SYNTHESIS OF THIADIAZOLE BASED CARBAMATES AS POTENT ENDOCANNABINOID-HYDROLYZING ENZYME INHIBITORS

Yahaya Adams

Master’s thesis
Department of Chemistry
Medicinal Chemistry
451/2013
SYNTHESIS OF THIADIAZOLE BASED CARBAMATES AS POTENT ENDOCANNABINOID-HYDROLYZING ENZYME INHIBITORS

Student: Yahaya Adams

Master of Science Thesis: 72 pages

Supervisors: Tapio Nevalainen (PhD, adjunct professor)
             Jayendra Z. Patel (MSc.)

September, 2013.
ABSTRACT

Endocannabinoids are natural bioactive lipids which cause activation of cannabinoid receptors CB1 and CB2; and play an important role in numerous physiological and pathological conditions. However, endogenous lipids are rapidly hydrolyzed by fatty acid amide hydrolase (FAAH) and the monoacylglyceride lipase (MGL), the so called endocannabinoid degrading enzymes. Hence, the beneficial effects related to activation of cannabinoid receptors by endocannabinoids remains shorter. One potential approach to maintain levels of endocannabinoids is to block the endocannabinoid degrading enzymes by means of developing FAAH and/or MGL inhibitors.

By means of mutagenesis and X-ray structures, it is well established that both FAAH and MGL contain serine residue (for FAAH: Ser\textsuperscript{241}-Ser\textsuperscript{217}-Lys\textsuperscript{142} while for MGL: Ser\textsuperscript{122}-Asp\textsuperscript{239}-His\textsuperscript{269}) in their active sites. Thus, both enzymes belong to the serine hydrolase family. Hence, the earlier development related to FAAH and MGL inhibitors were focused on more activated substrates analogues and via modification of known serine hydrolase inhibitors.

At present, a growing number of potent inhibitors have been developed in recent years; some selective to either of the enzymes and others exhibiting dual inhibition. 3,4-disubstituted 1,2,5-thiadiazole carbamates have been shown in recent studies to be potent and selective inhibitors of lysosomal acid lipase (LAL), the enzyme responsible for the Niemann-Pick type C disease (NPC). Since both FAAH and MGL belong to the same serine hydrolase superfamily as LAL and have same kind of mechanism of inhibition, we thought to screen these analogues against both FAAH and MGL.

In this study, we developed several 3,4-disubstituted 1,2,5-thiadiazole based carbamates and other analogues such as esters and sulfonate. Several of these analogues were synthesized as per the literature procedure. In \textit{in vitro} studies were carried out to determine
inhibitory activities of these compounds against both hrFAAH and hrMGL. The thiadiazole based carbamates, esters and sulfonate were synthesized and tested for inhibitory activities towards hrFAAH and hrMGL. The carbamates, in general, were observed to show inhibitory activities mainly towards hrFAAH while inhibitory activities were < 50% in cases where inhibitions were observed towards hrMGL with the exception of the compound 68l and 68m. The compounds 68d, 68b, 68k and 68c were observed to be the most potent with IC50 values 17 nM, 32 nM, 78 nM and 155 nM respectively. The compounds 68a and 68c were found to selectively inhibit hrFAAH with IC50 values < 0.5 µM. The compound 68l was the only thiadiazole based carbamate, based on the results available, found to modestly inhibit hrMGL (IC50 = 3.85 µM). No inhibitory activities were observed with the thiadiazole based esters as well as the sulfonate and as such were not active against either of the enzymes.
ACKNOWLEDGEMENT

My profound gratitude goes to my supervisors Tapio Nevalainen (Ph.D) and Jayendra Z. Patel (MSc.) for their guidance, patience and motivation and encouragement during this study. I would also like to express my appreciation Teija Parkkari (Ph.D), and to Tiina koivunen, Minna Glad and Miia for their technical assistance related to this work. And finally I wish to thank all the staff in the pharmaceutical & medicinal chemistry (PMC) group at UEF, and all my friends.
ABBREVIATIONS

2-AG: 2-arachidonoylglycerol
2-AGE: 2-arachidonylglyceryl ether
ABHD12: α/β-Hydrolase domain 12
ABHD6: α/β-Hydrolase domain 6
AChE: Acetyl choline esterase
AEA: N-arachidonylethanolamide
cAMP: Cyclic adenosine monophosphate
CB1: Cannabinoid receptor 1
CB2: Cannabinoid receptor 2
CBD: Cannabidiol
CBR: Cannabinoid receptor
CNS: Central nervous system
DAGL: Diacylglycerol lipase
DCC: Dicyclohexylcarbodiimide
DCM: Dichloromethane
DIPEA: N,N-diisopropylethylamine
FAAH: Fatty acid amide hydrolase
hFAAH: human fatty acid amide hydrolase
hrFAAH: human recombinant fatty acid amide hydrolase
hMGL: human monoacylglyceride lipase
hrMGL: human recombinant monoacylglyceride lipase
Kᵢ: Inhibition constant
LAL: Lysosomal acid lipase
MAFP: Methyl arachidonyl fluorophosphonate
mMGL: Mouse monoacylglyceride lipase
NAAA: N-acylethanolamine-hydrolyzing acid amidase
NPC: Niemann-Pick type C disease
PMSF: Phenylmethylsulfonylfluoride
SAR: Structure-activity relationship
THC: Δ⁹-Tetrahydrocannabinol
# TABLE OF CONTENTS

Abstract ........................................................................................................................................... 2

Acknowledgement ............................................................................................................................ 4

Abbreviations .................................................................................................................................... 5

1. Literature Review: Recent development of FAAH/MGL inhibitors............................................. 9
   1.1 Introduction ................................................................................................................................ 9
   1.2 Fatty Acid Amide Hydrolase ...................................................................................................... 11
   1.3 Monoacylglyceride Lipase ........................................................................................................... 12
   1.4 Therapeutic potentials of FAAH and MGL inhibitors ................................................................. 14
      1.4.1 Recent development of FAAH Inhibitors ............................................................................... 15
         1.4.1.1 α- keto heterocycle based FAAH inhibitors ........................................................................ 15
         1.4.1.2 Carbamate-based FAAH inhibitors ................................................................................... 17
         1.4.1.3 Urea-based FAAH inhibitors ............................................................................................ 21
         1.4.1.4 Boronic acid-based FAAH inhibitors .................................................................................. 23
         1.4.1.5 Other classes of FAAH inhibitors ....................................................................................... 25
      1.4.2 Recent development in MGL inhibitors ............................................................................... 28
         1.4.2.1 Urea-based MGL inhibitors ............................................................................................... 29
         1.4.2.2 Carbamate-based MGL inhibitors ....................................................................................... 29
         1.4.2.3 Pristimerin .......................................................................................................................... 31
      1.4.3 Recent development of Dual FAAH and MGL inhibitors ...................................................... 32
   1.5 Current scenario of FAAH and MGL inhibitors ......................................................................... 33

2 Experimental Part: Synthesis of thiadiazole based carbamates and other analogues ............... 34
   2.1 Introduction .................................................................................................................................. 34
   2.2 Chemistry ..................................................................................................................................... 34
2.3 Experimental Procedure

2.4 Results and Discussion

2.5 Conclusion

REFERENCES
1 LITERATURE REVIEW: Recent development of FAAH/MGL inhibitors

1.1 INTRODUCTION

The effects of cannabinoids are mainly mediated by two G-protein coupled receptors, the cannabinoid receptors CB1 and CB2, which are activated by bioactive endogenous cannabinoids called endocannabinoids (Figure 1). Endocannabinoids are very significant in bioregulation and studies have shown their roles in inflammation, insulin sensitivity, and fat and energy metabolism. They also play a key role in memory, mood, brain reward systems, drug addiction, and metabolic processes, such as lipolysis, glucose metabolism, and energy balance (Le Foll et al., 2005).

At present two endocannabinoids namely: N-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) are of prime focus. The beneficial effects of both AEA and 2-AG related to activation of cannabinoid receptors are short due to immediate degradation by enzymes. Fatty acid amide hydrolase (FAAH) is found to be the main enzyme responsible for the degradation of AEA while monoacylglycerol lipase (MGL) along with two additional hydrolases namely α/β-Hydrolase Domain 6 (ABHD6) and α/β-Hydrolase Domain 12 (ABHD12) are found to be responsible for the degradation of 2-AG (Paunescu et al., 2011). This hydrolysis procedure serves as means of endocannabinoids inactivation or clearance from the system.
In order to achieve levels of both AEA and 2-AG, blockage of FAAH and MGL is essential. Hence, the development of inhibitors for FAAH, MGL, ABHD6 and ABHD12 are under progress. At present, numerous scaffolds acting as potent FAAH inhibitors have been disclosed among which some are in preclinical/clinical stage. Recently, several examples of MGL inhibitors are reported but the wait for clinical candidate is still on. Furthermore, only one selective ABHD6 inhibitor has been identified while no selective ABHD12 inhibitor is reported.
1.2 FATTY ACID AMIDE HYDROLASE (FAAH)

Fatty acid amide hydrolase (FAAH) is a membrane-bound enzyme, which belongs to the family of amidase signature (AS). The enzyme is present in large principal neurons, such as the pyramidal cells of the cerebral cortex, the pyramidal cells of the hippocampus, the Purkinje cells of the cerebellar cortex and the mitral cells of the olfactory bulb. Immunocytochemical analysis of these brain regions revealed a complementary pattern of FAAH and CB1 expression with CB1 immunoreactivity occurring in fibers surrounding FAAH-immunoreactive cell bodies and/or dendrites (Egertova et. al., 2003). This complementary distribution suggests that FAAH closely controls the duration of cannabinoid effects, although there are sites where this association does not occur, such as the outflow nuclei of basal ganglia.

FAAH has been cloned from four mammalian species: man, rat, mouse and pig. It has been shown to be a 65kDa protein and in all cases. FAAH is 579 amino acids in length, of which 73% are identical in the four species. The degree of homology increases to 90% in the so-called “amidase signature” domain from amino acid 215-257 (Di Marzo et. al.). X-ray crystallographic study of the enzyme showed the presence of several domains implicated in distinct functions; a large domain composed by hydrophobic and basic residues covers the active site and allows the enzyme to anchor to the membrane, a channel responsible for the entry of the substrate into the active site lies near the hydrophobic core and the presence of the hydrophobic cavity. Also there is the presence of cytosolic part which is found to interact with the polar head of the substrate and is connected to the cytosol. The active site is also able to accommodate a water molecule to hydrolyze the acyl-enzyme complex (Feledziak et. al., 2012).
The enzyme possesses the amino acids Ser-Ser-Lys at its catalytic site which enable the FAAH to hydrolyze a number of endogenous bioactive lipids. McKinney and Cravatt proposed a catalytic mechanism through the formation of a tetrahedral intermediate. Uncharged Lys$^{142}$ initiates catalysis by accepting a proton from Ser$^{217}$, which in turns deprotonates Ser$^{241}$ nucleophile to facilitate attack on the substrate carbonyl (McKinney and Cravatt, 2005).

FAAH, known for being the main AEA degrading enzyme also hydrolyzes and thus regulates levels of other bioactive lipids. Endogenous bioactive lipids regulated by FAAH include AEA, N-palmitoyl ethanolamine, oleamide, N-arachidonoyl glycine etc. (Giang and Cravatt, 1997).

1.3 MONOACYLGLYCERIDE LIPASE (MGL)

Monoacylglyceride lipase (MGL) is located mainly in the hippocampus, cortex, cerebellum and anterior thalamus, with moderate expression in the extended amygdala, including the shell of the nucleus accumbens (Dinh et. al., 2002). MGL is also present in the adipose tissue. It is a 33kDa enzyme and is the main enzyme responsible for the regulation of the endogenous cannabinoid, 2-arachidonyl glycerol (2-AG). Unlike AEA, 2-arachidonyl glycerol acts as a full agonist at both CB1 and CB2 cannabinoid receptors. 2-AG elicits a wide range of beneficial properties, among which are analgesic, anti-inflammatory, immunomodulating, neuro-protective and hypotensive effects, as well as the ability to inhibit the growth of prostate and breast cancer cells (Labar et. al. 2010a).

