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JAYENDRA Z. PATEL

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ABSTRACT

This doctoral dissertation describes the design, synthesis and structure-activity relationships (SARs) of novel 1,3,4-oxadiazol-2-one, 1,2,5-thiadiazole carbamate, and loratadine analogues as promising inhibitors of the endocannabinoid hydrolases, such as FAAH, ABHD6, and MAGL. Inhibition of endocannabinoid hydrolases by specific inhibitors enhance endocannabinoid signaling, which in turn has been linked to various therapeutic effects of cannabinoids. In recent years, inhibitors of FAAH, MAGL and ABHD6 have shown potential to treat several diseases, such as pain, inflammation, CNS disorders and metabolic disorders without association of cannabimimetic side effects.

By adopting a hybridization approach, the earlier discovered 1,3,4-oxadiazol-2-ones were combined with nonsteroidal anti-inflammatory drugs (NSAIDs) to produce chiral 1,3,4-oxadiazol-2-ones of which JZP-327A was found to be a slowly reversible, potent FAAH inhibitor with an IC_{50} value of 11 nM. Systematic structural variations of 1,2,5-thiadiazole carbamates resulted in the identification of JZP-430 as an irreversible, potent ABHD6 inhibitor with an IC_{50} value of 44 nM. By increasing the electrophilicity on the carbonyl functionality of loratadine, several novel MAGL inhibitors were identified of which JZP-361 acted as the most potent MAGL inhibitor having an IC_{50} value of 46 nM. Interestingly, JZP-361 exhibited slowly reversible MAGL inhibition and it retained its histamine H_1 antagonistic affinity. The optimization of 1,3,4-oxadiazol-2-one by carrying out systematic structural variations led identification of JZP-169 as a potent ABHD6 inhibitor (IC_{50} value of 216 nM) having an irreversible mode of inhibition. All these compounds showed selectivity over endocannabinoid hydrolases, cannabinoid receptors, and other studied off-targets.

In summary, the results presented here demonstrate that 1,3,4-oxadiazol-2-one is a promising scaffold for the development of potent and selective inhibitors of serine hydrolases: JZP-327A for FAAH and JZP-169 for ABHD6. Interestingly, 1,3,4-oxadiazol-2-ones showed reversible inhibition mechanism against FAAH whereas they inhibited ABHD6 in an irreversible manner. The ABHD6 inhibitor JZP-430 may serve as an important lead compound when developing novel drugs for metabolic disorders. Finally, as a dual blocker of MAGL activity and the histamine H_1 receptor, the loratadine analogue JZP-361 may have potential to treat inflammation in chronic diseases, such as asthma.

National Library of Medicine Classification: QU 143, QV 126, QV 744

Medical Subject Headings: Chemistry, Pharmaceutical; Chemistry Techniques, Synthetic; Endocannabinoids; Enzyme Inhibitors; Hydrolases; Amidohydrolases; Monoacylglycerol Lipases; Drug Discovery; Oxadiazoles; Thiadiazoles; Loratadine/analogs & derivatives; Carbamates; Structure-Activity Relationship

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TIIVISTELMÄ

Tässä väitöskirjassa kuvataan uusia 1,3,4-oksadiatsol-2-oni, 1,2,5-tiadiatsolikarbamaatti- ja loratadiinijohdoksia lupaavina FAAH-, ABHD6- ja MAGL–inhibiittoreina sekä esitetään niiden suunnittelu, synteesi ja rakenne-aktiivisuussuhteet (SAR). Endokannabinoidijärjestelmän hydrolaasien toiminnan estäminen erityisillä ensyymi-inhibiittoreilla lisää endokannabinoidien signalointia, millä on yhteys kannabinoidien terapeuttisiin vaikutuksiin. Viime vuosina FAAH-, MAGL- ja ABHD6-inhibiittoreilla on osoitettu olevan terapeuttista tehoa useiden sairauksien, kuten kivun, tulehduksen ja aineenvaihdunnan häiriöiden hoidossa ilman kannabimimeettisiä sivuvaikutuksia.

Yhdistämällä aiemmin tunnettu 1,3,4-oksadiatsol-2-oni –rakenne tulehdus-kipulääkkeeseen (NSAID) saatiin aikaan kiraalisia 1,3,4-oksadiatsol-2-oni –rakenteita, joista yhdisteen JZP-327A havaittiin olevan hitaasti palautuva tehokas FAAH-estäjä IC_{50}-arvolla 11 nM. Systemaattinen 1,2,5-tiadiatsolikarbamaatti-rakenteiden rakenne-aktiivisuus tarkastelu johti yhdisteeseen JZP-430, joka on tehokas palautumaton ABHD6-inhibiittori IC_{50}-arvolla 44 nM. Lisäämällä loratadiinin karbonyyliryhmän elektrofiilisyyttä saatiin aikaiseksi useita uusia MAGL-estäjiä, joista JZP-361 oli voimakkain MAGL-estäjä IC_{50}-arvolla 46 nM. Tutkimuksessa JZP-361:n todettiin olevan mekanismiltaan hitaasti palautuva MAGL-estäjä ja se säilytti affiniteetin H1-reseptoriin. 1,3,4-Oksadiatsol-2-oni –rakennetta optimoitiin valmistamalla systemaattisesti erirakenteisia johdoksia, joista yhdisteen JZP-169 todettiin olevan tehokas palautumaton ABHD6-estäjä (IC_{50}-arvo 216 nM). Kaikkien näiden yhdisteiden todettiin olevan selektiivisiä endokannabinoidijärjestelmän hydrolaaseihin, kannabinoidireseptoreihin ja muihin tutkittuihin kohteisiin verrattuna.

Yhteenvetona voidaan todeta, että 1,3,4-oksadiatsol-2-oni –rakenne on käyttökelpoinen runkorakenne kehitettäessä tehokkaita ja selektiivisiä seriinihydrolaasiestäjiä, kuten työssä kehitetyt FAAH-estäjä JZP-327A ja ABHD6-estäjä JZP-169. Mielenkiintoinen tulos oli, että 1,3,4-oksadiatsol-2-oni –rakenteesta voitiin kehittää sekä palautuva FAAH-estäjä että palautumaton ABHD6-estäjä. Työssä löydetty ABHD6-estäjä JZP-430 voi toimia tärkeänä johtoyhdisteenä kehitettäessä uusia lääkehoitoja aineenvaihdunnan häiriöihin. Lopuksi, MAGL-entsyymin että histamiini-H1-reseptorin salpaaja, loratadiinianalogi JZP-361, voi olla käyttökelpoinen kroonisten tulehdussairauksien, kuten astman hoidossa.

Luokitus: QU 143, QV 126, QV 744
Yleinen suomalainen asiasanasto: orgaaninen kemia; lääkekemia; kemiallinen synteesi; endokannabinoidit; entsyymit; inhibiittorit; lääkesuunnittelu; rakenne; aktiivisuus

To My Family

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Kuopio, September 2015

Jayendra Z Patel

List of the original publications

This dissertation is based on the following original publications, denoted in the text as Roman numerals I-IV:

I Patel J Z, Parkkari T, Laitinen T, Kaczor A A, Saario S M, Savinainen J R, Navia-Paldanius D, Cipriano M, Leppänen J, Koshevoy I O, Poso A, Fowler C J, Laitinen J T and Nevalainen T. Chiral 1,3,4-Oxadiazol-2-ones as Highly Selective FAAH Inhibitors.
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II Patel J Z, Nevalainen T J, Savinainen J R, Adams Y, Laitinen T, Runyon R S, Vaara M, Ahenkorah S, Kaczor, A A, Navia-Paldanius D, Gynther M, Aaltonen N, Joharapurkar A A, Jain M R, Haka A S, Maxfield F R, Laitinen J T and Parkkari T. Optimization of 1,2,5-Thiadiazole Carbamates as Potent and Selective ABHD6 Inhibitors.
ChemMedChem 10: 253-265, 2015.

III Patel J Z, Ahenkorah S, Vaara M, Staszewski M, Adams Y, Laitinen T, Navia-Paldanius D, Parkkari T, Savinainen J R, Walczyński K, Laitinen J T and Nevalainen T J. Loratadine Analogues as MAGL Inhibitors.
Bioorg. Med. Chem. Lett. 25: 1436-1442, 2015.

IV Patel J Z, van Bruchem J, Laitinen T, Kaczor A A, Navia-Paldanius D, Parkkari T, Savinainen J R, Laitinen J T and Nevalainen T J. Revisiting 1,3,4-Oxadiazol-2-ones: Utilization in the Development of ABHD6 Inhibitors.
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Contents

Abbreviations

AA	arachidonic acid
AA-5-HT	arachidonoyl serotonin
ABHD	α/β-hydrolase domain containing
ABPP	activity-based protein profiling
ACN	acetonitrile
AChE	acetylcholinesterase
ADMK	arachidonoyl diazomethyl ketone
AEA	*N*-arachidonoyl-ethanolamine (anandamide)
1(3)-AG	1(3)-arachidonoyl-glycerol
2-AG	2-arachidonoyl-glycerol
2-AGE	2-arachidonyl glyceryl ether (noladin ether)
AMC-AA	7-amino-4-methyl coumarin arachidon-amide
AS	amidase signature
ATK	arachidonyl trifluoro-methyl ketone
ATP	adenosine triphosphate
BSA	bovine serum albumin
BuChE	butyrylcholinesterase
CB	cannabinoid
CB_1	cannabinoid receptor subtype-1
CB_2	cannabinoid receptor subtype-2
CCI	chronic constriction injury
CDTA	calcium-dependent transacylase
CEs	carboxylesterases
CFA	complete Freund's adjuvant
CHO	Chinese hamster ovary
p-CMB	*p*-chloromercuribenzoic acid
CNS	central nervous system
COX	cyclooxygenase
CRC	concentration response curve
CYP	cytochrome P450
Cys	cysteine
DAG	diacylglycerol
DAGL	diacylglycerol lipase
DCM	dichloromethane
DEA	docosatetraenoyl-ethanolamide

DHAP	dihydroxyacetone phosphate
DMAP	4-(dimethylamino)-pyridine
DMF	*N*,*N*-dimethylform-amide
DMSO	dimethylsulfoxide
DPP-IV	dipeptidyl peptidase-4
ECS	endocannabinoid system
EDTA	ethylenediamine-tetraacetic acid
EET-EA	epoxyeicosatrienoic ethanolamide
EMT	endocannabinoid membrane transporter
ERG	electron releasing group
EtOAc	ethyl acetate
EtOH	ethanol
EWG	electron withdrawing group
FAAH	fatty acid amide hydrolase
FAAH-2	fatty acid amide hydrolase-2
FABP	fatty acid binding protein
FAPA	fatty acid primary amide
FC	flash chromatography
FDA	Food and Drug Administration
FPs	fluorophosphonates
GDE1	glycerophosphodiester phosphodiesterase 1
GK	glycerol kinase
G-1-P	glycerol-1-phosphate
GPCR	G-protein coupled receptor
GPO	glycerol phosphate oxidase
HEK293	human embryonic kidney 293
hERG	human ether-a-go-go related gene
HETE-EA	hydroxyeicosatetra-enoic acid ethanol-amide
HPLC	high-performance liquid chromatography
HRP	horseradish peroxidase
IC_{50}	half maximal inhibitory concentration
i.p	intraperitoneal
IPA	isopropanol
*K*i	inhibition constant
LOX	lipoxygenase
LPS	lysophosphatidylserine
MAFP	methyl arachidonoyl fluorophosphonate

MAG/MAGL	monoacylglycerol lipase
MeOH	methanol
mSH	metabolic serine hydrolase
MTI	mild thermal injury
4-MUO	4-methylumbelliferone oleate
NAAA	*N*-acylethanolamine-hydrolyzing acid amidase
NADA	*N*-arachidonyl dopamine
NAE	*N*-acylethanolamine
NAGly	*N*-arachidonoyl-glycine
NAM	*N*-arachidonyl-maleimide
NAPE-PLD	*N*-acyl phosphatidyl-ethanolamine phospholipase D
NAT	*N*-acyltaurine
NAPE	*N*-arachidonoyl phosphatidylethanolamine
NEM	*N*-ethylmaleimide
NSAID	non-steroidal anti-inflammatory drug
OEA	*N*-oleoylethanolamine
2-OG	2-oleoylglycerol
PAP	phosphatidic acid
PBS	phosphate-buffered saline
PEA	*N*-palmitoylethanolamine (palmitoylethanolamide)
PE	petroleum ether
PG	prostaglandin
PG-EA	prostaglandin-ethanolamide
PGE2	prostaglandin E2
PG-GE	prostaglandin-glyceryl ester
PHARC	polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, cataract
PI-PLC	phosphatidyl inositides (PI)- selective phospholipase C
PL	phospholipase
PLC	phospholipase C
PLD	phospholipase D
PPAR	peroxisome proliferator activated receptor
PTPN22	protein tyrosine phosphatase, nonreceptor type 22
SAR	structure–activity relationship

S.E.M.	standard error of the mean	THC	(-)-Δ^9-tetrahydro-cannabinol
Ser	serine	THF	tetrahydrofuran
[^{35}S]-GTPγS	guanosine 5'-O-(3-[^{35}S]-thiotriphosphate	THL	tetrahydrolipstatin (orlistat)
SH	serine hydrolase	TMS	tetramethylsilane
SHIP	SH2 (Src Homology 2) domain-containing inositol 5'-phosphtase	ΔTM-rFAAH	transmembrane domain deleted rat FAAH
SNL	spinal nerve ligation	Tris-HCl	tris(hydroxymethyl)-aminomethane hydrochloride
sPLA2	soluble form of phospholipase A2	TRPV	transient receptor potential vanilloid
TAMRA-FP	carboxytetramethyl-rhodamine fluoro-phosphonate	TSRI	The Scripps Research Institute
TEA	triethylamine	UEF	The University of Eastern Finland
TGH	triacylglycerol hydrolase		

1 Introduction

Enzymes are biological catalysts responsible for mediating a wide variety of metabolic processes. They accelerate both the rate and specificity of metabolic chemical reactions and convert substrates into products. Both the specificity and their mechanistic diversity make enzymes appealing targets also for drug development (Patrick 2009) and today a large number of commonly used drugs are enzyme inhibitors. Currently, structure-based drug design is used, in which the three-dimensional (3D) structure of an enzyme's active site is used in the design of inhibitors of catalytic activity. A molecule acting as an inhibitor binds to the active site of enzyme in either a reversible or irreversible manner and when it is there, it is able to prevent a substrate from entering the enzyme's active site and/or hamper the enzymatic activity in some other way (Thomas 2007). Reversible inhibitors bind to enzymes noncovalently via ionic bonds, hydrogen bonds and hydrophobic interactions i.e. they do not undergo chemical reactions when bound to enzyme but have a tendency to form an equilibrium system with the enzyme and can thereby readily be removed by dilution or dialysis. There are some exceptions, a few reversible inhibitors bind by forming weak covalent bonds. In contrast, irreversible inhibitors predominantly form strong covalent bonds by modifying an essential residue present in the enzyme and thus their effect cannot be reversed. The reversible inhibitors can be classified as a competitive, uncompetitive, and noncompetitive while the irreversible inhibitor can be further classified as group-specific covalent modifying agents, affinity labels, transition state analogs, and suicide inhibitors (mechanism-based inhibitors). Earlier drug discovery attempts were focussed mainly on the development of compounds undergoing noncovalent interaction with targets. This is because of possible off-target interactions through the covalent bond which might result in long lasting detrimental effects. Nevertheless, it is possible to minimize undesirable interactions by modulating the electrophilic warhead reactivity of designed inhibitor. Indeed, several drugs forming covalent bonds with their targets with an acceptable side effect profiles have recently been approved by U.S. Food and Drug Administration (USFDA) (Mah *et al.,* 2014). Additionally, a few more compounds are currently in the preclinical or early-stage clinical phase (Singh *et al.,* 2011, Mah *et al.,* 2014). In fact, many essential and life saving drugs including aspirin, penicillin, omeprazole and clopidogrel were discovered later to act via the covalent mechanism (Mah *et al.,* 2014, Bauer 2015).

The serine hydrolase (SH) superfamily comprises ~240 enzymes in humans and generally they contain a conserved nucleophilic Ser residue which attacks ester, amide and thioester groups of substrate in order to facilitate hydrolysis. In general, SHs are categorized into two subfamilies - the serine proteases (~125 members, such as trypsin, thrombin, subtilisin enzymes) and the metabolic SHs (mSHs, ~115 members) (Simon & Cravatt 2010, Long &

Cravatt 2011). The mSHs include small-molecule hydrolases, such as amidases (e.g. FAAH), lipases (e.g. MAGL, ABHD6, ABHD12), esterases (e.g. acetylcholinesterase (AChE)), and peptidases (e.g. dipeptidyl peptidase-4 (DPP-IV)). Over 60% of mSHs adopt an α/β-fold and normally use a Ser-His-Asp triad for catalysis (Holmquist 2000) while some mSHs employ diverse folds and a catalytic dyad (e.g., Ser-Lys or Ser-Asp) or triad (e.g., Ser-Ser-Lys) (Dodson & Wlodawer 1998, Patricelli & Cravatt 1999). SHs play crucial roles in many biological processes, and inhibitors of several SHs are approved for clinical use, such as in type 2 diabetes (T2D), Alzheimer's disease (AD), and infectious diseases (Bachovchin & Cravatt 2012). However, there are still several poorly characterized SHs with respect to their substrate, inhibitors and (patho)physiological functions (Lord *et al.,* 2013). Due to presence of nucleophilic serine residue, the functional state of most SHs can be assessed by using active-site directed affinity labels such as fluorophosphonates (FPs) (Liu *et al.,* 1999, Simon & Cravatt 2010). At present, several selective inhibitors have been discovered by competitive ABPP (Bachovchin & Cravatt 2012). The competitive ABPP is also used extensively in the present study to confirm the selectivity of developed inhibitors toward specific endocannabinoid hydrolase (FAAH, MAGL, ABHD6/12) over other serine hydrolases (SHs) of mouse brain membrane proteomes (see subsection 5.3.12).

The biological significance of SHs has motivated numerous researchers to develop inhibitors for enzymes in this class, both for use as chemical tools to study enzyme function and as potential new therapeutic agents. This doctoral dissertation focuses on the serine hydrolases that belong to the so-called endocannabinoid system (ECS). Specific blockade of the endocannabinoid hydrolases, fatty acid amide hydrolase (FAAH), monoacyl glycerol lipase (MAGL), α/β-hydrolase domain containing 6 and 12 (ABHD6/12) prolongs the life-time of the endocannabinoids and thus increases their tissue levels. Even though inhibitors of endocannabinoid hydrolases are considered as compounds with great therapeutic potential to treat many common pathological conditions, such as pain, inflammation, central nervous system (CNS) disorders and metabolic disorders, none of the inhibitors have yet reached the clinics.

In this doctoral thesis, the design, synthesis and *in vitro* evaluation of compounds acting as potent and selective inhibitors of FAAH, MAGL and ABHD6 have been carried out. Moreover, a completely novel scaffold for the development of ABHD6 inhibitors has been identified and a dual-acting pharmacological tool possessing both MAGL inhibitory and antihistaminergic activities has been discovered. The inhibitors described in this thesis have great potential in clarifying the therapeutic potential of the ECS, for example, they can be considered as valuable leads for further drug development.

2 Review of the Literature

2.1 EMERGENCE OF THE ENDOCANNABINOID SYSTEM (ECS)

Twenty-five years of extensive research on the endocannabinoid system (ECS) has greatly improved our understanding of this highly complex signalling network. The ECS comprises two G-protein coupled cannabinoid receptors (GPCRs), their endogenous ligands (also known as endocannabinoids), and the proteins responsible for endocannabinoid biosynthesis, transportation and biodegradation.

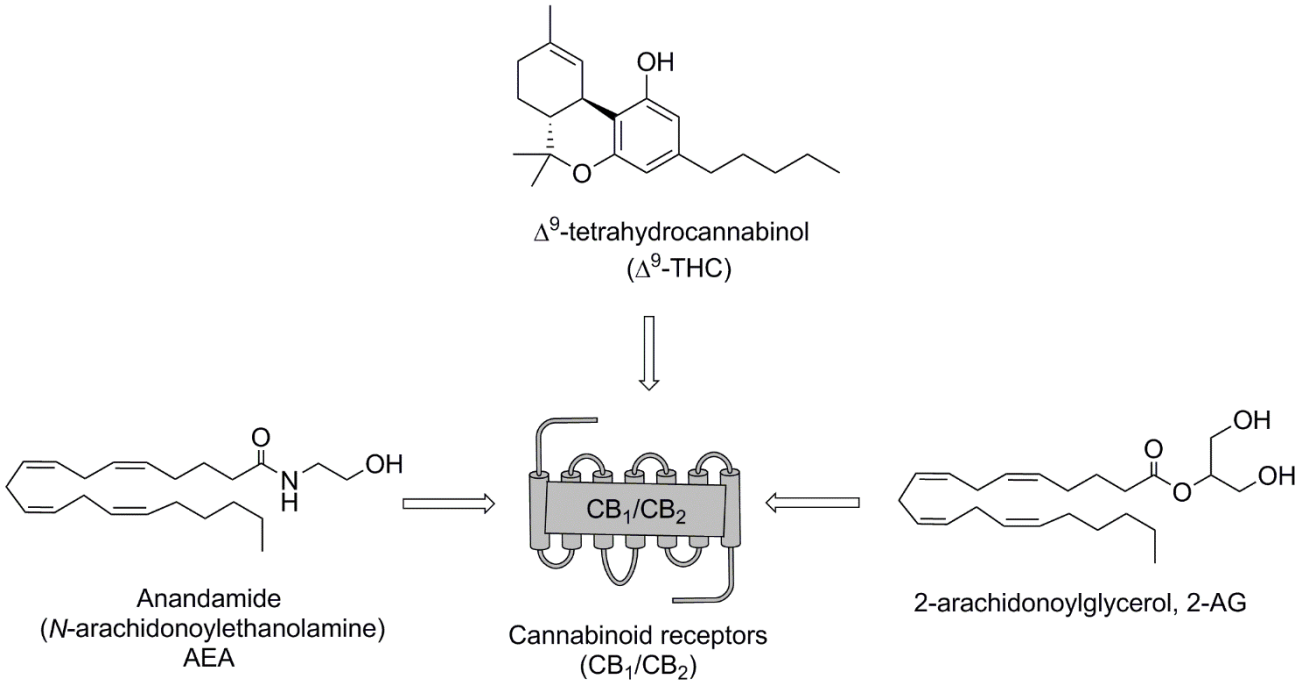


Figure 1. Activation of cannabinoid receptors by Δ^9-THC and endocannabinoids: AEA and 2-AG.

The hemp plant, *Cannabis sativa*, has been used for recreational and medicinal purposes for thousands of years, and it is known to affect mood, memory, cognition, pain etc. In 1964, researchers from Israel isolated the main psychoactive substance of *Cannabis sativa*, Δ^9-tetrahydrocannabinol (Δ^9-THC; Figure 1) (Gaoni & Mechoulam 1964). Later, in the early 1990s, the psychoactive effect of THC was found to be mediated through the activation of two GPCRs that were subsequently named cannabinoid receptor subtype-1, CB_1 (Devane *et al.,* 1988, Matsuda *et al.,* 1990) and cannabinoid receptor subtype-2, CB_2 (Munro *et al.,* 1993) (Figure 1). The CB_1 receptor is expressed mainly in the CNS, but is also present in peripheral tissues, including the lungs, liver, kidneys and adipose tissue (Pagotto *et al.,* 2006). The CB_2 receptor is expressed primarily by immune cells, including microglia in the brain (Munro *et al.,* 1993, Onaivi 2006). It has been found that the majority of the CNS effects of THC are

mediated by the CB_1 receptor (Zimmer *et al.,* 1999, Ledent *et al.,* 1999) while the immunosuppressive effects of cannabinoids are attributable to activation of the CB_2 receptor (Lunn *et al.,* 2006).

Soon after the discovery of the CB_1 receptor, the first endogenous cannabinoid ligand named as *N*-arachidonoylethanolamine (anandamide, AEA; Figure 1) was isolated in 1992 from porcine brain (Devane *et al.,* 1992). A second endocannabinoid, 2-arachidonoylglycerol (2-AG; Figure 1), was identified three years later from canine gut and rat brain (Mechoulam *et al.,* 1995, Sugiura *et al.,* 1995). AEA and 2-AG belong to a much larger class of lipids, so-called *N*-acylethanolamines (NAEs) and monoacylglycerols (MAGs), respectively. The lipids belonging to these classes differ in the length and degree of unsaturation of their acyl chains. Although, AEA and 2-AG have structural similarities and they bind to both cannabinoid receptors, they possess different efficacies *in vitro*: AEA acts as a partial agonist while 2-AG is considered to be a full agonist (Sugiura *et al.,* 1995, Stella *et al.,* 1997, Sugiura *et al.,* 1999, Sugiura *et al.,* 2000, Gonsiorek *et al.,* 2000, Savinainen *et al.,* 2001). Furthermore, the brain levels of 2-AG have been found to be 170-fold higher than those of AEA (Sugiura *et al.,* 1995, Stella *et al.,* 1997).

In addition to AEA and 2-AG, several other endocannabinoids have been described such as 2-arachidonyl glyceryl ether (2-AGE, noladin ether) (Sugiura *et al.,* 1999, Hanus *et al.,* 2001), *N*-arachidonoyl dopamine (NADA) (Bisogno *et al.,* 2000), *N*-stearoylethanolamine (Maccarrone *et al.,* 2002), *O*-arachidonoyl ethanolamine (virodhamine) (Porter *et al.,* 2002), homo-γ-linolenoyl ethanolamide and docosatetraenoylethanolamide (DEA) (Hanus *et al.,* 1993), although their biological activity and metabolism have not been fully clarified. Moreover, several endogenous fatty acid derivatives like *N*-palmitoylethanolamine (PEA) (Sheskin *et al.,* 1997, Lambert *et al.,* 1999), *N*-oleoylethanolamine (OEA) (Rodriguez *et al.,* 2001, Oveisi *et al.,* 2004, Nielsen *et al.,* 2004) and *N*-arachidonoylglycine (Sheskin *et al.,* 1997, Huang *et al.,* 2001) have been identified and termed as ''endocannabinoid-like'' compounds since they lack direct cannabinoid affinity. However, at present, out of all these endocannabinoid lipids, only AEA and 2-AG have been well-explored (Howlett *et al.,* 2002, Piomelli 2003, Pacher *et al.,* 2006).

Endocannabinoid biosynthesis

AEA and 2-AG are not prestored in vesicles but are produced ''on demand'' in postsynaptic nerve terminals and released only when and where necessary in response to physiological or pathological stimuli (Marsicano *et al.,* 2003, Alger & Kim 2011). Biochemical studies have revealed numerous biosynthetic pathways for AEA and 2-AG.

Biosynthetic pathways for AEA

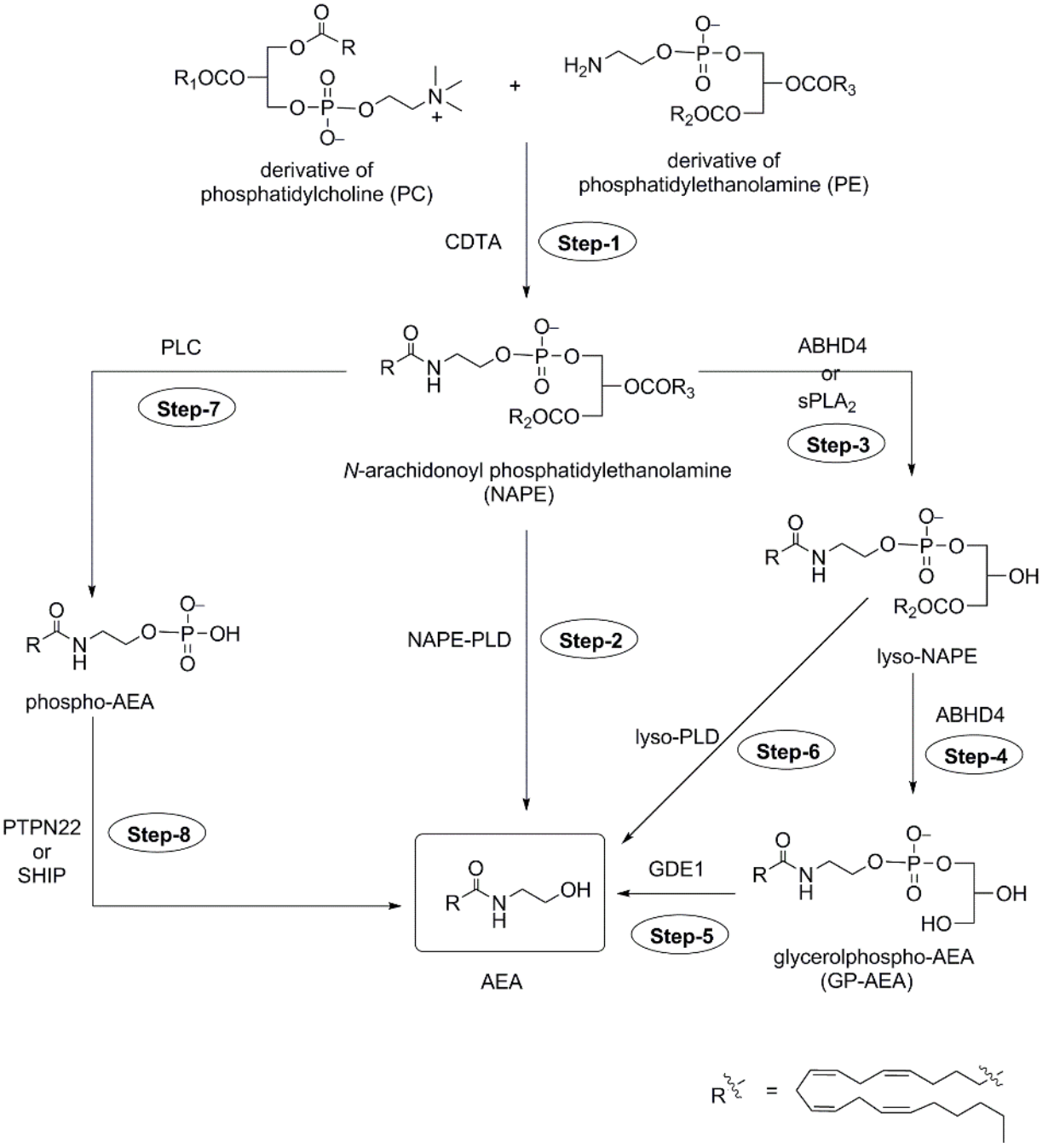


Figure 2. AEA biosynthesis. Abbreviations: CDTA, calcium-dependent transacylase; NAPE-PLD, *N*-arachidonoyl-phosphatidylethanolamine-specific phospholipase D; ABHD4, α/β-hydrolase domain containing 4; $sPLA_2$, soluble form of phospholipase A2; GDE1, glycerophosphodiester phosphodiesterase 1; PLC, phospholipase C; PTPN22, protein tyrosine phosphatase, non-receptor type 22; SHIP, SH2 (Src Homology 2) domain-containing inositol 5'-phosphatase; R_1, R_2 and R_3, fatty acids.

A number of pathways have been proposed for AEA biosynthesis from its phospholipid precursor, *N*-arachidonoyl-phosphatidylethanolamine (NAPE) (Figure 2). NAPE, in turn, is believed to originate from the calcium-dependent transacylase (CDTA) enzymatic transfer of

arachidonic acid (AA) from the *sn*-1 position of phosphatidylcholine (PC) to the primary amine of phosphatidylethanolamine (PE; Step-1) (Cadas *et al.,* 1996, Cadas *et al.,* 1997). Several alternative AEA biosynthesis pathways have been proposed: (i) direct synthesis of AEA from NAPE by NAPE-specific phospholipase D (NAPE-PLD; Step-2) enzyme (Okamoto *et al.,* 2004); (ii) sequential *O*-deacylation of NAPE by α/β-hydrolase domain containing 4 (ABHD4; Steps 3–4) (Simon & Cravatt 2006), followed by the phosphodiesterase-mediated hydrolysis by the glycerophosphodiester phosphodiesterase 1 (GDE1; Step 5) (Simon & Cravatt 2008) (iii) *O*-deacylation of NAPE by a soluble form of phospholipase A2 (sPLA$_{2,}$ Step 3), followed by hydrolysis of the phosphodiester bond by a lyso-PLD enzyme (step 6) (Sun *et al.,* 2004) ; and finally, (iv) conversion of NAPE to phospho-AEA by a phospholipase C (PLC)-like enzyme (step 7) followed by dephosphorylation by protein tyrosine phosphatase, non-receptor type 22 (PTPN22) (Liu *et al.,* 2006) or SH2 (Src Homology 2) domain-containing inositol 5'-phosphatase (SHIP) (step 8) (Liu *et al.,* 2008).

Biosynthetic pathways for 2-AG

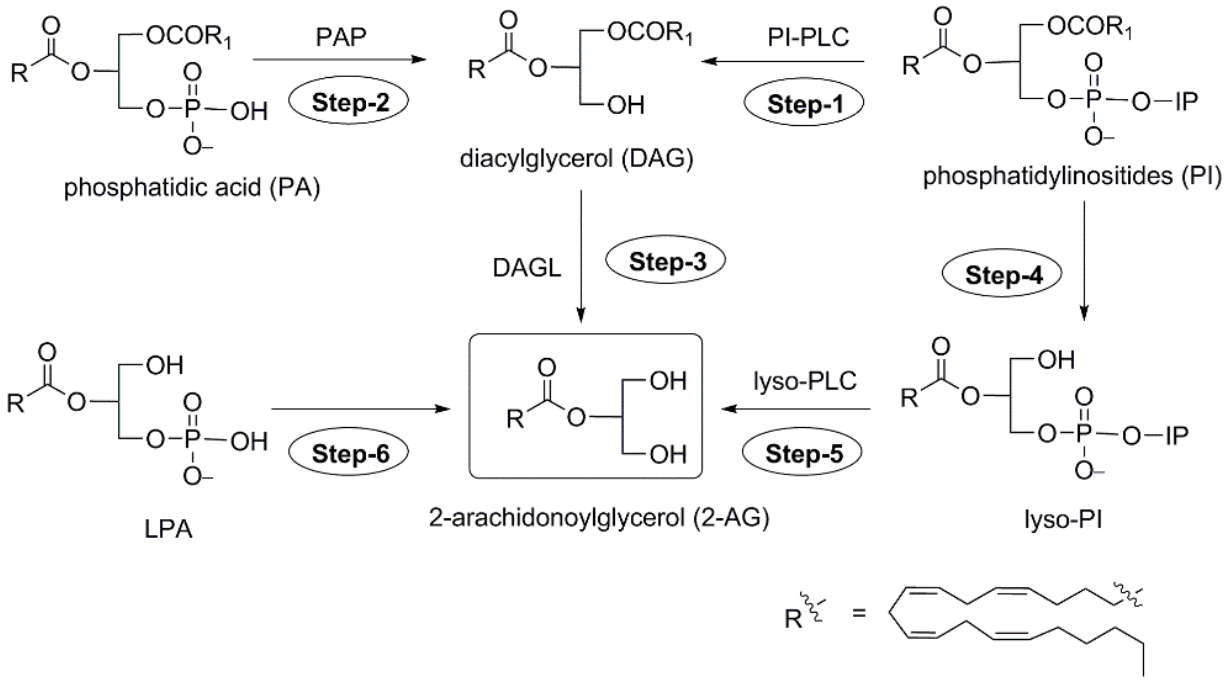


Figure 3. 2-AG biosynthesis. Abbreviations: PI-PLC, phosphatidylinositides (PI)-selective phospholipase C; PAP, phosphatidic acid (PA) phosphohydrolase; DAGL, diacylglycerol lipase; R_1, fatty acid.

The main pathway for 2-AG formation is from the hydrolysis of diacylglycerols (DAGs) in the presence of DAG lipase isoenzymes (DAGLα and DAGLβ; Step-3; Figure 3) (Bisogno *et al.,* 2003). DAGs, in turn, can be produced via the hydrolysis of either phosphatidylinositides (PI), catalyzed by a PI-selective phospholipase C (PI-PLC; Step-1) (Stella *et al.,* 1997, Kondo *et al.,* 1998), or from phosphatidic acid (PA), catalyzed by a PA phosphohydrolase (PAP; Step-

2) (Bisogno *et al.,* 1999). Two other pathways have been proposed: (i) the sequential actions of a phosphatidylinositol-preferring phospholipase A_1 (PLA_1), producing the 2-arachidonoyl-lysophosphatidylinositol (lyso-PI) intermediate, followed by lysoPI-specific phospholipase C (lyso-PLC) generating 2-AG (Steps 4-5) (Ueda *et al.,* 1993); (ii) the conversion from 2-arachidonoyl lysophosphatidic acid (LPA) to 2-AG by phosphatase (Step 6) (Nakane *et al.,* 2002).

Endocannabinoid biodegradation

Once synthesized, the endocannabinoids (AEA and 2-AG) are released from the cell to activate their target cell on presynaptic nerve terminals, and are then rapidly removed from the extracellular space by a selective cellular reuptake system followed by intracellular enzymatic degradation through two different pathways, hydrolysis and oxidation.

Cellular reuptake has been postulated to occur through endocannabinoid membrane transporter (EMT) (Mechoulam & Deutsch 2005, Moore *et al.,* 2005). The involvement of intracellular transporters of AEA, called fatty acid binding proteins (FABP5 and FABP7), have also been suggested (Kaczocha *et al.,* 2009, Maccarrone *et al.,* 2010).

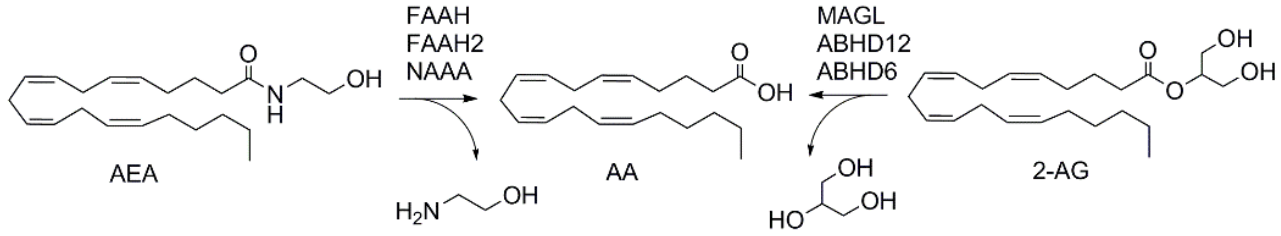


Figure 4. Hydrolytic degradation of AEA and 2-AG by some specific enzymes.

The enzymatic degradation of AEA and 2-AG is caused mainly by FAAH (Cravatt *et al.,* 1996) and MAGL (Dinh *et al.,* 2002, Dinh *et al.,* 2004), respectively (Figure 4). Fatty acid amide hydrolase-2 (FAAH-2) (Wei *et al.,* 2006, Kaczocha *et al.,* 2010) and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) (Tsuboi *et al.,* 2005, Tsuboi *et al.,* 2007, Zhao *et al.,* 2007) have also been claimed to be involved in AEA degradation. However, the main substrate for FAAH-2 is oleamide (Wei *et al.,* 2006) while NAAA hydrolyzes mainly PEA (Tsuboi *et al.,* 2005). In addition to MAGL, 2-AG degradation can be performed by a number of enzymes, such as FAAH (Goparaju *et al.,* 1998), neuropathy target esterase (NTE) (van Tienhoven *et al.,* 2002), and hormone-sensitive lipase (HSL) (Belfrage *et al.,* 1977). Moreover, two additional serine hydrolases, α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12) have been identified as 2-AG degrading enzymes (Figure 4) (Blankman *et al.,* 2007, Savinainen *et al.,* 2012). Additionally, under some specific conditions, 2-AG can be recognized as a substrate

of human carboxylesterases 1 and 2 (CES1 and CES2) (Xie *et al.,* 2010) while both AEA and 2-AG are potential substrates of butyrylcholinesterase (BuChE) (Romani *et al.,* 2011, Barricklow & Blatnik 2013).

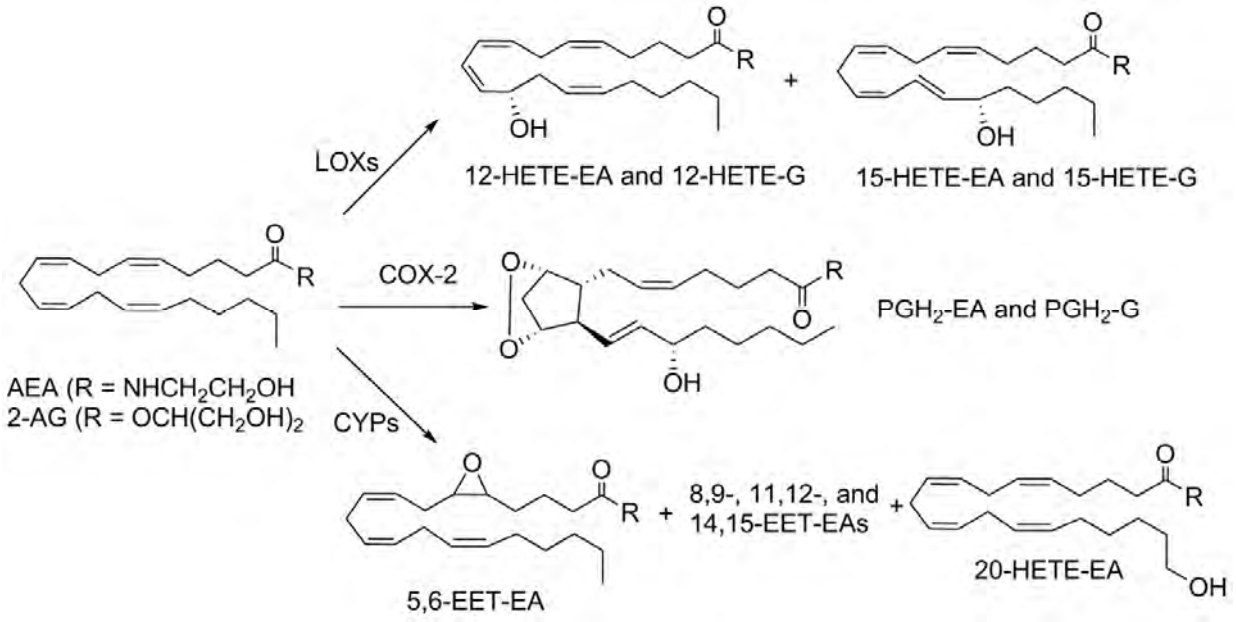


Figure 5. Oxidative degradation of AEA and 2-AG by some specific enzymes.

The endocannabinoids are susceptible to oxidation catalyzed by lipoxygenases (LOXs), cyclooxygenases (COXs), and cytochromes P450 (CYPs) (Kozak & Marnett 2002, Snider *et al.,* 2010, Rouzer & Marnett 2011) (Figure 5). Lipoxygenases, such as 12-LOX and 15-LOX, oxidize both AEA and 2-AG to HETE-EA and HETE-G, respectively, and of these, 15-HETE-G acts as a peroxisome proliferator-activated receptor alpha (PPARα) agonist (Rouzer & Marnett 2011) while 12-HETE-EA is considered to be an endocannabinoid since it can activate cannabinoid receptors (van *et al.,* 2002). If one considers the isoenzymes of COX, then only COX-2 can catalyze the dioxygenation of both AEA and 2-AG to the corresponding endoperoxides as direct precursors of prostaglandin ethanolamides (prostamides, PG-EAs) and prostaglandin glyceryl esters (PG-GEs). Since PG-EAs and PG-GEs have no affinity for the cannabinoid or prostanoid receptors, the endocannabinoid oxygenation by COXs is considered as a termination step (Matias *et al.,* 2004, Gatta *et al.,* 2012). P450-mediated endocannabinoid metabolism has been demonstrated since AEA, but not 2-AG, can be converted into four different epoxy analogues termed as epoxyeicosatrienoic ethanolamides (EET-EAs) and/or hydroxyl analogue, 20-hydroxyeicosatetraenoic acid ethanolamide (20-HETE-EA), by the 4F2, 3A4 and 2D6 classes of CYP P450 (Rouzer & Marnett 2011).

2.2 ENDOCANNABINOID HYDROLASES

2.2.1 Fatty acid amide hydrolase (FAAH)

FAAH is the principal enzyme responsible for the hydrolysis of AEA to AA and ethanolamine (Figure 6). FAAH (EC 3.5.1.4), a 63 kDa membrane-bound protein of 579 amino acid residues, belongs to the amidase signature (AS) family of enzymes (Giang & Cravatt 1997). Mutagenesis and enzymological studies have confirmed the presence of the unusual catalytic triad of Ser^{217}-Ser^{241}-Lys^{142} in the FAAH active site. The degradation step involves nucleophilic attack of the Ser^{241} residue on the carbonyl group of the substrate while Lys^{142} participates as acid/base catalyst to activate nucleophilic Ser^{241} (Figure 7). The Ser^{217} is also essential since it has been shown that its mutation to alanine will lower FAAH activity (Patricelli & Cravatt 1999, Patricelli *et al.,* 1999, Patricelli & Cravatt 2000, Bracey *et al.,* 2002, McKinney & Cravatt 2003).

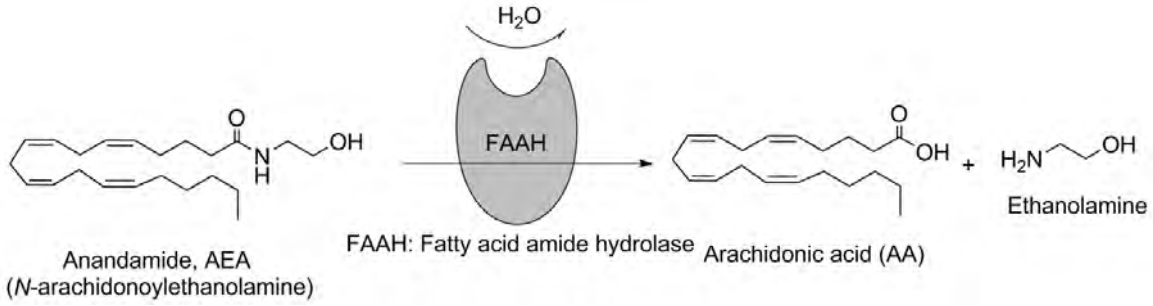


Figure 6. Inactivation of AEA by FAAH to arachidonic acid (AA) and ethanolamine.

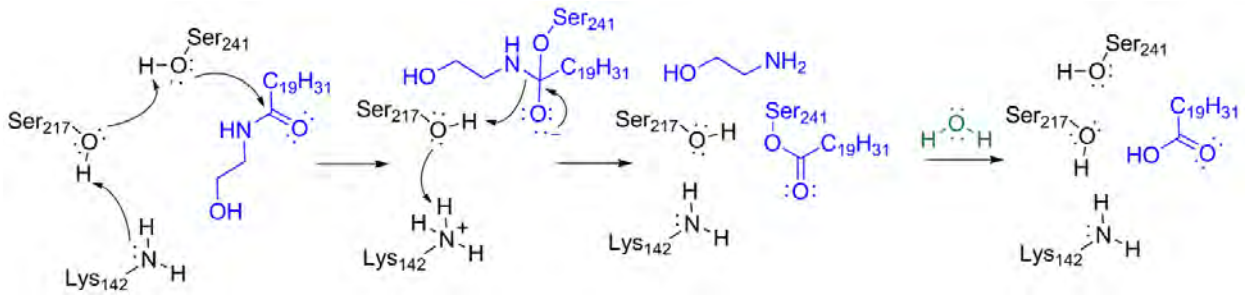


Figure 7. Mechanism for AEA hydrolysis by FAAH.

