initial steps of the reaction were carried out as described in General procedure B. After addition of **62** the mixture was stirred for 70 min at −10°C followed by 1 h at RT. The reaction was then cooled back

to −10°C and partially quenched by addition of DIPEA (20 eq, 450 µL). Desired substituted benzylamine (15 eq) dissolved in 2 mL DCM was added carefully and the mixture was stirred for 1 h, before being allowed to warm to RT and stirred for another 42 h. The mixture was diluted with EtOAc and the reaction was quenched with 0.1 M aq. HCl, after which the layers were separated. The organic layer was washed with 0.1 M aq. HCl and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*.

General procedure D

To an ice-cold solution of triphosgene (3 eq) and Na_2CO_3 or DIPEA (3 eq) in dry THF (5–10 mL) a solution of (substituted) 4-benzylpiperidine (1 eq) in dry THF (5–10 mL) was added dropwise (< 1 mL/min). The mixture was stirred on ice for 1 h, followed by 2 h at RT. The mixture was then diluted with EtOAc and washed with 1 M aq. HCl and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Water was removed by co-evaporation with toluene. The resulting oil was dissolved in dry DMF (2–5 mL), triazole thioether (1 eq) and K_2CO_3 (3 eq) were added and the reaction mixture was stirred overnight. The mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*.

General procedure E

To an ice-cold solution of $LiAlH_4$ (1.5 eq) in dry THF (20 mL/g of thioacetate) a solution of thioacetate (1 eq) in dry THF (10 mL/g of thioacetate) was added dropwise. The reaction mixture was allowed to warm to RT and stirred for 30 min, followed by 1 h at 50°C. The reaction was cooled and quenched by addition of 0.1 M aq. HCl on an ice bath. Solids were removed by filtration and washed with DCM. Combined filtrates were concentrated *in vacuo*. The resulting yellow oil was dissolved in DCM (20 mL/g of thioacetate) and added to a suspension of silica powder (5 g/g of thioacetate) in water (2.5 mL/g of thioacetate). 1 M Br_2 in DCM was added until the mixture started to color. Solids were removed by filtration. The filtrate was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Silica plug purification with pentane provided the disulfide.

General procedure F

1*H*-1,2,4-Triazole-3-thiol or sodium 1*H*-1,2,3-triazole-4-thiolate (1–1.1 eq), desired organohalide (1 eq) were dissolved in dry DMF (2–10 mL/mmol of triazole). To reactions with 1*H*-1,2,4-triazole-3-thiol K_2CO_3 (1–2 eq) was added. The mixture was stirred for at least 3 h. When full conversion was confirmed using TLC analysis EtOAc and water were added and the layers were separated. The aqueous layer was extracted with EtOAc, after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*.

General procedure G

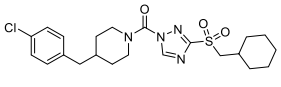
A solution of 1-(pyrrolidin-1-ylmethyl)-1*H*-1,2,4-triazole (2 eq) in dry THF (10 mL/mmol of triazole) was degassed by N_2 purging and cooled to −80°C. *n*-BuLi (2.3 M in THF, 2.2 eq) was added portion wise, after which the reaction mixture was stirred at −80°C for 30 min followed by 90 min at −30 to −25°C during which a white precipitate formed. The mixture was then cooled to −80°C and a solution of disulfide (1 eq) in dry THF (3 mL/mmol of disulfide) was added portion wise. The reaction was stirred for ≥ 3 h at −75°C, after which it was allowed to warm to RT and stirred overnight. Volatiles were removed under reduced pressure, after which the concentrate was diluted in DCM and washed with water. The organic layer was concentrated *in vacuo*. The residue was brought onto a silica gel column and washed with pentane and DCM. The product was then eluted using 1:1 DCM:MeOH. The fractions containing product were combined and concentrated *in vacuo*.

The product was treated with $NaBH_4$ in EtOH (20 eq) and stirred for 5 min. Volatiles were removed under reduced pressure, after which the residue was dissolved in DCM and washed with water and brine. The organic layer was concentrated *in vacuo*. The residue was dispersed in 5 M aq. KOH and extracted with chloroform. The pH of the aqueous layer was lowered to 7 using 12 M aq. HCl, after which it was again extracted with chloroform. The combined organic layers were dried over Na_2SO_4, filtrated and concentrated *in vacuo* yielding the triazole thioether, which was used without further purification.

General procedure H

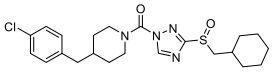
Diethyl 4-chlorobenzylphosphonate (1.2 eq) was dissolved in dry THF (2–3 mL/mmol of ketone) and cooled to −10°C. To this, freshly prepared 1 M KO*t*Bu in dry THF (1.5 eq) was added dropwise over the course of 10 min, upon which the mixture turned yellow and cloudy. The mixture was stirred for 1–3 h keeping the temperature below 5°C. Desired Boc-protected piperidinone or pyrrolidinone (1 eq) was dissolved in dry THF (1–2 mL/mmol) and added dropwise, upon which the color of the mixture slightly changed. The mixture was then allowed to warm to RT and stirred overnight. When full conversion was confirmed using TLC analysis, the reaction mixture was poured into ice-cold water and stirred for 1–2 h. The mixture was then extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((cyclohexylmethyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)-methanone (WEN091)



The title compound was synthesized as described in Chapter 2.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((cyclohexylmethyl)sulfinyl)-1*H*-1,2,4-triazol-1-yl)-methanone (1)



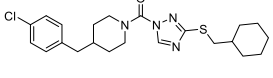
2 (7 mg, 0.016 mmol) was oxidized according General procedure A, but only 2 eq of AcOOH were used. Flash column chromatography (40 → 100% EtOAc in pentane) afforded the title compound (2 mg, 4.5 µmol, 28%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (s, 1H), 7.30 – 7.23 (m, 2H), 7.11 – 7.03 (m, 2H), 4.68 – 4.29 (m, 2H), 3.28 (dd, *J* = 13.0, 5.2 Hz, 1H), 3.19 – 2.82 (m, 2H), 2.97 (dd, *J* = 13.0, 8.8 Hz, 1H), 2.56 (d, *J* = 6.8 Hz, 2H), 2.06 – 2.00 (m, 2H), 2.02 – 1.91 (m, 1H), 1.86 – 1.72 (m, 3H), 1.68 (m, 2H), 1.47 – 1.22 (m, 6H), 1.26 – 1.06 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 147.73, 138.07, 130.51, 128.69, 61.43, 42.17, 37.98, 32.33, 30.46, 29.85, 26.09, 26.03.

HRMS: $[M+H]^+$ calculated for $C_{22}H_{29}ClN_4O_2S+H^+$ 449.1773, found 449.1774.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((cyclohexylmethyl)thio)-1*H*-1,2,4-triazol-1-yl)-methanone (2)

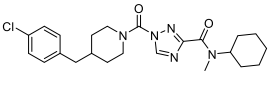


39 (1.1 eq, 20 mg, 0.095 mmol) was reacted with **40** (17 mg, 0.086 mmol) according to General procedure D. The residue was purified by flash column chromatography (0 → 30% EtOAc in pentane) yielding the title compound as a white gum (29 mg, 0.067 mmol, 78%).

Analytical data on next page.

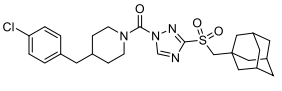
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.30 – 7.22 (m, 2H), 7.11 – 7.03 (m, 2H), 4.85 – 4.27 (m, 2H), 3.02 (d, *J* = 6.9 Hz, 2H), 2.99 – 2.89 (m, 2H), 2.56 (d, *J* = 7.0 Hz, 2H), 1.94 – 1.86 (m, 2H), 1.86 – 1.76 (m, 1H), 1.81 – 1.70 (m, 6H), 1.69 – 1.57 (m, 1H), 1.35 (qd, *J* = 12.5, 4.1 Hz, 2H), 1.24 – 1.08 (m, 2H), 1.00 (qd, *J* = 12.0, 3.4 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 163.40, 148.36, 147.39, 138.22, 132.08, 130.48, 128.61, 42.28, 38.87, 38.08, 37.90, 32.71, 31.98, 29.82, 26.37, 26.13.
HRMS: $[M+H]^+$ calculated for $C_{22}H_{29}ClN_4OS+H^+$ 433.18234, found 433.28218.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-cyclohexyl-*N*-methyl-1*H*-1,2,4-triazole-3-carboxamide (3)



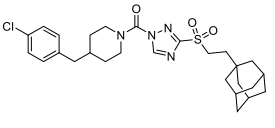
39 (1.1 eq, 111 mg, 0.53 mmol) was reacted with **41** (1 eq, 100 mg, 0.48 mmol) according to General procedure D. Flash column chromatography (EtOAc in pentane) yielded two rotationally restricted isomers (ratio ~1.7:1) of the title compound as slightly yellowish oil (127 mg, 0.29 mmol, 60%).
Rotamer 1:
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 7.30 – 7.23 (m, 2H), 7.11 – 7.04 (m, 2H), 4.79 – 4.31 (m, 2H), 3.68 (tt, *J* = 11.8, 3.6 Hz, 1H), 3.10 – 2.85 (m, 2H), 3.01 (s, 3H), 2.55 (d, *J* = 6.9 Hz, 2H), 1.90 – 1.71 (m, 7H), 1.66 – 1.62 (m, 1H), 1.63 – 1.51 (m, 2H), 1.35 (qd, *J* = 12.9, 3.8 Hz, 2H), 1.24 – 1.01 (m, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 161.50, 158.33, 148.26, 148.24, 138.12, 132.12, 130.49, 128.63, 58.12, 42.22, 38.00, 30.95, 27.96, 25.66, 25.60.
Rotamer 2:
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 7.30 – 7.23 (m, 2H), 7.11 – 7.04 (m, 2H), 4.79 – 4.31 (m, 2H), 4.60 – 4.49 (m, 1H), 3.10 – 2.85 (m, 2H), 2.95 (s, 3H), 2.55 (d, *J* = 6.9 Hz, 2H), 1.90 – 1.71 (m, 7H), 1.51 – 1.42 (m, 3H), 1.35 (qd, *J* = 12.9, 3.8 Hz, 2H), 1.24 – 1.01 (m, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 161.14, 158.61, 148.26, 148.24, 138.13, 132.12, 130.49, 128.63, 53.45, 42.18, 38.00, 31.23, 29.57, 25.64, 25.28.
HRMS: $[M+H]^+$ calculated for $C_{23}H_{30}ClN_5O_2+H^+$ 444.21608, found 444.21616.

(3-(((Adamant-1-yl)methyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)-methanone (4)



59 (30 mg, 0.062 mmol) was oxidized according to General procedure A. Flash column chromatography (33% EtOAc in pentane) afforded the title compound as a white solid (25 mg, 0.048 mmol, 81%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.29 – 7.24 (m, 2H), 7.10 – 7.06 (m, 2H), 4.55 – 4.34 (bs, 2H), 3.25 (s, 2H), 3.18 – 2.85 (m, 2H), 2.56 (d, *J* = 5.1 Hz, 2H), 2.01 – 1.96 (m, 3H), 1.88 – 1.61 (m, 15H), 1.37 (qd, *J* = 13.2, 4.3 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 163.42, 148.01, 147.32, 137.98, 132.17, 130.48, 128.65, 66.22, 47.83 (br), 46.62 (br), 42.07, 42.00, 37.87, 36.40, 34.76, 28.38.
HRMS: $[M+H]^+$ calculated for $C_{26}H_{33}ClN_4O_3S+H^+$ 517.20347, found 517.20322.

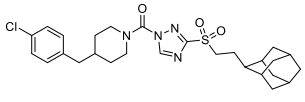
(3-((2-(Adamant-1-yl)ethyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)-methanone (5)



60 (25 mg, 0.050 mmol) was oxidized according to General procedure A. Flash column chromatography (33% EtOAc in pentane) afforded the title compound as a white solid (25 mg, 0.047 mmol, 94%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.29 – 7.24 (m, 2H), 7.11 – 7.05 (m, 2H), 4.56 – 4.36 (m, 2H), 3.41 – 3.32 (m, 2H), 3.20 – 2.86 (m, 2H), 2.57 (d, *J* = 6.8 Hz, 2H), 1.97 (p, *J* = 3.1 Hz, 3H), 1.87 – 1.52 (m, 9H), 1.47 – 1.43 (m, 6H), 1.37 (qd, *J* = 13.1, 4.1 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 161.81, 148.22, 147.25, 137.97, 132.19, 130.47, 128.66, 49.54, 47.77 (br), 46.21 (br), 42.07, 42.00, 37.87, 36.88, 35.33, 32.07, 28.51.
HRMS: $[M+H]^+$ calculated for $C_{27}H_{35}ClN_4O_3S+H^+$ 531.21912, found 531.21895.

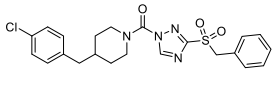
(3-((2-(Adamant-2-yl)ethyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)-methanone (6)



61 (28 mg, 0.056 mmol) was oxidized according to General procedure A. Flash column chromatography (33% EtOAc in pentane) afforded the title compound as a white solid (29 mg, 0.055 mmol, 97%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.29 – 7.25 (m, 2H), 7.11 – 7.04 (m, 2H), 4.53 – 4.36 (m, 2H), 3.41 – 3.34 (m, 2H), 3.20 – 2.87 (m, 2H), 2.61 – 2.52 (m, 2H), 2.02 – 1.92 (m, 2H), 1.91 – 1.60 (m, 16H), 1.55 – 1.48 (m, 2H), 1.37 (qd, *J* = 13.1, 4.1 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 161.89, 148.22, 147.23, 137.96, 132.18, 130.47, 128.66, 53.10, 47.98 (br), 46.45 (br), 43.44, 42.06, 38.94, 38.16, 37.87, 31.58, 31.38, 28.07, 27.86, 24.74.
HRMS: $[M+H]^+$ calculated for $C_{27}H_{35}ClN_4O_3S+H^+$ 531.21912, found 531.21893.

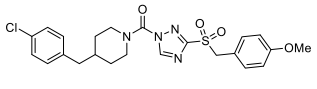
(3-(Benzylsulfonyl)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)methanone (7)



62 (30 mg, 0.070 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (28 mg, 0.063 mmol, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.33 – 7.24 (m, 7H), 7.10 – 7.02 (m, 2H), 4.63 (s, 2H), 4.44 – 3.99 (m, 2H), 3.08 – 2.78 (m, 2H), 2.68 – 2.40 (m, 2H), 1.87 – 1.70 (m, 1H), 1.86 – 1.53 (m, 2H), 1.39 – 1.15 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.75, 148.12, 147.12, 137.98, 132.16, 131.12, 130.47, 129.26, 128.97, 128.65, 126.50, 60.76, 47.90, 46.29, 42.03, 37.81, 32.12, 31.32.
HRMS: $[M+Na]^+$ calculated for $C_{22}H_{23}ClN_4O_3S+Na^+$ 481.1072, found 481.1070.

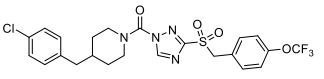
(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-methoxybenzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)-methanone (8)



63 (30 mg, 0.066 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (29 mg, 0.059 mmol, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.30 – 7.24 (m, 2H), 7.21 – 7.15 (m, 2H), 7.10 – 7.04 (m, 2H), 6.84 – 6.78 (m, 2H), 4.56 (s, 2H), 4.45 – 4.22 (m, 1H), 4.19 – 4.00 (m, 1H), 3.72 (s, 3H), 3.04 – 2.80 (m, 2H), 2.54 (d, *J* = 7.0 Hz, 2H), 1.86 – 1.70 (m, 2H), 1.70 – 1.52 (m, 1H), 1.38 – 1.29 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.82, 160.35, 148.10, 147.17, 138.00, 132.34, 132.15, 130.47, 128.65, 118.22, 114.42, 60.18, 55.33, 46.23 (br), 42.04, 37.82, 32.03 (br).
HRMS: $[M+Na]^+$ calculated for $C_{23}H_{25}ClN_4O_4S+Na^+$ 511.1177, found 511.1182.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (9, IK015)



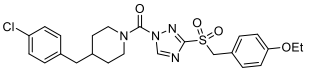
64 (40 mg, 0.078 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (40 mg, 0.074 mmol, 94%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.39 – 7.31 (m, 2H), 7.30 – 7.21 (m, 2H), 7.21 – 7.13 (m, 2H), 7.10 – 7.02 (m, 2H), 4.64 (s, 2H), 4.44 – 4.10 (m, 2H), 3.09 – 2.82 (m, 2H), 2.54 (d, *J* = 6.5 Hz, 2H), 1.86 – 1.72 (m, 1H), 1.72 – 1.58 (m, 2H), 1.38 – 1.20 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.76, 150.02 (q, *J* = 1.8 Hz), 148.28, 147.01, 137.92, 132.77, 132.20, 130.46, 128.67, 125.09, 121.32, 120.42 (q, *J* = 258.2 Hz), 59.75, 47.92 (br), 46.49 (br), 42.02, 37.81, 31.43 (br).