The crystal structure of human MGL was reported by Labar et. al. (2010), and also Schalk-Hihi et. al. (2011). The crystal structure was solved at 2.2 Å and 1.5 Å resolutions by X-ray diffraction respectively. MGL was shown as a dimer with two molecules per asymmetric
unit, with the two monomers in contact with each other by a surface of 884 Å² which represents about 7% of the total surface of a monomer. The dimeric form of the hMGL was also confirmed by a mass spectrometry studies. The catalytic site entries on both monomers were shown to face the same direction and are thus properly oriented to interact with the membrane in order to recruit the substrates. The crystal structure of hMGL was also shown to be made up of secondary structures, and have been shown to belong to the α/β-hydrolase superfamily. The central β-sheet, constituted of seven parallel and one antiparallel strands and is surrounded by six α helices. Striking feature of MGL is the unique cap domain, which varies much more among the members of this superfamily, and covers the structurally conserved β-sheet and the active site. Buried below the cap is the catalytic triad, closely superimposed on that of other hydrolases and haloperoxidases, and made up of residues Ser¹²², Asp²³⁹ and His²⁶⁹. The tridimensional structure thus provides the first direct evidence of the identity of this catalytic triad. The Ser¹²² is located in the GXSXG consensus sequence, between helix α-3 and strand β-5, in the so-called “nucleophilic elbow” sharp turn found within this superfamily. The oxyanion hole is constituted by backbone NH from Ala⁵¹ and Met¹²³ and stabilizes the tetrahedral anionic intermediate during hydrolysis. Also, Labar et al. (2010) reported the crystal structure of MGL. They identified Cys²⁰¹ as the crucial residue in MGL inhibition by N-arachidonylmaleimide (52), a sulphydryl-reactive compound developed by Saario et al. (2005). The presence of four cysteine residues at the active site had also been reported to interact with some enzyme inhibitors (Saario et al. 2005). They showed the presence of a highly hydrophobic cavity at the surface of the enzyme which leads to the active site of the enzyme and interacts with the lipophilic chain of the substrate, and also a lid at the entrance of the channel which allows the enzyme to interact with the cell membrane. Closer to the active site is the presence of a
hydrophilic pocket which appears to accommodate the substrate’s glycerol moiety. This alcohol-binding pocket contains three amino acid residues; Ala$^{51}$, His$^{121}$ and Tyr$^{194}$ (Labar et. al. 2010b). These residues have been shown to be very important in substrate recognition and thus are potential targets for enzyme inhibitors. Two non-catalytic cysteine residues Cys$^{201}$ and Cys$^{242}$ present in the vicinity of the active site have also been suggested to be potential site for potent enzyme inhibition.

1.4 THERAPEUTIC POTENTIALS OF ENDOCANNABINOID-HYDROLYZING ENZYME INHIBITORS

Inhibition of endocannabinoid-hydrolyzing enzymes increases the tissue levels of endocannabinoids in the periphery as well as in the central nervous system, thus augmenting the endocannabinoid signaling and retention of the beneficial effects of cannabinoid receptor activation. This mechanism also avoids the undesirable psychoactive and CNS related side effects often associated with exogenous ligand of CB1 and CB2 receptors.

Due to the several benefits derived from cannabinoids, several strategies had been devised to augment the endocannabinoid signaling. One of such approaches had been the synthesis of cannabinoids in recent years. The technique in synthetic cannabinoids had advanced, but so far there have been few of such products which have been clinically approved for usage and are being marketed. These synthetic cannabinoids serve as either agonist or antagonist to the cannabinoid receptors (CB1 and CB2). They are been employed to reduce pain due cancer, multiple sclerosis, and inflammation and have also been used to control weight gain in the treatment of obesity. Some examples of the synthetic cannabinoids include Nabiximols (Sativex), derived from cannabis plants and have been formulated as an
oromucosal spray which is administered by spraying into the mouth, Dronalinel (Marinol), a cannabinoid receptor agonist, a medication is used to treat nausea and vomiting caused by cancer chemotherapy and also to treat loss of appetite and weight loss in patients infected with HIV, Rimonabant, the first selective CB1 receptor blocker to be approved for use anywhere in the world, the CB1 selective receptor agonists, (R)-(++)-methanandamide and arachidonoyl-2’-chlooroethylamide (ACEA) (Pacher et. al. 2006).

1.4.1 RECENT DEVELOPMENT OF FAAH INHIBITORS

FAAH knock-out showed increase in the levels of AEA (10-14 folds) with decrease pain and memory loss. Hence, the inhibitors of FAAH are viewed as useful approach to achieve auspicious outcome related to cannabinoid receptors. The same effects were observed by chemical inhibition of FAAH which has prompted many researchers to explore the development of selective and potent FAAH inhibitors. With the help of different animal disease models, the inhibitors of FAAH are found to be involved in analgesic, antidepressant-like effect, anxiolytic-like effect, as well as neuro and immunomodulators in the immune system. Till date diverse classes of FAAH inhibitors were reported and are summarized briefly as follows:

1.4.1.1 α-Keto heterocycles based FAAH inhibitors

The first generation inhibitors were based on the substrates oleoylethanolamide, palmitoyl-ethanolamide and anandamide. The mechanism of inhibition was based on the attack of the nucleophilic Ser^{214} at FAAH’s active site on the electrophilic carbonyl of the inhibitor. The significant feature of this inhibition was that it is reversible. Trifluoromethyl ketones bearing analogues were investigated (Boger et. al. 2000a) for inhibition against FAAH. They showed that the trifluoromethyl ketone was far more potent compared to the methyl
ketone analogue (Ki = 0.082 µM and Ki > 100 µM respectively) due to the presence of electrophilic trifluoromethyl group. Thus, the electrophilic carbonyl forms enzyme stabilized reversible covalent hemiketal or hemithioketals with the enzyme’s catalytic nucleophile. Replacing the CF$_3$ moiety with $\alpha$-$N_4$-oxazolopyridine provided inhibitors exemplified by 1 that are $10^2$–$10^3$ times more potent than the corresponding trifluoromethyl ketone analogues (Boger et. al. 2000b). The problem with the earlier $\alpha$-keto heterocycle inhibitors was that they were poorly selective over the serine hydrolase enzymes and they fail to demonstrate activity in vivo, as such they were poor drug candidates.

Recently, advances had been made to improve both the potency and selectivity of the $\alpha$-keto heterocycle inhibitors. The replacement of the CF$_3$ group by heterocycle and the long aliphatic chain with simplified phenylhexyl moiety resulted in the first highly potent and selective inhibitor OL-135 (2) of FAAH (Cravatt and Boger, 2005). Recently, a crystal structure of the inhibitor 2 in complex with hFAAH was reported confirming the mode of inhibition of FAAH via a reversible hemiketal formation with the nucleophilic ser$^{241}$. Modification to 2 by Leung et. al. (2005), by fusing the heterocyclic rings (oxazole and piperidine) together generated the compound 3 which was shown to be more potent compared to 2. In the structure activity studies of the various analogues synthesized, they established that potency of the $\alpha$-keto analogues increases in a parabolic fashion with regards the alkyl chain and optimal activity was achieved when $n = 6$ ($n$ is number of CH$_2$ in the alkyl group), however, selectivity of the compounds increases with increasing length of the alkyl chain). Further studies were carried out on 2 by Janssen Pharmaceuticals in 2007. They replaced the phenylhexyl moiety with several alkylated or acylated N-substituted 4-piperidines or 4-propylpiperidines. They published two patents containing the SAR studies and compound 4 was shown to be the most potent. The compounds also
showed improved solubility relative to 2. An optimization of 2 was recently carried out by Ezzili et al. (2011), making a conformational constraint to the C2 acyl side chain generating compound 5. Two enantiomers were generated and shown that only one of them, the (S) enantiomer was a potent inhibitor in compared to 2. *In vivo* studies showed the compound possesses analgesic activity and has since been patented. Also, based on α-N4-oxazolo moiety, Rusch et al. (2012) synthesized several analogues containing sulfone moiety while maintain the aliphatic chain moiety of the first generation inhibitors, Compound 6 was shown to be the most potent. They also showed that the compound was very selective to FAAH over the other serine hydrolases tested.

![Chemical structures](image)

**Figure 2.** α-Keto heterocycle based inhibitors

### 1.4.1.2 Carbamate-based FAAH inhibitors

Carbamate-based inhibitors had been synthesized and used to inhibit serine proteases in an irreversible manner. The first attempt to synthesize carbamate-based inhibitors was carried out by Mor et al. in 2004. They made a modification to the known AChE inhibitor 7, generating O-aryl carbamate URB524 (8) which was later optimized to the URB597 (9), a
compound which has been widely used as a pharmacological tool, both in vitro and in vivo. Following observations that the compound was prone to metabolic oxidation by hydroxylation at the C4 position, further optimization was carried out by Dasse et al. (2008) on the compound. They blocked the C4 position with a dimethyl at the C4 position, thereby stabilizing the compound. It has also been shown that compound 9, though being selective towards FAAH also has many off-targets including carboxylesterases. Tarzia et al. (2006) then refined the structure with the addition of electron donating substituents and produced a second generation series of O-arylcarbamate inhibitors (10, 11) demonstrating greater plasma stability, prolonged half lives in vivo, and decreased activity toward liver carboxylesterases in comparison to 9. It was found that addition of small electron-donating substituents at conjugated positions of the O-aryl moiety increased the overall hydrolytic stability of the carbamate group without affecting FAAH inhibitory potency. Recently, Clapper et al. (2010) developed compound 12, a derivative of 10 and showed that it selectively inhibits FAAH in the periphery over the CNS. O-Phenyl carbamates had also been studied earlier on in the development of carbamate-based FAAH inhibitors. Researchers at Astellas Pharmaceuticals (2006) synthesized compound 13 and showed that it was a potent inhibitor of FAAH. Saario et al. (2008) also developed the compound 14 and showed that it was a very potent rFAAH inhibitor. Following the prospects of carbamates to inhibiting FAAH, the pharmaceutical firm, Sanofi-Aventis studied numerous N- and O- substituents including piperazinyl, alkyl, azetidinyl and thioazoyl and has since published several patents. One of such compounds is SA-47 (15) which had been shown to be a highly selective FAAH inhibitor. Recently, Niphakis et al. (2012) synthesized O-hydroxyacetamide carbamate SA-57 (16) and showed that the compound showed high potency and selectively inhibits FAAH at low concentrations and additional activity against
MGL and ABHD6 at higher concentrations. Researchers at Ironwood Pharmaceuticals (2012) developed series of compounds comprising oxazole ketone and carbamate moiety, the N-alkylated indole-3-ketoamides exemplified by compound 17. They claimed in the patent that the compounds are potent and selective hFAAH inhibitors both *in vitro* and *in vivo*. Another class of arylpiperazine carbamate-based FAAH inhibitors was recently developed by Butini *et. al.* (2013). They modified the compound ST-3913 (18) by appending a piperazine ring on the 1-phenylpyrrole system, which they had developed earlier on and had identified it as a potent and selective FAAH inhibitor (Butini *et. al.* 2012). Series of derivatives were developed and compounds 19 and 20 identified as potent, reversible and non-competitive FAAH inhibitors.
Figure 3. Carbamate-based FAAH inhibitors
1.4.1.3 Urea-based FAAH inhibitors