In addition to AEA, FAAH also hydrolyses several other bioactive amide and ester lipids thereby controlling their endogenous levels. For example with respect to the amides, FAAH regulates (i) *N*-acylethanolamines (NAEs), such as PEA and OEA, both of which are agonists of PPARα and involved in the regulation of inflammation and feeding, respectively (Fowler *et al.,* 2001, Bisogno *et al.,* 2002); (ii) *N*-acyltaurins (NATs) activating the transient receptor potential (TRP) family of calcium channels, including TRPV1 and TRPV4 (Saghatelian *et al.,* 2006); (iii) fatty acid primary amides (FAPAs), such as oleamide which induces sleep (Patricelli *et al.,* 1998). Esters, such as 2-AG are hydrolyzed by FAAH, although to a much lesser extent (Goparaju *et al.,* 1998). Consequently, FAAH inhibition increases the levels of

N-arachidonoyl dopamine, a TRPV1 agonist, and *N*-arachidonoylglycine, a GPR118 agonist (Hu *et al.,* 2009, Bradshaw *et al.,* 2009). Hence, inhibition of FAAH results in several effects which are not only mediated through the cannabinoid receptors but also via non-cannabinoid receptors, such as GPR118, ion channels (e.g. TRPV1) or nuclear receptors (like PPARα).

At present, several X-ray crystal structures of FAAH are available. Bracey *et al.* reported the first X-ray crystal structure of the transmembrane domain truncated rat FAAH (ΔTM-rFAAH) in complex with an irreversible inhibitor, methyl arachidonoyl fluorophosphonate (MAFP (2), see Table 1, subsection 2.3.1) (Bracey *et al.,* 2002). Later, researchers engineered a "humanized" rat FAAH (h/rFAAH) and obtained a second crystal structure in a complex with *N*-phenyl-4-(3-quinolinylmethyl)-1-piperidinecarboxamide (PF-750 (49), see Table 10, subsection 2.3.4). This was followed by several other h/rFAAH structures bound with different FAAH inhibitors such as *N*-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]methyl]-1-piperidinecarboxamide (PF-3845 (51), see Table 10, subsection 2.3.4) (Ahn *et al.,* 2009b), 1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-7-phenylheptane (OL-135 (12), see Table 3, subsection 2.3.2) and other related α-ketoheterocycles (Mileni *et al.,* 2009, Mileni *et al.,* 2010b, Otrubova *et al.,* 2013), and [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate (URB597 (23), also known as KDS-4103, see Table 4, subsection 2.3.3) (Mileni *et al.,* 2010a).

The FAAH knockout mouse has been developed. These animals display elevated AEA levels and exhibit several distinctive properties: (i) an analgesic phenotype in both the carrageenan model of inflammatory pain and the formalin model of spontaneous pain (Lichtman *et al.,* 2004b), (ii) reductions in inflammatory responses (Massa *et al.,* 2004, Cravatt *et al.,* 2004, Karsak *et al.,* 2007), (iii) improvements in sleep and memory (Huitron-Resendiz *et al.,* 2004, Varvel *et al.,* 2007). AEA has been shown to be involved in a number of physiological processes and several reports support the proposal that FAAH inhibition represents a promising novel approach in the treatment of pain, inflammation, anxiety, and depression (Fowler *et al.,* 2001, Labar & Michaux 2007, Ahn *et al.,* 2009a, Blankman & Cravatt 2013). This has stimulated the search for and the development of several classes of compounds acting as reversible or irreversible FAAH inhibitors; these compounds will be discussed in section 2.3.

2.2.2 Other AEA degrading enzymes (FAAH-2 and NAAA)

Fatty acid amide hydrolase-2 (FAAH2)

FAAH-2, a second amidase signature enzyme, is present in humans, but not in lower placental mammals, including mouse and rat (Wei *et al.,* 2006). FAAH-2 is a~60 kDa protein which shares the Ser-Ser-Lys catalytic triad with FAAH. However, it shares only 20%

homology with FAAH. FAAH-2 displays a substrate preference for primary fatty acid amides, such as oleamide, over NAEs, like AEA.

N-Acylethanolamine-hydrolyzing acid amidase (NAAA)

NAAA, a ~40 kDa protein of 359 amino acid residues, belongs to choloylglycine hydrolase family having catalytic triad of Cys^{131}-Asn^{292}-Asp^{150}. NAAA is highly expressed by both rodents and humans in immune cells, specifically in macrophages. In contrast to FAAH, NAAA is most active at acidic pH (Ueda *et al.,* 2001). Cys^{131} residue acts as a catalytic nucleophile in NAAA rather than the Ser moiety in FAAHs. The preferred substrate of NAAA is PEA (the levels of which are increased during inflammation) over AEA. Since NAAA is highly expressed in macrophages, it is considered as an interesting target to treat inflammation (Tsuboi *et al.,* 2005).

2.2.3 Monoacylglycerol lipase (MAGL/MGL)

MAGL is the main enzyme involved in the 2-AG hydrolysis to AA and glycerol (Figure 8) in the brain. MAGL, a 33-kDa membrane-associated protein with 303 amino acid residues, is classified as a member of α/β-hydrolase family. MAGL active site contains the classical catalytic triad (Ser^{122}-His^{269}-Asp^{239}) and the lipase motif GXSXG typical of serine hydrolases. The nucleophilic Ser^{122} is activated by the Asp^{239} and the His^{269} residues (Karlsson *et al.,* 1997). By undertaking ABPP of mouse brain 2-AG hydrolases, it was found that nearly 85% of 2-AG hydrolase activity is attributable to MAGL while remaining 15% is accounted for by two additional serine hydrolases, ABHD6 and ABHD12 (Blankman *et al.,* 2007). It is important to note that in addition to metabolizing 2-AG, all of these three enzymes are involved in the control of other bioactive MAGs levels. For example, MAGL inhibition increases levels of 2-oleoylglycerol (2-OG), an agonist of GPR119 (Hansen *et al.,* 2011).

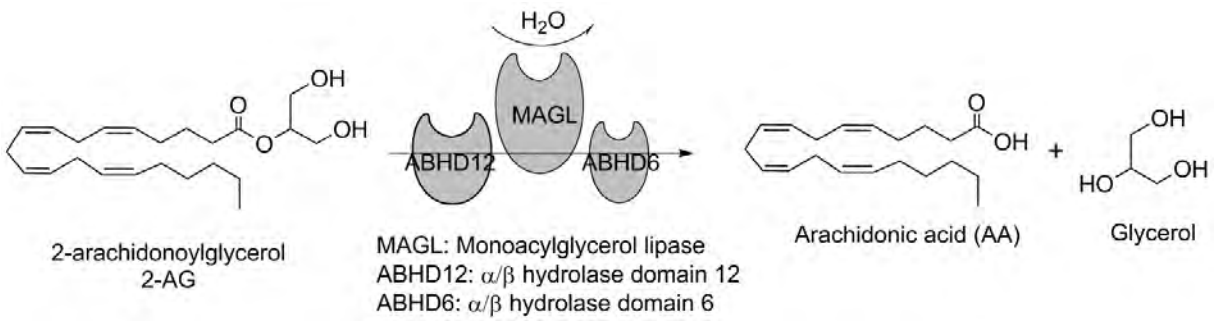


Figure 8. Inactivation of 2-AG by MAGL, ABHD6 and ABHD12 to arachidonic acid (AA) and glycerol

In comparison to FAAH, the first crystal structure of human MAGL (hMAGL) in its apo form and in complex with the covalent inhibitor SAR629 (118) (see Table 24, subsection 2.4.6), a derivative of earlier patented triazolo-carboxamide series, was only reported in 2010 by Sanofi-Aventis (Bertrand *et al.,* 2010). Subsequently, a few more crystal structures of MAGL

have been published (Labar *et al.,* 2010, Schubert *et al.,* 2009, Schalk-Hihi *et al.,* 2011). Like AEA, 2-AG has also been found to be involved in numerous physiological processes, and several reports suggest MAGL inhibition as a promising strategy to treat pain, inflammation, vomiting, nausea, cancer etc (Bisogno *et al.,* 2009, Kinsey *et al.,* 2011, Alhouayek *et al.,* 2011, Sticht *et al.,* 2012, Nomura *et al.,* 2010, Mulvihill & Nomura 2013). At present, several classes of MAGL inhibitors targeting either the cysteine residues (Cys^{201}, Cys^{208}, and Cys^{242}) located close to the catalytic site or the nucleophilic Ser^{122} residue of catalytic triad of MAGL have been described and will be reviewed in section 2.4.

2.2.4 Other 2-AG degrading enzymes (ABHD6 and ABHD12)

α/β-Hydrolase domain containing 6 (ABHD6)

ABHD6 accounts for minor degradation of 2-AG (~4%) to arachidonic acid (AA) and glycerol (Figure 8) (Blankman *et al.,* 2007). ABHD6, a 30-kDa integral membrane protein with 337 amino acid residues, belongs to the α/β-hydrolase family. The ABHD6 active site contains the classical catalytic triad (Ser^{148}-Asp^{278}-His^{306}) which is predicted to face into the cell interior (Blankman *et al.,* 2007, Savinainen *et al.,* 2012). This kind of alignment suggests that ABHD6 might be well-suited to guard the intracellular pool of 2-AG. Recently, a homology model of ABHD6 has been created and this may facilitate the development of ABHD6 inhibitors (Bowman & Makriyannis 2013). Recently, several reports have highlighted the potential importance of ABHD6 inhibitors in the treatment of inflammation, metabolic disorders, and epilepsy (Tchantchou & Zhang 2013, Alhouayek *et al.,* 2013, Thomas *et al.,* 2013, Naydenov *et al.,* 2014) but to date, only a few ABHD6 inhibitors have been described (see section 2.5).

α/β-Hydrolase domain containing 12 (ABHD12)

ABHD12 is responsible for only about 9% of total degradation of 2-AG to AA and glycerol (Figure 8) (Blankman *et al.,* 2007). ABHD12 is a ~ 45 kDa glycoprotein whose active site probably contains the classical catalytic triad (Ser^{246}-Asp^{333}-His^{372}) which is predicted to face the lumen and/or extracellular space. It has been speculated that ABHD12 may be involved in metabolizing the extracellular pool of 2-AG (Blankman *et al.,* 2007, Savinainen *et al.,* 2012). Genetically modified ABHD12 deficient mice were found to develop age-dependent symptoms that resembled a human neurodegenerative disorder, PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract) (Fiskerstrand *et al.,* 2010). Very recently, Blankman *et al.* revealed that ABHD12 controls brain lysophosphatidylserine (LPS) pathways and that these are deregulated in a murine model of the neurodegenerative disease PHARC (Blankman *et al.,* 2013). At present, no X-ray crystal structure of ABHD12 is available which makes it a challenging target for the development of novel inhibitors. However, recently triterpenoid-based compounds were identified as ABHD12 inhibitors (Parkkari *et al.,* 2014).

2.3 INHIBITORS OF FAAH

2.3.1. Substrate-based FAAH inhibitors

Substrate-based FAAH inhibitors are also described as the first generation FAAH inhibitors. The common design strategy utilized was the derivatization of a substrate (such as AEA) by making the carbonyl group more electrophilic/polar to facilitate the nucleophilic attack of reactive residues of an enzyme such as Ser^{241}. The concept was to mimic the tetrahedral intermediate state of an enzyme-substrate complex by forming stable enzyme adducts or hemiketal between the enzyme and the designed inhibitor (Figure 9).

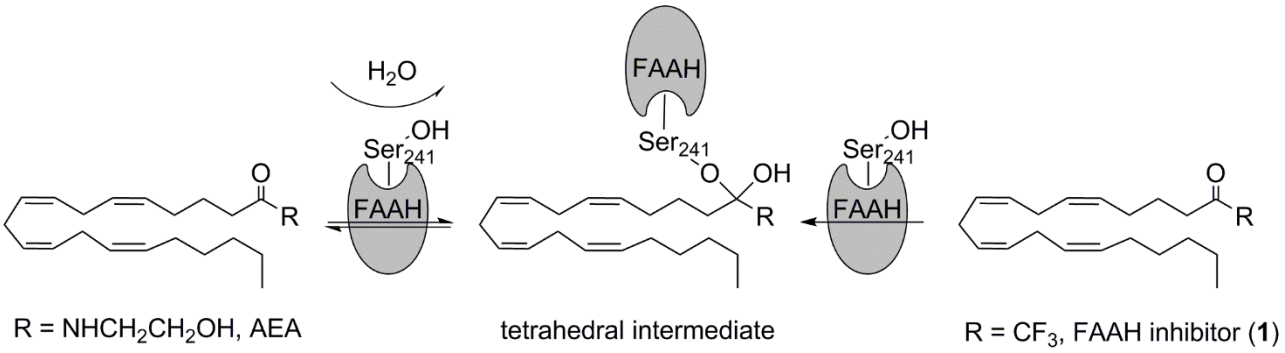
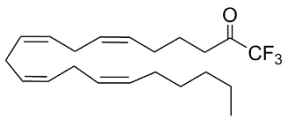
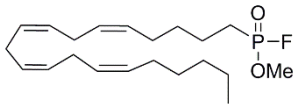
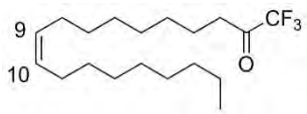
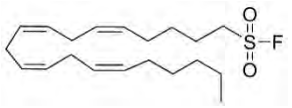
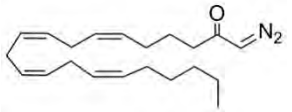
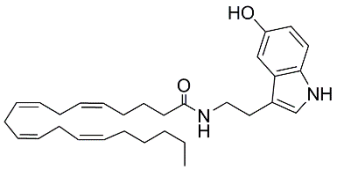


Figure 9. Formation of tetrahedral intermediate between FAAH and AEA / FAAH inhibitor (1)

Arachidonyltrifluoromethyl ketone (ATFMK or ATK, 1) was one of the first reported trifluoromethyl ketone analogues, being the most active reversible FAAH inhibitor at that time (IC_{50} = 1.9 µM, Table 1) (Koutek *et al.,* 1994). The discovery of ATFMK (1) was followed by the identification of several other anandamide and oleamide analogues (Table 1) and of these, the highly potent irreversible FAAH inhibitor methyl arachidonyl fluorophosphate (MAFP, 2) is still used widely as a non-specific pharmacological tool. (IC_{50} = 2.5 nM) (Deutsch *et al.,* 1997). Subsequently, Boger *et al.* synthesized oleoyltrifluoromethyl ketone (3), originally reported as an inhibitor of oleamide hydrolase, as a potent and competitive reversible FAAH inhibitor (IC_{50} = 0.082 µM) (Boger *et al.,* 1999). This research group also highlighted the importance of the presence, position, and stereochemistry of the $\Delta^{9,10}$ *cis* double bond of oleamide. A close analogue of MAFP (2), arachidonylsulfonyl fluoride (4) was shown to be as potent as MAFP (2) (IC_{50} = 0.1 nM) but it was a more selective FAAH inhibitor with respect to its binding to the other studied off-targets (Segall *et al.,* 2003). Arachidonoyl diazomethyl ketone (ADMK, 5) has been found to inhibit reversibly detergent-solubilized FAAH (IC_{50} = 2-6 µM) whereas it behaves as an irreversible inhibitor of native membrane FAAH (IC_{50} = 0.5 µM) (De *et al.,* 1997, Edgemond *et al.,* 1998). *N*-arachidonoyl amino acids, such as arachidonoyl serotonin (AA-5-HT, 6), *N*-arachidonoylglycine (NAGly), *N*-arachidonoyl

isoleucine, and enantiomers of *N*-arachidonoyl alanine also possess moderate abilities to inhibit FAAH activity (Bisogno *et al.,* 1998, Huang *et al.,* 2001, Grazia Cascio *et al.,* 2004).

Table 1. Inhibitory values for the substrate-based compounds 1-6 against FAAH and MAGL.

Compd	Structure	IC_{50} [µM]		References
		FAAH	MAGL	
1 ATFMK (ATK)		1.9[a]	66[b]	Koutek *et al.,* 1994 Saario *et al.,* 2004
2 MAFP		0.0025[a] 0.0010[c]	0.0022[b]	Deutsch *et al.,* 1997 Segall *et al.,* 2003 Saario *et al.,* 2004
3		K_i = 0.082[c]	NA[d]	Boger *et al.,* 1999
4		0.0011[c]	NA	Segall *et al.,* 2003
5 ADMK		2-6[e] 0.5[f]	NA	De *et al.,* 1997 Edgemond *et al.,* 1998
6 AA-5-HT		12-26[g] 5.6[h]	NI[i]	Bisogno *et al.,* 1998

Enzymatic assays were carried out using [a,f] rat brain homogenate protein and [^{3}H]-AEA as the substrate; [b] solubilized COS-7 membrane extracts from cells transiently transfected with human FAAH cDNA and 2-AG as the substrate; [c] solubilized rat liver plasma membrane extracts and ^{14}C-oleamide as the substrate; [d] Not analyzed. Enzymatic assays were carried out using [e] partially purified protein from mouse neuroblastoma N18TG2 or RBL-1 cells or porcine brain microsomal and [^{3}H]-AEA as the substrate; [g] mouse neuroblastoma N18TG2 cells and [^{14}C]-AEA as the substrate; [h] rat basophilic leukemia (RBL-2H3) cells and [^{3}H]-AEA as the substrate. [i] No inhibition.

However, these substrate-based FAAH inhibitors tend to lack selectivity i.e. they also inhibit other enzymes such as MAGL (Table 1). They also bind to the cannabinoid or TRPV1 receptors or have other unknown targets (Koutek *et al.,* 1994, Patterson *et al.,* 1996, Deutsch *et al.,* 1997, Segall *et al.,* 2003, Saario *et al.,* 2004). Nevertheless, both ATK (1) and MAFP (2) are considered as useful pharmacological tools and valuable reference compounds.

2.3.2 α- Ketoheterocycle-based inhibitors of FAAH

The α-ketoheterocycle-based compounds are potent and competitive reversible FAAH inhibitors synthesized mainly by Dale Boger's group. The design of these inhibitors was based on the earlier reported protease inhibitors (Edwards *et al.,* 1995). Extensive work on modifications of oleoyltrifluoromethyl ketone (3) was carried out by (i) replacing the trifluoromethyl group with various carbonyl activating heterocycles and, (ii) optimising the fatty acid chain. This led the identification of hundreds of potent and reversible FAAH inhibitors; some example structures 7-10 are shown in Table 2 (Boger *et al.,* 2000). The reversible, competitive inhibition has been proposed to occur via hemiketal formation with the nucleophilic Ser^{241} of FAAH (Figure 10). Several α-ketoheterocycle-based FAAH inhibitors (Boger *et al.,* 1999, Boger *et al.,* 2000) were later screened using the ABPP. Compound 11 was identified as a potent and highly selective FAAH inhibitor while others were found to react with 45- and 50-kDa brain enzyme KIAA1363 and 60-kDa heart enzyme triacylglycerol hydrolase (TGH) (Leung *et al.,* 2003).

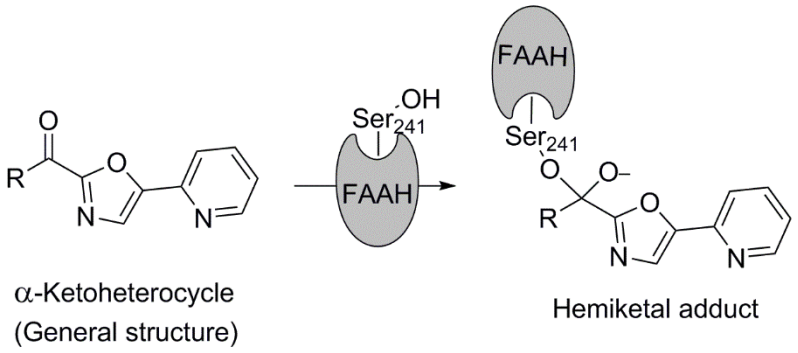
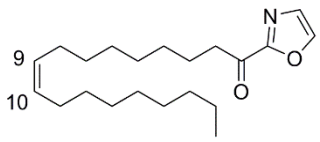
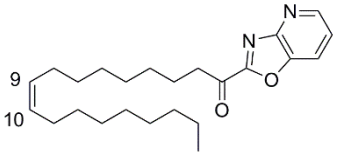
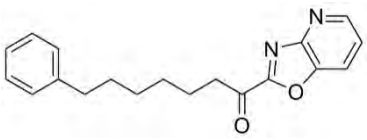
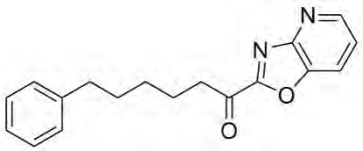
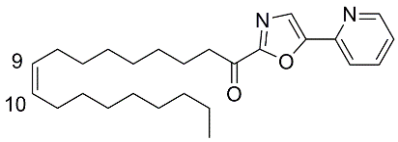


Figure 10. Hemiketal adduct formed between FAAH and an α-keto heterocycle inhibitor

Table 2. Inhibition activity of the α-ketoheterocycle-based compounds 7-11 against FAAH and MAGL.

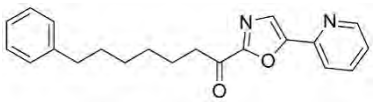
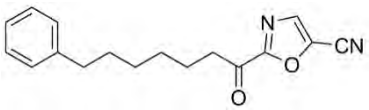
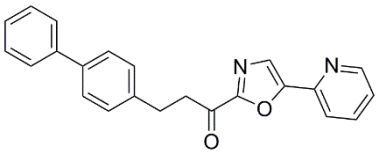
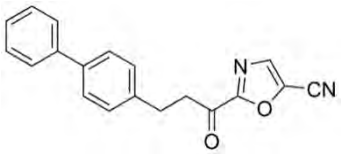
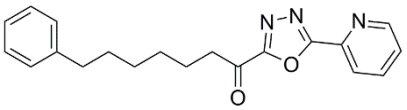
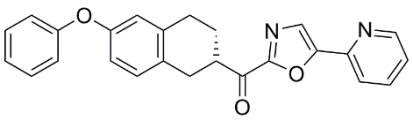
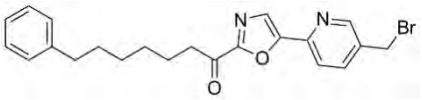
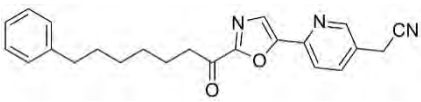
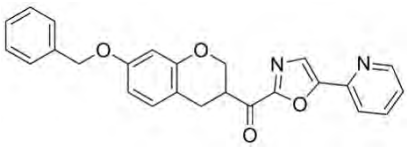
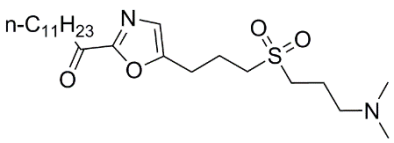
Compd	Structure	K_i (IC_{50}) [µM]		References
		FAAH[a]	MAGL	
7		0.017	NA[b]	Boger *et al.,* 2000
8		0.0023 (0.013)[c]	NA	Boger *et al.,* 2000

9 OL-92		0.00028	NA	Boger *et al.,* 2000
10		0.00020 (0.000094)[c]	NA	Boger *et al.,* 2000
11		IC_{50} = 0.150[d]	NI [e]	Leung *et al*., 2003

[a] Enzymatic assays were carried out by using solubilized rat liver plasma membrane extracts and ^{14}C-oleamide as the substrate. [b] Not analyzed. [c] Enzymatic assays were carried out by using solubilized COS-7 membrane extracts from cells transiently transfected with human FAAH cDNA and ^{14}C-oleamide as the substrate. [d] IC_{50} values determined by using the ABPP assay. [e] No inhibition.

A systematic exploration around the oxazole-based compound 7 was carried out which led to the discovery of 1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-7-phenylheptane (OL-135, 12) as a potent FAAH inhibitor (Ki = 4.7 nM, Table 3) (Boger *et al.,* 2005). In the ABPP assay, this compound showed selectivity for FAAH over other serine hydrolases of different mouse tissues, such as brain, heart, and kidney. After *i.p.* administration in mice, OL-135 (12) significantly elevated the endogenous levels of the AEA (and other lipids such as OEA and PEA) in the brain and spinal cord, and possessed an analgesic effect in multiple pain models (Lichtman *et al.,* 2004a, Chang *et al.,* 2006). However, subsequently it was shown that OL-135 (12) inhibited several carboxyesterases (CEs) (Zhang *et al.,* 2007). Then after, Boger's group conducted further variations on OL-135 (12) by modifying its C2 acyl side chain and/or heterocyclic core to afford several potent FAAH inhibitors 13-20 (Table 3) (Romero *et al.,* 2006, Romero *et al.,* 2007, Hardouin *et al.,* 2007, DeMartino *et al.,* 2008, Kimball *et al.,* 2008, Garfunkle *et al.,* 2008, Ezzili *et al.,* 2011, Otrubova & Boger 2012, Otrubova *et al.,* 2013, Otrubova *et al.,* 2014a, Duncan *et al.,* 2014). Among these, oral administration of (*S*)-enantiomer 17 (CE-12) significantly elevated the brain AEA levels and attenuated mechanical (> 6 h) and cold (> 9 h) allodynia for sustained periods. Interestingly, compounds 18 and 19 displayed interactions with an additional active site residue Cys^{269}. Compound 18 was reported to increase the levels of endogenous substrates to a greater extent and for a much longer duration (> 6 h) than OL-135 (12) and it reversed cold allodynia in the chronic constriction injury (CCI) model of neuropathic pain in mice for a sustained period (> 6 h). Janssen also described several modifications of OL-135 (12) in an attempt to improve its aqueous solubility and pharmacokinetic (PK) profile (Apodaca *et al.,* 2007a, Apodaca *et al.,* 2007b, Timmons *et al.,* 2008). In contrast to the reports from Boger's group, 2-ketooxazoles containing polar head groups (such as $–SO_2$ group in compound 21) in their C5-side chain were reported as FAAH inhibitors (Table 3) (Rusch *et al.,* 2012).

Table 3. Inhibitory activity of the α-ketoheterocycle-based compounds 12-21 against FAAH.

Compd	Structure	FAAH K_i (IC_{50}) [μM]	References
12 OL-135		0.0047[a] (0.0090)[b] [c]IC_{50} = 0.002 (FAAH) and 0.6 (TGH)	Boger *et al.*, 2005
13		0.0004[a] (0.001)[b] [c]IC_{50} = 0.0065 (FAAH) and 0.02 (TGH)	Romero *et al.*, 2006 Romero *et al.*, 2007
14		0.00075 [a] (0.0029)[b] [c]IC_{50} = 0.0007 (FAAH) and 1.2 (TGH)	Hardouin *et al.*, 2007
15		0.0002[a] (0.00026)[b] [c]IC_{50} = 0.001 (FAAH) and 0.026 (TGH)	Kimball *et al.*, 2008
16		0.00029[a] (0.0012)[b] [c]IC_{50} = 0.001 (FAAH) and 0.014 (TGH)	Garfunkle *et al.*, 2008
17 CE-12 (*S*)-enantiomer		0.0044[a] (0.0058)[b]	Ezzili *et al.*, 2011
18		0.0031[a] [c]IC_{50} = 0.20 (FAAH) and 0.9 (TGH)	Otrubova *et al.*, 2013 Otrubova *et al.*, 2014
19		0.0015[a] [c]IC_{50} = 0.090 (FAAH) and 0.450 (TGH)	Otrubova *et al.*, 2013 Otrubova *et al.*, 2014
20 *R* and *S* enantiomers		~0.018 [a]	Duncan *et al.*, 2014
21		0. 364[d]	Rusch *et al.*, 2012

Enzymatic assays were carried out by using [a] purified recombinant rat FAAH expressed in *Escherichia coli* (*E. coli*) and ^{14}C-oleamide as the substrate; [b] lysate of hFAAH-transfected COS-7 cells and ^{14}C-oleamide as the substrate. [c] IC_{50} values determined by the ABPP assay. [d] Enzymatic assays were carried out by using rat brain microsome FAAH and *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamid as the substrate.

To summarize, α-ketoheterocycle-based FAAH inhibitors have predominantly been explored by academic researchers. Due to their excellent potency and high FAAH selectivity with respect to possible off-targets, compounds 17 and 18 are considered to be valuable pharmacological tools. They could also serve as potential candidates for further drug development.

2.3.3 Carbamates as FAAH inhibitors

In 2003, Kathuria *et al.* utilized carbaryl, an anti-cholinesterase agent, and its positional isomer in the development of FAAH inhibitors. They produced highly potent compounds, such as [1,1'-biphenyl]-3-yl cyclohexylcarbamate (URB524, 22) and [1,1'-biphenyl]-3-yl cyclohexylcarbamate (URB597, also known as KDS-4103, 23) that had IC_{50} values of 0.063 and 0.0046 µM, respectively (Table 4) (Kathuria *et al.,* 2003). *P.o.* or *i.p.* administration of URB597 (23) exerted a combination of anxiolytic-like, anti-depressant-like, and analgesic effects by inhibiting FAAH both centrally and peripherally (Gobbi *et al.,* 2005, Piomelli *et al.,* 2006, Russo *et al.,* 2007), and it also reduced carrageenan induced edema (Jayamanne *et al.,* 2006). However, the data related to its efficacy on putative anti-anxiety and anti-depressive properties are controversial (Kathuria *et al.,* 2003, Naidu *et al.,* 2007). Later, it was shown that URB597 (23) also inhibited several CEs (Zhang *et al.,* 2007).

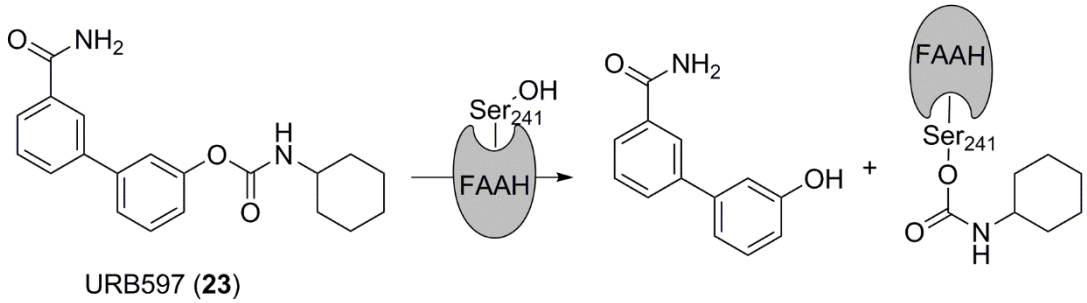
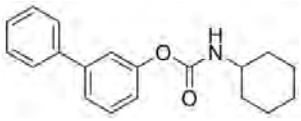
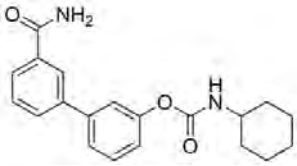
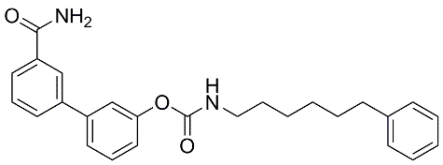
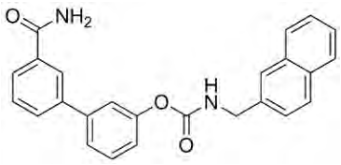
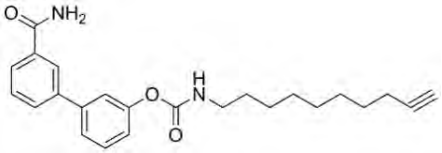
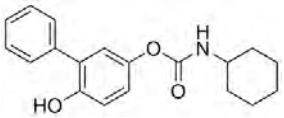
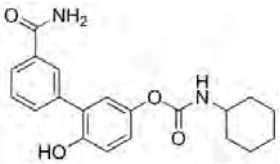
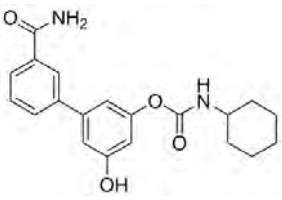


Figure 11. The *O*-biaryl group of URB597 (23) serves as a leaving group, resulting in the carbamylation of the FAAH enzyme

The discovery of URB597 (23) prompted other researchers to explore carbamate-based FAAH inhibitors. Modulation at the *N*-portion of URB597 (23) resulted in FAAH inhibitors (24-26, Table 4) with improved potency (Alexander & Cravatt 2005, Mor *et al.,* 2008). JP83 (24) and JP104 (25) were FAAH selective in the nervous system, but they bound to and interfered with several enzymes in peripheral tissues. Alexander *et al.* also provided experimental evidence that carbamates, such as URB597 (23) could inhibit FAAH via covalent carbamylation of Ser^{241} of FAAH (Figure 11). The details of the modifications made on the distal and proximal phenyl rings of URB524 (22) and URB597 (23) were also reported (Table 4) (Tarzia *et al.,* 2003, Mor *et al.,* 2004, Tarzia *et al.,* 2006, Clapper *et al.,* 2009, Clapper *et al.,* 2010, Moreno-Sanz *et al.,* 2013). This led to the identification of URB694 (27), a potent FAAH inhibitor having less reactivity

with off-target CEs, and URB937 (28), a peripherally restricted potent FAAH inhibitor which nonetheless exerted antinociceptive effects in mice and rats. Moreover, cyclohexylcarbamic acid 3'-carbamoyl-5-hydroxybiphenyl-3-yl ester (29) was identified as the most potent brain-impermeable FAAH inhibitor disclosed to date having an IC_{50} value of 0.0005 µM.

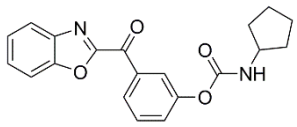
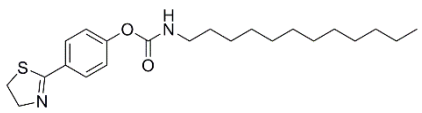
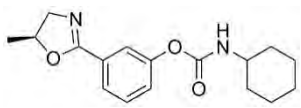
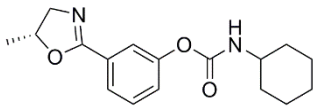
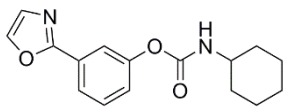
Table 4. Inhibitory activity of the carbamate compounds 22-29 against FAAH and MAGL.

Compd	Structure	IC_{50} [µM]		References
		FAAH	MAGL	
22 URB524		0.063[a]	NI[b]	Kathuria *et al.*, 2003
23 URB597 (KDS-4103)		0.0046[a]	NI[b,*]	Kathuria *et al.*, 2003
24 JP83		0.014[c]	NI	Alexander 2005
25 JP104		0.0073[c]	NI	Alexander 2005
26 URB880		0.00063[a]	100[d]	Mor *et al.*, 2008
27 URB694		0.030[a]	NA[e]	Clapper *et al.*, 2009
28 URB937		0.0028[a]	NA	Clapper *et al.*, 2010
29		0.0005[a]	NA	Moreno-Sanz *et al.*, 2013

Enzymatic assays were carried out by using [a] rat brain homogenates and [^{3}H]-AEA as the substrate. [b] No inhibition [*] (according to the ABPP assay (was found to inhibit CEs)). Enzymatic assays were carried out by using [c] purified recombinant enzyme and ^{14}C-oleamide as a substrate; [d] purified rat recombinant MAGL and 2-OG as the substrate. [e] Not analyzed.

Researchers from the University of Kuopio (now the University of Eastern Finland, UEF) have identified several carbamate-based potent FAAH inhibitors, such as 30-34 as having good selectivity over MAGL (Table 5) (Myllymäki *et al.,* 2007, Minkkilä *et al.,* 2009a, Myllymäki *et al.,* 2009, Käsnänen *et al.,* 2010). This includes the first report of chiral FAAH inhibitors where the *R*-enantiomer of 3-(5-methyl-4,5-dihydrooxazol-2-yl)phenyl cyclohexylcarbamate (FAAH IC_{50} = 0.0068 µM) was found 10-times more potent than its corresponding *S*-enantiomer (see compounds 32 and 33).

Table 5. Inhibitory activity of the carbamate compounds 30-34 against FAAH and MAGL.

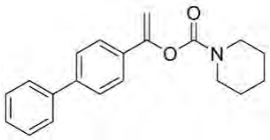
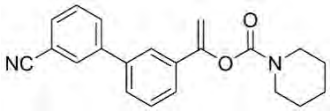
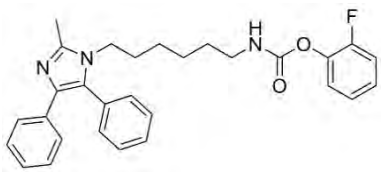
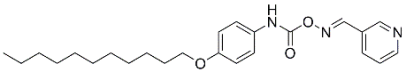
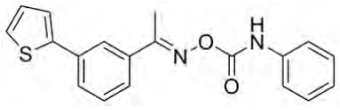
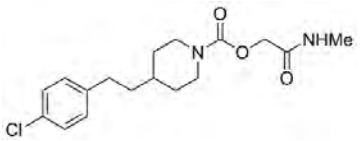
Compd	Structure	IC_{50} [µM] or % inhibition at 100 µM		References
		FAAH[a]	MAGL	
30		0.028	46% [b]	Myllymäki *et al.,* 2007
31		0.0063	2.1[c]	Minkkilä *et al.,* 2009
32		0.0068	14% [c]	Myllymäki *et al.,* 2009
33		0.073	13% [c]	Myllymäki *et al.,* 2009
34		0.0007	NI [b,d]	Käsnänen *et al.,* 2010

Enzymatic assays were carried out by using [a] brain homogenates and [^{3}H]-AEA as the substrate; [b] rat brain membranes and 2-AG as the substrate; [c] human recombinant MAGL and tritium-labelled 2-oleoylglycerol [glycerol-1,2,3-^{3}H] (i.e. [^{3}H]-2-OG) as the substrate. [d] No Inhibition

Several carbamates have been evaluated by pharmaceutical companies and also in collaboration with academic groups (Table 6). For example, Gattinoni *et al.* determined that enol carbamates acted as reversibly acting potent FAAH inhibitors (ST-4070 (35) and 36) (Gattinoni *et al.,* 2010a). Oral administration of ST-4070 (35) at 30 mg/kg produced a similar anti-anxiety effect as diazepam in a mouse model. Bristol-Myers Squibb (BMS) described derivatives of bisarylimidazoles and oximes, such as compounds 37 (BMS-1) and 38, as potent inhibitors of FAAH (Sit *et al.,* 2007, Sit *et al.,* 2010). Interestingly, Gattinoni *et al.* reported oxime carbamates, such as compound 39 (ST4020), having an inversed oxime carbamate bond compared to the BMS compounds (Gattinoni *et al.,* 2010b). Oral

administration of ST4020 (39) (10 mg/10 mL/kg) reduced anxiety but did not affect the locomotor activity in the elevated plus maze. ST4020 (39) also significantly reduced mechanical hyperalgesia in a rat model of neuropathic pain induced by vincristine (Minetti *et al.,* 2009). Niphakis *et al.* published SARs of *O*-hydroxyacetamide carbamates among which 2-(methylamino)-2-oxoethyl 4-(4-chlorophenethyl)piperidine-1-carboxylate (SA-57, 40) showed excellent potency towards mouse and human FAAH with IC_{50} values of 0.0032 and 0.0019 µM, respectively. SA-57 (40) exhibited selectivity for FAAH at low concentrations but at higher concentrations (at 10 µM) the drug inhibited MAGL and ABHD6. It was postulated that SA-57 could be a useful pharmacological tool to study the behavioral effects of complete FAAH blockade in combination with varying degrees of MAGL and ABHD6 inhibition (Niphakis *et al.,* 2012).

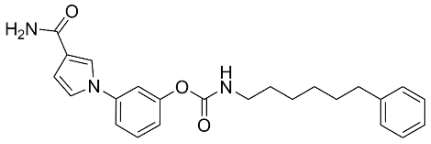
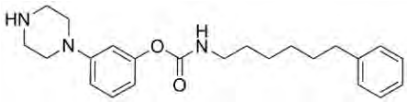
Table 6. Inhibitory values for the carbamate compounds 35-40 against FAAH and MAGL.

Compd	Structure	IC_{50} [µM]		References
		FAAH	MAGL	
35 ST-4070		0.009[a] (K_i = 0.054)[c]	NI[b]	Gattinoni *et al.,* 2010
36		0.013[a] (K_i = 0.046)[c]	NI	Gattinoni *et al.,* 2010
37 BMS-1		0.002[c]	NA[d]	Sit *et al.,* 2007
38		0.00015[c]	NA	Sit *et al.,* 2010
39 ST4020		0.0044[a]	NI	Gattinoni *et al.,* 2010
40 SA-57		0.0032 (mFAAH)[e] 0.0019 (hFAAH)[e]	0.41 (mMAGL)[e] 1.4 (hMAGL)[e]	Niphakis *et al.,* 2012

[a] Enzymatic assays were carried out by using mouse brain homogenates and [^{3}H]-AEA as the substrate. [b] No inhibition observed in mouse brain supernatants using [^{3}H]-2-OG as a substrate. [c] Enzymatic assays were carried out by using homogenates of crude membranes prepared from H4 cells that express transfected human FAAH (H4-FAAH) cells and [^{3}H]-AEA as the substrate. [d] Not analyzed. [e] human and mouse FAAH, MAGL, and ABHD6 orthologues assayed as recombinantly expressed proteins in transfected human embryonic kidney 293 (HEK293) cells and 2-AG as the substrate.

Butini *et al.* reported series of phenylpyrrole-based carbamates as potent FAAH inhibitors. Within the series compound 41 (ST3913, Table 7) showed excellent inhibition of FAAH (IC_{50} 0.6 nM) along with selectivity over other studied potential off-targets (Butini *et al.,* 2012). Moreover, compound ST3913 (41) was orally efficacious (30 mg/kg dose) producing an analgesic effect, and it did not exert any adverse effects. Later, they undertook structural modifications of ST3913 (41) and identified analogue 42 as a potent, reversible and noncompetitive mouse FAAH (mFAAH) inhibitor (Table 7) (Butini *et al.,* 2013).

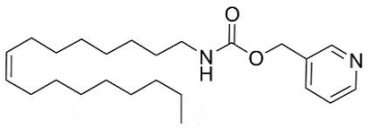
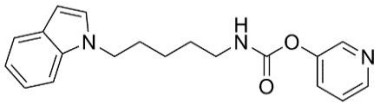
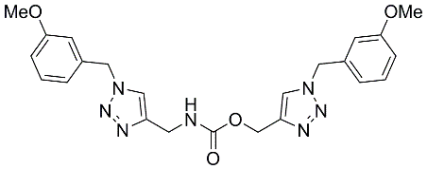
Table 7. Inhibitory activity of the carbamate compounds 41-42 against FAAH and MAGL.

Compd	Structure	K_i (IC_{50}) [nM]			References
		mFAAH[a]	hFAAH[b]	mMAGL[c]	
41 ST3913		0.16 (IC_{50} = 0.60)	NA[d]	NI[e]	Butini *et al.,* 2012
42		13	26 (IC_{50} = 44)	NA[d]	Butini *et al.,* 2013

Enzymatic assays were carried out by using [a] mouse brain homogenates and [^{3}H]-AEA as the substrate; [b] hFAAH and [^{3}H]-AEA as the substrate; [c] mouse brain supernatants and [^{3}H]-2-OG as the substrate. [d] Not analyzed. [e] No inhibition.

A carbamate analogue 43, structurally related to macamides, was shown to be an irreversible inhibitor of FAAH having an IC_{50} value of 0.153 µM (Table 8) (Wu *et al.,* 2013). Terwege *et al.* published detailed SARs of ω-heteroarylalkylcarbamate as FAAH inhibitors among which ω-indolylalkylcarbamate-based compound 44 was identified as a potent FAAH inhibitor (Terwege *et al.,* 2014). Very recently, *O*-(triazolyl)methyl carbamates were reported as a novel and potent class of FAAH inhibitors. In this class, compound 45 had a single-digit nanomolar IC_{50} value for both rat and human FAAH (Colombano *et al.,* 2015). Moreover, compound 45 showed a remarkable improvement over the *O*-arylcarbamate URB524 (22) in terms of its rat plasma stability.

Table 8. Inhibitory activity of the carbamate compounds 43-45 against FAAH and MAGL.

Compd	Structure	IC_{50} [µM]		References
		FAAH	MAGL	
43		0.153[a]	NA[b]	Wu *et al.,* 2013
44		0.0036[c]	0.22[d]	Terwege *et al.*, 2014
45		0.004[e]	NA	Colombano *et al.*, 2015

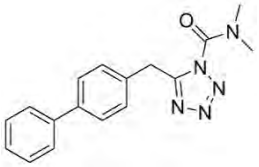
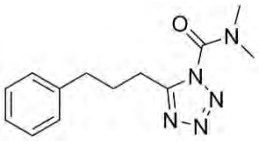
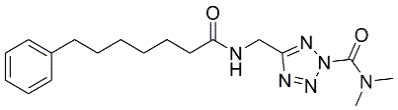
[a] Enzymatic assays were carried out by using human recombinant hFAAH and 7-amino-4-methyl coumarin-arachidonamide (AMC-AA) as the substrate. [b] Not analyzed. Enzymatic assays were carried out by using [c] rat brain microsome and *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide as the substrate; [d] human recombinant MAGL and 1,3-dihydroxypropan-1-yl 4-pyren-1-ylbutanoate as the substrate; [e] brain homogenates and [^{3}H]-AEA as the substrate.

In summary, systematic structural modifications of *O*-arylcarbamate-based irreversible FAAH inhibitors have led to the development of highly potent inhibitors with improved selectivity (e.g. URB597 (23) *vs* URB694 (27)) and biological stability (e.g. URB597 (23) *vs* compound 45). The carbamate scaffold has been proven to be a valuable tool that makes possible different mechanistic approaches when studying the *in vivo* effects of FAAH blockade in the CNS or even solely in the periphery.

2.3.4 Urea derivatives as FAAH inhibitors

The putative endocannabinoid transport inhibitor LY2183240 (46) was identified as a potent albeit non-selective FAAH inhibitor (IC_{50} = 13 nM, Table 9) (Alexander & Cravatt 2006). From the results of a mass spectrometry study it, was concluded that LY2183240 (46) bound covalently to FAAH by carbamylation of Ser^{241} of FAAH with tetrazole moiety acting as a leaving group. Maione *et al*. synthesized tetrazole urea-based, potent FAAH inhibitors 47 and 48 (Table 9) which showed selectivity over other tested endocannabinoid targets, such as MAGL, DAGL, CB_1 and CB_2 (Maione *et al.,* 2008). There are also several other reports describing tetrazole urea analogues as potent FAAH and/or MAGL inhibitors (Ortar *et al.,* 2008, Ortar *et al.,* 2013, Holtfrerich *et al.,* 2013).

