TKK Dissertations 203
Espoo 2009

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Doctoral Dissertation

Mikko Myllymäki

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Dissertation for the degree of Doctor of Philosophy to be presented with due permission of the Faculty of Chemistry and Materials Sciences for public examination and debate in Auditorium KE2 (Komppa Auditorium) at Helsinki University of Technology (Espoo, Finland) on the 19th of December, 2009, at 12 noon.

Helsinki University of Technology
Faculty of Chemistry and Materials Sciences
Department of Chemistry

Teknillinen korkeakoulu
Kemian ja materiaalitieteiden tiedekunta
Kemian laitos

Distribution:
Helsinki University of Technology
Faculty of Chemistry and Materials Sciences
Department of Chemistry
P.O. Box 6100 (Kemistintie 1)
FI - 02015 TKK
FINLAND
URL: http://chemistry.tkk.fi/
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Fax +358-9-470 22538
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ISBN 978-952-248-240-2
ISBN 978-952-248-241-9 (PDF)
ISSN 1795-2239
ISSN 1795-4584 (PDF)
URL: http://lib.tkk.fi/Diss/2009/isbn9789522482419/

TKK-DISS-2686

Multiprint Oy
Espoo 2009

ABSTRACT OF DOCTORAL DISSERTATION	HELSINKI UNIVERSITY OF TECHNOLOGY P.O. BOX 1000, FI-02015 TKK http://www.tkk.fi
Author Mikko Juhani Myllymäki	
Name of the dissertation Synthesis of 3-Heterocycle Phenyl *N*-Alkyl Carbamates and Their Activity as FAAH Inhibitors	
Manuscript submitted 7.8.2009	Manuscript revised 21.10.2009
Date of the defence 19.12.2009	
☒ Monograph	☐ Article dissertation (summary + original articles)
Faculty Faculty of Chemistry and Materials Sciences Department Department of Chemistry Field of research Organic chemistry Opponent(s) Prof Maurizio Botta Supervisor Prof Ari Koskinen Instructor Prof Ari Koskinen	

Abstract

Fatty acid amide hydrolase (FAAH) is an intracellular serine hydrolase that catalyzes the hydrolysis of the endocannabinoid *N*-arachidonoylethanolamide (AEA) to arachidonic acid and ethanolamine. FAAH also hydrolyze another important endocannabinoid, 2-arachidonoylglycerol (2-AG), although the main enzyme responsible for hydrolysis of 2-AG is MGL. Inhibition of FAAH or MGL enzymatic activity potentially leads to beneficial effects in many physiological disorders such as pain, inflammation and anxiety, due to increased endocannabinoid induced activation of cannabinoid receptors CB_1 and CB_2. In the present study a total of 101 compounds were designed, synthesized, characterized and tested against FAAH and MGL enzyme activity.

Altogether 47 compounds were found to inhibit FAAH with half-maximal inhibition concentrations (IC_{50}) between 0.74 and 100 nM. All potent compounds belong to the structural family of carbamates. Other carbonyl-containing compounds were prepared for comparison and they were found not to inhibit either FAAH or MGL. The synthesized carbamate derivatives were found to be selective for FAAH as the inhibition of MGL enzyme by these compounds was negligible. From the library of phenyl *N*-alkyl carbamates the most potent FAAH inhibitors were *meta*-substituted *N*-cyclohexylcarbamates. 4,5-Dihydrooxazol-2-yl (**221**), oxazol-2-yl (**242**), 2-methyltetrazol-5-yl (**273a**), imidazol-4-yl (**252**) and 1,2,3-thiadiazol-4-yl (**314**) were found to be the best heterocycle substituents of phenolic *N*-alkyl carbamate. Methyl ester (**276**) was the best acyclic substituent. These compounds inhibited FAAH with IC_{50} values between 0.74-3.9 nM.

Various synthesis methods were used to achieve the desired compounds. Microwave assisted novel or little known reactions applied in synthesis including condensation of acids and 2-aminophenol/2-amino-3-hydroxypyridine to prepare corresponding fused 2-oxazoles, oxazole formation via condensation of bromoketones and amides, and cleavage of benzyl and methyl ethers using ionic liquids.

In conclusion, the results of this work provide useful structure-activity relationship (SAR) information of carbamate compounds as FAAH inhibitors which can be utilized in further developments in this area.

Keywords FAAH, AEA, enzyme inhibitor, IC_{50}, carbamate, heterocycle	
ISBN (printed) 978-952-248-240-2	ISSN (printed) 1795-2239
ISBN (pdf) 978-952-248-241-9	ISSN (pdf) 1795-4584
Language English	Number of pages 148
Publisher Helsinki University of Technology, Department of Chemistry	
Print distribution Helsinki University of Technology, Department of Chemistry	
☒ The dissertation can be read at http://lib.tkk.fi/Diss/2009/isbn9789522482419/	

VÄITÖSKIRJAN TIIVISTELMÄ	TEKNILLINEN KORKEAKOULU PL 1000, 02015 TKK http://www.tkk.fi
Tekijä Mikko Juhani Myllymäki	
Väitöskirjan nimi 3-Heterosyklisten fenyyli-*N*-alkyylikarbamaattien synteesi ja niiden aktiivisuus FAAH-inhibiittoreina	
Käsikirjoituksen päivämäärä 7.8.2009	Korjatun käsikirjoituksen päivämäärä 21.10.2009
Väitöstilaisuuden ajankohta 19.12.2009	
☒ Monografia	☐ Yhdistelmäväitöskirja (yhteenveto + erillisartikkelit)
Tiedekunta Kemian ja materiaalitieteiden tiedekunta Laitos Kemian laitos (KE) Tutkimusala Orgaaninen kemia Vastaväittäjä(t) prof. Maurizio Botta Työn valvoja prof. Ari Koskinen Työn ohjaaja prof. Ari Koskinen	

Tiivistelmä

Rasvahappoamidihydrolaasi (FAAH) on solunsisäinen seriinihydrolaasi, joka katalysoi endokannabinoidin *N*-arakidonoyylietanoliamiini (AEA) hydrolyysiä arakidonihapoksi ja etanoliamiiniksi. FAAH hydrolysoi myös toista tärkeää endokannabinoidia, 2-arakidonoyyliglyserolia (2-AG), vaikka pääosin 2-AG:n hydrolyysi tapahtuu MGL:n katalysoimana. FAAH- ja MGL-entsyymien inhibointi johtaa endokannabinoidien pitoisuuden kasvuun. Endokannabinoidit aktivoivat CB_1- ja CB_2-reseptoreita, mikä johtaa useisiin lääkinnällisesti edullisiin tapahtumiin, kuten kivun, tulehduksen ja ahdistuksen vähenemiseen.

Tässä tutkimuksessa yhteensä 101 yhdistettä suunniteltiin, syntetisoitiin ja karakterisoitiin ja niiden *in vitro* aktiivisuus FAAH:a ja MGL:a inhiboivina entsyymeinä testattiin. Yhteensä 47 yhdistettä havaittiin inhiboivan FAAH:a puoleen maksimaalisesta inhibiitiosta (IC_{50}) konsentraatiovälillä 0.74-100 nM. Kaikki potentiaaliset yhdisteet kuuluvat karbamaattien rakenneperheeseen. Vertailun vuoksi myös muita karbonyylin sisältäviä yhdisteitä valmistettiin, ja mikään niistä ei inhiboinut kumpaakaan kohde-entsyymeistä. Syntetisoidut karbamaattijohdannaiset olivat selektiivisiä FAAH-inhibiittoreita siinä mielessä, että merkittävää MGL-inhibiitiota niillä ei havaittu. Fenyyli-*N*-alkyylikarbamaateista lupaavimpia FAAH inhibiittoreita olivat *meta*-substituoidut *N*-sykloheksyylikarbamaatit. Parhaat substituentit olivat 4,5-dihydro-oksatsol-2-yyli (**221**), oksatsol-2-yyli (**242**), 2-metyylitetratsol-5-yyli (**273a**), imidatsol-4-yyli (**252**) and 1,2,3-tiadiatsol-4-yyli (**314**). Metyyliesteri (**276**) oli paras asyklinen substituentti. Edellä mainitut yhdisteet inhiboivat FAAH:a IC_{50} arvoilla 0.74-3.9 nM.

Yhdisteiden valmistamisessa käytettiin useita erilaisia synteesimenetelmiä. Työn aikana kehitettiin ja sovellettiin mikroaaltoavusteisia uusia tai harvoin raportoituja reaktiomenetelmiä, kuten fuusioituneiden bentso- ja pyridiinioksatsolien valmistus kondensoimalla happoja ja 2-aminofenolia tai 2-amino-3-hydroksipyridiiniä, oksatsolien valmistus bromiketonien ja amidien kondensaatiolla sekä katkaistiin aryylisiä bentsyyli- ja metyylieettereitä ionisilla liuottimilla.

Tämä väitöskirjatyö sisältää runsaasti rakenne-aktiivisuus-suhde (SAR) tietoa fenolisista karbamaateista FAAH-inhibiittoreina, mitä voidaan hyödyntää uusien entsyymi-inhibiittoriyhdisteiden kehittämisessä.

Asiasanat FAAH, AEA, entsyymi-inhibiittori, IC_{50}, karbamaatti, heterosykli	
ISBN (painettu) 978-952-248-240-2	ISSN (painettu) 1795-2239
ISBN (pdf) 978-952-248-241-9	ISSN (pdf) 1795-4584
Kieli englanti	Sivumäärä 148
Julkaisija Teknillinen korkeakoulu, Kemian laitos	
Painetun väitöskirjan jakelu Teknillinen korkeakoulu, Kemian laitos	
☒ Luettavissa verkossa osoitteessa http://lib.tkk.fi/Diss/2009/isbn9789522482419/	

ACKNOWLEDGEMENTS

The present work was carried out in the Department of Chemistry, Helsinki University of Technology (TKK), during the years 2004-2009. The study was financially supported by the National Technology Agency of Finland (TEKES) until the end of 2006. From 2007 to 2009 I held the position of teaching assistant in TKK. In addition, I want to thank the Finnish Cultural Foundation for their financial support.

I owe my most sincere thanks to my principal supervisor, Professor Ari Koskinen. I admire the great depth of his knowledge and experience in organic synthesis, and from him I have learned a lot about both traditional and modern ways of looking into chemistry.

During the first years of my study I had the luxury of also having another supervisor, Dr. Joel Castillo-Melendez, who encouraged me to focus into FAAH study.

In addition to my supervisors, I want to express my gratitude to all those who have made a significant scientific contribution to this work: Dr. Susanna Saario, M.Sc. Anna Minkkilä, M.Sc. Heikki Käsnänen, M.Sc. Antti Kataja, Dr. Tapio Nevalainen, Professor Antti Poso and Dr. Tomi Järvinen.

I want to thank undergraduate students Mia Puhakainen, Minni Pirttimaa and Tiina Rautalin for help in syntheses of some compounds of this thesis.

I am greatly indebted to Professor Jari Yli-Kauhaluoma and Professor Lise-Lotte Gundersen, the official referees of this thesis, for their constructive criticism towards my manuscript, and again to Antti Kataja for revising the language of this thesis.

I had the pleasure of having a number of highly skilled chemists as good friends in the lab: Dr. Markku Oila, Dr. Jan Tois, Dr. Vesa Rauhala, Dr. Juho Helaja, M.Sc. Aapo Tammisto, M.Sc. Antti Pohjakallio, M.Sc. Esa Kumpulainen and M.Sc. Mikko Passiniemi, among many others. I learned a lot about chemistry from all you guys.

Tiia Juhala and Anna-Maija Horko are gratefully acknowledged for carrying out HRMS and elemental analyses, and Dr. Jari Koivisto for having all the answers to any NMR-spectroscopy related questions.

My six-year stay in TKK would not have been as enjoyable without extremely nice people in the lab. Thank you all for everything!

I want to thank my parents Liisa and Juhani and my parents-in-law Helena and Matti for their support during the whole process. Finally, the utmost support I got from my loving wife, Lotta. Thank you for being there no matter what. Our beautiful and cheerful daughter Essi has also been extremely important by bringing a meaning and perspective to the work and life.

TABLE OF CONTENTS

ABBREVIATIONS

2-AG	2-arachidonoylglycerol
ABPP	activity based protein profiling
ACB	acyl chain binding (channel)
AChE	acetylcholinesterase
AEA	*N*-arachidonoylethanolamide
aq.	aqueous
AS	amidase signature
CA	cytoplasmic access (channel)
CB_1	cannabinoid receptor 1
CB_2	cannabinoid receptor 2
cDNA	complementary DNA
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
FAAH	fatty acid amide hydrolase
FAAH-2	second fatty acid amide hydrolase
FC	flash (silica) chromatography
HEK293	human embryonic kidney 293
hex	hexanes
HPLC	high pressure liquid chromatography
HSL	hormone sensitive lipase
IBX	*o*-iodoxybenzoic acid
IPA	isopropyl alcohol
LAH	lithium aluminum hydride
MAFP	methyl arachidonyl fluorophosphonate
MGL	monoglyceride lipase
MW	microwave
NAAA	*N*-acylethanolamine-hydrolyzing acid amidase

NAE	*N*-acylethanolamide
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PE	petroleum ether (60-95 ^{o}C)
PEA	*N*-palmitoylethanolamine
QSAR	quantitative structure-activity relationship
R_f	retention factor for TLC
rfx	refluxing
rt	room temperature
SAR	structure-activity relationship
SDS-PAGE	sodium dodecyl sulphate acrylamide gel electrophoresis
TEA	triethylamine
TGH	triacylglycerol hydrolase
Δ^9-THC	Δ^9-tetrahydrocannabinol
THF	tetrahydrofuran(yl)
TLC	thin (silica) layer chromatography
ΔTM	transmembrane domain-deleted
TRPV1	transient receptor potential vanilloid type 1 channels
%ee	enantiomeric excess

AUTHOR'S CONTRIBUTION

The author, Mikko Myllymäki, has designed, prepared and chemically characterized majority of the 101 end compounds presented in this dissertation. To specify, 14 compounds (**287**-**289**, **292**-**302)** were prepared in collaboration with Dr. Joel Castillo-Melendez. In addition, the assistance of pre-graduate students was used in the preparation of 18 compounds (**192-194**, **223**, **225-234**, **273a-b**). Compounds **306-316**, **319-326**, **329**-**332** were prepared by M. Sc. Anna Minkkilä, which is mentioned in the text (addition to 101). These compounds are included to this dissertation since they where published together with compounds prepared by the author.[153]

Majority of the compounds have been published, [153,197,206,207] and the author has contributed to the analyzing the results and writing the manuscripts.