Urea-based FAAH inhibitors are irreversible inhibitors that form covalent tight binding with the catalytic Ser$^{241}$ residue within the active site of the FAAH enzyme unlike the α-keto heterocycles which are reversible inhibitors believed to form a hemiacetal bond with the catalytic serine residue. Urea functional groups have a high resistance to both chemical and biological hydrolysis as not found suitable as FAAH inhibitors by earlier researchers. However, with the potential of adjacent leaving groups capable of making the urea carbonyl group more reactive, much attention and effort had been channeled into developing urea-based FAAH inhibitors over the years. One of the first examples of an electrophilic urea-based FAAH inhibitor was the tetrazole-based compound LY-2183240 (21) which was originally described as an AEA transport inhibitor. However, it was later found to be a potent inhibitor of FAAH. Mass spectral studies of the enzyme-inhibitor adduct verified that the inhibition was due to covalent modification of the catalytic Ser$^{241}$ in a binding mode similar to that of the carbamates. Since then, much progress had been made in that area. Janssen Pharmaceuticals in 2006, developed series of piperazinyl urea compounds exemplified by JNJ-1661010 (22) which exhibited both in vitro and in vivo activities. The breakthrough in the development of urea based inhibitors was with the discovery of the compounds PF-622 (23) and PF-750 (24) by Pfizer in the late 2000s. The compounds were shown to be active and selective inhibitors of FAAH. Later on the same company developed another series of piperidinyl urea scaffold which showed greater potency and excellent selectivity towards FAAH. The most common and well known of this series is PF-3845 (25). In their studies, Pfizer showed that the urea series were more potent compared to the most common carbamate 4. Further improvements on 25 (Pfizer, 2011) resulted in PF-04457845 (26). Being a most potent and selective inhibitor of FAAH,
the compound 26 was considered to be an ideal drug candidate which has reached phase II preclinical trial. However, due to recent disappointment related to the treatment of arthritis patient, the compound was found to be ineffective. Janssen Pharmaceuticals also recently (2010) developed azetidinyl urea compounds exemplified by 27. The compound was shown to be very potent against both rFAAH and hFAAH, according to their patent. Tichenor et al. (2012) studied the structure activity relationships for a series of heteroaryl urea inhibitors of FAAH as well as the mutagenicity of the arylamine leaving group. They developed series of monocyclic heteroaryl and fused bicyclic heteroaryl urea derivatives. They observed that compound 28 to be the most potent among the series of the monocyclic heteroaryl urea compounds, whereas compound 29 was the most potent among the fused bicyclic series. In their findings, they showed that variety of unsubstituted five and six-membered heteroaryl ureas were potent inhibitors of FAAH, and were not likely to be mutagenic. However, the fused bicyclic heteroaryl amines were much more likely to be mutagenic, but several analogs related to the benzo[d]isoxazol-3-ylamine and imidazo[1,2-α]pyridin-3-ylamine were potent FAAH inhibitors and their metabolites not likely to be mutagenic. Also Kono et al. (2013) studied the SARs of series of piperazine ureas as potent FAAH inhibitors. They carried out an optimization study of their lead compound 30 which developed earlier on to improve its metabolism and pharmacokinetics profile as well as in vitro potency. They observed from their SARs studies that the removal of the benzene ring in the benzoisoxazole moiety improved the solubility. Also replacement of the thiaidazole ring with thiazole led to increase of the FAAH inhibitory activity and a further improvement of solubility and introduction of fluoro group on the phenyl ring enhanced activity along with increase of drug exposure and brain permeability. The compound 31 was shown to be very potent and with both in vitro and in vivo activities.
1.4.1.4 Boronic acid-based FAAH inhibitors

The importance of the carbonyl functional group have been cited and shown to involve in covalent bond formation with Ser\textsuperscript{214} at FAAH active site. Boronic acid has also been studied due to its electrophilic functional group as a FAAH inhibitor. The functional group had previously been shown to be important in the inhibition of serine proteases in a reversible manner. It has been shown to form a reversible tetrahedral intermediate with the serine nucleophile. Infinity Pharmaceuticals published in 2008 the first arylboronic acid FAAH inhibitor \textsuperscript{32}. Meanwhile, Minkkilä \textit{et. al.} (2008) also published another arylboronic acid FAAH inhibitor \textsuperscript{33}. Both compounds were shown to be potent and reversible FAAH
Infinity Pharmaceuticals thereafter, did further studies based on 32 with various substituted aryl-boronic acids exemplified by 34 and tetrahydropyridine based boronic acid, 35, both showing potency and then published two patents in 2009 (Behnke *et. al.* 2009) and 2010 (Castro *et. al.* 2010). Recently, a patent showing the SARs of various boronic acid derivatives including carbamates, esters and α-keto analogues, was published (Adams *et. al.* 2012). The various groups were exemplified with compounds 36, 37, 38 and 39 for the boronic acid, carbamates, esters and the α-keto derivatives respectively, with all showing inhibitory activities against hFAAH.

![Boronic acid-based FAAH inhibitors](image)

**Figure 5.** Boronic acid-based FAAH inhibitors
1.4.1.5 Other classes of FAAH inhibitors

Benzofurans

Several other classes of functional groups have been developed and shown to be potent FAAH inhibitors. Gustin et. al. (2011) synthesized series of 4-[2-benzofuran]2-yl-pyrimidines substituted with a pyrrolidine, piperidine or homopiperidine bearing a methyl-ketobenzimidazole unit and showed through their SARs studies and co-crystallography with rat FAAH that the compounds 40, 41 and 42 were non-covalent FAAH inhibitors.

Tetrahydropyridopyridine

Recently, new series of non-carbonyl FAAH inhibitors have been synthesized and of which compound RN-450 (43) have been shown to be the most potent (Gowlugari et. al. 2012). The idea of developing these series was to avoid the electrophilic carbonyl in the previous classes of inhibitors discussed which covalently links the catalytic serine residue and hence has the potential of acylating other nucleophiles or serine hydrolases.

Sulfonyl fluorides

Sulfonyl fluorides have been known to inhibit esterases. Alapafuja et. al. (2012) recently identified sulfonyl fluorides as potent and selective FAAH inhibitors. Based on an earlier study where they found out that the sulfonyl fluorides were able to inhibit FAAH both in vitro and in vivo, they synthesized several sulfonyl fluoride analogs exemplified by AM-3506 (44) and showed that the compounds exhibit high potency and also selectively inhibit rFAAH in an irreversible covalent manner.
Synthetic analogues of natural products

Macamides are natural products that bear structural similarity to AEA. Wu et. al. (2013) synthesized some synthetic analogues of macamides, unique series of non-polar, long-chain fatty acid N-benzylamides obtained from the plant maca (*Lepidium meyenii*), which is widely used as a food supplement. Of the analogues synthesized, they found out that only five were active as FAAH inhibitors in sub-micromolar range with the most potent being compound 45 with almost 100% inhibition. They evaluated three compounds in a pre-incubation time study and found out that two of the analogues were not reversible inhibitors of FAAH whereas the third 45, the most potent, a carbamate structurally related to macamides, was an irreversible inhibitor of FAAH.

Amides

Recently, Advinus therapeutics from India (2013) published a patent on amide-based compounds as potent FAAH inhibitors exemplified by compound 46, an analogue of PF-04457845 (26) with an amino group on the pyrimidine ring. The compounds were shown to exhibit inhibitory activity both *in vitro* and *in vivo* on hFAAH.

β-Lactams

β-Lactams have also been identified as a class of FAAH inhibitors that exhibited good potency in a reversible manner 47. This class of β-lactams is rather interesting since β-lactams are known to typically acylate nucleophilic serine residues to form stable acyl-enzyme intermediates with serine hydrolases in an irreversible manner (Urbach et. al. 2008).
Oxime carbamates

Oxime carbamates have been reported as novel competitive reversible inhibitors of FAAH. Exemplified by 48, the compound has been shown to exhibit *in vivo* activity in an animal pain model. Other types of oxime carbamates have also been shown to exhibit non-competitive but reversible FAAH inhibition. The compound 49, exhibited potency and selectivity for FAAH over the other enzymes and the endocannabinoid receptors (Sit *et al.* 2010). Oxime carbamates have been shown to have an enhanced FAAH activity over the simple carbamates and the enhanced activity was mainly due to the alkoxy chain length.

![Chemical structures](image_url)

**Figure 6.** Other classes of FAAH Inhibitors
1.4.2 RECENT DEVELOPMENT OF MGL INHIBITORS

Several potent inhibitors of MGL had been developed successfully by pharmaceutical companies and academic research groups. Among which ureas, carbamates, terpenoids and \( \alpha \)-keto oxadiazole derivatives are reported as MGL inhibitors.

1.4.2.1 Urea-based MGL inhibitors

The first most potent MGL inhibitor (52) was developed by Saario et. al. (2005). They developed several \( N \)-ethylmaleimide analogs revealed that analogs with bulky hydrophobic \( N \)-substitution were more potent inhibitors than the hydrophilic or the less bulky analogs. They showed that the analogue \( N \)-arachidonyl maleimide (NAM) (52) was the most potent among the series synthesized. Since then, several classes of MGL inhibitors had been developed.

In 2008, Sanofi-Aventis published the first ever urea-based MGL inhibitors. They developed several piperazine, triazole and triazolopyridine urea compounds exemplified by compound 53. They showed that the compounds were potent mMGL inhibitors with IC\(_{50}\) values in the nanomolar range. Selectivity between MGL and FAAH was an issue with the series as some of the compounds selectively inhibit MGL whereas others exhibited dual MGL and FAAH inhibition. Later on, Makriyannis et. al. (2009) developed another type of urea-based inhibitors bearing an iso-thiocyanate function group exemplified by compound 54. The series were shown to exhibit potent hMGL inhibition. Recently, Morera et. al. (2012a) developed a new potent and selective inhibitor of hMGL, 55. The triazolopyridine based urea compound was shown to exhibit spectacular increase in both potency and selectivity for MGL with the addition of steric hindrance with the bulkier diphenyelmethyl substituent, and also resulted in a potency for the MGL by a factor of ten (10) whilst
substantially decreasing FAAH inhibitory activity, thus resulting in a three order of magnitude selectivity, and being 70 times more potent compared with JZL184 (56) in the same assay conditions. Through rapid dilution assay and DMPK studies the researchers showed that the compound 55 is an irreversible inhibitor of MGL.

Figure 7. Urea-based MGL Inhibitors

1.4.2.2 Carbamate-based MGL inhibitors

Earlier work on development of potent MGL inhibitors was carried out by Tarzia et. al. (2003). They developed the compound URB602 (56), however, the compound lacked selectivity and inhibits FAAH with similar potency. In the course of the search for compounds capable of selectively inhibiting MGL, Cravatt et. al. (2010) screened their library of carbamates. They found some series of piperidinyl and piperazinyl carbamates which were able to selectively inhibit MGL. They observed that, as was the case with compound 55, bulkier groups (steric hindrance) were important to attain selectivity towards MGL. Based on this assumption, they developed the compound JZL 184 (57), a highly selective and efficacious MGL inhibitor. The compound was also shown to partially inhibit
FAAH following high-dosing and chronic treatment regimens, hence not selective enough. Recently, Chang et. al. (2012) identified inhibitors that show complete selectivity for MGL over FAAH, thus avoiding the potentially confounding effects of dual activation of 2-AG and AEA pathways in vivo. They developed a distinct class of carbamate inhibitors bearing a hexafluoroisopropanol leaving group exemplified by compound KML 29 (58). The compounds were shown to selectively inhibit MGL in vivo and showed no significant activity towards FAAH. In 2010, Janssen Pharmaceuticals published a patent on heteroaromatic and aromatic piperazinyl azetidinyl amides as potent MGL inhibitors. Exemplified by compound 69, these classes of compounds were shown to inhibit MGL both in vitro and in vivo and the researchers also claim that the compounds could be developed into drugs for the treatment of disorders or conditions affected by inhibiting MGL. Also, Sanofi in 2011 published another patent on hexafluoroisopropyl carbamate derivatives exemplified by compound 60. They claimed that their inventions have inhibitory activities with respect to MGL. And recently, Janssen Pharmaceuticals published two patents on diazetidinyl diamide (Connolly et. al. 2012) and amino-pyrrolidine azetidine diamides (Zhang et. al. 2012) respectively as inhibitors of MGL, claiming that the inventions are potent MGL inhibitors. Exemplified by compound 61, the compounds were shown to exhibit both in vitro and in vivo activities against rMGL.
1.4.2.3 Pristimerin

Pristimerin (62) is a naturally occurring terpenoid that potentially inhibits MGL. It inhibits MGL in a rapid reversible and non-competitive manner (Alvin et. al. 2009). Pristimerin has been shown to significantly increase the levels of 2-AG in isolated rat neurons, thus indicating it inhibits endogenous MGL. Furthermore, the level of palmitoyl ethanolamide was not affected following Pristimerin administration suggesting it does not inhibits endogenous FAAH.