Table 9. Inhibitory activity of the tetrazole urea-based compounds 46-48 against FAAH and MAGL.

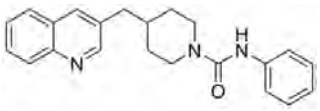
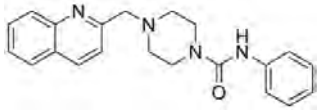
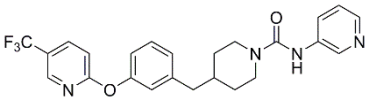
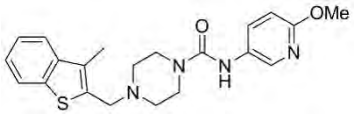
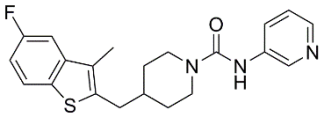
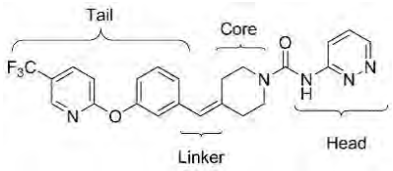
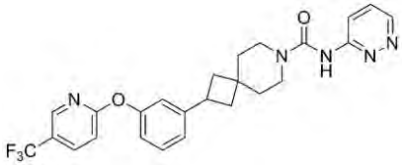
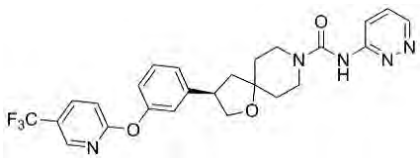
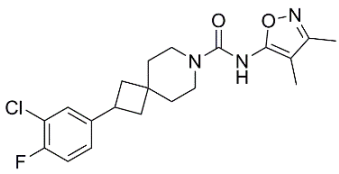
Compd	Structure	IC_{50} [μM]		References
		FAAH	MAGL	
46 LY2183240		0.013[a]	0.0053[a]	Alexander and Cravatt 2006
47 OMDM119		0.0027[b]	10[c]	Maione *et al.*, 2008
48 OMDM122		0.0021[b]	>10[c]	Maione *et al.*, 2008

IC_{50} values were measured by [a] competitive ABPP assays using mice brain membrane proteomes; [b] using rat brain membranes and [^{14}C]-AEA as the substrate; [C] using COS cell cytosolic fractions and [^{3}H]-2-AG as the substrate.

Pfizer and/or the Scripps Research Institute (TSRI) have produced numerous potent and highly selective FAAH inhibitors (49-57, Table 10). Both PF-750 (49) and PF-622 (50) inhibited FAAH in a time-dependent manner by covalently modifying the nucleophile Ser^{241} residue (Ahn *et al.,* 2007). Compound 51 (PF-3845) elevated brain levels of AEA for up to 24 h (Ahn *et al.,* 2009b). It also produced significant CB_1/CB_2-dependent antihyperalgesic effects in the complete Freund's adjuvant (CFA) model of inflammatory pain. Another compound they discovered, PF-465 (52) showed anti-inflammatory activity in a rat CFA model (Johnson *et al.,* 2009). In 2011, they provided details about the highly potent and selective FAAH inhibitor PF-04457845 (54) (Johnson *et al.,* 2011). Oral administration of PF-04457845 (54) at 0.1 mg/kg resulted in efficacy comparable to that obtained with naproxen at 10 mg/kg in a rat model of inflammatory pain. Although being well tolerated in the phase I trials, during phase II trials PF-04457845 (54) was found to be ineffective since it did not reduce osteoarthritis (OA) pain in the patients' knees (Huggins *et al.,* 2012). Pfizer undertook several modifications of PF-04457845 (54) by replacing its methylenepiperidine core with different spirocyclic cores as well as simultaneous optimization at its other modular units i.e. a tail group, an optional hydrocarbon linker and a urea head group; this led the discovery of compounds 55 and 56 (Meyers *et al.,* 2011a). Although selective towards FAAH in the ABPP assay, these compounds were not efficacious *in vivo*. Hence further variations were carried out aimed at improving the drug's PK properties which resulted in the identification of PF-04862853 (57) as a potent and selective FAAH inhibitor (Meyers *et al.,* 2011b). PF-04862853 (57) was finally

taken to the phase I clinical trials as it exhibited good PK profile and it was effective in a pain model.

Table 10. Inhibitory activity of the piperazine/piperidine urea-based compounds 49-57 against FAAH.**

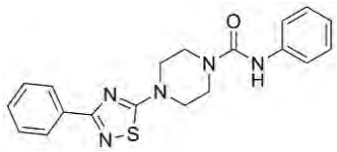
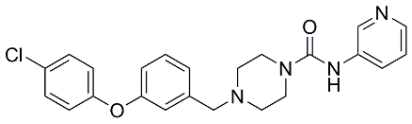
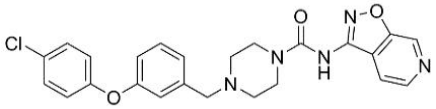
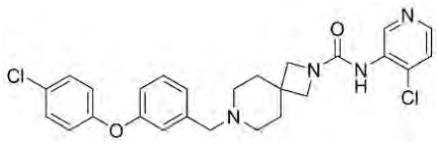
Compd	Structure	k_{inact}/K_i ($M^{-1}s^{-1}$)[a]		References
		rFAAH	hFAAH	
49 PF-750		104	791 IC_{50} = 0.0162 µM[b]	Ahn *et al.*,2007
50 PF-622		154	NA[c] IC_{50} = 0.0330 µM[b]	Ahn *et al.*,2007
51 PF-3845		3900 K_i = 0.23 µM	12,600	Ahn *et al.*,2009
52 PF-465		1060	2931	Johnson *et al.*,2009
53 PF-946		729	4474	Johnson *et al.*,2009
54 PF-04457845		32400	40300	Johnson *et al.*,2011
55		NA	1570	Meyers *et al.*,2011a
56		NA	3040	Meyers *et al.*,2011a
57 PF-04862853		5820	4190	Meyers *et al.*,2011b

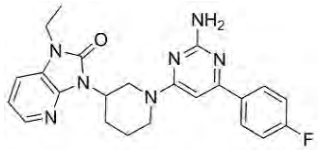
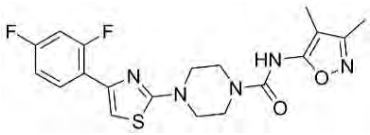
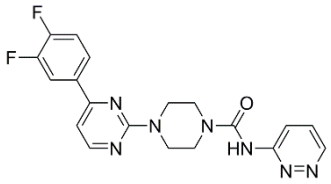
** In the ABPP most of the compounds exhibited high selectivity for FAAH over other serine hydrolases. [a] Determination of inhibitory potencies k_{inact}/K_i($M^{-1}s^{-1}$) was carried out by GDH-coupled FAAH assay (De Bank *et al.,* 2005). Briefly, the production of ammonia generated from the hydrolysis of oleamide by FAAH was monitored. GDH catalyzes the condensation of ammonia and α-ketoglutarate to glutamate with a

concomitant conversion of NADH to nicotinamide adenine dinucleotide, oxidized form (NAD^+), which is spectrophotometrically measured at 340 nm. [b] IC_{50} values were measured for hFAAH and rFAAH (1h pre-incubation with test compound) by GDH-coupled FAAH assay. [c] Not analyzed.

Johnson & Johnson provided details of a series of thiadiazolopiperazinyl aryl and heterocyclic ureas with good PK profiles as a novel class of potent FAAH inhibitors such as compounds 58 (JNJ-1661010) and 59 (JNJ-40355003), respectively (Table 11) (Keith *et al.,* 2008, Keith *et al.,* 2012). JNJ-1661010 (58) exhibited analgesic activity in two models of neuropathic pain i.e. mild thermal injury (MTI) and spinal nerve ligation (SNL) (Karbarz *et al.,* 2008). Oral administration of JNJ-40355003 (59) in rats, dogs and monkeys elevated the plasma and brain concentrations of three FAAs, namely AEA, PEA, and OEA. The elevation of FAAs in a monkey which expressed FAAH-2, strongly suggested that FAAH-2 was not contributing greatly to the breakdown of fatty acid ethanolamides in the plasma. Later, they identified two potent FAAH inhibitors compound 60 and JNJ-42119779 (61) (Tichenor *et al.,* 2012, Keith *et al.,* 2014b). Amgen described a series of novel, noncovalent FAAH inhibitors, such as compound 62 (Table 11) having *in vitro* potency comparable to known covalent FAAH inhibitors and good PK profiles along with reasonable CNS penetration (Gustin *et al.,* 2011). Takeda reported a series of piperazine ureas and identified compounds 63 and 64 as potent FAAH inhibitors having good brain permeability (Table 11) (Kono *et al.,* 2013, Kono *et al.,* 2014). Oral administration of compound 63 was efficacious in the acetic acid-induced writhing test in mice and compound 64 showed robust and dose-dependent analgesic efficacy in animal models of both neuropathic and inflammatory pain.

Table 11. Inhibitory activity of the piperazine/piperidine urea-based compounds 58-64 against FAAH.

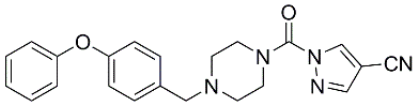
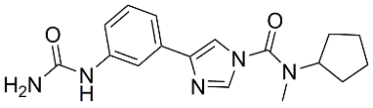
Compd	Structure	IC_{50} (nM)[a]		References
		rFAAH	hFAAH	
58 JNJ-1661010		34	33	Keith *et al.*,2008
59 JNJ-40355003		33	1.4	Keith *et al.*,2012
60		0.7	0.5	Tichenor *et al.*,2012
61 JNJ-42119779		9	8	Keith *et al.*,2014

62		43[b]	14[b]	Gustin *et al.*,2011
63		0.46[c]	0.43[c]	Kono *et al.*,2013
64		0.28[d]	0.72[d]	Kono *et al.*,2014

Values were measured using [a] a 1h preincubation and [^{3}H]-AEA as a substrate; [b] purified rat or human FAAH (with a 2h preincubation) and AMC-AA as the substrate; [C] human and rat FAAH enzyme fractions and [^{3}H]-AEA as the substrate (with 30 min preincubation); [d] purified rat or human FAAH enzyme fractions (with 30 min preincubation) and AMC-AA as the substrate

Recently, Otrubova *et al.* identified several pyrazole-based ureas such as compound 65 (Table 12) as potent irreversible inhibitors of FAAH (Otrubova *et al.,* 2014b). BIAL-Portela & Ca. disclosed in a patent that a urea-based compound 66 was a potent, peripherally selective and metabolically stable FAAH inhibitor (Rosa *et al.,* 2014).

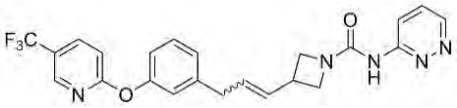
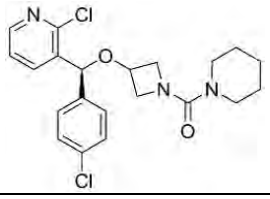
Table 12. Inhibitory activity of the urea-based compounds 65 and 66 against FAAH.

Compd	Structure	K_i (nM) rFAAH	References
65		0.1 [a]	Otrubova *et al.* 2014b
66		0.7 % [b,c]	Rosa *et al*., 2014

[a] Values were measured with a 3h preincubation using the ABPP assay. [b] Values were measured with brain, liver or lung protein and [^{3}H]-AEA as the substrate. [c] Remaining activity after 8 h in mouse liver (oral administration of compound 66 (3 mg/kg)).

Pfizer designed novel FAAH inhibitors by replacing piperidine of PF-3845 (51) with azetidine (exemplified by compound 67, Table 13) (Wang *et al.,* 2009a). The *in vitro* ABPP of both *cis* and *trans* stereoisomers of 67 showed similar selectivity profiles as observed for PF-3845 (51). However, they failed to achieve comparable *in vivo* efficacy to PF-3845 (51). Vernalis produced a chiral azetidine urea-based compound VER-156084 (68) having an *S*-configuration (Hart *et al.,* 2009). Its *R*-isomer was inactive at inhibiting both enzymes. However, VER-156084 (68) suffered from a poor PK profile (Roughley *et al.,* 2012).

Table 13. Inhibitory activity of the azetidine urea-based compounds 67 and 68 against FAAH.

Compd	Structure	k_{inact}/K_i ($M^{-1}s^{-1}$)[a]		References
		rFAAH	hFAAH	
67 (cis and trans isomers)		*cis* 5390 *trans* 2380	*cis* 9000 *trans* 9340	Wang *et al.,* 2009
68 VR-156084		0.078[b]	0.531[b]	Hart *et al.,* 2009 Roughley *et al.,* 2012

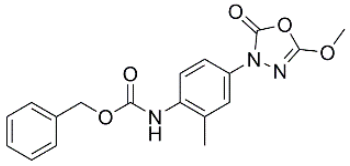
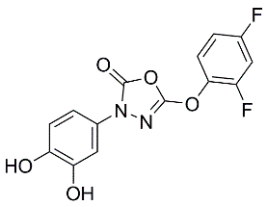
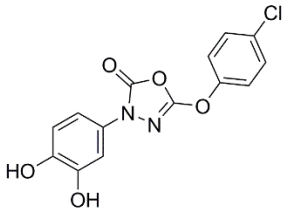
Inhibitory activies have been determined by using [a] rat or human FAAH and AMC-AA as the substrate; [b] rat or human FAAH (pre-incubation 3h) and AMC-AA probe.

In general, piperidine/piperazine urea-based irreversible FAAH inhibitors have been mainly developed by pharmaceutical companies. It appears that these types of compounds offer an excellent balance of potency, selectivity, and PK profiles, and hence, have been considered as promising drug candidates.

2.3.5 1,3,4-Oxadiazol-2-ones as FAAH inhibitors

Commercially available hormone sensitive lipase (HSL) inhibitor, CAY10499 (69, Table 14) was identified as a potent inhibitor of both FAAH and MAGL (Minkkilä *et al.,* 2009b). Since inhibition of both FAAH and MAGL was attributed to the 1,3,4-oxadiazol-2-one pharmacophore, numerous 1,3,4-oxadiazol-2-ones were synthesized and identified as potent and reversible inhibitors of both FAAH and MAGL (Minkkilä *et al.,* 2009b, Käsnänen *et al.,* 2013). BIAL - Portela & Ca. described several 1,3,4-oxadiazol-2-ones as potent FAAH inhibitors. For example, catechols 70 and 71 at doses of 10 mg/kg were found to be peripherally restricted FAAH inhibitors and it was proposed that these compounds could be useful in the treatment of certain cardiovascular disorders such as hypertension and heart failure (Kiss *et al.,* 2011). In short, 1,3,4-oxadiazol-2-one represents a promising scaffold for further exploration.

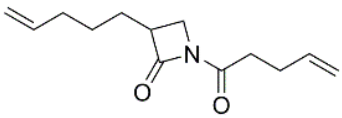
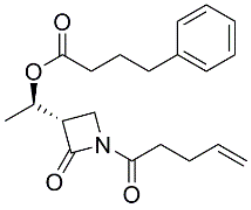
Table 14. Inhibitory activity of the 1,3,4-oxadiazol-2-ones 69-71 against FAAH and MAGL.

Compd	Structure	IC_{50} [µM]		References
		FAAH	MAGL	
69 CAY10499		0.086[a]	0.092[b]	Minkkilä *et al.*,2009
70		0.004[c]	NA[d]	Kiss *et al.*,2011
71		0.006[c]	NA	Kiss *et al.*,2011

Values were measured with [a] rat brain homogenate (10 min preincubation) and [^{3}H]-AEA as the substrate; [b] human recombinant MAGL (hMAGL) activity with 2-AG as the substrate. [c] Values were measured with rat brain (without cerebellum) (15 min preincubation) and [^{3}H]-AEA as the substrate. [d] Not analyzed.

2.3.6 β-Lactam-based FAAH inhibitors

Table 15. Inhibitory activities of β-lactam compounds 72 and 73 against FAAH and MAGL.

Compd	Structure	IC_{50} [µM]		References
		hFAAH[a]	hMAGL[b]	
72		4.5	657	Urbach *et al.*,2008
73		0.005	4.06	Feledziak *et al.*,2009

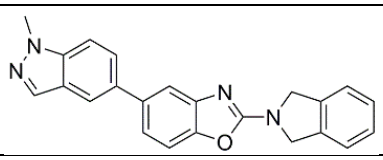
[a] Values were measured with hFAAH (10 min preincubation) and [^{3}H]-AEA as the substrate. [b] hMAGL activity (10 min preincubation) was measured using [^{3}H]-OG as the substrate.

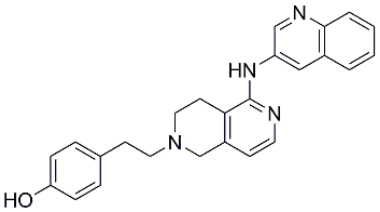
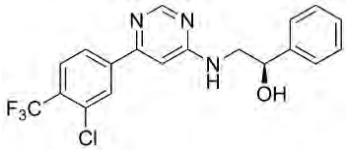
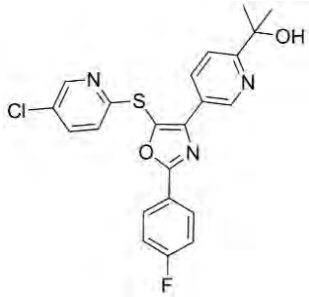
Urbach *et al*. have described the properties of β-lactams/2-azetidinones as inhibitors of hFAAH (Urbach *et al.,* 2008). The best compound in this series, 3-(pent-4-en-1-yl)-1-(pent-4-enoyl)azetidin-2-one (72), had an IC_{50} value of 4.5 µM and ~146 fold selectivity for hFAAH versus hMAGL (Table 15). Later, they identified several potent inhibitors of hFAAH, such as compound 73 which exhibited an IC_{50} value in the nanomolar range and higher selectivity (~ 800-fold) over MAGL (Feledziak *et al.,* 2009). Interestingly, these kinds of analogues were found to have a reversible mechanism of hFAAH inhibition as determined by time-dependent preincubation and rapid dilution studies and confirmed in docking analyses in a homology model of FAAH. Due to their reversible mode of FAAH inhibition, the β-lactam class of compounds should receive further attention.

2.3.7 FAAH inhibitors lacking electrophile

Renovis investigated a series of 2,5-disubstituted benzoxazole and tetrahydropyridopyridine analogues and claimed compounds 74 and RN-450 (75) were potent and reversibly acting FAAH inhibitors (Table 16) (Gowlugari *et al.,* 2012, Estiarte *et al.,* 2012). Very recently, researchers from Janssen Pharmaceutical produced a series of 1-aryl-2-[(6-aryl)pyrimidin-4-yl]aminoethanols as competitive inhibitors of FAAH (Keith *et al.,* 2014a). The best compound of the series (*R*)-2-({6-[3-chloro-4-(trifluoromethyl)phenyl]pyrimidin-4-yl}amino)-1-phenylethan-1-ol (JNJ-40413269, 76) showed excellent inhibitory potency towards FAAH with optimal PK properties. It was also efficacious in the SNL model of neuropathic pain in the rat. Merck has also developed the oxazole-based compound MK-4409 (77) as a potent, selective, and reversible noncovalent modifying FAAH inhibitor (Chobanian *et al.,* 2014). MK-4409 (77) showed a promising preclinical profile and excellent efficacy in pain models such as CFA and SNL. Infinity Pharmaceuticals patented several isoxazoline heterocyclic FAAH inhibitors lacking electrophile (Behnke *et al.,* 2010). In summary, this unique class of compounds lacking electrophile has shown encouraging outcomes in preclinical studies and holds potential for further development as a drug candidates.

Table 16. Inhibitory activities of the compounds 74-77 lacking electrophile against FAAH.

Compd	Structure	IC_{50} [µM]		References
		rFAAH	hFAAH	
74		NA[a]	0.0012[b]	Estiarte *et al.*, 2012

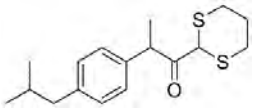
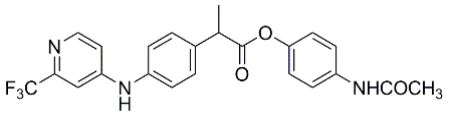
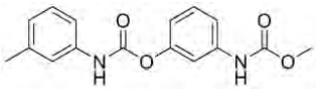
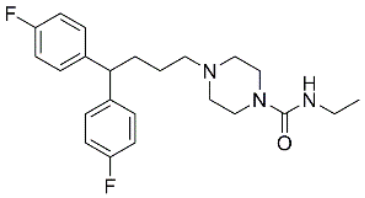
75 RN-450		0.025[c]	0.013[c]	Gowlugari *et al.*,2012
76 JNJ-40413269		0.0063[d]	0.0053[d]	Keith *et al.*,2014
77 MK-4409		0.011[e]	0.011[e]	Chobanian *et al.*,2014

[a] Not analyzed. Values were measured [b] with human FAAH expressed in HEK293 cells with a 3h preincubation and AMC-AA as the substrate; [c] by using HTS assay: rat brain homogenates or human FAAH expressed in Chinese hamster ovary (CHO) cells (with a 2 min preincubation) and AMC-AA as the substrate; [d] with human FAAH or rat FAAH (1h preincubation) and [^{3}H]-AEA as the substrate; [e] cell lysate or microsome fractions with the fluorescent substrate AMC-AA or [^{3}H]-AEA.

2.3.8 Marketed drugs and its derivatives as FAAH inhibitors

Several non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, flurbiprofen, naproxen and carprofen have been utilized in the development of FAAH inhibitors (like compound 78, Table 17) (De Wael *et al.,* 2010), dual FAAH-COX inhibitors (compounds 132-137, Table 29, section 2.6) (Holt *et al.,* 2007, Favia *et al.,* 2012, Fowler *et al.,* 2013, Cipriano *et al.,* 2013) or multi-target compounds (like 145 and 146, Table 29, section 2.6) (Rose *et al.,* 2014). Attempts to reveal new properties of already marketed compounds have resulted in the identification of phenmedipham (79) and amperozide (80) as inhibitors of human FAAH, (Vincent *et al.,* 2009). In addition, some ester analogues of paracetamol have been identified as FAAH inhibitors (see compound 81) (Onnis *et al.,* 2010). In summary, screening of marketed drugs has led to the successful identification of several potent FAAH inhibitors. Many of them are being studied for their dual blockade of FAAH-COX or as multiple target acting compounds.

Table 17. Inhibitory values for the marketed drugs and its derivatives 78-81 against FAAH and MAGL.

Compd	Structure	IC_{50} [μM]		References
		FAAH	MAGL	
78		1.38[a]	NA[b]	De Wael *et al.*,2010
79		0.377[c]	NA	Vincent *et al.*,2009
80 Phenmedipham		1.34[c]	NA	Vincent *et al.*,2009
81 Amperozide		0.10[d]	1.9[e]	Onnis *et al.*,2010

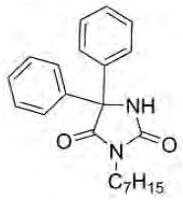
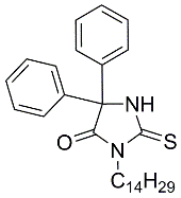
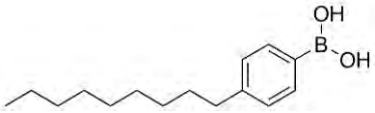
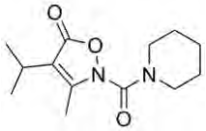
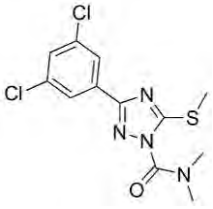
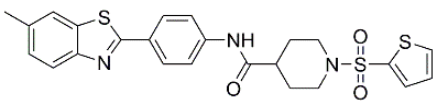
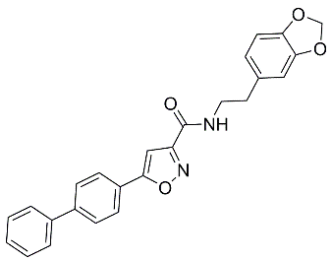
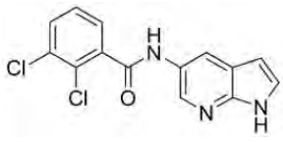
[a] Inhibitory activities were measured by using recombinant human FAAH (10 min preincubation) and [^{3}H]-AEA as a substrate. [b] Not analyzed. Values were measured by using [c] rat brain homogenates (HTS assay) or human FAAH expressed in CHO cells (with a 2 min preincubation) and AMC-AA as the substrate; [d] rat brain homogenate (10 min preincubation) and [^{3}H]-AEA as the substrate; [e] human recombinant MAGL (hMAGL) activity with 4-nitrophenylacetate as the substrate.

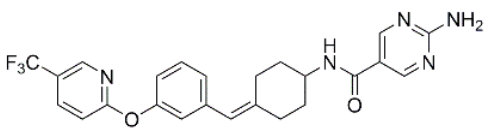
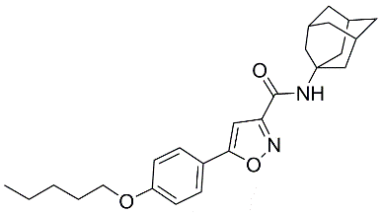
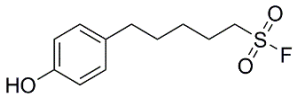
2.3.9 Miscellaneous FAAH inhibitors

Several substituted 2-thioxoimidazolidin-4-ones (thiohydantoin) and imidazolidine-2,4-diones (hydantoin) have been reported to be reversible and competitive FAAH inhibitors; examples are compounds 82 and 83, which showed pI_{50} values of 5.12 and 5.94, respectively (Table 18) (Muccioli *et al.,* 2006). Minkkilä *et al.* described boronic acid, 2*H*-isoxazol-5-one and carbamoyltriazole containing analogues 84-86 (Table 18), respectively, as potent FAAH inhibitors having selectivity over MAGL (Minkkilä *et al.,* 2008, Minkkilä *et al.,* 2009b). Infinity Pharmaceuticals has been granted several patents describing boronic acid analogues as potent FAAH inhibitors (Adams *et al.,* 2008, Behnke *et al.,* 2009, Castro *et al.,* 2010b, Castro *et al.,* 2010a). Abbott has described the identification through HTS of the benzothiazole analogue 87 (Table 18) which they claimed to be a potent, selective and reversible FAAH inhibitor (Wang *et al.,* 2009b). In addition, several diverse amide analogues 88-91 (Table 18) have been described as potent FAAH inhibitors (Scott *et al.,* 2011, Andrzejak *et al.,* 2011, Kharul *et al.,* 2013, Tourteau *et al.,* 2014) and of these, compound 89 (AZ513) acted as a noncovalent, reversible, and noncompetitive inhibitor of FAAH. The structural features of compound 90 resemble a well-known FAAH inhibitor, PF-04457845 (54). The sulfonyl fluoride-based analogue 5-(4-hydroxyphenyl)pentanesulfonyl fluoride (AM3506, 92, Table

18) exhibited good selectivity for FAAH over other serine hydrolases in the ABPP assay and it normalized cardiovascular function in hypertension without causing adverse metabolic effects (Godlewski *et al.,* 2010, Alapafuja *et al.,* 2012). The rapid dilution assay and mass spectrometry analysis suggested that AM3506 inhibited FAAH in an irreversible manner by forming a covalent bond with Ser^{241} of FAAH.

Table 18. Inhibitory values for the miscellaneous compounds 82-92 against FAAH and MAGL.

Compd	Structure	IC_{50} [µM]		References
		FAAH	MAGL	
82		7.58[a] (pI_{50} = 5.12)	NA[b]	Muccioli *et al.*,2006
83		1.15[a] (pI_{50} = 5.94)	NA	Muccioli *et al.*,2006
84		0.0091[a]	7.9[c]	Minkkilä *et al.*,2008
85		0.00045[a]	6[c]	Minkkilä *et al.*,2009
86		0.030[a]	4.6[c]	Minkkilä *et al.*,2009
87		0.018[d]	NA	Wang *et al.*,2009
88		0.088[a]	NI[e] at 30 µM	Andrzejak *et al.*,2011
89 AZ513		0.027[a] (0.551)[f]	NA	Scott *et al.*,2011

90		0.0006 for hFAAH[g] 0.007 for rFAAH[h]	NA	Kharul *et al.*, 2013
91		0.008[i]	NA	Tourteau *et al.*,2014
92 AM3506		0.0028[j]	0.383[k]	Alapafuja *et al.*,2012

[a] Values were measured by using rat brain homogenate protein (10 min preincubation) and [^{3}H]-AEA as the substrate. [b] Not analyzed. Inhibitory activity was measured by using [c] human recombinant MAGL and 2-AG as the substrate; [d] rat FAAH enzyme expressed in HEK293 cells and [^{3}H]-AEA as the substrate; [e] human recombinant MAGL and [^{3}H]-2-OG as the substrate; human FAAH expressed in CHO cells (with a 30 min preincubation) and AMC-AA as the substrate; [g] human FAAH as enzyme source and AMC-AA as the substrate; [h] rat FAAH as the enzyme source and AMC-AA as the substrate; [i] recombinant human FAAH preparation (expressed in *E. coli*) and [^{3}H]-AEA as the substrate. For the inhibition assay [j] rat ΔTM FAAH was expressed in *E. coli* cells (with a 15 min preincubation) and AMC-AA was used as the substrate; [k] recombinant hexahistidine-tagged human MAGL (hMAGL) was expressed in *E. coli* cells (with a 15 min preincubation) and AMC-AA as the substrate.

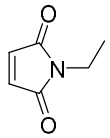
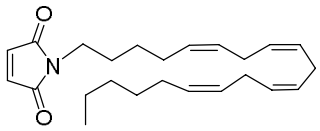
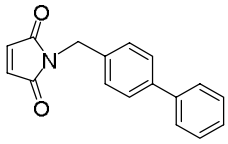
2.4 INHIBITORS OF MAGL

To date, numerous classes of MAGL inhibitors targeting either the nucleophilic cysteine residues (Cys^{201}, Cys^{208}, and Cys^{242}) located close to the catalytic site or the nucleophilic Ser^{122} residue of MAGL have been described.

2.4.1 Maleimide-based MAGL inhibitors

Inhibition of MAGL by *p*-chloromercuribenzoic acid (*p*-CMB), mercury chloride ($HgCl_2$), and *N*-ethylmaleimide (NEM, 93) pointed to the existence of essential thiol containing residues in the active site of MAGL (Table 19) (Tornqvist & Belfrage 1976, Sakurada & Noma 1981). Saario *et al.* developed several NEM analogues, among which *N*-arachidonylmaleimide (NAM, 94) was found to be the most potent MAGL inhibitor (IC_{50} = 0.14 µM) (Saario *et al.*, 2005). They also investigated binding of NAM with a comparison model of MAGL and provided an initial insight into the location of the cysteine residues (Cys^{208} and Cys^{242}) near the binding site. Site mutagenisis and mass spectrometry studies revealed that NAM formed a Michael conjugate addition product with Cys^{242} of human MAGL thereby causing its inhibition (Zvonok *et al.*, 2008). Matuszak *et al.* also synthesized *N*-substituted maleimide derivatives among which 1-biphenyl-4-ylmethylmaleimide (95) inhibited MAGL with an IC_{50} value of 790 nM in an irreversible manner (Matuszak *et al.*, 2009).

Table 19. Inhibitory activities of the maleimide compounds 93-95 against MAGL and FAAH.

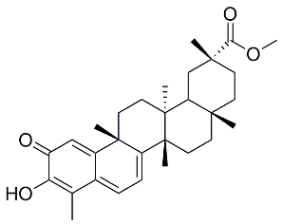
Compd	Structure	IC_{50} [µM]		References
		MAGL	FAAH	
93 NEM		53[a]	NA[b]	Saario *et al.*,2005
94 NAM		0.140[a] (1.10)[c]	3.3[d]	Saario *et al.*,2005
95		0.79[c]	16.6[d]	Matuszak *et al.*,2009

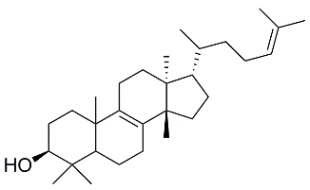
[a] Inhibitory activity has been determined by using rat cerebellar membranes and 2-AG as the substrate. [b] Not analyzed. Inhibitory activity has been determined by using [c] human recombinant MAGL and [^{3}H]-2-OG as the substrate; [d] hFAAH and [^{3}H]-AEA as the substrate.

2.4.2 Natural compounds as MAGL inhibitors

King *et al.* screened a natural compound library and identified two bioisosteric terpenes, pristimerin (96) and euphol (97) as being potent and reversible MAGL inhibitors (IC_{50} = 93 nM and 315 nM, respectively) (Table 20) (King *et al.,* 2009b). Moreover, with the help of site mutagenesis studies, they demonstrated that pristimerin and euphol could interact with Cys^{208} and Cys^{201} respectively. Interestingly, both pristimerin and euphol were also found to inhibit ABHD6 with IC_{50} values of 98 nM and 9 µM, respectively. However, they did not affect the activity of ABHD12.

Table 20. Inhibitory activity of the triterpenoid compounds 96 and 97 against MAGL and FAAH.

Compd	Structure	IC_{50} [µM]		References
		rMAGL[a]	rFAAH[b]	
96 Pristimerin		0.093	NI[c]	King *et al.,* 2009

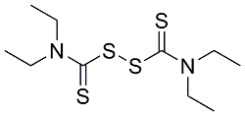
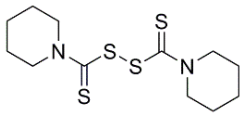
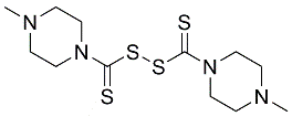
97 Euphol		0.315	NA[d]	King *et al.,* 2009

Inhibitory activity has been determined by using [a] purified *E. coli* recombinant rat MAGL and 2-OG as the substrate; [b] rat brain FAAH and [^{3}H]-AEA as the substrate. [c] No inhibition. [d] Not analyzed.

2.4.3 Disulfide-based MAGL inhibitors

Labar *et al*. found disulfiram (98), a marketed drug used for decades to treat alcoholism, and related analogues (such as 99) could act as inhibitors of MAGL (Table 21). They inhibited MAGL in an irreversible manner through an interaction with Cys^{208} and/or Cys^{242} of hMAGL (Labar *et al.,* 2007). Later, Kapanda *et al*. published a detailed SAR study of bis(dialkylaminethiocarbonyl)disulfide derivatives and identified them as potent hMAGL inhibitors having high selectivity over FAAH such as compound 100 (Kapanda *et al.,* 2009). Their MAGL activity was attributed to the presence of both thiocarbonyl groups and disulfide moiety which interacted with Cys^{208} and Cys^{242} of hMAGL.

Table 21. Inhibitory activity of the disulfide compounds 98-100 against MAGL and FAAH.

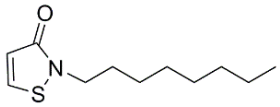
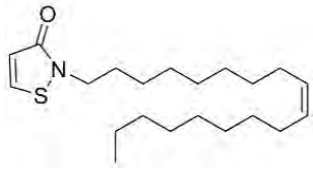
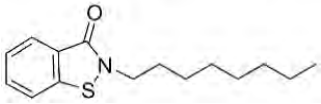
Compd	Structure	IC_{50} [μM]		References
		hMAGL[a]	hFAAH[b]	
98 Disulfiram		0. 36	NA[c]	Labar *et al.,*2007
99		0.13	NA	Labar *et al.,*2007
100		0.109	> 1000	Kapanda *et al.,*2009

Inhibitory activity was determined using [a] human recombinant MAGL and [^{3}H]-2-OG as the substrate; [b] hFAAH and [^{3}H]-AEA as the substrate. [c] Not analyzed.

2.4.4 Isothiazolinone-based MAGL inhibitors

King *et al.* described a new family of isothiazolinone-based MAGL inhibitors (101-103, Table 22) (King *et al.,* 2009a). Kinetic and site-directed mutagenesis studies showed that octhilinone (101) inhibited MAGL through a partially reversible mechanism that involved a specific interaction with Cys^{208}.

Table 22. Inhibitory activity of the isothiazolinone and benzisothiazolinone compounds 101-103 against MAGL and FAAH.

Compd	Structure	IC_{50} [µM]		References
		MAGL	FAAH	
101 Octhilinone		0. 088	NA[b]	King *et al.*,2009
102		0.043	NA	King *et al.*,2009
103		0.059	NA	King *et al.*,2009

[a] Inhibitory activity was determined using purified rat MAGL and 2-OG as the substrate. [b] Not analyzed.

2.4.5 Carbamate-based MAGL inhibitors

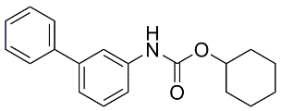
Hohmann *et al.* stated that the carbamate-based compound URB602 (104) was a selective MAGL inhibitor (IC_{50} = 28 µM) (Table 23) (Hohmann *et al.,* 2005). However, subsequently several research groups showed that it also inhibited FAAH with almost similar potency (IC_{50} = 17 µM) (Vandevoorde *et al.,* 2007). Later, Szabo *et al.* investigated a series of URB602 analogues and of these, compound 105 displayed 74% inhibition of MAGL at 100 µM compared to 27% for the parent compound URB602 (Szabo *et al.,* 2011).

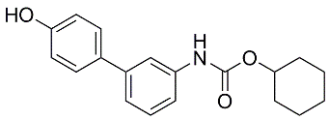
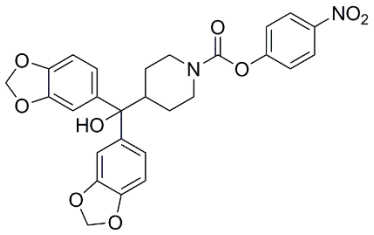
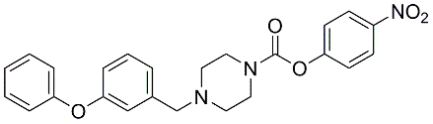
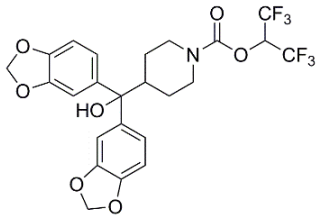
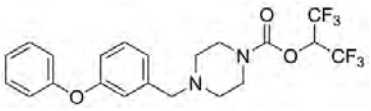
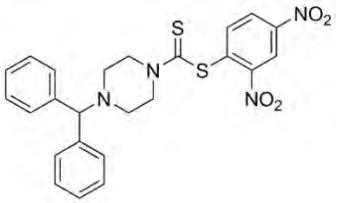
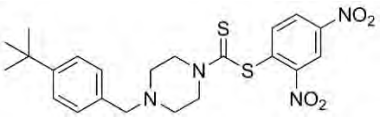
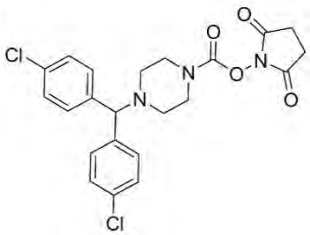
Long *et al.* used competitive ABPP to screen a structurally diverse library of carbamates and discovered 4-bisarylcarbinol analogue JZL184 (106) as a potent MAGL inhibitor having good selectivity (>100-fold) over other serine hydrolases (Table 23) (Long *et al.,* 2010). Moreover, they identified 4-aryloxybenzyl-based analogue JZL195 (107) as a dual FAAH-MAGL

inhibitor. JZL184 (106) treated mice had increased brain 2-AG levels and exhibited a broad array of CB_1-dependent behavioral effects, including analgesia, hypothermia and hypomotility (Long *et al.,* 2009a). JZL184 (106) irreversibly inhibited MAGL via carbamoylation of the enzyme's serine nucleophile (Long *et al.,* 2009b). However, due to the residual low-level cross-reactivity with FAAH and peripheral carboxylesterases (CESs), JZL184 (106) may not be a suitable compound for investigating in biological studies with high doses or chronic treatment. JZL195 (107) was found to produce a CB_1 agonist like effect in the mouse tetrad test. JZL195 (107) also evoked THC like responses in a drug discrimination behavior test and these could be reversed by treatment with a CB_1 antagonist (Long *et al.,* 2009a, Long *et al.,* 2009b, Schlosburg *et al.,* 2010). Chang *et al.* produced an *O*-hexafluoroisopropyl (HFIP) carbamate analog of JZL184 (106), KML29 (108, Table 23) which potently and selectively inhibited MAGL *in vitro* and *in vivo* with minimal cross-reactivity with central and peripheral serine hydrolases, including no detectable activity against FAAH (Chang *et al.,* 2012). Furthermore, a dual FAAH-MAGL inhibitor JZL195 (107) was converted to an HFIP carbamate analog, leading to identification of potent MAGL inhibitor JW642 (109).

Kapanda *et al.* reported 2,4-dinitroaryldithiocarbamate-based compound 110 (CK16) to be a potent MAGL inhibitor, and compound 111 (CK37) as a dual FAAH-MAGL inhibitor (Table 23) (Kapanda *et al.,* 2012). They found that these compounds behaved as irreversible inhibitors of MAGL, interacting with Cys^{208} or Cys^{242} and Ser^{122} residues of the enzyme. When tested in a murine melanoma cell line that endogenously expresses MAGL, CK16 (110) only slightly increased 2-AG levels in contrast to the robust increase observed with CK37 (111). Niphakis *et al.* reported that MJN110 (112, Table 23), an *N*-hydroxysuccinimidyl (NHS) carbamate analog of JZL184 (106) /KML29 (108), possessed superior potency and comparable selectivity to the previously developed MAGL inhibitors, such as KML29 (108), with both acute and chronic dosing regimens (Niphakis *et al.,* 2013). Moreover, MJN110 (112) could reverse established mechanical allodynia in a rat model of diabetic neuropathy, pointing to a potential clinical application for MAGL inhibitors in the treatment of chronic pain caused by diabetes.

Table 23. Inhibitory activities of the carbamate compounds 104-112 against MAGL, ABHD6 and FAAH.

Compd	Structure	IC_{50} [µM]			References
		MAGL	ABHD6	FAAH	
104 URB602		28[a]	NA[b]	17[c]	Hohmann *et al.*,2005 Vandevoorde *et al.*,2007

105		26%[d]	NA	NA	Szabo *et al.*,2011
106 JZL184		0.010[e]	3.27[e]	4.7[e]	Long *et al.*,2009 Long *et al.*,2010
107 JZL195		0.013[e]	0.050[e]	0.019[e]	Long *et al.*,2009 Long *et al.*,2010
108 KML29		0.015[e]	4.87[e]	>50[e]	Chang *et al.*,2012
109 JW642		0.0076[e]	0.107[e]	31[e]	Chang *et al.*,2012
110 CK16		0.354[f]	NA	>1000[g]	Kapanda *et al.*,2012
111 CK37		0.154[f]	NA	1.73[g]	Kapanda *et al.*,2012
112 MJN110		0.0095[e]	0.260[e]	>100[e]	Niphakis *et al.*,2013

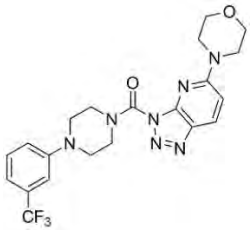
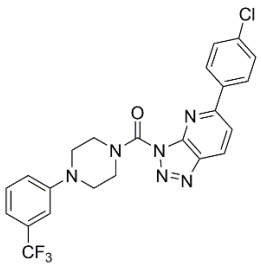
[a] Inhibitory activity was determined by using cytosolic fractions from rat brain and 2-OG as the substrate. [b] Not analyzed. [c] Inhibitory activity was determined by using rat brain homogenate and [^{3}H]-AEA as the substrate. [d] Remaining activity at 100 μM compared to 73% for the URB602 (human recombinant MAGL used as the enzyme source and 4-nitrophenylacetate (4-NPA) as the substrate). [e] Inhibitory activity was determined by using competitive ABPP: reductions in FP-Rh labeling of MAGL, ABHD6, and FAAH were quantified following preincubation of proteomic samples with the test compound. Brain membrane proteomes were used to measure the inhibition of mouse and rat enzymes, whereas human enzymes were evaluated from proteomes of transiently transfected HEK293T cells. Inhibitory activity was determined by using [f] human recombinant MAGL and [^{3}H]-2-OG as the substrate; [g] human recombinant FAAH and [^{3}H]-AEA as the substrate.

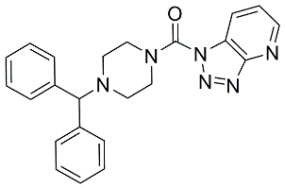
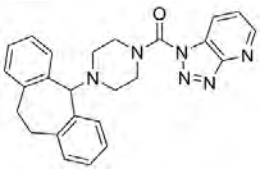
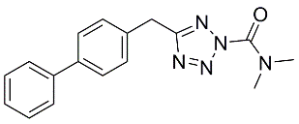
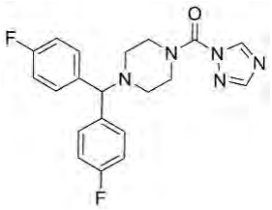
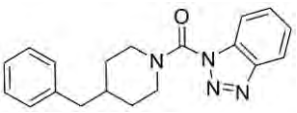
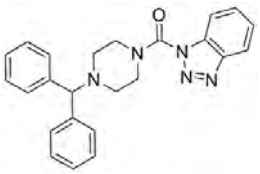
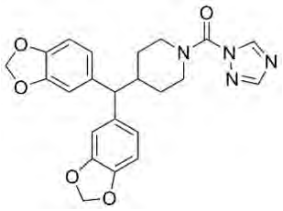
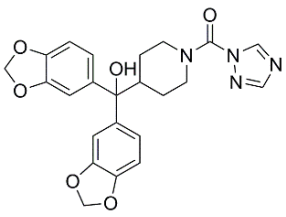
2.4.6 Urea-based MAGL inhibitors

Sanofi-Aventis possess several patents describing triazolopyridine carboxamide derivatives as dual FAAH-MAGL inhibitors (such as 113 and 114, Table 24) (Even & Hoornaert 2008a, Even & Hoornaert 2008b) or MAGL inhibitors (such as 115 and 116, Table 24) (Hoornaert 2008c). Zvonok *et al.* identified the 2,5-LY2183240 isomer 5-[(biphenyl-4-yl)methyl]-*N*,*N*-dimethyl-*2H*-tetrazole-2-carboxamide (AM6701, 117) as a potent inhibitor of MAGL (Table 24) (Zvonok *et al.,* 2008). Later Bertrand *et al*. from Sanofi-Aventis published the first crystal structure of human MAGL in its apo form and in a complex with the covalent inhibitor SAR629 (118, Table 24), a derivative of an earlier patented triazolo-carboxamide series (Bertrand *et al.,* 2010).

Morera *et al.* reported that benzotriazol-1-yl carboxamide derivatives functioned as dual FAAH-MAGL inhibitors (such as 119) or MAGL inhibitors (like compound 120, ML30) (Table 24) (Morera *et al.,* 2012). Our group revealed the importance of the 1,2,4-triazole leaving group in piperidine/piperazine ureas if one wanted to achieve excellent potency and selectivity (Aaltonen *et al.,* 2013, Korhonen *et al.,* 2014). Compound 121 (JJKK-048, Table 24) was found to be potent inhibitor of both human and rodent MAGL (IC_{50} < 0.4 nM). The 1,2,4-triazole analogue of JZL184 (106) (compound 122, Table 24) was also identified as a potent MAGL inhibitor with good selectivity over FAAH.