1. INTRODUCTION

Medicinal chemistry is mainly based on synthesis. Without synthetic chemists to prepare, purify and characterize novel compounds or commercially unavailable known compounds they simply do not exist. In addition, during this survey to prepare target compounds there is a great opportunity to find new reactions and synthesis methods. Although medicinal chemistry belongs to the field of target oriented synthesis, like total synthesis of natural products its contribution to the science of synthetic chemistry is noticeable.

Cannabis *(Cannabis sativa* L.*)* has had a large role in medicine and recreation for over 4000 years.[1] The main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**), was isolated and characterized by Mechoulam in 1964.[2] After this it took almost 30 years to find the receptor which is activated by these compounds called cannabinoids. Shortly after that, the endogenous cannabinoid system was presented, opening a new group of target proteins for pharmacological manipulation. Since then the cannabinoid receptors, hydrolyzing enzymes of endogenous cannabinoids and their transport mechanisms have been extensively studied.[3]

Arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) are considered to be the most important endogenous agonists for the G-protein-coupled cannabinoid receptors CB_1 and CB_2.[4,5,6] The endocannabinoids are inactivated rapidly by cellular reuptake, followed by intracellular hydrolysis by specific enzymes.[7,8] AEA is rapidly hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH; EC 3.5.1.4).[7,9] Also, like AEA, 2-AG is thought to be removed from its sites of action by cellular reuptake and to be then hydrolyzed enzymatically. Although 2-AG can be hydrolyzed by FAAH,[10] the main enzyme responsible for 2-AG hydrolysis *in vivo* is probably monoglyceride lipase[11] (MGL; EC 3.1.1.23) or MGL-like enzyme.[12] Due to the rapid inactivation, the cannabimimetic effects of the endocannabinoids remain very weak. The increase of concentration of endocannabinoids in the extracellular space can lead to several beneficial therapeutic

effects[13] such as alleviation of pain[14,15] and anxiety,[16] reduction of intraocular pressure,[17] as well as increase of appetite.[18] The activation of CB_2 receptors is involved in the decrease of inflammation, lowering of blood pressure and suppression of peripheral pain.[19]

The main objective of this study was to design and prepare novel compounds which would efficiently inhibit fatty acid amide hydrolase. The second objective was to gather structure-activity relationship (SAR) data of both FAAH and MGL inhibitors for further development of inhibitors against these enzymes.

2. GENERAL BACKGROUND OF THE CANNABINOID SYSTEM

A large number of reports concerning physiological signaling system called the cannabinoid system is released monthly. To date, this system has been elucidated to consist of at least two receptors named CB_1 and CB_2, numerous endogenous ligands as their agonists (endocannabinoids), and enzymes involved in biosynthesis and degradation of these ligands.

2.1. CB-receptors

Both CB_1 and CB_2 are seven-transmembrane proteins belonging to the superfamily of G-protein coupled receptors.[20,21] They share a 68% sequence homology at the amino acid level in domains involved in ligand identification, and a 44% homology throughout the proteins.

Plant C_{21} terpenophenols found from *Cannabis sativa* and their synthetic analogues are classified as cannabinoids (**1**-**6**, **Figure 1**).[1] Due to the lipophilic nature of cannabinoids, their psychotropic activity was first thought to be based on pertubation of lipid membranes.[22] When a group of stereo- and other isomers of Δ^9-THC was prepared and found to raise different psychotropic responses within test animals, the idea of specific cannabinoid receptor was raised.[23] The first cannabinoid receptor (CB_1) was then identified utilizing a tritium labeled derivative of the synthetic non-classical cannabinoid CP-55940 (**4**).[24] CP-55940 was developed by Pfizer during their cannabinoid project before discovery of cannabinoid receptor.[25]

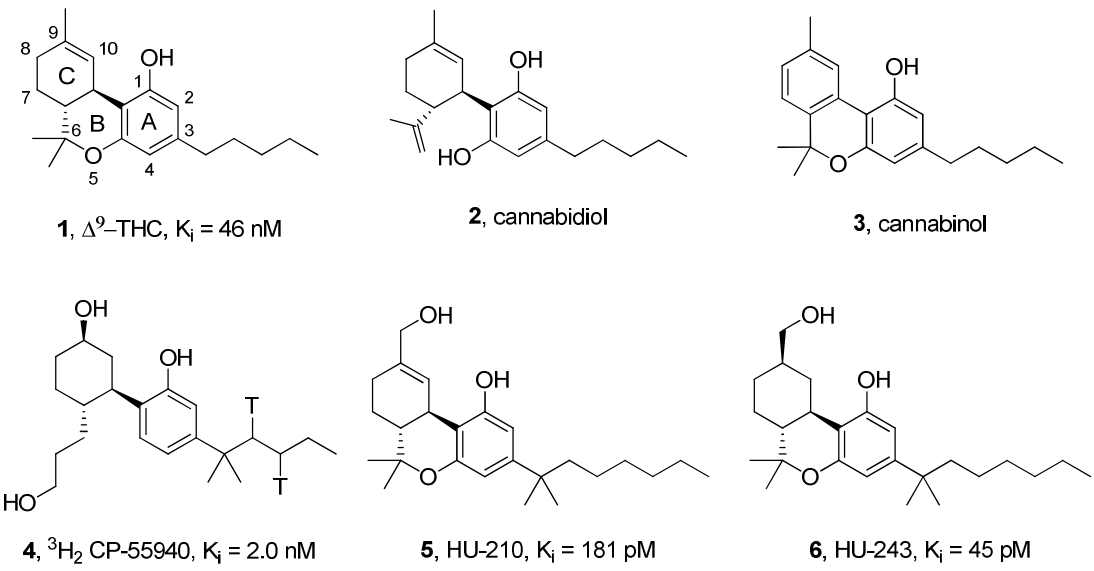


Figure 1. Structures and CB_1-affinities[30] of some natural (**1-3**) and synthetic (**4-6**) cannabinoids.

CB_1

CB_1 has been cloned from rat[20] (cerebral cortex cDNA library) and from human[26] (brain stem cDNA library). CB_1, constituting of 473 amino acids, is mainly expressed in the central nervous system and, to a much lower extent, in several peripheral tissues such as adrenal gland, heart, lung, prostate, uterus, ovary, testis, bone marrow, thymus and tonsils.[27,28]

Several enantiopure cannabinoid derivatives have been synthesized and studied as CB_1 agonists. Some of the most important are shown in **Figure 1**. Addition of a hydroxy group and changing the alkyl chain from pentyl to the slightly more lipophilic dimethylheptyl resulted in very active compound HU-210[29] (**5**, **Figure 1**). The hydrogenated derivative HU-243 (**6**, **Figure 1**) was found to be four times more active than HU-210, which in turn is already 250-fold more active than THC in terms of binding to CB_1.[30]

CB_2

Existence of peripheral effects of cannabis together with discovery of CB_1- receptor led to the cloning (cDNA of human promyelotic leukaemic line HL60) and identification of the second cannabinoid receptor, called CB_2, by Munro *et al.*[21] CB_2 (360 amino acids) is predominantly located in peripheral tissues and cells involved

in immune system like spleen, tonsils, mast cells, B-cells, macrophages and killer cells.[27,31,32] However, later it has been reported that CB_2 receptors are expressed also in the central nervous system.[33]

CB_1 and CB_2 receptor activation

The general role of CB_1 and CB_2 is to regulate the release of chemical messengers. The activation of these receptors is coupled with several signal transduction pathways via the $G_{i/o}$ family of G-protein coupled receptor superfamily. These pathways include negative coupling to adenylyl cyclase and activation of focal adhesion kinase, mitogen activated protein kinase and phosphatidylinositol-3-kinase. CB_1 is also coupled to ion channels regulating Ca^{2+} and K^+ -release through membranes. To summarize, the actions resulting from CB_1 activation and cannabinoid administration in general are ubiquitous and complex because of the wide distribution of cannabinoid receptors in the body.[34]

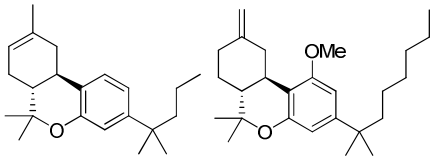
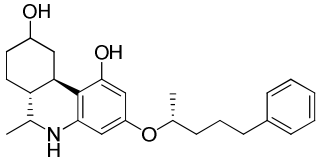
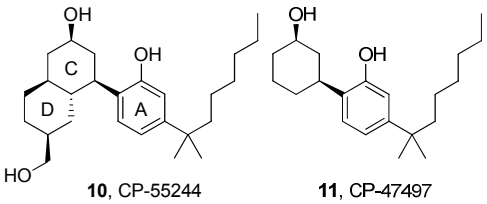
Other cannabinoid receptors

Most of the cannabinoid-induced physiological effects can be explained to occur by binding to CB_1 or CB_2. However, there are examples where these receptors do not explain observed pharmacology. This, together with studies using mice genetically deleted for the known receptors, has confirmed the hypothesis of additional receptors.[35] So far, the only identified receptor candidate responsible of these non-CB_1/CB_2 cannabinoid ligand effects is an orphan G-protein coupled receptor GPR55.[36,37]

2.2. Cannabinoid ligands

The most studied and/or effective ligands binding to cannabinoid receptors can be divided in five main groups (**Table 1**): Classical cannabinoids (e.g. **1-3**, **Figure 1**), non-classical cannabinoids, aminoalkylindoles, eicosanoids, and diarylpyrazoles. These groups can be further subdivided as agonists (first four groups) and antagonists/inverse agonists. [34] In addition, several other potent CB_1/CB_2 binding compounds that do not fit into these categories are presented in the literature.[25,38]

Table 1. Five groups of typical ligands that bind to cannabinoid receptors.[34,39,40]

<table>
<tr>
<td>Classical cannabinoids
Δ^9-THC (1), Δ^8-THC, HU-210 (5)
 
7, JWH-133
CB_1 selective
8, L-759656
CB_1 selective
 
9, Desacetyl-L-nantradol</td>
<td>Non-classical cannabinoids
CP-55940 (4)
 
10, CP-55244
11, CP-47497</td>
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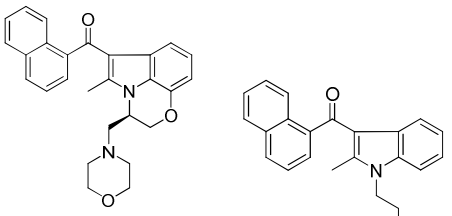
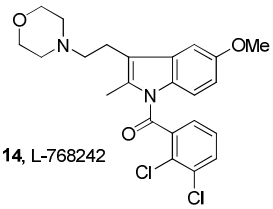
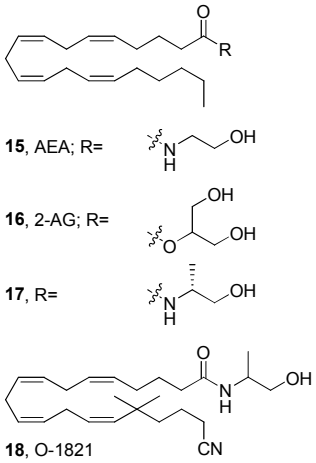
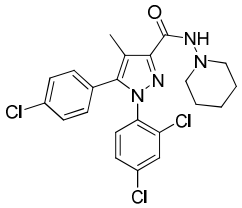
<td>Aminoalkylindoles
 
12, R-(+)-WIN55212
13, JWH-015
 
14, L-768242</td>
<td>Eicosanoids
 
15, AEA; R=
16, 2-AG; R=
17, R=
18, O-1821</td>
</tr>
<tr>
<td>Diarylpyrazoles
 
19, SR141719A
CB_1 antagonist
Rimonabant</td>
<td></td>
</tr>
</table>

2.3. Endocannabinoids

The identification of CB_1 launched the search for specific endogenous ligands (endocannabinoids). The first endocannabinoid, *N*-arachidonoylethanolamide (**15**, AEA) was identified and isolated from porcine brain.[4] It is also called anandamide after the Sanskrit word *ananda* for "bliss".[25] Search for endogenous agonists for the periferal CB_2 led to the isolation of the second known endocannabinoid, 2-arachidonoyl glycerol (**16**, 2-AG)[5]. At the same time, 2-AG was also isolated from rat brain.[6] Other reported endocannabinoids include *O*-arachidonoylethanolamide (virodhamine, **20**)[41], *N*-arachidonoyl-dopamine (NADA, **21**)[42,43], 2-arachidonyl glyceryl ether (noladinether, **22**)[44], homo-γ-linoleoylethanolamide (**23**) and 7,10,13,16-docosatetraenoylethanolamide (**24**)[45]. The physiological role of these endocannabinoids **20-24** has not been thoroughly evaluated, and for example the existence of noladin ether (**22**) as endogenous compound in mammalian brain tissue has been questioned.[46]

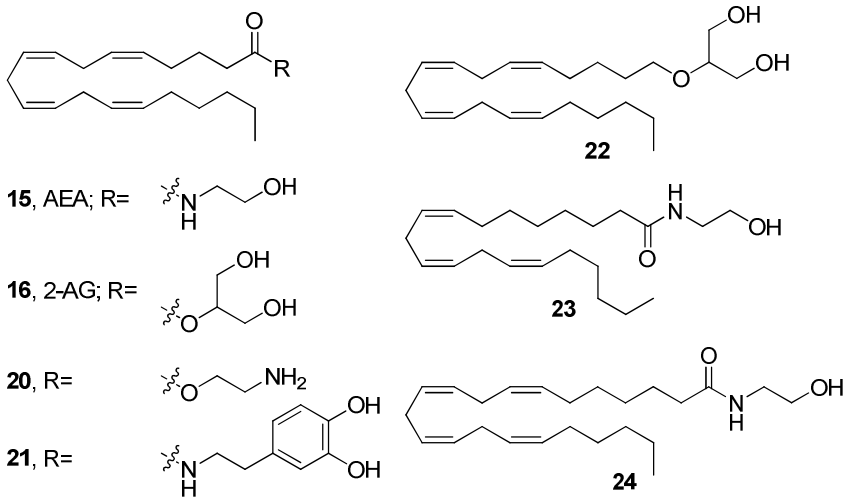


Figure 2. The structures of known endocannabinoids.