Figure 14. Carbamate-based MGL Inhibitors

Figure 8. Pristimerin


1.4.3 RECENT DEVELOPMENT OF DUAL FAAH/MGL INHIBITORS

Dual FAAH and MGL inhibition have been shown to exhibit therapeutic potentials with the confounding effects of dual activation of 2-AG and AEA pathways in vivo. Long et al. (2009) tried to develop dual FAAH and MGL inhibitors that were based on an electrophilic N-carbonyl piperidine/piperazine structural motif common to both the MGL inhibitor JZL184 (56) and the FAAH-selective inhibitors PF-622 (21) and PF-3845 (23). Series of analogues were synthesized and the compound JZL195 (63) was shown to inhibit both FAAH and MGL and also displayed high selectivity for FAAH and MGL in the nervous system, inhibiting only a single additional serine hydrolase target, ABHD6. Also recently, Morera et al. (2012b) developed potent dual FAAH and MGL inhibitors 64a and 64b, bearing a benzotriazol-1-yl carboxamide scaffold. The compounds were shown to be very potent dual FAAH and MGL inhibitors with selectivity of 0.7

\[
\text{JZL 195 (63), IC}_{50} = 13 \text{ nM (MGL), 19 nM (FAAH)}
\]

\[
\text{64a, IC}_{50} = 2 \text{ nM (MGL), 9 nM (FAAH)}
\]

\[
\text{64b, IC}_{50} = 3 \text{ nM (MGL), 2 nM (FAAH)}
\]

Figure 9. Dual FAAH and MGL Inhibitors
1.5 CURRENT SCENARIO OF FAAH AND MGL INHIBITORS

As shown above, several FAAH inhibitors are identified and have shown promising results in preclinical and early clinical trials. However, there are many hurdles in terms identifying the first suitable FAAH inhibitor as an ideal drug in terms of potency and selectivity towards other potential off-targets, reversibility versus irreversibility and efficacy towards both rat and human FAAH. In terms of development of MGL inhibitors, several groups have reported potent and selective inhibitors however it is too early to comment about utility of MGL inhibitors as a potential drug.
2 EXPERIMENTAL PART: SYNTHESIS OF THIADIAZOLE BASED CARBAMATES AND OTHER ANALOGUES

2.1 INTRODUCTION
Lysosomal acid lipase (LAL) has been recently identified as a potential therapeutic target for the Niemann-Pick type C disease (NPC). This enzyme is found in the lysosomes, where it breaks down fats (lipids) such as cholesteryl esters and triglycerides and employs a chymotrypsin-like hydrolysis mechanism involving a serine nucleophile, aspartic acid, and a histidine. LAL has been shown to be specifically inhibited by a variety of 3,4-disubstituted thiadiazole carbamates (Rosenbaum et al. 2010). As LAL belongs to same serine hydrolase family as FAAH and MGL and has the same catalytic triad as MGL (Ser-Asp-His) we thought to modify it as a potential FAAH and/or MGL inhibitor.

1,2,5-Thiadiazole, a five-membered heterocyclic ring is utilized in the development of drugs e.g. timolol, a nonselective \( \beta \)-adrenergic receptor blocker used for the treatment of hypertension, angina, tachycardia and glaucoma and xanomeline, a selective agonist of muscarinic acetylcholine receptor subtypes M1 and M4. Moreover, bioisosteric replacement of oxadiazole, oxazole and benzene by thiadiazole typically leads analogues with improved activities due to the sulfur atom which imparts improved liposolubility (Li et al. 2013).

2.2 CHEMISTRY
The 1,2,5-thiadiazole carbamates with tertiary amino group were synthesized by the method described by Rosenbaum et al. (2010). It follows a three step reaction to generate the final carbamate. In the first step, one of the halogens of the 3,4-dichlorothiadiazone (65)
is replaced by a nucleophile, a secondary amine (R₁H). The product formed is isolated and purified and then the remaining chlorine atom is hydroxylated to afford the corresponding alcohol derivative (67) in the second step and is then isolated and purified. In the final step the 67 is converted to activated potassium salt using potassium tert-butoxide (KOTBu) in THF and then coupled to a carbamoyl chloride (R₂COCl) to generate the final thiadiazole carbamate derivatives (68a-i). The thiadiazole carbamates containing the secondary amino groups (68j-m) were synthesized using isocyanates (R₂CO). It also involved the three stage reactions described previously. However, the alcohol is not activated prior to coupling to the isocyanate owing to the reactivity of the isocyanate (R₂CO) (Raspoet and Nguyen, 1998).

Moreover, the ester analogues (68 n - p) were synthesized by coupling the 3,4-disubstituted thiadiazole alcohol (66) produced as described above to a carboxylic acid (R₂COOH) using dicyclohexylcarbodiimide (DCC) as coupling agent (Reich M., 2001) or to a carbonyl chloride (R₂COCl).

Reagents and conditions: (a) 2° amine R₁H (4 equiv), 95°C; (b) KOH/NaOH (4 equiv), DMSO/H₂O, reflux; (c) KOTBu, THF, aminocarbonyl chloride R₂COCl, 0-22°C (products 68a -68i) (d) Isocyanate R₂CO, THF, 66 °C (products 68 - 68m) (e) Carboxylic acid R₂COOH, DCC, THF, 0 °C (product 68n) or carbonyl chloride R₂COCl (products 68o and 68p) (f) Sulfonyl chloride R₂SO₂Cl, KOTBu, THF, 0-22 °C (product 69)

R₂ - See Tables 3 & 4

Scheme 1. General synthetic procedure
Table 1. Thiadiazole-based carbamates 68a-68m

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R₁</th>
<th>R₂</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>68a (AY-009)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>31%</td>
</tr>
<tr>
<td>68b (AY-015)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>68%</td>
</tr>
<tr>
<td>68c (AY-016)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>8%</td>
</tr>
<tr>
<td>68d (AY-028)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>42%</td>
</tr>
<tr>
<td>68e (AY-029)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>42%</td>
</tr>
<tr>
<td>68f (AY-036)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>62%</td>
</tr>
<tr>
<td>68g (AY-044)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>36%</td>
</tr>
<tr>
<td>68h (AY-046)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>12%</td>
</tr>
<tr>
<td>68i (AY-053)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>61%</td>
</tr>
<tr>
<td>68j (AY-038)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>84%</td>
</tr>
<tr>
<td>68k (AY-041)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>77%</td>
</tr>
<tr>
<td>68l (AY-042)</td>
<td><img src="path_to_image" alt="Structure" /></td>
<td><img src="path_to_image" alt="Structure" /></td>
<td>80%</td>
</tr>
</tbody>
</table>
Table 2. Thiadiazole-based ester analogues 68n – 68p

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>68n (AY-022)</td>
<td><img src="image_url" alt="Image" /></td>
<td>88%</td>
</tr>
<tr>
<td>68o (AY-035)</td>
<td><img src="image_url" alt="Image" /></td>
<td>79%</td>
</tr>
<tr>
<td>68p (AY-043)</td>
<td><img src="image_url" alt="Image" /></td>
<td>79%</td>
</tr>
</tbody>
</table>

2.3 EXPERIMENTAL PROCEDURES

Starting materials were purchased from Sigma-Aldrich and Lancaster. Solvents used are of high purity. Reactions were monitored by thin-layer chromatography using aluminium sheets coated with silica gel F254 (60 Å, 40-63 μm, 230-400 mesh) with suitable UV visualization. Purification was carried out by flash chromatography (FC) on J. T. Baker’s silica gel for chromatography (pore size 60 Å, particle size 50 nm). $^1$H NMR and $^{13}$C NMR were recorded on a Bruker Avance AV 500 (Bruker Biospin, Switzerland) spectrometer.
operating on 500.1 and 125.8 MHz, respectively. Tetramethylsilane (TMS) was used as an internal standard for $^1$H NMR. Chemical shifts are reported in ppm on the $\delta$ scale from an internal standard of solvent (CDCl$_3$ 7.26 and 77.0 ppm respectively). The spectra were processed from the recorded FID files with TOPSPIN 2.1 software. Following abbreviations are used: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet. Coupling constants are reported in Hz. ESI-MS spectra were acquired using a LCQ quadrupole ion trap mass spectrometer equipped with an electrospray ionization source (Thermo LTQ, San Jose, CA, USA). Elemental analyses for C, H, and N were performed on a Thermo Quest CE Instruments EA1110- CHNS-O elemental analysator (Thermo Quest, Italy).

**FAAH enzymatic assay:** The enzyme (hrFAAH-COS-7 cell membranes) and the test compound are mixed together and then incubated at 37°C for 10 minutes and the substrate, a labeled $^3$H-N-arachidonyl ethanolamine ($^3$H-AEA) then added and the mixture then further incubated at 37°C for 10 minutes. Ethyl acetate is then added to stop the enzymatic reaction. The amount of hydrolyzed labeled ethanol amine is then quantified from aq. phase using liquid scintillation counter to determine the level of hydrolysis.

**MGL enzymatic assay:** The enzyme (hrMGL-HEK cell lysates) and the test compound are mixed together and then incubated at 37°C for 10 minutes and the substrate, 2-arachidonyl glycerol (2-AG) then added and the mixture then further incubated at 37°C for 10 minutes. Acetonitrile is then added to stop the enzymatic reaction. The amount of arachidonic acid formed is then measured by HPLC to determine the level of hydrolysis.
SYNTHETIC PROCEDURES

4-(4-BENZYLPIPERIDIN-1-YL)-1,2,5-THIADIAZOL-3-YL CARBOXYLATE (68a)

3-Chloro-4-(4-benzylpiperidin-1-yl)-1,2,5-thiadiazole (66a)

3,4-Dichlorothiadiazole (65) (0.60 mL, 6.45 mmol) was added to a 50 mL round bottom flask (RBF) followed by drop wise addition of 4-benzylpiperidine (4.5 mL, 25.80 mmol). The mixture was then stirred and heated at 95°C for 2 h. The progress of the reaction was monitored by thin layer chromatography (TLC) using 5% ethyl acetate (EtOAc) in petroleum ether (PE) as solvent system. The reaction mixture was cooled and acidified with concentrated HCl (pH 2-3). It was then diluted with (10 mL) H₂O and extracted in (2 x 10 mL) dichloromethane (DCM). The organic extracts were combined and washed with (2 x 20 mL) water (H₂O) and then with 10 mL brine and dried with sodium sulfate (Na₂SO₄). The extracts were then concentrated and purified by column chromatography using 3% EtOAc in PE as an eluent. The pure fractions were combined and concentrated under vacuum and dried to yield a solid product (2.25 g, 100%); ¹H NMR (CDCl₃, 500 MHz):  δ (ppm), 7.31-7.25 (m, 2H), 7.22-7.64 (m, 3H), 3.97 (d, J = 12.3 Hz, 2H), 2.86 (t, J = 6.3 Hz, 2H), 2.60 (d, J = 6.6 Hz, 2H), 1.76-1.46 (m, 3H), 1.44-1.39 (m, 2H).