Table 24. Inhibitory activity of the urea compounds 113-122 against MAGL and FAAH.

Compd	Structure	IC_{50} [μM]		References
		MAGL	FAAH	
113		0.004[a]	0.010[b]	Even 2008a
114		0.002[a]	0.002[b]	Even 2008b

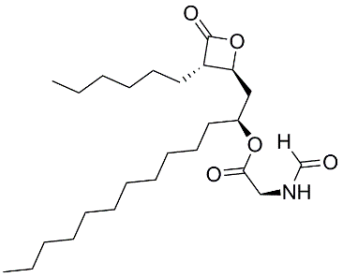
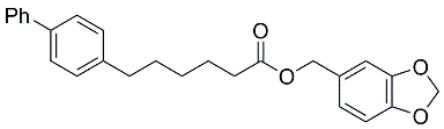
115		0.004[a]	NA[c]	Hoornaert 2008c
116		0.025[a]	NA	Hoornaert 2008c
117 AM6701		0.0017[d] (0.0009)[e]	NA	Zvonok *et al.*,2008
118 SAR629		0.0011[f] (219 pM)[g]	NA	Bertrand *et al.*,2010
119		0.003[h]	0.002[i]	Morera *et al.*,2012
120 ML30		0.0054[h] (0.0044)[f] (0.0019)[g] (0.0015)[j]	0.562[i]	Morera *et al.*,2012
121 JJKK-048		214 pM[f] (275 pM)[g] (363 pM)[j]	4.8[k]	Aaltonen *et al.*,2013
122		0.0007[j]	1.6[k]	Korhonen *et al.*,2014

Inhibitory activity was determined by using [a] mice brain homogenate (cerebellum and medulla eliminated) and [^{3}H]-2-OG as the substrate; [b] mouse brain homogenate (cerebellum and medulla eliminated) and [^{3}H]-AEA as the substrate. [c] Not analyzed. [d] Inhibitory activity was determined by using fluorescence-based assay by using either rMAGL or [e] hMAGL and AMC-AA as the substrate. Inhibitory activity was determined by using [f] rat or [g] mouse brain membranes and 2-AG as the substrate; [h] human MAGL and [^{3}H]-2-OG as the substrate; [i] human FAAH and [^{3}H]-AEA as a substrate; [j] HEK293 cell lysates overexpressing hMAGL and 2-AG as the substrate; [k] COS-7 cells transiently overexpressing human recombinant FAAH and [^{3}H]-AEA as the substrate.

2.4.7 Miscellaneous MAGL inhibitors

Bisogno *et al.* reported on the modification of tetrahydrolipstatin (THL, orlistat) and identified OMDM169 (123) as a reversible MAGL inhibitor (Table 25) (Bisogno *et al.,* 2009). However, this compound was also found to inhibit other enzymes such as pancreatic lipase, FAAH and DAGL. Janssen Pharmaceuticals have been awarded three patents for amide analogues as MAGL inhibitors (Bian *et al.,* 2010, Chevalier *et al.,* 2010, Flores *et al.,* 2010). Very recently, López-Rodríguez *et al.* described compound 124 (Table 25) as a potent (IC_{50} = 0.18 µM) and reversible MAGL inhibitor having good selectivity towards the other endocannabinoid targets and several other tested receptors and enzymes (Hernández-Torres *et al.,* 2014). Compound 124 also improved the clinical progression of the disease in a mouse model of multiple sclerosis (MS) without inducing undesirable CB_1 -mediated side effects.

Table 25. Inhibitory activity of the compounds 123 and 124 against MAGL and FAAH.

Compd	Structure	IC_{50} [µM]		References
		hMAGL[a]	hFAAH[b]	
123 OMDM169		0.89[a]	3[b]	Bisogno *et al.,* 2009
124		0.24[c]	18[d]	Hernández-Torres *et al.,* 2014

Inhibitory activity was determined by using [a] human recombinant MAGL and [^{3}H]-2-AG as the substrate; [b] rat brain as enzyme source and [^{14}C]-AEA as the substrate; [c] cytosolic and membrane homogenates of rat brain as MAGL source and [^{3}H]-2-OG as the substrate; [d] rat brain homogenate as the enzyme source and [^{3}H]-AEA as the substrate.

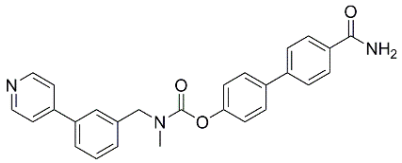
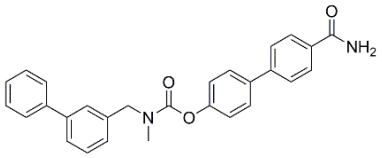
2.5 INHIBITORS OF ABHD6

To date, very little success has been achieved in the identification of potent and selective ABHD6 inhibitors (see subsections 2.5.1-2.5.3). There are several examples of non-selective ABHD6 inhibitors e.g. MAFP (2, Table 1), THL, RHC-80267, and the triterpene pristimerin (Navia-Paldanius *et al.,* 2012).

2.5.1 Carbamate-based ABHD6 inhibitors

In 2007, Li *et al.* screened a library of carbamate derivatives using a functional proteomic strategy and identified compound WWL70 (125) as a potent (IC_{50} = 70 nM) and selective inhibitor of ABHD6 (Table 26) (Li *et al.,* 2007). WWL70 (125) exerted anti-inflammatory and beneficial effects in metabolic disorders (Thomas *et al.,* 2013, Tchantchou & Zhang 2013). A brain penetrating and selective ABHD6 inhibitor, WWL123 (126, Table 26) (Bachovchin *et al.,* 2010), an isoster analogue of WWL70 (125), showed an antiepileptic effect in pentylenetetrazole (PTZ)-induced epileptiform seizures and spontaneous seizures in R6/2 mice (Naydenov *et al.,* 2014).

Table 26. Inhibitory values for the carbamate compounds 125 and 126 against ABHD6, MAGL and FAAH.

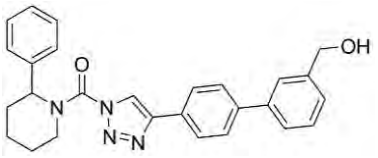
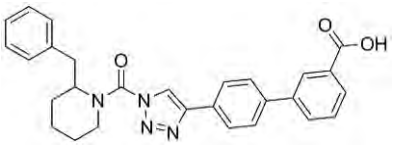
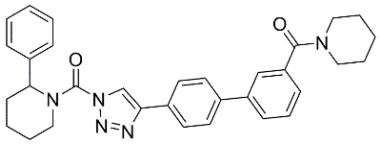
Compd	Structure	IC_{50} [μM]			References
		ABHD6	MAGL	FAAH	
125 WWL70		0.070[a] (0.085)[b]	NI[c]	NI	Li *et al.,* 2007 Navia-Paldanius *et al.,* 2012
126 WWL123		0.43[a]	NI	NI	Bachovchin *et al.,* 2010

[a] Inhibitory activity was determined by using transiently transfected COS-7 cells with the human ABHD6 cDNA and using competitive ABPP reductions in FP-Rh labeling of ABHD6 was quantified (in ABPP assay, these compounds are ABHD6 selective). [b] Glycerol liberated from 1-AG hydrolysis was determined with a sensitive fluorescent glycerol assay using lysates of HEK293 cells expressing hABHD6 (Navia-Paldanius *et al*., 2012). [c] No inhibition via ABPP.

2.5.2 Triazole urea-based ABHD6 inhibitors

Hsu *et al.* recently claimed that (2-substituted)-piperidyl-1,2,3-triazole ureas (127-129) were ABHD6 inhibitors (Table 27) (Hsu *et al.,* 2013). KT182 (127) and KT203 (128) showed exceptional potency and selectivity in Neuro2A cells (<5 nM), and at equivalent doses in mice (1 mg/kg), these compounds acted as systemic and peripherally restricted ABHD6 inhibitors, respectively. Moreover, KT185 (129) was found to be an orally bioavailable ABHD6 inhibitor having excellent selectivity against other brain and liver serine hydrolases *in vivo*.

Table 27. Inhibitory activity of the carbamate compounds 127-129 against ABHD6, MAGL and FAAH.

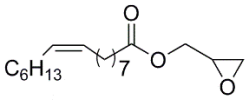
Compd	Structure	IC_{50} [µM]			References
		ABHD6	MAGL	FAAH	
127 KT182		0.0017[a] (0.015)[b]	NI[c]	NI	Hsu *et al.*,2013
128 KT203		0.00082[a] (0.0039)[b]	NI	NI	Hsu *et al.*,2013
129 KT185		0.0013[a] (0.0136)[b]	NI	NI	Hsu *et al.*,2013

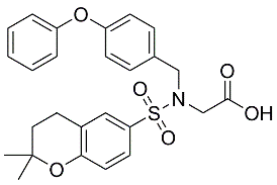
Inhibitory activity was determined by using [a] competitive ABPP in mouse brain membrane proteomes. The reductions in HT-01 (Hsu *et al.*, 2012) probe labeling of ABHD6 were quantified after preincubation of a proteomic sample with the inhibitor; [b] recombinant mouse ABHD6 protein overexpressed in HEK293T cells and 2-AG as a substrate. [c] No inhibition in competitive ABPP of mouse brain membrane proteomes.

2.5.3 Miscellaneous ABHD6 inhibitors

Marrs and colleagues described UCM710 (130) as a dual inhibitor of ABHD6 (IC_{50} = 2.4 µM) and FAAH (IC_{50} = 4 µM) (Table 28) (Marrs *et al.*, 2011). Very recently, Janssen *et al.* described a glycine sulfonamide analogue LEI-106 (131, Table 28) as a potent and selective dual inhibitor of sn-1-diacylglycerol lipase α (DAGL-α) and ABHD6 (Janssen *et al.*, 2014).

Table 28. Inhibitory activity of the carbamate compounds 130 and 131 against ABHD6, MAGL and FAAH.

Compd	Structure	IC_{50} [µM]			References
		ABHD6	MAGL	FAAH	
130 UCM710		2.4[a]	NI[b]	4[c]	Marrs *et al.*, 2011

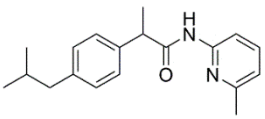
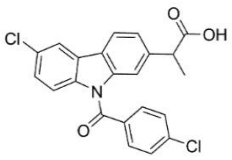
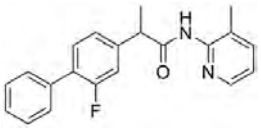
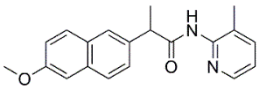
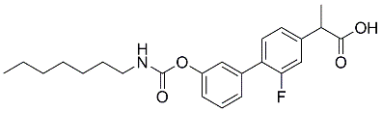
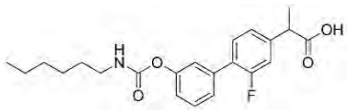
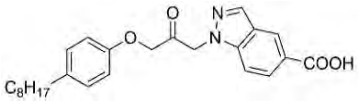
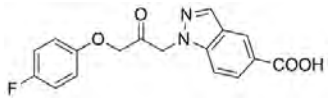
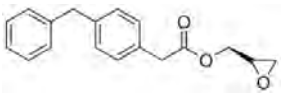
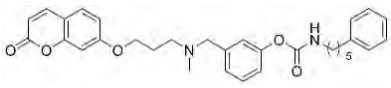
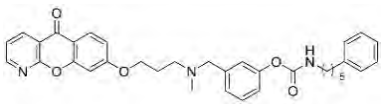
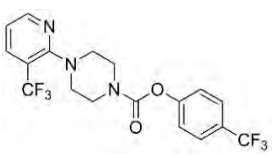
131 LEI-106		K_i = 0.8[d]	NI[e]	NI[e]	Janssen *et al.,* 2014

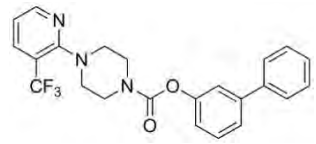
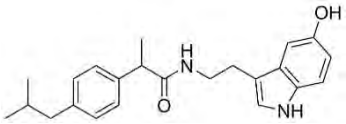
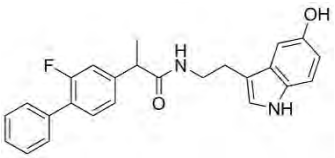
[a] Inhibitory activities were determined by using homogenates prepared from COS-7 cells heterologously expressing ABHD6 and [^{3}H]-2-AG as the substrate. [b] No inhibition IC_{50} > 1mM (homogenates prepared from COS-7 cells heterologously expressing MAGL and [^{3}H]-2-AG as the substrate). Inhibitory activities were determined by using [c] homogenates prepared from COS-7 cells heterologously expressing FAAH and [^{3}H]-AEA as the substrate; [d] HEK293T cells transiently transfected with hABHD6 and 2-AG as the substrate. (Note: K_i ($DAGL_\alpha$) = 0.7 µM. This was determined using HEK293T cells transiently transfected with hDAGL-α and *para*-nitrophenylbutyrate (PNP-butyrate) as the substrate).

2.6 COMPOUNDS WITH DUAL OR MULTIPLE TARGETS

Several research groups have reported dual FAAH-COX inhibitors, such as compounds 132-137 (Table 29) (Holt *et al.,* 2007, Favia *et al.,* 2012, Fowler *et al.,* 2013, Cipriano *et al.,* 2013, De Vivo *et al.,* 2014a, De Vivo *et al.,* 2014b). Instead of targeting one enzyme, dual inhibition has been considered as a potential approach to achieve a sufficent degree of analgesia at a lower dose (Naidu *et al.,* 2009, Sasso *et al.,* 2012). Indazole-5-carboxylic acid derivatives 138 and 139 were identified as dual cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$)-FAAH inhibitors (Forster *et al.,* 2010, Zahov *et al.,* 2011). The authors speculated that dual inhibition could lead to improved analgesic and anti-inflammatory properties in comparison with selective $cPLA_2\alpha$ and FAAH inhibition. As described above in sections 2.3 and 2.4, several compounds such as CAY10499 (69, Table 14), JZL195 (107, Table 23), 113 , 114 and 119 (Table 24) act as dual FAAH-MAGL inhibitors. Furthermore, a series of dual acting FAAH-MAGL inhibitors has been investigated and of these, (2*R*)-(−)-oxiran-2-ylmethyl(4-benzylphenyl)acetate (140, Table 29) potently inhibited human recombinant MAGL (IC_{50} = 2.4 µM), rat brain MAGL hydrolysis (IC_{50} = 0.68 µM), and rat brain FAAH (IC_{50} = 0.29 µM) (Cisneros *et al.,* 2012). This compound acted as a noncompetitive, reversible MAGL inhibitor. Dual acting FAAH-cholinesterase (ChE) inhibitors 141 and 142 have also been identified (Table 29). By targeting both the cholinenergic and endocannabinoid systems, it has been proposed that they may be able to prevent the progression of Alzheimer's disease (AD) (Rampa *et al.,* 2012). AA-5-HT (6), a dual FAAH-TRPV1 blocker, was found to be more efficacious at inhibiting pain and anxiety in rodents than either a high-potency, FAAH-selective inhibitor or a TRPV1-selective inhibitor (Costa *et al.,* 2010, Micale *et al.,* 2009). Maione *et al*. reported a similar study using the FAAH-TRPV1 blocker OMDM198 (143, Table 29) and the FAAH inhibitor-TRPA1 agonist, OMDM202 (144, Table 29), where they found these compounds had improved analgesic effects similar to those observed with AA-5-HT (Maione *et al.,* 2013). Very recently, NSAID–serotonin conjugates ibuprofen-5-HT (145) and flurbiprofen-5-HT (146) were developed and they were able to inhibit three targets, FAAH, TRPV1, and COX2 (Table 29). This kind of triple inhibition may offer effective pain relief with a high therapeutic index (Rose *et al.,* 2014).

Table 29. Inhibitory activity of the compounds 132-146 acting at dual or multiple targets.

Compd	Structure	IC_{50} [μM] or %inhibition		References
		FAAH	target(s)	
132 ibu-am5		4.7[a,b] 2.5[a,c]	NI (at 300 μM) for MAGL[d] 60 for COX-1[e] NI for COX-2[f]	Holt *et al.*,2007 Fowler *et al.*,2013
133		22[g]	NA[h] for MAGL 74.3 for COX-1[e] 72.3 for COX-2[f]	Favia *et al.*,2012
134 Flu-AM1		0.44[a]	NA for MAGL 6.6 for COX-1[e] 42 for COX-2[f]	Cipriano *et al.*,2013
135 Nap-AM1		0.74[a]	NA for MAGL 56 for COX-1[e] > 100 for COX-2[f]	Cipriano *et al.*,2013
136		0.015[i]	0.3 for COX-1[e] 0.32 for COX-2[f]	De Vivo *et al.*, 2014a
137 (+)-isomer		0.094[i] 0.097[j]	0.00001 for COX-1[e] 0.010 for COX-2[f]	De Vivo *et al.*, 2014b
138		0.16[k]	0.005[l] for $cPLA_2\alpha$	Forster *et al.*, 2010
139		0.090[k]	0.015[l] for $cPLA_2\alpha$	Zahov *et al.*, 2011
140		0.29[a]	0.64[m] for MAGL 2.4[n] for hrMAGL	Cisneros *et al.*, 2012
141		0.050[o]	0.075[p] for AChE 0.0016[q] for BuChE	Rampa *et al.*, 2012
142		0.040[o]	0.090[p] for AChE 0.0017[q] for BuChE	Rampa *et al.*, 2012
143 OMDM198		3.36[o]	1[r] for hTRPV1	Maione *et al.*, 2013

144 OMDM202		0.38[o]	1[r] for rTRPA1	Maione *et al.,* 2013
145 Ibuprofen-5-HT		5[s]	10[t] for COX2 6[u] for TRPV1	Rose *et al.,* 2014
146 Flurbiprofen-5-HT		15[s]	8[t] for COX2 9[u] for TRPV1	Rose *et al.,* 2014

Values were measured by using [a] rat brain homogenate protein and [^{3}H]-AEA as the substrate at [b] pH = 8 or [c] pH = 6; [d] rat brain cytosolic fractions or human recombinant MAGL and [^{3}H]-2-OG as the substrate; [e] ovine COX-1 or [f] human recombinant COX-2 enzyme and AA as the substrate; [g] 30 min preincubation with protein and [^{3}H]-AEA as the substrate. [h] Not analyzed. Values were measured by using [i] rat brain homogenate and [^{3}H]-AEA as the substrate; [j] human FAAH expressed in HEK293 cells with a 50 min preincubation and [^{3}H]-AEA as the substrate; [k] rat brain microsomes and *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide as the substrate; [l] cPLA$_2$α isolated from human platelets and 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine sonicated with 1,2-dioleoyl-sn-glycerol as the substrate; [m] cytosolic and membrane homogenates of rat brain and [^{3}H]-2-OG as the substrate; [n] lysates of human recombinant MAGL and 4-nitrophenyl acetate as the substrate; [o] rat brain FAAH and [^{14}C]-AEA as the substrate; [p] human recombinant AChE using acetylthiocholine iodide as the substrate; [q] human serum BuChE using butyrylthiocholine iodide as the substrate; [r] intracellular Ca^{2+} elevation mediated by TRPV1 and TRPA1 channels overexpressed in HEK293 cells; [s] human recombinant FAAH and AMC-AA as the substrate; [t] human recombinant COX2 and 10-acetyl-3,7-dihydroxyphenoxazine (ADHP) as the substrate; [u] BEAS-2B human TRPV1 over-expressing cells and Fluo-4 AM, a fluorogenic calcium indicator using the Fluo-4 Direct Kit (Invitrogen).

3 Therapeutic Utility of Endocannabinoid Hydrolase Inhibitors

During the past few years, major progress has been made in the development of endocannabinoid hydrolase inhibitors. The field has evolved from substrate-based potent albeit non-selective FAAH inhibitors to diverse classes of FAAH inhibitors having excellent potency and significantly improved selectivity profiles (Seierstad & Breitenbucher 2008, Vandevoorde 2008, Petrosino & Di Marzo 2010, Deng 2010, Minkkilä *et al.,* 2010, Otrubova *et al.,* 2011, Feledziak *et al.,* 2012, Blankman & Cravatt 2013, Bisogno & Maccarrone 2013). Several α-ketoheterocyclic, carbamate and urea-based FAAH inhibitors have been used widely in diverse preclinical models to validate the therapeutic potential of FAAH inhibition in pain, inflammation, CNS disorders etc. Some of these compounds have even progressed to clinical trials. Compounds, such as OL-135 (12, Table 3) and URB597 (23, Table 4) are known to cause significant elevations of the endogenous levels of the AEA (as well as OEA and PEA) and they have been found to be highly effective in different pain models, including inflammatory and neuropathic models (Lichtman *et al.,* 2004b, Chang *et al.,* 2006, Piomelli *et al.,* 2006, Jayamanne *et al.,* 2006, Russo *et al.,* 2007). Analogues of OL-135 (12) such as CE-12 (17, Table 3) and 18 (Table 3) have shown extended duration of FAAH inhibition thereby achieving long lasting analgesic effects (Ezzili *et al.,* 2011, Otrubova *et al.,* 2013). A peripherally restricted inhibitor URB937 (28, Table 4) along with its improved selectivity and biological stability was found to be effective in pain models such as SNL (Clapper *et al.,* 2010). Several other *in vivo* efficacious compounds ST4070 (35, Table 6) (Gattinoni *et al.,* 2010a) and ST4020 (39, Table 6) (Minetti *et al.,* 2009) have emerged as potential anxiolytic drugs while ST3913 (41, Table 7) has elicited an analgesic effect (Butini *et al.,* 2012). Some of the most remarkable *in vivo* efficacious FAAH inhibitors are PF-3845 (51, Table 10) (Ahn *et al.,* 2009b), PF-465 (52, Table 10) (Johnson *et al.,* 2009), PF-04457845 (54, Table 10) (Johnson *et al.,* 2011), and PF-04862853 (57, Table 10) (Meyers *et al.,* 2011b). Both PF-3845 (51) and PF-465 (52) have been shown to increase AEA levels and demonstrated to possess anti-inflammatory activity in a rat CFA model. PF-04457845 (54) was well tolerated in the phase I clinical study, however, it failed to show efficacy in patients in phase II clinical trials (Huggins *et al.,* 2012). Other ongoing or completed studies with PF-04457845 (54) include trials evaluating the potential of these types of drugs for treating Tourette syndrome, cannabinoid dependence, fear conditioning, sleep and acute and chronic pain (https://clinicaltrials.gov/ct2/results?term=PF-04457845&Search=Search). PF-04862853 (57) is currently under consideration for human trials. All of the three compounds JNJ-1661010 (58, Table 11) (Keith *et al.,* 2008), JNJ-40355003 (59, Table 11) (Keith *et al.,* 2012) and JNJ-42119779 (61, Table 11) (Keith *et al.,* 2014b) have been reported to elevate the levels of AEA, PEA, and OEA. JNJ-1661010 (58) exerted analgesic activity in both MTI and SNL models of neuropathic

pain (Karbarz *et al.,* 2008), and JNJ-40355003 (59) treated rats exposed to chronic stress did not develop any significant increases in anxiety-like behavior (Hill *et al.,* 2013) while JNJ-42119779 (61) showed modest efficacy in a neuropathic pain model. Takeda's compounds 63 and 64 (Table 11) possessed analgesic efficacy in animal models of pain (Kono *et al.,* 2013, Kono *et al.,* 2014). AM3506 (92, Table 18) has been reported to reduce blood pressure, heart rate and to improve cardiac contractility in hypertensive animals through activation of CB1 receptors, which correlates with the blockade of FAAH activity and the subsequent increase in tissue levels of AEA (Godlewski *et al.,* 2010). In contrast to the above FAAH inhibitors, compounds lacking a reactive carbonyl group such as JNJ-40413269 (76, Table 16) and MK-4409 (77, Table 16) (Chobanian *et al.,* 2014, Keith *et al.,* 2014a) has shown promising preclinical profiles and excellent efficacy in pain models such as SNL. In addition to the above classes, 1,3,4-oxadiazol-2-one and β-lactam have emerged as promising scaffolds for the development of potent and selective FAAH inhibitors (see subsections 2.3.5 and 2.3.6). Several other candidates are under clinical investigations but their chemical structures have not been disclosed. For example, Janssen's JNJ-42165279 recently completed phase 1 clinical studies (https://clinicaltrials.gov/ct2/show/NCT01650597?term=FAAH&rank=6) and Vernalis' V158866 has passed through a First-in-Human (FIH) study (https://clinicaltrials.gov/ct2/show/NCT01634529?term=FAAH&rank=8).

In contrast to FAAH blockers, very few potent MAGL inhibitors have been tested (Minkkilä *et al.,* 2010, Feledziak *et al.,* 2012, Blankman & Cravatt 2013, Kapanda *et al.,* 2013). However, some compounds have shown promising results in preclinical models of pain, inflammation, vomiting, cancer, etc. (Mulvihill & Nomura 2013). The earlier developed maleimide, dithio-, triterpenoid- and octhilinone analogues targeted the cysteine residues near to the catalytic site of MAGL. However, these analogues are likely to react with other cysteine containing proteins. The appearance of X-ray structures of MAGL has facilitated the development of piperidine/piperazine containing carbamates/ureas targeting mainly the nucleophilic Ser^{122} residue of MAGL. Brain 2-AG levels were elevated in JZL184 (106, Table 23) treated mice and the drug exhibits a broad array of CB_1-dependent behavioral effects, including analgesia, hypothermia and hypomotility (Long *et al.,* 2009a). It is known that genetic inactivation of MAGL or chronic treatment with the irreversible MAGL inhibitor JZL184 (106) causes a prompt increase in 2-AG levels thereby leading functional antagonism, pharmacological tolerance, and receptor desensitization that eventually weakens the antinociceptive properties of the inhibitor (Lichtman *et al.,* 2010, Chanda *et al.,* 2010, Schlosburg *et al.,* 2010). Functional antagonism associated with chronic MAGL blockade may be avoided by partially inhibiting MAGL. Thus, partial blockade of MAGL by treatment with a low dose of JZL184 (106) has maintained the antinociceptive and anxiolytic-like responses after chronic treatment (Busquets-Garcia *et al.,* 2011). In KML29 (108, Table 23) treated mouse, 10-fold elevations in 2-AG (and reductions in AA) have been detected without any accompanying alterations in the FAAH substrates, AEA, OEA, or PEA (Chang *et al.,* 2012). MJN110 (112, Table 23) has been reported to be able to reverse established mechanical allodynia in a rat

model of diabetic neuropathy (Niphakis *et al.,* 2013). In comparison to the irreversible MAGL inhibitors described above, the first potent and selective reversible MAGL inhibitor 124 (Table 25) has been found to be effective in the treatment of multiple sclerosis (MS) without any sign of CNS side effects (Hernández-Torres *et al.,* 2014).

At present, very limited pharmacological data of ABHD6 inhibitors are available. ABHD6 is considered as an emerging target to treat inflammation, metabolic disorders, and epilepsy (Tchantchou & Zhang 2013, Alhouayek *et al.,* 2013, Thomas *et al.,* 2013, Naydenov *et al.,* 2014). Chronic treatment with WWL70 (125, Table 26) has exerted anti-inflammatory and neuroprotective effects in a mouse model of traumatic brain injury (TBI) (Tchantchou & Zhang 2013). Inhibition of ABHD6 with WWL70 (125) has also been reported to protect mice against high-fat-diet-induced obesity and glucose intolerance (Thomas *et al.,* 2013). WWL123 (126, Table 26), a brain penetrating selective ABHD6 inhibitor, exerted an antiepileptic effect in pentylenetetrazole (PTZ)-induced epileptiform seizures and also against spontaneous seizures in R6/2 mice (Naydenov *et al.,* 2014). Furthermore, ABHD6 blockade retained its antiepileptic activity even with chronic dosing and no tolerance was observed in contrast to the situation reported after chronic treatment with CB1 receptor agonists (Blair *et al.,* 2009) and MAGL inhibitors (Schlosburg *et al.,* 2010).

The development of selective ABHD12 inhibitors is eagerly awaited. However, a genetic mutation of ABHD12 has been associated with neurodegenerative disease, and hence, it is possible that inhibitors of ABHD12 may cause long term adverse effects.

As mentioned in subsection 2.2.1, inhibition of FAAH not only causes an increase in the levels of AEA but the concentrations of other lipids are also affected; for example levels of OEA and PEA increase which results in several effects mediated by non-cannabinoid receptors such as GPR118, TRPV1 or PPARα. These kinds of effects might enhance or reduce the favourable properties of FAAH, for example, activation of PPARs could also modulate inflammatory effects whereas activation of TRPV1 could evoke effects that are opposite to those mediated by cannabinoid receptor activation. As discussed in section 2.6, compounds with either dual targets, such as FAAH-COX inhibitors, cPLA$_2\alpha$-FAAH inhibitors, FAAH-ChE inhibitors, FAAH-TRPV1 blocker, FAAH inhibitor/TRPA1 agonist or compounds acting on multiple targets, such as FAAH, TRPV1, and COX2 might be beneficial since this may be one way to reduce any potential side effects.

4 Aims of the Study

This thesis belongs to the medicinal chemistry research field and it involves the development of potent and selective inhibitors of endocannabinoid hydrolases (FAAH, MAGL and ABHD6). The main objectives of this research work have been as follows:

1. To design, synthesize and identify novel 1,3,4-oxadiazol-2-ones as potent and selective inhibitors of FAAH and ABHD6.
2. To exploit 1,2,5-thiadizole carbamate scaffold in the identification of novel potent and selective ABHD6 inhibitors.
3. To identify potential lead structures and utilize them in the development of potent and selective MAGL inhibitors.

5 Experimental Section

5.1 MATERIALS AND METHODS

Reagents and solvents were purchased from commercial suppliers and were used without further purification. Most of the reactions were monitored by thin-layer chromatography (TLC) with suitable UV visualization. Purification was carried out by flash chromatography (FC) or by CombiFlash Companion (Teledyne Isco, USA). ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Avance AV 500 (Bruker Biospin, Switzerland) spectrometer and processed from the recorded FID files with TOPSPIN 2.1 software or ACD/NMR Processor Academic Edition. ESI-MS spectra were acquired using an LCQ quadrupole ion trap mass spectrometer equipped with an electrospray ionization source (Thermo LTQ, San Jose, CA, USA). Elemental analyses were performed on a ThermoQuest CE instrument (EA 1110 CHNS-O) or a Perkin-Elmer PE 2400 Series II CHNS-O Analyzer. For a racemic compound and its pure enantiomers, optical rotation angles ($[\alpha]_D$) were measured using a Jasco model P-2000 polarimeter. The enantiomers of selected racemic compounds described in subsection 5.2.1 were separated by preparative high-performance liquid chromatography (HPLC; Shimadzu LC- 10Avp, Fennolab, Fenno Medical Oy) on a chiral column (Lux 5µ Cellulose-2, 250 x 21.2 mm, Phenomenex).

5.2 DESIGN AND GENERAL SYNTHESIS PROCEDURES

The general synthetic pathways of 1,3,4-oxadiazol-2-ones as inhibitors of FAAH and ABHD6, 1,2,5-thiadiazole carbamates as inhibitors of ABHD6, and loratadine analogues as inhibitors of MAGL, are briefly described below. The detailed synthetic procedures have been reported in the original publications (I-IV).

5.2.1 Chiral 1,3,4-oxadiazol-2-ones (I)

Earlier our group has defined 1,3,4-oxadiazol-2-ones (i, Figure 12) as potent and reversible inhibitors of both FAAH and MAGL (Minkkilä *et al.,* 2009b, Käsnänen *et al.,* 2013). However, in the ABPP assay, they showed poor selectivity for the serine hydrolases in the mouse brain membrane proteomes (unpublished work). To improve the selectivity, it was decided to adopt a new approach where the 1,3,4-oxadiazol-2-one scaffold (i) was combined with non-steroidal anti-inflammatory drugs (NSAIDs, ii, Figure 1), ibuprofen (Fowler *et al.,* 2013, Holt *et al.,* 2007, De Wael *et al.,* 2010) and carprofen (Favia *et al.,* 2012) which both known to have

activity towards FAAH and/or COX isoenzymes. Ibuprofen and carprofen both have a chiral center and the *R*-enantiomer of ibuprofen is known to be slightly more potent than the *S*-enantiomer with respect to its ability to inhibit FAAH (Fowler *et al.,* 1999). Hence, it was thought that by introducing a chiral center into the 1,3,4-oxadiazol-2-ones, it would be possible to study its effect on the selectivity towards either FAAH or MAGL. Figure 12 illustrates the hybridization approach where our earlier disclosed 1,3,4-oxadiazol-2-ones (i) are combined with derivatives of phenylalkanoic acid present in marketed NSAIDs (ii) to afford novel chiral 1,3,4-oxadiazol-2-ones (iii).

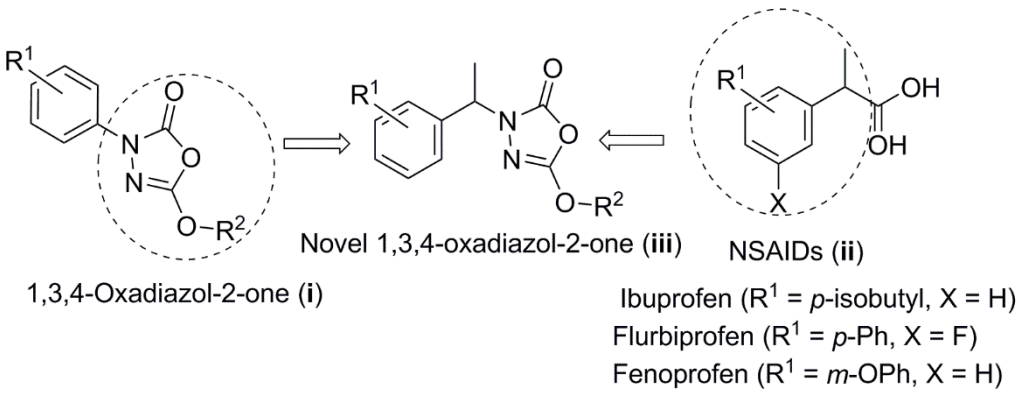
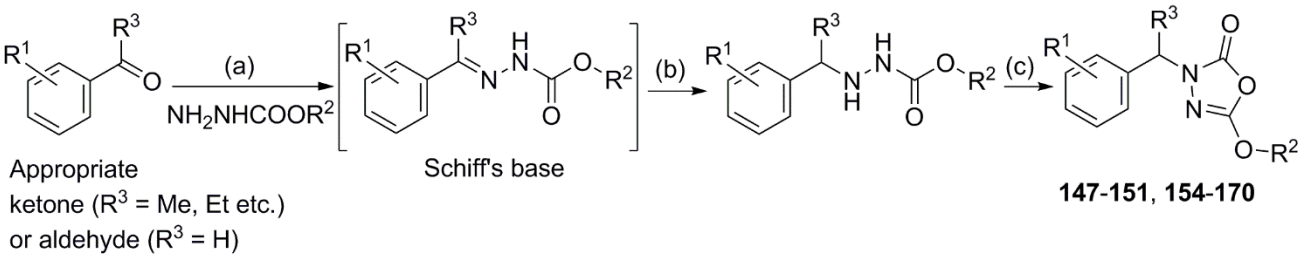
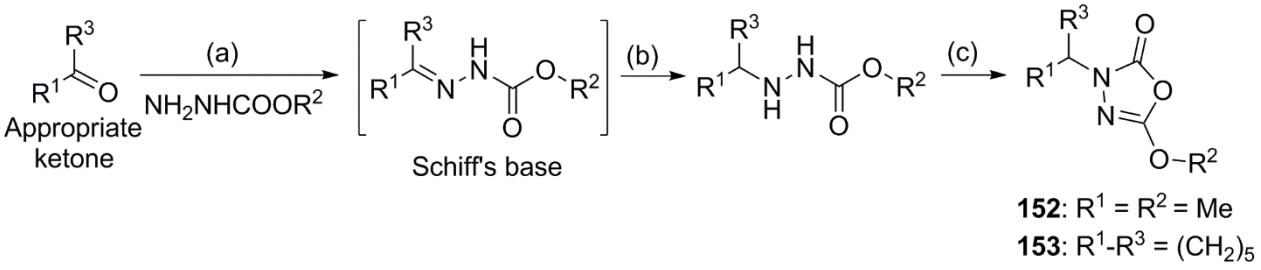


Figure 12. Chiral 1,3,4-oxadiazol-2-ones via the hybridization approach.

The general synthetic routes of 1,3,4-oxadiazol-2-ones 147-170 are listed below (Schemes 1 & 2, Table 30). The detailed synthetic procedures for the compounds 147-170 are described in the original publication (I). Briefly, an appropriate ketone or aldehyde was condensed with methyl carbazate to afford Schiff's base which was reduced *in situ* by either sodium borohydride or 10% Pd/C to the corresponding hydrazine carboxylate derivative. Finally, a cyclization in the presence of phosgene gave the desired 1,3,4-oxadiazol-2-ones 147-170.

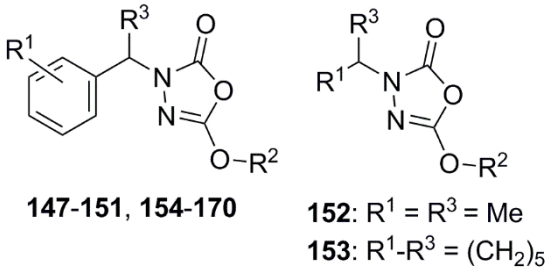


Scheme 1. Synthesis of 1,3,4-oxadiazol-2-ones 147-151 and 154-170. Reagents and conditions: (a) $NH_2NHCOOR^2$ (R^2 = Me), MeOH, 4 Å molecular sieves, 3-4 drops of AcOH, reflux, 2-16 h; (b) $NaBH_3CN$, MeOH, methanolic HCl, 0-25 °C, 24-72 h or 10% Pd/C, H_2 (atm), 20-25 °C, 6-12 h, MeOH; (c) $COCl_2$, CH_2Cl_2, pyridine, 0-25 °C, 6-16 h.



Scheme 2. Synthesis of 1,3,4-oxadiazol-2-ones 152 and 153. Reagents and conditions: (a) $NH_2NHCOOR^2$ (R^2 = Me), acetone (for 152) and MeOH (for 153), 4 Å molecular sieves, 3-4 drops of AcOH, reflux, 2-16 h; (b) & (c) same as Scheme 1.

Table 30. List of the synthesized 1,3,4-oxadiazol-2-ones 147-170 as per Schemes 1 and 2.



R^2 = Me for compounds **147-170**

Compd	R^1	R^3	Compd	R^1	R^3
147	*p*-isobutyl	Me	159	(-)-*p*-Ph	Me
148	*p*-Ph	Me	160	(+)-*p*-Ph	Me
149	*m*-Ph	Me	161	(-)-*p*-Ph_2N	Me
150	*p*-OPh	Me	162	(+)-*p*-Ph_2N	Me
151	*m*-OPh	Me	163	H	H
152	------	------	164	*p*-isobutyl	H
153	------	------	165	*p*-Ph	H
154	H	Me	166	*m*-Ph	H
155	H	Ph	167	*o*-OPh	H
156	*p*-Ph_2N	Me	168	*m*-OPh	H
157	(-)-*p*-isobutyl	Me	169	*p*-OPh	H
158	(+)-*p*-isobutyl	Me	170	*p*-Ph_2N	H

5.2.2 1,2,5-Thiadiazole carbamates (II)

In 2010, Helquist and colleagues reported 1,2,5-thiadiazole carbamates (i, Figure 13) as potent inhibitors of lysosomal acid lipase (LAL, also known as LIPA) (Rosenbaum *et al.,* 2010). They noted that 1,2,5-thiadiazole carbamate bound covalently to LAL by carbamylation of Ser of LAL with 1,2,5-thiadiazole alcohol group serving as the leaving group. Till date, numerous carbamate compounds have been reported as inhibitors of endocannabinoid hydrolases (Mor *et al.,* 2004, Li *et al.,* 2007, Long *et al.,* 2010, Bachovchin *et al.,* 2010, Chang *et al.,* 2012, Niphakis *et al.,* 2013) (for recent reviews, see (Minkkilä *et al.,* 2010, Feledziak *et al.,* 2012, Kapanda *et al.,* 2013, Blankman & Cravatt 2013). Hence, it was intended to utilize the 1,2,5-thiadiazole carbamate scaffold for the development of inhibitors of the endocannabinoid hydrolases. Since only limited structure-activity relationship (SAR) studies based on this scaffold have been reported (Rosenbaum *et al.,* 2010), it was decided to carry optimization by introducing different cyclic and non-cyclic secondary amines at the main core while a small set of different cyclic amines were introduced as potential leaving groups (ii, Figure 13).

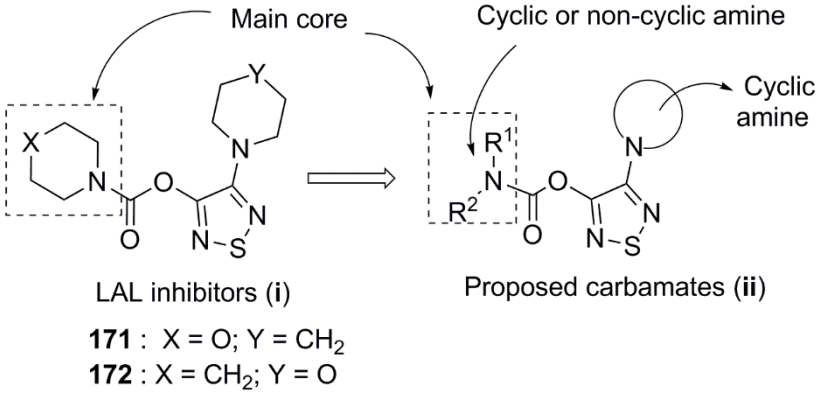
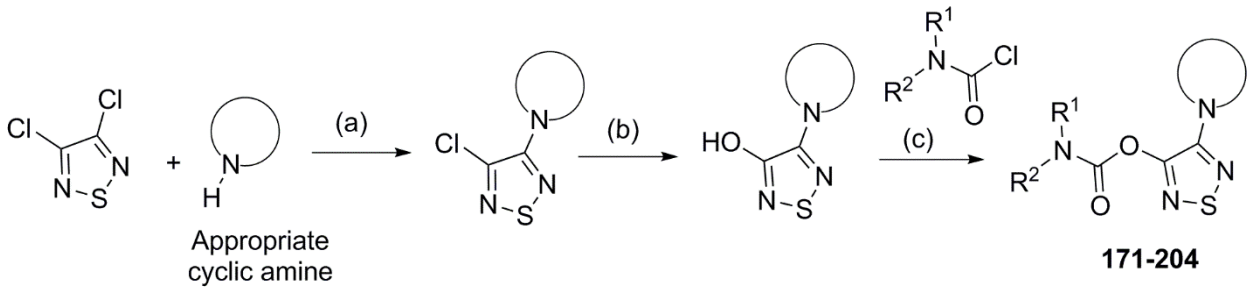


Figure 13. Optimization of 1,2,5-thiadiazole carbamates.

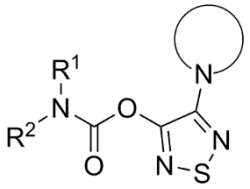
The general synthetic procedure of 1,2,5-thiadiazole carbamate 171-204 is presented below (Scheme 3, Tables 31 & 32). The detailed synthetic procedures are described in the original publication (II).

In accordance with the procedure described in the literature, commercially available 3,4-dichloro-1,2,5-thiadiazole was coupled with the appropriate secondary amine to obtain the corresponding monochloro 1,2,5-thiadiazole derivative, which was then converted to 1,2,5-thiadiazole alcohol via treatment with aqueous alkali. Finally, coupling with the appropriate carbamoyl chloride gave the desired 1,2,5-thiadiazole carbamates 171-204.



Scheme 3. Synthesis of 1,2,5-thiadiazole carbamate derivatives 171-204. Reagents and conditions: (a) 110-120 ^{o}C, 2-6 h or K_2CO_3, DMF, 100-110 ^{o}C, 6-10 h; (b) aq. NaOH or KOH, DMSO, reflux, 1-6 h; (c) dry THF, KOtBu, 0-25 ^{o}C, 16-24 h.

Table 31. List of the synthesized 1,2,5-thiadiazole carbamates 171-179 as per Scheme 3 (cyclic amine at the main core).