2-AG and AEA are no doubt the most important endocannabinoids due to their abundance, activity and selectivity.[3,47,48] 2-AG is more efficacious *in vitro* than AEA, latter being in activity in the same level as Δ^9-THC. Hence, AEA is

considered to be a partial agonist and 2-AG a full agonist of both CB_1 and CB_2.[49,50] In addition, concentrations of 2-AG in brain tissues are higher than those of AEA. The concentration differences vary between the reports from 170-fold to 2-fold in favor to 2-AG. The extracellular concentrations are close to equivalent (2-5-fold in favor to 2-AG), which is probably closer to the truth when comparing the active concentrations of these endocannabinoids.[51,52] In addition, it has been reported that 2-AG is rapidly generated in rat brain after decapitation, which obviously has had influence on the findings.[53]

Endocannabinoids AEA and 2-AG have also other targets in addition to extracellular CB-receptors. Both are reported to act as agonists of nuclear peroxisome proliferator-activated receptor (PPAR) family members PPARα and PPARγ. AEA is also an endogenous agonist for the vanilloid receptor channel TRPV1.[54,55]

2.3.1. Anandamide (AEA)

Shortly after the discovery of AEA, its biosynthesis was also described.[56,57] AEA is generated from membrane phospholipids by a two-step biosynthesis (**Figure 3**). In fact, all *N*-acylamides are synthesized in a similar way in animal tissues. First, the acyl chain is transferred from a glycerophospholipid, e.g. *N*-phosphatidylcholine (**25**, **Figure 4**), to the amino group of phosphaditylethanolamine (PE, **26**) by *N*-acyltransferase. The *N*-arachidonylphosphatidylethanolamine (NArPE, **27**) formed in the reaction is then hydrolyzed to AEA by a selective phospholipase D (NAPE-PLD).[58]

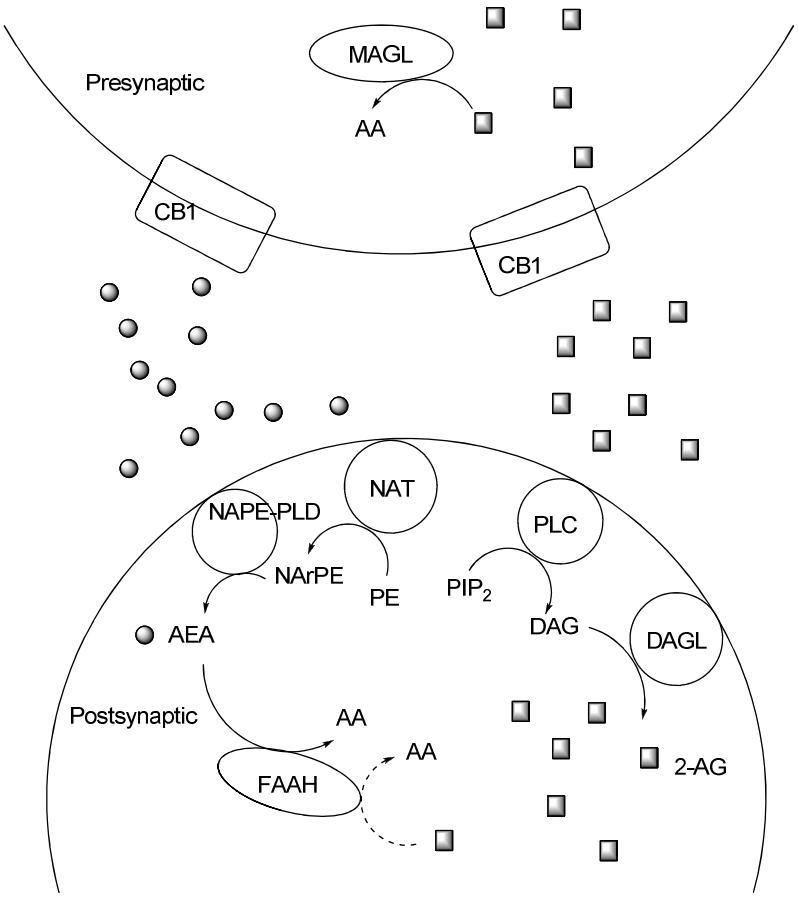
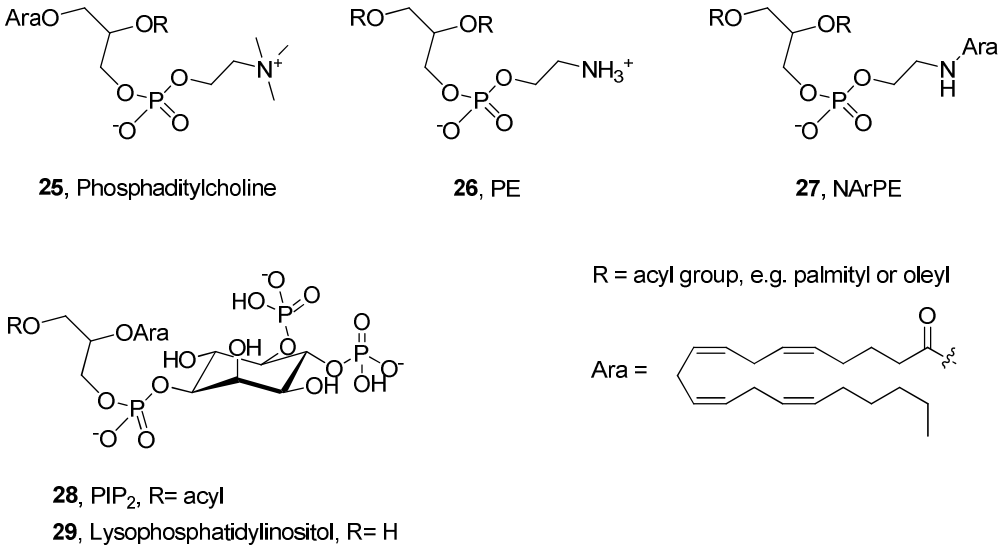


Figure 3. Life cycle of AEA and 2-AG. *N*-acyltransferase (NAT) is also known as calcium-dependent transacylase (CDTA). Other abbreviations: PE, phosphatidylethanolamine, NArPE, *N*-arachidonylphosphatidylethanolamine; NAPE-PLD, *N*-acylphosphatidylethanolamine-selective phospholipase D; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C, DAG, diacylglycerol; DAGL, diacylglycerol lipase. Dashed line for hydrolysis of 2-AG to arachidonic acid (AA) by FAAH designates minor route of hydrolysis. Major hydrolyzing enzyme for 2-AG is MGL.

The production of AEA takes place in postsynaptic neurons in response to a stimulus, e.g. elevation of intracellular Ca^{2+}-levels. Thus, AEA is not stored but synthesized "on demand".[47] AEA is simultaneously released to extracellular space where its binding sites (CB-receptors) are located. AEA is then rapidly removed from its site of action by cellular reuptake, followed by intracellular hydrolysis by fatty acid amide hydrolase (FAAH), an enzyme localized on the endoplasmic reticulum. Reuptake is thought to be carried out by either passive diffusion[59],

facilitated diffusion[60], endocytosis,[61] or by transport protein[62,63]. The existence of these different theories[64] inherits from the fact that as a very lipophilic molecule, AEA is most likely able to diffuse through membranes readily without aiding mechanisms. In addition, some inhibitors of the yet unidentified transport protein have been reported to inhibit FAAH as well.[65,66,67] The question should rather be that how does such a lipophilic molecule as AEA (or 2-AG) travel through an aqueous medium from the membrane to the site of catabolism. Indeed, in recent report it was suggested that a chaperon mechanism could be involved. It was found that overexpression of two known lipid carrier proteins FABP5 and FABP7 enhanced the hydrolysis of FAAH.[68] It will not come as surprise if inhibition studies against these chaperon-proteins are reported in near future.



25, Phosphaditylcholine

26, PE

27, NArPE

28, PIP_2, R= acyl

29, Lysophosphatidylinositol, R= H

Figure 4. Structures of the main intermediates of AEA and 2-AG biosynthesis.

2.3.2. 2-Arachidonoylglycerol (2-AG)

Biosynthesis of 2-AG (as AEA) is initiated by Ca^{2+} flux into the postsynaptic cell. The predominant routes involve the usage of arachidonic acid-enriched membrane phospholipid such as phosphatidylinositol-4,5-bisphosphonate (PIP_2, **28**, **Figure 4**) as starting material. PIP_2 is dephosphorylated by phospholipase C (PLC), forming diacylglycerol (DAG), which is then hydrolyzed to 2-AG by *sn*-1 selective diacylglyceride lipase. The alternative route is mediated by phospholipase A which

cleaves the 1-acyl from PIP_2, resulting in lysophosphatidylinositol (**29**), which in turn is transformed to 2-AG by PLC.[69] The cellular release and uptake of the 2-AG is suggested to occur via same mechanisms as with AEA; the cellular accumulation of 2-AG was prevented by AEA in human astrocytoma cells.[10] By contrast, the corresponding inhibition was not detected in macrophages.[70] The main hydrolyzing enzyme for 2-AG is MGL, which is responsible for ~80% of overall 2-AG hydrolysis.[11,71] The remaining 2-AG hydrolyzing activity is attributed to FAAH along with some other lipases and esterases. In a recent study, 98% of 2-AG hydrolysis in brain was accounted for: MGL hydrolyzed 85%, and two previously uncharacterized enzymes, α/β-hydrolases ABHD6 (4%) and ABHD12 (9%) were responsible for 13% of 2-AG hydrolyzing activity.[72]

2.4. Hydrolyzing enzymes

2.4.1. FAAH

N-Acylethanolamide-specific membrane-associated enzyme activity in rat liver microsomes and mitochondria was described already in 1985, indicating the occurrence of NAEs as natural cell constituents.[73] Later, an enzyme activity converting AEA to arachidonic acid was characterized from cell membrane fractions of rat brain tissue.[74,75] Around the same time oleamide, *cis*-9-octadeceneamide (**30**, **Figure 5**), was described as a sleep-inducing substance. Oleamide was suggested to be hydrolyzed by the same enzyme as NAEs.[76,77] Indeed, shortly afterwards Cravatt and colleagues succeeded in purifying this hydrolase activity to near homogeneity from rat liver membranes, using a column covalently modified with an oleoyl trifluoromethyl ketone inhibitor derivative.[7] This enzyme was named fatty acid amide hydrolase (FAAH), since it was found to hydrolyze numerous fatty acid amides, including anandamide and oleamide, subsequently proven via cloning and transfection of the corresponding cDNA.[7,9] Substrate specificity and selectivity studies have been conducted, revealing that FAAH hydrolyzes primary amides more rapidly than ethanolamides.[78]

Increase of acyl chain saturation decreases the rate of hydrolysis, possibly due to requirement of a hairpin conformation for substrate recognition at the active site.[79] This hairpin conformation is most preferred by four non-conjugated *cis* double

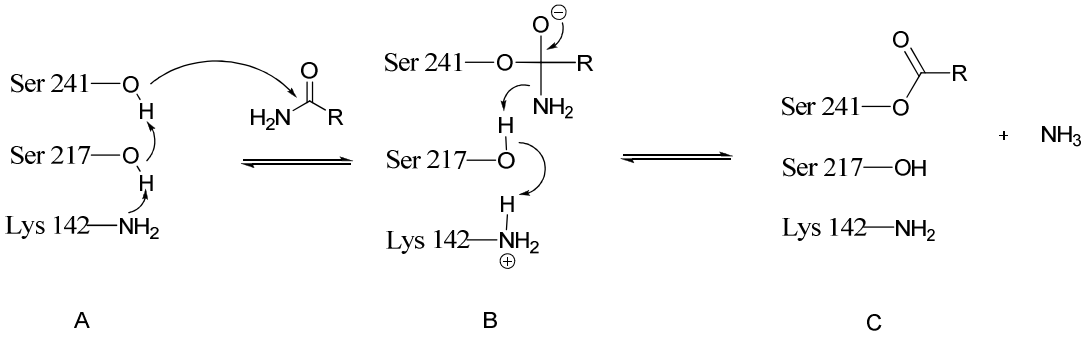
bonds as in arachidonyl carbon skeleton.[80] AEA-analogues containing a tertiary amide nitrogen are poor enzyme substrates. Increased steric hinderance around the carbonyl also decreases the rate of hydrolysis. In addition, substrates containing H-bonding and/or electronegative headgroup substituents or atoms, such as hydroxyl or fluoro, are in turn more prone to hydrolysis. Interestingly, the stereoselectivity with methylanandamide substrates is opposite to that observed for the CB_1 receptor: the enantiomer with the lower enzyme turnover shows higher receptor affinity.[79]

Human, mouse, and porcine FAAH genes have since been characterized and shown to be highly conserved in their primary structure. Both human and rat FAAH are expressed broadly and at high levels in the nervous system, where the enzyme is localized predominantly to intracellular membranes (e.g., smooth endoplasmic reticulum, outer membrane of the mitochondria) in the somatodendritic compartment of neurons.[52] The active pH range of FAAH is 7.0-9.5, the enzyme being most active at pH 9.[81]

FAAH is comprised of 579 amino acids and it was characterized by sequence analysis as the first mammalian member of amidase signature (AS) enzyme family, containing over 100 mainly fungal and bacterial enzymes. AS enzymes share a characteristic feature of having a highly conserved region that is rich in serine, glycine, and alanine residues.[52] In order to gain sufficient amounts of pure enzyme for further mechanistic and configurational studies, a bacterial expression system was developed for the rat enzyme. Both wild-type FAAH and transmembrane domain-deleted FAAH (ΔTM-FAAH) were expressed in *E. coli*. Unlike most AS enzymes, FAAH is an integral membrane protein, having a transmembrane domain of first 29 amino acids. However, deletion of these amino acids (ΔTM-FAAH) resulted in an enzyme that had the same catalytic activity and binding property to membranes as wild type FAAH. These results indicate that FAAH possesses multiple domains for membrane association and is capable of being active in other than its natural configuration. Further comparison of wild type and ΔTM-FAAH showed that the latter had smaller solution size.[82] Recombinant human FAAH has also been expressed using baculovirus-insect cell and *E. coli* systems.[52]

In 2002 the X-ray crystalline structure of ΔTM-FAAH was determined in 2.8 Å resolution opening a new era in FAAH study.[83] The results revealed FAAH to be a

dimeric enzyme. The enzyme was treated with the irreversible inhibitor methoxy arachidonyl fluorophosphonate (MAFP) prior to crystallization. MAP was bound by covalent phosphorus-oxygen bond to Ser241 in FAAH active site. Ser241 forms an unusual Ser-Ser-Lys catalytic triad with Ser217 and Lys142 (**Scheme 1**).[84,85] The same catalytic triad was found from two bacterial AS-family enzymes malonamidase E2 (MAE2)[86] and peptide amidase (PAM)[87] by X-ray crystal structures published around the same time as that of FAAH.