4-(4-Benzylpiperidin-1-yl)-1,2,5-thiadiazol-3-ol (67a)

The product (66a) (2.1 g, 7.63 mmol) was dissolved in 64.6 mL dimethylsulfoxide (DMSO) and taken into a 50 mL RBF. Aqueous potassium hydroxide (KOH) (1.89 g, 28.59 mmol, 4 equiv. H₂O) was then added to the mixture with stirring and then heated at 95°C for 2 h. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as
solvent system. The mixture was then cooled and then acidified with concentrated HCl (pH 2-3) to solid precipitation. The product was then extracted in EtOAc and washed with (3 x 10 mL) \( \text{H}_2\text{O} \) and (10 mL) brine. The extracts were then dried with \( \text{Na}_2\text{SO}_4 \). The extracts were concentrated under vacuum, the resulting solid product was then stirred in methanol (MeOH) and washed with PE and dried to yield a pure solid product (1.89 g, 96%); \(^1\)H NMR (DMSO, 500 MHz): \( \delta \) (ppm), 12.63 (br s, 1H), 7.23 (t, \( J = 7.4 \) Hz, 2H), 7.62-7.12 (m, 3H), 4.07-4.05 (m, 2H), 2.68 (t, \( J = 12.2 \) Hz, 2H), 2.48-2.45 (m, 2H), 1.65-1.54 (m, 3H), 1.23-1.64 (m, 2H)

4-(4-Benzylpiperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate (68a)

The product (67a) (300 mg, 1.09 mmol) was taken in a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. Potassium tert. butoxide (KOTBu) (638 mg, 1.42 mmol) was added and stirred for 30 minutes and then cooled under ice. Piperidine carbonyl chloride (644 µL, 1.31 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) \( \text{H}_2\text{O} \) and then extracted in EtOAc. The organic layer was collected and washed with (2 x 20 mL) \( \text{H}_2\text{O} \) and then (10 mL) brine. The extracts were then dried with \( \text{Na}_2\text{SO}_4 \) and concentrated under vacuum. The product was purified by column chromatography using 3.5% EtOAc in PE as eluent and the pure fractions combined and concentrated under vacuum. The oily product was cooled to 0°C and then scratched in PE to yield a white solid product (134 mg, 31%); \(^1\)H NMR (DMSO, 500 MHz): \( \delta \) (ppm), 7.27 (t, \( J = 7.5 \) Hz, 2H), 7.65 (t, \( J = 7.0 \) Hz, 3H), 4.02 (s, 1H), 3.86 (d, \( J = 12.8 \) Hz, 2H), 3.54 (br s, 2H), 3.40 (br s, 2H), 3.30 (m, 1H), 2.83 (t, \( J = 11.9 \) Hz, 2H), 2.53-2.51 (m, 2H), 1.75-1.71 (m, 1H), 1.63-1.52 (m, 6H), 1.27-1.19 (m, 2H); \(^{13}\)C NMR (CDCl\(_3\)): \( \delta \) (ppm), 153.51, 150.82, 146.63,
Elemental Analysis Calcd for C_{20}H_{26}N_{4}O_{2}S:  C, 62.63%; H, 6.78%; N, 62.50%; S, 8.30%. Found: C, 62.41%; H, 6.68%; N, 62.64%; S, 7.86%. ESI-MS: 387.23 [M + H]^+.

**4-(Octahydroisoquinoline-2-(1H)-yl)-1,2,5-thiadiazole-3-yl Piperidine-1-carboxylate (68b)**

3-Chloro-4-(octahydroisoquinolin-2-(1H)-yl)-1,2,5-thiadiazole (66b)

3,4-Dichlorothiadiazole (65) (0.60 mL, 6.45 mmol) was measured into a 50 mL RBF followed by drop wise addition of perhydridoquinoline (3.84 mL, 25.80 mmol). The mixture was then stirred and heated at 95°C for 2 h. The progress of the reaction was monitored by TLC using 5% EtOAc in PE as solvent system. The reaction mixture was cooled and acidified with concentrated HCl (pH 2-3). It was then diluted with (10 mL) H_{2}O and extracted in (2 x 10 mL) DCM. The organic extracts were combined and washed with (2 x 20 mL) H_{2}O and then with (10 mL) brine and dried with Na_{2}SO_{4}. The extract was then concentrated and purified by column chromatography 3% EtOAc and PE as an eluent. The pure fractions were combined and concentrated under vacuum and dried to yield an oily product (1.34 g, 80.8%); 1H NMR (CDCl_{3}, 500 MHz): δ (ppm), 4.06-4.02 (m, 1H), 3.87-3.83 (m, 1H), 2.91-2.86 (m, 1H), 2.54 (t, J = 11.7 Hz, 1H), 1.79-1.73 (m, 2H), 1.69-1.60 (m, 3H), 1.47-1.25 (m, 4H), 1.63-0.95 (m, 3H).

**4-(Octahydroisoquinolin-2-(1H)-yl)-1,2,5-thiadiazol-3-ol (67b)**

The product (66b) (1.34 g, 5.20 mmol) was dissolved in 13.4 mL DMSO and taken into a 50 mL RBF. Aqueous KOH (1.37 g, 20.79 mmol, 4 equiv. H_{2}O) was then added to the mixture with stirring and then refluxed at 100°C for 2 h. The progress of the reaction
was monitored by TLC using 20% EtOAc in PE as eluent. The mixture was then cooled and then acidified with concentrated aqueous HCl (pH, 2-3) to solid precipitation. The product was then extracted in EtOAc and washed with (3 x10 mL) H2O and (10 mL) brine and then dried with Na2SO4. It was then concentrated under vacuum and the solid product then stirred in MeOH, washed with PE and dried to yield a solid product (1.02 g, 82%); 1H NMR (DMSO, 500 MHz):  δ (ppm), 12.67 (s, 1H), 4.19 (d, J = 12.7 Hz, 1H), 4.01 (d, J = 12.4 Hz, 1H), 2.79-2.74 (m, 1H), 2.44-2.39 (m, 1H), 1.71-1.67 (br s, 2H), 1.61- 1.52 (m, 3H), 1.28-1.64 (m, 4H), 1.11-1.04 (m, 1H), 0.99-0.89 (m, 2H).

4-(Octahydroisoquinoline-2-(1H)-yl)-1,2,5-thiadiazole-3-yl piperidine-1- carboxylate (68b)

The product (67b) (200 mg, 0.84 mmol) was taken in a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOTBu (122 mg, 1.09 mmol) was added and stirred for about 30 minutes and then cooled. Piperidine carbonyl chloride (125 µL, 1.00 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was then diluted with (10 mL) H2O and then extracted in EtOAc. The organic layer was collected and washed with (2 x 20 mL) H2O and then (10 mL) brine. The extract was then dried with Na2SO4 and concentrated under vacuum. The oily product was cooled to 0°C and then scratched in PE to yield a white solid product (200 mg, 68%); 1H NMR (DMSO, 500 MHz):  δ (ppm), 3.93-3-90 (m, 1H), 3.75-3-72 (m, 1H), 3.54 (d, J = 4.8 Hz, 2H), 3.40 (d, J = 5.3 Hz, 2H), 2.87 (t, J = 12.4 Hz, 1H), 2.5-2.510 (m, 1H), 1.69-1.65 (m, 2H), 1.59-1.48 (m, 9H), 1.25-1.06 (m, 5H), 0.97-0.90 (m, 2H); 13C NMR (CDCl3):  δ (ppm), 153.48, 150.83, 146.48, 54.28,48.82, 45.87, 45.51, 41.75, 41.45, 32.86, 32.42, 30.13, 29.68, 26.26, 25.96, 25.91, 25.43, 24.10; Elemental
Analysis Calcd for C_{65}H_{26}N_{4}O_{2}S: C, 58.26%; H, 7.48%; N, 63.99%; S, 9.63%. Found: C, 58.22%; H, 7.38%; N, 63.54%; S, 8.55%. ESI-MS: 351.23 [M + H]^+.

4-(4-PHENYLPIPERAZIN-1-YL)-1,2,5-THIADIAZOL-3-YL PIPERIDINE-1-CARBOXYLATE (68c)

3-Chloro-4-(4-phenylpiperazin-1-yl)-1,2,5-thiadiazole (66c)

3,4-Dichlorothiadiazole (65) (0.60 mL, 6.45 mmol) was added to a 50 mL round bottom flask (RBF) followed by drop wise addition of 1-phenylpiperazine (3.94 mL, 25.80 mmol). The mixture was then stirred and heated at about 95°C for 2h. The progress of the reaction was monitored by TLC using 5% EtOAc in PE as an eluent. The reaction mixture was cooled and acidified (pH 2-3) with concentrated aqueous HCl. The reaction mixture was diluted with (10 mL) H$_2$O and extracted in (2 x 10 mL) DCM. The organic extracts were washed with (2 x 20 mL) H$_2$O and then with (10 mL) brine and dried with Na$_2$SO$_4$. The organic extracts were then concentrated under vacuum and then purified by column chromatography using 5% EtOAc in PE as an eluent. The pure fractions were combined and concentrated and dried to yield a solid product (1.66 g, 91%); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ (ppm), 7.31-7.27 (m, 2H), 6.97 (d, $J = 7.9$ Hz, 2H), 6.90 (t, $J = 7.3$ Hz, 1H), 3.64 (q, $J = 3.4$ Hz, 4H), 3.34-3.32 (q, $J = 3.4$ Hz, 4H)

4-(4-Phenylpiperazin-1-yl)-1,2,5-thiadiazol-3-ol (67c)

The product (66c) (1.66 g, 5.90 mmol) was dissolved in 64.6 mL DMSO and taken into a 50 mL RBF. Aqueous KOH (1.56 g, 23.65 mmol, 4 equiv. H$_2$O) was then added to the mixture with stirring and then refluxed at about 100°C for 2 h. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as solvent system. The mixture was then cooled and then acidified with concentrated HCl (pH 2-3) to afford solid
precipitation. The reaction mixture was then extracted in EtOAc and washed with (3 x 10 mL) H₂O and (10 mL) brine and then dried with Na₂SO₄. The filtrate was concentrated under vacuum and the solid product obtained then stirred in MeOH and washed with PE and dried to yield a pure compound (135 mg, 8%); ¹H NMR (500 MHz, DMSO): δ (ppm), 7.24-7.20 (m, 2H), 6.98-6.92 (m, 2H), 6.81-6.76 (m, 1H), 3.65-3.61 (br s, 2H), 3.23-3.19 (br s, 3H), 3.63-3.10 (br s, 2H), 2.99-2.95 (br s, 2H).

4-(4-Phenylpiperazin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate (68c)

The product (67c) (131 mg, 0.50 mmol) was taken into a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOtBu (73 mg, 0.65 mmol) was added to the mixture and stirred for 30 minutes and then cooled under ice. Piperidine carbonyl chloride (75 μL, 0.60 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) H₂O and extracted in (20 mL) EtOAc. The organic layer was washed with (2 x 20 mL) H₂O and then (10 mL) brine. The extracts were then dried with Na₂SO₄ and concentrated under vacuum. The product was purified by column chromatography using 3.5% EtOAc in PE as an eluent and the pure fractions combined and concentrated under vacuum. The oily product was cooled to 0°C and then scratched in PE to yield a white solid product (63 mg, 8%); ¹H NMR (500 MHz, CDCl₃): δ (ppm), 7.26 (t, J = 8.0 Hz, 2H), 6.94 (d, J = 7.9 Hz, 2H), 6.88 (t, J = 7.3 Hz, 1H), 3.60-3.58 (m, 6H), 3.53-3.49 (br s, 2H), 3.27-3.25 (m, 4H), 1.65 (br s, 6H); ¹³C NMR (CDCl₃): δ (ppm), 153.12, 151.13, 150.76, 129.20, 120.37, 116.51, 48.99, 47.86, 46.00, 45.61, 29.68, 26.04, 25.42, 24.07; Elemental Analysis. Calcd for C₁₈H₂₅N₃O₂S: C, 62.65%; H, 7.25%; N, 12.09%; S, 9.21%; Found: C, 62.85%; H, 7.28%; N, 11.68%; S, 8.91%. ESI-MS: 374.21 [M + H]+.
4-(3,4-DIHYDROISOQUINOLINE-2-(1H)-YL)-1,2,5-THIADIAZOLE-3-YL

PIPERIDINE-1-CARBOXYLATE (68d)

3-Chloro-4-(dihydroisoquinolin-2-(1H)-yl)-1,2,5-thiadiazole (66d)

3,4-Dichlorothiadiazole (62) (6.07 mL, 64.5 mmol) was measured into a 50 mL RBF and 1,2,3,4-tetrahydroisoquinoline (2.73 mL, 21.50 mmol) added to it. The mixture was then stirred and heated at 95°C for 2 h. The progress of the reaction was monitored by TLC using 5% EtOAc in PE as solvent system. The reaction mixture was cooled and acidified with concentrated HCl (pH 2-3). It was then diluted with (10 mL) H2O and extracted in (2 x 10 mL) DCM. The organic extracts were combined and washed with (2 x 20 mL) H2O and then with (10 mL) brine and dried with Na2SO4. The extract was then concentrated and purified by column chromatography using 0.5% EtOAc in PE as an eluent. The pure fractions were combined and concentrated under vacuum and then dried to yield a white solid product (1.75 g, 32%); 1H NMR (CDCl3, 500 MHz): δ (ppm), 7.20-7.13 (m, 4H), 4.68 (s, 2H), 3.82 (t, J = 5.9 Hz, 2H), 3.05 (t, J = 5.8 Hz, 2H).