171-179

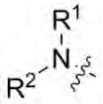
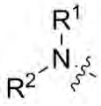
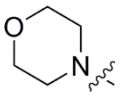
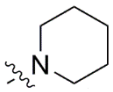
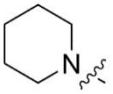
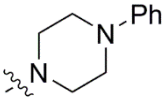
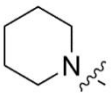
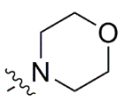
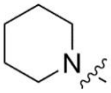
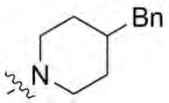
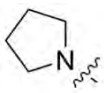
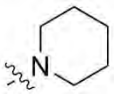
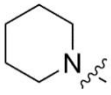
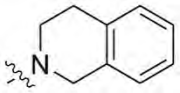
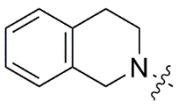
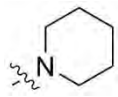
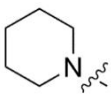
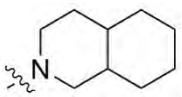
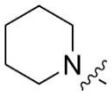
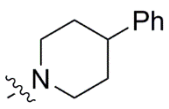
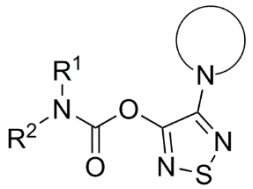
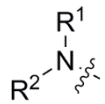
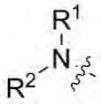
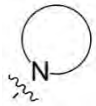
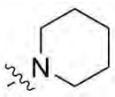
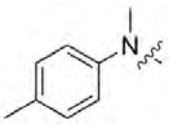
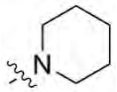
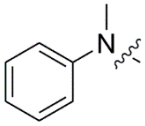
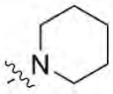
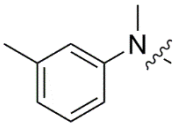
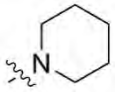
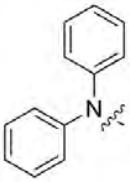
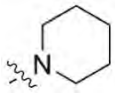
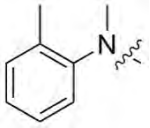
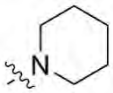
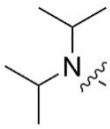
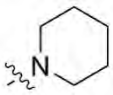
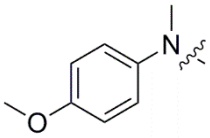
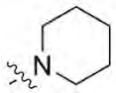
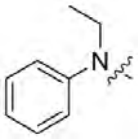
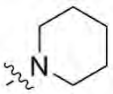
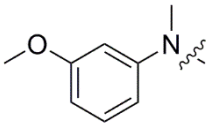
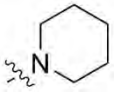
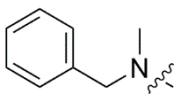
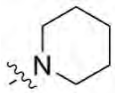
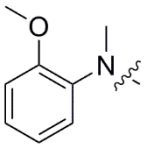
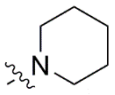
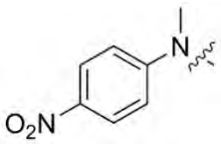
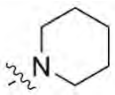
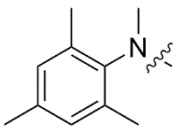
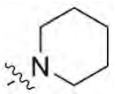
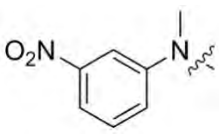
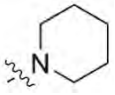
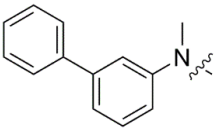
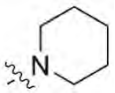
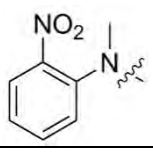
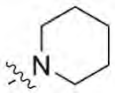
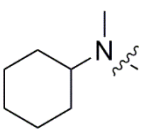
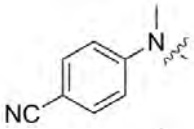
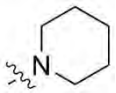
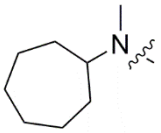
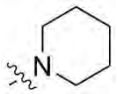
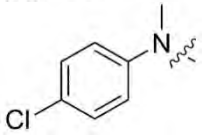
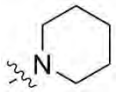
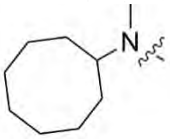
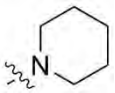
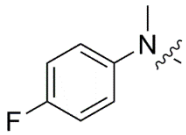
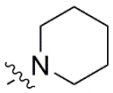
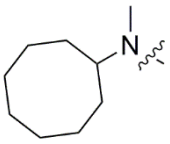
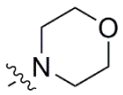
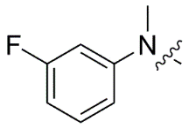
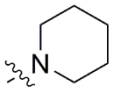
Compd			Compd		
171			176		
172			177		
173			178		
174			179		
175			-----	-----	-----

Table 32. List of the synthesized 1,2,5-thiadiazole carbamates 180-204 as per Scheme 3 (non-cyclic amine at the main core).



180-204

Compd			Compd		
180			193		
181			194		
182			195		
183			196		
184			197		
185			198		
186			199		
187			200		
188			201		

189			202		
190			203		
191			204		
192			-----	-----	-----

5.2.3 Loratadine analogues (III)

At present, several different classes of MAGL inhibitors have been reported and of these, some piperidine/piperazine-based carbamates/ureas have been described as the most potent and selective MAGL inhibitors (see subsections 2.4.5 and 2.4.6). Compounds JZL184 (106) (Long *et al.,* 2010), KML29 (108) (Chang *et al.,* 2012), CK16 (110) (Kapanda *et al.,* 2012), MJN110 (112) (Niphakis *et al.,* 2013), 115 and 116 (Hoornaert 2008c), SAR629 (118) (Bertrand *et al.,* 2010, Aaltonen *et al.,* 2013), ML30 (120) (Morera *et al.,* 2012), JJKK-048 (121) (Korhonen *et al.,* 2014) and 122 (Korhonen *et al.,* 2014), share common structural features such as two aromatic rings, a piperidine/piperazine linker, and a polar tail. These features are crucial in achieving good potency and selectivity towards MAGL over other possible off-targets, particularly FAAH and/or ABHD6 (Kapanda *et al.,* 2012, Long *et al.,* 2010, Chang *et al.,* 2012, Minkkilä *et al.,* 2010, Feledziak *et al.,* 2012, Blankman & Cravatt 2013, Kapanda *et al.,* 2013). Based on the structural similarity with the above mentioned class of MAGL inhibitors, it was decided to select the well-known histamine H_1 antagonist loratadine (205), for further optimization (Figure 14). As the carbamate functionality present in loratadine was not reactive, a small series of loratadine analogues 206-217 was synthesized with a more electrophilic carbamate/urea moiety.

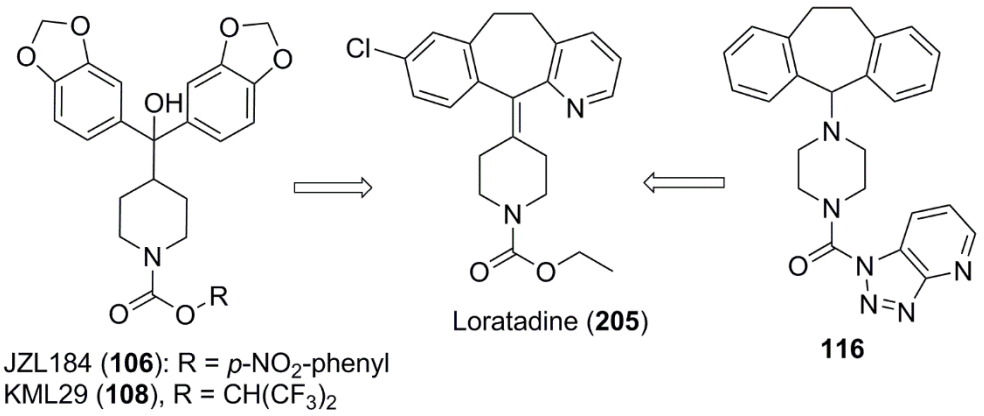
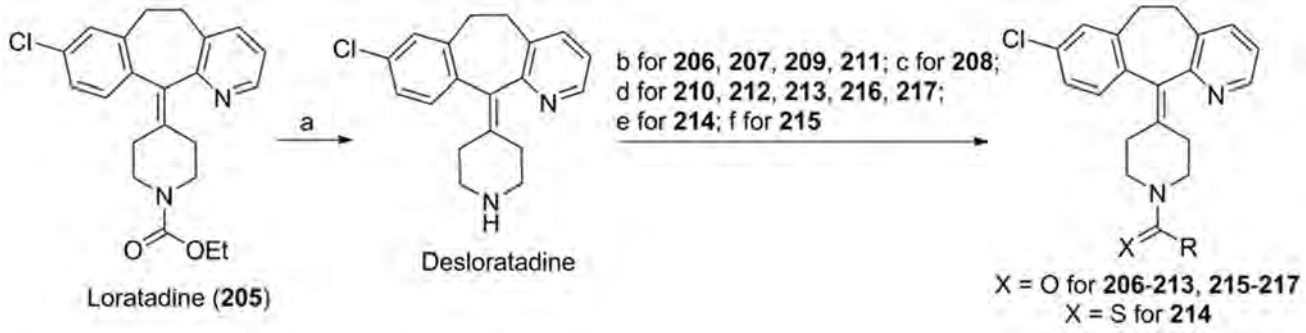


Figure 14. Structural similarities between known MAGL inhibitors, such as JZP184 (106), KML29 (108) and 116, with the well-known drug loratadine (205).

The general synthetic procedure for obtaining the loratadine analogues 206-217 is presented below (Scheme 4, Table 33). Additionally, the 1,2,4-triazole urea analogue 219 of the pharmaceutical drug, cyproheptadiene (218), was also synthesized (Scheme 5). The detailed synthetic procedures are described in the original publication (III).

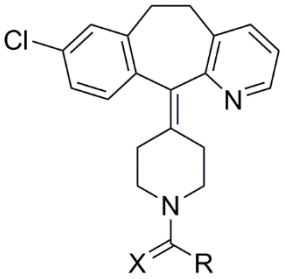
In agreement with the literature procedure, commercially available loratadine was converted into desloratadine by the addition of aqueous alkali. Finally, desloratadine was coupled with the appropriate hetetocyclic urea or carbamoyl chloride or isocyanate to obtain the desired loratadine urea or carbamate analogues 206, 207, 209-213, 215-217. For the synthesis of the urea analogue 208, desloratadine was first treated with triphosgene followed by in situ coupling with 1*H*-1,2,3-triazolo[4,5-*b*]pyridine. The dithiocarbamate derivative 214 was obtained by the reaction with a dithiocarbamic acid salt; prepared *in situ* via the reaction of desloratadine with CS_2 in the presence of triethylamine, with 2,4-dinitrofluorobenzene using the well-known Sanger reaction.



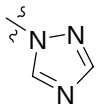
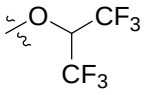
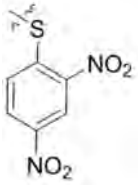
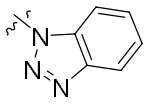
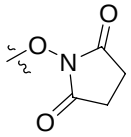
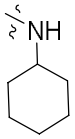
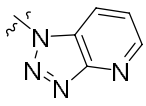
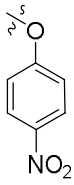
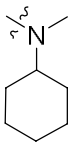
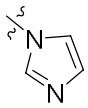
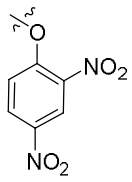
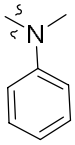
Scheme 4. Synthesis of loratadine analogues 206-217. Reagents and conditions: (a) Aq. KOH, EtOH, reflux, 16-18 h; (b) DIPEA, RCOR (R = 206a, 207a, 209a, 211a, respectively), DCM, 22-25 °C, 24-26 h; (c) (i) pyridine, DCM, triphosgene, 0-25 °C, 2-3 h; (ii) carbamoyl chloride from (i) followed by RH (R = 208a), DIPEA, DMAP, DCM, 0-25 °C, 24-26 h; (d) DIPEA or pyridine, RCOCl (R = 210a, 212a, 213a, 216a, 217a, respectively), DCM, 0-25 °C, 16-24 h; (e) (i) CS_2,

Et_3N, 22-25 °C, 16 h; (ii) R-F (R = 2,4-dinitrophenyl), DMF, 22-25 °C, 24 h; (f) RNCO (R = cyclohexyl), THF, reflux, 4 h.

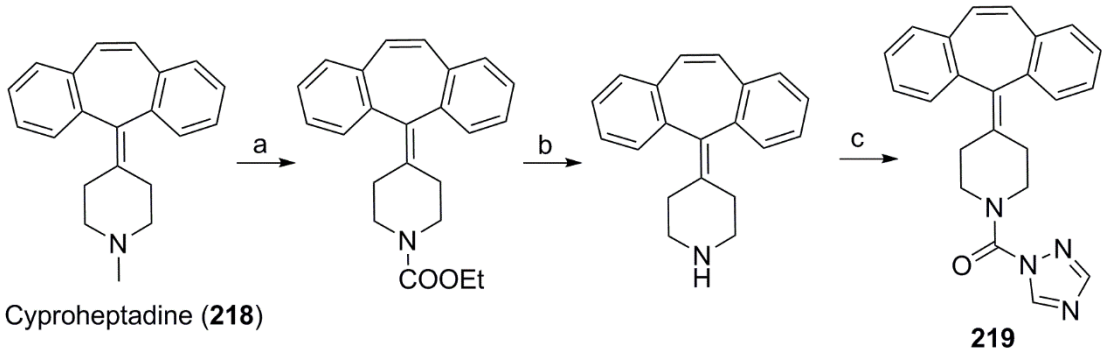
Table 33. List of the intermediates 206a-213a, 216a and 217a, and synthesized compounds 206-217 as per the Scheme 4.



X = O for **206-213**, **215-217**
X = S for **214**

Compd	R	Compd	R	Compd	R
206a & 206		210a & 210		214	
207a & 207		211a & 211		215	
208a & 208		212a & 212		216a & 216	
209a & 209		213a & 213		217a & 217	

The 1,2,4-triazole urea bearing cyproheptadine 219 was synthesized through a three-step procedure (Scheme 5). First, the commercially available cyproheptadine (218) was converted into an ethyl ester analogue by reaction with ethylchloroformate which was subsequently treated with alkali to obtain the amine. Finally, the amine was coupled with 1,1‘-carbonyl-di-(1,2,4-triazole) (CDT) to produce the desired triazole urea analogue 218.



Scheme 5. Synthesis of triazole analogue of cyproheptadine 219. Reagents and conditions: Reagents and conditions: (a) Toluene, ClCOOEt, reflux, 3 h; (b) Aq. KOH, EtOH, reflux, 16-18 h; (c) DIPEA, RCOR (R = 206a), DMSO, 22-25 °C, 24 h.

5.2.4 3-Benzyl-1,3,4-oxadiazol-2-ones (IV)

Several research groups including our own have described the use of the 1,3,4-oxadiazol-2-one scaffold (Figure 15, general structures i and ii) in the development of serine hydrolase inhibitors (Ben Ali *et al.,* 2006, Minkkilä *et al.,* 2009b, Point *et al.,* 2012, Delorme *et al.,* 2012, Kiss *et al.,* 2011, Käsnänen *et al.,* 2013, Patel *et al.,* 2013, Savinainen *et al.,* 2014). Recently, we have shown that the removal of the methyl group at the 3-position of 1,3,4-oxadiazol-2-ones led to the identification of 3-benzyl-1,3,4-oxadiazol-2-ones (Figure 15, general structure iii) as dual FAAH-MAGL inhibitors (Patel *et al.,* 2013). While screening 1,3,4-oxadiazol-2-ones against other possible targets, it was observed that compound 163 (see Table 30, subsection 5.2.1) achieved nearly 40% inhibition of hABHD6 at 1 μM and importantly no inhibition was observed towards either hFAAH or hMAGL at 10 μM (Patel *et al.,* 2013). This finding prompted the design and synthesis of another series of 3-benzyl-1,3,4-oxadiazol-2-ones.

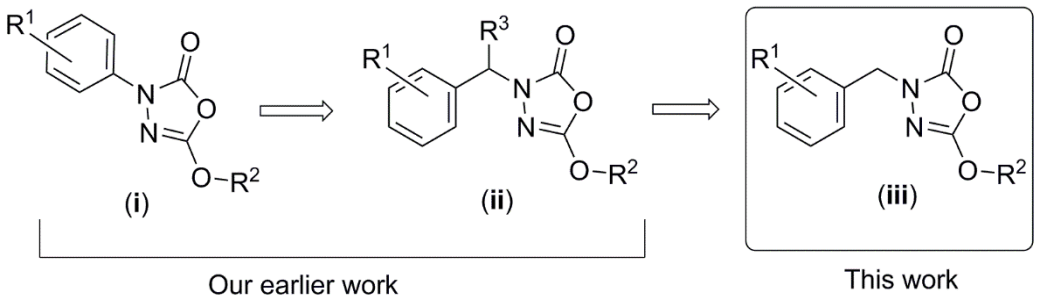
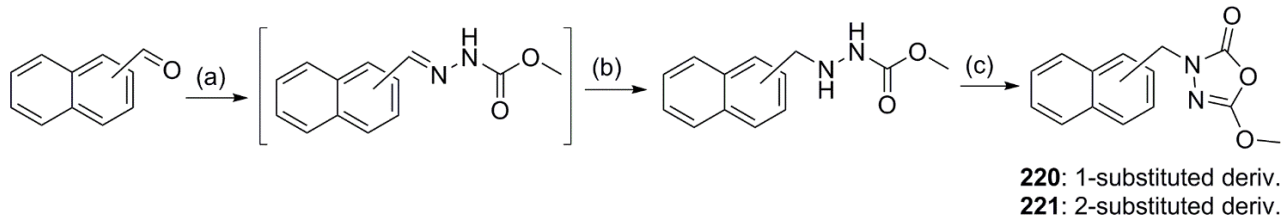


Figure 15. Advancement in 1,3,4-oxadiazol-2-ones (i-iii).

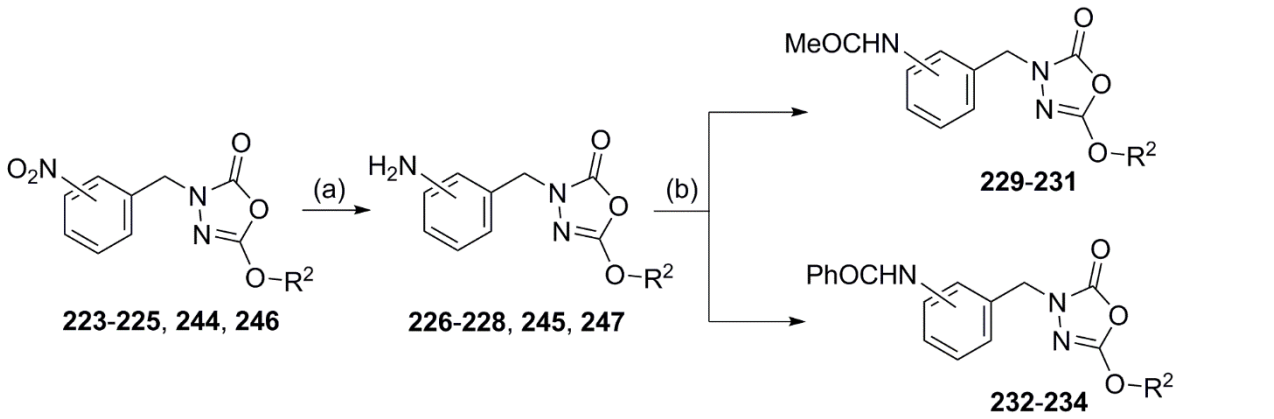
The synthetic procedures of 1,3,4-oxadiazol-2-ones (220-223, 225 and 235-247, Table 34) are similar as described in the original publication (I). Compound 224 was disclosed in our

earlier report (Savinainen *et al.,* 2014) while the detailed synthetic procedures of analogues (226-234, Table 34) is described in the original publication (IV).

The targeted naphthalene analogues (220 and 221, see Scheme 6) were synthesized using the appropriate naphthaldehyde in a similar manner as described for Scheme 1 (Note: R^2 = Me). The targeted 3-benzyl-1,3,4-oxadiazol-2-ones 222, 223, 225 and 235-247 (Note: R^2 = Me, except compounds 244 and 246 where R^2 = Et and Ph, repectively, and for all compounds R^3 = H in Scheme 1) were obtained through a similar procedure as described in Scheme 1 (see section 5.2.1). Nitro-containing 1,3,4-oxadiazol-2-ones (223-225, 244 and 246) obtained through Scheme 1 were reduced by 10% Pd/C to the corresponding amino derivatives (226-228, 245 and 247, respectively, see Scheme 7). Selected amino derivatives (226-228) were coupled with either acetyl chloride or benzoyl chloride to produce the desired acetamides 229-231 and benzamides 232-234, respectively.



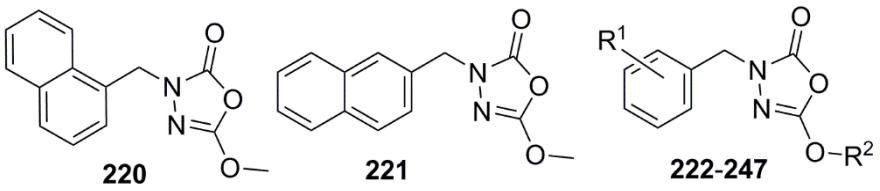
Scheme 6. Synthesis of 1,3,4-oxadiazol-2-ones 220 and 221. Reagents and conditions: see Scheme 1.



For compounds: **223-225**, **226-228, 229-231**, **232-234**: R^2 = Me; **244** & **245**: R^2 = Et; **246** & **247**: R^2 = Ph

Scheme 7. Synthesis of 1,3,4-oxadiazol-2-ones 226-234, 245 & 247. Reagents and conditions: (a) 10% Pd/C, H_2 (atm), 20-25 ^oC, 4-8 h, MeOH; (b) MeCOCl or PhCOCl, DCM, Et_3N, 0-25 °C, 16-24 h

Table 34. List of the disclosed 1,3,4-oxadiazol-2-ones 220-247.



Compd	R^1	R^2	Compd	R^1	R^2	Compd	R^1	R^2
220	-----	-----	230	*m*-NHCOMe	Me	240	*m*-Cl	Me
221	-----	-----	231	*p*-NHCOMe	Me	241	*m*-OMe	Me
222	*o*-Ph	Me	232	*o*-NHCOPh	Me	242	*m*-OBn	Me
223	*o*-NO_2	Me	233	*m*-NHCOPh	Me	243	*p*-OBn	Me
224	*m*-NO_2	Me	234	*p*-NHCOPh	Me	244	*m*-NO_2	Et
225	*p*-NO_2	Me	235	*m*-CN	Me	245	*m*-NH_2	Et
226	*o*-NH_2	Me	236	*p*-CN	Me	246	*m*-NO_2	Ph
227	*m*-NH_2	Me	237	*m*-COOMe	Me	247	*m*-NH_2	Ph
228	*p*-NH_2	Me	238	*p*-COOMe	Me			
229	*o*-NHCOMe	Me	239	*m*-CF_3	Me			

5.3 *IN VITRO* BIOLOGICAL SCREENING

Substrates such as *N*-arachidonoylethanolamine (AEA), 2-arachidonoylglycerol (2-AG), and 1(3)-arachidonoylglycerol (1(3)-AG) were purchased from Cayman Chemical (Ann Arbor, MI, USA). Tritium-labelled *N*-arachidonoyl ethanolamine [ethanolamine 1-^{3}H] (i.e. ^{3}H-AEA) was procured from American Radiolabeled Chemicals Inc. (St Louis, MO, USA). Hydrolase inhibitors were from the following sources: 1-oxazolo[4,5-*b*]pyridin-2-yl-1-dodecanone (CAY10435), *N*-phenyl-4-(quinolin-2-ylmethyl)piperidine-1-carboxamide (PF-750, 49) were obtained from Cayman Chemicals; MAFP (2), URB597 (23), WWL70 (125) and THL (orlistat) from Sigma-Aldrich; 5-ethoxy-3-(3-phenoxyphenyl)-1,3,4-oxadiazol-2(3*H*)-one (ATM-114), JZL195 (107) and JJKK-048 (121) were synthesized in house.

5.3.1 *In vitro* assay for FAAH activity

FAAH inhibitory values of the synthesized compounds 147-217 and 219-247 described in sections 5.2.1-5.2.4 were determined using membranes of COS-7 cells expressing human recombinant FAAH (hFAAH-COS-7) which has the ability to hydrolyze tritium-labelled

anandamide [ethanolamine 1-^{3}H] (i.e. [^{3}H]-AEA) (Figure 16), essentially as in the previously described radioactivity-based enzymatic assay (Saario *et al.,* 2006).

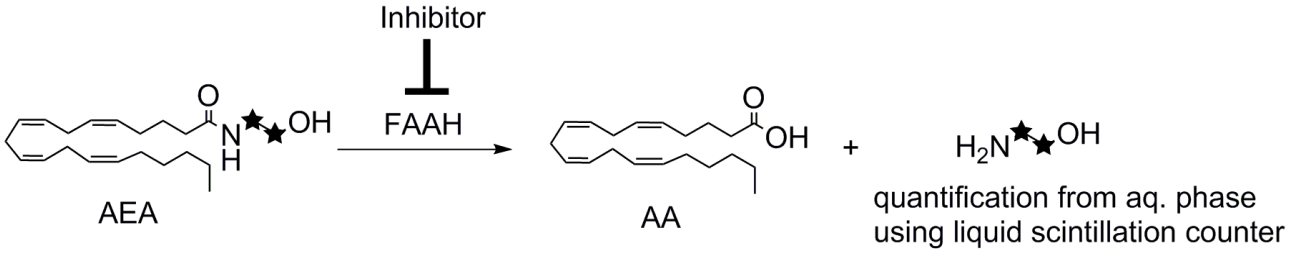


Figure 16. Schematic representation of radioactivity-based FAAH assay. FAAH causes hydrolysis of [^{3}H]-AEA to AA and radioactive ethanolamine; in the presence of an inhibitor of FAAH this process is either blocked or slowed down.

The assay buffer was 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.4); 1 mM ethylenediaminetetraacetic acid (EDTA) and the test compounds as well as reference standard/s were dissolved in DMSO (the final DMSO concentration was max 5% v/v). The incubations were performed in the presence of 0.5% (w/v) bovine serum albumin (BSA), essentially fatty acid free. Test compound or vehicle (as control, 5 μL) was preincubated with protein (1 μg, 55 μL) for 10 min at 37 °C (60 μL). The protein concentration was determined by the method of Bradford with BSA as a standard (Bradford 1976). At the 10 min time point, 20 μM AEA was added so that its final concentration was 2 μM in 100 μL (containing 10 nM of [^{3}H]-AEA having specific activity of 60 Ci/mmol and concentration of 1 mCi/mL). The incubations proceeded for 10 min at 37 °C. Ethyl acetate (EtOAc, 400 μL) was added at the 20 min time point to stop the enzymatic reaction. Additionally, 100 μL of 50 mM Tris-HCl, pH 7.4; 1 mM EDTA was added. Samples were centrifuged at 13000 rpm for 4 min at RT, and aliquots (100 μL) from the aqueous phase containing [ethanolamine 1-^{3}H] were measured for radioactivity by liquid scintillation counting (Wallac 1450 MicroBeta; Wallac Oy, Finland). Non-specific binding was subtracted from all the values. Those inhibitors causing more than 50% FAAH inhibition at 10 μM were tested with at least five to six different concentrations (10^{-9} M to 10^{-4} M) to obtain a dose response curve and the IC_{50} values calculated from non-linear regressions using GraphPad Prism 5.0 for Windows.

5.3.2 *In vitro* assay for MAGL activity

The ability of synthesized compounds 147-217 and 219-247 described in sections 5.2.1-5.2.4 to inhibit MAGL was determined using lysates of human MAGL (hMAGL-HEK) overexpressing HEK cells which has the capability to hydrolyze 2-arachidonoylglycerol (2-AG) (Figure 17), this being assayed by the previously described HPLC-based enzymatic method (Saario *et al.,* 2004, Minkkilä *et al.,* 2008).

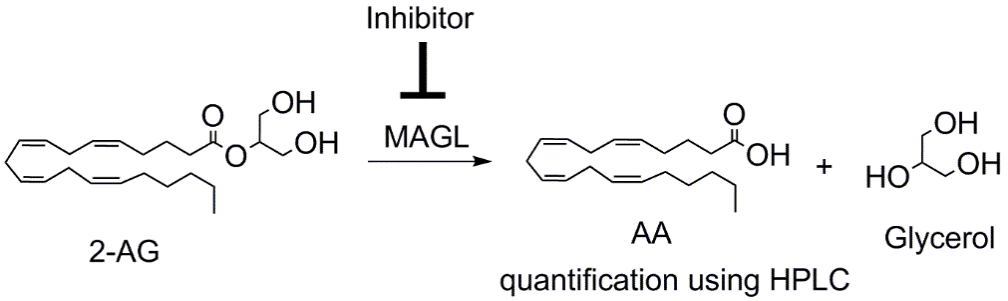


Figure 17. Schematic representation of HPLC-based MAGL assay. MAGL causes hydrolysis of 2-AG to AA and glycerol; in the presence of an inhibitor of MAGL this process is either blocked or slowed down.

Incubations were conducted in 50 mM Tris–HCl (pH 7.4), 1 mM EDTA with 0.5% (w/v) BSA. Test compounds were dissolved in DMSO (the final DMSO concentration was not more than 5% v/v). Test compound or vehicle (as control, 5 μL) was preincubated with protein (2.5 μg, 55 μL) for 10 min at 37 °C (60 μL). The hrMAGL concentration chosen was within the initial rate velocity for the enzyme reaction. At the 10 min time point, 2-AG (125 μM) was added to achieve a final concentration of 50 μM with the final incubation volume of 100 μL. The incubations proceeded for 10 min at 37 °C. To stop the enzymatic reaction against 2-AG, 400 μL of cold 11 mM phosphate buffer (H_3PO_4) in acetonitrile (ACN) (the pH of the samples was simultaneously decreased to 3.0) added to stabilize 2-AG against acyl migration to 1(3)-AG. All samples were centrifuged at 13000 rpm for 4 min at RT. The formation of arachidonic acid (AA) and depletion of 2-AG (and 1(3)-AG) were measured by HPLC according to the previously reported method with minor modifications (Saario *et al.,* 2004).

The analytical HPLC system consisted of an Agilent (Agilent Technologies, Santa Clara, California, USA) G1322A vacuum degasser, G1312A binary pump, G1313A autosampler, G1316A Colcom thermostatted column compartment, G1315B diode array UV/Vis detector (set at 211 nm) and 35900E interface module. The separations were accomplished on a Zorbax XDB-C18 Rapid Resolution HT column (4.6 x 50 mm, 1.8 μm) (Agilent, U.S.A). The injection volume was 20 μL. A mobile phase mixture of 34% H_3PO_4 (30 mM, pH 3.0) in ACN was used at a flow rate of 2 mL/min at 50 °C. Retention times were 2.57 min for 2-AG, 2.81 min for 1(3)-AG and 4.41 min for AA. The relative concentrations of 2-AG, 1(3)-AG and AA were determined by the corresponding peak areas. This was justified by the equivalence of response factors for the studied compounds, and was supported by the observation that the sum of the peak areas was constant throughout the experiments. Any inhibitor causing more than 50% MAGL inhibition at 10 μM was tested with at least five to six different

concentrations (10^{-9} M to 10^{-4} M) to obtain a dose response curve and the IC_{50} values calculated from non-linear regressions using GraphPad Prism 5.0 for Windows.

5.3.3 Determination of ABHD6/12 activity

ABHD6/12 inhibitory values of the selected synthesized compounds described in sections 5.2.1-5.2.4 were determined using lysates of HEK293 cells expressing human ABHD6 (hABHD6) or human ABHD12 (hABHD12) and its ability to hydrolyze 1(3)-AG (or 2-AG) to AA and glycerol (Figure 18) where the production of the latter was quantified by a sensitive fluorometric enzymatic method according to the validated method described previously (Navia-Paldanius *et al.,* 2012, Aaltonen *et al.,* 2013).

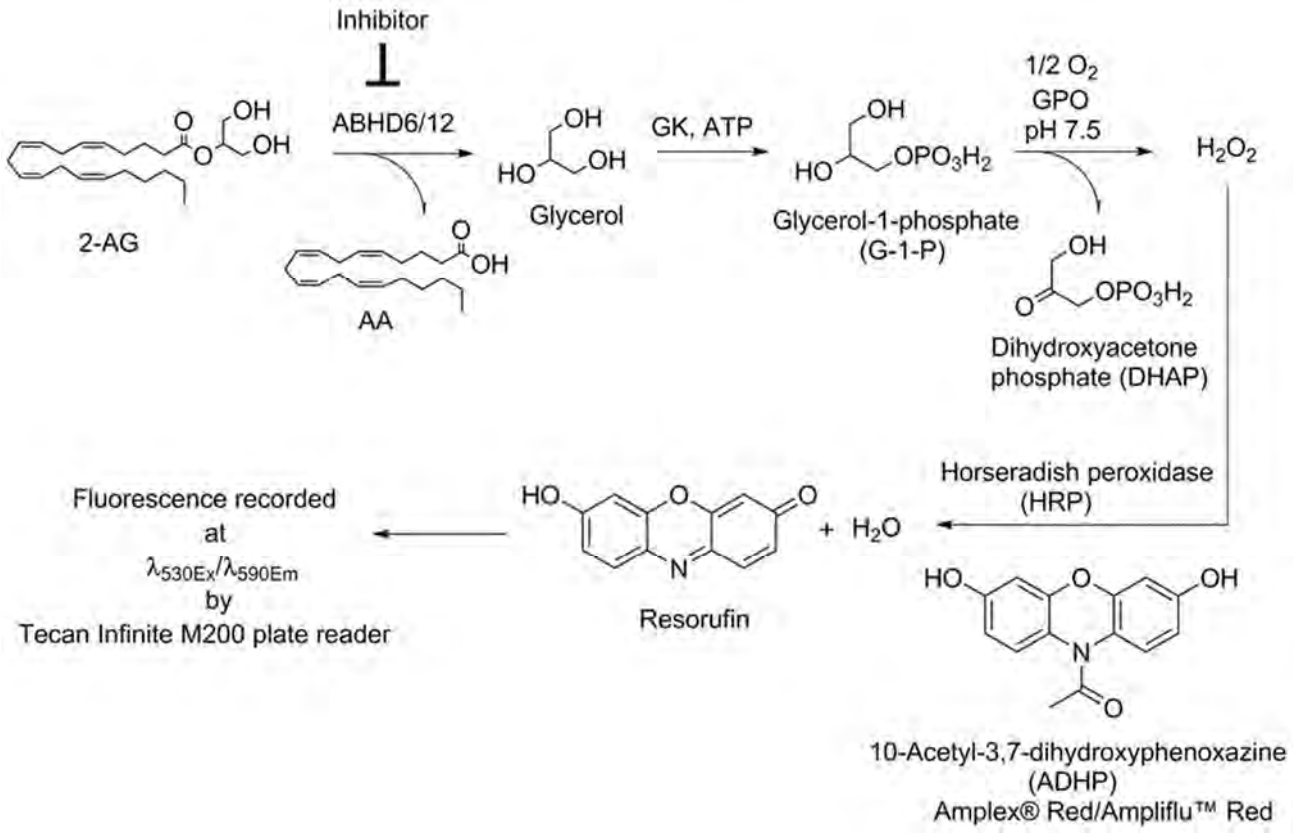


Figure 18. Schematic representation of a sensitive fluorometric glycerol assay for ABHD6 and ABHD12. ABHD6/12 causes hydrolysis of 1(3)-AG (or 2-AG) to AA and glycerol. The glycerol production was coupled via a three-step enzymatic cascade to hydrogen peroxide (H_2O_2) dependent generation of resorufin whose fluorescence was monitored. Abbreviations: GK, glycerol kinase; G-1-P, glycerol-1-phosphate; ATP, adenosine triphosphate; GPO, glycerol phosphate oxidase; DHAP, dihydroxyacetone phosphate; HRP, horseradish peroxidase.

Incubations were conducted in the assay buffer consisting of 50 mM Tris–HCl (pH 7.4), 1 mM EDTA with 0.5% BSA (w/v, essentially fatty acid free), 5 mM $MgCl_2$, 100 mM NaCl. Cellular

lysates (99 µL, 0.3 µg/well, minimum amount required to obtain a optimal signal) were pre-incubated for 30 min at RT with 1 µL DMSO (control) or test compound. Thereafter, the glycerol assay mix containing 1(3)-AG or 2-AG (100 µL, 12.5 µM final concentration) was added and glycerol production monitored kinetically for 90 min at RT. Glycerol is converted to glycerol-1-phosphate (G-1-P) by glycerol kinase (GK) in the presence of adenosine triphosphate (ATP). Glycerol phosphate oxidase (GPO)-catalyzed oxidation of G-1-P generates H_2O_2 which in the presence of horseradish peroxidase (HRP) converts AmplifuTM Red into the fluorescent product resorufin. Resorufin fluorescence (λ_{ex} 530; λ_{em} 590 nm) was kinetically monitored using a Tecan Infinite M200 plate reader (Tecan Group Ltd., Männedorf, Switzerland). The assays routinely contained 0.5% (w/v) BSA (essentially fatty acid free) as a carrier for lipophilic compounds. 1-AG was used instead of 2-AG, as this is the preferred endocannabinoid isomer for hABHD6 and hABHD12 (Navia-Paldanius *et al.,* 2012). The IC_{50} values at the time-point 90 min were calculated after nonlinear fitting of the inhibitor dose–response curves. To monitor assay performance, assay blanks without enzyme, cellular background (HEK293 cell lysates) as well as a glycerol standard were included for each tested substrate. Fluorescence of the assay blank was subtracted before calculation of the final results. Inhibitors causing more than 50% ABHD6/12 inhibition at 1 or 10 µM were tested with at least four to five different concentrations (10^{-9} M to 10^{-4} M) to obtain dose response curve and the IC_{50} values calculated from non-linear regressions using GraphPad Prism 5.0 for Windows.

5.3.4 CB_1 and CB_2 receptor assays

Cannabinoid receptor-mediated G-protein activity of the selected compounds described in sections 5.2.1-5.2.4 was measured according to earlier reported methods (Savinainen *et al.,* 2003, Savinainen *et al.,* 2005). CB_1 receptor-dependent activity was determined in rat cerebellar membranes and CB2 receptor-dependent activity in membranes prepared from CHO cells stably transfected with the human CB2 receptor using guanosine 5'-*O*-(3-[^{35}S]-thio)-triphosphate ([^{35}S]-GTPγS) membrane-binding assay.

5.3.5 FAAH reversibility studies and kinetics

To determine the reversibility of FAAH inhibition by enantiomers 157, 159 and 161 (see section 5.2.1), the effect of preincubation time and the recovery of enzymatic activity after a rapid and large dilution (500-fold) of enzyme-inhibitor complex was studied, as described previously (Minkkilä *et al.,* 2009a). In the pre-incubation mixture, the amount of hFAAH-COS-7 cell membranes was 20-fold higher than the amount required for the activity assay, and the inhibitor concentration was 20-fold higher than the IC_{50} value (i.e. 11 nM (157), 23 nM (159), 98 nM (161)), and the concentrations of selected reference inhibitors were as follows, 17 nM for CAY10435, an established reversible inhibitor (Leung *et al.,* 2003), and 321

nM for PF-750 (49), a known irreversible inhibitor (Ahn *et al.,* 2007). hFAAH-COS-7 cell membranes (40 µg) were preincubated with the inhibitor (or DMSO) in 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA at 37°C for 60 min. Then 1.8 µL of enzyme-inhibitor mixture was taken and immediately diluted 500-fold into the assay buffer containing 30 µM AEA (total volume 900 µL). Incubations were continued at 37°C and 100 µL samples were taken at 0,10, 20, 30, 40, 50, 60 and 70 min time points. The enzymatic reaction was stopped by adding 400 µL of cold 11 mM H_3PO_4 in ACN. Tubes were centrifuged for 4 min at RT 14000 rpm. Then 150 µL aliquots were taken and 50 µL of 30 mM H_3PO_4 (pH 3.0) was added. The relative concentration of arachidonic acid was analyzed by the HPLC method (see *in vitro* assay for MAGL activity).

Kinetic studies of enantiomers 157, 159 and 161

In the time-dependency studies, hrFAAH-COS-7 cell membranes (1 µg, 55 µL) were preincubated at different time points of 0, 10 or 60 minutes with an inhibitor (or DMSO, 5 µL) in 50 mM Tris-HCl (pH 7.4) and 1 mM EDTA at 37°C for 10 minutes and then 40 µL at the appropriate AEA dilution was added giving a final volume of 100 µL. The incubation was continued for 10 min at 37°C. The reaction was stopped and the samples were analyzed as described in the FAAH reversibility assay.

5.3.6 MAGL reversibility studies

The reversibility of MAGL inhibition by compound 206 (see section 5.2.3) was determined in the presence of reference compounds; MAFP (2), an established irreversible inhibitor (Savinainen *et al.,* 2010) and ATM-114, a known reversible inhibitor (Savinainen *et al.,* 2010), through a 96-well plate format based fluorescent assay by quantifying the glycerol liberated from the 1(3)-AG by a sensitive fluorometric enzymatic method according to the validated method described previously (Aaltonen *et al.,* 2013). The reversibility of compounds (in volume of 0.5 µL) to inhibit hMAGL in HEK cells (in volume of 4.5 µL) was tested after a rapid 40-fold dilution of the preincubated enzyme-inhibitor complex with the substrate solution (195 µL). The inhibition of enzymatic hydrolysis and the reversibility of responses were followed at 0, 10, 30 and 60 min time-points at 25 °C. The statistically significant difference in the IC_{50} concentrations between 10 and 60 min time-points was taken as an indication of compound reversibility whereas a stable IC_{50} value between 0 and 60 min was considered as irreversible behaviour.

5.3.7 ABHD6 reversibility studies

The reversibility of ABHD6 inhibition by compounds 204 and 227 (see sections 5.2.2 and 5.2.4) was determined in the presence of reference compound WWL70 (125), an established irreversible inhibitor, in a 96-well plate format based fluorescent assay by quantifying

glycerol with a sensitive fluorometric enzymatic method according to the validated method described for the determination of reversibility for hMAGL (see subsection 5.3.6) (Aaltonen *et al.,* 2013).

5.3.8 Cyclooxygenase (COX) isoenzymes assay

The ability of compounds 157 and 158 (see section 5.2.1) to inhibit COX isoenzymes were determined by the earlier reported method with minor modifications (Meade *et al.,* 1993, Onnis *et al.,* 2010). Briefly, buffer (1 µM hematin, 2 mM phenol, 5 mM EDTA, substrate [AA or 2-AG, 10 µM final concentrations], 0.1 M Tris-HCl, pH 7.4) and test compound (in DMSO) was added to an oxygen electrode chamber (Oxygraph System, Hansatech Instruments, King's Lynn, U.K.). A baseline was established over a period of 5 min before reactions were started by the addition of COX (ovine COX-1 or human recombinant COX-2, as appropriate), and the oxygen consumption was measured in 10 second blocks. Data are presented as the change in oxygen consumption from the point of addition of the enzyme.

5.3.9 Lysosomal lipase (LAL) assay

The ability of selected synthesized compounds described in section 5.2.2 to inhibit LAL was determined using a previously described fluorescence-based enzymatic method (Figure 19) (Rosenbaum *et al.,* 2010).

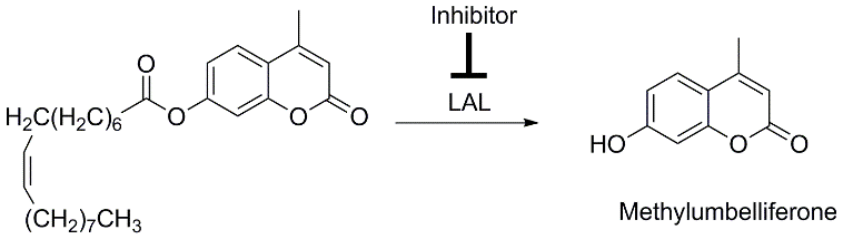


Figure 19. Schematic representation of the fluorescence-based assay for LAL. LAL causes hydrolysis of 4-methylumbelliferone oleate (4-MUO) to 4-methylumbelliferone. The generation of 4-methylumbelliferone fluorescence was monitored.

In short, purified human LAL overexpressed in Pichia pastoris (phLAL, 0.01 U/mL, 105 U/mg) was mixed with test compounds at 10 µM and preincubated for 20 minutes at 37°C. The reaction was started by addition of 4-methylumbelliferone oleate (4-MUO), which was cleaved by enzymatic activity to 4-methylumbelliferone. The reaction was allowed to proceed for 1 h at 37°C, and enzymatic activity was quantified by 4-methylumbelliferone fluorescence (λ_{ex} 355 nm/λ_{em} 450 nm) monitoring using a SpectraMaxM2 fluorometer (MDS

Inc.,Toronto, Canada). Background fluorescence was subtracted from all the values, and results were normalized to the DMSO control value.

5.3.10 Determination of histamine H_1 antagonistic activity

Compounds 206 and 219 described in section 5.2.3 were tested for histamine H_1 antagonistic effects *in vitro*, following standard methods, using the guinea pig ileum (Arunlakshana & Schild 1959). For tested compounds, concentrations up to 2 x 10^{-4} mol/dm^3 were prepared using DMSO as a solvent. Further dilutions were prepared in water. Aqueous solutions were prepared for pyrilamine. The compounds were tested a minimum of two times on the two different animal preparations and at a minimum of three different concentrations.

Male guinea pigs weighing 300-400 g were sacrificed by a blow to the head. The ileum was excised and placed in phosphate buffer at room temperature (pH 7.4) containing (mM) NaCl (136.9); KCl (2.68); $NaHPO_4$ (7.19). After flushing the intraluminal contents, segments of about 2 cm long were cut and mounted for isotonic contractions in water jacketed 20 mL organ baths filled with oxygenated (O_2:CO_2 = 95:5, v/v) Krebs buffer containing (mM) NaCl (117.5); KCl (5.6); $MgSO_4$ (1.18); $CaCl_2$ (2.5); NaH_2PO_4 (1.28); $NaHCO_3$ (25); glucose (5.5) and indomethacin (1 x 10^{-6} mol/L) at 37 °C under a constant load of 0.5 g. After a 30 min equilibration period with washings every 10 mins, a sub-maximal priming dose of histamine (1 mM) was given and washed out (standard washing procedure: 3 changes of buffer during 30 mins). After washing, the antagonistic activity of given compounds was measured by recording a concentration response curve (CRC) for histamine in the presence of the tested compounds which was added 5 mins before histamine. This procedure was repeated with higher concentrations of the compounds. The antagonism was of a competitive nature causing a parallel shift of the CRC. The pA_2-values were calculated according to Arunlakshana and Schild (Arunlakshana *et al.*, 1959). The pA_2 values were compared with the affinity of pyrilamine and loratadine.

5.3.11 Data analysis

The results from the enzyme inhibition experiments are presented as mean ± 95% confidence intervals of at least two independent experiments performed in duplicate. The results from enzyme reversibility studies are presented as mean ± S.E.M. of at least three independent experiments performed in duplicate. The inhibitor dose–response curves (DRCs) and IC_{50} values were calculated from nonlinear regressions using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, CA (USA): www.graphpad.com) and Matlab.

5.3.12 Activity-based protein profiling (ABPP) assay

The selected compounds described in sections 5.2.1-5.2.4 were tested using *in vitro* ABPP assay for its selectivity towards specific endocannabinoid hydrolyzing enzyme/s (i.e. FAAH, MAGL, ABHD6 and 12) over other serine hydrolases of mouse whole brain proteomes prepared from brain tissue of 4-week-old male mice (Figure 20), in accordance with the previously reported assay (Navia-Paldanius *et al.,* 2012, Aaltonen *et al.,* 2013). The ethical approval was obtained from the local welfare officer of UEF.