HRMS: $[M+H]^+$ calculated for $C_{23}H_{22}ClF_3N_4O_4S+H^+$ 543.10751, found 543.10753.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-ethoxybenzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)-methanone (10)



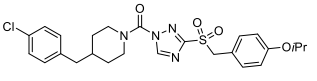
65 (40 mg, 0.085 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (35 mg, 0.070 mmol, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.29 – 7.23 (m, 2H), 7.20 – 7.12 (m, 2H), 7.10 – 7.03 (m, 2H), 6.83 – 6.75 (m, 2H), 4.56 (s, 2H), 4.42 – 4.26 (m, 1H), 4.16 – 4.01 (m, 1H), 3.94 (q, *J* = 7.1 Hz, 2H), 3.03 – 2.79 (m, 2H), 2.53 (d, *J* = 7.0 Hz, 2H), 1.85 – 1.69 (m, 1H), 1.83 – 1.56 (m, 2H), 1.36 (t, *J* = 7.0 Hz, 3H), 1.33 – 1.18 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.76, 159.69, 148.06, 147.14, 137.98, 132.28, 132.08, 130.44, 128.59, 117.96, 114.85, 63.52, 60.18, 47.73 (br), 46.22 (br), 41.99, 37.76, 31.99 (br), 31.35 (br), 14.80.

HRMS: $[M+H]^+$ calculated for $C_{24}H_{27}ClN_4O_4S+H^+$ 503.15143, found 503.15122.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-isopropoxybenzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)-methanone (11)



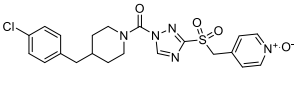
66 (40 mg, 0.082 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (33 mg, 0.064 mmol, 77%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.31 – 7.23 (m, 2H), 7.20 – 7.11 (m, 2H), 7.13 – 7.03 (m, 2H), 6.83 – 6.74 (m, 2H), 4.56 (s, 2H), 4.47 (hept, *J* = 6.1 Hz, 1H), 4.42 – 4.25 (m, 1H), 4.22 – 4.03 (m, 1H), 3.07 – 2.81 (m, 2H), 2.54 (d, *J* = 6.9 Hz, 2H), 1.82 – 1.70 (m, 1H), 1.85 – 1.58 (m, 2H), 1.38 – 1.22 (m, 2H), 1.28 (d, *J* = 6.0 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.92, 158.79, 148.08, 147.21, 138.00, 132.38, 132.19, 130.49, 128.68, 117.77, 116.07, 70.00, 60.21, 47.67 (br), 42.08, 37.89, 32.28 (br), 31.38 (br), 22.07.

HRMS: $[M+H]^+$ calculated for $C_{25}H_{29}ClN_4O_4S+H^+$ 517.16708, found 517.16687.

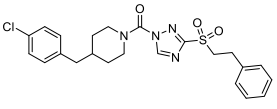
4-(((1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-1*H*-1,2,4-triazol-3-yl)sulfonyl)methyl)-pyridine 1-oxide (12)



67 (50 mg, 0.12 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (53 mg, 0.011 mmol, 95%).

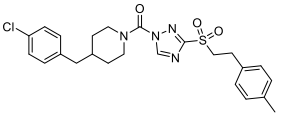
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.19 – 8.11 (m, 2H), 7.31 – 7.21 (m, 4H), 7.11 – 7.03 (m, 2H), 4.61 (s, 2H), 4.42 – 4.26 (m, 2H), 3.15 – 2.85 (m, 2H), 2.65 – 2.46 (m, 2H), 1.87 – 1.71 (m, 3H), 1.41 – 1.28 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.46, 146.81, 139.68, 137.91, 132.24, 130.50, 128.71, 128.51, 124.52, 58.49, 42.03, 37.82.
HRMS: $[M+H]^+$ calculated for $C_{21}H_{22}ClN_5O_4S+H^+$ 476.11538, found 476.11525.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-(phenethylsulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (13)



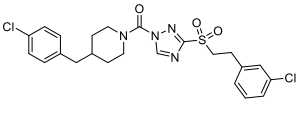
68 (40 mg, 0.091 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (16 mg, 0.033 mmol, 36%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.32 – 7.24 (m, 5H), 7.19 – 7.14 (m, 2H), 7.10 – 7.04 (m, 2H), 4.54 – 4.33 (m, 2H), 3.80 – 3.57 (m, 2H), 3.27 – 3.13 (m, 2H), 3.13 – 2.83 (m, 2H), 2.57 (d, *J* = 6.8 Hz, 2H), 1.94 – 1.68 (m, 3H), 1.37 (qd, *J* = 13.3, 4.1 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 161.73, 148.27, 147.15, 137.96, 136.99, 132.21, 130.49, 128.99, 128.69, 128.52, 127.25, 55.62, 46.85 (br), 42.09, 37.90, 28.44.
HRMS: $[M+Na]^+$ calculated for $C_{23}H_{25}ClN_4O_3S+Na^+$ 495.1228, found 473.1232.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-methylphenethyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)-methanone (14)



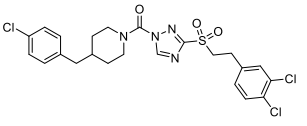
69 (40 mg, 0.088 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (22 mg, 0.045 mmol, 51%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.32 – 7.22 (m, 2H), 7.14 – 6.99 (m, 6H), 4.60 – 4.27 (m, 2H), 3.76 – 3.58 (m, 2H), 3.22 – 3.09 (m, 2H), 3.09 – 2.84 (m, 2H), 2.57 (d, *J* = 6.8 Hz, 2H), 2.31 (s, 3H), 1.94 – 1.68 (m, 3H), 1.36 (qd, *J* = 13.2, 4.1 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 161.74, 148.26, 147.15, 137.96, 136.91, 133.87, 132.21, 130.49, 129.63, 128.69, 128.38, 55.74, 47.84 (br), 42.09, 37.89, 28.02, 21.15.
HRMS: $[M+Na]^+$ calculated for $C_{24}H_{27}ClN_4O_3S+Na^+$ 509.1385, found 509.1382.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((3-chlorophenethyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)-methanone (15)



70 (40 mg, 0.084 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (32 mg, 0.063 mmol, 75%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.30 – 7.24 (m, 2H), 7.23 – 7.19 (m, 2H), 7.18 – 7.16 (m, 1H), 7.12 – 7.01 (m, 3H), 4.51 – 4.35 (m, 2H), 3.73 – 3.61 (m, 2H), 3.20 – 3.13 (m, 2H), 3.11 – 2.87 (m, 2H), 2.57 (d, *J* = 6.8 Hz, 2H), 1.89 – 1.71 (m, 3H), 1.37 (qd, *J* = 13.2, 4.1 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 161.57, 148.30, 147.05, 138.89, 137.95, 134.64, 132.16, 130.48, 130.22, 128.75, 128.66, 127.46, 126.74, 55.17, 42.05, 37.86, 32.02, 28.18.
HRMS: $[M+H]^+$ calculated for $C_{23}H_{24}Cl_2N_4O_3S+H^+$ 507.1019, found 507.1016.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((3,4-dichlorophenethyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)-methanone (16)



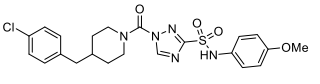
71 (28 mg, 0.055 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (12 mg, 0.023 mmol, 41%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.31 – 7.23 (m, 3H), 7.11 – 7.04 (m, 2H), 7.02 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.52 – 4.36 (m, 2H), 3.75 – 3.60 (m, 2H), 3.27 – 3.12 (m, 2H), 3.12 – 2.83 (m, 2H), 2.66 – 2.51 (m, 2H), 1.91 – 1.69 (m, 3H), 1.37 (qd, *J* = 13.2, 4.2 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 161.63, 147.03, 137.95, 137.14, 132.93, 132.24, 131.50, 130.89, 130.64, 130.50, 128.70, 127.99, 55.01, 42.08, 37.88, 27.73.

HRMS: $[M+Na]^+$ calculated for $C_{23}H_{23}Cl_3N_4O_3S+Na^+$ 563.0449, found 563.0455.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(4-methoxyphenyl)-1*H*-1,2,4-triazole-3-sulfonamide (17)



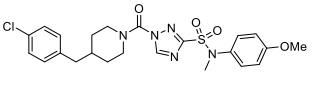
62 (1 eq, 50 mg, 0.12 mmol) was reacted with *p*-anisidine (**92**, 50 eq, 721 mg dissolved in 1.3 mL DCM, 5.86 mmol) according to General procedure B. Flash column chromatography (30 → 60% EtOAc in pentane) afforded the title compound as off-white gum (15 mg, 0.031 mmol, 26%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.28 – 7.23 (m, 2H), 7.21 – 7.16 (m, 2H), 7.09 – 7.04 (m, 2H), 6.82 – 6.78 (m, 2H), 4.45 – 4.23 (m, 2H), 3.74 (s, 3H), 3.45 (s, 3H), 3.07 – 2.80 (m, 2H), 2.53 (d, *J* = 6.4 Hz, 2H), 1.82 – 1.55 (m, 3H), 1.39 – 1.16 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 160.83, 158.44, 148.06, 147.28, 138.04, 132.17, 130.49, 128.67, 127.80, 125.81, 114.61, 55.50, 47.81 (br), 46.26 (br), 42.07, 37.83, 32.05 (br), 31.39 (br).

HRMS: $[M+H]^+$ calculated for $C_{22}H_{24}ClN_5O_4S+H^+$ 419.13103, found 419.13099.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(4-methoxyphenyl)-*N*-methyl-1*H*-1,2,4-triazole-3-sulfonamide (18)



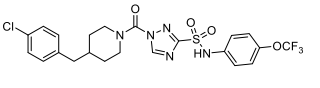
62 (1 eq, 50 mg, 0.12 mmol) was reacted with *N*-methyl-*p*-anisidine (**93**, 35 eq, 562 mg dissolved in 2 mL DCM, 4.10 mmol) according to General procedure B. Flash column chromatography (30 → 55% EtOAc in pentane) afforded the title compound as slightly brown oil (43 mg, 0.085 mmol, 73%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.29 – 7.23 (m, 4H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.06 (d, *J* = 8.0 Hz, 3H), 6.81 (d, *J* = 8.3 Hz, 2H), 4.34 (s, 2H), 3.74 (s, 3H), 3.45 (s, 3H), 3.07 – 2.80 (m, 2H), 2.53 (d, *J* = 6.4 Hz, 2H), 1.82 – 1.55 (m, 3H), 1.39 – 1.16 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 161.03, 159.12, 147.82, 147.37, 137.97, 133.01, 132.01, 130.43, 128.60, 128.55, 114.44, 55.49, 47.77 (br), 46.19 (br), 41.97, 40.03, 37.73, 31.95 (br), 31.36 (br).

HRMS: $[M+H]^+$ calculated for $C_{23}H_{26}ClN_5O_4S+H^+$ 504.14668, found 504.14631.

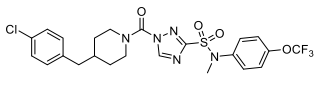
1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(4-(trifluoromethoxy)phenyl)-1*H*-1,2,4-triazole-3-sulfonamide (19, WEN222)



62 (1 eq, 50 mg, 0.12 mmol) was reacted with 4-(trifluoromethoxy)aniline (**94**, 35 eq, 550 µL, 4.10 mmol) according to General procedure B. Flash column chromatography (25 → 40% EtOAc in pentane) afforded the title compound as slightly brown oil (33 mg, 0.061 mmol, 52%).

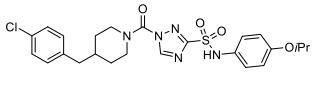
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.89 (s, 1H), 7.37 – 7.31 (m, 2H), 7.32 – 7.22 (m, 2H), 7.14 – 7.08 (m, 2H), 7.09 – 7.03 (m, 2H), 4.47 – 3.95 (m, 2H), 3.03 – 2.80 (m, 2H), 2.53 (d, *J* = 6.6 Hz, 2H), 1.85 – 1.49 (m, 3H), 1.38 – 1.15 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.51, 148.38, 147.08, 146.90 (d, *J* = 1.9 Hz), 137.92, 134.33, 132.18, 130.44, 128.65, 123.53, 122.08, 120.43 (q, *J* = 257.4 Hz), 47.74 (br), 46.35 (br), 41.99, 37.75, 32.03 (br), 31.33 (br).
HRMS: $[M+H]^+$ calculated for $C_{22}H_{21}ClF_3N_5O_4S+H^+$ 544.10276, found 544.10268.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-methyl-*N*-(4-(trifluoromethoxy)phenyl)-1*H*-1,2,4-triazole-3-sulfonamide (20)



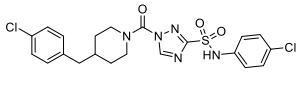
62 (1 eq, 49 mg, 0.12 mmol) was reacted with *N*-methyl-4-(trifluoromethoxy)aniline (**95**, 10 eq, 175 µL, 1.17 mmol) according to General procedure C. Flash column chromatography (20 → 50% EtOAc in pentane) afforded the title compound as grayish gum (8 mg, 0.014 mmol, 12%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.40 – 7.33 (m, 2H), 7.32 – 7.22 (m, 2H), 7.20 – 7.14 (m, 2H), 7.11 – 7.03 (m, 2H), 4.42 – 4.30 (m, 2H), 3.48 (s, 3H), 3.09 – 2.83 (m, 2H), 2.54 (d, *J* = 6.1 Hz, 2H), 1.85 – 1.74 (m, 1H), 1.85 – 1.64 (m, 2H), 1.39 – 1.20 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 161.02, 148.40 (q, *J* = 1.4 Hz), 147.95, 147.33, 139.08, 137.95, 132.22, 130.47, 128.80, 128.68, 121.74, 120.46 (q, *J* = 258.1 Hz), 47.85 (br), 46.35 (br), 42.06, 39.76, 37.87, 32.17 (br), 31.45 (br).
HRMS: $[M+H]^+$ calculated for $C_{23}H_{23}ClF_3N_5O_4S+H^+$ 558.11841, found 558.11824.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(4-isopropoxyphenyl)-1*H*-1,2,4-triazole-3-sulfonamide (21)



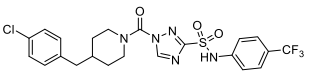
62 (1 eq, 51 mg, 0.12 mmol) was reacted with **96** (10 eq, 177 mg dissolved in 1 mL DCM, 1.17 mmol) according to General procedure C. Flash column chromatography (20 → 60% EtOAc in pentane) afforded the title compound as brown oil (3 mg, 6 µmol, 5%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.31 (bs, 1H), 7.30 – 7.24 (m, 2H), 7.16 – 7.10 (m, 2H), 7.08 – 7.03 (m, 2H), 6.78 – 6.71 (m, 2H), 4.42 (hept, *J* = 6.1 Hz, 1H), 4.39 – 4.09 (m, 2H), 2.91 (s, 2H), 2.53 (d, *J* = 6.9 Hz, 2H), 1.82 – 1.71 (m, 1H), 1.82 – 1.56 (m, 2H), 1.36 – 1.20 (m, 2H), 1.27 (d, *J* = 6.1 Hz, 6H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 161.07, 156.90, 147.30, 138.03, 132.20, 130.49, 128.69, 127.30, 125.92, 116.43, 70.28, 68.13, 47.93 (br), 46.26 (br), 42.11, 37.91, 22.07.
HRMS: $[M+H]^+$ calculated for $C_{24}H_{28}ClN_5O_4S+H^+$ 518.16233, found 518.16209.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(4-chlorophenyl)-1*H*-1,2,4-triazole-3-sulfonamide (22)



62 (1 eq, 49 mg, 0.12 mmol) was reacted with 4-chloroaniline (**97**, 53 eq, 781 mg dissolved in 1 mL DCM, 4.10 mmol) according to General procedure B. Flash column chromatography (20 → 40% EtOAc in pentane) afforded the title compound as white solid (10 mg, 0.020 mmol, 18%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.10 (s, 1H), 7.31 – 7.24 (m, 2H), 7.24 – 7.20 (m, 4H), 7.10 – 7.05 (m, 2H), 4.43 – 4.02 (m, 2H), 3.00 – 2.81 (m, 2H), 2.54 (d, *J* = 7.0 Hz, 2H), 1.84 – 1.73 (m, 1H), 1.85 – 1.53 (m, 2H), 1.36 – 1.17 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 160.59, 148.16, 147.08, 137.97, 134.14, 132.24, 131.90, 130.49, 129.60, 128.71, 123.79, 47.80 (br), 46.35 (br), 42.06, 37.82, 32.09 (br), 31.01 (br).
HRMS: $[M+H]^+$ calculated for $C_{21}H_{21}Cl_2N_5O_3S+H^+$ 494.08149, found 494.08133.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(4-(trifluoromethyl)phenyl)-1*H*-1,2,4-triazole-3-sulfonamide (23)



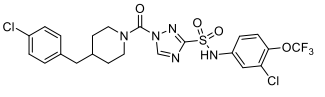
62 (1 eq, 67 mg, 0.16 mmol) was reacted with 4-(trifluoromethyl)aniline (**98**, 35 eq, 650 µL diluted to 1 mL with DCM, 5.15 mmol) according to General procedure B. Flash column chromatography (20 → 40% EtOAc in pentane) afforded the title compound as off-white gum (66 mg, 0.13 mmol, 80%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (s, 1H), 7.54 – 7.49 (m, 2H), 7.43 – 7.37 (m, 2H), 7.30 – 7.24 (m, 2H), 7.09 – 7.04 (m, 2H), 4.44 – 4.03 (m, 2H), 3.02 – 2.84 (m, 2H), 2.54 (d, *J* = 5.7 Hz, 2H), 1.84 – 1.72 (m, 1H), 1.85 – 1.53 (m, 2H), 1.38 – 1.16 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 160.58, 148.35, 147.00, 139.11, 137.91, 132.25, 130.46, 128.70, 127.65 (q, *J* = 32.9 Hz), 126.75 (q, *J* = 3.7 Hz), 123.91 (d, *J* = 272.2 Hz), 120.80, 47.83 (br), 46.36 (br), 42.02, 37.79, 32.08 (br).