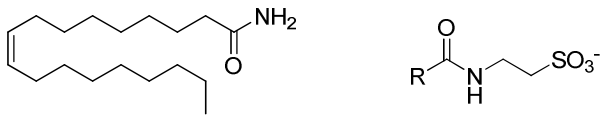
Scheme 1. Proposed mechanism for the FAAH active site acylation step in the amide hydrolysis.[85]

Serine hydrolases typically have a serine-histidine-aspartic acid catalytic triad, where the nucleophilicity of the serine is based on histidine's imidazole group functioning as a proton shuttle between anionic aspartate and serine -OH.[88] The unique Ser-Ser-Lys catalytic triad of FAAH seems to be the basis of its ability to hydrolyze amides in the presence of otherwise similar esters. FAAH has been found to hydrolyze both oleamide and oleoyl methyl ester at an equal rate, which is not typical for serine hydrolases. They normally prefer to hydrolyze esters much faster than amides.[85] FAAH also hydrolyzes the second endocannabinoid 2-AG at a similar rate as AEA.[89]

The FAAH structure contains channels that allow the enzyme to access both the membrane and cytosolic compartments of the cell. One of these channels is called the acyl chain-binding (ACB) channel leading from the membrane-binding surface of the protein to the enzyme active site. ACB channel is comprised almost entirely of hydrophobic residues and is thought to participate in substrate recognition.[90] Another channel emerges from the active site at an angle of approximately 80° from the substrate-binding cavity to create a solvent-exposed "cytoplasmic port". The

FAAH structure suggests a model where fatty acid amide substrates gain access to the active site by first entering into the membrane. After hydrolysis, the fatty acid would exit the enzyme via ACB channel to membrane. Hydrophilic amine products would be excreted directly to cytosolic space via the cytoplasmic port (also called cytoplasmic access (CA) channel).[83] The cytoplasmic port may also play a role by providing an entry for the molecular water required in the hydrolysis.[52]

In addition to *N*-acyl ethanolamides, FAAH is suggested to hydrolyze *N*-acyl taurines (NAT, **31**) which activate the transient receptor potential (TRP) family of calcium channels.[91]



30, Oleamide **31**, NAT

Figure 5. Structures of oleamide and NAT. Acyl (RCO) in most abundant compounds is C18:1 (oleoyl), C18:2 (linoleoyl), C20:4 (arachidonoyl), C22:6 (docosahaexenoyl).

In order to further study the pharmacology of FAAH, gene depletion was used and FAAH knockout mice were developed. These FAAH(-/-) mice have 15-fold higher brain levels of anandamide than wild type mice.[92] In addition, FAAH(-/-) mice exhibit a CB_1 receptor mediated analgesic phenotype in a variety of acute and inflammatory pain models.[93]

2.4.2. MGL

Several findings indicated that FAAH is not the enzyme responsible for most of the 2-AG degradation. The rate of monoacylglycerol hydrolysis in brain extracts is nearly two orders of magnitude higher than the rate of hydrolysis of NAEs.[94] In addition, porcine brain fractions possessing hydrolysis activities of 2-AG and anandamide were separated chromatographically.[95] Finally, the inactivation of FAAH did not change the endogenous levels or hydrolysis of 2-AG and other monoacylglycerides in brain tissue, similar results having been achieved with both FAAH (-/-) mice and pharmacological inactivation.[52]

Monoglyceride lipase (MGL; EC 3.1.1.23, also called monoacylglycerol lipase) was originally purified from rat adipose tissue. This enzyme was found to hydrolyze both 1- and 2- isomers of monoacylglycerol at equal rates.[96] MGL catalyzes the last step in the hydrolysis of stored triglycerides in the adipocyte, and presumably also complements the action of lipoprotein lipase in degrading triglycerides from chylomicrons and very low density lipoproteins. Monoglyceride lipase was cloned from a mouse adipocyte cDNA library. The predicted amino acid sequence consisted of 302 amino acids, corresponding to a molecular weight of 33.218 kDa. The sequence showed no homology to other known mammalian proteins, but a number of microbial proteins, including two bacterial lysophospholipases and a family of haloperoxidases, were found to be distantly related to this enzyme. The structural elements, as well as the putative catalytic triad of MGL, were identified by multiple sequence alignment and secondary structure prediction. The residues of the proposed triad, Ser122, Asp239, and His269, were confirmed by site-directed mutagenesis experiments. This is a typical triad for serine hydrolases.[97] MGL is ubiquitously expressed among tissues, including the brain where it is located in presynaptic terminals of neurons acting as the major (>80%) degrading enzyme of 2-AG.[71] MGL is capable of hydrolyzing other long chain glyceryl esters as well, still having the fastest rate for hydrolysis of arachidonoyl glycerols.[98,99] The crystallization of MGL has not been reported to date.

2.4.3. FAAH-2 and NAAA

FAAH-2

During activity based protein profiling (ABPP) studies on a human cancer cell lines, a second AS family enzyme with fatty acid amide hydrolyzing activity was discovered.[100] This enzyme, named FAAH-2, has a transmembrane domain and a serine-serine-lysine catalytic triad like FAAH, despite that these enzymes share only 20% sequence homology. FAAH-2 exhibits different features in regard to its substrate specificity and tissue distribution compared with those of FAAH. FAAH-2 hydrolyzes primary fatty acid amide substrates such as oleamide at equivalent rates as FAAH, but FAAH hydrolyzes NAEs, including anandamide, significantly more efficiently than FAAH-2. Tissue distributions for human are also different: FAAH-2

appears at higher relative levels in peripheral tissues (e.g., heart, ovary) in contrast to FAAH showing robust expression in the nervous system. The FAAH-2 gene has not been found in a number of lower placental mammals including mouse and rat. Interestingly, both enzymes are inhibited by selective FAAH inhibitors *α*-ketoheterocycles (OL-135) and *O*-aryl carbamates (URB597) (for structures see chapter 3.1.2 and 3.1.3). The discovery of a second FAAH enzyme selectively present in higher mammals suggests that fatty acid amide metabolism may differ between humans and rodents.[100]

NAAA

Fully saturated *N*-palmitoylethanolamine (PEA) is an anti-inflammatory and immunosuppressive endogenous substance which is not efficiently degraded by FAAH. In year 2001 Ueda *et al.* reported the discovery of an amidase that was more active in hydrolyzing PEA than AEA in contrast to FAAH. The enzyme with a molecular mass of 31 kDa was purified from rat lung, where it was also found to possess the highest specific enzyme activity. Relative reactivities of the enzyme with various *N*-acylethanolamines (100 μM) were as follows (acyl skeletons in parentheses): *N*-palmitoylethanolamine (Acyl: C16:0) > *N*-myristoylethanolamine (C14:0) > *N*-stearoylethanolamine (C18:0) = *N*-oleoylethanolamine (C18:1(*cis*-9)) > *N*-linoleoylethanolamine (C18:2(*all cis*-9, 12)) > AEA (C20:4(*all cis*-4, 8, 11, 14)).[101] This NAE hydrolase was cloned, characterized, and termed as *N*-acylethanolamine-hydrolyzing acid amidase (NAAA), reflecting its acidic pH optimum of pH 5. High sequence similarity between NAAA and acid ceramidase was detected in the lysate when human NAAA cDNA was transfected into HEK293 cells. NAAA is not a serine hydrolase and is therefore much less sensitive to general serine hydrolase inhibitors such as MAFP. NAAA is completely inhibited by 10 μM *p*-chloromercuribenzoic acid, a thiol-blocking reagent.[102] In human, NAAA shows highest relative expression in peripheral tissues like prostate, leukocyte, liver, spleen, kidney and pancreas.[103] The high expression of the NAAA in macrophages and certain peripheral tissues indicates to a potential role of this enzyme in fatty acid amide hydrolysis *in vivo* at these sites.[52]

3. ENZYME INHIBITORS

By inhibiting the main endocannabinoid hydrolyzing enzymes FAAH or MGL, the effect of endocannabinoids could be enhanced and more selective therapeutic effects achieved.[38,104,105] Thus the inhibition of these enzymes is of great interest to medicinal chemists nowadays.

3.1. FAAH inhibitors

FAAH inhibitors represent several classes of structures and functionalities but most of them fall in the two most studied compound groups: α-ketoheterocycles and *N*-alkyl carbamates.

3.1.1. Early FAAH inhibitors

First FAAH inhibitors were typical covalent non-selective serine hydrolase derived compounds (**Figure 6**). Albeit being selective, some of them have had a significant role as tools for pharmacological studies. Phenylmethylsulfonyl fluoride (**32**), is a well-known serine protease, and the first FAAH inhibitor described having IC_{50} value of 290 nM.[106] Combining the fatty acid moiety and the sulfonyl fluoride group resulted in more selective inhibitors: saturated acyl chains from laurylsulfonyl (C12, **33**, IC_{50} 3 nM) and stearylsulfonyl fluoride (C18, IC_{50} 4 nM) were equally potent, but a drop in activity was found with arachidylsulfonyl fluoride (C20, IC_{50} 48 nM).[107] Change to a substrate-like unsaturated arachidonyl skeleton gave even higher inhibition activity (**34**, IC_{50} 0.1 nM).[108] Methylarachidonyl fluorophosphonate (MAFP, **35**), already mentioned in the context of FAAH crystal studies[83], was found to inhibit FAAH with the IC_{50} value of 2.5 nM.[109] Series of analogues of MAFP with *n*-chain lengths varying from C8 to C20 and number of *cis*-double bonds from 0 to 4 have also been developed: the most potent of these was C12:0-compound methyldodecylfluorophosphonate (IC_{50} 1-3 nM).[110] In addition, an alternative polar functionality containing diazomethylarachidonyl ketone (**36**, IC_{50} 0.5-6 μM) was presented.[111,112] All these compounds have significant agonist affinity

for the CB_1 receptor, which limits their usefulness in studies of cannabinoid system. Compound **34**, for example, inhibits [^{3}H]-CP-55,940 binding to mouse CB_1 receptors with an IC_{50} value of 304 nM.[108] These results suggest that arachidonyl tail can be replaced from already potent FAAH inhibitor by saturated C12-*n*-chain without significant loss of inhibitory potency.

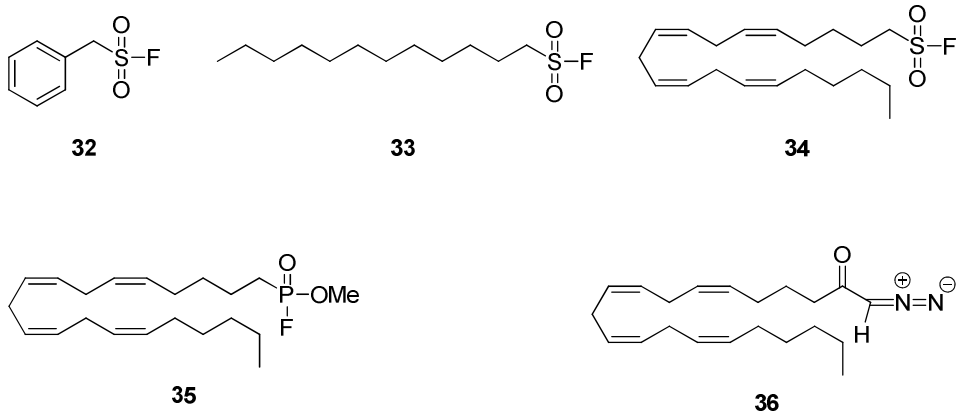


Figure 6. Structures of some early FAAH inhibitors derived from typical serine hydrolase inhibitors.

Due to their low selectivity towards serine hydrolases, compounds **32**-**36** were considered as pharmacological tools rather than potential *in vivo* substances. First truly designed FAAH inhibitors were based on the idea to use the activated carbonyl compounds as putative active site traps. Different long carbon chain possessing amides, α-ketoamides, α-ketoesters and trifluoroketones were tested against FAAH. It is noteworthy that these studies were conducted before the enzyme was fully characterized or the catalytic mechanism understood. To this end, trifluoromethyl ketone, α-ketoester, and α-ketoamide derivatives of arachidonic acid or similar fatty acids were made and tested for inhibition of AEA hydrolysis. Most potent of these were arachidonyl trifluoromethyl ketone **37** and ketoester **39** with a C18:0 chain, both inhibiting roughly 80% of FAAH activity in 100 μM concentration (**Figure 7**).[113]

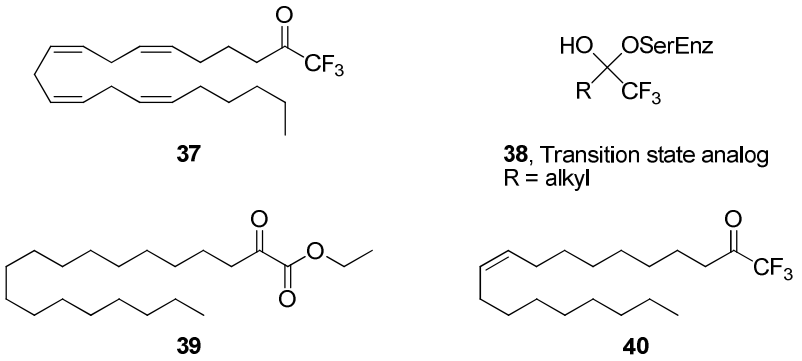


Figure 7. Structures of first designed FAAH inhibitors and the transition state analog (**38**) for which the design was based.

The Boger group studied oleamide hydrolyzing activity inhibitors and published a series of very similar compounds. Oleyl trifluoroketone **40** was the most potent in this study. Oleyl ketoester derivative of **39** was also found equipotent inhibitor of this activity.[114] Oleamide hydrolyzing enzyme had already been suggested to be hydrolyzed by the same enzyme as AEA,[77] and was indeed later revealed to be FAAH.

A logical approach to FAAH inhibition is the modification of substrates to create inhibitors. Early work in this area identified a number of FAAH inhibitors (**Figure 8**).[104]

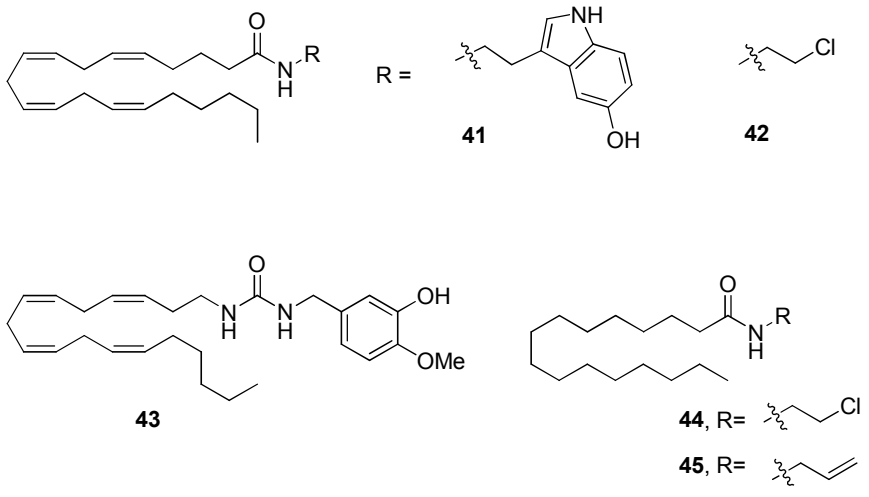
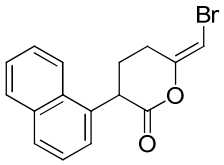


Figure 8. Examples of substrate inspired FAAH inhibitors.