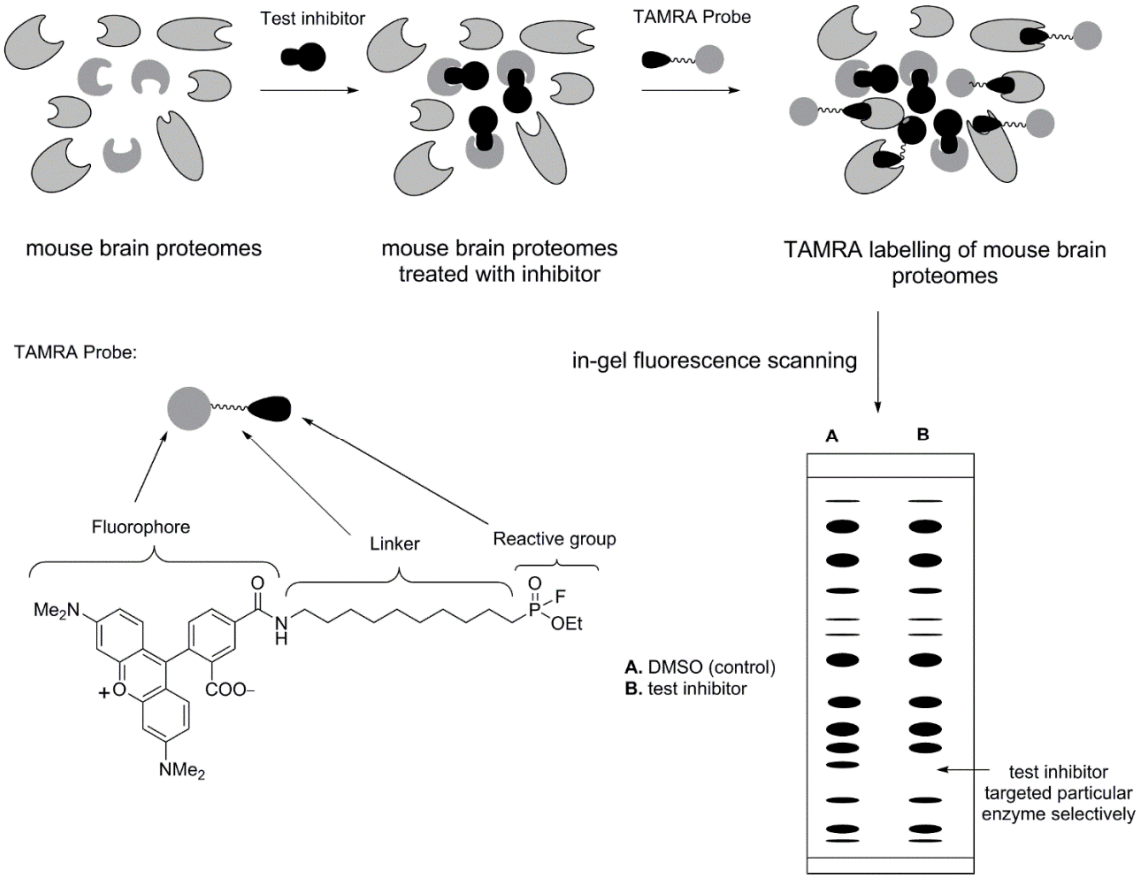


Figure 20. Schematic representation of a competitive ABPP of mouse brain membrane proteomes in the absence (Panel A) or presence of the test inhibitor (Panel B). The structure of the TAMRA probe also shown.

ABPP was conducted using carboxytetramethylrhodamine fluorophosphonate (TAMRA-FP; ActivX Fluorophosphonate Probes (FPs), Thermo Fisher Scientific Inc., Rockford, IL) probes. Briefly, brain membranes (100 µg) diluted in phosphate-buffered saline (PBS) were treated for 1 h with vehicle (DMSO) or the selected inhibitors (with appropriate concentrations ranging from 0.010-10 µM) at 25 °C. Then, serine hydrolases were labeled with 0.5 µL of 100 µM TAMRA-FP for 1 h at 25 °C (final probe concentration 2 µM). The reaction was quenched by addition of 2 x gel loading buffer, after which 10-20 µg protein was loaded per lane and the proteins were resolved in 10% SDS-PAGE together with molecular weight standards.

TAMRA-FP labeling was visualized (λ_{ex} 552; λ_{em} 575 nm) using a fluorescent scanner (FLA-3000 laser fluorescence scanner, Fujifilm, Tokyo, Japan).

5.4 SINGLE CRYSTAL X-RAY DIFFREACTION ANALYSIS

Enantiomerically pure solid compound 159 (see section 5.2.1) was crystallized by slow evaporation of its dichloromethane (DCM)/ethanol (EtOH) solution at 278 K. Subsequently, a suitable crystal was immersed in cryo-oil, mounted in a Nylon loop, and measured at a temperature of 120 °K. Data concerning the X-ray diffraction was collected on a Bruker Kappa Apex II Duo diffractometer using CuKα radiation (λ = 1.54178 Å). The APEX2 program package was used for cell refinements and data reductions. The structures were solved by direct methods using the SHELXS-97 programs with the WinGX graphical user interface. A semi-empirical absorption correction (SADABS) was applied to all data. Structural refinements were carried out using SHELXL-2013. All hydrogen atoms in enantiomer 159 were positioned geometrically and constrained to ride on their parent atoms, with C-H = 0.95-1.00 Å, and Uiso = 1.2-1.5 Ueq (parent atom).

Analysis of the absolute structure of enantiomer 159 using likelihood methods (Hooft, Straver & Spek, 2008) was performed using PLATON. The absolute structure parameter *y* was calculated to yield 0.06(8) indicating that the absolute structure (*S*) has probably been determined correctly. The method calculated that the probability that the structure is inverted was smaller than 4×10^{-28}. The Flack *x* parameter was refined to give a value of 0.08(9) that also corresponds to a correct absolute structure determination.

6 Results and Discussion

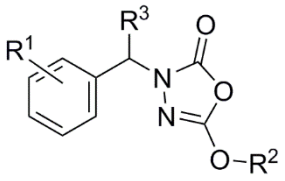
The *in vitro* biological evaluation of 1,3,4-oxadiazol-2-ones (147-170, 220-247), 1,2,5-thiadiazole carbamates (171-247), loratadine (205) and its analogues (206-217), and cyproheptadine analogue 219 was initiated by screening the inhibitory activities against hFAAH and hMAGL at 10 µM concentrations (see subsections 5.3.1-5.3.2 for assay protocols). Selected compounds were further screened at 1 or 10 µM concentrations against hABHD6 and hABHD12 using the assay protocols discussed above (see subsection 5.3.3). The IC_{50} values were calculated for those compounds having ⩾50% inhibition at the mentioned concentration against specific enzymes. The detailed results are discussed in the original publication (I-IV) and only a brief overview is presented here.

Some of the potent inhibitors found through the above screening procedures were further tested towards cannabinoid receptors (see subsection 5.3.4 for assay protocol) and other possible off-targets, such as COX isoenzymes for 1,3,4-oxadiazol-2-ones (see subsection 5.3.8 for assay protocol), LAL for 1,2,5-thiadiazole carbamates (see subsection 5.3.9 for assay protocol), histamine H_1 antagonistic affinity for loratadine analogues and cyproheptadiene analogue (see subsection 5.3.10 for assay protocols). The detailed results are discussed in the original publication (I-IV) and only outcome is revealed here. Finally, the best inhibitors in terms of potency and selectivity were tested further to determine their binding properties and/or inhibition mechanism towards specific enzyme (see subsections 5.3.5-5.3.7 for reversibility assay protocol), and selectivity over serine hydrolases of mouse brain membrane proteomes (see subsection 5.3.12). The detailed results are discussed in the original publication (I-IV) and only outcome is stated here.

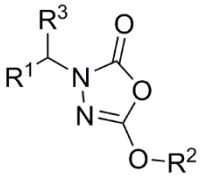
6.1 Chiral 1,3,4-oxadiazol-2-ones (I)

The inhibitory activity data of 1,3,4-oxadiazol-2-ones 147-170 tested at 10 µM concentration towards hFAAH and hMAGL are presented in Table 35. All of these compounds achieved ⩽ 50% inhibition for hABHD6 and hABHD12 at 1 µM. Hence, inhibition data towards these enzymes are not shown here, however, in the original publication (I) they were reported as supplementary information.

Table 35. Inhibitory activities of the 1,3,4-oxadiazol-2-ones 147-170 against FAAH and MAGL.



147-151, **154-170**



152: $R^1 = R^3 = Me$

153: $R^1\text{-}R^3 = (CH_2)_5$

R^2 = Me for compounds **147-170**

Compd	R^1	R^3	pI_{50} (range) [IC_{50}, μM][a] or % inhibition at 10 μM[b]	
			hFAAH	hMAGL
147	*p*-isobutyl	Me	7.64 (7.63-7.65) [0.023]	48%
148	*p*-Ph	Me	7.33 (7.32-7.33) [0.048]	34%
149	*m*-Ph	Me	5.93 (5.92-5.94) [1.2]	42%
150	*p*-OPh	Me	7.47 (7.45-7.49) [0.034]	5.68 (5.62-5.75) [2.0]
151	*m*-OPh	Me	5.28 (5.25-5.32) [5.2]	14%
152	------	------	12%	6%
153	------	------	40%	NI[c]
154	H	Me	5.22 (5.20-5.24) [6.0]	NI
155	H	Ph	NI	NI
156	*p*-Ph_2N	Me	6.94 (6.92-6.96) [0.12]	6.20 (6.17-6.23) [0.63]
157 (JZP-327A)	(-)-*p*-isobutyl	Me	7.94 (7.91-7.97) [0.011]	16%
158	(+)-*p*-isobutyl	Me	6.61 (6.57-6.66) [0.24]	4.87 (4.51-5.24) [4.0]
159	(-)-*p*-Ph	Me	7.63 (7.60-7.66) [0.023]	18%
160	(+)-*p*-Ph	Me	5.54 (5.50-5.58) [3.0]	49%
161	(-)-*p*-Ph_2N	Me	7.0 (6.99-7.01) [0.098]	41%
162	(+)-*p*-Ph_2N	Me	5.21 (5.03-5.40) [6.3]	6.47 (6.43-6.50) [0.35]
163	H	H	NI	NI
164	*p*-isobutyl	H	7.05 (7.02-7.08) [0.091]	5.87 (5.86-5.89) [1.3]
165	*p*-Ph	H	6.79 (6.78-7.79) [0.16]	5.79 (5.69-5.88) [1.6]
166	*m*-Ph	H	46%	32%
167	*o*-OPh	H	5.20 (5.15-5.26) [6.3]	NI
168	*m*-OPh	H	6.01 (5.97-6.03) [0.98]	15%

169	*p*-OPh	H	7.0 (6.89-7.14) [0.10]	6.40 (6.39-6.41) [0.40]
170	*p*-Ph_2N	H	6.34 (6.30-7.37) [0.47]	6.60 (6.55-6.65) [0.26]
CAY10435[d]	------	------	7.78 ± 0.09 [0.017][e]	NA[f]
PF-750 (49)[g]	------	------	6.49 ± 0.09 [0.32][e]	NA

[a] pI_{50} values ($-\log_{10}$ [IC_{50}]) represent the mean (range) from two independent experiments performed in duplicate. IC_{50} values are calculated for those compounds having $\geqslant$ 50% inhibition at the tested concentrations and are derived from the mean pI_{50} values as shown in brackets. [b] The percentage (%) of inhibition is represented as the mean from two independent experiments performed in duplicates. [c] NI indicates no inhibition. [d] 1-oxazolo[4,5-*b*]pyridin-2-yl-1-dodecanone has a commercial supplier code CAY10435 and was used as a reference FAAH inhibitor as has been reported by Boger *et al*., 2000. [e] pI_{50} values ($-\log_{10}$ [IC_{50}]) represent the mean ± S.E.M. from three independent experiments performed in duplicates. [f] NA indicates not analyzed. [g] *N*-phenyl-4-(quinolin-2-yl-methyl)piperidine-1-carboxamide (PF-750, 49) was used as reference FAAH inhibitor and reported by Ahn *et al*., 2007.

SARs of 1,3,4-oxadiazol-2-ones 147-170

As shown in Table 35, the hybridized 1,3,4-oxadiazol-2-ones of ibuprofen (147) and simplified flurbiprofen (148) showed excellent inhibitory activities against FAAH in low nanomolar range (IC_{50} = 23 nM and 48 nM, respectively) while fenoprofen analogue (151) showed moderate inhibitory activity (IC_{50} = 5.2 μM). These compounds showed good selectivity towards FAAH since they only weakly inhibited MAGL at 10 μM concentration (48%, 34%, 14%, respectively). A similar trend in the inhibitory activities against FAAH was observed for the *meta*-substituted analogue of 148 (compound 149) and *para*-substituted analogue of 151 (compound 150) as *meta*-substitution (149) decreased and *para*-substitution (150) improved FAAH inhibition (IC_{50} = 1.2 and 34 nM, respectively). Compound 149 showed very weak inhibition (42%) of MAGL at 10 μM concentration while compound 150 exhibited a weak inhibitory activity against MAGL (IC_{50} = 2 μM). The difference in the binding of compounds 149 and 150 toward FAAH and MAGL was attributable to the different substituents. The 1,3,4-oxadiazol-2-ones of acetone (152), cyclohexanone (153), acetophenone (154), and benzophenone (155) showed a dramatic loss of inhibitory activities against both FAAH and MAGL. The results of analogues 152 and 153 highlight the importance of the aromatic ring while compounds 154 and 155 emphasize the importance of proper fitting of a smaller methyl group compared to bulky phenyl ring at the 3-position of 1,3,4-oxadiazol-2-ones to FAAH binding site. Compound 156 which had a bulkier hydrophobic substituent at the *para*-position of the phenyl ring exhibited slightly less potency (IC_{50} = 0.12 μM) toward FAAH. However, improvement in MAGL activity (IC_{50} = 0.63 μM) was observed.

Selected chiral 1,3,4-oxadiazol-2-ones 147, 148 and 156 were separated using a chiral column (see subsection 5.1). An improved FAAH inhibition was observed for (-)-enantiomers 157, 159 and 161, with IC_{50} values of 11, 23 and 98 nM, respectively, compared to their racemic

analogues. At the same time, improved selectivity of these analogues over MAGL was observed. In contrast, (+)-enantiomers 158, 160 and 162 show decreased FAAH inhibition (IC_{50} = 0.24, 3.0 and 6.3 μM, respectively) as compared to both racemic mixture and (-)-enantiomers though their potency to inhibit MAGL was slightly improved. For example, the IC_{50} value of the MAGL inhibition for the compound 162 was 0.35 μM while its (-)-enantiomer 161 showed only 41% inhibition at 10 μM concentration. Finally, the absolute configuration of the solid compound 159 was determined through the single-crystal X-ray diffraction and it was found to be an *S*-enantiomer (subsection 5.4). Since the configuration will remain the same for other (-)-enantiomers, we concluded that 157 and 161 have the same configuration i.e. *S*-configuration.

Compounds 163-170 lacking the methyl group at 3-position of 1,3,4-oxadiazol-2-one ring showed either a drastic drop or a complete loss of the inhibitory activities against FAAH and MAGL. Moreover, several of these analogues showed loss of selectivity over MAGL.

Overview towards other potential off-targets

The hybridized analogues of ibuprofen, i.e. (-) and (+)-enantiomers 157 (JZP-327A) and 158, were found to be inactive towards COX isoenzymes at 10 μM concentration. The best FAAH inhibitors, (-)-enatiomers 157 (JZP-327A) and 159 did not show any blockade or activation of cannabinoid receptors 10 μM concentration. Moreover, detailed inhibition mechanism studies of (-)-enantiomers 157 (JZP-327A), 159 and 161 suggested that they were tight binding, slowly reversible inhibitors of the hFAAH. Nonetheless, the mechanistic studies provided evidence of the noncompetitive nature of these compounds, in the molecular modeling studies they showed favorable interactions within the active site of hFAAH comprising the catalytic triad Ser^{217}-Ser^{241}-Lys^{142} including important hydrogen-bonding of the carbonyl oxygen to the oxyanion hole Ile^{238}-Gly^{239}-Gly^{240}-Ser^{241}. Finally, when compound 157 (JZP-327A) was tested against other serine hydrolases labelled by TAMRA-FP in mouse brain membrane proteome using competitive ABPP, it exhibited a dose-dependent inhibition of FAAH, achieving complete inhibition at 1 μM. Moreover, the high selectivity of compound 157 (JZP-327A) towards FAAH was also evident as it did not show any inhibition against the other brain serine hydrolases including MAGL, ABHD6 and ABHD12 at 10 μM concentration.

Conclusion

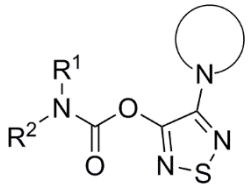
In short, with respect to the disclosed analogues, *S*-enantiomer of 3-(1-(4-isobutylphenyl)ethyl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (JZP-327A, 157), was found to be a potent FAAH inhibitor (IC_{50} 11 nM) with a slowly reversible mode of inhibition. Moreover, it was found to be > 900 fold FAAH selective over the main putative off-targets, MAGL, cannabinoid receptors, and COX isoenzymes. Finally, in proteomic analysis,

compound 157 displayed high selectivity among the serine hydrolases of mouse brain membrane proteome.

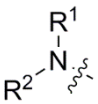
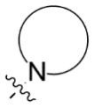
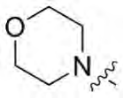
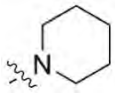
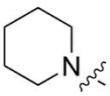
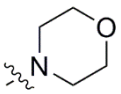
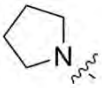
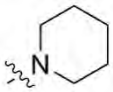
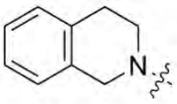
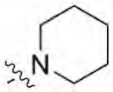
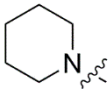
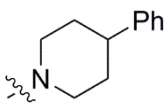
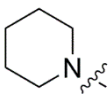
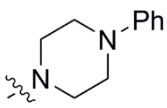
6.2 1,2,5-thiadiazole carbamates (II)

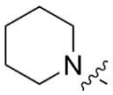
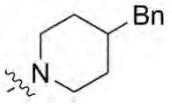
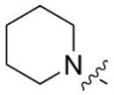
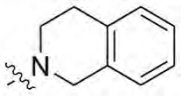
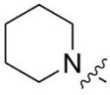
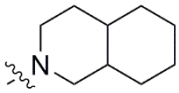
The inhibitory activity data of 1,2,5-thiadiazole carbamates 171-204 screened at 1 and 10 µM towards hABHD6 and hFAAH, respectively, are presented in Tables 36 and 37. All compounds, except compound 185 showed ⩽50% inhibition at 10 µM concentration for hMAGL. Moreover, all of these compounds were found to be inactive toward hABHD12 at 1 µM. Hence, inhibition data towards hMAGL and hABHD12 enzymes are not shown here, however, in original publication (II) they were reported as supplementary information.

Table 36. ABHD6 and FAAH inhibitory activities of the 1,2,5-thiadiazole carbamates 171-179 having cyclic amine at the main core.



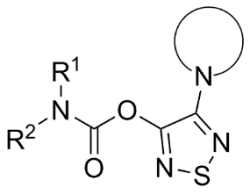
171-179

Compd			pI_{50} (range) [IC_{50}, µM][a] or % inhibition at 1 µM[b]	pI_{50} (range) [IC_{50}, µM][a] or % inhibition at 10 µM[b]
			hABHD6	hFAAH
171			7.28 (7.23-7.32) [0.052]	6.39 (6.29-6.49) [0.40]
172			7.07 (7.03-7.10) [0.085]	6.48 (6.41-6.55) [0.30]
173			6.58 (6.43-6.73) [0.26]	6.09 (6.01-6.18) [0.81]
174			6.88 (6.80-6.95) [0.13]	6.25 (6.23-6.27) [0.56]
175			41 %	5.83 (5.34-6.31) [1.47]
176			40 %	6.68 (6.51-6.84) [0.21]

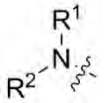
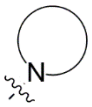
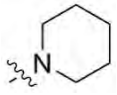
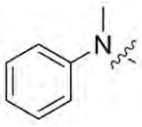
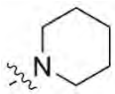
177			15 %	6.49 (6.30-6.67) [0.32]
178			6.34 (6.22-6.45) [0.46]	7.77 (7.71-7.83) [0.017]
179			6.25 (6.19-6.31) [0.56]	7.51 (7.48-7.53) [0.031]
WWL70 (125)[c]	-----	-----	7.07 ± 0.05 [0.085][d]	30 %
THL[e]	-----	-----	7.32 ± 0.11 [0.048]	NA[f]
JZP-327A (157)[g]	-----	-----	NI[h]	7.94 (7.91−7.97) [0.011]

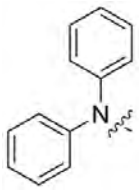
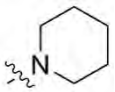
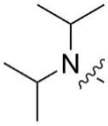
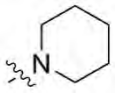
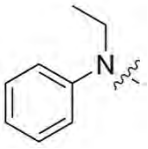
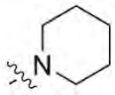
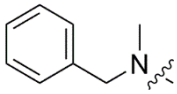
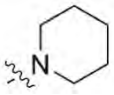
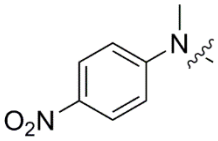
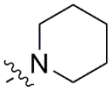
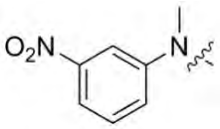
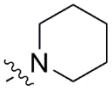
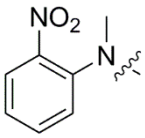
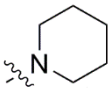
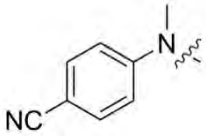
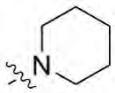
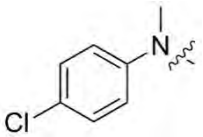
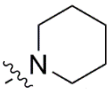
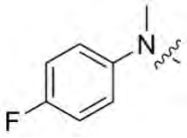
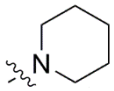
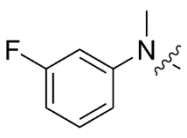
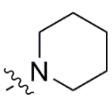
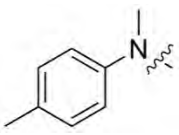
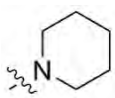
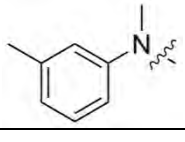
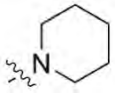
[a,b] See footnotes of Table 35. [c] *N*-Methyl-*N*-[[3-(4-pyridinyl)phenyl]methyl]-4'-(aminocarbonyl)[1,1'-biphenyl]-4-yl carbamic acid ester (WWL70, 125) used as reference ABHD6 inhibitor and reported by Li *et al*., 2007. [d] pI_{50} values (-log$_{10}$ [IC$_{50}$]) represent the mean ± S.E.M. from three independent experiments performed in duplicates and reported by Navia-Paldanius *et al*., 2012. [e] THL, tetrahydrolipstatin (orlistat) and its ABHD6 inhibitory values are reported by Navia-Paldanius *et al*., 2012. [f] NA indicates not analyzed. [g] *S*-(-)-3-(1-(4-isobutylphenyl)ethyl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (JZP-327A, 157) used as reference FAAH inhibitor reported by Patel *et al*. 2013. [h] NI indicates no inhibition.

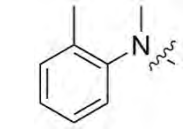
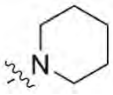
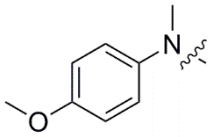
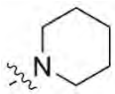
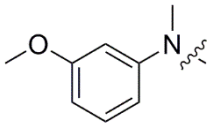
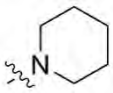
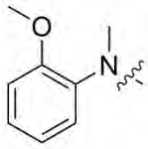
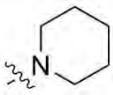
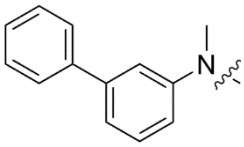
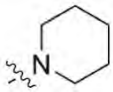
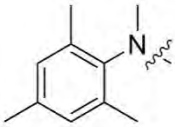
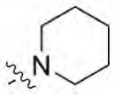
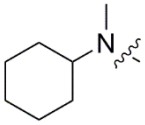
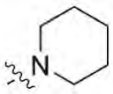
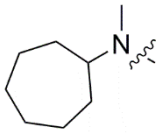
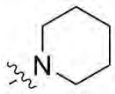
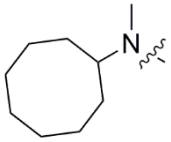
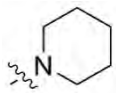
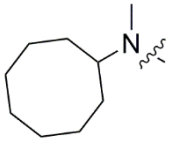
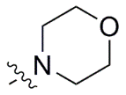
Table 37. ABHD6 and FAAH inhibitory activities of the 1,2,5-thiadiazole carbamates 180-204 having non-cyclic amine at the main core.



180-204

Compd			pI_{50} ± SEM [IC$_{50}$, μM][a] or % inhibition at 1 μM[b]	pI_{50} (range) [IC$_{50}$, μM][c] or % inhibition at 10 μM[b]
			hABHD6	hFAAH
180			10 %	5.19 (5.17-5.20) [6.45]
181			7.66 ± 0.07 [0.022]	5.06 (5.05-5.07) [8.91]

182			NI[d]	NI
183			NI	NI
184			6.33 ± 0.13 [0.47]	24 %
185			8.01 ± 0.03 [0.010]	7.20 (7.17-7.23) [0.063]
186			5.90 ± 0.08 [1.25]	19 %
187			5.92 ± 0.05 [1.20]	11 %
188			NI	46 %
189			15 %	19 %
190			6.39 ± 0.03 [0.41]	16 %
191			7.11 ± 0.07 [0.078]	22 %
192			7.22 ± 0.05 [0.060]	48 %
193			6.83 ± 0.04 [0.15]	21 %
194			7.27 ± 0.07 [0.054]	9 %

195			17 %	40 %
196			6.58 ± 0.04 [0.26]	17 %
197			6.71 ± 0.07 [0.19]	17 %
198			11 %	18 %
199			6.04 ± 0.10 [0.91]	13 %
200			NI	7 %
201			7.36 ± 0.05 [0.044]	16 %
202			7.37 ± 0.05 [0.043]	21 %
203			7.14 ± 0.06 [0.072]	13 %
204 (JZP-430)			7.36 ± 0.05 [0.044]	18 %

[a] pI_{50} values (-$\log_{10}$ [IC_{50}]) represent the mean ± S.E.M. from three independent experiments performed in duplicates. IC_{50} values are calculated for those compounds having ⩾50% inhibition at 1 μM for hABHD6 and are derived from the mean pI_{50} values as shown in brackets. [b] The percentage (%) inhibition is represented as the mean from two independent experiments performed in duplicate. [c] pI_{50} values (-$\log_{10}$ [IC_{50}]) represent the mean (range) from two independent experiments performed in duplicate. IC_{50} values are calculated for those compounds having ⩾50% inhibition at 10 μM for hFAAH and are derived from the mean pI_{50} values as shown in brackets. [d] NI indicates no inhibition.

SARs of 1,2,5-thiadiazole carbamates 171-204

As shown in Table 36, 1,2,5-thiadiazole carbamates 171-174 having diverse cyclic amines at the main core were found to be slightly better inhibitors of ABHD6 compared to FAAH at the tested concentration. For example, analogue 171 showed excellent ABHD6 inhibition in the low nanomolar range (IC_{50} 52 nM) while moderate inhibitory activity was observed against FAAH (IC_{50} 0.40 μM). With respect to the 1,2,5-thiadiazole carbamates 175-179 which had an intact piperidine at the main core and a different bulky substituent as a possible leaving group, analogues 175-177 showed moderate FAAH inhibition, while only weak inhibition of ABHD6 was observed at selected concentration. However, fused bicyclic analogues 178 and 179 showed improved FAAH inhibition (IC_{50} 17 nM and 31 nM, respectively) while moderate inhibition ABHD6 was observed (IC_{50} 0.46 and 0.56 μM, respectively). In general, several of analogues 171-179 were found to be potent ABHD6 inhibitors but no satisfactory selectivity over FAAH was observed (selectivity-ratio < 30-fold).

Subsequently, 1,2,5-thiadiazole carbamates 180-204 having non-cyclic amines at the main core were tested and the results are shown in Table 37. The *N*,*N*-dimethyl analogue 180 showed weak FAAH inhibition (IC_{50} 6.45 μM) while no inhibition was observed toward ABHD6. However, replacement of one methyl group of 180 with a phenyl group (compound 181) resulted in the creation of a highly potent ABHD6 inhibitor (IC_{50} 22 nM) which had 404-fold selectivity over FAAH (IC_{50} 8.9 μM). However, the addition of another phenyl group into compound 181 (compound 182) resulted in a complete loss of activity. A similar result was observed with the *N*,*N*-diisopropyl analogue (compound 183) as it also showed a loss of activity. The alteration of the methyl group of compound 181 for an ethyl (compound 184) resulted in a ~20-fold drop in potency while selectivity was still evident as it possessed weak FAAH inhibition. The replacement of the phenyl ring of compound 181 into benzyl (compound 185) resulted in a 2-fold increase in ABHD6 inhibitory activity (IC_{50} 10 nM). However, the loss of selectivity was observed as it also showed excellent FAAH inhibition (IC_{50} 67 nM) as well as moderate MAGL inhibition (IC_{50} 5.6 μM).

Substitution at the *para* or *meta* position on the phenyl ring of compound 181 by different electron withdrawing groups (EWGs; i.e. compounds 186, 187, 189-192) or electron releasing groups (ERGs; i.e. compounds 193, 194, 196, 197) generally displayed a 4- to 55-fold drop of ABHD6 inhibitory activity while *ortho* substitution by either EWG (compound 188) or ERGs (compounds 195 and 198) resulted in a complete loss of activity. Although, the majority of these analogues still maintain significant selectivity over FAAH. Compound 199 which had a *meta*-phenyl substituent led to a 40-fold decline in the inhibitory activity against ABHD6. However, it still maintains marginal selectivity over FAAH. The bulky trimethyl substituted compound 200 showed complete loss of activity towards both ABHD6 and FAAH. Since no further improvements in ABHD6 inhibitory activity or selectivity were obtained with the *N*-methyl-*N*-aryl analogues 186-200, the phenyl ring of compound 181 was replaced by different cycloalkyl rings (compounds 201-203). These *N*-methyl-*N*-benzyl analogues (201-203)

resulted in approximately a 2-4-fold loss of ABHD6 inhibition, while interestingly no inhibition of FAAH was observed at 10 µM. Finally, the lipophilicity of compound 203 (cLogP = 5.5) was reduced by replacing its piperidine ring with a morpholine ring (compound 204). Compound 204 exerted comparable ABHD6 inhibitory activity as compounds 201 and 202 but in addition it was less lipophilic (cLogP = 4.1).

Overview towards other potential off-targets

Selected potent ABHD6 inhibitors were further screened at 10 µM towards the main off-target LAL. Briefly, cyclic analogues (171, 172, 178 and 179) were found to inhibit LAL activity almost completely while among the non-cyclic analogues (181, 191, 194 and 201-204), *N*-methyl-*N*-aryl analogues 181, 191 and 194, and *N*-methyl-*N*-cycloalkyl analogues 201-204 were found to be weak LAL inhibitors. Interestingly, the *N*-methyl-*N*-benzyl analogue 185 achieved > 99% inhibition. The IC_{50} values were determined for those compounds having > 50% inhibition in the original publication (II). In the ABPP assay, using mouse brain membrane proteomes, cyclic analogues (171, 172, 178 and 179) as well as non-cyclic analogues (181, 185, 191, 194 and 201) tested at 1 µM were found to inhibit ABHD6 along with inhibition of an unidentified ~30 kDa serine hydrolase as an off-target. However, non-cyclic analogues 202-204 were found to inhibit ABHD6 selectively at 1 µM. Finally, due to less lipophilicity of analogue 204 (JZP-430) compared to analogues 202 and 203, it was decided to test it in the ABPP assay with different concentrations varying from 0.25-5 µM. It was found that 204 (JZP-430) inhibited ABHD6 dose-dependently, being effective even at 0.25 µM concentration. Selective inhibition of ABHD6 was detected even at the 1 µM concentration while negligible inhibition of FAAH was obtained with the 2.5 µM concentration. At a 20-fold (5 µM) concentration, partial inhibition of FAAH was detected. In short, when tested at a concentration below 2.5 µM, JZP-430 (204) appeared to be selective for ABHD6 over the other assayed brain serine hydrolases, including FAAH, MAGL and ABHD12. Compound JZP-430 (204) was inactive towards cannabinoid receptors at 10 µM. Moreover, in the reversibility study, it was found to inhibit ABHD6 in an irreversible manner. Furthermore, in the molecular modeling studies, compound JZP-430 (204) revealed favorable interactions within the active site of hABHD6 comprising the catalytic triad Ser^{148}- His^{306}-Asp^{278} including important hydrogen-bonding of the carbonyl oxygen to the oxyanion hole Met^{149}-Phe^{80}.

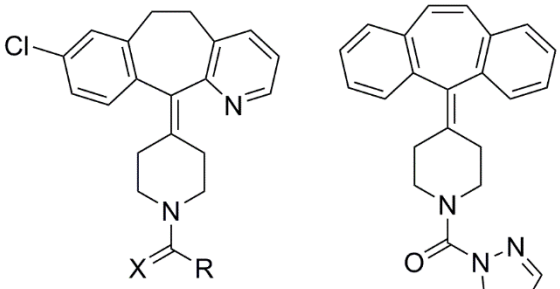
Conclusion

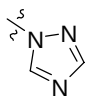
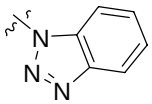
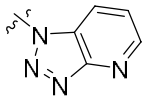
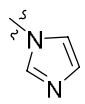
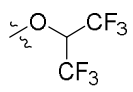
In short, the best compound of the series 4-morpholino-1,2,5-thiadiazol-3-yl cyclooctyl(methyl)carbamate (JZP-430, 204), potently and irreversibly inhibited hABHD6 (IC_{50} 44 nM) and was found to be > 200-fold selective over other potential off-targets such as FAAH, LAL and cannabinoid receptors. Additionally, proteomic analysis indicated that compound 204 has good selectivity i.e. it does not inhibit the other serine hydrolases of mouse brain membrane proteome.

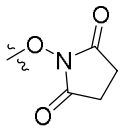
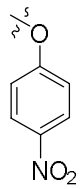
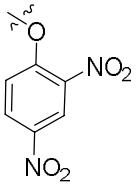
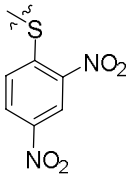
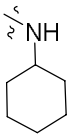
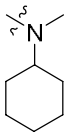
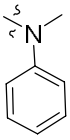
6.3 Loratadine analogues (III)

The inhibitory activity data of loratadine (205) and its analogues 206-217, and cyproheptadine analogue 219 screened at 10 µM towards hMAGL and hFAAH are presented in Table 38. Selected analogues 206 and 219 were also tested at 10 µM against hABHD6 and hABHD12, and only the outcome towards these enzymes has been described. However, in the original publication (III) they were reported in detail.

Table 38. Inhibitory activities of the loratadine (205) and its analogues 206-217, and cycproheptadine analogue 219 against MAGL and FAAH.



Compd	R	pI_{50} (range) [IC_{50}, µM][a] or % inhibition at 10 µM[b]	
		hMAGL	hFAAH
205 Loratadine	----	NI[c]	11%
206 (JZP-361)		7.34 (7.21-7.47) [0.046]	5.14 (5.12-5.16) [7.24]
207		6.68 (6.66-6.69) [0.208]	22%
208		7.01 (7.01-7.02) [0.098]	30%
209		NI	7%
210		6.19 (6.11-6.26) [0.645]	15%

211		5.59 (5.58-5.59) [2.57]	12%
212		27%	6%
213		NI	14%
214		14%	12%
215		NI	9%
216		NI	NI
217		NI	11%
219	----	7.33 (7.32-7.34) [0.047]	5.16 (5.14-5.19) [6.91]
JJKK-048 (121)[d]	-----	9.44 ± 0.05 [0.00036][e]	5.32 ± 0.05 [4.8]
JZP-327A (157)[f]	-----	NI	7.94 (7.91−7.97) [0.011]

[a] pI_{50} values (-$\log_{10}$ [IC_{50}]) represent the mean (range) from two independent experiments performed in duplicates. IC_{50} values were calculated for those compounds having ⩾50% inhibition at 10 μM and were derived from the mean pI_{50} values as shown in brackets. [b] The percentage (%) of inhibition is represented as the mean from two independent experiments performed in duplicates. [c] NI indicates no inhibition. [d] ({4-[bis-(benzo[*d*][1,3]dioxol-5-yl)methyl]-piperidin-1-yl}(1*H*-1,2,4-triazol-1-yl)methanone) (JJKK-048, 121) is used as MAGL reference standard and reported by Aaltonen *et al*., 2013. [e] pI_{50} values (-$\log_{10}$ [IC_{50}]) represent the mean ± S.E.M. from three independent experiments performed in duplicate. [f] *S*-(-)-3-(1-(4-isobutylphenyl)ethyl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (JZP-327A, 157) used as reference FAAH inhibitor reported by Patel *et al*. 2013.

SARs of loratadine analogues 206-217 and the cyproheptadine analogue 219

As shown in Table 38, loratadine (205) was found to be inactive towards both MAGL and FAAH. However, replacement of the ethoxy group present in loratadine with the 1,2,4-triazole group (compound 206) resulted in potent MAGL inhibitor (IC_{50} = 46 nM) having ~150-fold selectivity over hFAAH (IC_{50} = 7.24 μM). Two urea analogues, triazolopyridine and benzotriazole (compounds 207 and 208) were also found to be good MAGL inhibitors although 2-4 fold less potent than compound 206. However, the imidazole urea analogue 209 was found to be inactive. The carbamate analogues, hexafluoroisopropyl (HFIP) and succinimide (210 and 211) showed weak MAGL inhibition with IC_{50} values of 0.64 and 2.57 μM, respectively. Compounds 212-214 which possessed a nitro substituted phenyl ring showed a loss of MAGL inhibitory activity. This result indicates that these compounds had inappropriate dimensions due to their bulky phenyl group and were incapable of fitting into the MAGL binding pocket. As expected, analogues with the less electrophilic carbonyl groups (compounds 215-217) were found to be inactive towards MAGL. Moreover, most of the analogues 207-217 did not show any appreciable FAAH inhibition. The 1,2,4-triazole urea analogue of cyproheptadine (compound 219) was found to be equally potent with compound 206 as an MAGL inhibitor and had comparable selectivity over FAAH.

Overview towards other potential targets

1,2,4-Triazole urea analogues 206 and 219 caused moderate inhibition of ABHD6 with IC_{50} values of 1.78 and 0.75 μM, respectively. However, they were inactive towards ABHD12 at 10 μM. Interestingly, they showed histamine H_1 antagonistic properties (pA_2 = 6.81 and 6.50, respectively). Compound JZP-361 (206) was found to be inactive towards cannabinoid receptors at 10 μM. Moreover, in the reversibility study, it was found to inhibit hMAGL in a slowly reversible manner. Interestingly, in the molecular modeling studies, compound 206 was found to be capable of having favorable interactions within the active site of hMAGL comprising the catalytic triad Ser^{122}- His^{169}-Asp^{239} including important hydrogen-bonding of the carbonyl oxygen to the oxyanion hole Met^{123}-Ala^{51}.

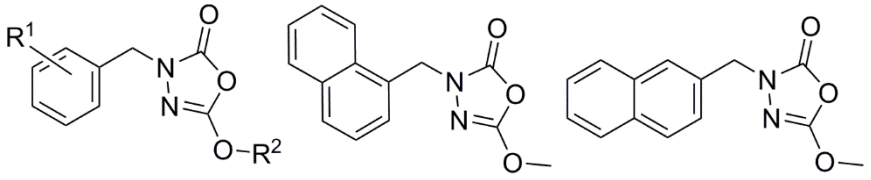
Conclusion

In conclusion, a small set of analogues was synthesized and from these, (4-(8-chloro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11(6*H*)-ylidene)piperidin-1-yl)(1*H*-1,2,4-triazol-1-yl)methanone (JZP-361, 206) potently and reversibly inhibited hMAGL (IC_{50} 46 nM) and was found to be > 150-fold selective for inhibiting hMAGL in preference to other potential off-targets, such as FAAH and cannabinoid receptors. Moreover, compound 206 retained histamine H_1 antagonistic affinity, hence, it emerged as a novel dual-acting pharmacological tool possessing both MAGL inhibitory and antihistaminergic activities.

6.4 3-Benzyl-1,3,4-oxadiazol-2-ones (IV)

The inhibitory activity data of 1,3,4-oxadiazol-2-ones 220-247 screened at 1 µM towards ABHD6 and, 10 µM towards hFAAH and hMAGL are presented in Table 39. All compounds were found to be inactive toward hABHD12 at 1 µM. Hence, inhibition data towards this enzyme are not shown, however, in the original publication (IV) they were reported as supplementary information.

Table 39. Inhibitory activities of the 1,3,4-oxadiazol-2-ones 163-169* and 220-247* against FAAH, MAGL and ABHD6.



163-169, **222-247** **220** **221**

Compd	R^1	R^2	pI_{50} (range) [IC_{50}, µM][a] or % inhibition at 10 µM[b]		pI_{50} (range) [IC_{50}, µM] [a] or % Remaining ABHD6 activity at 1 µM[c]
			hFAAH	hMAGL	hABHD6
163	H	Me	NI[d]	NI	61%
164	*p*-isobutyl	Me	7.05 (7.02-7.08) [0.091]	5.87 (5.86-5.89) [1.3]	NI
165	*p*-Ph	Me	6.79 (6.78-7.79) [0.16]	5.79 (5.69-5.88) [1.6]	NI
166	*m*-Ph	Me	46%	32%	NI
167	*o*-OPh	Me	5.20 (5.15-5.26) [6.3]	NI	60%
168	*m*-OPh	Me	6.01 (5.97-6.03) [0.98]	15%	70%
169	*p*-OPh	Me	7.0 (6.89-7.14) [0.10]	6.40 (6.39-6.41) [0.40]	77%
220	-----	-----	35%	11%	64%
221	-----	-----	5.40 (5.26-5.54) [3.98]	NI	NI
222	*o*-Ph	Me	23%	8%	NI
223	*o*-NO_2	Me	36%	43%	57%

224	*m*-NO_2	Me	7%	32%	65%
225	*p*-NO_2	Me	18%	36%	NI
226	*o*-NH_2	Me	8%	4%	NI
227 (JZP-169)	*m*-NH_2	Me	18%	21%	6.66 ± 0.04 [0.216][e]
228	*p*-NH_2	Me	34%	23%	NI
229	*o*-NHCOMe	Me	11%	3%	NI
230	*m*-NHCOMe	Me	NI	12%	NI
231	*p*-NHCOMe	Me	37%	16%	NI
232	*o*-NHCOPh	Me	NI	NI	NI
233	*m*-NHCOPh	Me	NI	48%	79%
234	*p*-NHCOPh	Me	6.21 (6.10-6.32) [0.62]	6.25 (6.21-6.29) [0.56]	88%
235	*m*-CN	Me	NI	23%	63%
236	*p*-CN	Me	NI	25%	NI
237	*m*-COOMe	Me	20%	7%	6.35 (6.24-6.47) [0.45]
238	*p*-COOMe	Me	5.17 (5.14-5.20) [6.76]	15%	NI
239	*m*-CF_3	Me	12%	48%	6.06 (5.94-6.19) [0.87]
240	*m*-Cl	Me	37%	43%	52%
241	*m*-OMe	Me	NI	6%	70%
242	*m*-OBn	Me	5.82 (5.80-5.84) [1.51]	5.49 (5.48-5.50) [3.23]	53%
243	*p*-OBn	Me	6.39 (6.37-6.41) [0.41]	6.28 (6.25-6.31) [0.52]	NI
244	*m*-NO_2	Et	NI	30%	63%
245	*m*-NH_2	Et	10%	15%	NI

246	*m*-NO_2	Ph	38%	5.48 (5.47-5.49) [3.31]	6.88 (6.7-6.75) [0.13]
247	*m*-NH_2	Ph	25%	5.1 (5.0-5.1) [7.94]	7.13 ± 0.05 [0.073][e]
JZP-327A (157)[f]	-----	-----	7.94 (7.91-7.97) [0.011]	16%	NI
JZP-430 (204) [g]	-----	-----	18%	NI	7.36 ± 0.05 [0.044][e]
JZP-361 (206)[h]	-----	-----	5.14 (5.12-5.16) [7.24]	7.34 (7.21-7.47) [0.046]	5.75 (5.66-5.84) [1.78]

[*] Compounds 163-169 are from the section 6.1 and are reported in original publication (I) and included here to provide a better clarification of SARs while compound 224 was reported in our published work (Savinainen *et al.,* 2014). [a] pI_{50} values (-$\log_{10}$ [IC_{50}]) represent the mean (range) from two independent experiments performed in duplicates. IC_{50} values were calculated for those compounds causing ⩾ 50% inhibition at the stated concentration and were derived from the mean pI_{50} values as shown in brackets. [b] The percentage (%) of inhibition is calculated from the mean of two independent experiments performed in duplicates. [c] Remaining activity (%) at 1 μM is given when it is <90%.[d] NI indicates no inhibition. [e] pI_{50} values (-$\log_{10}$ [IC_{50}]) represent the mean ± S.E.M. from three independent experiments performed in duplicates. [f] *S*-(-)-3-(1-(4-isobutylphenyl)ethyl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (JZP-327A, 157) was used as reference FAAH inhibitor as reported by Patel *et al*. 2013. [g] 4-morpholino-1,2,5-thiadiazol-3-yl cyclooctyl(methyl)carbamate (JZP-430, 204) was used as reference ABHD6 inhibitor as previously reported (Patel *et al.,* 2015b). [h] (4-(8-chloro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11(6*H*)-ylidene)piperidin-1-yl)(1*H*-1,2,4-triazol-1-yl)methanone (JZP-361, 206) was used as a reference MAGL inhibitor as reported previously (Patel *et al.,* 2015a).

SARs of 1,3,4-oxadiazol-2-ones 220-247

As shown in Table 39, among the analogues 163-169 and 222, the unsubstituted analogue 163 was inactive towards both FAAH and MAGL. However, at 1 μM concentration analogue 163 exhibited nearly 40% ABHD6 inhibition. Analogues with bulky substituents on the *para* position of the benzyl moiety, such as isobutyl (compound 164), Ph (compound 165), and OPh (compound 169) exhibited dual FAAH-MAGL inhibition. Shifting the substituent from *para* (compounds 165 and 169) to *meta* (compounds 166 and 168) positions resulted in a drop of FAAH inhibitory activity. However, this change resulted in a complete loss of MAGL inhibition. Similarly shifting of *para* substituents (compounds 165 and 169) to the *ortho* (compounds 167 and 222) position led to very weak or even complete loss of FAAH and/or MAGL inhibition. Of the bicyclic analogues, the 1-naphthyl analogue 220 was determined to be inactive towards FAAH while the 2-naphthyl analogue 221 (IC_{50} 3.98 μM) was a weak FAAH inhibitor. However, both of these analogues were found to be inactive towards MAGL while only 1-naphthyl analogue weakly inhibited ABHD6 at 1 μM concentration.

Analogues with a smaller EWG nitro and ERG amino substituents (compounds 223-225 and 226-228, respectively) at different positions on the benzyl moiety were found inactive towards FAAH and MAGL. However, in these analogues, only the *meta*-amino analogue 227

showed significant ABHD6 inhibition with an IC_{50} value of 216 nM. Protection of the amino group in compounds 226-228 with an acetyl (compounds 229-231) achieved no improvement in FAAH and MAGL inhibition, and loss of ABHD6 inhibition by *meta* substituted compound 230 revealed the importance of the free amino of compound 227. On the contrary, protection with the bulky benzoyl group of compounds 226-228 (compounds 232-234, repectively) resulted in enhanced FAAH and MAGL inhibition for *para* substituted analogue 234 over its *ortho* (compound 232) and *meta* (compound 233) counterparts, a consistent outcome previously observed with *para* bulky substituted compounds 165 and 169. The majority of the analogues having either smaller EWGs at either *meta* (compounds 235, 237, 239 and 240) or *para* positions (compound 236) or smaller ERG at the *meta* position (compound 241) were found to be inactive towards FAAH and MAGL although analogue 238 which had a *para* substituted EWG exhibited weak FAAH inhibition. Moreover, among these analogues, only *meta*-substituted analogues 237 and 239 showed weak ABHD6 inhibition with IC_{50} values of 0.45 and 0.87 µM, respectively. As expected, analogues 242 and 243 which had bulky benzyloxy substituents at their *meta* and *para* position respectively, resulted in an improved FAAH and MAGL inhibition.