HRMS: $[M+H]^+$ calculated for $C_{22}H_{21}ClF_3N_5O_3S+H^+$ 528.10785, found 528.10765.

***N*-(3-Chloro-4-(trifluoromethoxy)phenyl)-1-(4-(4-chlorobenzyl)piperidine-1-carbonyl)-1*H*-1,2,4-triazole-3-sulfonamide (24)**



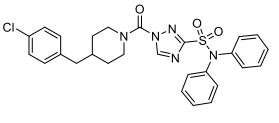
62 (1 eq, 49 mg, 0.12 mmol) was reacted with 3-chloro-4-(trifluoromethoxy)aniline (**99**, 14 eq, 228 µL diluted to 1 mL with DCM, 1.57 mmol) according to General procedure C. Flash column chromatography (20 → 50% EtOAc in pentane) afforded the title compound as white solid (8 mg, 0.014 mmol, 12%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.53 (s, 1H), 7.49 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.30 – 7.25 (m, 2H), 7.23 – 7.17 (m, 2H), 7.10 – 7.04 (m, 2H), 4.45 – 4.13 (m, 2H), 3.08 – 2.83 (m, 2H), 2.55 (d, *J* = 3.9 Hz, 2H), 1.85 – 1.74 (m, 1H), 1.84 – 1.60 (m, 2H), 1.37 – 1.18 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 160.49, 146.95, 142.86 (q, *J* = 1.7 Hz), 137.91, 135.18, 132.24, 130.47, 128.69, 128.46, 123.58, 123.53, 120.93, 47.83 (br), 46.43 (br), 42.03, 37.82, 32.07 (br), 41.44 (br).

HRMS: $[M+H]^+$ calculated for $C_{22}H_{20}Cl_2F_3N_5O_4S+H^+$ 578.06379, found 578.06321.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*,*N*-diphenyl-1*H*-1,2,4-triazole-3-sulfonamide (25)



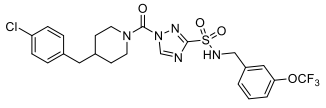
62 (1 eq, 50 mg, 0.12 mmol) was reacted with diphenylamine (**100**, 35 eq, 578 µL dissolved in 2 mL DCM, 4.10 mmol) according to General procedure B. Preparative HPLC afforded the title compound as dark oil (5 mg, 9 µmol, 8%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.51 – 7.44 (m, 4H), 7.36 – 7.28 (m, 4H), 7.29 – 7.23 (m, 4H), 7.09 – 7.03 (m, 2H), 4.38 – 4.23 (m, 2H), 3.01 – 2.85 (m, 2H), 2.54 (d, *J* = 6.9 Hz, 2H), 2.03 – 1.85 (m, 3H), 1.83 – 1.72 (m, 1H), 1.70 – 1.55 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 162.11, 147.40, 140.82, 138.02, 132.23, 130.50, 129.49, 128.69, 128.30, 127.80, 77.42, 77.16, 76.91, 42.11, 37.89.

HRMS: $[M+H]^+$ calculated for $C_{27}H_{26}ClN_5O_3S$ 536.15176, found 536.15163.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(3-(trifluoromethoxy)benzyl)-1*H*-1,2,4-triazole-3-sulfonamide (26)



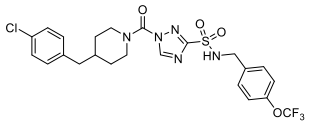
62 (1 eq, 50 mg, 0.12 mmol) was reacted with 3-(trifluoromethoxy)benzylamine (**101**, 15 eq, 263 µL, 1.78 mmol) according to General procedure C. Flash column chromatography (5 → 50% EtOAc in pentane) afforded the title compound as grayish gum (13 mg, 0.023 mmol, 20%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (s, 1H), 7.36 – 7.30 (m, 1H), 7.29 – 7.22 (m, 3H), 7.19 – 7.16 (m, 1H), 7.16 – 7.10 (m, 1H), 7.11 – 7.03 (m, 2H), 5.99 – 5.80 (m, 1H), 4.41 (app. d, *J* = 6.2 Hz, 4H), 3.13 – 2.83 (m, 2H), 2.56 (d, *J* = 6.9 Hz, 2H), 1.87 – 1.75 (m, 1H), 1.68 (s, 2H), 1.34 (qd, *J* = 13.3, 4.1 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 162.15, 149.53 (q, *J* = 1.9 Hz), 147.28, 138.47, 137.99, 132.19, 130.49, 130.31, 128.68, 126.32, 125.74, 120.63, 120.56, 120.50 (q, *J* = 257.4 Hz), 47.15, 44.47, 42.07, 37.88, 31.81.

HRMS: $[M+H]^+$ calculated for $C_{23}H_{23}ClF_3N_5O_4S+H^+$ 558.11841, found 558.11832.

1-(4-(4-Chlorobenzyl)piperidine-1-carbonyl)-*N*-(4-(trifluoromethoxy)benzyl)-1*H*-1,2,4-triazole-3-sulfonamide (27)



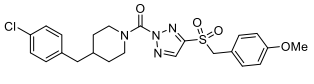
62 (1 eq, 50 mg, 0.12 mmol) was reacted with 4-(trifluoromethoxy)benzylamine (**102**, 15 eq, 268 µL, 1.78 mmol) according to General procedure C. Preparative HPLC afforded the title compound as colorless oil (< 1 mg, < 2 µmol, < 2%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.38 – 7.31 (m, 2H), 7.30 – 7.26 (m, 2H), 7.21 – 7.15 (m, 2H), 7.11 – 7.04 (m, 2H), 4.50 – 4.34 (m, 2H), 4.40 (d, *J* = 6.2 Hz, 2H), 3.16 – 2.85 (m, 2H), 2.57 (d, *J* = 6.8 Hz, 2H), 1.87 – 1.71 (m, 3H), 1.36 (qd, *J* = 12.4, 4.2 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 147.33, 140.58, 132.24, 130.51, 129.57, 128.71, 121.45, 47.17, 42.12, 37.92.

HRMS: $[M+H]^+$ calculated for $C_{23}H_{23}ClF_3N_5O_4S+H^+$ 558.11841, found 558.11810.

(4-(4-Chlorobenzyl)piperidin-1-yl)(4-((4-methoxybenzyl)sulfonyl)-2*H*-1,2,3-triazol-2-yl)-methanone (28)



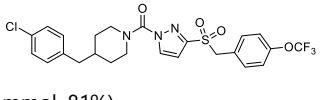
72 (15 mg, 0.033 mmol) was oxidized according to General procedure A. Flash column chromatography afforded the title compound (9.7 mg, 0.020 mmol, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.29 – 7.24 (m, 2H), 7.18 – 7.11 (m, 2H), 7.11 – 7.05 (m, 2H), 6.86 – 6.79 (m, 2H), 4.51 (s, 2H), 4.50 – 4.41 (m, 1H), 3.76 (s, 3H), 3.70 – 3.59 (m, 1H), 3.11 – 2.94 (m, 2H), 2.57 (d, *J* = 7.0 Hz, 2H), 1.90 – 1.75 (m, 2H), 1.75 – 1.54 (m, 1H), 1.40 – 1.32 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.48, 147.65, 147.52, 137.98, 136.53, 132.36, 132.22, 130.48, 128.70, 118.32, 114.53, 61.75, 55.40, 48.02, 46.23, 42.09, 37.91, 32.06.

HRMS: $[M+Na]^+$ calculated for $C_{23}H_{25}ClN_4O_4S+Na^+$ 511.1177, found 511.1177.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-pyrazol-1-yl)-methanone (29)

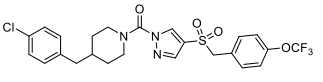


73 (36 mg, 0.071 mmol) was oxidized according to General procedure A. Flash column chromatography (10 → 40% EtOAc in pentane) afforded the title compound as gray gum (31 mg, 0.057 mmol, 81%).

Analytical data on next page.

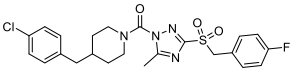
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, *J* = 2.7 Hz, 1H), 7.31 – 7.22 (m, 4H), 7.20 – 7.13 (m, 2H), 7.11 – 7.05 (m, 2H), 6.64 (d, *J* = 2.8 Hz, 1H), 4.48 (s, 2H), 4.45 – 4.22 (m, 2H), 3.09 – 2.84 (m, 2H), 2.56 (d, *J* = 7.1 Hz, 2H), 1.86 – 1.68 (m, 3H), 1.40 – 1.27 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 151.11, 149.84 (q, *J* = 1.9 Hz), 149.47, 138.11, 133.78, 132.60, 132.10, 130.47, 128.62, 126.01, 121.18, 120.42 (q, *J* = 257.8 Hz), 108.77, 60.99, 42.11, 37.96, 29.80.
HRMS: $[M+NH_4]^+$ calculated for $C_{24}H_{23}ClF_3N_3O_4S+NH_4^+$ 559.13881, found 559.13867.

(4-(4-Chlorobenzyl)piperidin-1-yl)(4-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-pyrazol-1-yl)-methanone (30, WEN258)



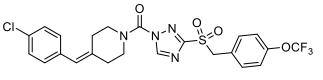
74 (28 mg, 0.055 mmol) was oxidized according to General procedure A. Flash column chromatography (15 → 40% EtOAc in pentane) afforded the title compound as white solid (25 mg, 0.046 mmol, 84%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (d, *J* = 0.8 Hz, 1H), 7.50 (d, *J* = 0.8 Hz, 1H), 7.31 – 7.23 (m, 4H), 7.23 – 7.16 (m, 2H), 7.10 – 7.04 (m, 2H), 4.42 – 4.33 (m, 2H), 4.36 (s, 2H), 3.00 – 2.91 (m, 2H), 2.56 (d, *J* = 7.1 Hz, 2H), 1.86 – 1.67 (m, 3H), 1.34 (qd, *J* = 12.8, 4.1 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 149.97 (d, *J* = 1.9 Hz), 149.28, 140.32, 138.13, 135.45, 132.56, 132.11, 130.48, 128.63, 126.63, 122.07, 121.28, 120.45 (q, *J* = 257.4 Hz), 62.90, 42.19, 37.99, 31.91 (br).
HRMS: $[M+Na]^+$ calculated for $C_{24}H_{23}ClF_3N_3O_4S+Na^+$ 564.09421, found 564.09377.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-fluorobenzyl)sulfonyl)-5-methyl-1*H*-1,2,4-triazol-1-yl)methanone (31)



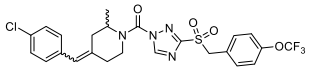
75 (49 mg, 0.107 mmol) was oxidized according General procedure A. Flash column chromatography (30 → 80% EtOAc in pentane) afforded the title compound (52 mg, 0.106 mmol, quant.).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.21 (m, 4H), 7.10 – 7.03 (m, 2H), 7.06 – 6.96 (m, 2H), 4.56 (s, 2H), 4.41 – 4.33 (m, 1H), 3.49 (d, *J* = 13.5 Hz, 1H), 2.97 – 2.83 (m, 2H), 2.67 (s, 3H), 2.61 – 2.47 (m, 2H), 1.81 – 1.65 (m, 2H), 1.65 – 1.57 (m, 1H), 1.39 – 1.18 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 164.56, 160.61 (d, *J* = 296.7 Hz), 158.54, 147.97, 137.96, 132.99 (d, *J* = 8.5 Hz), 132.14, 130.45, 128.63, 122.40 (d, *J* = 3.3 Hz), 116.05 (d, *J* = 21.8 Hz), 59.86, 47.98, 45.57, 41.96, 37.78, 32.04, 31.29, 13.73.
HRMS: $[M+H]^+$ calculated for $C_{24}H_{24}ClFN_4O_3S$ 491.13144, found 491.13150.

(4-(4-Chlorobenzylidene)piperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (32)



114 (125 mg, 0.39 mmol) was reacted with **123** (1.2 eq, 143 mg, 0.47 mmol) according to General procedure D. The residue was purified by flash column chromatography (0 → 30% EtOAc in pentane) yielding the title compound as off-white gum (27 mg, 0.050 mmol, 13%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.38 – 7.33 (m, 2H), 7.33 – 7.28 (m, 2H), 7.21 – 7.16 (m, 2H), 7.14 – 7.09 (m, 2H), 6.39 (s, 1H), 4.65 (s, 2H), 3.81 – 3.74 (m, 2H), 3.69 – 3.61 (m, 2H), 2.67 – 2.37 (m, 4H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 160.95, 150.06, 148.37, 147.09, 136.14, 135.17, 132.80, 132.75, 130.22, 128.65, 125.38, 125.08, 122.17, 121.35, 120.43 (q, *J* = 258.2 Hz), 77.41, 77.16, 76.91, 59.76.
HRMS: $[M+Na]^+$ calculated for $C_{23}H_{20}ClF_3N_4O_4S$ 563.07381, found 563.07342.

(4-(4-Chlorobenzylidene)-2-methylpiperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (33)



115 (58 mg, 0.17 mmol) was reacted with **123** (1.2 eq, 65 mg, 0.21 mmol) according to General procedure D. The residue was purified by flash column chromatography (10 → 40% EtOAc in pentane) yielding the title compound as mixture of two *E/Z* isomers (ratio ~4:3) as yellow-gray gum (35 mg, 0.063 mmol, 37%).

Isomer 1:

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (s, 1H), 7.39 – 7.33 (m, 2H), 7.33 – 7.28 (m, 2H), 7.21 – 7.15 (m, 2H), 7.15 – 7.07 (m, 2H), 6.50 (s, 1H), 4.80 – 4.55 (m, 1H), 4.64 (s, 2H), 4.32 – 4.15 (m, 1H), 3.29 (td, *J* = 13.3, 3.3 Hz, 1H), 2.75 – 2.60 (m, 1H), 2.50 (td, *J* = 13.5, 4.8 Hz, 1H), 2.45 – 2.38 (m, 2H), 1.19 (d, *J* = 5.7 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 160.80, 149.98 (q, *J* = 1.4 Hz), 148.36, 147.15, 135.23, 134.04, 132.78, 132.64, 130.02, 128.63, 127.03, 125.10, 121.29, 120.06 (q, *J* = 258.3 Hz), 59.71, 49.95, 42.82, 35.46, 34.26, 16.88.

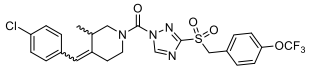
Isomer 2:

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (s, 1H), 7.39 – 7.33 (m, 2H), 7.33 – 7.28 (m, 2H), 7.21 – 7.15 (m, 2H), 7.15 – 7.07 (m, 2H), 6.37 (s, 1H), 4.80 – 4.55 (m, 1H), 4.64 (s, 2H), 4.14 – 3.96 (m, 1H), 3.23 – 3.14 (m, 1H), 2.84 – 2.75 (m, 1H), 2.75 – 2.60 (m, 1H), 2.35 – 2.14 (m, 2H), 1.30 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 160.80, 149.98 (q, *J* = 1.4 Hz), 148.36, 147.15, 135.27, 133.91, 132.78, 132.66, 130.07, 128.62, 126.83, 125.08, 121.29, 120.06 (q, *J* = 258.3 Hz), 59.71, 50.36, 41.92, 40.63, 29.15, 16.65.

HRMS: $[M+Na]^+$ calculated for $C_{24}H_{22}ClF_3N_4O_4S+Na^+$ 577.08946, found 577.08896.

(4-(4-Chlorobenzylidene)-3-methylpiperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (34)

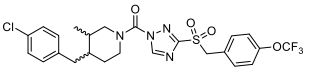


116 (80 mg, 0.24 mmol) was reacted with **123** (1.1 eq, 81 mg, 0.26 mmol) according to General procedure D. The residue was purified by flash column chromatography (10 → 50% EtOAc in pentane) yielding a mixture of *E/Z* isomers (ratio ~2:1) of the title compound as colorless oil (79 mg, 0.14 mmol, 60%).

Analytical data on next page.