Arachidonylserotonin **41** (AA5HT) was initially described as FAAH inhibitor[115] but it was later described to have antagonist properties towards transient receptor potential vanilloid-1 (TRPV1). Compound **41** was highly effective against both acute and chronic peripheral pain possibly due to its dual activity as a FAAH inhibitor and TRPV1 antagonist.[116] The manipulation of the ethanolamine portion of AEA resulted in useful early inhibitors of FAAH of which the most potent was the alkyl chloride derivative **42** (IC_{50} 0.9 μM). It was speculated that most of these analogues were probably substrates for FAAH.[117] In 2001 DiMarzo described the urea compound **43** (O-1987, IC_{50} 2 μM), found by preparing derivatives of arvanil.[118] The series of palmitoylamide analogues (e.g. **44** and **45**) similar to the one previously described with arachidonic acid amides were prepared and some weak inhibitors were identified.[119] In the *in vivo* evaluation **45** was shown to be effective in reversing thermal and tactile hypersensitivity in a number of neuropathic pain models in rats.[120]



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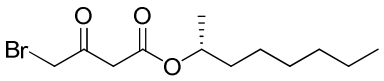

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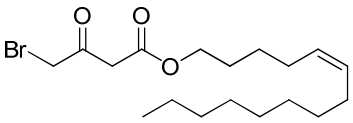

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Figure 9. Miscellaneous early FAAH inhibitors.

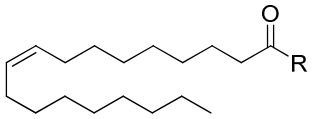
As an early study, some non-substrate like inhibitors were also prepared (**Figure 9**). (*E*)-6-(Bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one (**46**), which was reported to inhibit chymotrypsin-like proteases and cytosolic Ca^{2+}-independent phospholipase A_2, was found to inhibit FAAH with an IC_{50} value of 0.8 μM.[121] Another miscellaneous class early inhibitor was 2-octyl γ-bromoacetoacetate **47**, which was suggested as an endogenous sleep inducing substance, since it was isolated from human cerebrospinal fluid. A small series of derivatives of **47** was also developed, resulting in compound **48** as the most potent of this series. FAAH-IC_{50} for **47** and **48** were 2.6 and 0.6 μM respectively.[122]

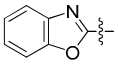
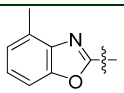
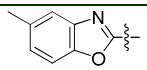
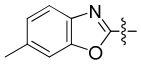
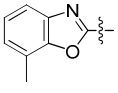
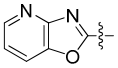
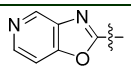
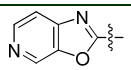
3.1.2. α-Ketoheterocycles

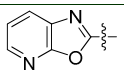
The α-ketoheterocycle functionality contains a moderately electrophilic ketone carbonyl possessing reactivity that is adjustable by small changes in the electron withdrawing properties of the heterocycle. It also provides a rigid structural template for introducing new interactions in the enzyme active-site close to the actual nucleophilic attack to the carbonyl. Potent inhibitors containing this group have been presented for at least for serine proteases (elastase, thrombin, factor X_a, tryptase, chymase), cysteine proteases (cathepsin K and cathepsin S), and hydrolases (FAAH).[123]

After introducing trifluoromethyl oleyl ketone (**40**) the Boger group replaced the trifluoromethyl group with various heterocycles identifying highly potent and selective FAAH inhibitors (**Table 2**).[124]

Table 2. Replacement of CF_3 by heterocycle.[124] N.a.; No affinity detected by 100 μM.

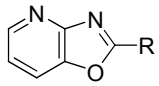


Compd	R	K_i (nM)	Compd	R	K_i (nM)
40	CF_3	82	**56**		370
49		17	**57**		N.a.
50		n.a.	**58**		N.a.
51		n.a.	**59**		N.a.
52		65	**60**		13 000
53		N.a.	**61**		2.3
54		N.a.	**62**		7.2
55a		110	**63**		3.7

55b		130	**64**		11

The most active ketones were the ones possessing a clearly electron withdrawing heterocycle. Oxazoles were found to be superior to thiazoles or imidazoles, and oxazole ketone **49** (17 nM) had a moderately improved activity over the trifluoromethyl ketone **40** (82 nM). Benzoxazole **56** (370 nM) was significantly weaker FAAH inhibitor than pyridyloxazoles **61**-**64** (2.3-11 nM). Albeit the fact that the position of the pyridyl nitrogen had only a slight effect to inhibitory activity, **61** (2.3 nM) was chosen as parent compound for next modifications focusing on alkyl tail. These results indicated that the pyridyl nitrogen is interacting with enzymes active site via hydrogen bonds or is enhancing the electrophilicity of the ketone, or both. The steric hindrance survey of benzoxazoles by compounds **56-60** suggests that oxazole-nitrogen serves as important hydrogen bond acceptor in active site.[124]

Table 3. Optimizing the alkyl chain of **61** (2.3 nM).[124]



Compd	R	K_i (nM)
61	C18:1 Δ^9, *cis* (oleoyl)	2.3
65	C18:1 Δ^9, *trans*	3.2
66	C20:4 (arachidonoyl)	1.0 (unstable)
67	C18:1 Δ^9, triple bond	0.14
68	C9:1 Δ^9, double bond	0.15
69	C18:0	11
70	C12:0	0.57
71	C8:0	0.69
72	C6:0	2.1
73	$Ph(CH_2)_3CO$	6.9
74	$Ph(CH_2)_5CO$	0.20
75, OL-92	$Ph(CH_2)_6CO$	0.28
76	$Ph(CH_2)_8CO$	0.50

Optimization of the alkyl chain of **61** revealed several important facts (**Table 3**). Compound **65**, the *trans*-analogue of **61**, was as active as parent compound **61**, and **66** bearing arachidonyl tail was slightly more active than its oleyl derivative **61**, but not stable enough to be considered as a lead compound for further studies. The triple bond oleoyl analogue **67** and terminal double bond compound **68** were the most active in these series (140 and 150 pM). The authors commented compounds **67** and **68** very briefly, and derivatives of these moieties have not been reported since. By adding the screening of saturated acyls and acylphenyls (**69-76**) to this table it was concluded that the optimal lipophilic part contains a saturated acyl chain with length of 8-12 carbons or an alkyl phenyl with 5-8 carbon long CH_2-bridge between the phenyl and ketone. Compound **75** is also known as OL-92, and it is one of the most potent and selective FAAH inhibitors reported to date (IC_{50} 0.3 nM[125]).[124] Unfortunately, in animal studies this compound failed to demonstrate FAAH inhibition or analgesia *in vivo*, possibly because of poor pharmacokinetics.[126] The importance of the carbonyl group was further verified by preparing non-ketone analogues of **61**: oxime-, methyloxime-, alcohol-, CH_2-, and dimethylhydrazone derivatives were found to be at least 200-fold less effective inhibitors than the parent ketone **61**. In addition, a methyl substituent in α-position (racemic) of ketone **61** resulted in 4-fold decrease of the inhibition activity.[127]

After these results Boger continued the optimization of α-ketoheterocycles with oxazole as the heterocycle and $(CH_2)_6Ph$ as the alkyl group from parent compounds **49** and **75**. A large number of compounds with varying 5-substituents in 2-oxazolyl ring were prepared, resulting in compound OL-135 (**77**, **Figure 10**), which is one of the best characterized FAAH inhibitor so far.[104,125,128]

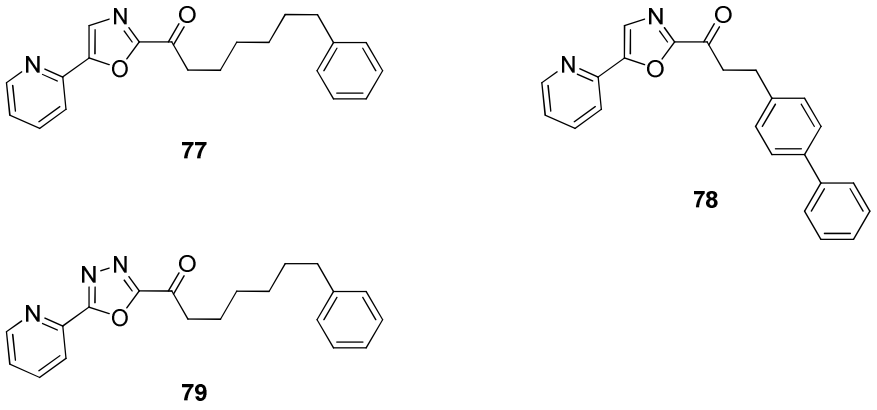


Figure 10. Some of the most potent and selective α-ketoheterocycle FAAH-inhibitors.

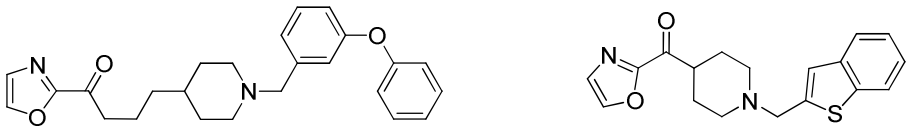
Compound **77** inhibited FAAH with an IC_{50} value of 2.0 nM and was 300:1 selective towards heart TGH enzyme, which is a typical competing target of α-ketoheterocycles. For comparison, trifluoromethyl oleyl ketone **40** had no selectivity and OL-92 (**75**) had 10:1 selectivity towards TGH.[125] Some improvement in terms of selectivity towards serine hydrolases TGH and KIAA1363[129] was gained by compounds **78** (IC_{50} 0.7 nM) and **79** (IC_{50} 1.0 nM). The selectivities towards TGH were 1700:1 for **78** and 140:1 for **79**.[130,131] Extensive proteomic analysis has demonstrated that **77** is highly selective for FAAH. Compound **77** was found to increase endogenous endocannabinoid levels in the brain and spinal cord of mice and to produce significant analgesic effects in tail immersion, hot plate, and formalin tests in mice, without the associated motor impairment typical of CB_1 agonism.[126] Subsequently **77** was found to be effective in both mild thermal injury induced tactile allodynia (acute tissue injury) and spinal nerve ligation (neuropathic pain) models in rats.[132]

The substituent effect of the ketone carbonyl and its correlation to the inhibitory potency have also been studied. Clear correlation was demonstrated between the Hammett σ_p constant and FAAH inhibition in simple substituted oxazoles.[133,134]

Extensive SAR studies have been reported, covering more or less the chemical space around the core molecule OL-135, compound **77**.[135] Latest reports have been focused on modifications of the central heterocycle (oxazole in **77**) such as screening the various heterocycles and exploring the effect of 4-substituents of the oxazole.[136,137] Heterocycle modifications resulted in a large amount of new SAR

data and some improvement in selectivity.[136] 4-Substituted derivatives of **77** were found to be practically equally potent and selective as corresponding 5-substituted, which indicates that compounds do flipping in the active site to fit in.[137]

In order to decrease the lipophilicity and to create novel structures, two patent applications have been published[138,139] applying propylpiperidine- and piperidine moieties instead of alkylphenyl chain with 2-carbonyloxazole in FAAH inhibitors. The most potent of these compounds **80** and **81** inhibited FAAH with IC_{50} values of 0.4 nM and 2.0 nM respectively (**Figure 11**).



80 **81**

Figure 11. Examples of compounds having piperidine containing tails.

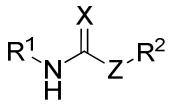
In conclusion, the activated ketone series of FAAH inhibitors has been widely explored and valuable compounds and essential SAR data has been revealed. However, the pharmacokinetics of this type of molecules are still under investigation.[140] This will be an important factor in evaluating their suitability as clinical candidates.[104]

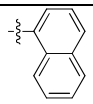
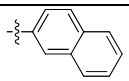
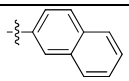
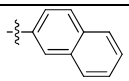
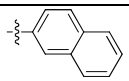
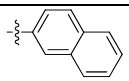
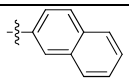
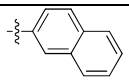
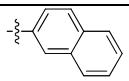
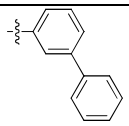
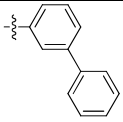
3.1.3. Carbamate- and urea based irreversible FAAH inhibitors

Reactive carbamates

Second large class of traditional FAAH inhibitors consists of carbamate compounds. The development of carbamate-based FAAH inhibitors started by preparing the derivatives of known AChE inhibitor **82** (**Table 4**).[141]

Table 4. Examples of the first carbamate-based FAAH inhibitors. [a]No inhibition detected by 30 μM.



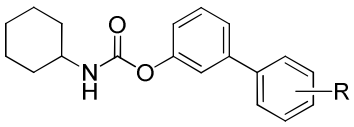
Compd	R^1	X	Z	R^2	IC_{50} (nM)
82	CH_3	O	O		n.a.[a]
83	CH_3	O	O		18290
84	*c*-hexyl	O	O		324
85	*c*-hexyl	O	CH_2		n.a.
86	*c*-hexyl	O	NH		n.a.
87	*c*-hexyl	O	S		15580
88	*c*-hexyl	S	NH		n.a.
89	*c*-hexyl	S	S		n.a.
90	*c*-hexyl	S	O		n.a.
91		O	O	*c*-hexyl	n.a.
92	*c*-hexyl	O	O		3776
93, URB524	*c*-hexyl	O	O		63

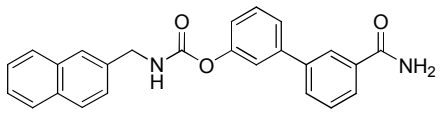
SAR and QSAR studies of the first carbamate compounds were also conducted.[141] Briefly, the AChE inhibitor (**82**) and its analogues did not show inhibitory activity except for a weak inhibitory activity by 2-naphthyl compound (**83**). Replacing the

methyl group of **83** with more lipophilic cyclohexyl resulted in a 60-fold decrease in IC_{50} (compound **84**). To examine the role of the carbamate function in FAAH inhibition, several bioisosters of **84** were prepared (**88**-**93**). Both the ester **85** and the urea **86** were ineffective and replacement of oxygen with sulfur (compounds **87-90**) also led to inactive analogues, with the partial exception of the thiocarbamic acid *S*-naphthyl ester **87**. Interestingly, also the "reversed" carbamate **91** appeared to be inactive.[142]

These results suggest that the carbamate group with *O*-aryl and *N*-alkyl is essential for inhibition of FAAH activity by this series of compounds. Less lipophilic *O*-phenyl moiety (compound **92**) decreased the activity significantly compared to 2-naphthyl. Derivatives of **84** were then prepared, containing two phenyls or phenyl and alkyl as lipophilicity-increasing groups, leading to biphenyl compound **93**, dubbed as URB524, which was found to be superior in this series.[141] URB524 was subsequently used as the parent compound in a study of the substituent effect on the distal phenyl group (**Table 5**).[16,143] The distal ring was selected for modifications since previous 3D-QSAR analyses suggested that this moiety was critical for achieving significant inhibitory potency.[141] Additionally, substitutions on the distal ring do not change the electrophilicity of the carbamate group directly via resonance. The activity differences raised by different substituents were then assumed to correlate directly with non-covalent binding interactions in the active site.[143]

Table 5. The effect of different *meta*- or *para*-substituents in the distant phenyl ring of URB524 (**93**, R = H, 63 nM).