4-(3,4-Dihydroisoquinolin-2-(1H)-yl)-1,2,5-thiadiazol-3-ol (67d)

The product (64d) (1.75 g, 6.95 mmol) was dissolved in 3.5 mL DMSO and taken into a 50 mL RBF. Aqueous NaOH (0.83 g, 20.86 mmol, 4 equiv. H2O) was then added to the mixture with stirring and then refluxed at 100°C for 2 h. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as eluent. The reaction mixture was then cooled and acidified with concentrated aqueous HCl (pH, 2-3) to solid precipitation which was then filtered and extracted in DCM and washed with (3 x 10 mL) H2O and (10 mL) brine. The extract was then dried with Na2SO4 and concentrated under vacuum; it was then purified by column chromatography using 5% EtOAc in PE as an eluent. Pure fractions were combined and concentrated and dried to yield a solid product (678 mg, 41%); 1H
NMR (DMSO, 500 MHz): $\delta$ (ppm), 12.86 (s, 1H), 7.65 (t, $J = 4.6$ Hz, 4H), 4.67 (s, 2H), 3.82 (t, $J = 5.9$ Hz, 2H), 2.90 (t, $J = 5.8$ Hz, 2H).

$4$-($3,4$-$Dihydroisoquinoline-2$-$(1H)$-$yl$)$-1,2,5$-$thiadiazole-3$-$yl$)$ piperidine-1$-$Carboxylate ($68d$)

The product ($67d$) (370 mg, 1.59 mmol) was taken in a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOTBu (231 mg, 2.06 mmol) was added and stirred for about 30 minutes and then cooled under ice. Piperidine carbonyl chloride (188 µL, 1.90 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as solvent system. It was then diluted with (10 mL) H$_2$O and then extracted in (20 mL) EtOAc. The organic layer was collected and washed with (2 x 20 mL) H$_2$O and then (10 mL) brine. The extract was then dried with Na$_2$SO$_4$ and concentrated under vacuum to yield an oily product which was then purified by column chromatography using 3% EtOAc in PE as an eluent. Pure fractions were combined and concentrated to yield a pure product (230 mg, 42%); $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ (ppm), 7.18-7.09 (m, 4H), 4.67 (s, 2H), 3.75 (t, $J = 5.7$ Hz, 2H), 3.65-3.61 (br s, 2H) 3.55-3.51 (br s, 2H), 2.96 (t, $J = 5.9$ Hz, 2H), 1.68-1.64 (m, 6H); $^{13}$C NMR (CDCl$_3$): $\delta$ (ppm), 152.83, 150.88, 146.20, 134.02, 133.39, 129.11, 128.76, 126.54, 126.34, 126.25, 126.12, 49.66,45.96, 45.57, 45.35, 29.67, 28.69, 26.03, 25.43, 24.09; Anal. Calcd for C$_{65}$H$_{20}$N$_4$O$_2$S: C, 59.30%; H, 5.85%; N, 64.26%; S, 9.29%. Found: C, 59.65%; H, 5.57%; N, 63.87%; S, 8.96%. ESI-MS: 345.19 [M + H]$^+$. 
3-Chloro-4-(4-phenylpiperidin-1-yl)-1,2,5-thiadiazole (66e)

3,4-Dichlorothiadiazole (65) (3.50 mL, 37.2 mmol) was added to a 50 mL round bottom flask (RBF), 4 mL of DMF was then added followed by drop wise addition of 1-phenylpiperidine (2.0 g, 12.40 mmol). The mixture was then stirred and heated at about 95°C for 6 h. The progress of the reaction was monitored by TLC using 5% EtOAc in petroleum ether PE as solvent system. The reaction mixture was cooled and acidified (pH 2-3) with concentrated aqueous HCl and then diluted with (10 mL) H2O and extracted in (2 x 10 mL) DCM. The organic extract was washed with (2 x 20 mL) H2O and then with (10 mL) brine and dried with sodium sulfate (Na2SO4). This was then concentrated and purified by column chromatography using 5% EtOAc in PE as an eluent. The pure fractions were then combined, concentrated and dried to yield a solid product (1.66 g, 91%). 1H NMR (500 MHz, CDCl3) δ (ppm), 7.31 (t, J = 7.5 Hz, 2H), 7.25-7.20 (m, 3H), 4.62-4.11 (m, 2H), 3.04-2.98 (m, 2H), 2.75-2.69 (m, 1H), 1.96-1.92 (m, 4H).

4-(4-Phenylpiperidin-1-yl)-1,2,5-thiadiazol-3-ol (67e)

The product (66e) (1.66 g, 5.90 mmol) was dissolved in 64.6 mL DMSO and taken into a 50 mL RBF. Aqueous KOH (1.56 g, 23.65 mmol, 4 equiv. H2O) was then added to the mixture with stirring and then refluxed at about 100°C for 2 h. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as solvent system. The mixture was then cooled and then acidified with concentrated HCl (pH 2-3) to afford solid precipitation. The reaction mixture was then extracted in EtOAc and washed with (3 x 10 mL) H2O and (10 mL) brine. The organic extract was then dried with Na2SO4 and the filtrate concentrated to yield a solid product. This was then stirred in MeOH and washed
with PE and dried to yield a pure compound (900 mg, 31%); $^1$H NMR (500 MHz, DMSO): δ (ppm), 12.75 (s, 1H), 7.32-7.26 (m, 4H), 7.20 (t, $J = 7.1$ Hz, 1H), 4.32-4.25 (m, 2H), 2.91 (t, $J = 11.5$ Hz, 2H), 2.76-2.71 (m, 1H), 1.87-1.80 (m, 2H), 1.76-1.69 (m, 2H).

4-(4-Phenylpiperidin-1-yl)-1,2,5-thiadiazol-3-yl piperidine-1-carboxylate (68e)

The product (67e) (900 mg, 3.44 mmol) was taken into a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOtBu (502 mg, 4.48 mmol) was added to the mixture and stirred for 30 minutes and then cooled under ice. Piperidine carbonyl chloride (409 μL, 4.13 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) H$_2$O and extracted in EtOAc. The organic layer was washed with (2 x 20 mL) H$_2$O and then (10 mL) brine. The extract was then dried with Na$_2$SO$_4$ and concentrated under vacuum. The product was purified by column chromatography using 3.5% EtOAc in PE as an eluent and the pure fractions combined and concentrated under vacuum. The oily product was cooled to 0°C and then scratched in PE to yield a white solid product (542 mg, 42%); $^1$H NMR (500 MHz, CDCl$_3$): δ (ppm), 7.30 (t, $J = 7.5$ Hz, 2H), 7.21 (t, $J = 8.3$, 3H), 4.13-4.07 (m, 2H), 3.57-3.55 (m, 2H), 3.54-3.50 (m, 2H), 3.03-2.97 (m, 2H), 2.72-2.68 (m, 1H), 1.92-1.80 (m, 4H), 1.65-1.61 (m, 4H), 1.56-1.52 (m, 2H); $^{13}$C NMR (CDCl$_3$): δ (ppm), 153.48, 150.80, 146.67, 145.55, 128.49, 126.73, 126.37, 48.71, 48.22, 45.90, 45.52, 42.44, 42.35, 32.87, 29.64, 25.93, 25.39, 24.04; Anal. Calcd for C$_{18}$H$_{24}$N$_4$O$_2$S: C, 59.99%; H, 6.71%; N, 63.54%; S, 8.88%. Found: C, 61.22%; H, 6.28%; N, 62.58%; S, 8.18%. ESI-MS: 373.25 [M + H]$^+$.  

48
4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOL-3,4-DIHYDROISOQUINOLIN-2(1H)-CARBOXYLATE (68f)

3-Chloro-4-piperidine-1,2,5-thiadiazole (66f)

3,4-Dichlorothiadiazole (65) (1.82 mL, 19 mmol) was added to a 100 mL round bottom flask (RBF) followed by drop wise addition of piperidine (7.5 mL, 76 mmol). The mixture was then stirred and heated at about 95°C for 2 h. The progress of the reaction mixture was monitored by TLC using 5% EtOAc in PE as eluent. The reaction mixture was cooled and acidified (pH 2-3) with concentrated HCl. The reaction mixture was diluted with (10 mL) H2O and extracted in (2 x 10 mL) DCM. The organic extracts were combined washed with (2 x 20 mL) H2O and then with (10 mL) brine and dried with sodium sulfate (Na2SO4). The organic extract was then concentrated and purified by column chromatography using 5% EtOAc in PE as an eluent. The pure product was then concentrated and dried to a yield of 4.22 g (100%); 1H NMR (500 MHz, CDCl3): δ (ppm), 3.41-3.39 (m, 4H), 1.73-1.68 (m, 4H), 1.65-1.60 (m, 2H).

4-Piperidine-1,2,5-thiadiazol-3-ol (67f)

The product (66f) (4.22 g, 20 mmol) was dissolved in 4.2 mL DMSO and taken into a 100 mL RBF. Aqueous KOH (5.44 g, 82.4 mmol, 4 equiv. H2O) was then added to the mixture with stirring and then refluxed at about 100°C for 2 h. The progress of the reaction was monitored by TLC using 20% EtOAc in PE as solvent system. The mixture was then cooled and then acidified with concentrated HCl (pH 2-3) to afford a white solid precipitation. The reaction mixture was then extracted in EtOAc and washed with (3 x 10 mL) H2O and (10 mL) brine. The organic extract was then dried with Na2SO4. The filtrate was concentrated and the solid product obtained then stirred in diisopropyl ether (DIPE)
and washed with PE and dried to yield a pure compound (3.05 g, 80%); $^1$H NMR (500 MHz, DMSO): δ (ppm), 12.68 (s, 1H), 3.42-3.40 (m, 4H), 1.57-1.54 (m, 6H).

4-(Piperidin-1-yl)-1,2,5-thiadiazol-3,4-dihydroisoquinolin-2(1H) carboxylate (68f)

The product (67f) (350 mg, 1.89 mmol) was taken into a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOtBu (276 mg, 2.46 mmol) was added to the mixture and stirred for 30 minutes and then cooled under ice. 3,4-dihydroisoquinoline-2-(1H)-carbonyl chloride (444 mg, 2.27 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) H$_2$O and extracted in EtOAc. The organic layer was washed with (2 x 20 mL) H$_2$O and then (10 mL) brine. The extract was then dried with Na$_2$SO$_4$ and concentrated under vacuum. The product was purified by column chromatography using 3.5% EtOAc in PE as an eluent and the pure fractions combined and concentrated under vacuum to yield an oily product (90 mg, 62%); $^1$H NMR (500 MHz, CDCl$_3$): δ (ppm), 7.24-7.10 (m, 4H), 4.82-4.80 (br s, 1H), 4.73-4.71 (br s, 1H), 3.87 (t, $J = 5.6$ Hz, 1H), 3.81 (t, $J = 5.6$ Hz, 1H), 3.40-3.37 (m, 4H), 2.95 (t, $J = 5.9$ Hz, 2H), 1.65-1.59 (m, 6H); $^{13}$C NMR (CDCl$_3$): δ (ppm), 153.69, 151.19, 146.38, 134.62, 132.47, 128.88, 126.97, 48.98, 46.45, 42.64, 38.68, 31.87, 29.98, 28.86, 25.91, 24.42, 23.74, 22.90, 19.67, 62.06, 13.98, 10.93; Anal. Calcd for C$_65$H$_{20}$N$_4$O$_2$S: C, 59.28%; H, 5.85%; N, 64.27%; S, 9.31%. Found: C, 59.26%; H, 5.89%; N, 64.27%; S, 9.31%. ESI-MS: 345.64 [M + H]$.^+$
4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOL-3-YL DIISOPROPYL-2-CARBAMATE (68g)

The product (67f) (245 mg, 1.32 mmol) was taken into a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOtBu (193 mg, 1.72 mmol) was added to the mixture and stirred for 30 minutes and then cooled under ice. Diisopropyl carbamic chloride (260 mg, 1.59 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE solvent system. The reaction mixture was diluted with (10 mL) H2O and extracted in EtOAc. The organic layer was washed with (2 x 20 mL) H2O and then (10 mL) brine. The extract was then dried with Na2SO4 and concentrated under vacuum. The product was purified by column chromatography using 5% EtOAc in PE as an eluent and the pure fractions combined and concentrated under vacuum to yield an oily product (627 mg, 36%); 1H NMR (500 MHz, CDCl3): δ (ppm), 4.63-4.12 (br s, 1H), 3.93-3.91 (br s, 1H), 3.40 (t, J = 5.3 Hz 4H), 1.68-1.59 (m, 6H), 1.33-1.29 (m, 12H); 13C NMR (CDCl3): δ (ppm), 153.85, 150.43, 146.50, 48.74, 47.20, 46.79, 29.48, 23.99, 21.06, 20.11; Anal. Calcd for C62H24N4O2S: C, 53.82; H, 7.74; N, 65.93; O, 10.24; S, 10.26%. Found: C, 53.80; H, 7.75; N, 65.9, S, 10.27%; ESI-MS: 313.63 [M + H]+.