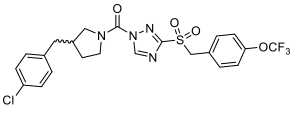
Isomer 1:
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.38 – 7.33 (m, 2H), 7.33 – 7.28 (m, 2H), 7.20 – 7.14 (m, 2H), 7.13 – 7.06 (m, 2H), 6.37 (s, 1H), 4.65 (s, 2H), 4.01 – 3.90 (m, 1H), 3.78 – 3.67 (m, 1H), 3.63 – 3.46 (m, 1H), 3.38 – 3.28 (m, 1H), 2.84 – 2.69 (m, 1H), 2.66 – 2.58 (m, 1H), 2.40 – 2.24 (m, 1H), 1.32 – 1.18 (m, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.78, 149.94, 148.42, 147.25, 140.76, 135.41, 132.75, 132.55, 130.26, 128.53, 125.07, 123.14, 121.24, 120.35 (q, *J* = 258.1 Hz), 59.76, 52.96 (br), 48.26 (br), 37.95 (br), 27.83 (br), 16.32.
Isomer 2:
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.38 – 7.33 (m, 2H), 7.33 – 7.28 (m, 2H), 7.20 – 7.14 (m, 2H), 7.13 – 7.06 (m, 2H), 6.29 (s, 1H), 4.65 (s, 2H), 3.90 – 3.80 (m, 1H), 3.78 – 3.67 (m, 1H), 3.63 – 3.46 (m, 1H), 3.44 – 3.37 (m, 1H), 2.84 – 2.69 (m, 1H), 2.59 – 2.50 (m, 1H), 2.51 – 2.41 (m, 1H), 1.13 – 1.03 (m, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.78, 149.94, 148.42, 147.25, 140.65, 135.15, 132.75, 132.55, 129.82, 128.63, 125.07, 124.33, 121.24, 120.35 (q, *J* = 258.1 Hz), 59.76, 54.09 (br), 46.94 (br), 38.51 (br), 26.94 (br), 17.11 (br).
HRMS: $[M+Na]^+$ calculated for $C_{24}H_{22}ClF_3N_4O_4S+Na^+$ 577.08946, found 577.08839.

(4-(4-Chlorobenzyl)-3-methylpiperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (35)



118 (75 mg, 0.22 mmol) was reacted with **123** (1.1 eq, 75 mg, 0.24 mmol) according to General procedure D. The residue was purified by flash column chromatography (25 → 50% EtOAc in pentane) yielding the title compound as colorless oil (88 mg, 0.17 mmol, 71%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.38 – 7.29 (m, 2H), 7.31 – 7.22 (m, 2H), 7.20 – 7.13 (m, 2H), 7.12 – 7.02 (m, 2H), 4.64 (s, 2H), 4.35 – 3.95 (m, 2H), 3.16 (s, 2H), 2.62 – 2.42 (m, 2H), 2.06 – 1.70 (m, 2H), 1.66 – 1.48 (m, 2H), 1.04 – 0.73 (m, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 160.51, 149.84 (q, *J* = 1.7 Hz), 148.23, 148.21, 138.22, 132.66, 131.94, 130.22, 128.58, 125.12, 120.30 (q, *J* = 257.9 Hz), 121.15, 59.66, 53.65 (br), 52.40 (br), 47.64 (br), 46.33 (br), 40.83, 38.10 (br), 31.95 (br), 26.28 (br), 25.28 (br), 11.08 (br).
HRMS: $[M+Na]^+$ calculated for $C_{24}H_{24}ClF_3N_4O_4S+Na^+$ 579.10511, found 579.10457.

(3-(4-Chlorobenzyl)pyrrolidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (36)



117 (50 mg, 0.16 mmol) was reacted with **123** (1.2 eq, 60 mg, 0.20 mmol) according to General procedure D. The residue was purified by flash column chromatography (30 → 50% EtOAc in pentane) yielding a mixture of two conformationally restricted isomers of the title compound (ratio ~3:2) as gray gum (31 mg, 0.059 mmol, 36%).
Conformer 1:
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.36 – 7.31 (m, 2H), 7.31 – 7.22 (m, 2H), 7.20 – 7.13 (m, 2H), 7.13 – 7.06 (m, 2H), 4.63 (s, 2H), 3.85 – 3.78 (m, 1H), 3.81 – 3.70 (m, 1H), 3.64 – 3.55 (m, 1H), 3.35 – 3.28 (m, 1H), 2.73 – 2.69 (m, 2H), 2.53 – 2.39 (m, 1H), 2.09 – 2.02 (m, 1H), 1.73 – 1.57 (m, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 161.00, 149.98, 147.70, 145.93 (d, *J* = 2.6 Hz), 137.85, 132.74, 132.50, 130.07, 128.91, 125.17, 121.30, 120.35 (q, *J* = 257.2 Hz), 59.70, 53.93, 49.53, 38.71, 38.07, 31.89.

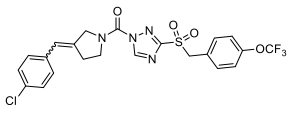
Conformer 2:

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.97 (s, 1H), 7.36 – 7.31 (m, 2H), 7.31 – 7.22 (m, 2H), 7.20 – 7.13 (m, 2H), 7.13 – 7.06 (m, 2H), 4.63 (s, 2H), 3.93 – 3.86 (m, 2H), 3.81 – 3.70 (m, 1H), 3.55 – 3.48 (m, 1H), 2.79 – 2.66 (m, 1H), 2.61 – 2.53 (m, 1H), 2.53 – 2.39 (m, 1H), 2.02 – 1.95 (m, 1H), 1.73 – 1.57 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 161.00, 149.98, 147.64, 145.93 (d, *J* = 2.6 Hz), 137.90, 132.74, 132.50, 130.07, 128.91, 125.13, 121.30, 120.35 (q, *J* = 257.2 Hz), 59.70, 54.74, 48.77, 41.61, 38.01, 29.21.

HRMS: $[M+Na]^+$ calculated for $C_{22}H_{20}ClF_3N_4O_4S+Na^+$ 529.09186, found 529.09164.

(3-(4-Chlorobenzylidene)pyrrolidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (37)



119 (114 mg, 0.37 mmol) was reacted with **123** (1.1 eq, 127 mg, 0.41 mmol) according to General procedure D. The residue was purified by preparative HPLC yielding a mixture of two *E*/*Z* isomers of the title compound (ratio ~2:1) as white powder (24 mg, 0.046 mmol, 12%).

Isomer 1:

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (s, 1H), 7.40 – 7.31 (m, 4H), 7.23 – 7.15 (m, 2H), 7.16 – 7.11 (m, 2H), 6.49 (s, 1H), 4.64 (s, 2H), 4.52 – 4.49 (s, 1H), 4.49 – 4.47 (m, 1H), 3.98 – 3.92 (m, 1H), 3.85 – 3.79 (m, 1H), 2.93 – 2.81 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 161.22, 150.02, 147.81, 145.93, 137.04, 134.58, 133.30, 132.76, 132.69, 129.53, 128.94, 125.11, 123.41, 121.34, 59.72, 54.70, 51.16, 48.08, 47.18, 34.42, 31.58.

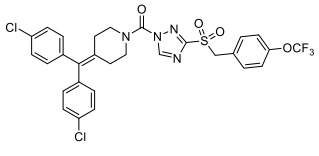
Isomer 2:

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.40 – 7.31 (m, 4H), 7.23 – 7.15 (m, 2H), 7.11 – 7.06 (m, 2H), 6.45 (s, 1H), 4.72 – 4.69 (m, 1H), 4.64 (s, 2H), 4.62 – 4.60 (m, 1H), 4.05 – 4.00 (m, 1H), 3.92 – 3.87 (m, 1H), 2.93 – 2.81 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 161.22, 150.02, 147.81, 145.93, 136.88 (br), 134.79 (br), 133.17 (br), 132.69, 129.73, 129.70, 128.84, 128.80, 125.14, 123.07, 121.34, 59.84, 55.10, 52.28, 49.71, 49.10, 29.88, 27.50.

HRMS: $[M+Na]^+$ calculated for $C_{22}H_{18}ClF_3N_4O_4S+Na^+$ 527.07621, found 527.07611.

(4-(Bis(4-chlorophenyl)methylene)piperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazol-1-yl)methanone (38)



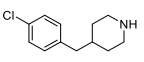
122 (81 mg, 0.19 mmol) was reacted with **123** (1.1 eq, 63 mg, 0.21 mmol) according to General procedure D. The residue was purified by flash column chromatography (10 → 40% EtOAc in pentane) yielding the title compound as white crystalline solid (84 mg, 0.13 mmol, 69%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 4H), 7.14 (d, *J* = 7.7 Hz, 2H), 7.02 (d, *J* = 6.5 Hz, 4H), 4.63 (s, 2H), 3.72 – 3.67 (m, 4H), 2.58 – 2.31 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.77, 149.89 (q, *J* = 1.6 Hz), 148.39, 147.02, 139.45, 136.82, 133.17, 132.87, 132.72, 130.88, 128.66, 125.07, 121.24, 120.22 (q, *J* = 257.1 Hz), 59.70, 48.26 (br), 46.83 (br), 31.37 (br), 30.83 (br).

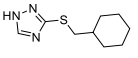
HRMS: $[M+Na]^+$ calculated for $C_{29}H_{23}Cl_2F_3N_4O_4S+Na^+$ 651.08419, found 651.08416.

4-(4-Chlorobenzyl)piperidine (39)



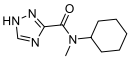
4-(4-Chlorobenzyl)pyridine (**124**, 1 eq, 1.71 mL, 9.82 mmol), PtO_2 (4 mol%, 89 mg, 0.39 mmol) and hydrochloric acid (1 eq, 818 µL 12 M, 9.82 mmol) were added to EtOH (30 mL) and shaken for 24h under 3 bar H_2 in a Parr reaction vessel. Catalyst was removed by filtration and volatiles under reduced pressure. Flash column chromatography (5 → 15% 7M methanolic ammonia in EtOAc) afforded the title compound as yellow oil (1.79 g, 8.53 mmol, 87%)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 – 7.19 (m, 2H), 7.10 – 7.02 (m, 2H), 3.03 (dt, *J* = 12.6, 3.0 Hz, 2H), 2.54 (td, *J* = 12.3, 2.1 Hz, 2H), 2.49 (d, *J* = 6.5 Hz, 2H), 1.66 (bs, 1H), 1.64 – 1.55 (m, 2H), 1.58 – 1.51 (m, 1H), 1.13 (qd, *J* = 13.8, 13.3, 3.8 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 139.09, 131.58, 130.55, 128.31, 46.80, 43.22, 38.48, 33.44.

3-((Cyclohexylmethyl)thio)-1*H*-1,2,4-triazole (40)



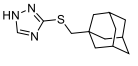
1*H*-1,2,4-Triazole-3-thiol (**76**, 2.00 g, 19.8 mmol) was dissolved in 70 mL dry DMF and (bromomethyl)cyclohexane (1 eq, 2.76 mL, 19.8 mmol) was added slowly. K_2CO_3 (0.73 eq, 2.00 g, 14.4 mmol) was added and the mixture was stirred for 6 h, during which it turned light purple and finally white and cloudy. The mixture was diluted with EtOAc and washed with water. The aqueous layer was extracted with EtOAc, after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Flash column chromatography (20 → 40% EtOAc in pentane) afforded the title compound as white crystalline solid (3.43 g, 17.4 mmol, 88%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 13.13 (bs, 1H), 8.20 (s, 1H), 3.08 (d, *J* = 7.0 Hz, 2H), 1.91 – 1.80 (m, 2H), 1.76 – 1.61 (m, 3H), 1.69 – 1.52 (m, 1H), 1.31 – 1.05 (m, 3H), 0.98 (qd, *J* = 11.7, 3.4 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 157.29, 147.61, 39.96, 37.84, 32.49, 26.22, 25.95.

***N*-cyclohexyl-*N*-methyl-1*H*-1,2,4-triazole-3-carboxamide (41)**



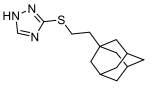
To a solution of 1*H*-1,2,4-triazole-3-carboxylic acid (**77**, 1 eq, 100 mg, 0.88 mmol) in dry THF (10 mL) with a few drops of DMF, $SOCl_2$ (78 eq, 5.0 mL, 68.5 mmol) was added and the mixture was refluxed for 14 h. All volatiles were removed *in vacuo*, after which the residual lime green oil was dissolved in dry DCM (5 mL) and added dropwise to an ice-cold solution of *N*-methylcyclohexanamine (**78**, 13 eq, 1.5 mL, 11.5 mmol) in dry DCM (5 mL). The mixture was allowed to warm to RT and stirred overnight. Because TLC showed little reaction progress DIPEA (2 eq, 300 µL, 1.72 mmol) was added and stirring was continued for 110 h. All volatiles were removed *in vacuo*. Flash column chromatography of the residue (40 → 70% EtOAc in petroleum ether) afforded the title compound as white solid (129 mg, 0.62 mmol, 70%).
LC-MS: $[M+H]^+$ calculated for $C_{10}H_{16}N_4O+H^+$ 209.14, found 209.07. RT = 7.78 min (0 ⟶ 50% CH_3CN in H_2O)

3-(((Adamant-1-yl)methyl)thio)-1*H*-1,2,4-triazole (42)



88 (405 mg, 1.12 mmol) was treated according to General procedure G to obtain the title compound as a white solid (89 mg, 0.36 mmol, 65%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.80 (bs, 1H), 8.15 (s, 1H), 3.08 (s, 1H), 2.01 – 1.95 (m, 4H), 1.74 – 1.53 (m, 11H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 157.87, 148.01, 46.99, 41.54, 36.78, 33.89, 28.51.

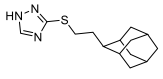
3-((2-(Adamant-1-yl)ethyl)thio)-1*H*-1,2,4-triazole (43)



89 (462 mg, 1.18 mmol) was treated according to General procedure G to obtain the title compound as a white solid (100 mg, 0.380 mmol, 64%).

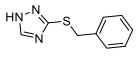
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.68 (bs, 1H), 8.12 (s, 1H), 3.19 – 3.10 (m, 2H), 1.96 (p, *J* = 3.1 Hz, 3H), 1.75 – 1.57 (m, 6H), 1.54 – 1.44 (m, 8H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 44.08, 42.22, 37.15, 33.06, 28.70, 27.47.

3-((2-(Adamant-2-yl)ethyl)thio)-1*H*-1,2,4-triazole (44)



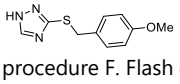
90 (393 mg, 1.01 mmol) was treated according to General procedure G to obtain the title compound as a white solid (220 mg, 0.835 mmol, 83%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 3.21 – 3.14 (m, 2H), 1.90 – 1.75 (m, 9H), 1.75 – 1.68 (m, 6H), 1.54 – 1.47 (m, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 157.19, 148.10, 43.73, 39.16, 38.40, 32.62, 31.76, 31.72, 31.42, 28.30, 28.09, 0.13.

3-(Benzylthio)-1*H*-1,2,4-triazole (45)



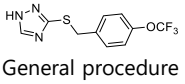
1,2,4-Triazole-3-thiol (**76**, 1.05 eq, 500 mg, 4.94 mmol) was benzylated with benzyl bromide (1 eq, 560 µL, 4.70 mmol) according to General procedure F. Flash column chromatography (40 → 70% EtOAc in pentane) afforded the title compound as white crystalline solid (669 mg, 3.50 mmol, 74%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 13.49 (bs, 1H), 8.09 (s, 1H), 7.31 – 7.26 (m, 2H), 7.26 – 7.17 (m, 3H), 4.33 (s, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 136.82, 128.92, 128.73, 127.72, 37.48.

3-((4-Methoxybenzyl)thio)-1*H*-1,2,4-triazole (46)



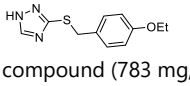
1*H*-1,2,4-Triazole-3-thiol (**76**, 1.4 eq, 106 mg, 1.05 mmol) was benzylated with 4-methoxybenzyl chloride (1 eq, 105 µL, 0.77 mmol) according to General procedure F. Flash column chromatography afforded the title compound (116 mg, 0.52 mmol, 64%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.95 (bs, 1H), 8.12 (s, 1H), 7.26 – 7.17 (m, 2H), 6.82 – 6.74 (m, 2H), 4.30 (s, 2H), 3.74 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 159.10, 156.43, 147.53, 130.16, 128.71, 114.11, 55.35, 37.06.

3-((4-(Trifluoromethoxy)benzyl)thio)-1*H*-1,2,4-triazole (47)



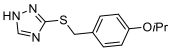
1*H*-1,2,4-Triazole-3-thiol (**76**, 1.05 eq, 400 mg, 3.96 mmol) was benzylated with 4-(trifluoromethoxy)benzyl bromide (1 eq, 958 mg, 3.76 mmol) according to General procedure F. This afforded the title compound (433 mg, 1.57 mmol, 40%), which was used without further purification.
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.31 (bs, 1H), 8.16 (s, 1H), 7.41 – 7.33 (m, 2H), 7.15 – 7.07 (m, 2H), 4.36 (s, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 148.66 (q, *J* = 2.1 Hz), 135.93, 130.42, 121.18, 120.53 (q, *J* = 257.3 Hz), 36.32.