Compd	R	IC_{50} (nM)	Compd	R	IC_{50} (nM)
94	4-CH_3	155	**103**	3-F	97
95	4-CF_3	1587	**104**	3-C_6H_5O	420
96	4-$C(O)NH_2$	5909	**105**	3-*n*-C_3H_7	110
97	4-NH_2	360	**106**	3-NO_2	50
98	4-F	65	**107**	3-SO_2NH_2	27
99	3-CH_3	62	**108**	3-$C(O)CH_3$	9.1
100	3-CF_3	146	**109**	3-CH_2OH	8.7
101, URB579	3-$C(O)NH_2$	4.6	**110**	3-CH_2NH_2	21177
102	3-NH_2	65	**111**	3-OH	8.7
112, IC_{50} 0.63 nM 					

These results clearly indicated that *meta* substitution in the distal ring was more favourable than *para* (**94-98** vs. **99-103**). The set of *meta*-substituents was then enlarged to contain total of 19 different compounds including hydrogen (**93**, URB524). QSAR model of this series was built, and the activity of the compounds was found to inversely correlate with the lipophilicity of the phenol. This was unexpected, since the initial hypothesis was that biphenyl group was mimicking the alkyl tail of the substrate and pointing towards the ACB-channel. These findings were in contrast with this, indicating that phenol points to a CA channel. Very soon this controversy was explained; Alexander and Cravatt proposed the mechanism where the *O*-aryl carbamate compounds are carbamoylating the active site Ser241 (**Figure 12**).[144] Less lipophilic biphenyls would then serve as better leaving groups in carbamoylation, thus explaining the correlation found. The phenol's role as a

leaving group also explains for the loss of the activity with "inversion" of carbamate functionality (**91** vs **93, Table 4**). The most well studied carbamate-based FAAH inhibitor, URB597 (**101**), was presented within this study. URB597 has been shown to display activity in rodent models of acute,[16] inflammatory,[145,146] and neuropathic pain,[147,148] as well as anxiety[16] and depression[142]. In addition, URB597 also enhances non-opioid, stress-induced analgesia.[149] The optimization of the *N*-alkyl moiety of the biphenyl compounds URB524 and URB597 resulted in the compound **112** with almost 10-fold improvement in IC_{50} value.[150]

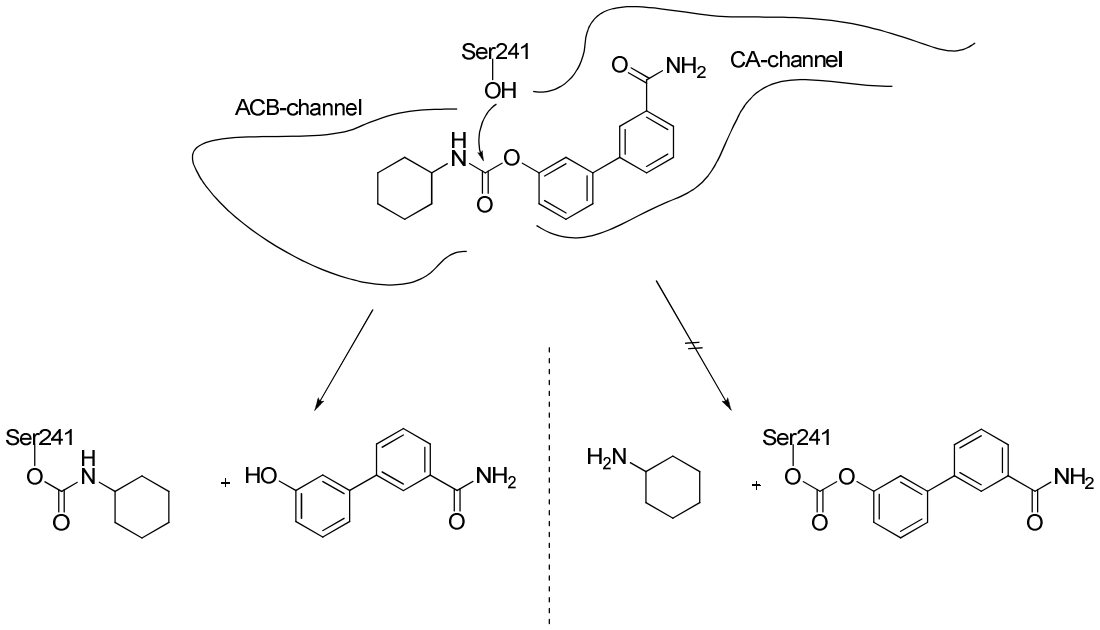


Figure 12. Ser241 carbamoylation by the carbamates.

The proposed Ser241 carbamoylation mechanism was judged by mass spectrometry studies and *in vivo* activity based protein profiling (ABPP). For this purpose four new carbamate-based inhibitors **113**-**116** (**Figure 13**), derived from URB597, were developed. Compound **113** (IC_{50} 58 nM) was found as potent as URB597 which inhibited FAAH with an IC_{50} value of 48 nM in this study. Compound **114** was less potent (190 nM) and **115** (14 nM) and **116** (7.3 nM) more potent than URB597.[144]

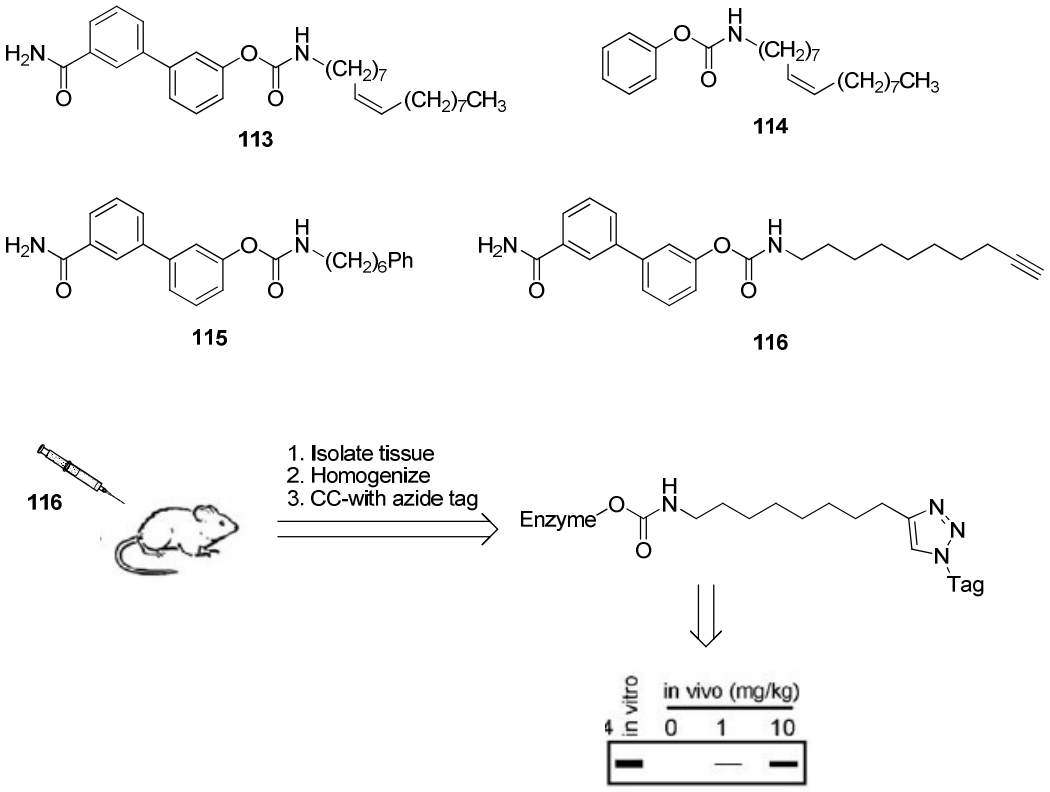


Figure 13. Compounds **113-116** and general method for characterizing the proteome reactivity of **116** *in vivo*. Mice were administered **116** at escalating doses (e.g., 0.25–10 mg/kg, i.p.). After 1 h, the animals were sacrificed, and their tissues were removed, homogenized, and reacted under click chemistry (CC) conditions with an azide modified rhodamine reporter tag (RhN_3). Labeled proteins were visualized in SDS-PAGE by in-gel fluorescence scanning.[144]

The research group in University of Kuopio, Finland, conducted a virtual screening of commercial compounds to a comparative model of the CB_2 receptor and human MGL enzyme, resulting in moderate hits for the original targets.[151,152] Group of these hits were also tested against FAAH revealing 11 compounds considered as FAAH inhibitors with IC_{50} values in the range of 44-0.52 µM. Compounds **117**-**120** are the most potent of these, of which **120** inhibited FAAH with an IC_{50} value of 0.52 µM (**Figure 14**).[152]

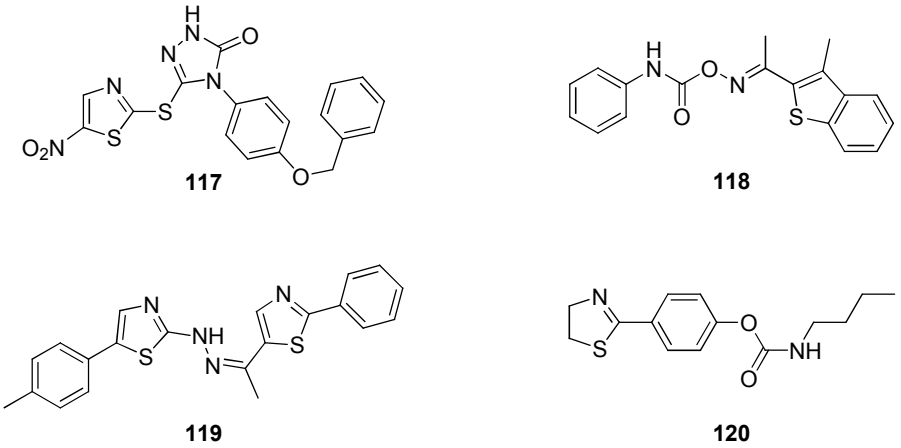


Figure 14. Some FAAH inhibitors found by virtual screen in University of Kuopio.

Compounds **117** and **118** (IC_{50} 4 µM, for both) were MGL hit molecules and compounds **119** (IC_{50} 0.69 µM) and **120** (IC_{50} 0.52 µM) CB_2 hits. All hit compounds were also evaluated as MGL inhibitors and **120** was found to inhibit MGL with an IC_{50} value of 31 µM.[152] Using **120** as a parent compound, a group of *para*-substituted phenyl ***N***-alkyl carbamates were prepared.[153] The results and SAR of these compounds will be presented more detailed in Chapter 4.

High-throughput screen on a targeted subset of Bristol-Myers Squibb's proprietary library revealed a class of bisarylazole derivatives as potent inhibitors FAAH. After the modifications made on lead **121** (IC_{50} 64 nM), the authors concluded with a carbamate derivative **122**, having the IC_{50} value of 2 nM (**Figure 15**).[154]

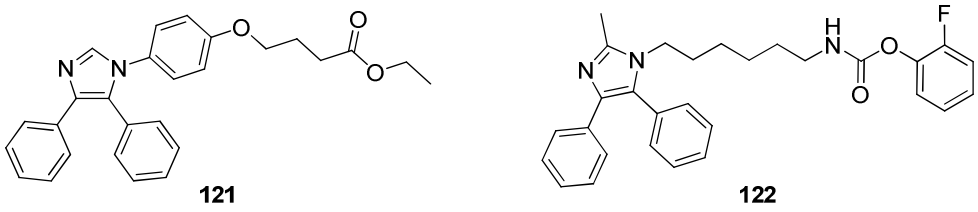


Figure 15. BMS hit **121** and carbamate **122** derived from it.

Reactive ureas

In addition to carbamates, several urea group containing compounds have been found to inhibit FAAH efficiently in terms of potency and selectivity. Thiadiazolopiperazinyl urea **123** (**Figure 16**) was found to be a potential FAAH-inhibitor (IC_{50} 34 nM) by high throughput screening of the chemical library of Johnson & Johnson Pharmaceuticals. Analogues of **123** revealed to be more potent than the parent compound, if the polarity of aromatic substituent of urea -NH was modified to be less lipophilic. This correlates to the mechanism, where the leaving group properties of aryl in urea or carbamate dominate the SAR. Cl- or F-atom in 2-position of the ureyl phenyl increased the potency about 5-fold, as well as replacing the phenyl to 3-pyridyl, 4-pyrimidyl or benzothiadiazolyl (**124**).[155] These compounds belong to the family of FAAH inhibitors which have also been reported in two patent applications.[156,157]

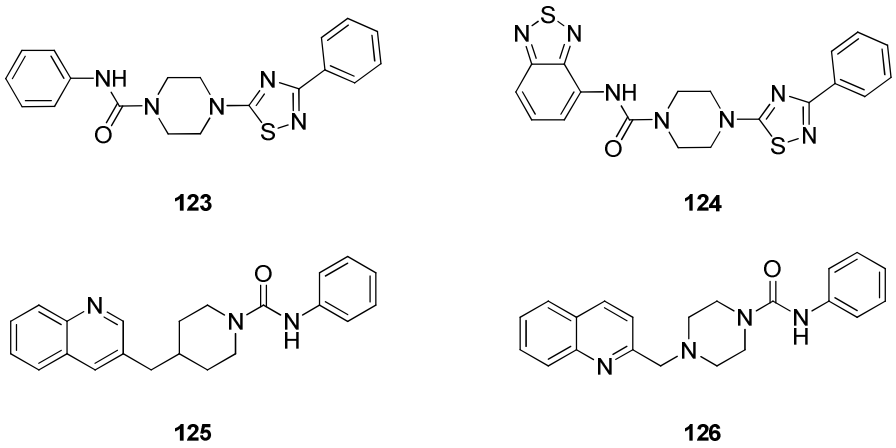


Figure 16. First piperidinyl- and piperazidinyl ureas as FAAH inhibitors.