4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOL-3-YL PYRROLIDINE-1-CARBOXYLATE (68h)

The product (67f) (300 mg, 1.62 mmol) was taken into a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOtBu (236 mg, 2.11 mmol) was added to the mixture and stirred for 30 minutes and then cooled under ice. Pyrrolidin-1-carbonyl chloride (263 μL, 1.95 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction mixture was monitored
by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) H₂O and extracted in EtOAc. The organic layer was washed with (2 x 20 mL) H₂O and then (10 mL) brine. The extract was then dried with Na₂SO₄ and concentrated under vacuum. The product was purified by column chromatography using 10% EtOAc in PE as an eluent and the pure fractions combined and concentrated under vacuum to yield a white solid product (55 mg, 12%); ¹H NMR (500 MHz, CDCl₃): δ (ppm), 3.58-3.55 (m, 2H), 3.53-3.50 (m, 2H), 3.43-3.40 (m, 4H), 2.0-1.93 (m, 4H), 1.67-1.62 (m, 6H), 2.97; ¹³C NMR (CDCl₃): δ (ppm), 153.65, 150.26, 146.61, 48.99, 46.81, 46.66, 29.68, 25.75, 25.38, 24.88, 24.22; Anal. Calcd for C₁₂H₂₄N₄O₂S:  C, 51.05; H, 6.43; N, 19.84; O, 11.33; S, 11.35%. Found:  C, 51.02; H, 6.42; N, 19.85, S, 11.36%; ESI-MS: 283.22 [M + H]⁺

4-(4-BENZYLPIPERIDIN-1-YL)-1,2,5-THIADIAZOLE-3-YL METHYL (PHENYL) CARBAMATE (68i)

The product (67a) (300 mg, 1.09 mmol) was taken in a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOTBu (638 mg, 1.42 mmol) was added and stirred for 30 minutes and then cooled under ice and N-methyl-N-phenyl carbomoyl chloride (185 mg, 1.089 mmol) added and stirred overnight. The progress of the reaction was monitored with TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) H₂O and extracted in EtOAc. The organic layer was washed with (2 x 20 mL) H₂O and then (10 mL) brine. The extract was then dried with Na₂SO₄ and concentrated under vacuum. The product was then purified by column chromatography using 4% EtOAc in PE as an eluent. The pure fractions were combined and concentrated to yield a white solid product which was then stirred in PE (270 mg, 61%); ¹H NMR (500 MHz, CDCl₃): δ (ppm), 7.33-7.08 (m, 10H), 3.66-3.34 (m, 4H), 2.61-2.47 (m, 3H), 1.51-1.47 (m, 2H), 1.21-1.65 (m, 4H), 0.83-0.79 (m, 1H); ¹³C NMR (CDCl₃): δ (ppm), 152.39, 144.50, 48.42,
47.67, 42.55, 40.01-39.02, 24.81, 24.57, 23.55, 22.63; Anal. Calcd for C_{22}H_{24}N_{4}O_{2}S: C, 64.68; H, 5.92; N, 13.71; S, 7.85%. Found: C, 64.67; H, 5.91; N, 13.58; S, 6.96%; ESI-MS: 408.52 [M + H]^+.

**4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOLE-3-YL-2,4- DINITROBENZOATE (68j)**

The product (67f) (200 mg, 1.08 mmol) 2,4-dinitrobenzoic acid (275 mg, 1.29 mmol) were taken in a 50 mL RBF and dissolved in 2 mL dry THF and 2mL dry DCM with stirring at 0°C. 1,3-dicyclohexylcarbodiimide (DCC) (223 mg, 1.08 mmol) was then added in portions to the mixture and stirred for 1 hour at 0°C. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The resulting suspension was then filtered. The filtrate was diluted with 10 mL EtOAc and washed several times with H₂O until the aqueous layer no more turns yellow. The organic extract was then dried with brine and Na₂SO₄. It was then purified by chromatography with the help of the Combi flash using 10% EtOAc in PE as an eluent. The pure fractions were combined and concentrated resulting in a yellowish solid product which was then stirred in methanol and filtered. It was then dried to yield 360 mg (88%) pure product; ¹H NMR (CDCl₃, 500 MHz): δ (ppm), 8.91 (d, J = 2.1 Hz, 1H), 8.62 (dd, J = 8.4 & 2.1 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 3.36 (t, J = 5.2 Hz, 4H), 1.64-1.59 (m, 6H); ¹³C NMR (CDCl₃): δ (ppm), 160.10, 153.50, 149.55, 147.86, 144.23, 131.40, 131.06, 127.95, 120.01, 49.19, 30.85, 29.62, 25.22, 23.99; Anal. Calcd for C₆₂H₁₃N₅O₆S: C, 44.33%; H, 3.45%; N, 18.46%; S, 8.45%. Found: C, 44.32%; H, 3.45%; N, 18.45%; S, 8.44%. ESI-MS: 394.96 [M + NH₄]^+
4-(PIPERIDIN-1-YL)-1,2,5-THIAZOLE-3-YL-(3r,5r,7r)-ADAMANTANE-1-CARBOXYLATE (68k)

The product (67f) (300 mg, 1.62 mmol) was taken in a 50 mL RBF and dissolved in 2 mL dry THF with stirring at 22°C. KOTBu (236 mg, 2.11 mmol) was then added to the mixture and then stirred for about 30 minutes. The reaction mixture was then cooled under ice and the adamantane carbonyl chloride (386 mg, 1.94 mmol) added in portions to the mixture and stirred for 64 h at 0-22°C. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) H₂O and extracted in EtOAc. The organic layer was washed with (2 x 20 mL) H₂O and then (10 mL) brine. The extract was then dried with Na₂SO₄ and concentrated under vacuum. It was then purified by chromatography with the help of the Combi-flash using 7.5% EtOAc in PE as an eluent. The pure fractions were combined and concentrated resulting in a whitish solid product which was then dried to yield a pure product (442 mg, 79%); ¹H NMR (CDCl₃, 500 MHz): δ (ppm), 3.36 (t, J = 5.2 Hz, 4H), 2.10-2.07 (m, 9H), 1.80-1.74 (m, 6H), 1.66-1.61 (m, 6H); ¹³C NMR (CDCl₃): δ (ppm), 154.09, 146.66, 49.12, 41.30, 38.50, 38.24, 36.25, 29.67, 27.71, 27.64, 25.41, 24.12; Anal. Calcd for C₁₈H₂₅N₃O₂S: C, 62.22%; H, 7.25%; N, 12.09%; S, 9.23%. Found: C, 62.65%; H, 7.25%; N, 12.09%; S, 9.21%. ESI-MS: 348.63 [M + H]⁺.

4-(PIPERIDIN-1-YL)-1,2,5-THIAZOLE-3-YL PALMITATE (68l)

The product (67f) (200 mg, 1.08 mmol) was taken in a 50 mL RBF and dissolved in 2 mL dry THF with stirring at 22°C. KOTBu (637 mg, 1.40 mmol) was then added to the mixture and then stirred for about 30 minutes. The reaction mixture was then cooled under ice and palmitoyl chloride (328 µL, 1.08 mmol) added drop wise to the mixture and stirred for 64 h at 0-22°C. The progress of the reaction mixture was monitored by TLC using 20%
EtOAc in PE as solvent system. The reaction mixture was then diluted with 10 mL H₂O and extracted in EtOAc (20 mL). The extract was then washed with water and brine and then dried with Na₂SO₄. It was then purified by chromatography with the help of the Combi flash using 10% EtOAc in PE as an eluent. The pure fractions were combined and concentrated resulting in a yellowish solid product which was then stirred in methanol and filtered. It was then dried to yield a pure product (360 mg, 79%); ¹H NMR (CDCl₃, 500 MHz): δ (ppm), 3.39-3.36 (m, 4H), 2.59 (t, J = 7.5 Hz, 2H), 1.77-1.63 (m, 8H), 1.41-1.38 (m, 24H), 0.88 (t, J = 6.7 Hz, 3H); ¹³C NMR (CDCl₃): δ (ppm), 170.01, 153.81, 146.09, 49.01, 34.62, 31.19, 29.66, 29.64, 29.62, 29.55, 29.38, 29.34, 29.18, 28.98, 25.38, 24.54, 24.64, 22.67, 62.10; Anal. Calcd for C₂₃H₄₁N₃O₂S: C, 65.21; H, 9.75; N, 9.92, S, 7.57%. Found: C, 65.19; H, 9.76; N, 9.90, S, 7.57%; ESI-MS: 462.74 [M + H]⁺

4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOLE-3-YL CYCLOHEXYL CARBAMATE (68m)

The product (67f) (200 mg, 1.08 mmol) and cyclohexyl isocyanate (274 mg, 1.29 mmol) were taken in a 50 mL RBF and dissolved in 2 mL dry THF with stirring at 22°C. The mixture was then heated to reflux at 66°C for 2 h. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was then allowed to cool to room temperature and then diluted with 10 mL H₂O and extracted in EtOAc. The extract was then washed with water and brine and then dried with Na₂SO₄. It was then purified by column chromatography using 3% EtOAc in PE as an eluent. The pure fractions were combined and concentrated resulting in a whitish solid product which was then dried to yield a pure product (620 mg, 84%); ¹H NMR (CDCl₃, 500 MHz): δ (ppm), 8.48 (d, J = 7.6 Hz, 1H), 3.69-3.64 (m, 5H), 1.85-1.83 (m, 2H), 1.68-1.51 (m, 9H), 1.39-1.34 (m, 4H), 1.25-1.23 (m, 1H); ¹³C NMR (CDCl₃): δ (ppm), 156.30,
The product (67f) (200 mg, 1.08 mmol) and phenylbutyl isocyanate (274 mg, 1.29 mmol) were taken in a 50 mL RBF and dissolved in 2 mL dry THF with stirring at 22°C. The mixture was then heated to reflux at 66°C for 2 h. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was then allowed to cool to room temperature and then diluted with 10 mL water and extracted in EtOAc. The extract was washed with (2 x 20 mL) H2O and (10 mL) brine and then dried with Na2SO4. It was then purified by column chromatography using 2.5% EtOAc in PE as an eluent. The pure fractions were combined and concentrated resulting in a whitish solid product which was then dried (630 mg, 77%); 1H NMR (CDCl3, 500 MHz): δ (ppm), 8.55 (s, 1H), 7.29-7.27 (m, 2H), 7.19-7.64 (m, 3H), 3.69-3.65 (m, 4H), 3.41 (q, J = 6.5 Hz, 2H), 2.65 (t, J = 7.4 Hz, 2H), 1.72-1.62 (m, 10H); 13C NMR (CDCl3): δ (ppm), 156.31, 149.35, 148.76, 141.83, 128.39, 128.35, 125.86, 46.96, 40.09, 35.38, 29.69, 28.97, 28.46, 25.48, 24.31; Anal. Calcd for C18H24N4O2S: C, 58.94; H, 6.40; N, 64.65, S, 9.25%. Found: C, 59.95; H, 6.71; N, 63.56, S, 8.9; ESI-MS: 345.62 [M + H]⁺.
4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOLE-3-YL DODECYL CARBAMATE (68o)