3-((4-Ethoxybenzyl)thio)-1*H*-1,2,4-triazole (48)



1*H*-1,2,4-Triazole-3-thiol (**76**, 1 eq, 500 mg, 4.94 mmol) was reacted with **126** (1 eq, 844 mg, 4.94 mmol) according to General procedure F. This afforded the title compound (783 mg, 3.33 mmol, 67%), which was used without further purification.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (bs, 1H), 8.14 (s, 1H), 7.28 – 7.20 (m, 2H), 6.86 – 6.75 (m, 2H), 4.32 (s, 2H), 3.99 (q, *J* = 7.0 Hz, 2H), 1.39 (t, *J* = 7.0 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 158.62, 130.19, 128.58, 114.76, 63.60, 37.07, 14.95.

3-((4-Isopropoxybenzyl)thio)-1*H*-1,2,4-triazole (49)

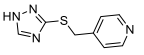


1*H*-1,2,4-Triazole-3-thiol (**76**, 1 eq, 400 mg, 3.96 mmol) was reacted with **130** (1 eq, 730 mg, 3.96 mmol) according to General procedure F. This afforded the title compound (348 mg, 1.40 mmol, 35%), which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.25 – 7.17 (m, 2H), 6.82 – 6.74 (m, 2H), 4.49 (hept, *J* = 6.1 Hz, 1H), 4.31 (s, 2H), 1.30 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.48, 130.20, 128.48, 116.03, 70.05, 37.07, 22.12.

4-(((1*H*-1,2,4-triazol-3-yl)thio)methyl)pyridine (50)

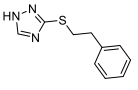


1*H*-1,2,4-Triazole-3-thiol (**76**, 1 eq, 500 mg, 4.94 mmol) was reacted with 4-(bromomethyl)pyridine hydrobromide (1 eq, 1.25 g, 4.94 mmol) according to General procedure F. This afforded the title compound (630 mg, 3.28 mmol, 66%), which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.93 (bs, 1H), 8.49 – 8.43 (m, 2H), 8.16 (s, 1H), 7.34 – 7.29 (m, 2H), 4.31 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 149.24, 147.92, 124.27, 35.76.

3-(Phenethylthio)-1*H*-1,2,4-triazole (51)

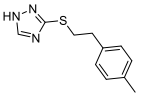


1*H*-1,2,4-Triazole-3-thiol (**76**, 1.05 eq, 100 mg, 0.99 mmol) was reacted with phenethyl bromide (1 eq, 128 µL, 0.94 mmol) according to General procedure F. Flash column chromatography (30 → 70% EtOAc in pentane) afforded the title compound (126 mg, 0.61 mmol, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.44 (bs, 1H), 8.17 (s, 1H), 7.31 – 7.23 (m, 2H), 7.23 – 7.14 (m, 3H), 3.43 – 3.33 (m, 2H), 3.05 – 2.96 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.86, 147.12, 139.66, 128.67, 128.55, 126.61, 35.98, 34.00.

3-((4-Methylphenethyl)thio)-1*H*-1,2,4-triazole (52)

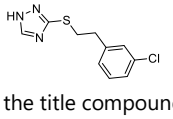


1*H*-1,2,4-Triazole-3-thiol (**76**, 1.05 eq, 100 mg, 0.99 mmol) was reacted with 4-methylphenethyl bromide (1 eq, 143 µL, 0.94 mmol) according to General procedure F. Flash column chromatography (30 → 70% EtOAc in pentane) afforded the title compound (181 mg, 0.83 mmol, 88%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.35 (bs, 1H), 8.18 (s, 1H), 7.08 – 7.01 (m, 4H), 3.39 – 3.31 (m, 2H), 2.97 – 2.90 (m, 2H), 2.27 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.82, 146.96, 136.43, 136.05, 129.15, 128.41, 35.42, 34.11, 20.99.

3-((3-Chlorophenethyl)thio)-1*H*-1,2,4-triazole (53)

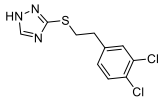


1*H*-1,2,4-Triazole-3-thiol (**76**, 1.05 eq, 100 mg, 0.99 mmol) was reacted with 3-chlorophenethyl bromide (1 eq, 138 µL, 0.94 mmol) according to General procedure F. Flash column chromatography (30 → 70% EtOAc in pentane) afforded the title compound (131 mg, 0.55 mmol, 58%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.34 (bs, 1H), 8.23 (s, 1H), 7.22 – 7.13 (m, 3H), 7.11 – 7.02 (m, 1H), 3.42 – 3.31 (m, 2H), 3.04 – 2.93 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.00, 147.03, 141.60, 134.25, 129.85, 128.81, 126.92, 126.86, 35.68, 33.65.

3-((3,4-Dichlorophenethyl)thio)-1*H*-1,2,4-triazole (54)

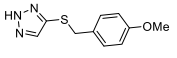


1*H*-1,2,4-Triazole-3-thiol (**76**, 1.05 eq, 100 mg, 0.99 mmol) was reacted with **132** (1 eq, 197 mg, 0.94 mmol) according to General procedure F. Flash column chromatography (30 → 50% EtOAc in pentane) afforded the title compound (160 mg, 0.58 mmol, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.34 (d, *J* = 8.2 Hz, 1H), 7.31 (d, *J* = 2.0 Hz, 1H), 7.05 (dd, *J* = 8.2, 2.1 Hz, 1H), 3.45 – 3.31 (m, 2H), 3.08 – 2.91 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 147.26, 139.93, 132.50, 130.78, 130.75, 130.54, 128.30, 35.28, 33.45.

4-((4-Methoxybenzyl)thio)-2*H*-1,2,3-triazole (55)

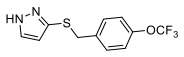


Sodium 2*H*-1,2,3-triazole-4-thiolate (1.05 eq, 100 mg, 0.81 mmol) was benzylated with 4-methoxybenzyl chloride (1 eq, 105 µL, 0.77 mmol) according to General procedure F, with the exception that no base was added. Flash column chromatography (5 → 40% EtOAc in pentane) afforded the title compound (111 mg, 0.50 mmol, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.89 (bs, 1H), 7.48 (s, 1H), 7.19 – 7.08 (m, 2H), 6.83 – 6.74 (m, 2H), 4.06 (s, 2H), 3.75 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 158.91, 139.43, 133.59, 130.11, 129.22, 113.99, 55.29, 39.00.

3-((4-(Trifluoromethoxy)benzyl)thio)-1*H*-pyrazole (56)

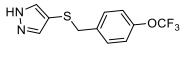


3-Aminopyrazole (**133**, 1.25 eq, 200 mg, 2.41 mmol) was dissolved in 40% H_2SO_4 in water (10 mL) and cooled on ice. $NaNO_2$ (1.2 eq, 200 mg, 2.89 mmol) dissolved in water (10 mL) was added dropwise over the course of 15 min. After stirring for 35 min the pH of the yellow mixture was adjusted to 5 with sat. aq. NaOAc. This mixture was then added dropwise to an ice-cold stirring solution of 4-trifluoromethoxybenzyl mercaptan (**91**, 1 eq, 308 µL, 1.93 mmol) in 1 M aq. NaOH (2 mL) over the course of 35 min. Immediately precipitate formed and the mixture turned orange. The mixture was stirred on ice for 1 h. Water and EtOAc were added and the layers were separated. The aqueous layer was extracted with EtOAc until the organic layer remained colorless. The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Flash column chromatography (10 → 40% EtOAc in pentane) afforded the title compound as yellow oil (131 mg, 0.48 mmol, 25%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (bs, 1H), 7.53 (d, *J* = 2.2 Hz, 1H), 7.28 – 7.19 (m, 2H), 7.08 (d, *J* = 7.9 Hz, 2H), 6.22 (d, *J* = 2.2 Hz, 1H), 4.04 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.44 (q, *J* = 1.9 Hz), 141.51, 136.79, 132.65, 130.29, 121.05, 120.54 (q, *J* = 257.1 Hz), 109.35, 39.23.

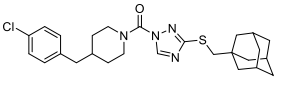
4-((4-(Trifluoromethoxy)benzyl)thio)-1*H*-pyrazole (57)



135 (1.25 eq, 200 mg, 2.41 mmol) was dissolved in 40% H_2SO_4 in water (10 mL) and cooled on ice. $NaNO_2$ (1.2 eq, 200 mg, 2.89 mmol) dissolved in water (10 mL) was added dropwise over the course of 10 min. After stirring for 30 min the pH of the yellow mixture was adjusted to 5 with sat. aq. NaOAc. This mixture was then added dropwise to an ice-cold stirring solution of 4-trifluoromethylbenzyl mercaptan (**91**, 1 eq, 308 µL, 1.93 mmol) in 1 M aq. NaOH (2 mL) over the course of 30 min. Immediately precipitate formed and the mixture turned orange. The mixture was stirred on ice for 1 h. Water and EtOAc were added and the layers were separated. The aqueous layer was extracted with EtOAc until the organic layer remained colorless. The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Flash column chromatography (15 → 40% EtOAc in pentane) afforded the title compound as yellowish crystalline solid (49 mg, 0.18 mmol, 9%). *Analytical data on next page.*

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.49 (bs, 1H), 7.42 – 7.36 (m, 2H), 7.20 – 7.07 (m, 4H), 3.78 (s, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 148.35 (q, *J* = 2.0 Hz), 138.40, 137.28, 130.44, 120.98, 120.56 (q, *J* = 257.1 Hz), 41.27.

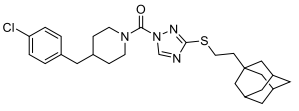
(3-(((Adamant-1-yl)methyl)thio)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)-methanone (59)



39 (1.1 eq, 92 mg, 0.44 mmol) and **42** (1 eq, 99 mg, 0.40 mmol) were coupled according to General procedure D to obtain the title compound as a white solid (120 mg, 0.247 mmol, 62%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.29 – 7.23 (m, 2H), 7.11 – 7.04 (m, 2H), 4.71 – 4.31 (m, 2H), 3.03 (s, 2H), 2.99 – 2.90 (m, 2H), 2.56 (d, *J* = 7.1 Hz, 2H), 1.98 (p, *J* = 3.1 Hz, 3H), 1.88 – 1.76 (m, 1H), 1.76 – 1.54 (m, 14H), 1.36 (qd, *J* = 12.5, 4.2 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 163.92, 148.42, 138.24, 132.10, 130.49, 128.62, 45.71, 42.29, 41.62, 38.09, 36.85, 33.93, 32.01, 28.56.

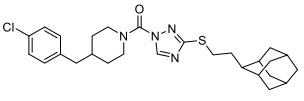
(3-((2-(Adamant-1-yl)ethyl)thio)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)-methanone (60)



39 (1.2 eq, 44 mg, 0.21 mmol) and **43** (1 eq, 46 mg, 0.18 mmol) were coupled according to General procedure D to obtain the title compound as a white solid (57 mg, 0.11 mmol, 65%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.29 – 7.22 (m, 2H), 7.10 – 7.04 (m, 2H), 4.91 – 4.28 (m, 2H), 3.13 – 3.06 (m, 2H), 3.01 – 2.89 (m, 2H), 2.55 (d, *J* = 7.1 Hz, 2H), 1.96 (p, *J* = 3.2 Hz, 3H), 1.86 – 1.68 (m, 6H), 1.66 – 1.58 (m, 3H), 1.55 – 1.48 (m, 8H), 1.36 (qd, *J* = 12.9, 4.2 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 163.11, 148.36, 138.21, 132.09, 130.42, 128.70, 128.60, 44.27, 44.16, 42.28, 42.25, 38.03, 37.16, 33.02, 32.00, 28.70, 26.22.

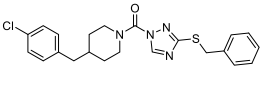
(3-((2-(Adamant-2-yl)ethyl)thio)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)-methanone (61)



39 (1.2 eq, 44 mg, 0.21 mmol) and **44** (1 eq, 46 mg, 0.18 mmol) were coupled according to General procedure D to obtain the title compound as a white solid (64 mg, 0.13 mmol, 73%).
Analytical data on next page.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.28 – 7.23 (m, 2H), 7.10 – 7.05 (m, 2H), 4.85 – 4.14 (m, 2H), 3.15 – 3.07 (m, 2H), 2.99 – 2.90 (m, 2H), 2.55 (d, *J* = 7.2 Hz, 2H), 1.92 – 1.65 (m, 18H), 1.54 – 1.48 (m, 2H), 1.34 (qd, *J* = 12.5, 4.2 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 163.05, 148.32, 138.18, 132.03, 130.41, 128.55, 43.90, 42.18, 39.13, 38.35, 37.97, 32.72, 31.91, 31.73, 30.26, 28.26, 28.04.

(3-(Benzylthio)-1*H*-1,2,4-triazol-1-yl)(4-(4-chlorobenzyl)piperidin-1-yl)methanone (62)

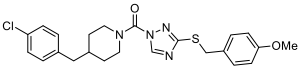


39 (1 eq, 600 mg, 2.86 mmol) was reacted with **45** (1.1 eq, 602 mg, 3.15 mmol) according to General procedure D. Flash column chromatography (10 → 40% EtOAc in pentane) yielded the title compound as slightly yellowish oil (915 mg, 2.14 mmol, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.42 – 7.34 (m, 2H), 7.33 – 7.18 (m, 5H), 7.09 – 7.02 (m, 2H), 4.57 – 4.30 (m, 2H), 4.35 (s, 2H), 2.94 – 2.83 (m, 2H), 2.52 (d, *J* = 7.1 Hz, 2H), 1.84 – 1.72 (m, 1H), 1.72 – 1.60 (m, 2H), 1.28 (qd, *J* = 13.7, 13.2, 4.2 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.17, 148.11, 147.39, 138.12, 136.94, 131.89, 130.38, 128.79, 128.50, 128.45, 127.42, 42.06, 37.82, 35.96, 31.79 (br).

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-methoxybenzyl)thio)-1*H*-1,2,4-triazol-1-yl)-methanone (63)

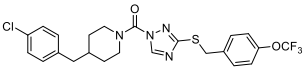


39 (1 eq, 95 mg, 0.45 mmol) was reacted with **46** (1 eq, 100 mg, 0.45 mmol) according to General procedure D. Flash column chromatography yielded the title compound (86 mg, 0.19 mmol, 42%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.36 – 7.26 (m, 2H), 7.29 – 7.22 (m, 2H), 7.12 – 7.02 (m, 2H), 6.85 – 6.77 (m, 2H), 4.61 – 4.36 (m, 2H), 4.31 (s, 2H), 3.75 (s, 3H), 3.02 – 2.78 (m, 2H), 2.54 (d, *J* = 7.1 Hz, 2H), 1.86 – 1.74 (m, 1H), 1.70 (m, 2H), 1.31 (qd, *J* = 12.7, 3.9 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.35, 158.98, 148.23, 147.41, 138.17, 131.97, 130.43, 130.06, 128.87, 128.54, 113.95, 55.30, 47.23 (br), 42.15, 37.93, 35.59, 31.93 (br).

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)thio)-1*H*-1,2,4-triazol-1-yl)methanone (64)

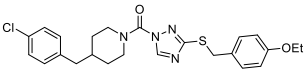


39 (1.1 eq, 126 mg, 0.60 mmol) was reacted with **47** (1 eq, 150 mg, 0.55 mmol) according to General procedure D. Flash column chromatography yielded the title compound (167 mg, 0.33 mmol, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.46 – 7.39 (m, 2H), 7.30 – 7.22 (m, 2H), 7.16 – 7.10 (m, 2H), 7.10 – 7.03 (m, 2H), 4.47 – 4.42 (m, 2H), 4.35 (s, 2H), 2.98 – 2.85 (m, 2H), 2.54 (d, *J* = 7.1 Hz, 2H), 1.86 – 1.74 (m, 1H), 1.74 – 1.66 (m, 2H), 1.29 (qd, *J* = 12.8, 3.5 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.90, 148.56 (q, *J* = 1.8 Hz), 148.22, 147.61, 138.16, 136.06, 132.14, 130.47, 130.32, 128.64, 121.13, 120.54 (q, *J* = 257.2 Hz), 42.21, 38.01, 35.23, 31.94 (br).

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-ethoxybenzyl)thio)-1*H*-1,2,4-triazol-1-yl)methanone (65)

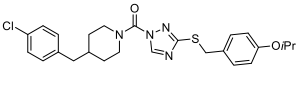


39 (1.1 eq, 294 mg, 1.40 mmol) was reacted with **48** (1 eq, 300 mg, 1.28 mmol) according to General procedure D. Flash column chromatography yielded the title compound (465 mg, 0.99 mmol, 77%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.33 – 7.21 (m, 4H), 7.10 – 7.04 (m, 2H), 6.84 – 6.77 (m, 2H), 4.60 – 4.35 (m, 2H), 4.31 (s, 2H), 3.98 (q, *J* = 7.0 Hz, 2H), 2.97 – 2.86 (m, 2H), 2.54 (d, *J* = 7.0 Hz, 2H), 1.85 – 1.75 (m, 1H), 1.75 – 1.65 (m, 2H), 1.39 (t, *J* = 7.0 Hz, 3H), 1.31 (qd, *J* = 12.5, 4.1 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.46, 158.41, 148.30, 147.46, 138.20, 132.04, 130.46, 130.09, 128.73, 128.59, 114.55, 63.53, 47.25 (br), 42.21, 37.99, 35.67, 31.93 (br), 14.92.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-isopropoxybenzyl)thio)-1*H*-1,2,4-triazol-1-yl)-methanone (66)

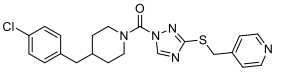


39 (1.1 eq, 139 mg, 0.66 mmol) was reacted with **49** (1 eq, 150 mg, 0.60 mmol) according to General procedure D. Flash column chromatography yielded the title compound (267 mg, 0.55 mmol, 91%).