Replacement of the methoxy group present at the 5-position of 1,3,4-oxadiazol-2-ones in compounds 224 and 227 with an ethoxy group (compounds 244 and 245) resulted in the loss of inhibitory activities towards all studied enzymes, the sole exception being compound 244 which at 1 µM concentration weakly inhibited ABHD6. However, when a phenoxy group was incorporated (compounds 246 and 247), it was found that the compounds exhibited weak inhibition toward FAAH and moderate inhibition towards MAGL while excellent inhibition towards ABHD6 was detected i.e. IC_{50} values of 0.13 and 0.071 µM, respectively. Overall, majority of analogues with a bulky or smaller *meta* and *ortho* substituent displayed weak to noticeable ABHD6 inhibition.

Overview towards other potential off-targets

In the ABPP assay, using mouse brain membrane proteomes, compound 227 was found to inhibit ABHD6 selectively at 10 µM without affecting the other brain serine hydrolases, including FAAH, MAGL and ABHD12. However, in the same assay, compound 247 targeted ABHD6 in a non-selective manner as two other serine hydrolases were found as an off-targets at 10 µM. Compound 227 caused no blockade or activation of cannabinoid receptors at 10 µM. The detail inhibition mechanism study of compound 227 (JZP-169) suggested that it bound to hABHD6 in an irreversible manner and the molecular modeling studies suggested favorable interactions with the active site of hABHD6 which contains the catalytic triad Ser^{148}-His^{306}-Asp^{278} and where there is important hydrogen-bonding of the carbonyl oxygen to the oxyanion hole Met^{149}-Phe^{80}.

Conclusion

In summary, many analogues were prepared and of these, 3-(3-aminobenzyl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (JZP-169, 227) was found to be a potent (IC_{50} 216 nM) and irreversible inhibitor of ABHD6. Moreover, it displayed no inhibition towards MAGL, FAAH, ABHD12, nor did it bind to cannabinoid receptors. Furthermore, in proteomic analysis, compound 227 inhibited only ABHD6 band without affecting the other serine hydrolases of mouse brain membrane proteomes.

7 General Discussion

7.1 SUMMARY OF THE DEVELOPMENT OF POTENT AND SELECTIVE INHIBITORS OF ENZYMES INVOLVED IN ENDOCANNABINOID INACTIVATION

Inhibition of FAAH has been shown to have therapeutic potential in several disorders such as pain, inflammation, anxiety and depression. The majority of FAAH inhibitors have been designed through structural modifications of other serine hydrolase inhibitors. In general, these compounds share a reactive carbonyl which undergoes a nucleophilic attack by the nucleophilic Ser^{241} residue of FAAH. However, one recent report revealed that it is also possible to target an additional active site residue Cys^{269} of FAAH through which one can prolong FAAH inhibition and in turn extend the duration of the pharmacological effect. Moreover, some promising FAAH inhibitors even lack electrophile. At present, several chemical tools are available with which to study beneficial and detrimental effects of pharmacological modulation of FAAH. It is clear that much more work will be needed before it will be possible to transfer any potential FAAH inhibitor from the preclinical to the clinical stages. With respect to clinical utility, one major challenge for FAAH inhibitor design is to optimize the following requirements i.e. excellent potency, selectivity over other potential off-targets, reversibility or irreversibility, and efficacy toward human rather than non-human FAAH. However these hurdles can be overcome and a few FAAH inhibitors are currently undergoing clinical trials. Moreover, recent published reports have highlighted the importance of targeting FAAH simultaneously with other targets such as COX, TRPV, etc. to achieve desired effects with reduced side effects.

In contrast to FAAH inhibitors, the utility of MAGL inhibitors has only been validated in preclinical studies. Inhibition of MAGL is considered as a promising approach to treat pain, inflammation, vomiting, nausea and cancer. Some of the early developed inhibitors have several sulfhydryl-reacting compounds mainly targeting the cysteine residues (Cys^{201}, Cys^{208}, and Cys^{242}) located close to the catalytic site of MAGL. In recent years, a few potent and selective inhibitors targeting nucleophilic Ser^{122} of MAGL have been developed. It may be possible to avoid the undesired side effects associated with chronic treatment with irreversible MAGL inhibitors by developing reversible MAGL inhibitors. In addition to FAAH and MAGL inhibitors, only a limited number of potent and selective ABHD6 inhibitors have been identified while the search for the potent and selective inhibitors of ABHD12 is still underway. Very recently, it has been claimed that ABHD6 inhibitors have therapeutic potential in the treatment of neuroinflammation, epilepsy and metabolic disorders.

The present study was aimed at the development of potent and selective inhibitors of the endocannabinoid hydrolases. Previously known scaffolds were optimized to obtain inhibitors which not only blocked the target enzymes with improved potency but also possessed good selectivity with respect to the other off-targets. The research results of this study are discussed below based on the main objectives listed in section 4.

In the first subproject, the earlier disclosed potent but non-selective 1,3,4-oxadiazol-2-ones were hybridized with derivatives of phenylalkanoic acid present in marketed NSAIDs to develop novel chiral 1,3,4-oxadiazol-2-ones. The best compound identified in this series, JZP-327A, was able to inhibit FAAH in a reversible manner with IC_{50} values in the nanomolar range as well as demonstrating high selectivity over other studied potential off-targets (other ECS members, COX-isoenzymes, and serine hydrolases of mouse brain membrane proteomes). Hence, JZP-327A is an important lead which should be tested in animal models to confirm its *in vivo* efficacy and to elucidate its PK profile.

The emerging therapeutic potential of ABHD6 in the treatment of inflammation, epilepsy and metabolic disorders, was the reason to develop inhibitors of ABHD6 as described in the second subproject. Only limited SARs had been carried out on the recently reported 1,2,5-thiadiazole carbamate-based LAL inhibitors. Hence, it was decided to develop novel ABHD6 inhibitors by undertaking systematic structural variations around the 1,2,5-thiadiazole carbamate scaffold. Many compounds were produced and of these, JZP-430 was identified as a potent ABHD6 inhibitor with an IC_{50} value of 44 nM while displaying a good selectivity profile over the other studied potential targets such as other members of ECS, LAL and mouse brain membrane proteomes. Therefore, JZP-430 is an important lead with which to clarify the effects of ABHD6 inhibition in animal models.

The third subproject was based on structural similarity of piperidine/piperazine- based carbamate/urea inhibitors of MAGL with the well-known histamine H_1 antagonist, loratadine. A small set of carbamate/urea analogues was produced having a polar/electrophilic carbonyl group, and from these, the 1,2,4-triazole-based loratadine analogue, JZP-361, was discovered to be a potent MAGL inhibitor with an IC_{50} value of 46 nM and it was observed to be selective over FAAH and cannabinoid receptors. In contrast to the earlier reported 1,2,4-triazole containing irreversible inhibitor JJKK-048, compound JZP-361 was found to inhibit MAGL in a slow reversible manner. Moreover, JZP-361 retained its histamine H_1 antagonistic affinity, making it an interesting dual-acting pharmacological tool to study the consequences of concomitant blockade of MAGL and histamine H_1 receptor activities.

The fourth subproject involved the further optimization of 1,3,4-oxadiazol-2-ones as ABHD6 inhibitors. The introduction of different substitutions on the benzyl moiety of 1,3,4-oxadiazol-2-ones led to the identification of JZP-169 as a potent ABHD6 inhibitor (IC_{50} = 216 nM) while also displaying a good selectivity profile over the studied off-targets such as other members of ECS and serine hydrolases of mouse brain membrane proteomes. In contrast to reversible inhibition of FAAH achieved by the 1,3,4-oxadiazol-2-one-based compound JZP-327A, this new compound, i.e. JZP-169, inhibited ABHD6 in an irreversible manner.

All of the lead compounds described in this study achieved the goals set at the start of this research project. In addition to striking a good balance between potency and selectivity toward other potential off-targets, these compounds also exhibited optimal molecular weight and lipophilicity; the next step will be to investigate their PK profile and determine their *in vivo* efficacy. Clearly, further study will be needed to explore their therapeutic potential.

7.2 FUTURE PERSPECTIVES

Several developed potent and selective FAAH inhibitors have shown promising outcomes in preclinical studies and a few have even progressed to clinical trials. However, the disappointing results of PF-04457845 in phase II trials in patients suffering from osteoarthritis pain raised several questions about the clinical effectiveness of selective FAAH inhibitors. One major question still needing to be answered is whether administration of a FAAH inhibitor alone will be sufficient to achieve efficacious therapies or whether simultaneous modulation of several targets will be needed. Indeed, several promising results have been reported, at least in animal models, with dual FAAH-COX inhibitors and with a dual FAAH-TRPV1 blocker as well as with a compound having dual action as FAAH inhibitor/TRPA1 agonist. It is possible that irreversible MAGL inhibitors may cause excessive blockade of the MAGL leading to clinically unacceptable side effects. However, one recent study indicated that it may be advantageous to devise reversibly acting MAGL inhibitors for situations when rapid clearance of drug is needed and thus it may be beneficial to focus on the development of these kinds of compounds in the future. Although, ABHD6 inhibitors have shown promising results in combatting the metabolic syndrome, their utility as efficacious therapies still needs to be clarified in further studies.

8 Conclusions

The general aim of the studies described in this thesis was to develop potent and selective inhibitors of FAAH, MAGL and ABHD6. This goal was achieved by conducting the design, synthesis, structural characterization, and the *in vitro* pharmacological evaluation of these novel compounds. The following conclusions can be drawn from the results of this thesis:

1. Chiral 1,3,4-oxadiazol-2-ones have been identified as inhibitors of FAAH. Compound JZP-327A exhibited excellent FAAH potency and selectivity over other off-targets, and can be considered as a promising compound to be studied further in various *in vivo* tests, such as in models of pain and inflammation.

2. The 1,2,5-thiadiazole carbamate scaffold has been successfully utilized in the development of ABHD6 inhibitors. The excellent potency towards ABHD6 and the good selectivity over the potential off-targets found for the best compound in this series, JZP-430, make it a promising candidate for further investigation.

3. A close stuctural analogue of loratadine, JZP-361 was identified as a potent MAGL inhibitor. Since JZP-361 also retained its histamine H_1 antagonistic affinity, it can be considered as an intriguing pharmacological tool with which to study inflammation in chronic diseases, such as asthma. Finally, the structural similarity of loratadine and JZP-361 raises the possibility that some of the other known MAGL inhibitors may also possess H_1 antagonistic properties.

4. The 1,3,4-oxadiazol-2-one-based compound, JZP-169, was identified as a potent and selective ABHD6 inhibitor displaying good selectivity toward other off-targets. Furthermore, its irreversible inhibition of ABHD6 highlights that 1,3,4-oxadiazol-2-one is a versatile scaffold for the development of serine hydrolase inhibitors. Additionally, 1,3,4-oxadiazol-2-one scaffold constitutes a novel class of ABHD6 inhibitors.

9 References

Aaltonen N, Savinainen JR, Ribas CR, Rönkkö J, Kuusisto A, Korhonen J, Navia-Paldanius D, Häyrinen J, Takabe P, Käsnänen H, Pantsar T, Laitinen T, Lehtonen M, Pasonen-Seppänen S, Poso A, Nevalainen T, Laitinen JT: Piperazine and piperidine triazole ureas as ultrapotent and highly selective inhibitors of monoacylglycerol lipase. Chem Biol 20: 379-390, 2013.

Adams J, Behnke ML, Castro AC, Evans CA, Grenier L, Grogan MJ, Liu T, Snyder DA, Tibbitts TT: Preparation of arylboronates as inhibitors of fatty acid amide hydrolase. WO2008063300A2, 2008.

Ahn K, Johnson DS, Fitzgerald LR, Liimatta M, Arendse A, Stevenson T, Lund ET, Nugent RA, Nomanbhoy TK, Alexander JP, Cravatt BF: Novel mechanistic class of fatty acid amide hydrolase inhibitors with remarkable selectivity. Biochemistry 46: 13019-13030, 2007.

Ahn K, Johnson DS, Cravatt BF: Fatty acid amide hydrolase as a potential therapeutic target for the treatment of pain and CNS disorders. Expert Opin Drug Discovery 4: 763-784, 2009a.

Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK, Weerapana E, Sadagopan N, Liimatta M, Smith SE, Lazerwith S, Stiff C, Kamtekar S, Bhattacharya K, Zhang Y, Swaney S, Van B, Keri, Stevens RC, Cravatt BF: Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. Chem Biol 16: 411-420, 2009b.

Alapafuja SO, Nikas SP, Bharathan IT, Shukla VG, Nasr ML, Bowman AL, Zvonok N, Li J, Shi X, Engen JR, Makriyannis A: Sulfonyl fluoride inhibitors of fatty acid amide hydrolase. J Med Chem 55: 10074-10089, 2012.

Alexander JP & Cravatt BF: Mechanism of carbamate inactivation of FAAH: implications for the design of covalent inhibitors and in vivo functional probes for enzymes. Chem Biol 12: 1179-1187, 2005.

Alexander JP & Cravatt BF: The putative endocannabinoid transport blocker LY2183240 is a potent inhibitor of FAAH and several other brain serine hydrolases. J Am Chem Soc 128: 9699-9704, 2006.

Alger BE & Kim J: Supply and demand for endocannabinoids. Trends Neurosci 34: 304-315, 2011.

Alhouayek M, Lambert DM, Delzenne NM, Cani PD, Muccioli GG: Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. FASEB J 25: 2711-2721, 10.1096/fj.10-176602, 2011.

Alhouayek M, Masquelier J, Cani PD, Lambert DM, Muccioli GG: Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. Proc Natl Acad Sci USA 110: 17558-17563, 2013.

Andrzejak V, Muccioli GG, Body-Malapel M, El Bakali J, Djouina M, Renault N, Chavatte P, Desreumaux P, Lambert DM, Millet R: New FAAH inhibitors based on 3-carboxamido-5-aryl-isoxazole scaffold that protect against experimental colitis. Bioorg Med Chem 19: 3777-3786, 2011.

Apodaca R, Breitenbucher JG, Epperson MT, Fried AK, Pippel DJ, Seierstad M: Preparation of piperidinylbutanoyl oxazoles as fatty acid amide hydrolase (FAAH) modulators. WO2007061862A2, 2007a.

Apodaca R, Breitenbucher JG, Chambers AL, Seierstad M, Xiao W: Preparation of oxazolyl piperidinyl methanones as modulators of fatty acid amide hydrolase. WO2007140005A2, 2007b.

Arunlakshana O & Schild HO: Some quantitative uses of drug antagonists. Br J Pharmacol Chemother 14: 48-58, 1959.

Bachovchin DA, Ji T, Li W, Simon GM, Blankman JL, Adibekian A, Hoover H, Niessen S, Cravatt BF: Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. Proc Natl Acad Sci USA 107: 20941-20946, S20941/1-S20941/172, 2010.

Bachovchin DA & Cravatt BF: The pharmacological landscape and therapeutic potential of serine hydrolases. Nat Rev Drug Discovery 11: 52-68, 2012.

Barricklow J & Blatnik M: 2-Arachidonoylglycerol is a substrate for butyrylcholinesterase: a potential mechanism for extracellular endocannabinoid regulation. Arch Biochem Biophys 536: 1-5, 2013.

Bauer RA: Covalent inhibitors in drug discovery: from accidental discoveries to avoided liabilities and designed therapies. Drug Discov Today doi:10.1016/j.drudis.2015.05.005, 2015.

Behnke ML, Castro AC, Evans CA, Grenier L, Grogan MJ, Liu T, Snyder DA, Tibbitts TT: Preparation of oxadiazolylphenylboronic acid derivatives and analogs for use as fatty acid amide hydrolase inhibitors. WO2009126691A1, 2009.

Behnke ML, Castro AC, Chan LK, Evans CA, Grenier L, Grogan MJ, Leblanc Y, Liu T, Peluso S, Snyder DA, Tibbitts TT: Preparation of isoxazoline derivatives for use as fatty acid amide hydrolase inhibitors. WO2010135360A1, 2010.

Belfrage P, Jergil B, Stralfors P, Tornqvist H: Hormone-sensitive lipase of rat adipose tissue: identification and some properties of the enzyme protein. FEBS Lett 75: 259-64, 1977.

Ben Ali Y, Chahinian H, Petry S, Muller G, Lebrun R, Verger R, Carrière F, Mandrich L, Rossi M, Manco G, Sarda L, Abousalham A: Use of an inhibitor to identify members of the hormone-sensitive lipase family. Biochemistry 45: 14183-14191, 2006.

Bertrand T, Auge F, Houtmann J, Rak A, Vallee F, Mikol V, Berne PF, Michot N, Cheuret D, Hoornaert C, Mathieu M: Structural basis for human monoglyceride lipase inhibition. J Mol Biol 396: 663-73, 2010.

Bian H, Chevalier KM, Connolly PJ, Flores CM, Lin S, Liu L, Mabus J, Macielag MJ, McDonnell ME, Pitis PM, Zhang Y, Zhang S, Clemente J, Zhu B: Piperazinylazetidinyl diamides as monoacylglycerol lipase inhibitors and their preparation and use in the treatment of diseases. WO2010124114A1, 2010.

Bisogno T, Melck D, De Petrocellis L, Bobrov MY, Gretskaya NM, Bezuglov VV, Sitachitta N, Gerwick WH, Di Marzo V: Arachidonoylserotonin and other novel inhibitors of fatty acid amide hydrolase. Biochem Biophys Res Commun 248: 515-522, 1998.

Bisogno T, Melck D, De Petrocellis L, Di Marzo V: Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. J Neurochem 72: 2113-2119, 1999.

Bisogno T, Melck D, Bobrov MY, Gretskaya NM, Bezuglov VV, De Petrocellis L, Di Marzo V: *N*-Acyl-dopamines: novel synthetic CB1 cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem J 351: 817-824, 2000.

Bisogno T, De Petrocellis L, Di Marzo V: Fatty acid amide hydrolase, an enzyme with many bioactive substrates. Possible therapeutic implications. Curr Pharm Des 8: 533-547, 2002.

Bisogno T, Howell F, Williams G, Minassi A, Grazia Cascio M, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams E, Gangadharan U, Hobbs C, Di Marzo V, Doherty P: Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163: 463-468, 2003.

Bisogno T, Ortar G, Petrosino S, Morera E, Palazzo E, Nalli M, Maione S, Di Marzo V: Development of a potent inhibitor of 2-arachidonoylglycerol hydrolysis with antinociceptive activity in vivo. Biochim Biophys Acta, Mol Cell Biol Lipids 1791: 53-60, 2009.

Bisogno T & Maccarrone M: Latest advances in the discovery of fatty acid amide hydrolase inhibitors. Expert Opin Drug Discov 8: 509-522, 2013.

Blair RE, Deshpande LS, Sombati S, Elphick MR, Martin BR, DeLorenzo RJ: Prolonged exposure to WIN55,212-2 causes downregulation of the CB1 receptor and the development of tolerance to its anticonvulsant effects in the hippocampal neuronal culture model of acquired epilepsy. Neuropharmacol 57: 208-218, 2009.

Blankman JL, Simon GM, Cravatt BF: A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 14: 1347-1356, 2007.

Blankman JL & Cravatt BF: Chemical probes of endocannabinoid metabolism. Pharmacol Rev 65: 849-871, 2013.

Blankman JL, Long JZ, Trauger SA, Siuzdak G, Cravatt BF: ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. Proc Natl Acad Sci USA 110: 1500-1505, S1500/1-S1500/20, 2013.

Boger DL, Sato H, Lerner AE, Austin BJ, Patterson JE, Patricelli MP, Cravatt BF: Trifluoromethyl ketone inhibitors of fatty acid amide hydrolase: a probe of structural and conformational features contributing to inhibition. Bioorg Med Chem Lett 9: 265-270, 1999.

Boger DL, Sato H, Lerner AE, Hedrick MP, Fecik RA, Miyauchi H, Wilkie GD, Austin BJ, Patricelli MP, Cravatt BF: Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. Proc Natl Acad Sci USA 97: 5044-5049, 2000.

Boger DL, Miyauchi H, Du W, Hardouin C, Fecik RA, Cheng H, Hwang I, Hedrick MP, Leung D, Acevedo O, Guimarães CR, Jorgensen WL, Cravatt BF: Discovery of a potent, selective, and efficacious class of reversible α-ketoheterocycle inhibitors of fatty acid amide hydrolase effective as analgesics. J Med Chem 48: 1849-1856, 2005.

Bowman AL & Makriyannis A: Highly predictive ligand-based pharmacophore and homology models of ABHD6. Chem Biol Drug Des 81: 382-388, 2013.

Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF: Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. Science 298: 1793-1796, 2002.

Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254, 1976.

Bradshaw HB, Rimmerman N, Hu SS, Benton VM, Stuart JM, Masuda K, Cravatt BF, O'Dell DK, Walker JM: The endocannabinoid anandamide is a precursor for the signaling lipid *N*-arachidonoyl glycine by two distinct pathways. BMC Biochem 10: 14, 2009.

Busquets-Garcia A, Puighermanal E, Pastor A, de lT, Maldonado R, Ozaita A: Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. Biol Psychiatry 70: 479-486, 2011.

Butini S, Brindisi M, Gemma S, Minetti P, Cabri W, Gallo G, Vincenti S, Talamonti E, Borsini F, Caprioli A, Stasi MA, Di Serio S, Ros S, Borrelli G, Maramai S, Fezza F, Campiani G, Maccarrone M: Discovery of potent inhibitors of human and mouse fatty acid amide hydrolases. J Med Chem 55: 6898-6915, 2012.

Butini S, Gemma S, Brindisi M, Maramai S, Minetti P, Celona D, Napolitano R, Borsini F, Cabri W, Fezza F, Merlini L, Dallavalle S, Campiani G, Maccarrone M: Identification of a novel arylpiperazine scaffold for fatty acid amide hydrolase inhibition with improved drug disposition properties. Bioorg Med Chem Lett 23: 492-495, 2013.

Cadas H, Gaillet S, Beltramo M, Venance L, Piomelli D: Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. J Neurosci 16: 3934-3942, 1996.

Cadas H, di Tomaso E, Piomelli D: Occurrence and biosynthesis of endogenous cannabinoid precursor, *N*-arachidonoyl phosphatidylethanolamine, in rat brain. J Neurosci 17: 1226-1242, 1997.

Castro AC, Evans CA, Grenier L, Grogan MJ: Preparation of piperidineboronic acid derivatives for use as fatty acid amide hydrolase inhibitors. WO2010118159A1, 2010a.

Castro AC, Grogan MJ, Snyder DA: Inhibitors of fatty acid amide hydrolase. WO2010118155A1, 2010b.

Chanda PK, Gao Y, Mark L, Btesh J, Strassle BW, Lu P, Piesla MJ, Zhang M, Bingham B, Uveges A, Kowal D, Garbe D, Kouranova EV, Ring RH, Bates B, Pangalos MN, Kennedy JD, Whiteside GT, Samad TA: Monoacylglycerol lipase activity is a critical modulator of the tone and integrity of the endocannabinoid system. Mol Pharmacol 78: 996-1003, 2010.

Chang J, Niphakis M, Lum K, Cognetta III A, Wang C, Matthews M, Niessen S, Buczynski M, Parsons L, Cravatt B: Highly selective inhibitors of monoacylglycerol lipase bearing a reactive group that is bioisosteric with endocannabinoid substrates. Chem Biol 19: 579-588, 2012.

Chang L, Luo L, Palmer JA, Sutton S, Wilson SJ, Barbier AJ, Breitenbucher JG, Chaplan SR, Webb M: Inhibition of fatty acid amide hydrolase produces analgesia by multiple mechanisms. Br J Pharmacol 148: 102-113, 2006.

Chevalier KM, Dax SL, Flores CM, Liu L, Macielag MJ, McDonnell ME, Nelen MI, Prouty S, Todd M, Zhang S, Zhu B, Nulton EL, Clemente J: Preparation of (hetero)aromatic piperazinyl azetidinyl amides as monoacylglycerol lipase (MGL) inhibitors. WO2010124121A1, 2010.

Chobanian HR, Guo Y, Liu P, Chioda MD, Fung S, Lanza TJ, Chang L, Bakshi RK, Dellureficio JP, Hong Q, McLaughlin M, Belyk KM, Krska SW, Makarewicz AK, Martel EJ, Leone JF, Frey L, Karanam B, Madeira M, Alvaro R, Shuman J, Salituro G, Terebetski JL, Jochnowitz N, Mistry S, McGowan E, Hajdu R, Rosenbach M, Abbadie C, Alexander JP, Shiao L, Sullivan KM, Nargund RP, Wyvratt MJ, Lin LS, DeVita RJ: Discovery of MK-4409, a novel oxazole FAAH inhibitor for the treatment of inflammatory and neuropathic pain. ACS Med Chem Lett 5: 717-721, 2014.

Cipriano M, Björklund E, Wilson AA, Congiu C, Onnis V, Fowler CJ: Inhibition of fatty acid amide hydrolase and cyclooxygenase by the *N*-(3-methylpyridin-2-yl)amide derivatives of flurbiprofen and naproxen. Eur J Pharmacol 720: 383-390, 2013.

Cisneros JA, Björklund E, González-Gil I, Hu Y, Canales Ã, Medrano FJ, Romero A, Ortega-Gutiérrez S, Fowler CJ, López-Rodíguez ML: Structure-activity relationship of a new series of reversible dual monoacylglycerol lipase/fatty acid amide hydrolase inhibitors. J Med Chem 55: 824-836, 2012.

Clapper JR, Vacondio F, King AR, Duranti A, Tontini A, Silva C, Sanchini S, Tarzia G, Mor M, Piomelli D: A second generation of carbamate-based fatty acid amide hydrolase inhibitors with improved activity in vivo. ChemMedChem 4: 1505-1513, 2009.

Clapper JR, Moreno-Sanz G, Russo R, Guijarro A, Vacondio F, Duranti A, Tontini A, Sanchini S, Sciolino NR, Spradley JM, Hohmann AG, Calignano A, Mor M, Tarzia G, Piomelli D: Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. Nat Neurosci 13: 1265-1270, 2010.

Colombano G, Albani C, Ottonello G, Ribeiro A, Scarpelli R, Tarozzo G, Daglian J, Jung K, Piomelli D, Bandiera T: *O*-(Triazolyl)methyl carbamates as a novel and potent class of fatty acid amide hydrolase (FAAH) inhibitors. ChemMedChem 10: 380-395, 2015.

Costa B, Bettoni I, Petrosino S, Comelli F, Giagnoni G, Di Marzo V: The dual fatty acid amide hydrolase/TRPV1 blocker, *N*-arachidonoyl-serotonin, relieves carrageenan-induced inflammation and hyperalgesia in mice. Pharmacol Res 61: 537-546, 2010.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB: Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384: 83-87, 1996.

Cravatt BF, Saghatelian A, Hawkins EG, Clement AB, Bracey MH, Lichtman AH: Functional disassociation of the central and peripheral fatty acid amide signaling systems. Proc Natl Acad Sci USA 101: 10821-10826, 2004.

De Bank PA, Kendall DA, Alexander SPH: A spectrophotometric assay for fatty acid amide hydrolase suitable for high-throughput screening. Biochem Pharmacol 69: 1187-1193, 2005.

De Petrocellis L, Melck D, Ueda N, Maurelli S, Kurahashi Y, Yamamoto S, Marino G, Di M, V.: Novel inhibitors of brain, neuronal, and basophilic anandamide amidohydrolase. Biochem Biophys Res Commun 231: 82-88, 1997.

De Vivo M, Scarpelli R, Cavalli A, Migliore M, Piomelli D, Habrant D, Favia A: Preparation of phenylpropanoic acid derivatives as multi-target FAAH and COX inhibitors. WO2014023325A1, 2014a.

De Vivo M, Scarpelli R, Cavalli A, Migliore M, Piomelli D, Habrant D, Favia A: Preparation of phenylpropanoic acid derivatives as multi-target FAAH and COX inhibitors. WO2014023643A1, 2014b.

De Wael F, Muccioli GG, Lambert DM, Sergent T, Schneider YJ, Rees JF, Marchand-Brynaert J: Chemistry around imidazopyrazine and ibuprofen: discovery of novel fatty acid amide hydrolase (FAAH) inhibitors. Eur J Med Chem 45: 3564-3574, 2010.

Delorme V, Diomandé SV, Dedieu L, Cavalier J, Carrière F, Kremer L, Leclaire J, Fotiadu F, Canaan S: MmPPOX inhibits *Mycobacterium tuberculosis* lipolytic enzymes belonging to the hormone-sensitive lipase family and alters mycobacterial growth. PLoS One 7: e46493, 2012.

DeMartino JK, Garfunkle J, Hochstatter DG, Cravatt BF, Boger DL: Exploration of a fundamental substituent effect of α-ketoheterocycle enzyme inhibitors: potent and selective inhibitors of fatty acid amide hydrolase. Bioorg Med Chem Lett 18: 5842-5846, 2008.

Deng H: Recent advances in the discovery and evaluation of fatty acid amide hydrolase inhibitors. Expert Opin Drug Discovery 5: 961-993, 2010.

Deutsch DG, Omeir R, Arreaza G, Salehani D, Prestwich GD, Huang Z, Howlett A: Methyl arachidonyl fluorophosphonate: a potent irreversible inhibitor of anandamide amidase. Biochem Pharmacol 53: 255-260, 1997.

Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC: Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34: 605-613, 1988.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R: Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258: 1946-1949, 1992.

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D: Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci USA 99: 10819-10824, 2002.

Dinh TP, Kathuria S, Piomelli D: RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. Mol Pharmacol 66: 1260-1264, 2004.

Dodson G & Wlodawer A: Catalytic triads and their relatives. Trends Biochem Sci 23: 347-352, 1998.

Duncan KK, Otrubova K, Boger DL: α-Ketoheterocycle inhibitors of fatty acid amide hydrolase: Exploration of conformational constraints in the acyl side chain. Bioorg Med Chem 22: 2763-2770, 2014.

Edgemond WS, Greenberg MJ, McGinley PJ, Muthian S, Campbell WB, Hillard CJ: Synthesis and characterization of diazomethylarachidonyl ketone: an irreversible inhibitor of *N*-arachidonylethanolamine amidohydrolase. J Pharmacol Exp Ther 286: 184-90, 1998.

Edwards PD, Zottola MA, Davis M, Williams J, Tuthill PA: Peptidyl α-ketoheterocyclic inhibitors of human neutrophil elastase. 3. In vitro and in vivo potency of a series of peptidyl α-ketobenzoxazoles. J Med Chem 38: 3972-3982, 1995.

Estiarte MA, Johnson RJ, Kaub CJ, Gowlugari S, O'Mahony DJR, Nguyen MT, Emerling DE, Kelly MG, Kincaid J, Vincent F, Duncton MAJ: 2-Amino-5-arylbenzoxazole derivatives as potent inhibitors of fatty acid amide hydrolase (FAAH). MedChemComm 3: 611-619, 2012.

Even L & 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 monoacyl glycerol lipase (MGL) inhibitors or mixed MGL and fatty acid amide hydrolase inhibitors. WO2008145839A1, 2008a.

Even L & Hoornaert C: Derivatives of triazolopyridine carboxamides, particularly (piperazin-1-yl)(1,2,3-triazolo[4,5-*b*]pyridin-1-yl)methanones, their preparation and use as selective monoacyl glycerol lipase (MGL) inhibitors or mixed MGL and fatty acid amide hydrolase inhibitors. WO2008145843A1, 2008b.

Ezzili C, Mileni M, McGlinchey N, Long JZ, Kinsey SG, Hochstatter DG, Stevens RC, Lichtman AH, Cravatt BF, Bilsky EJ, Boger DL: Reversible competitive α-ketoheterocycle inhibitors of fatty acid amide hydrolase containing additional conformational constraints in the acyl side chain: orally active, long-acting analgesics. J Med Chem 54: 2805-2822, 2011.

Favia AD, Habrant D, Scarpelli R, Migliore M, Albani C, Bertozzi SM, Dionisi M, Tarozzo G, Piomelli D, Cavalli A, De Vivo M: Identification and characterization of carprofen as a multitarget fatty acid amide hydrolase/cyclooxygenase inhibitor. J Med Chem 55: 8807-8826, 2012.

Feledziak M, Michaux C, Urbach A, Labar G, Muccioli GG, Lambert DM, Marchand-Brynaert J: β-Lactams derived from a carbapenem chiron are selective inhibitors of human fatty acid amide hydrolase versus human monoacylglycerol lipase. J Med Chem 52: 7054-7068, 2009.

Feledziak M, Lambert DM, Marchand-Brynaert J, Muccioli GG: Inhibitors of the endocannabinoid-degrading enzymes, or how to increase endocannabinoid's activity by preventing their hydrolysis. Recent Pat CNS Drug Discov 7: 49-70, 2012.

Fiskerstrand T, H'mida-Ben Brahim D, Johansson S, M'zahem A, Haukanes BI, Drouot N, Zimmermann J, Cole AJ, Vedeler C, Bredrup C, Assoum M, Tazir M, Klockgether T, Hamri A, Steen VM, Boman H, Bindoff LA, Koenig M, Knappskog PM: Mutations in ABHD12 cause the neurodegenerative disease PHARC: an inborn error of endocannabinoid metabolism. Am J Hum Genet 87: 410-417, 2010.

Flores CM, Nelen MI, Nulton EL, Prouty S, Todd M, Zhang S: Heteroaromatic and aromatic piperazinyl azetidinyl amides as monoacylglycerol lipase inhibitors. WO2010124122A1, 2010.

Forster L, Ludwig J, Kaptur M, Bovens S, Elfringhoff AS, Holtfrerich A, Lehr M: 1-Indol-1-yl-propan-2-ones and related heterocyclic compounds as dual inhibitors of cytosolic phospholipase $A_{2\alpha}$ and fatty acid amide hydrolase. Bioorg Med Chem 18: 945-952, 2010.

Fowler CJ, Janson U, Johnson RM, Wahlström G, Stenström A, Norstrom K, Tiger G: Inhibition of anandamide hydrolysis by the enantiomers of ibuprofen, ketorolac, and flurbiprofen. Arch Biochem Biophys 362: 191-196, 1999.

Fowler CJ, Jonsson KO, Tiger G: Fatty acid amide hydrolase: biochemistry, pharmacology, and therapeutic possibilities for an enzyme hydrolyzing anandamide, 2-arachidonoylglycerol, palmitoylethanolamide, and oleamide. Biochem Pharmacol 62: 517-526, 2001.

Fowler CJ, Björklund E, Lichtman AH, Naidu PS, Congiu C, Onnis V: Inhibitory properties of ibuprofen and its amide analogues towards the hydrolysis and cyclooxygenation of the endocannabinoid anandamide. J Enzyme Inhib Med Chem 28: 172-182, 2013.

Gaoni Y & Mechoulam R: Isolation, structure, and partial synthesis of an active constituent of hashish. J Am Chem Soc 86: 1646-1647, 1964.

Garfunkle J, Ezzili C, Rayl TJ, Hochstatter DG, Hwang I, Boger DL: Optimization of the central heterocycle of α-ketoheterocycle inhibitors of fatty acid amide hydrolase. J Med Chem 51: 4392-4403, 2008.

Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, Maione S, Di Marzo V: Discovery of prostamide $F_{2\alpha}$ and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. PLoS One 7: e31111, 2012.

Gattinoni S, De Simone C, Dallavalle S, Fezza F, Nannei R, Amadio D, Minetti P, Quattrociocchi G, Caprioli A, Borsini F, Cabri W, Penco S, Merlini L, Maccarrone M: Enol carbamates as inhibitors of fatty acid amide hydrolase (FAAH)

endowed with high selectivity for FAAH over the other targets of the endocannabinoid system. ChemMedChem 5: 357-360, 2010a.

Gattinoni S, Simone CD, Dallavalle S, Fezza F, Nannei R, Battista N, Minetti P, Quattrociocchi G, Caprioli A, Borsini F, Cabri W, Penco S, Merlini L, Maccarrone M: A new group of oxime carbamates as reversible inhibitors of fatty acid amide hydrolase. Bioorg Med Chem Lett 20: 4406-4411, 2010b.

Giang DK & Cravatt BF: Molecular characterization of human and mouse fatty acid amide hydrolases. Proc Natl Acad Sci USA 94: 2238-2242, 1997.

Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D: Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci USA 102: 18620-18625, 2005.

Godlewski G, Alapafuja SO, Batkai S, Nikas SP, Cinar R, Offertaler L, Osei-Hyiaman D, Liu J, Mukhopadhyay B, Harvey-White J, Tam J, Pacak K, Blankman JL, Cravatt BF, Makriyannis A, Kunos G: Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. Chem Biol 17: 1256-1266, 2010.

Gonsiorek W, Lunn C, Fan X, Narula S, Lundell D, Hipkin RW: Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. Mol Pharmacol 57: 1045-1050, 2000.

Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S: Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. FEBS Lett 422: 69-73, 1998.

Gowlugari S, DeFalco J, Nguyen MT, Kaub C, Chi C, Duncton MAJ, Emerling DE, Kelly MG, Kincaid J, Vincent F: Discovery of potent, non-carbonyl inhibitors of fatty acid amide hydrolase (FAAH). Med Chem Commun 3: 1258-1263, 2012.

Grazia Cascio M, Minassi A, Ligresti A, Appendino G, Burstein S, Di Marzo V: A structure-activity relationship study on *N*-arachidonoyl-amino acids as possible endogenous inhibitors of fatty acid amide hydrolase. Biochem Biophys Res Commun 314: 192-196, 2004.

Gustin DJ, Ma Z, Min X, Li Y, Hedberg C, Guimaraes C, Porter AC, Lindstrom M, Lester-Zeiner D, Xu G, Carlson TJ, Xiao S, Meleza C, Connors R, Wang Z, Kayser F: Identification of potent, noncovalent fatty acid amide hydrolase (FAAH) inhibitors. Bioorg Med Chem Lett 21: 2492-2496, 2011.

Hansen KB, Rosenkilde MM, Knop FK, Wellner N, Diep TA, Rehfeld JF, Andersen UB, Holst JJ, Hansen HS: 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans. J Clin Endocrinol Metab 96: E1409-E1417, 2011.

Hanus L, Gopher A, Almog S, Mechoulam R: Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J Med Chem 36: 3032-4, 1993.

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R: 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. Proc Natl Acad Sci USA 98: 3662-3665, 2001.

Hardouin C, Kelso MJ, Romero FA, Rayl TJ, Leung D, Hwang I, Cravatt BF, Boger DL: Structure-activity relationships of α-ketooxazole inhibitors of fatty acid amide hydrolase. J Med Chem 50: 3359-3368, 2007.

Hart T, Macias AT, Benwell K, Brooks T, D'Alessandro J, Dokurno P, Francis G, Gibbons B, Haymes T, Kennett G, Lightowler S, Mansell H, Matassova N, Misra A, Padfield A, Parsons R, Pratt R, Robertson A, Walls S, Wong M, Roughley S: Fatty acid amide hydrolase inhibitors. Surprising selectivity of chiral azetidine ureas. Bioorg Med Chem Lett 19: 4241-4244, 2009.

Hernández-Torres G, Cipriano M, Hedén E, Björklund E, Canales Á, Zian D, Feliú A, Mecha M, Guaza C, Fowler CJ, Ortega-Gutiérrez S, López-Rodríguez ML: A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis. Angew Chem Int Ed Engl 53: 13765-13770, 2014.

Hill MN, Kumar SA, Filipski SB, Iverson M, Stuhr KL, Keith JM, Cravatt BF, Hillard CJ, Chattarji S, McEwen BS: Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. Mol Psychiatry 18: 1125-1135, 2013.

Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Michael Walker J, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D: An endocannabinoid mechanism for stress-induced analgesia. Nature 435: 1108-1112, 2005.

Holmquist M: Alpha/beta-hydrolase fold enzymes: structures, functions and mechanisms. Curr Protein Pept Sci 1: 209-235, 2000.

Holt S, Paylor B, Boldrup L, Alajakku K, Vandevoorde S, Sundström A, Cocco MT, Onnis V, Fowler CJ: Inhibition of fatty acid amide hydrolase, a key endocannabinoid metabolizing enzyme, by analogues of ibuprofen and indomethacin. Eur J Pharmacol 565: 26-36, 2007.

Holtfrerich A, Hanekamp W, Lehr M: (4-Phenoxyphenyl)tetrazolecarboxamides and related compounds as dual inhibitors of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Eur J Med Chem 63: 64-75, 2013.

Hoornaert C: Derivatives of triazolopyridine carboxamides, particularly (piperazin-1-yl)(1,2,3-triazolo[4,5-*b*]pyridin-1-yl)methanones, their preparation and use as monoacyl glycerol lipase (MGL) inhibitors for treating MGL-related pathologies. WO2008145842A2, 2008c.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG: International union of pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54: 161-202, 2002.

Hsu K, Tsuboi K, Adibekian A, Pugh H, Masuda K, Cravatt BF: DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. Nat Chem Biol 8: 999-1007, 2012.

Hsu K, Tsuboi K, Chang JW, Whitby LR, Speers AE, Pugh H, Cravatt BF: Discovery and optimization of piperidyl-1,2,3-triazole ureas as potent, selective, and in vivo-active inhibitors of α/β-hydrolase domain containing 6 (ABHD6). J Med Chem 56: 8270-8279, 2013.

https://clinicaltrials.gov/ct2/results?term=PF-04457845&Search=Search (URL checked on 04.06.2015)

https://clinicaltrials.gov/ct2/show/NCT01650597?term=FAAH&rank=6 (URL checked on 04.06.2015)

https://clinicaltrials.gov/ct2/show/NCT01634529?term=FAAH&rank=8 (URL checked on 04.06.2015)

Hu SS, Bradshaw HB, Benton VM, Chen JS, Huang SM, Minassi A, Bisogno T, Masuda K, Tan B, Roskoski R, Cravatt BF, Di M, Vincenzo, Walker JM: The biosynthesis of *N*-arachidonoyl dopamine (NADA), a putative endocannabinoid and endovanilloid, via conjugation of arachidonic acid with dopamine. Prostaglandins Leukot Essent Fatty Acids 81: 291-301, 2009.

Huang SM, Bisogno T, Petros TJ, Chang SY, Zavitsanos PA, Zipkin RE, Sivakumar R, Coop A, Maeda DY, De Petrocellis L, Burstein S, Di Marzo V, Walker JM: Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. J Biol Chem 276: 42639-42644, 2001.

Huggins JP, Smart TS, Langman S, Taylor L, Young T: An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. Pain 153: 1837-1846, 2012.

Huitron-Resendiz S, Sanchez-Alavez M, Wills DN, Cravatt BF, Henriksen SJ: Characterization of the sleep-wake patterns in mice lacking fatty acid amide hydrolase. Sleep 27: 857-65, 2004.

Janssen FJ, Deng H, Baggelaar MP, Allarà M, van dW, den Dulk H, Ligresti A, van Esbroeck, Annelot C. M., McGuire R, Di Marzo V, Overkleeft HS, van dS: Discovery of glycine sulfonamides as dual inhibitors of sn-1-diacylglycerol lipase α and α/β-hydrolase domain 6. J Med Chem 57: 6610-6622, 2014.

Jayamanne A, Greenwood R, Mitchell VA, Aslan S, Piomelli D, Vaughan CW: Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. Br J Pharmacol 147: 281-288, 2006.

Johnson DS, Ahn K, Kesten S, Lazerwith SE, Song Y, Morris M, Fay L, Gregory T, Stiff C, Dunbar JB, Liimatta M, Beidler D, Smith S, Nomanbhoy TK, Cravatt BF: Benzothiophene piperazine and piperidine urea inhibitors of fatty acid amide hydrolase (FAAH). Bioorg Med Chem Lett 19: 2865-2869, 2009.

Johnson DS, Stiff C, Lazerwith SE, Kesten SR, Fay LK, Morris M, Beidler D, Liimatta MB, Smith SE, Dudley DT, Sadagopan N, Bhattachar SN, Kesten SJ, Nomanbhoy TK, Cravatt BF, Ahn K: Discovery of PF-04457845: a highly potent, orally bioavailable, and selective urea FAAH inhibitor. ACS Med Chem Lett 2: 91-96, 2011.

Kaczocha M, Glaser ST, Deutsch DG: Identification of intracellular carriers for the endocannabinoid anandamide. Proc Natl Acad Sci USA 106: 6375-6380, 2009.

Kaczocha M, Glaser ST, Chae J, Brown DA, Deutsch DG: Lipid droplets are novel sites of *N*-acylethanolamine inactivation by fatty acid amide hydrolase-2. J Biol Chem 285: 2796-2806, 2010.

Kapanda CN, Muccioli GG, Labar G, Poupaert JH, Lambert DM: Bis(dialkylaminethiocarbonyl)disulfides as potent and selective monoglyceride lipase inhibitors. J Med Chem 52: 7310-7314, 2009.

Kapanda CN, Masquelier J, Labar G, Muccioli GG, Poupaert JH, Lambert DM: Synthesis and pharmacological evaluation of 2,4-dinitroaryldithiocarbamate derivatives as novel monoacylglycerol lipase inhibitors. J Med Chem 55: 5774-5783, 2012.

Kapanda CN, Poupaert JH, Lambert DM: Insight into the medicinal chemistry of the endocannabinoid hydrolase inhibitors. Curr Med Chem 20: 1824-1846, 2013.

Karbarz MJ, Luo L, Chang L, Tham C, Palmer JA, Wilson SJ, Wennerholm ML, Brown SM, Scott BP, Apodaca RL, Keith JM, Wu J, Breitenbucher JG, Chaplan SR, Webb M: Biochemical and biological properties of 4-(3-phenyl-[1,2,4] thiadiazol-5-yl)-piperazine-1-carboxylic acid phenylamide, a mechanism-based inhibitor of fatty acid amide hydrolase. Anesth Analg 108: 316-329, 2008.

Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C: cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem 272: 27218-27223, 1997.

Karsak M, Gaffal E, Date R, Wang-Eckhardt L, Rehnelt J, Petrosino S, Starowicz K, Steuder R, Schlicker E, Cravatt B, Mechoulam R, Buettner R, Werner S, Di M, Vincenzo, Tueting T, Zimmer A: Attenuation of allergic contact dermatitis through the endocannabinoid system. Science 316: 1494-1497, 2007.

Käsnänen H, Myllymäki MJ, Minkkilä A, Kataja AO, Saario SM, Nevalainen T, Koskinen AMP, Poso A: 3-Heterocycle-phenyl *N*-alkylcarbamates as FAAH inhibitors: design, synthesis and 3D-QSAR studies. ChemMedChem 5: 213-231, 2010.