In year 2007 Cravatt *et al*. reported very similar compounds as highly selective FAAH inhibitors (**125** and **126**, **Figure 16**), found by a high-throughput screen of Pfizer chemical library.[158] Piperidinyl urea PF-750 (**125**) and piperazidinyl urea PF-622 (**126**) were 7- and 3- times more potent FAAH inhibitors than URB597 with the incubation time of 60 min used. The authors showed by using four different preincubation times that this parameter has a relatively large influence on detected inhibitory activities with covalent irreversible inhibitors. Covalent bonding to FAAH

was proven by several methods, e.g. time-dependent inhibition and the rapid dilution studies. In addition, compounds **125** and **126** were tritium-labeled from different sides of the urea bond in order to see which part is attached to enzyme and which is excreted via the CA channel. Both radiometric study and MS-MALDI studies revealed that the anilinic moiety was not present in the enzyme/enzyme fraction after the assay. Prior to MS-MALDI, the sample was tryptase-cleaved to contain amino acid 213-214 fraction of the enzyme. The selectivity of PF-750 (**125**) against several other serine hydrolases was determined by competitive ABPP and MS studies. The selectivity of PF-750 was compared to those of several other FAAH inhibitors including URB-597 (**101**), OL-135 (**77**), and **74** (dubbed here CAY-10402), which were profiled at 100 μM. Consistent with previous reports of serine hydrolase targets for URB-597[159], OL-135[126], and CAY-10402[160], multiple off-targets for these inhibitors were observed. In contrast, no off-targets were observed for PF-750 (**125**) when tested at 500 μM. The remarkable specificity of the piperidine/piperazine urea inhibitors for FAAH may derive, at least in part, from the FAAH's ability to function as a C(O)-N bond hydrolase, which distinguishes it from the majority of metabolic serine hydrolases in mammals that are restricted to hydrolyzing esters or thioesters.[160]

To gain insight into the active-site features responsible for the high FAAH specifity of PF-750 (**125**), the Cravatt group determined the crystal structure of the h/rFAAH protein bound to **125**.[161] Not surprisingly, modifications on **125** were reported by the Cravatt group, resulting in improvements in potency and valuable SAR data by preparing 57 novel compounds. The first change in structure was replacing the 2- or 3-quinolyl moiety by 3-methylbenzothiophene and modifying the "right hand side" of the urea. The aromatic moiety was found to be crucial for activity, and altogether the structure was relatively sensitive to changes on this side of the molecule (compounds **127**-**130**, **Figure 17**). A three-fold drop in activity was found by removing the methyl from the benzothiophene of **130**. On the other hand, replacement of piperazidinyl urea with piperidinyl urea gave over two-fold improvement compared to **130**. Interestingly, the substituent effect on 6-position of pyridyl-ring of **130** (**130a-j**) did not follow the earlier pattern. With these compounds the potency did not correlate with the leaving group ability, indicating

that the active site is rather hindered near the 6-position of the pyridine.[162] These findings then led to discovery of PF-3845 (**131**), providing improved potency and pharmacokonetics. In animal studies, PF-3845 raised brain anandamide levels for up to 24 hours and produced a significant cannabinoid receptor-dependent reduction in inflammatory pain.[163]

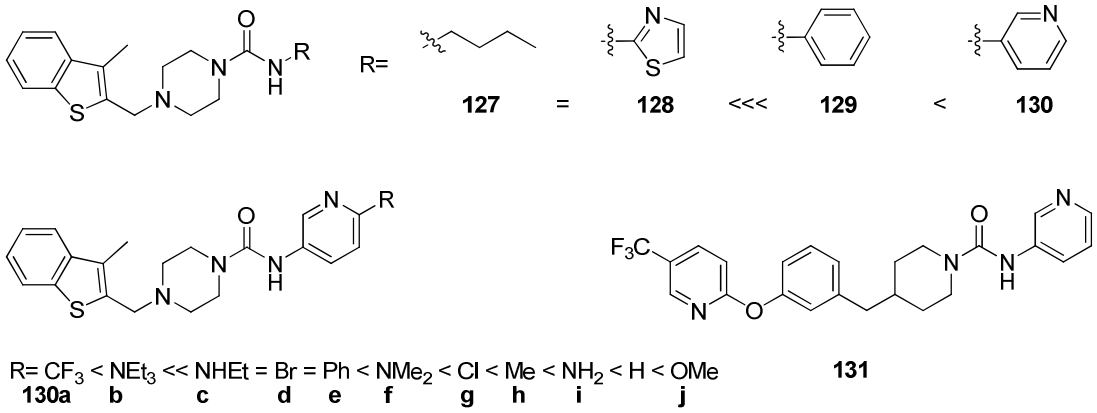


Figure 17. The evolution of the most potent urea-based FAAH inhibitor PF-3845 (**131**).

In addition to highly selective urea based FAAH inhibitors, there are examples of ureas that are not so selective. LY2183240 (**132**, **Figure 18**) was first reported as a putative AEA transport protein inhibitor,[164] but was later revealed to be a irreversible covalent FAAH inhibitor with the IC_{50} value of 18 nM.[67]

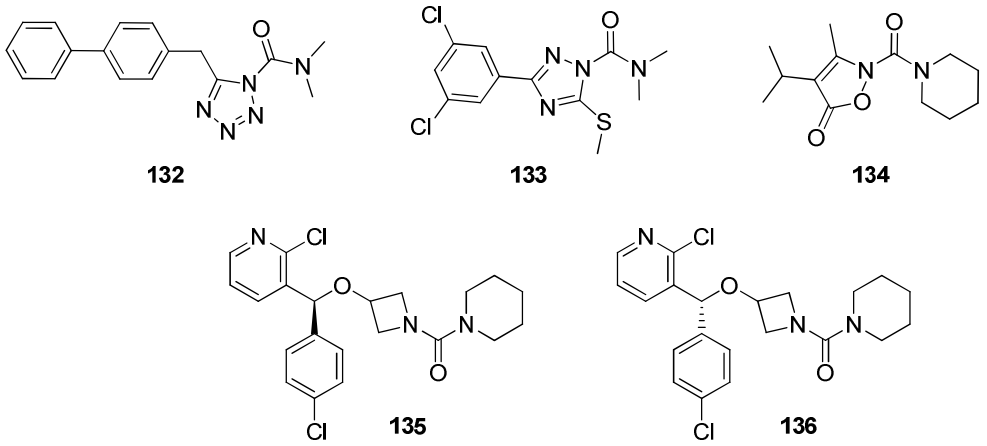


Figure 18. LY2183240 (**132**) and the recent urea compounds **133**-**136**.

In a very recent paper Minkkilä *et al*. tested a selection of known hormone sensitive lipase (HSL) inhibitors against FAAH and MGL.[165] HSL (EC 3.1.1.79) is an intracellular serine hydrolase catalyzing the hydrolysis of diglycerides in adipose tissues. It has emerged as a promising target for treatment of insulin resistance and dyslipidemic disorders.[166] FAAH IC_{50} value for carbamoyl triazole **133** was 30 nM, and for carbamoyl isooxazolone **134** exceptionally high 0.45 nM. IC_{50} values against MGL were 1.1-4.6 µM for **133** and 6.1-71 µM for **134**, indicating selectivity against another serine hydrolase operating in the same region. The variation in MGL IC_{50} values rises from two different assays used. Original target HSL was inhibited by *para*-chloro analogue of **133** with an IC_{50} value of 20 nM, being also unselective towards acetyl- and butyrylcholine esterases, though.[167] HSL-IC_{50} value for **134** was 2 nM.[168]

In another very recent paper, chiral azetidinyl ureas **135** and **136** were reported, providing high selectivity based on enantioselectivity. The (*S*)-enantiomer **135** inhibited FAAH with an IC_{50} value of 145 nM, and the (*R*)-enantiomer **136** did not show inhibition activity at 10 µM concentration. The racemic mixture had an IC_{50} value of 291 nM.[169]

As a conclusion, these urea compounds **132-136** deserve further development in order to gain selectivity.

3.1.4. Activity based protein profiling (ABPP)

The selectivity determination of FAAH inhibitors towards other serine hydrolases by conventional substrate-based assays would be a laborous task considering the huge size of the serine hydrolase superfamily (>200 members in humans) as well as the large number of these enzymes that represent uncharacterized proteins (i.e., enzymes without known substrates). For this purpose, a functional proteomic screen based on the activity-based protein profiling (ABPP)[170,171] technology has been implemented to evaluate the selectivity of FAAH inhibitors against serine hydrolases directly in native cell/tissue samples. Competitive ABPP for serine hydrolases involves the coordinated application of a candidate inhibitor and a reporter-tagged fluorophosphonate,[159] which serves as a general activity-based probe for the serine hydrolase superfamily[172,173]. Serine hydrolases that show significant reduction in probe labeling intensity in the presence of an inhibitor are scored as targets of the compound. In this way, competitive ABPP provides a global view of the proteome-wide selectivity of serine hydrolase inhibitors and has been successfully employed to address the selectivity of various FAAH inhibitors. [125,126,130,144,158,159,160]

3.1.5. Other FAAH inhibitor classes

(Thio)hydantoins

Hydantoin structure (**137, Figure 19)** was reported to provide a new template for reversible FAAH inhibitor development.[174] The compounds were not highly active, and at best reached micromolar IC_{50} level in FAAH inhibition (**138**, pIC_{50} 5.94). SAR revealed that thiohydantoins (X = S) were roughly 10-fold more active than corresponding hydantoins (X = O) and that the best compounds contained phenyls in both R^1 and R^2. Having a chlorine or bromine substituent in 4-position of the phenyls surprisingly reduced the potency significantly. The optimal N-3 substituent was a C6-C14 alkyl, and N-1 substituents were found to decrease the activity.

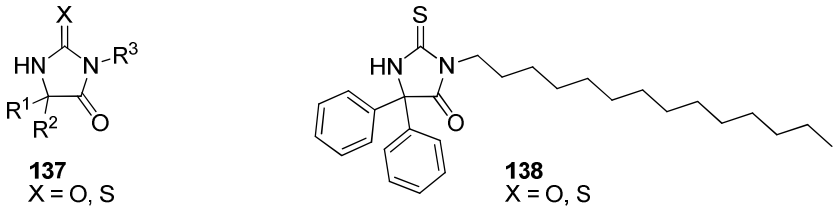


Figure 19. (Thio)hydantoins as FAAH inhibitors template.

Sulfonyls

Sulfonyl-containing benzothiazole compound **139** (**Figure 20**) was found to be a potential FAAH inhibitor by using high-throughput screening.[175] Derivatives of **139** were prepared by varying each part of the molecule, but no major improvement in potency was gained. Structure-activity relationship (SAR) studies indicated that the sulfonyl group, the piperidine ring and the benzothiazole were the key components to the activity. Compound **139** was found to be a reversible and highly selective inhibitor of FAAH, proven by time-dependent preincubation study and ABPP evaluation in brain, liver, heart and intestine tissues of rat. The modeling study indicated that hydrophobic interactions of the benzothiazole ring with the enzyme contributed to its extraordinary potency.[175]

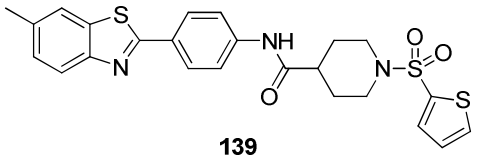


Figure 20. Sulfonyl containing selective FAAH inhibitor

Boronic acids

In 2008 Minkkilä *et al.* reported boronic acid (compounds **140**-**144** as examples**,** **Figure 21**) as a novel functional moiety in FAAH inhibitors.[176] Similar findings with a huge number of compound examples were published in a patent application around the same time. Roughly 300 different aryl boronic acids, including a few boronic esters (**145**), were reported to inhibit FAAH with K_i value less than 100 nM.[177] Boronic acid is a strong Lewis acid because of the boron empty orbital. Most phenylboronic acids have a pK_a in the range of 4.5–8.8 depending on the phenyl substitution. This means that with appropriate substitution, boronic acids would have the right properties for ready conversion from a neutral and trigonal planar sp^2 boron

to an anionic tetrahedral sp^3 boron under physiological conditions. The process of cleaving an amide bond also requires similar conversion of a sp^2 carbonyl carbon to a tetrahedral sp^3 carbon. This makes boronic acids good transition state analogues for the inhibition of hydrolytic enzymes. Thus, boronic acids have been used extensively as inhibitors of various hydrolytic enzymes such as peptidases and lipases.[178] The activity of boronic acids as FAAH inhibitors seems to depend mostly on boronic acid being the aromatic substituent, or at least to be attached to a sp^2-carbon (**140**, IC_{50} 14 nM). The second major SAR driving force is the lipophilicity, activity correlating positively with increasing lipophilicity (**141**, IC_{50} 9 nM). Lowering the pK_a of the boronic acid seems to have a minor improving effect to the activity compared to the lipophilicity. As an example, trifluoro compound **142** had an IC_{50} value of 36 nM and a calculated pK_a 8.1, even though the IC_{50} values of more Lewis acidic nitro- (pK_a 7.4) and cyano- (pK_a 7.7) analogues were 210 nM and 290 nM, respectively. In addition, *ortho*-substituents of phenylboronic acids seem to interfere with the formation of the tetrahedral sp^3 adduct, thus decreasing the potency. Compound **143,** having a *para*-phenyl substituent (IC_{50} 21 nM), was clearly more active than its *ortho*-analogue (IC_{50} 110 μM). Interestingly, also *meta*-substituted phenylboronic acids (**144,** IC_{50} 130 nM) were less potent than corresponding *para*-analogues, as shown by three pairs.[176] As a conclusion, the best activities can be reached with phenylboronic acids, having one small strongly electron withdrawing substituent in *ortho*- or *meta*-position and a lipophilic moiety in *para*-position. Fluorine is very electronegative and small enough not to disturb the tetrahedral intermediate in *ortho*-position. In fact, this was widely exploited by the authors of the patent, and numerous this kind of compounds reached K_i value below 10 nM.[177]

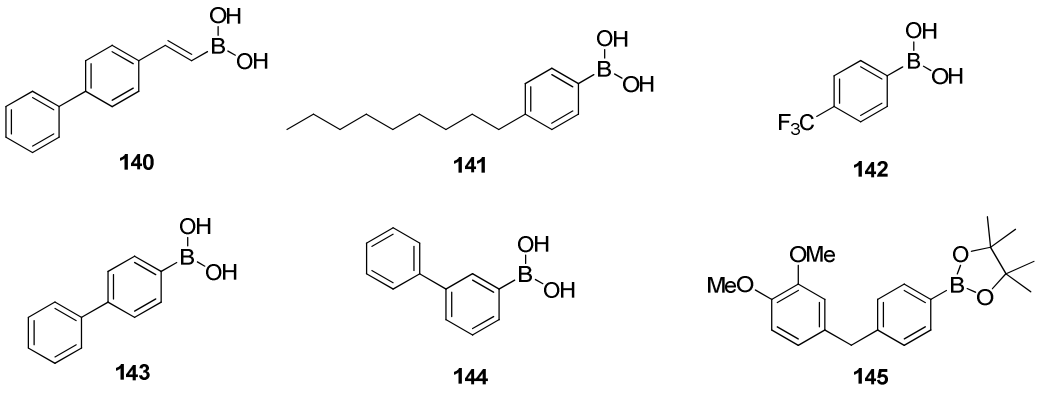


Figure 21. Boronic acids and ester as FAAH inhibitors.