Analytical data on next page.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.33 – 7.21 (m, 4H), 7.11 – 7.03 (m, 2H), 6.83 – 6.76 (m, 2H), 4.60 – 4.37 (m, 2H), 4.49 (hept, *J* = 6.1 Hz, 1H), 4.31 (s, 2H), 2.97 – 2.86 (m, 2H), 2.54 (d, *J* = 7.0 Hz, 2H), 2.17 (s, 2H), 1.85 – 1.76 (m, 1H), 1.76 – 1.67 (m, 2H), 1.38 – 1.24 (m, 2H), 1.31 (d, *J* = 6.1 Hz, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 162.49, 157.37, 148.31, 147.46, 138.20, 132.05, 130.47, 130.12, 128.63, 128.59, 115.88, 69.95, 42.21, 38.01, 35.67, 31.93 (br), 22.12.

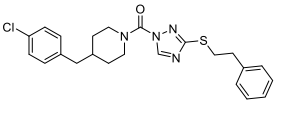
(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((pyridin-4-ylmethyl)thio)-1*H*-1,2,4-triazol-1-yl)-methanone (67)



39 (1.1 eq, 240 mg, 1.14 mmol) was reacted with **50** (1 eq, 200 mg, 1.04 mmol) according to General procedure D. Flash column chromatography yielded the title compound (320 mg, 0.75 mmol, 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.55 – 8.49 (m, 2H), 7.37 – 7.31 (m, 2H), 7.31 – 7.23 (m, 2H), 7.11 – 7.03 (m, 2H), 4.45 – 4.37 (m, 2H), 4.31 (s, 2H), 2.94 – 2.83 (m, 2H), 2.54 (d, *J* = 7.1 Hz, 2H), 1.84 – 1.71 (m, 1H), 1.74 – 1.65 (m, 2H), 1.31 – 1.23 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 161.37, 149.96, 148.07, 147.65, 146.62, 138.12, 132.10, 130.46, 128.62, 123.78, 46.63 (br), 42.17, 37.94, 34.72, 31.89 (br).

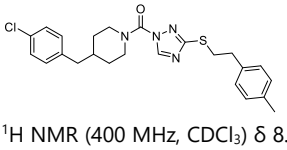
(4-(4-Chlorobenzyl)piperidin-1-yl)(3-(phenethylthio)-1*H*-1,2,4-triazol-1-yl)methanone (68)



39 (1 eq, 102 mg, 0.49 mmol) was reacted with **51** (1 eq, 100 mg, 0.49 mmol) according to General procedure D. Flash column chromatography (5 → 30% Et_2O in pentane) yielded the title compound (217 mg, 0.49 mmol, quant.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.32 – 7.26 (m, 2H), 7.26 – 7.16 (m, 5H), 7.07 – 7.01 (m, 2H), 4.85 – 4.15 (m, 2H), 3.39 – 3.30 (m, 2H), 3.13 – 3.01 (m, 2H), 3.01 – 2.87 (m, 2H), 2.52 (d, *J* = 7.1 Hz, 2H), 1.86 – 1.75 (m, 1H), 1.75 – 1.64 (m, 2H), 1.40 – 1.26 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 162.45, 148.12, 147.37, 139.86, 138.07, 131.81, 130.33, 128.51, 128.47, 128.41, 126.51, 47.09, 42.02, 37.81, 36.04, 32.81, 31.78.

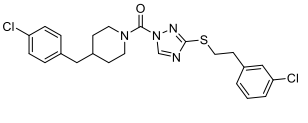
(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-methylphenethyl)thio)-1*H*-1,2,4-triazol-1-yl)-methanone (69)



39 (1 eq, 102 mg, 0.49 mmol) was reacted with **52** (1 eq, 107 mg, 0.49 mmol) according to General procedure D. Flash column chromatography (5 → 30% Et_2O in pentane) yielded the title compound (221 mg, 0.49 mmol, quant.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.28 – 7.20 (m, 2H), 7.14 – 7.07 (m, 4H), 7.07 – 7.01 (m, 2H), 4.76 – 4.36 (m, 2H), 3.37 – 3.29 (m, 2H), 3.05 – 2.96 (m, 2H), 2.98 – 2.86 (m, 2H), 2.53 (d, *J* = 7.1 Hz, 2H), 2.32 (s, 3H), 1.88 – 1.76 (m, 1H), 1.76 – 1.65 (m, 2H), 1.41 – 1.30 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 162.52, 148.12, 147.38, 138.08, 136.80, 136.01, 131.82, 130.33, 129.15, 128.40, 128.38, 47.10, 42.04, 37.83, 35.60, 32.95, 31.79, 21.03.

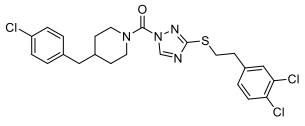
(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((3-chlorophenethyl)thio)-1*H*-1,2,4-triazol-1-yl)-methanone (70)



39 (1 eq, 88 mg, 0.42 mmol) was reacted with **53** (1 eq, 100 mg, 0.42 mmol) according to General procedure D. Flash column chromatography (5 → 30% Et_2O in pentane) yielded the title compound as slightly yellowish oil (130 mg, 0.27 mmol, 66%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.29 – 7.17 (m, 5H), 7.12 – 7.08 (m, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 4.90 – 4.14 (m, 2H), 3.38 – 3.29 (m, 2H), 3.10 – 3.00 (m, 2H), 3.00 – 2.84 (m, 2H), 2.54 (d, *J* = 7.1 Hz, 2H), 1.89 – 1.77 (m, 1H), 1.77 – 1.68 (m, 2H), 1.34 (qd, *J* = 12.9, 4.2 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 162.29, 148.18, 147.48, 141.90, 138.12, 134.22, 131.91, 130.39, 129.81, 128.73, 128.49, 126.85, 126.77, 47.38, 42.08, 37.90, 35.79, 32.54, 31.85.

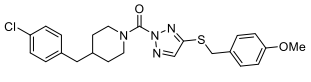
(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((3,4-dichlorophenethyl)thio)-1*H*-1,2,4-triazol-1-yl)-methanone (71)



39 (2 eq, 160 mg, 0.76 mmol) was reacted with **54** (1 eq, 100 mg, 0.37 mmol) according to General procedure D. Flash column chromatography yielded the title compound (56 mg, 0.11 mmol, 30%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.40 – 7.32 (m, 2H), 7.30 – 7.22 (m, 2H), 7.13 – 7.04 (m, 3H), 4.46 – 4.33 (m, 2H), 3.50 – 3.37 (m, 2H), 3.10 – 3.01 (m, 2H), 3.01 – 2.84 (m, 2H), 2.55 (d, *J* = 7.0 Hz, 2H), 1.85 – 1.77 (m, 1H), 1.77 – 1.69 (m, 2H), 1.36 (qd, *J* = 12.7, 4.0 Hz, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 158.57, 151.05, 149.60, 140.05, 138.29, 134.31, 132.09, 130.76, 130.58, 130.51, 128.63, 128.27, 42.28, 38.10, 34.67, 33.43, 31.98.

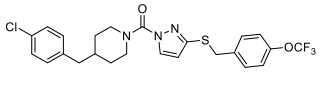
(4-(4-Chlorobenzyl)piperidin-1-yl)(4-((4-methoxybenzyl)thio)-2*H*-1,2,3-triazol-2-yl)-methanone (72)



39 (1 eq, 95 mg, 0.425 mmol) was reacted with **55** (1 eq, 100 mg, 0.425 mmol) according to General procedure D. Flash column chromatography (5 → 30% Et_2O in pentane) yielded the title compound (30 mg, 0.066 mmol, 15%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.28 – 7.21 (m, 4H), 7.12 – 7.03 (m, 2H), 6.85 – 6.78 (m, 2H), 4.59 – 4.28 (m, 1H), 4.21 (s, 2H), 4.13 – 3.88 (m, 1H), 3.76 (s, 3H), 3.05 – 2.85 (m, 2H), 2.55 (d, *J* = 7.0 Hz, 2H), 1.90 – 1.72 (m, 1H), 1.71 – 1.51 (m, 2H), 1.39 – 1.30 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 159.13, 148.88, 145.09, 138.24, 136.42, 132.05, 130.48, 130.21, 128.60, 114.10, 55.36, 42.22, 38.06, 37.30, 32.06.

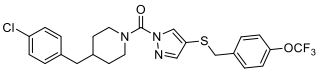
(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-(trifluoromethoxy)benzyl)thio)-1*H*-pyrazol-1-yl)-methanone (73)



39 (1 eq, 46 mg, 0.22 mmol) was reacted with **56** (2.2 eq, 131 mg, 0.48 mmol) according to General procedure D. Flash column chromatography (10 → 40% EtOAc in pentane) yielded the title compound as slightly yellowish gum (62 mg, 0.12 mmol, 55%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, *J* = 2.7 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.28 – 7.23 (m, 2H), 7.16 – 7.09 (m, 2H), 7.09 – 7.03 (m, 2H), 6.21 (d, *J* = 2.7 Hz, 1H), 4.51 – 4.39 (m, 2H), 4.22 (s, 2H), 2.89 (td, *J* = 12.9, 2.6 Hz, 2H), 2.52 (d, *J* = 7.2 Hz, 2H), 1.83 – 1.70 (m, 1H), 1.69 – 1.60 (m, 2H), 1.29 (qd, *J* = 12.7, 4.2 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 150.55, 148.75, 148.38, 138.36, 136.40, 133.43, 131.96, 130.45, 130.19, 128.53, 121.04, 120.51 (q, *J* = 257.2 Hz), 108.12, 42.28, 38.11, 36.16, 32.00.

(4-(4-Chlorobenzyl)piperidin-1-yl)(4-((4-(trifluoromethoxy)benzyl)thio)-1*H*-pyrazol-1-yl)-methanone (74)

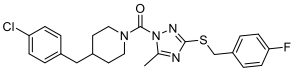


39 (1.2 eq, 46 mg, 0.22 mmol) was reacted with **57** (1 eq, 49 mg, 0.18 mmol) according to General procedure D. Flash column chromatography (15 → 50% Et_2O in pentane) yielded the title compound as yellowish gum (44 mg, 0.086 mmol, 48%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, *J* = 0.7 Hz, 1H), 7.33 (d, *J* = 0.7 Hz, 1H), 7.28 – 7.22 (m, 2H), 7.22 – 7.17 (m, 2H), 7.15 – 7.10 (m, 2H), 7.09 – 7.04 (m, 2H), 4.46 – 4.40 (m, 2H), 3.84 (s, 2H), 2.92 (td, *J* = 12.5, 6.3 Hz, 2H), 2.54 (d, *J* = 7.1 Hz, 2H), 1.84 – 1.74 (m, 1H), 1.74 – 1.66 (m, 2H), 1.33 (qd, *J* = 12.8, 4.2 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 150.52, 148.45, 145.17, 138.34, 136.73, 135.28, 131.99, 130.49, 130.40, 128.56, 121.06, 120.53 (q, *J* = 257.2 Hz), 112.31, 42.29, 40.56, 38.14, 31.99.

(4-(4-Chlorobenzyl)piperidin-1-yl)(3-((4-fluorobenzyl)thio)-5-methyl-1*H*-1,2,4-triazol-1-yl)-methanone (75)

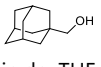


39 (1 eq, 65 mg, 0.31 mmol) was reacted with 3-((4-fluorobenzyl)thio)-1*H*-1,2,4-triazole (**58**, 70 mg, 0.31 mmol, kindly provided by Anthe Janssen) according to General procedure D. The residue was purified by flash column chromatography (0 → 40% EtOAc in pentane) yielding the title compound as gray gum (84 mg, 0.18 mmol, 59%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.31 (m, 2H), 7.31 – 7.22 (m, 2H), 7.10 – 7.02 (m, 2H), 7.02 – 6.91 (m, 2H), 4.43 – 4.25 (m, 1H), 4.29 (s, 2H), 4.04 – 3.82 (m, 1H), 2.96 – 2.79 (m, 2H), 2.58 (s, 3H), 2.54 (d, *J* = 7.0 Hz, 2H), 1.84 – 1.68 (m, 1H), 1.70 – 1.56 (m, 2H), 1.36 – 1.14 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.55 (d, *J* = 359.9 Hz), 160.89, 157.58, 149.37, 138.16, 132.99 (d, *J* = 3.2 Hz), 132.03, 130.53 (d, *J* = 8.2 Hz), 130.43, 128.56, 115.41 (d, *J* = 21.5 Hz), 47.91 (br), 45.32 (br), 42.13, 37.93, 35.24, 31.97, 13.85.

1-Adamantanemethanol (82)

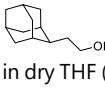


A solution of 1-adamantanecarboxylic acid (**79**, 5.0 g, 28 mmol) in dry THF (10 mL/g) was added dropwise to an ice-cold suspension of $LiAlH_4$ (2.5 eq, 35 mL 2.0 M in THF, 70 mmol) in dry THF (20 mL/g). The reaction mixture was warmed to RT and stirred for 30 min, followed by reflux for 30 min. The reaction was then quenched by addition of 10% aq. NaOH on an ice bath. Solids were removed by filtration and washed with DCM. Combined filtrates were dried over Na_2SO_4, filtrated and concentrated *in vacuo* to obtain the title compound as a white solid (4.7 g, 27 mmol, 99%), which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.20 (s, 2H), 2.00 (p, *J* = 3.1 Hz, 3H), 1.77 – 1.61 (m, 7H), 1.51 (d, *J* = 2.9 Hz, 6H).

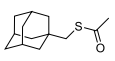
^{13}C NMR (126 MHz, $CDCl_3$) δ 74.03, 39.17, 37.31, 34.62, 28.31.

2-Adamantaneethanol (84)



Methyl 2-(adamant-2-yl)acetate (**81**, 32.0 g, 154 mmol) in dry THF (10 mL/g) was added dropwise to an ice-cold suspension of $LiAlH_4$ (2.5 eq, 193 mL 2.0 M in THF, 385 mmol) in dry THF (20 mL/g). The reaction mixture was warmed to RT and stirred for 30 min, followed by reflux for 30 min. The reaction was then quenched by addition of 10% aq. NaOH on an ice bath. Solids were removed by filtration and washed with DCM. Combined filtrates were dried over Na_2SO_4, filtrated and concentrated *in vacuo* to obtain the title compound as a white solid (27.0 g, 150 mmol, 97%) which was used without characterization.

_S_-(adamant-1-ylmethyl)thioacetate (85)

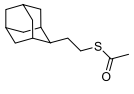


Triflic anhydride (1.05 eq, 8.9 g, 32 mmol) was added portionwise to a solution of **82** (1 eq, 5.0 g, 30 mmol) and pyridine (1.2 eq, 2.9 mL, 36 mmol) in dry DCM (60 mL), while maintaining the temperature between −15 and −5°C. The mixture was stirred for 15 min followed by 30 min at RT. The mixture was then diluted with hexane (150 mL), cooled to 0°C and ice-cold 1 M aq. H_2SO_4 was added until pH < 7. The layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The resulting brown liquid was dissolved in CH_3CN (50 mL) and cooled to 0°C. AcSK (2 eq, 6.9 g, 60 mmol) and 18-Crown-6 (0.3 eq, 1.9 mL, 9 mmol) were added, the mixture was warmed to RT and stirred for ≥ 72 h. Solids were removed by filtration and washed with hexane until they were colorless. Combined filtrates were concentrated *in vacuo*. Flash column chromatography (150:1 hexane:EtOAc) provided the thioacetate as a red solid (4.3 g, 19 mmol, 64%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.73 (s, 2H), 2.35 (s, 3H), 1.96 (hept, *J* = 3.0 Hz, 3H), 1.72 – 1.58 (m, 6H), 1.50 (d, *J* = 2.9 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 196.16, 42.62, 41.56, 36.88, 33.37, 30.89, 28.57.