Käsnänen H, Minkkilä A, Taupila S, Patel JZ, Parkkari T, Lahtela-Kakkonen M, Saario SM, Nevalainen T, Poso A: 1,3,4-Oxadiazol-2-ones as fatty-acid amide hydrolase and monoacylglycerol lipase inhibitors: synthesis, in vitro evaluation and insight into potency and selectivity determinants by molecular modelling. Eur J Pharm Sci 49: 423-433, 2013.

Kathuria S, Gaetani S, Fegley D, Valiño F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D: Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med (NY) 9: 76-81, 2003.

Keith JM, Apodaca R, Xiao W, Seierstad M, Pattabiraman K, Wu J, Webb M, Karbarz MJ, Brown S, Wilson S, Scott B, Tham C, Luo L, Palmer J, Wennerholm M, Chaplan S, Breitenbucher JG: Thiadiazolopiperazinyl ureas as inhibitors of fatty acid amide hydrolase. Bioorg Med Chem Lett 18: 4838-4843, 2008.

Keith JM, Apodaca R, Tichenor M, Xiao W, Jones W, Pierce J, Seierstad M, Palmer J, Webb M, Karbarz M, Scott B, Wilson S, Luo L, Wennerholm M, Chang L, Brown S, Rizzolio M, Rynberg R, Chaplan S, Breitenbucher JG: Aryl piperazinyl

ureas as inhibitors of fatty acid amide hydrolase (FAAH) in rat, dog, and primate. ACS Med Chem Lett 3: 823-827, 2012.

Keith JM, Hawryluk N, Apodaca RL, Chambers A, Pierce JM, Seierstad M, Palmer JA, Webb M, Karbarz MJ, Scott BP, Wilson SJ, Luo L, Wennerholm ML, Chang L, Rizzolio M, Chaplan SR, Breitenbucher JG: 1-Aryl-2-((6-aryl)pyrimidin-4-yl)amino)ethanols as competitive inhibitors of fatty acid amide hydrolase. Bioorg Med Chem Lett 24: 1280-1284, 2014a.

Keith JM, Jones WM, Pierce JM, Seierstad M, Palmer JA, Webb M, Karbarz MJ, Scott BP, Wilson SJ, Luo L, Wennerholm ML, Chang L, Brown SM, Rizzolio M, Rynberg R, Chaplan SR, Breitenbucher JG: Heteroarylureas with spirocyclic diamine cores as inhibitors of fatty acid amide hydrolase. Bioorg Med Chem Lett 24: 737-741, 2014b.

Kharul R, Bhuniya D, Mookhtiar KA, Singh U, Hazare A, Patil S, Datrange L, Thakkar M: Preparation of amide compounds as FAAH inhibitors, compositions and applications thereof. WO2013042139A1, 2013.

Kimball FS, Romero FA, Ezzili C, Garfunkle J, Rayl TJ, Hochstatter DG, Hwang I, Boger DL: Optimization of α-ketooxazole inhibitors of fatty acid amide hydrolase. J Med Chem 51: 937-947, 2008.

King AR, Lodola A, Carmi C, Fu J, Mor M, Piomelli D: A critical cysteine residue in monoacylglycerol lipase is targeted by a new class of isothiazolinone-based enzyme inhibitors. Br J Pharmacol 157: 974-983, 2009a.

King AR, Dotsey EY, Lodola A, Jung KM, Ghomian A, Qiu Y, Fu J, Mor M, Piomelli D: Discovery of potent and reversible monoacylglycerol lipase inhibitors. Chem Biol 16: 1045-1052, 2009b.

Kinsey SG, Nomura DK, O'Neal ST, Long JZ, Mahadevan A, Cravatt BF, Grider JR, Lichtman AH: Inhibition of monoacylglycerol lipase attenuates nonsteroidal anti-inflammatory drug-induced gastric hemorrhages in mice. J Pharmacol Exp Ther 338: 795-802, 2011.

Kiss LE, Ferreira HS, Beliaev A, Torrão L, Bonifáciob MJ, Learmonth DA: Design, synthesis, and structure-activity relationships of 1,3,4-oxadiazol-2(3*H*)-ones as novel FAAH inhibitors. Med Chem Commun 2: 889-894, 2011.

Kondo S, Kondo H, Nakane S, Kodaka T, Tokumura A, Waku K, Sugiura T: 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through Ca^{2+}-dependent and -independent mechanisms. FEBS Lett 429: 152-6, 1998.

Kono M, Matsumoto T, Kawamura T, Nishimura A, Kiyota Y, Oki H, Miyazaki J, Igaki S, Behnke CA, Shimojo M, Kori M: Synthesis, SAR study, and biological evaluation of a series of piperazine ureas as fatty acid amide hydrolase (FAAH) inhibitors. Bioorg Med Chem 21: 28-41, 2013.

Kono M, Matsumoto T, Imaeda T, Kawamura T, Fujimoto S, Kosugi Y, Odani T, Shimizu Y, Matsui H, Shimojo M, Kori M: Design, synthesis, and biological evaluation of a series of piperazine ureas as fatty acid amide hydrolase inhibitors. Bioorg Med Chem 22: 1468-1478, 2014.

Korhonen J, Kuusisto A, van Bruchem J, Patel JZ, Laitinen T, Navia-Paldanius D, Laitinen JT, Savinainen JR, Parkkari T, Nevalainen TJ: Piperazine and piperidine carboxamides and carbamates as inhibitors of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Bioorg Med Chem 22: 6694-6705, 2014.

Koutek B, Prestwich GD, Howlett AC, Chin SA, Salehani D, Akhavan N, Deutsch DG: Inhibitors of arachidonoyl ethanolamide hydrolysis. J Biol Chem 269: 22937-40, 1994.

Kozak KR & Marnett LJ: Oxidative metabolism of endocannabinoids. Prostaglandins Leukot Essent Fatty Acids 66: 211-220, 2002.

Labar G, Bauvois C, Muccioli GG, Wouters J, Lambert DM: Disulfiram is an inhibitor of human purified monoacylglycerol lipase, the enzyme regulating 2-arachidonoylglycerol signaling. ChemBioChem 8: 1293-1297, 2007.

Labar G & Michaux C: Fatty acid amide hydrolase: from characterization to therapeutics. Chem Biodiversity 4: 1882-1902, 2007.

Labar G, Bauvois C, Borel F, Ferrer J, Wouters J, Lambert DM: Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. ChemBioChem 11: 218-227, 2010.

Lambert DM, DiPaolo FG, Sonveaux P, Kanyonyo M, Govaerts SJ, Hermans E, Bueb JL, Delzenne NM, Tschirhart EJ: Analogues and homologues of *N*-palmitoylethanolamide, a putative endogenous CB2 cannabinoid, as potential ligands for the cannabinoid receptors. Biochim Biophys Acta, Mol Cell Biol Lipids 1440: 266-274, 1999.

Ledent C, Valverdej O, Cossu G, Petitet F, Aubert J, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M: Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283: 401-404, 1999.

Leung D, Hardouin C, Boger DL, Cravatt BF: Discovering potent and selective reversible inhibitors of enzymes in complex proteomes. Nat Biotechnol 21: 687-691, 2003.

Li W, Blankman JL, Cravatt BF: A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. J Am Chem Soc 129: 9594-9595, 2007.

Lichtman AH, Leung D, Shelton CC, Saghatelian A, Hardouin C, Boger DL, Cravatt BF: Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: Evidence for an unprecedented combination of potency and selectivity. J Pharmacol Exp Ther 311: 441-448, 2004a.

Lichtman AH, Shelton CC, Advani T, Cravatt BF: Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. Pain 109: 319-327, 2004b.

Lichtman AH, Blankman JL, Cravatt BF: Endocannabinoid overload. Mol Pharmacol 78: 993-995, 2010.

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chang AC, Zhou Z, Huang BX, Kim H, Kunos G: A biosynthetic pathway for anandamide. Proc Natl Acad Sci USA 103: 13345-13350, 2006.

Liu J, Wang L, Harvey-White J, Huang BX, Kim H, Luquet S, Palmiter RD, Krystal G, Rai R, Mahadevan A, Razdan RK, Kunos G: Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacol 54: 1-7, 2008.

Liu Y, Patricelli MP, Cravatt BF: Activity-based protein profiling: the serine hydrolase. Proc Natl Acad Sci USA 96: 14694-14699, 1999.

Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavon FJ, Serrano AM, Selley DE, Parsons LH, Lichtman AH, Cravatt BF: Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat Chem Biol 5: 37-44, 2009a.

Long JZ, Nomura DK, Cravatt BF: Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. Chem Biol 16: 744-753, 2009b.

Long JZ, Jin X, Adibekian A, Li W, Cravatt BF: Characterization of tunable piperidine and piperazine carbamates as inhibitors of endocannabinoid hydrolases. J Med Chem 53: 1830-1842, 2010.

Long JZ & Cravatt BF: The metabolic serine hydrolases and their functions in mammalian physiology and disease. Chem Rev 111: 6022-6063, 2011.

Lord CC, Thomas G, Brown JM: Mammalian alpha beta hydrolase domain (ABHD) proteins: Lipid metabolizing enzymes at the interface of cell signaling and energy metabolism. Biochim Biophys Acta, Mol Cell Biol Lipids 1831: 792-802, 2013.

Lunn CA, Reich E, Bober L: Targeting the CB2 receptor for immune modulation. Expert Opin Ther Targets 10: 653-663, 2006.

Maccarrone M, Cartoni A, Parolaro D, Margonelli A, Massi P, Bari M, Battista N, Finazzi-Agro A: Cannabimimetic activity, binding, and degradation of stearoylethanolamide within the mouse central nervous system. Mol Cell Neurosci 21: 126-140, 2002.

Maccarrone M, Dainese E, Oddi S: Intracellular trafficking of anandamide: new concepts for signaling. Trends Biochem Sci 35: 601-608, 2010.

Mah R, Thomas JR, Shafer CM: Drug discovery considerations in the development of covalent inhibitors. Bioorg Med Chem Lett 24: 33-39, 2014.

Maione S, Morera E, Marabese I, Ligresti A, Luongo L, Ortar G, Di Marzo V: Antinociceptive effects of tetrazole inhibitors of endocannabinoid inactivation: cannabinoid and non-cannabinoid receptor-mediated mechanisms. Br J Pharmacol 155: 775-782, 2008.

Maione S, Costa B, Piscitelli F, Morera E, De Chiaro M, Comelli F, Boccella S, Guida F, Verde R, Ortar G, Di Marzo V: Piperazinyl carbamate fatty acid amide hydrolase inhibitors and transient receptor potential channel modulators as "dual-target" analgesics. Pharmacological Research 76: 98-105, 2013.

Marrs WR, Horne EA, Ortega-Gutierrez S, Cisneros JA, Xu C, Lin YH, Muccioli GG, Lopez-Rodriguez M, Stella N: Dual inhibition of α/β-hydrolase domain 6 and fatty acid amide hydrolase increases endocannabinoid levels in neurons. J Biol Chem 286: 28723-28728, 2011.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutierrez SO, van dS, Lopez-Rodriguez M, Casanova E, Schuetz G, Zieglgaensberger W, Di Marzo V, Behl C, Lutz B: CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 302: 84-88, 2003.

Massa F, Marsicano G, Hermann H, Cannich A, Monory K, Cravatt BF, Ferri G, Sibaev A, Storr M, Lutz B: The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest 113: 1202-1209, 2004.

Matias I, Chen J, De Petrocellis L, Bisogno T, Ligresti A, Fezza F, Krauss AH-, Shi L, Protzman CE, Li C, Liang Y, Nieves AL, Kedzie KM, Burk RM, Di Marzo V, Woodward DF: Prostaglandin ethanolamides (prostamides): in vitro pharmacology and metabolism. J Pharmacol Exp Ther 309: 745-757, 2004.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI: Structures of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346: 561-564, 1990.

Matuszak N, Muccioli GG, Labar G, Lambert DM: Synthesis and in vitro evaluation of *N*-substituted maleimide derivatives as selective monoglyceride lipase inhibitors. J Med Chem 52: 7410-7420, 2009.

McKinney MK & Cravatt BF: Evidence for distinct roles in catalysis for residues of the serine-serine-lysine catalytic triad of fatty acid amide hydrolase. J Biol Chem 278: 37393-37399, 2003.

Meade EA, Smith WL, DeWitt DL: Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 268: 6610-6614, 1993.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z: Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50: 83-90, 1995.

Mechoulam R & Deutsch DG: Toward an anandamide transporter. Proc Natl Acad Sci USA 102: 17541-17542, 2005.

Meyers MJ, Long SA, Pelc MJ, Wang JL, Bowen SJ, Walker MC, Schweitzer BA, Madsen HM, Tenbrink RE, McDonald J, Smith SE, Foltin S, Beidler D, Thorarensen A: Discovery of novel spirocyclic inhibitors of fatty acid amide hydrolase (FAAH). Part 1: Identification of 7-azaspiro[3.5]nonane and 1-oxa-8-azaspiro[4.5]decane as lead scaffolds. Bioorg Med Chem Lett 21: 6538-6544, 2011a.

Meyers MJ, Long SA, Pelc MJ, Wang JL, Bowen SJ, Schweitzer BA, Wilcox MV, McDonald J, Smith SE, Foltin S, Rumsey J, Yang Y, Walker MC, Kamtekar S, Beidler D, Thorarensen A: Discovery of novel spirocyclic inhibitors of fatty acid amide hydrolase (FAAH). Part 2. Discovery of 7-azaspiro[3.5]nonane urea PF-04862853, an orally efficacious inhibitor of fatty acid amide hydrolase (FAAH) for pain. Bioorg Med Chem Lett 21: 6545-6553, 2011b.

Micale V, Cristino L, Tamburella A, Petrosino S, Leggio GM, Drago F, Di M, Vincenzo.: Anxiolytic Effects in Mice of a Dual Blocker of Fatty Acid Amide Hydrolase and Transient Receptor Potential Vanilloid Type-1 Channels. Neuropsychopharmacology 34: 593-606, 2009.

Mileni M, Garfunkle J, DeMartino JK, Cravatt BF, Boger DL, Stevens RC: Binding and inactivation mechanism of a humanized fatty acid amide hydrolase by α-ketoheterocycle inhibitors revealed from cocrystal structures. J Am Chem Soc 131: 10497-10506, 2009.

Mileni M, Kamtekar S, Wood DC, Benson TE, Cravatt BF, Stevens RC: Crystal structure of fatty acid amide hydrolase bound to the carbamate inhibitor URB597: discovery of a deacylating water molecule and insight into enzyme inactivation. J Mol Biol 400: 743-754, 2010a.

Mileni M, Garfunkle J, Ezzili C, Kimball FS, Cravatt BF, Stevens RC, Boger DL: X-ray crystallographic analysis of α-Ketoheterocycle inhibitors bound to a humanized variant of fatty acid amide hydrolase. J Med Chem 53: 230-240, 2010b.

Minetti P, Cabri W, Borsini F, Caprioli A, Penco S, Dallavalle S, Merlini L, Maccarrone M: Preparation of oxime carbamoyl derivatives as modulators of fatty acid amides hydrolase. WO2009138416A1, 2009.

Minkkilä A, Saario SM, Käsnänen H, Leppänen J, Poso A, Nevalainen T: Discovery of boronic acids as novel and potent inhibitors of fatty acid amide hydrolase. J Med Chem 51: 7057-7060, 2008.

Minkkilä A, Myllymäki MJ, Saario SM, Castillo-Melendez J, Koskinen AMP, Fowler CJ, Leppänen J, Nevalainen T: The synthesis and biological evaluation of para-substituted phenolic *N*-alkyl carbamates as endocannabinoid hydrolyzing enzyme inhibitors. Eur J Med Chem 44: 2994-3008, 2009a.

Minkkilä A, Savinainen JR, Käsnänen H, Xhaard H, Nevalainen T, Laitinen JT, Poso A, Leppänen J, Saario SM: Screening of various hormone-sensitive lipase inhibitors as endocannabinoid-hydrolyzing enzyme inhibitors. ChemMedChem 4: 1253-1259, 2009b.

Minkkilä A, Saario SM, Nevalainen T: Discovery and development of endocannabinoid-hydrolyzing enzyme inhibitors. Curr Top Med Chem 10: 828-858, 2010.

Moore SA, Nomikos GG, Dickason-Chesterfield AK, Schober DA, Schaus JM, Ying BP, Xu YC, Phebus L, Simmons RMA, Li D, Lyengar S, Felder CC: Identification of a high-affinity binding site involved in the transport of endocannabinoids. Proc Natl Acad Sci USA 102: 17852-17857, 2005.

Mor M, Rivara S, Lodola A, Plazzi PV, Tarzia G, Duranti A, Tontini A, Piersanti G, Kathuria S, Piomelli D: Cyclohexylcarbamic acid 3'- or 4'-substituted biphenyl-3-yl esters as fatty acid amide hydrolase inhibitors: synthesis, quantitative structure-activity relationships, and molecular modeling studies. J Med Chem 47: 4998-5008, 2004.

Mor M, Lodola A, Rivara S, Vacondio F, Duranti A, Tontini A, Sanchini S, Piersanti G, Clapper JR, King AR, Tarzia G, Piomelli D: Synthesis and quantitative structure-activity relationship of fatty acid amide hydrolase inhibitors: modulation at the *N*-portion of biphenyl-3-yl alkylcarbamates. J Med Chem 51: 3487-3498, 2008.

Moreno-Sanz G, Duranti A, Melzig L, Fiorelli C, Ruda GF, Colombano G, Mestichelli P, Sanchini S, Tontini A, Mor M, Bandiera T, Scarpelli R, Tarzia G, Piomelli D: Synthesis and structure-activity relationship studies of *O*-biphenyl-3-yl carbamates as peripherally restricted fatty acid amide hydrolase inhibitors. J Med Chem 56: 5917-5930, 2013.

Morera L, Labar G, Ortar G, Lambert DM: Development and characterization of endocannabinoid hydrolases FAAH and MAGL inhibitors bearing a benzotriazol-1-yl carboxamide scaffold. Bioorg Med Chem 20: 6260-6275, 2012.

Muccioli GG, Fazio N, Scriba GKE, Poppitz W, Cannata F, Poupaert JH, Wouters J, Lambert DM: Substituted 2-thioxoimidazolidin-4-ones and imidazolidine-2,4-diones as fatty acid amide hydrolase inhibitors templates. J Med Chem 49: 417-425, 2006.

Mulvihill MM & Nomura DK: Therapeutic potential of monoacylglycerol lipase inhibitors. Life Sci 92: 492-497, 2013.

Munro S, Thomas KL, Abu-Shaar M: Molecular characterization of a peripheral receptor for cannabinoids. Nature 365: 61-65, 1993.

Myllymäki MJ, Saario SM, Kataja AO, Castillo-Melendez J, Nevalainen T, Juvonen RO, Järvinen T, Koskinen AMP: Design, synthesis, and in vitro evaluation of carbamate derivatives of 2-benzoxazolyl- and 2-benzothiazolyl-(3-hydroxyphenyl)-methanones as novel fatty acid amide hydrolase inhibitors. J Med Chem 50: 4236-4242, 2007.

Myllymäki MJ, Käsnänen H, Kataja AO, Lahtela-Kakkonen M, Saario SM, Poso A, Koskinen AMP: Chiral 3-(4,5-dihydrooxazol-2-yl)phenyl alkylcarbamates as novel FAAH inhibitors: Insight into FAAH enantioselectivity by molecular docking and interaction fields. Eur J Med Chem 44: 4179-4191, 2009.

Naidu PS, Varvel SA, Ahn K, Cravatt BF, Martin BR, Lichtman AH: Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. Psychopharmacology 192: 61-70, 2007.

Naidu PS, Booker L, Cravatt BF, Lichtman AH: Synergy between enzyme inhibitors of fatty acid amide hydrolase and cyclooxygenase in visceral nociception. J Pharmacol Exp Ther 329: 48-56, 2009.

Nakane S, Oka S, Arai S, Waku K, Ishima Y, Tokumura A, Sugiura T: 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain. Arch Biochem Biophys 402: 51-58, 2002.

Navia-Paldanius D, Savinainen JR, Laitinen JT: Biochemical and pharmacological characterization of human α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). J Lipid Res 53: 2413-2424, 2012.

Naydenov A, Horne E, Cheah C, Swinney K, Hsu K, Cao J, Marrs W, Blankman J, Tu S, Cherry A, Fung S, Wen A, Li W, Saporito M, Selley D, Cravatt B, Oakley J, Stella N: ABHD6 blockade exerts antiepileptic activity in PTZ-induced seizures and in spontaneous seizures in R6/2 mice. Neuron 83: 361-371, 2014.

Nielsen MJ, Petersen G, Astrup A, Hansen HS: Food intake is inhibited by oral oleoylethanolamide. J Lipid Res 45: 1027-1029, 2004.

Niphakis MJ, Johnson DS, Ballard TE, Stiff C, Cravatt BF: *O*-Hydroxyacetamide carbamates as a highly potent and selective class of endocannabinoid hydrolase inhibitors. ACS Chem Neurosci 3: 418-426, 2012.

Niphakis MJ, Cognetta AB, Chang JW, Buczynski MW, Parsons LH, Byrne F, Burston JJ, Chapman V, Cravatt BF: Evaluation of NHS carbamates as a potent and selective class of endocannabinoid hydrolase inhibitors. ACS Chem Neurosci 4: 1322-1332, 2013.

Nomura DK, Long JZ, Niessen S, Hoover HS, Ng S, Cravatt BF: Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell 140: 49-61, 2010.

Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N: Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 279: 5298-5305, 2004.

Onaivi ES: Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. Neuropsychobiology 54: 231-246, 2006.

Onnis V, Congiu C, Björklund E, Hempel F, Söderström E, Fowler CJ: Synthesis and evaluation of paracetamol esters as novel fatty acid amide hydrolase inhibitors. J Med Chem 53: 2286-2298, 2010.

Ortar G, Schiano Moriello A, Cascio MG, De Petrocellis L, Ligresti A, Morera E, Nalli M, Di Marzo V: New tetrazole-based selective anandamide uptake inhibitors. Bioorg Med Chem Lett 18: 2820-2824, 2008.

Ortar G, Morera E, De Petrocellis L, Ligresti A, Schiano Moriello A, Morera L, Nalli M, Ragno R, Pirolli A, Di Marzo V: Biaryl tetrazolyl ureas as inhibitors of endocannabinoid metabolism: modulation at the *N*-portion and distal phenyl ring. Eur J Med Chem 63: 118-132, 2013.

Otrubova K, Ezzili C, Boger DL: The discovery and development of inhibitors of fatty acid amide hydrolase (FAAH). Bioorg Med Chem Lett 21: 4674-4685, 2011.

Otrubova K & Boger DL: α-Ketoheterocycle-based inhibitors of fatty acid amide hydrolase (FAAH). ACS Chem Neurosci 3: 340-348, 2012.

Otrubova K, Brown M, McCormick MS, Han GW, O'Neal ST, Cravatt BF, Stevens RC, Lichtman AH, Boger DL: Rational design of fatty acid amide hydrolase inhibitors that act by covalently bonding to two active site residues. J Am Chem Soc 135: 6289-6299, 2013.

Otrubova K, Cravatt BF, Boger DL: Design, synthesis, and characterization of α-ketoheterocycles that additionally target the cytosolic port Cys269 of fatty acid amide hydrolase. J Med Chem 57: 1079-1089, 2014a.

Otrubova K, Srinivasan V, Boger DL: Discovery libraries targeting the major enzyme classes: The serine hydrolases. Bioorg Med Chem Lett 24: 3807-3813, 2014b.

Oveisi F, Gaetani S, Eng KT, Piomelli D: Oleoylethanolamide inhibits food intake in free-feeding rats after oral administration. Pharmacol Res 49: 461-466, 2004.

Pacher P, Batkai S, Kunos G: The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 58: 389-462, 2006.

Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R: The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr Rev 27: 73-100, 2006.

Parkkari T, Haavikko R, Laitinen T, Navia-Paldanius D, Rytilahti R, Vaara M, Lehtonen M, Alakurtti S, Yli-Kauhaluoma J, Nevalainen T, Savinainen JR, Laitinen JT: Discovery of triterpenoids as reversible inhibitors of α/β-hydrolase domain containing 12 (ABHD12). PLoS One 9: e98286/1-e98286/12, 12 pp., 2014.

Patel JZ, Parkkari T, Laitinen T, Kaczor AA, Saario SM, Savinainen JR, Navia-Paldanius D, Cipriano M, Leppänen J, Koshevoy IO, Poso A, Fowler CJ, Laitinen JT, Nevalainen T: Chiral 1,3,4-oxadiazol-2-ones as highly selective FAAH inhibitors. J Med Chem 56: 8484-8496, 2013.

Patel JZ, Ahenkorah S, Vaara M, Staszewski M, Adams Y, Laitinen T, Navia-Paldanius D, Parkkari T, Savinainen JR, Walczyński K, Laitinen JT, Nevalainen TJ: Loratadine analogues as MAGL inhibitors. Bioorg Med Chem Lett 25: 1436-1442, 2015a.

Patel JZ, Nevalainen TJ, Savinainen JR, Adams Y, Laitinen T, Runyon RS, Vaara M, Ahenkorah S, Kaczor AA, Navia-Paldanius D, Gynther M, Aaltonen N, Joharapurkar AA, Jain MR, Haka AS, Maxfield FR, Laitinen JT, Parkkari T: Optimization of 1,2,5-thiadiazole carbamates as potent and selective ABHD6 inhibitors. ChemMedChem 10: 253-265, 2015b.

Patricelli MP, Patterson JE, Boger DL, Cravatt BF: An endogenous sleep-inducing compound is a novel competitive inhibitor of fatty acid amide hydrolase. Bioorg Med Chem Lett 8: 613-618, 1998.

Patricelli MP & Cravatt BF: Fatty acid amide hydrolase competitively degrades bioactive amides and esters through a nonconventional catalytic mechanism. Biochemistry 38: 14125-14130, 1999.

Patricelli MP, Lovato MA, Cravatt BF: Chemical and mutagenic investigations of fatty acid amide hydrolase: evidence for a family of serine hydrolases with distinct catalytic properties. Biochemistry 38: 9804-9812, 1999.

Patricelli MP & Cravatt BF: Clarifying the catalytic roles of conserved residues in the amidase signature family. J Biol Chem 275: 19177-19184, 2000.

Patrick GL. An Introduction to medicinal chemistry, 4th Edition. Oxford University Press 2009.

Patterson JE, Ollmann IR, Cravatt BF, Boger DL, Wong C, Lerner RA: Inhibition of oleamide hydrolase catalyzed hydrolysis of the endogenous sleep-inducing lipid *cis*-9-octadecenamide. J Am Chem Soc 118: 5938-5945, 1996.

Petrosino S & Di Marzo V: FAAH and MAGL inhibitors: therapeutic opportunities from regulating endocannabinoid levels. Curr Opin Invest Drugs 11: 51-62, 2010.

Piomelli D: The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4: 873-884, 2003.

Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton TR, Dasse O, Monaghan EP, Parrott JA, Putman D: Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). CNS Drug Rev 12: 21-38, 2006.

Point V, Pavan Kumar, K. V. P., Marc S, Delorme V, Parsiegla G, Amara S, Carriere F, Buono G, Fotiadu F, Canaan S, Leclaire J, Cavalier J: Analysis of the discriminative inhibition of mammalian digestive lipases by 3-phenyl substituted 1,3,4-oxadiazol-2(3*H*)-ones. Eur J Med Chem 58: 452-463, 2012.

Porter AC, Sauer J, Knierman MD, Becker GW, Berna MJ, Bao J, Nomikos GG, Carter P, Bymaster FP, Leese AB, Felder CC: Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. J Pharmacol Exp Ther 301: 1020-1024, 2002.

Rampa A, Bartolini M, Bisi A, Belluti F, Gobbi S, Andrisano V, Ligresti A, Di Marzo V: The first dual ChE/FAAH inhibitors: new perspectives for Alzheimer's disease? ACS Med Chem Lett 3: 182-186, 2012.

Rodriguez dF, Navarro M, Gomez R, Escuredo L, Nava F, Fu J, Murillo-Rodriguez E, Gluffrida A, Lo Verme J, Gaetani S, Kathurla S, Gall C, Piomell D: An anorexic lipid mediator regulated by feeding. Nature 414: 209-212, 2001.

Romani R, Galeazzi R, Rosi G, Fiorini R, Pirisinu I, Ambrosini A, Zolese G: Anandamide and its congeners inhibit human plasma butyrylcholinesterase. Possible new roles for these endocannabinoids? Biochimie 93: 1584-1591, 2011.

Romero FA, Hwang I, Boger DL: Delineation of a fundamental α-ketoheterocycle substituent effect for use in the design of enzyme inhibitors. J Am Chem Soc 128: 14004-14005, 2006.

Romero FA, Du W, Hwang I, Rayl TJ, Kimball FS, Leung D, Hoover HS, Apodaca RL, Breitenbucher JG, Cravatt BF, Boger DL: Potent and selective α-ketoheterocycle-based inhibitors of the anandamide and oleamide catabolizing enzyme, fatty acid amide hydrolase. J Med Chem 50: 1058-1068, 2007.

Rosa C, Gusmao dN, Kiss LE, Soares da Silva, Patricio Manuel,Vieira Araujo, Russo D, Wahnon JBR, Maton W: Preparation of cyclopentylureidophenylimidazolecarboxamide derivatives for use as enzyme inhibitors. WO2014017936A2, 2014.

Rose TM, Reilly CA, Deering-Rice C, Brewster C, Brewster C: Inhibition of FAAH, TRPV1, and COX2 by NSAID-serotonin conjugates. Bioorg Med Chem Lett 24: 5695-5698, 2014.

Rosenbaum AI, Cosner CC, Mariani CJ, Maxfield FR, Wiest O, Helquist P: Thiadiazole carbamates: potent inhibitors of lysosomal acid lipase and potential niemann-pick type C disease therapeutics. J Med Chem 53: 5281-5289, 2010.

Roughley SD, Browne H, Macias AT, Benwell K, Brooks T, D'Alessandro J, Daniels Z, Dugdale S, Francis G, Gibbons B, Hart T, Haymes T, Kennett G, Lightowler S, Matassova N, Mansell H, Merrett A, Misra A, Padfield A, Parsons R, Pratt R, Robertson A, Simmonite H, Tan K, Walls SB, Wong M: Fatty acid amide hydrolase inhibitors. 3: Tetra-substituted azetidine ureas with in vivo activity. Bioorg Med Chem Lett 22: 901-906, 2012.

Rouzer CA & Marnett LJ: Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. Chem Rev 111: 5899-5921, 2011.

Rusch M, Zahov S, Vetter IR, Lehr M, Hedberg C: Design, synthesis and evaluation of polar head group containing 2-keto-oxazole inhibitors of FAAH. Bioorg Med Chem 20: 1100-1112, 2012.

Russo R, LoVerme J, La R, Giovanna, Compton TR, Parrott J, Duranti A, Tontini A, Mor M, Tarzia G, Calignano A, Piomelli D: The fatty acid amide hydrolase inhibitor URB597 (cyclohexylcarbamic acid 3'-carbamoylbiphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. J Pharmacol Exp Ther 322: 236-242, 2007.

Saario SM, Savinainen JR, Laitinen JT, Jarvinen T, Niemi R: Monoglyceride lipase-like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. Biochem Pharmacol 67: 1381-1387, 2004.

Saario SM, Salo OMH, Nevalainen T, Poso A, Laitinen JT, Järvinen T, Niemi R: Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. Chem Biol 12: 649-656, 2005.

Saario SM, Poso A, Juvonen RO, Järvinen T, Salo-Ahen OMH: Fatty acid amide hydrolase inhibitors from virtual screening of the endocannabinoid system. J Med Chem 49: 4650-4656, 2006.

Saghatelian A, McKinney MK, Bandell M, Patapoutian A, Cravatt BF: A FAAH-regulated class of *N*-acyl taurines that activates TRP ion channels. Biochemistry 45: 9007-9015, 2006.

Sakurada T & Noma A: Subcellular localization and some properties of monoacylglycerol lipase in rat adipocytes. J Biochem 90: 1413-19, 1981.

Sasso O, Bertorelli R, Bandiera T, Scarpelli R, Colombano G, Armirotti A, Moreno-Sanz G, Reggiani A, Piomelli D: Peripheral FAAH inhibition causes profound antinociception and protects against indomethacin-induced gastric lesions. Pharmacol Res 65: 553-563, 2012.

Savinainen JR, Järvinen T, Laine K, Laitinen JT: Despite substantial degradation, 2-arachidonoylglycerol is a potent full efficacy agonist mediating CB1 receptor-dependent G-protein activation in rat cerebellar membranes. Br J Pharmacol 134: 664-672, 2001.

Savinainen JR, Saario SM, Niemi R, Järvinen T, Laitinen JT: An optimized approach to study endocannabinoid signaling: evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1 receptors. Br J Pharmacol 140: 1451-1459, 2003.

Savinainen JR, Kokkola T, Salo OMH, Poso A, Järvinen T, Laitinen JT: Identification of WIN55212-3 as a competitive neutral antagonist of the human cannabinoid CB2 receptor. Br J Pharmacol 145: 636-645, 2005.

Savinainen JR, Yoshino M, Minkkilä A, Nevalainen T, Laitinen JT: Characterization of binding properties of monoglyceride lipase inhibitors by a versatile fluorescence-based technique. Anal Biochem 399: 132-134, 2010.

Savinainen JR, Saario SM, Laitinen JT: The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. Acta Physiol 204: 267-276, 2012.

Savinainen JR, Patel JZ, Parkkari T, Navia-Paldanius D, Marjamaa JJT, Laitinen T, Nevalainen T, Laitinen JT: Biochemical and pharmacological characterization of the human lymphocyte antigen B-associated transcript 5 (BAT5/ABHD16A). PLoS One 9: e109869/1-e109869/17, 17 pp., 2014.

Schalk-Hihi C, Schubert C, Alexander R, Bayoumy S, Clemente JC, Deckman I, DesJarlais RL, Dzordzorme KC, Flores CM, Grasberger B, Kranz JK, Lewandowski F, Liu L, Ma H, Maguire D, Macielag MJ, McDonnell ME, Mezzasalma Haarlander T, Miller R, Milligan C, Reynolds C, Kuo LC: Crystal structure of a soluble form of human monoglyceride lipase in complex with an inhibitor at 1.35 Å resolution. Protein Sci 20: 670-683, 2011.

Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, Nguyen PT, Ramesh D, Booker L, Burston JJ, Thomas EA, Selley DE, Sim-Selley L, Liu Q, Lichtman AH, Cravatt BF: Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. Nat Neurosci 13: 1113-1119, 2010.

Schubert C, Grasberger BL, Schalk-Hihi C, Maguire DM, Lewandowski FA, Milligan CM, Alexander RS: Protein engineering and crystal structure of human monoacylglycerol lipase. WO2009132267A1, 2009.

Scott CW, Tian G, Yu XH, Paschetto KA, Wilkins DE, Meury L, Cao CQ, Varnes J, Edwards PD: Biochemical characterization and in vitro activity of AZ513, a noncovalent, reversible, and noncompetitive inhibitor of fatty acid amide hydrolase. Eur J Pharmacol 667: 74-79, 2011.

Segall Y, Quistad GB, Nomura DK, Casida JE: Arachidonylsulfonyl derivatives as cannabinoid CB1 receptor and fatty acid amide hydrolase inhibitors. Bioorg Med Chem Lett 13: 3301-3303, 2003.

Seierstad M & Breitenbucher JG: Discovery and development of fatty acid amide hydrolase (FAAH) inhibitors. J Med Chem 51: 7327-7343, 2008.

Sheskin T, Hanuš L, Slager J, Vogel Z, Mechoulam R: Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. J Med Chem 40: 659-667, 1997.

Simon GM & Cravatt BF: Endocannabinoid biosynthesis proceeding through glycerophospho-*N*-acyl ethanolamine and a role for α/β-hydrolase 4 in this pathway. J Biol Chem 281: 26465-26472, 2006.

Simon GM & Cravatt BF: Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-*N*-acyl ethanolamine precursors in mouse brain. J Biol Chem 283: 9341-9349, 2008.

Simon GM & Cravatt BF: Activity-based proteomics of enzyme superfamilies: serine hydrolases as a case study. J Biol Chem 285: 11051-11055, 2010.

Singh J, Petter RC, Baillie TA, Whitty A: The resurgence of covalent drugs. Nat Rev Drug Discov 10: 307-317, 2011.

Sit SY, Conway C, Bertekap R, Xie K, Bourin C, Burris K, Deng H: Novel inhibitors of fatty acid amide hydrolase. Bioorg Med Chem Lett 17: 3287-3291, 2007.

Sit SY, Conway CM, Xie K, Bertekap R, Bourin C, Burris KD: Oxime carbamate-discovery of a series of novel FAAH inhibitors. Bioorg Med Chem Lett 20: 1272-1277, 2010.

Snider NT, Walker VJ, Hollenberg PF: Oxidation of the endogenous cannabinoid arachidonoyl ethanolamide by the cytochrome P450 monooxygenases: physiological and pharmacological implications. Pharmacol Rev 62: 136-154, 2010.

Stella N, Schweitzer P, Piomelli D: A second endogenous cannabinoid that modulates long-term potentiation. Nature 388: 773-8, 1997.

Sticht MA, Long JZ, Rock EM, Limebeer CL, Mechoulam R, Cravatt BF, Parker LA: Inhibition of monoacylglycerol lipase attenuates vomiting in suncus murinus and 2-arachidonoyl glycerol attenuates nausea in rats. Br J Pharmacol 165: 2425-2435, 2012.

Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K: 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 215: 89-97, 1995.

Sugiura T, Kodaka T, Nakane S, Miyashita T, Kondo S, Suhara Y, Takayama H, Waku K, Seki C, Baba N, Ishima Y: Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogs, and related compounds. J Biol Chem 274: 2794-2801, 1999.

Sugiura T, Kondo S, Kishimoto S, Miyashita T, Nakane S, Kodaka T, Suhara Y, Takayama H, Waku K: Evidence that 2-arachidonoylglycerol but not *N*-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J Biol Chem 275: 605-612, 2000.

Sun Y, Tsuboi K, Okamoto Y, Tonai T, Murakami M, Kudo I, Ueda N: Biosynthesis of anandamide and *N*-palmitoylethanolamine by sequential actions of phospholipase A_2 and lysophospholipase D. Biochem J 380: 749-756, 2004.

Szabo M, Agostino M, Malone DT, Yuriev E, Capuano B: The design, synthesis and biological evaluation of novel URB602 analogues as potential monoacylglycerol lipase inhibitors. Bioorg Med Chem Lett 21: 6782-6787, 2011.

Tarzia G, Duranti A, Tontini A, Piersanti G, Mor M, Rivara S, Plazzi PV, Park C, Kathuria S, Piomelli D: Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. J Med Chem 46: 2352-2360, 2003.

Tarzia G, Duranti A, Gatti G, Piersanti G, Tontini A, Rivara S, Lodola A, Plazzi PV, Mor M, Kathuria S, Piomelli D: Synthesis and structure-activity relationships of FAAH inhibitors: cyclohexylcarbamic acid biphenyl esters with chemical modulation at the proximal phenyl ring. ChemMedChem 1: 130-139, 2006.

Tchantchou F & Zhang Y: Selective inhibition of α/β-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. J Neurotrauma 30: 565-79, 2013.

Terwege T, Dahlhaus H, Hanekamp W, Lehr M: ω-Heteroarylalkylcarbamates as inhibitors of fatty acid amide hydrolase (FAAH). Med Chem Commun 5: 932-936, 2014.

Thomas G. Medicinal chemistry: an introduction, second edition. Oxford University Press 2007.

Thomas G, Betters JL, Lord CC, Brown AL, Marshall S, Ferguson D, Sawyer J, Davis MA, Melchior JT, Blume LC, Howlett AC, Ivanova PT, Milne SB, Myers DS, Mrak I, Leber V, Heier C, Taschler U, Blankman JL, Cravatt BF, Lee RG, Crooke RM, Graham MJ, Zimmermann R, Brown HA, Brown JM: The serine hydrolase ABHD6 is a critical regulator of the metabolic syndrome. Cell Rep 5: 508-520, 2013.

Tichenor MS, Keith JM, Jones WM, Pierce JM, Merit J, Hawryluk N, Seierstad M, Palmer JA, Webb M, Karbarz MJ, Wilson SJ, Wennerholm ML, Woestenborghs F, Beerens D, Luo L, Brown SM, Boeck MD, Chaplan SR, Breitenbucher JG: Heteroaryl urea inhibitors of fatty acid amide hydrolase: structure-mutagenicity relationships for arylamine metabolites. Bioorg Med Chem Lett 22: 7357-7362, 2012.

Timmons A, Seierstad M, Apodaca R, Epperson M, Pippel D, Brown S, Chang L, Scott B, Webb M, Chaplan SR, Breitenbucher JG: Novel ketooxazole based inhibitors of fatty acid amide hydrolase (FAAH). Bioorg Med Chem Lett 18: 2109-2113, 2008.

Tornqvist H & Belfrage P: Purification and some properties of a monoacylglycerol-hydrolyzing enzyme of rat adipose tissue. J Biol Chem 251: 813-9, 1976.

Tourteau A, Leleu-Chavain N, Body-Malapel M, Andrzejak V, Barczyk A, Djouina M, Rigo B, Desreumaux P, Chavatte P, Millet R: Switching cannabinoid response from CB_2 agonists to FAAH inhibitors. Bioorg Med Chem Lett 24: 1322-1326, 2014.

Tsuboi K, Sun Y, Okamoto Y, Araki N, Tonai T, Ueda N: Molecular characterization of *N*-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. J Biol Chem 280: 11082-11092, 2005.

Tsuboi K, Zhao L, Okamoto Y, Araki N, Ueno M, Sakamoto H, Ueda N: Predominant expression of lysosomal *N*-acylethanolamine-hydrolyzing acid amidase in macrophages revealed by immunochemical studies. Biochim Biophys Acta, Mol Cell Biol Lipids 1771: 623-632, 2007.

Ueda H, Kobayashi T, Kishimoto M, Tsutsumi T, Okuyama H: A possible pathway of phosphoinositide metabolism through EDTA-insensitive phospholipase A1 followed by lysophosphoinositide-specific phospholipase C in rat brain. J Neurochem 61: 1874-81, 1993.

Ueda N, Yamanaka K, Yamamoto S: Purification and characterization of an acid amidase selective for *N*-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. J Biol Chem 276: 35552-35557, 2001.

Urbach A, Muccioli GG, Stern E, Lambert DM, Marchand-Brynaert J: 3-Alkenyl-2-azetidinones as fatty acid amide hydrolase inhibitors. Bioorg Med Chem Lett 18: 4163-4167, 2008.

van Tienhoven M, Atkins J, Li Y, Glynn P: Human neuropathy target esterase catalyzes hydrolysis of membrane lipids. J Biol Chem 277: 20942-20948, 2002.

van dS, van Kuik JA, Bari M, van Zadelhoff G, Leeflang BR, Veldink GA, Finazzi-Agrò A, Vliegenthart JFG, Maccarrone M: Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase. J Med Chem 45: 3709-3720, 2002.

Vandevoorde S, Jonsson K, Labar G, Persson E, Lambert DM, Fowler CJ: Lack of selectivity of URB602 for 2-oleoylglycerol compared to anandamide hydrolysis in vitro. Br J Pharmacol 150: 186-191, 2007.

Vandevoorde S: Overview of the chemical families of fatty acid amide hydrolase and monoacylglycerol lipase inhibitors. Curr Top Med Chem 8: 247-267, 2008.

Varvel SA, Wise LE, Niyuhire F, Cravatt BF, Lichtman AH: Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. Neuropsychopharmacology 32: 1032-1041, 2007.

Vincent F, Nguyen MT, Emerling DE, Kelly MG, Duncton MAJ: Mining biologically-active molecules for inhibitors of fatty acid amide hydrolase (FAAH): Identification of phenmedipham and amperozide as FAAH inhibitors. Bioorg Med Chem Lett 19: 6793-6796, 2009.

Wang JL, Bowen SJ, Schweitzer BA, Madsen HM, McDonald J, Pelc MJ, Tenbrink RE, Beidler D, Thorarensen A: Structure based design of novel irreversible FAAH inhibitors. Bioorg Med Chem Lett 19: 5970-5974, 2009a.

Wang X, Sarris K, Kage K, Zhang D, Brown SP, Kolasa T, Surowy C, El K, Odile F., Muchmore SW, Brioni JD, Stewart AO: Synthesis and evaluation of benzothiazole-based analogues as novel, potent, and selective fatty acid amide hydrolase inhibitors. J Med Chem 52: 170-180, 2009b.

Wei BQ, Mikkelsen TS, McKinney MK, Lander ES, Cravatt BF: A second fatty acid amide hydrolase with variable distribution among placental mammals. J Biol Chem 281: 36569-36578, 2006.

Wu H, Kelley CJ, Pino-Figueroa A, Vu HD, Maher TJ: Macamides and their synthetic analogs: Evaluation of in vitro FAAH inhibition. Bioorg Med Chem 21: 5188-5197, 2013.

Xie S, Borazjani A, Hatfield MJ, Edwards CC, Potter PM, Ross MK: Inactivation of lipid glyceryl ester metabolism in human THP1 monocytes/macrophages by activated organophosphorus insecticides: role of carboxylesterases 1 and 2. Chem Res Toxicol 23: 1890-1904, 2010.

Zahov S, Drews A, Hess M, Elfringhoff AS, Lehr M: 1-(3-Biaryloxy-2-oxopropyl)indole-5-carboxylic acids and related compounds as dual inhibitors of human cytosolic phospholipase $A_2\alpha$ and fatty acid amide hydrolase. ChemMedChem 6: 544-549, 2011.

Zhang D, Saraf A, Kolasa T, Bhatia P, Zheng GZ, Patel M, Lannoye GS, Richardson P, Stewart A, Rogers JC, Brioni JD, Surowy CS: Fatty acid amide hydrolase inhibitors display broad selectivity and inhibit multiple carboxylesterases as off-targets. Neuropharmacol 52: 1095-1105, 2007.

Zhao L, Tsuboi K, Okamoto Y, Nagahata S, Ueda N: Proteolytic activation and glycosylation of *N*-acylethanolamine-hydrolyzing acid amidase, a lysosomal enzyme involved in the endocannabinoid metabolism. Biochim Biophys Acta, Mol Cell Biol Lipids 1771: 1397-1405, 2007.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI: Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci USA 96: 5780-5785, 1999.

Zvonok N, Pandarinathan L, Williams J, Johnston M, Karageorgos I, Janero DR, Krishnan SC, Makriyannis A: Covalent inhibitors of human monoacylglycerol lipase: ligand-assisted characterization of the catalytic site by mass spectrometry and mutational analysis. Chem Biol 15: 854-862, 2008.

JAYENDRA Z. PATEL

Development of Potent and Selective Inhibitors of Enzymes Involved in Endocannabinoid Inactivation

In recent years, inhibitors of FAAH, MAGL and ABHD6 have shown potential to treat several diseases, such as pain, inflammation, CNS disorders and metabolic disorders without association of cannabimimetic side effects. This doctoral thesis reports identification of novel 1,3,4-oxadiazol-2-one, 1,2,5-thiadiazole carbamate, and loratadine analogues as potent and selective inhibitors of FAAH, ABHD6, and MAGL, respectively. Moreover, 1,3,4-oxadiazol-2-one scaffold was successfully utilized in the development of ABHD6 inhibitors.

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