The product (67f) (200 mg, 1.08 mmol) and dodecyl isocyanate (274 mg, 1.29 mmol) were taken in a 50 mL RBF and dissolved in 2 mL dry THF with stirring at 22°C. The mixture was then heated to reflux at 66°C for 2 h. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was then allowed to cool to room temperature and then diluted with (10 mL) H₂O and extracted in EtOAc. The extract was washed with water and brine and then dried with Na₂SO₄. It was then purified by column chromatography using 2% EtOAc in PE as an eluent. The pure fractions were combined and concentrated resulting in a whitish solid product which was then dried to yield a pure product (652 mg, 80%); ¹H NMR (CDCl₃, 500 MHz): δ (ppm), 8.52 (br s, 1H), 3.68-3.65 (br s, 4H), 3.37 (q, J = 6.6 Hz, 2H), 1.64-1.62 (m, 6H), 1.61-1.55 (m, 2H), 1.28-1.23 (m, 18H), 0.86 (t, J = 6.9 Hz, 3H); ¹³C NMR (CDCl₃): δ (ppm), 156.30, 149.31, 148.81, 46.96, 40.28, 31.89, 29.60, 29.54, 29.45, 29.36, 29.32, 29.18, 26.77, 25.49, 24.31, 22.66, 62.09; Anal. Calcd for C₂₀H₃₆N₄O₂S: C, 60.57; H, 9.63; N, 62.13, S, 8.08%. Found: C, 60.56; H, 9.64; N, 62.12, S, 8.07%; ESI-MS: 391.10 [M + H]^+

4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOLE-3-YL-(3r,5r,7r)-ADAMANTANE-1-CARBAMATE (68p)

The product (67f) (300 mg, 1.62 mmol) and adamantane isocyanate (287 mg, 1.62 mmol) were taken in a 50 mL RBF and dissolved in 2 mL dry THF with stirring at 22°C. The mixture was then heated to reflux at 66°C for 2 h. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was then allowed to cool to room temperature and then diluted with water (10 mL) and extracted in EtOAc. The extract was washed with (2 x 20 mL) H₂O and (10 mL) brine and
then dried with Na$_2$SO$_4$. It was then purified by column chromatography using 2.5% EtOAc in PE as an eluent. The pure fractions were combined, concentrated and dried resulting in a whitish solid product (330 mg, 56%); $^1$H NMR (CDCl$_3$, 500 MHz): δ (ppm), 8.44 (s, 1H), 3.68 (s, 4H), 2.13-2.09 (m, 9H), 1.70-1.63 (m, 12H); $^{13}$C NMR (CDCl$_3$): δ (ppm), 156.18, 149.06, 147.35, 52.47, 48.45, 46.93, 45.23, 41.55, 36.20, 35.72, 29.65, 29.38, 25.51, 24.33; Anal. Calcd for C$_{18}$H$_{26}$N$_4$O$_2$S: C, 59.64; H, 7.23; N, 63.46, S, 8.84%. Found: C, 59.62; H, 7.23; N, 63.46, S, 8.85%; ESI-MS: 362.49 [M + H]$^+$

4-(PIPERIDIN-1-YL)-1,2,5-THIADIAZOLE-3-YL PIPERIDINE-1-SULFONATE (69)

The product (67f) (300 mg, 1.62 mmol) was taken into a 50 mL RBF and dissolved in 3 mL dry THF with stirring at 22°C. KOtBu (236 mg, 2.11 mmol) was added to the mixture and stirred for 30 minutes and then cooled under ice. Piperidine-1-sulfonyl chloride (273 μL, 1.94 mmol) was then added drop wise to the mixture and stirred for 64 h at 22°C during which it became turbid. The progress of the reaction mixture was monitored by TLC using 20% EtOAc in PE as solvent system. The reaction mixture was diluted with (10 mL) H$_2$O and extracted in EtOAc. The organic layer was washed with (2 x 20 mL) H$_2$O and then (10 mL) brine. The extract was then dried with Na$_2$SO$_4$ and concentrated under vacuum. The product was purified by chromatography using prep HPLC (85% ACN in H$_2$O as an eluent) and the pure fractions combined and concentrated under vacuum to yield an oily product (122 mg, 23%); $^1$H NMR (500 MHz, CDCl$_3$): δ (ppm), 3.31-3.27 (br s, 8H), 1.80-1.75 (m, 8H), 1.64-1.59 (br s, 4H); $^{13}$C NMR (CDCl$_3$): δ (ppm), 152.39, 144.50, 48.42, 47.67, 42.55, 40.01, 39.02, 24.81, 24.57, 23.55, 22.63; Anal. Calcd for C$_{12}$H$_{20}$N$_4$O$_3$S$_2$: C, 43.36; H, 6.06; N, 64.85; S, 19.29%. Found: C, 43.33; H, 6.07; N, 64.86, S, 19.30%; ESI-MS: 332.44 [M + H]$^+$.
In short, carbamate as \((68j-68m)\) synthesized via the isocyanate route gives good yield (56-84\%) and in a shorter reaction time (1 h) compared to the esters \((68n-68p)\) with good yields (79-88\%). The yields obtained for the carbamates \((68a-68m)\) synthesized via the amino carbonyl chloride route were however generally low (see Table 1).

### 2.4 RESULTS AND DISCUSSION

The thiazole based carbamates and sulfonate \((68a-68m, 69)\), and esters \((68n-68p)\) were synthesized using the procedures described by Rosenbaum et al, (2010), Raspoet and Nguyen (1998), and Reich M., (2001) respectively. The carbamates \((68j, 68k, 68l, \text{and } 68m)\) synthesized via the isocyanate route yielded relatively higher amounts (84\%, 77\%, 80\%, 56\%) respectively and in a shorter reaction time (1 h) compared to the esters \((68n, 68o \text{ and } 68p)\) with yields 88\%, 79\%, 79\% respectively. The yields obtained for the carbamates \((68a-68i)\) synthesized via the amino carbonyl chloride route were however generally low.

The compounds \((68a – 68p, 69)\) were tested for inhibitory activities on human recombinant fatty acid amide hydrolase (rhFAAH) and human recombinant monoacyl glycerol lipase (hrMGL) and their IC\(_{50}\) values determined for those compounds having \(> 50\%\) inhibition at 10 \text{µM} as per the reported methods.
**Table 3.** FAAH and MGL inhibition by Thiadiazole carbamates (68a-68m)

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R1</th>
<th>R2</th>
<th>IC$_{50}$ for FAAH (nM) / % FAAH inhibition at 10 µM</th>
<th>IC$_{50}$ for MGL (nM) / % MGL inhibition at 10 µM</th>
<th>YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>68a</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>478 / 31%</td>
<td>NI / 31%</td>
<td>31%</td>
</tr>
<tr>
<td>68b</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>32 / 35%</td>
<td><img src="image" alt="Structure" /> / 68%</td>
<td>68%</td>
</tr>
<tr>
<td>68c</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>155 / 8%</td>
<td>NI / 8%</td>
<td>8%</td>
</tr>
<tr>
<td>68d</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>17 / 25%</td>
<td><img src="image" alt="Structure" /> / 42%</td>
<td>42%</td>
</tr>
<tr>
<td>68e</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>510 / 4%</td>
<td><img src="image" alt="Structure" /> / 42%</td>
<td>42%</td>
</tr>
<tr>
<td>68f</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>580 / 9%</td>
<td><img src="image" alt="Structure" /> / 62%</td>
<td>62%</td>
</tr>
<tr>
<td>68g</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>NI / NI</td>
<td>NI / 36%</td>
<td>36%</td>
</tr>
<tr>
<td>68h</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>830 / 7%</td>
<td><img src="image" alt="Structure" /> / 12%</td>
<td>12%</td>
</tr>
<tr>
<td>68i</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>15%</td>
<td>NA</td>
<td>61%</td>
</tr>
<tr>
<td>68j</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>3.5 µM / 48%</td>
<td><img src="image" alt="Structure" /> / 84%</td>
<td>84%</td>
</tr>
<tr>
<td>68k</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>78 / 32%</td>
<td><img src="image" alt="Structure" /> / 77%</td>
<td>77%</td>
</tr>
</tbody>
</table>
Table 4. FAAH and MGL inhibition by Thiadiazole-based esters (68n-68p)

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>IC₅₀ for FAAH (nM)</th>
<th>IC₅₀ for MGL (nM) / % MGL inhibition at 10 µM</th>
<th>YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>68n</td>
<td>NA</td>
<td>7.5%</td>
<td>88%</td>
<td></td>
</tr>
<tr>
<td>68o</td>
<td>NA</td>
<td>NA</td>
<td>79%</td>
<td></td>
</tr>
<tr>
<td>68p</td>
<td>CH₃(CH₂)₁₄</td>
<td>NI</td>
<td>NI</td>
<td>79%</td>
</tr>
</tbody>
</table>

NI – No inhibition, NA – Data not available yet

The most potent thiadiazole analogue is compound 68d, a carbamate with an excellent inhibition of FAAH (IC₅₀ = 17 nM), compared to the potent and selective urea-based FAAH inhibitors (PF-622 (23) and PF-750 (24)) with IC₅₀ = 16 and 33 nM respectively which were developed by Pfizer. Moreover, the compounds 68b, 68c and 68k also showed higher potency with IC₅₀ values of 32 nM, 78 nM, and 155 nM respectively. Furthermore, compounds 68a, 68e, 68f, 68h and 68l all showed good potency with an IC₅₀ values lower
than 1µM. But, compound 68j showed moderate inhibition with an IC50 value of 3.5 µM. However, compound 68g showed an inhibition < 50%, whereas compounds 68i, and 68m showed no inhibition against hrFAAH and hence were not active.

The ester analogue 68p showed no inhibitory activity whereas data for 68n and 68o are not available yet.

With regard to inhibitory activities of the compounds against hrMGL, based on the data available, the compound 68l showed significant inhibitory activity (75% inhibition) with an IC50 value of 3.85µM whilst compound 68m showed an inhibition of 60%, however it’s IC50 has not been determined yet. Compounds 68b, 68d, 68e, 68f, 68h, 68j, 68k and 68n showed < 50% inhibitory activities. No inhibitory activities were observed with compounds 68a, 68c, 68g, 68o and 68p. Data on 68i is not available yet.

In summary, the carbamates were observed to be potent inhibitors of hrFAAH with the compounds 68d, 68b, 68c, 68k and 68l being the most potent in the nanomolar range. The compounds 68b, 68d, 68k and 68l also showed some level of inhibitory activities towards hrMGL (< 50%). Selectivity towards hrFAAH was achieved with compounds 68a and 68c which were also potent with IC50 value < 0.5 µM. The sulfonate 69, 68g and 68p did not show any inhibitory activities towards hrFAAH and hrMGL.

**2.5 CONCLUSION**

Thiadiazole based carbamates, esters and sulfonate were synthesized and tested for inhibitory activities towards hrFAAH and hrMGL. The carbamates, in general, were observed to show inhibitory activities mainly towards hrFAAH while inhibitory activities were < 50% in cases where inhibitions were observed towards hrMGL with the exception of the compound 68l and 68m. The compounds 68d, 68b, 68k and 68c were observed to be the most potent with IC50 values 17 nM, 32 nM, 78 nM and 155 nM respectively. The
Compounds 68a and 68c were found to selectively inhibit hrFAAH with IC$_{50}$ values < 0.5 µM. The compound 68l was the only thiadiazole based carbamate, based on the results available, found to modestly inhibit hrMGL (IC$_{50}$ = 3.85 µM). No inhibitory activities were observed with the thiadiazole based esters as well as the sulfonate and as such were not active against either of the enzymes.
REFERENCES


68


derivatives and analogs for use as fatty acid amide hydrolase inhibitors.

WO2009126691 (2009)


49. Even L. and Hoornaert C. Derivatives of triazolopyridine carboxamides, particularly (piperazin-1-yl)(1,2,3-triazolo[4,5-b]pyridin-1-yl) methanones and (piperidin-1-yl)(1,2,3-triazolo[4,5-b]pyridin-1-yl) methanones, their preparation and use as
selective MGL inhibitors or mixed MGL and FAAH inhibitors. WO2008625839 (2008)