_S_-(2-(adamant-2-yl)ethyl)thioacetate (87)

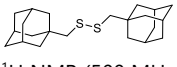


Triflic anhydride (1.05 eq, 5.0 g, 18 mmol) was added portionwise to a solution of **84** (1 eq, 3.0 g, 17 mmol) and pyridine (1.2 eq, 1.6 mL, 20 mmol) in dry DCM (34 mL), while maintaining the temperature at −70°C. The mixture was stirred for 15 min followed by 30 min at RT. The mixture was then diluted with hexane (85 mL), cooled to 0°C and ice-cold 1 M aq. H_2SO_4 was added until pH < 7. The layers were separated, the organic layer was washed with water and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The resulting brown liquid was dissolved in CH_3CN (28 mL) and cooled to 0°C. AcSK (2 eq, 3.9 g, 34 mmol) and 18-Crown-6 (0.3 eq, 1.1 mL, 5 mmol) were added, the mixture was warmed to RT and stirred for ≥ 72 h. Solids were removed by filtration and washed with hexane until they were colorless. Combined filtrates were concentrated *in vacuo*. Flash column chromatography (150:1 hexane:EtOAc) provided the thioacetate as a red liquid (2.2 g, 9.2 mmol, 56%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89 – 2.81 (m, 2H), 2.32 (s, 3H), 1.92 – 1.64 (m, 15H), 1.57 – 1.47 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 195.91, 43.80, 39.07, 38.30, 32.43, 31.60, 31.57, 28.21, 27.98, 27.57.

Bis(adamant-1-ylmethyl)disulfide (88)

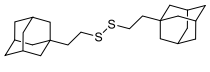


85 (1.0 g, 4.5 mmol) was treated according to General procedure E to obtain the title compound as a white solid (790 g, 2.18 mmol, 98%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.63 (s, 4H), 1.98 (p, *J* = 3.1 Hz, 6H), 1.73 – 1.59 (m, 12H), 1.57 (d, *J* = 2.9 Hz, 12H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 56.23, 41.90, 36.97, 34.33, 28.61.

Bis(2-(Adamant-1-yl)ethyl)disulfide (89)

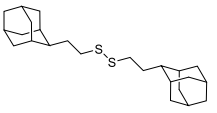


S-(2-(adamant-1-yl)ethyl)thioacetate (**86**, 1.5 g, 6.3 mmol, kindly provided by Alexander Pashenko) was treated according to General procedure E to obtain the title compound as a white crystalline solid (800 mg, 2.05 mmol, 65%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.70 – 2.63 (m, 4H), 1.96 (p, *J* = 3.1 Hz, 6H), 1.74 – 1.59 (m, 12H), 1.49 (d, *J* = 2.9 Hz, 12H), 1.47 – 1.41 (m, 4H).

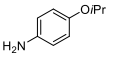
^{13}C NMR (126 MHz, $CDCl_3$) δ 44.24, 42.44, 37.23, 33.57, 32.89, 28.78.

Bis(2-(adamant-2-yl)ethyl)disulfide (90)



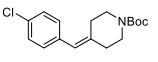
87 (1.05 g, 4.40 mmol) was treated according to General procedure E to obtain the title compound as a white crystalline solid (430 g, 1.10 mmol, 50%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.73 – 2.65 (m, 4H), 1.92 – 1.66 (m, 30H), 1.52 (d, *J* = 12.6 Hz, 4H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 43.58, 39.24, 38.46, 37.68, 32.47, 31.86, 31.79, 28.37, 28.16.

4-Isopropoxyaniline (96)



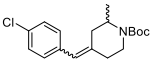
137 (1 eq, 491 mg, 2.71 mmol) was dissolved in MeOH (8 mL), Pd/C (1.7 mol%, 49 mg 10%, 0.046 mmol) was added and the mixture was purged with N_2. HCl (1.3 eq, 300 µL 12 M, 3.60 mmol) was added and purging was continued. The mixture was then purged with H_2 and stirred for 5 h. The mixture was purged with N_2 before it was filtrated over celite, treated with activated charcoal and filtrated over celite again. The filtrate was concentrated *in vacuo*, affording the title compound as brown solid (392 mg, 2.59 mmol, 96%), which was used without further purification.
^{1}H NMR (400 MHz, MeOD) δ 7.32 (m, 2H), 7.03 (m, 2H), 4.63 (p, *J* = 6.0 Hz, 1H), 1.30 (d, *J* = 5.8 Hz, 6H).
^{13}C NMR (101 MHz, MeOD) δ 159.57, 125.22, 125.10, 123.81, 117.93, 71.35, 22.10.

***tert*-Butyl 4-(4-chlorobenzylidene)piperidine-1-carboxylate (108)**



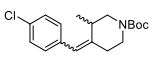
N-Boc-4-piperidinone (**103**, 500 mg, 2.51 mmol) was reacted with diethyl (4-chlorobenzyl)phosphonate (**107**, 1.1 eq, 609 µL, 2.76 mmol) according to General procedure H. Flash column chromatography (0 → 10% EtOAc in pentane) yielded the title compound as white crystalline solid (482 mg, 1.57 mmol, 62%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.24 (m, 2H), 7.14 – 7.07 (m, 2H), 6.30 (s, 1H), 3.54 – 3.47 (m, 2H), 3.43 – 3.36 (m, 2H), 2.45 – 2.38 (m, 2H), 2.36 – 2.28 (m, 2H), 1.48 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.82, 139.33, 135.91, 132.12, 130.27, 128.42, 123.48, 79.72, 36.26, 29.24, 28.54.

***tert*-Butyl 4-(4-chlorobenzylidene)-2-methylpiperidine-1-carboxylate (109)**



N-Boc-2-methyl-4-piperidinone (**104**, 285 mg, 1.34 mmol) was reacted with diethyl (4-chlorobenzyl)phosphonate (**107**, 1.3 eq, 373 µL, 1.69 mmol) according to General procedure H. Flash column chromatography (0 → 15% Et_2O in pentane) yielded a mixture of *E*/*Z* isomers (ratio ~3:2) of the title compound as colorless oil (195 mg, 0.61 mmol, 45%).
Isomer 1:
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.21 (m, 2H), 7.16 – 7.04 (m, 2H), 6.41 (s, 1H), 4.54 – 4.41 (m, 1H), 4.12 – 4.03 (m, 1H), 2.98 (td, *J* = 12.8, 3.6 Hz, 1H), 2.60 – 2.51 (m, 1H), 2.41 – 2.29 (m, 1H), 2.29 – 2.21 (m, 2H), 1.47 (s, 9H), 1.02 (d, *J* = 6.9 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.80, 136.95, 135.99, 132.01, 130.01, 128.35, 125.15, 79.48, 47.25 (br), 39.78 (br), 35.96, 33.81, 28.51, 16.94.
Isomer 2:
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.21 (m, 2H), 7.16 – 7.04 (m, 2H), 6.27 (s, 1H), 4.54 – 4.41 (m, 1H), 4.02 – 3.94 (m, 1H), 2.89 (td, *J* = 12.8, 3.5 Hz, 1H), 2.70 (dt, *J* = 14.4, 3.3 Hz, 1H), 2.64 – 2.57 (m, 1H), 2.20 – 2.07 (m, 2H), 1.47 (s, 9H), 1.13 (d, *J* = 6.8 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.71, 136.86, 135.94, 132.01, 130.13, 128.35, 124.98, 79.50, 47.83 (br), 41.26, 38.84 (br), 28.95, 28.51, 16.94.

***tert*-Butyl 4-(4-chlorobenzylidene)-3-methylpiperidine-1-carboxylate (110)**



NaH (1.6 eq, 300 mg 60% dispersion in mineral oil, 7.50 mmol) was suspended in dry THF (10 mL) and cooled on ice. Diethyl (4-chlorobenzyl)phosphonate (**107**, 1.1 eq, 1.20 mL, 5.16 mmol) dissolved in dry THF (10 mL) was added dropwise and the mixture was stirred for 1.5 h. *N*-Boc-3-methyl-4-piperidinone (**105**, 1 eq, 1.0 g, 4.69 mmol) dissolved in dry THF (10 mL) was added dropwise. The mixture was allowed to warm to RT and stirred for 2 d. The reaction was cooled on ice and quenched with sat. aq. NH_4Cl. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Flash column chromatography (0 → 15% Et_2O in pentane) yielded a mixture of *E/Z* isomers (ratio ~2:1) of the title compound as white crystalline solid (900 mg, 2.80 mmol, 60%).

Isomer 1:

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 – 7.21 (m, 2H), 7.15 – 7.02 (m, 2H), 6.27 (s, 1H), 3.66 (dd, *J* = 13.0, 3.1 Hz, 1H), 3.48 (dt, *J* = 11.4, 5.7 Hz, 1H), 3.31 (d, *J* = 4.4 Hz, 1H), 3.08 (dd, *J* = 12.6, 7.4 Hz, 1H), 2.63 – 2.49 (m, 1H), 2.48 – 2.34 (m, 1H), 2.32 – 2.16 (m, 1H), 1.47 (s, 9H), 1.15 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 155.10, 143.94, 136.47, 132.32, 130.43, 128.48, 121.61, 79.58, 51.62, 45.04, 38.68, 28.64, 27.91, 16.52.

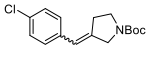
Isomer 2:

^{1}H NMR (300 MHz, DMSO) δ 7.32 – 7.22 (m, 2H), 7.15 – 7.05 (m, 2H), 6.21 (s, 1H), 4.35 – 4.16 (m, 1H), 3.96 – 3.86 (m, 1H), 3.00 – 2.88 (m, 1H), 2.85 – 2.68 (m, 2H), 2.61 (tdd, *J* = 13.0, 5.0, 1.8 Hz, 1H), 2.05 (dt, *J* = 13.3, 1.9 Hz, 1H), 1.47 (s, 9H), 1.17 (d, *J* = 6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 155.44, 144.00, 136.22, 132.44, 130.08, 128.61, 122.91, 79.64, 50.17, 45.83, 32.70, 32.54, 28.67, 17.42.

NB: NMR recorded at 60 °C.

***tert*-Butyl 3-(4-chlorobenzylidene)pyrrolidine-1-carboxylate (111)**



Boc-3-pyrrolidinone (**106**, 500 mg, 2.70 mmol) was reacted with diethyl (4-chlorobenzyl)phosphonate (**107**, 1.1 eq, 655 µL, 2.97 mmol) according to General procedure H. Flash column chromatography (0 → 8% EtOAc in pentane) yielded a mixture of *E/Z* isomers (ratio ~3:2) of the title compound as colorless oil (357 mg, 1.22 mmol, 45%).

Isomer 1:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.22 (m, 2H), 7.21 – 7.14 (m, 2H), 6.37 – 6.31 (m, 1H), 4.24 – 4.06 (m, 2H), 3.50 – 3.39 (m, 2H), 2.81 – 2.68 (m, 2H), 1.49 (s, 9H).

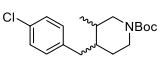
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.35, 138.93, 135.70, 135.48 (br), 135.40 (br), 132.34, 129.47, 128.45, 121.47, 79.45, 48.63, 44.27, 43.85, 34.13, 33.39, 28.48.

Isomer 2:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.22 (m, 2H), 7.13 – 7.05 (m, 2H), 6.37 – 6.31 (m, 1H), 4.24 – 4.06 (m, 2H), 3.61 – 3.49 (m, 2H), 2.81 – 2.68 (m, 2H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 154.45, 139.94, 135.40 (br), 132.26, 129.25, 128.53, 120.87 (br), 79.53 (br), 52.08 (br), 51.89 (br), 46.08 (br), 45.74 (br), 29.68, 29.06, 28.48.

***tert*-Butyl 4-(4-chlorobenzyl)-3-methylpiperidine-1-carboxylate (112)**



A solution of **110** (200 mg, 0.62 mmol) in EtOAc (1 mL) was purged with N_2 (10 min). Pd/C (5 mol%, 33 mg 10%, 0.031 mmol) was added and purging was continued (10 min). The mixture was purged with H_2 and stirred overnight. Solids were removed by filtration over celite, volatiles *in vacuo*. Flash column chromatography (0 → 20% Et_2O in pentane) afforded the title compound as mixture of isomers (178 mg, 0.55 mmol, 88%, ratio ~3:1).

Isomer 1:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.17 (m, 2H), 7.10 – 7.00 (m, 2H), 4.18 – 4.04 (m, 1H), 3.89 – 3.74 (m, 1H), 2.90 – 2.82 (m, 1H), 2.68 – 2.55 (m, 1H), 2.55 – 2.38 (m, 3H), 1.89 – 1.76 (m, 1H), 1.75 – 1.64 (m, 1H), 1.45 (s, 9H), 1.34 – 1.20 (m, 1H), 0.91 (d, *J* = 6.1 Hz, 3H).

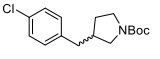
^{13}C NMR (101 MHz, $CDCl_3$) δ 155.01, 138.77, 131.37, 130.09, 128.19, 78.85, 50.69 (br), 43.27 (br), 41.05, 38.71, 38.57 (br), 31.72, 28.26, 10.94.

Isomer 2:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.17 (m, 2H), 7.10 – 7.00 (m, 2H), 4.03 – 3.88 (m, 2H), 3.06 – 2.96 (m, 1H), 2.79 – 2.68 (m, 1H), 2.55 – 2.38 (m, 2H), 2.38 – 2.25 (m, 1H), 2.18 – 2.09 (m, 1H), 1.45 (s, 9H), 1.34 – 1.20 (m, 2H), 1.00 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 154.42, 138.67, 131.37, 130.36, 128.11, 78.99, 49.35 (br), 44.17, 43.96 (br), 38.71, 38.07 (br), 35.63, 28.26, 16.67.

***tert*-Butyl 3-(4-chlorobenzyl)pyrrolidine-1-carboxylate (113)**

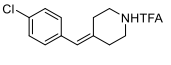


A solution of **111** (51 mg, 0.17 mmol) in EtOAc (4 mL) was purged with N_2 (15 min). Pd/C (5 mol%, 9 mg 10%, 8.5 µmol) was added and purging was continued (15 min). The mixture was purged with H_2 and stirred for 5 h. Solids were removed by filtration over celite, volatiles *in vacuo*, providing the title compound as colorless oil (50 mg, 0.17 mmol, quant.), which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.22 (m, 2H), 7.09 (d, *J* = 8.2 Hz, 2H), 3.57 – 3.36 (m, 2H), 3.32 – 3.16 (m, 1H), 3.05 – 2.87 (m, 1H), 2.76 – 2.54 (m, 2H), 2.44 – 2.29 (m, 1H), 1.97 – 1.85 (m, 1H), 1.73 – 1.37 (m, 10H).

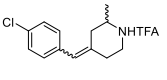
^{13}C NMR (101 MHz, $CDCl_3$) δ 154.69, 138.86, 132.01, 130.09, 128.77, 128.63, 128.52, 79.19, 51.22, 50.96, 45.66, 45.24, 44.46, 40.74, 40.00, 38.64, 31.44, 30.74, 28.62, 28.11.

4-(4-Chlorobenzylidene)piperidin-1-ium 2,2,2-trifluoroacetate (114)



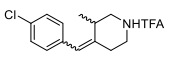
108 (252 mg, 0.82 mmol) was dissolved in DCM (2 mL) and cooled on ice. To this, TFA (5.6 eq, 350 µL, 4.59 mmol) was added dropwise. The reaction was allowed to warm to RT and stirred for 1 h. Volatiles were removed *in vacuo*. The residual off-white crystalline solid was used immediately in the next reaction.

4-(4-Chlorobenzylidene)-2-methylpiperidin-1-ium 2,2,2-trifluoroacetate (115)



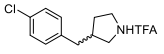
109 (65 mg, 0.20 mmol) was dissolved in DCM (2 mL). To this, TFA (10 eq, 166 µL, 2.17 mmol) was added carefully, after which the mixture was stirred for 26 h. All volatiles were removed *in vacuo*. The residue was used immediately in the next reaction.

4-(4-Chlorobenzylidene)-3-methylpiperidin-1-ium 2,2,2-trifluoroacetate (116)



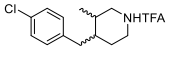
110 (200 mg, 0.62 mmol) was dissolved in DCM (1 mL). To this, TFA (5.3 eq, 250 µL, 3.27 mmol) was added dropwise and the reaction was stirred for 5 h. All volatiles were removed *in vacuo*. The resulting oil was triturated with 9:1 *n*-hexane:EtOAc, yielding the title compound as white crystalline solid, which was used immediately in the next reaction.

3-(4-Chlorobenzyl)pyrrolidin-1-ium 2,2,2-trifluoroacetate (117)



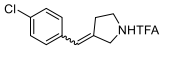
113 (50 mg, 0.17 mmol) was dissolved in DCM (3 mL). To this, TFA (10 eq, 130 µL, 1.70 mmol) was added dropwise and the mixture was stirred for 3 d. Solids were removed by filtration over celite, volatiles *in vacuo*. The residue was used immediately in the next reaction.

4-(4-Chlorobenzyl)-3-methylpiperidin-1-ium 2,2,2-trifluoroacetate (118)



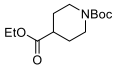
112 (178 mg, 0.55 mmol) was dissolved in DCM (1 mL). To this, TFA (5 eq, 210 µL, 2.75 mmol) was added dropwise and the mixture was stirred for 18 h. All volatiles were removed *in vacuo*. The resulting brown crystalline solid was triturated with 9:1 *n*-hexane:EtOAc, affording the title compound as white crystalline solid (150 mg, 0.44 mmol, 81%), which was used immediately in the next reaction.

3-(4-Chlorobenzylidene)pyrrolidin-1-ium 2,2,2-trifluoroacetate (119)



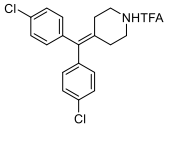
111 (109 mg, 0.37 mmol) was dissolved in DCM (2.2 mL). To this TFA (6 eq, 163 µL, 2.13 mmol) was added dropwise, after which the mixture was stirred for 19 h. All volatiles were removed *in vacuo* and the residual brown solid was used immediately in the next reaction.

1-(*tert*-Butyl) 4-ethyl piperidine-1,4-dicarboxylate (121)



Ethyl isonipecotate (**138**, 1 eq, 500 mg, 3.18 mmol) and Et_3N (1.5 eq, 665 µL, 4.77 mmol) were dissolved in DCM (7 mL) and cooled on ice. Boc_2O (1.2 eq, 833 mg, 3.82 mmol) was added portionwise. The mixture was stirred on ice for 30 min, after which it was allowed to warm to RT and stirred for 50 h. Water was added and the layers were separated. The organic layer was washed with water and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The residue was used immediately in the next reaction.

4-(Bis(4-chlorophenyl)methylene)piperidin-1-ium 2,2,2-trifluoroacetate (122)

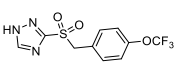


139 (350 mg, 0.80 mmol) was dissolved in DCM (5 mL). To this, TFA (10 eq, 607 µL, 7.93 mmol) was added dropwise, after which the reaction was stirred for 6 d. All volatiles were removed *in vacuo*. The resulting brown oil was mixed with 9:1 *n*-hexane:EtOAc (0.5 mL) causing white precipitation. The brown solution was discarded. This procedure was repeated until no more white precipitate formed. Solids were isolated by filtration and eluted with MeOH, after which they were concentrated *in vacuo*, affording the title compound as white crystalline solid (285 mg, 0.66 mmol, 82%).

^{1}H NMR (400 MHz, MeOD) δ 7.39 – 7.31 (m, 4H), 7.18 – 7.10 (m, 4H), 3.29 – 3.21 (m, 4H), 2.61 – 2.54 (m, 4H).

^{13}C NMR (101 MHz, MeOD) δ 140.79, 138.84, 134.35, 132.05, 131.53, 129.69, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 46.09, 29.26.

3-((4-(Trifluoromethoxy)benzyl)sulfonyl)-1*H*-1,2,4-triazole (123)

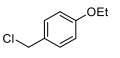


47 (345 mg, 1.25 mmol) was oxidized according General procedure A, affording the title compound as white crystalline solid (347 mg, 1.13 mmol, 90%) of sufficient purity to use in the next reaction.

^{1}H NMR (400 MHz, MeOD) δ 8.69 (s, 1H), 7.40 – 7.33 (m, 2H), 7.25 – 7.19 (m, 2H), 4.76 (s, 2H).

^{13}C NMR (101 MHz, MeOD) δ 161.54, 150.93, 147.02, 134.09, 128.00, 122.04, 121.84 (q, *J* = 258.6 Hz), 60.65.

1-(Chloromethyl)-4-ethoxybenzene (126)

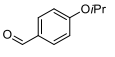


To an ice-cold solution of 4-ethoxybenzyl alcohol (**125**, 1.0 g, 6.57 mmol) in dry Et_2O with a few drops of DMF, $SOCl_2$ (2 eq, 953 µL, 13.1 mmol) was added dropwise. The mixture was allowed to warm to RT and stirred overnight. Volatiles were removed *in vacuo*, after which the residue was dissolved in DCM and washed with water and 1 M aq. Na_2CO_3. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*, yielding the title compound (965 mg, 5.66 mmol, 86%) in sufficient purity to be used as such.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.25 (m, 2H), 6.90 – 6.82 (m, 2H), 4.56 (s, 2H), 4.02 (q, *J* = 7.0 Hz, 2H), 1.41 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.17, 130.17, 129.63, 114.76, 63.62, 46.49, 14.91.

4-Isopropoxybenzaldehyde (128)



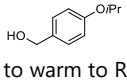
4-Hydroxybenzaldehyde (**127**, 1 eq, 2.0 g, 16.4 mmol), K_2CO_3 (1.2 eq, 2.72 g, 19.7 mmol), KI (1.01 eq, 2.75 g, 16.5 mmol) and isopropyl iodide (2.4 eq, 3.92 mL, 39.3 mmol) were dissolved in dry DMF (50 mL), heated to 75°C and stirred overnight. Volatiles were removed *in vacuo*, after which the residue was dissolved in $CHCl_3$ and washed with water. The aqueous layer was extracted with $CHCl_3$ and the combined organic layers were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Flash column chromatography (20% EtOAc in pentane) yielded the title compound (1.89 g, 11.5 mmol, 70%).

Analytical data on next page.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 7.85 – 7.77 (m, 2H), 7.01 – 6.93 (m, 2H), 4.67 (p, *J* = 6.1 Hz, 1H), 1.37 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 190.81, 163.23, 132.08, 129.56, 115.63, 70.35, 21.92.

(4-Isopropoxyphenyl)methanol (129)

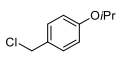


128 (1.5 g, 9.13 mmol) was dissolved in 1:1 THF:H_2O (20 mL) and cooled on ice. $NaBH_4$ (3 eq, 1.0 g, 27.4 mmol) was added portionwise, after which the mixture was allowed to warm to RT and stirred overnight. Volatiles were removed *in vacuo*, EtOAc and water were added to the residue and the layers were separated. The aqueous layer was extracted with EtOAc, after which the organic layer was washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Flash column chromatography (EtOAc in pentane) yielded the title compound (1.45 g, 8.72 mmol, 96%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.23 (m, 2H), 6.91 – 6.83 (m, 2H), 4.60 (s, 2H), 4.54 (hept, *J* = 6.1 Hz, 1H), 1.72 (bs, 1H), 1.33 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.50, 133.03, 128.75, 116.00, 70.03, 64.97, 22.09.

1-(Chloromethyl)-4-isopropoxybenzene (130)

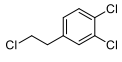


To an ice-cold solution of **129** (1.0 g, 6.02 mmol) in dry Et_2O with a few drops of DMF, $SOCl_2$ (2 eq, 873 µL, 12.0 mmol) was added dropwise. The mixture was allowed to warm to RT and stirred overnight. Volatiles were removed *in vacuo*, after which the residue was dissolved in DCM and washed with water and 1 M aq. Na_2CO_3. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*, yielding the title compound (647 mg, 3.65 mmol, 61%) in sufficient purity to be used as such.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.24 (m, 2H), 6.88 – 6.82 (m, 2H), 4.55 (s, 2H), 4.54 (hept, *J* = 6.0 Hz, 1H), 1.33 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 158.14, 130.19, 129.48, 116.00, 70.00, 46.49, 22.10.

1,2-Dichloro-4-(2-chloroethyl)benzene (132)



3,4-Dichlorophenethyl alcohol (**131**, 128 µL, 1.05 mmol) was dissolved in dry DCM (11 mL) with a few drops of DMF and cooled on ice. $SOCl_2$ (9 eq, 684 µL, 9.42 mmol) was added dropwise, after which the mixture was heated to 40°C and stirred for a week. When TLC confirmed full conversion the reaction was quenched with water and the layers were separated. The aqueous layer was extracted with $CHCl_3$, after which the combined organic layers were dried over $MgSO_4$, filtrated and concentrated *in vacuo*, affording the title compound (179 mg, 0.85 mmol, 81%), which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 2.0 Hz, 1H), 7.05 (dd, *J* = 8.2, 2.1 Hz, 1H), 3.68 (t, *J* = 7.0 Hz, 2H), 3.00 (t, *J* = 7.0 Hz, 2H).

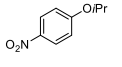
No ^{13}C NMR recorded.

1*H*-Pyrazol-4-amine (135)

4-Nitropyrazole (**134**, 1 eq, 1.60 g, 14.2 mmol) was dissolved in EtOH (13 mL), Pd/C (2 mol%, 282 mg 10%, 0.27 mmol) was added and the mixture was purged with N_2 for 30 min. The mixture was then purged with H_2 and stirred for 12 h. H_2 was removed by purging with N_2, solids by filtration over celite and volatiles *in vacuo*. This yielded the title compound as dark red crystalline solid (1.17 g, 14.1 mmol, quant.), which was used without further purification.

^{1}H NMR (400 MHz, MeOD) δ 7.22 (s, 2H).

1-Isopropoxy-4-nitrobenzene (137)

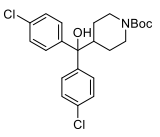


In a MW vial 4-nitrophenol (**136**, 1 eq, 1.0 g, 7.19 mmol) was dissolved in dry DMF (2.5 mL), to which then K_2CO_3 (1.5 eq, 1.5 g, 10.8 mmol) was added. To the stirring suspension then isopropyl bromide (1.5 eq, 1.0 mL, 10.8 mmol) was added carefully. The vial was sealed and heated to 120°C overnight. Upon completion all volatiles were removed *in vacuo*. The residue was dissolved in EtOAc and washed with brine, 1 M aq. NaOH and brine again, dried over $MgSO_4$, filtrated and concentrated *in vacuo*, yielding the title compound as yellowish runny oil (1.07 g, 5.89 mmol, 82%), which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 – 8.12 (m, 2H), 6.97 – 6.88 (m, 2H), 4.68 (p, *J* = 6.1 Hz, 1H), 1.39 (d, *J* = 6.1 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 163.24, 140.90, 125.88, 115.17, 70.95, 21.72.

***tert*-Butyl 4-(bis(4-chlorophenyl)(hydroxy)methyl)piperidine-1-carboxylate (139)**



121 (381 mg, 1.48 mmol) was dissolved in dry THF (16 mL) and cooled on ice. 4-chlorophenylmagnesium bromide (**120**, 6.7 eq, 10 mL 1.0 M in Et_2O, 10 mmol) was added dropwise to the cloudy mixture over the course of 15 min. The mixture became clear when the ice bath was removed after 20 min, after which it was allowed to warm to RT and stirred for 4 d, during which it became cloudy again. The reaction was cooled on ice and quenched with sat. aq. NH_4Cl. EtOAc was added and the layers were separated. The aqueous layer was extracted with EtOAc, after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Flash column chromatography (0 → 15% EtOAc in pentane) afforded the title compound as white crystalline solid (570 mg, 1.31 mmol, 88%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.33 (m, 4H), 7.32 – 7.22 (m, 4H), 4.21 – 4.01 (m, 2H), 2.75 – 2.58 (m, 2H), 2.46 (tt, *J* = 11.8, 3.0 Hz, 1H), 1.50 – 1.37 (m, 2H), 1.41 (s, 9H), 1.29 (qd, *J* = 12.5, 4.2 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 154.73, 143.97, 132.80, 128.56, 127.35, 79.64, 79.13, 77.48, 77.36, 77.16, 76.84, 44.30, 43.49 (br), 28.49, 26.38.

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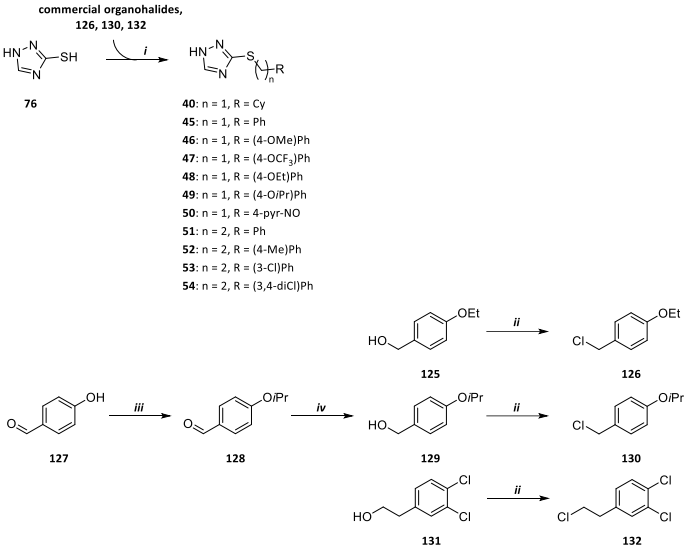
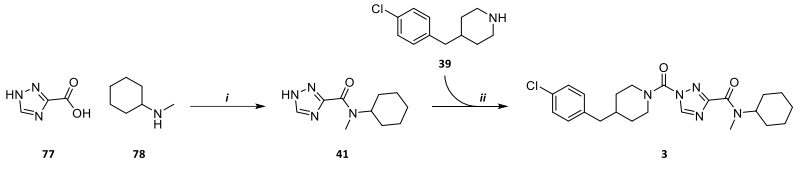
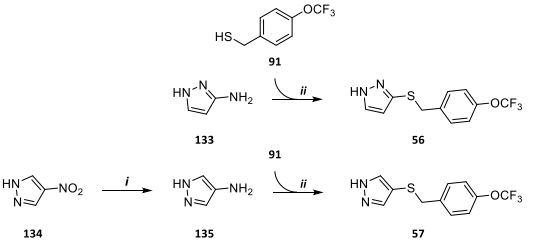
Supplementary information



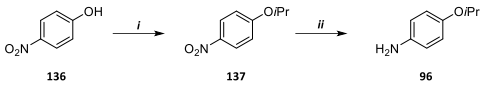
Supplementary Scheme S4.1. Synthesis of 1,2,4-triazole building blocks 40, 45–54. Reagents and conditions: *i*) K_2CO_3, DMF, 3 h RT; *ii*) $SOCl_2$, cat. DMF, Et_2O, o/n 0°C → RT; *iii*) Isopropyl iodide, KI, K_2CO_3, DMF, o/n 75°C; *iv*) $NaBH_4$, THF:H_2O, o/n 0°C → RT.



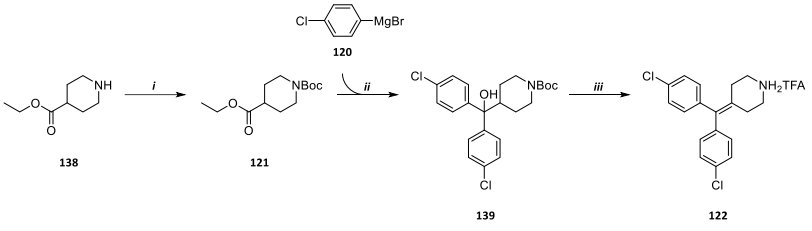
Supplementary Scheme S4.2. Synthesis of triazole amide inhibitor 3. Reagents and conditions: *i*) 1. **77**, $SOCl_2$, cat. DMF, THF, 14 h relux, then 2. **78**, DIPEA, DCM, > 110 h 0°C → RT; *ii*) 1. **39**, triphosgene, DIPEA, THF, 3 h 0°C → RT, then 2. **41**, K_2CO_3, DMF, o/n RT.



Supplementary Scheme S4.3. Synthesis of pyrazole building blocks 56 and 57. Reagents and conditions: *i*) 1 atm H_2, Pd/C, EtOH, 12 h RT; *ii*) 1. **133** or **135**, H_2SO_4, $NaNO_2$, H_2O, 30 min 0°C, then 2. **91**, NaOH, H_2O, 1.5 h 0°C.



Supplementary Scheme S4.4. Synthesis of aniline building block 96. Reagents and conditions: *i*) Isopropyl bromide, K_2CO_3, o/n 120°C; *ii*) H_2 (1 atm), Pd/C, HCl, MeOH, 5 h RT.



Supplementary Scheme S4.5. Synthesis of amine building block 122. Reagents and conditions: *i*) Et_3N, Boc_2O, DCM, 50 h 0°C → RT; *ii*) THF, 4 d 0°C → RT; *iii*) TFA, DCM, 6 d RT.

Supplementary Table S4.1. Physicochemical properties of 9, 19 and 30. Potency on PLA2G4E determined using gel-based cABPP (N ≥ 2) [a]Molecular weight (MW) and topological polar surface area (tPSA) calculated using ChemDraw Professional 16.0; [b]Partition coefficient (clogP) calculated using DataWarrior 5.0.0; [c]HAC = number of heavy atoms; [d]HBA = number of hydrogen bond acceptors; [e]HBD = number of hydrogen bond donors; [f]RB = number of rotatable bonds; [g]Lipophilic efficiency LipE = pIC_{50} – clogP; [h]Ligand efficiency LE = 1.4pIC_{50}/HAC.

	pIC_{50} ± SEM	MW (Da)[a]	tPSA (Å^2)[a]	clogP[b]	HAC[c]	HBA[d]	HBD[e]	RB[f]	LipE[g]	LE[h]
9	8.10 ± 0.02	543	92	4.98	36	8	0	8	3.12	0.32
19	7.04 ± 0.05	544	104	4.80	36	9	1	7	2.24	0.27
30	< 5.0	542	79	4.86	36	7	0	8	< 0.14	< 0.19

Supplementary Table S4.2. CB_1 and CB_2 receptor binding by 9, 19 and 30. Percentage displacement of [^{3}H]CP55,940 by 1 µM of inhibitor expressed as mean ± SEM (N = 2).

	CB_1	CB_2
9	18 ± 14%	14 ± 22%
19	31 ± 7%	28 ± 20%
30	33 ± 8%	20 ± 23%