3.2. MGL-inhibitors

Although MGL is agreed to be the major enzyme responsible for degradation of 2-AG, which in turn is considered at least as important an endocannabinoid as AEA, selective inhibitors have not been discovered until very recently.

Covalent irreversible inhibitors

As other serine hydrolases, non-selective covalent inhibitors MAFP (**35**, IC_{50} 2.2 nM) and hexadecylsulphonylfluoride (IC_{50} 245 nM) have also been reported to efficiently inhibit MGL.[12] Another interesting class of covalent inhibitors was reported by Saario *et al.*, by testing a group of maleimides against MGL. The maleimide group is a general reactive electrophile for thiols, thus this work suggests that MGL possess sulfhydryl sensitive site. The most potent compound was a substrate analogue, *N*-arachidonylmaleimide (NAM, **146, Figure 22**) inhibiting MGL with IC_{50} value of 140 nM. The corresponding succinimide derivative did not inhibit MGL (1 mM), indicating that the activity was based on reactivity of the maleimide double bond. Modelling studies conducted within this study suggested that MGL contains a non-catalytic cysteine residue in its active site, which could account for its unusual sensitivity to maleimide reagents.[179] NAM (**146**), despite containing a highly reactive maleimide group, exhibits rather high selectivity for MGL relative to other brain serine hydrolases.[72] In addition, NAM has been shown to potentiate the pharmacological effects of 2-AG *in vivo*.[180] In a recent paper, an

extension to the structural library and SAR of maleimides as FAAH and MGL inhibitors was reported, supporting earlier findings.[181] However, as a general thiol-sensitive group, maleimides will most likely react with other cysteine-containing proteins and small molecules *in vivo*.

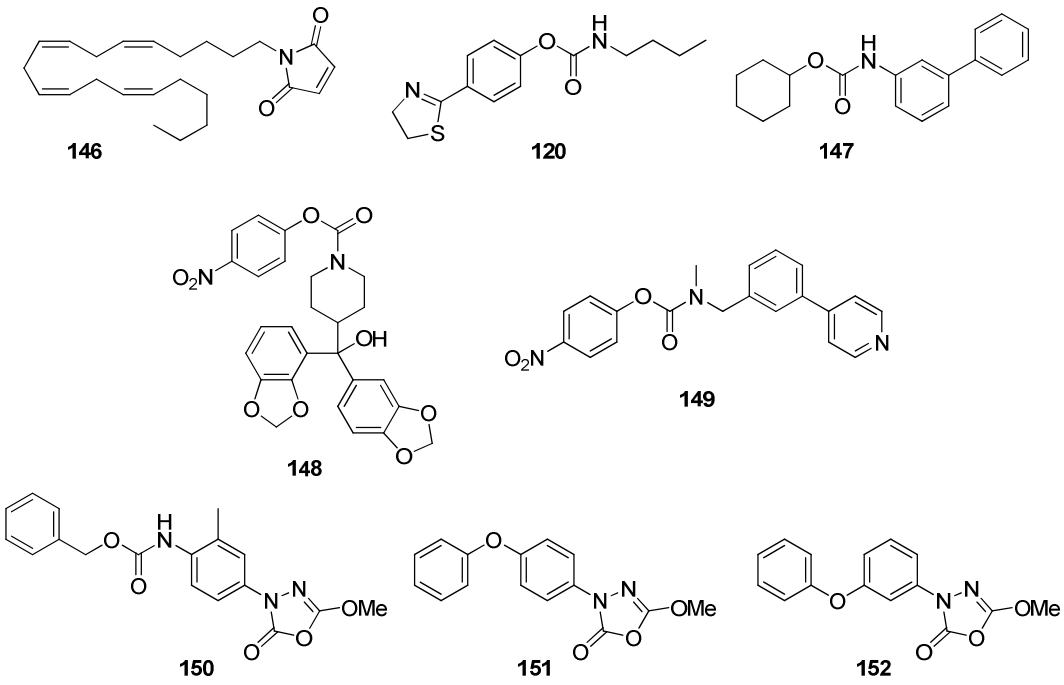


Figure 22. Examples of MGL-inhibitors reported so far.

Carbamates

So far the largest group of MGL inhibitors are carbamates (**120**, **147**-**150, Figure 22**). Compound **120**, previously mentioned in context with FAAH inhibitors (IC_{50} for FAAH, 520 nM), inhibited MGL with an IC_{50} value of 31 μM.[152] URB602 (**147**) was reported initially to inhibit rat brain MGL selectively with an IC_{50} value of 28 μM through a non-competitive mechanism.[149,182] When purified rat recombinant MGL enzyme was used, the activity was decreased (IC_{50} 223 μM). Derivatives of **147** were also evaluated, resulting in slight improvement in potency with corresponding urea analogue (IC_{50} 115 μM).[183]

In a recent report Cravatt *et al.* introduced an exceptionally potent and selective MGL inhibitor, JZL184 (**148**, IC_{50} 6 nM), displaying also robust activity *in vivo*.[184]

The authors commented that its discovery was based on implementation of activity-based proteomic methods to broadly evaluate and optimize the selectivity of inhibitors across the serine hydrolase superfamily. Compound **148** (JZL184) was modified from the non-selective carbamate **149**, which inhibited several serine hydrolases selectively and was found during validation studies of the ABPP-method.[185,186] JZL184 was found to produce a rapid and sustained blockade of brain 2-AG hydrolase activity in mice, resulting in eight-fold elevations in endogenous 2-AG levels that were maintained for at least 8 h. Moreover, AEA levels were unaffected by JZL184 (**148**). JZL184-treated mice showed a wide array of CB_1-dependent behavioural effects, including analgesia, hypomotility and hypothermia, suggesting a broad role for 2-AG–mediated endocannabinoid signaling throughout the mammalian nervous system.[184]

Oxadiazolones

In the same recent paper where Minkkilä *et al.* reported urea compounds **132** and **133** as novel FAAH inhibitors, they described another class of potential FAAH and MGL inhibitors, oxadiazolones (**150-152**, **Figure 22**). Commercially available carbamate CAY10499 (**150**) was used as a lead, since it inhibited MGL with IC_{50} value of 330 nM.[165,187] Another group reported an IC_{50} of 400 nM for CAY10499 against MGL.[188] Minkkilä prepared a small series of derivatives of **150,** and concluded that the inhibitory activity was based on the 1,3,4-oxadiazol-2(3*H*)-one moiety rather than on the carbamate. The most potent MGL inhibitor, albeit not selective over FAAH (IC_{50} 65 nM), was the *para*-substituted oxadiazolone **151** with an IC_{50} value of 78 nM against 2-AG hydrolysis. Corresponding *meta*-substituted **152** was ten-fold more potent against FAAH (IC_{50} 6.1 nM) with similar activity against MGL (IC_{50} 110 nM) as **152**. Moreover, most of the oxadiazolone compounds were shown to inhibit both FAAH and MGL in reversible manner, as determined by rapid dilution studies. Since these compounds were derivatized from known HSL inhibitors, no selectivity among other serine hydrolases was expected.[165] As an example, **152** has been reported to inhibit HSL with an IC_{50} value of 70 nM.[189] Anyhow, oxadiazolones as non-carbamate structures are a very interesting novel template for inhibitor development against both FAAH and MGL.

4. THIS STUDY

In addition to review of the literature, this thesis contains the design, synthesis and characterization of over 100 novel compounds which were prepared and tested against FAAH and MGL enzymes in order to find better inhibitors.

4.1. Initial plan

By the time this study was initiated, only two groups had presented potential FAAH inhibitors: Dale Boger's (α-ketoheterocycles like **74)** and Daniele Piomelli's group (*meta*-substituted *N*-alkyl phenyl carbamates like URB597 **101**).[16,124] These structures have very different structural motifs, and were used as the starting point in this study (**Figure 23**).

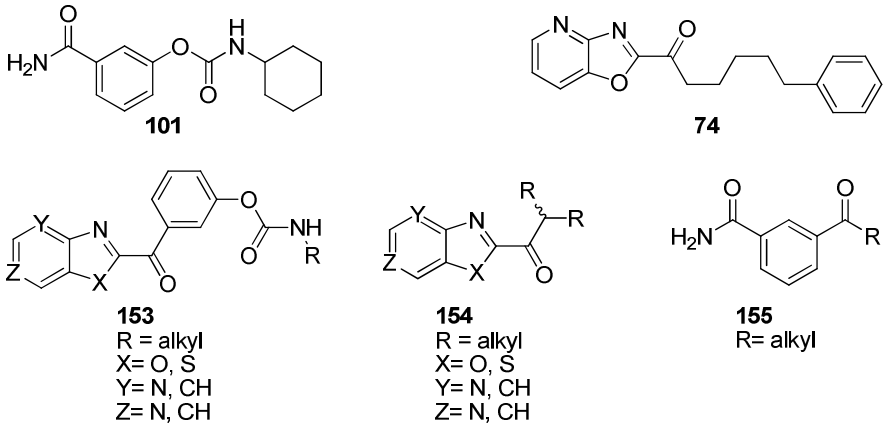


Figure 23.

The structural motifs of **101** and **74** were combined, and a general structure **153** was constructed in order to gain SAR data for further development of potent FAAH and possibly MGL inhibitors. Compounds of this structure type were named hybrids. In addition, the plan was to study the activity of chiral compounds of a general structure like **154**, in order to gain information on FAAH active site stereochemistry**.** Moreover, 3-phenyl amide **155**, containing the alkyl part similar to one in **74**, was designed to see what kind of activity would this type of combination result in. Most

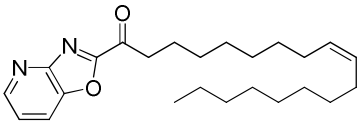
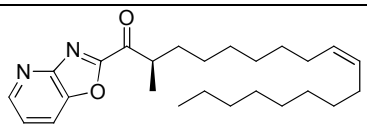
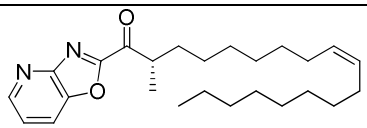
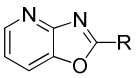
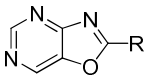
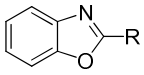
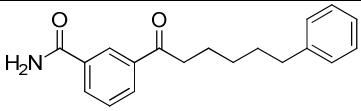
importantly, the idea was to elaborate the plans based on activity data found during the study.

4.2. Results and discussion

4.2.1. Acyl and alkyl heterocycles

In order to find out whether the enantiomers of 2'-methylated 2-oleyl oxazolopyridines have significantly different activity against FAAH, compounds **156** and **157** were designed and prepared. In addition, it was decided to prepare compound **61** for study-to-study comparison of FAAH bioassay as reference. Surprisingly, compounds **158a** and **158b** showed FAAH inhibition activity (IC_{50} ~ 400 nM), and derivatives **158c**-**160d** were added to the series. The activity of **158a-b** was later revealed as an unknown error in assay. Compound **161** was also prepared and tested within this series. (**Table 6**).

Table 6. IC_{50} or percentage of inhibition of FAAH and MGL by compounds **61** and **156**-**161**.

Compd	Structure	FAAH (nM)[a]	MGL[b]
61		0.213 (0.162-0.280)	0%
156		5.8 (4.3-7.9) (n=4)	0%
157		4.3 (2.9-6.3)	0%
158a-d	 **a**; R= H **b**; R= Me **c**; R= Et **d**; R= Pr	0%	0%
159a-d	 **a**; R= H **b**; R= Me **c**; R= Et **d**; R= Pr	0%	0%
160a-d	 **a**; R= H **b**; R= Me **c**; R= Et **d**; R= Pr	0%	0%
161		0%	0%
101	see **Fig. 22**, lit. 4.6 nM[16]	3.8 (2.9-5.0)[152]	30%[d]

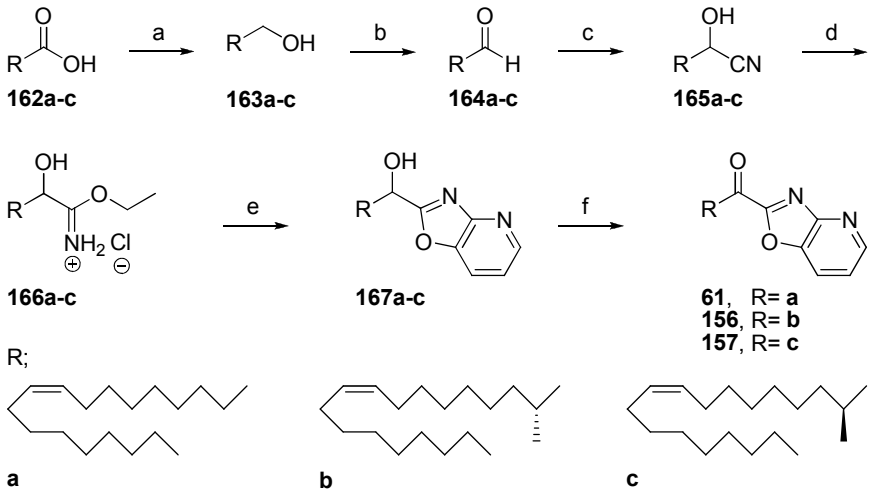
[a] IC_{50} values represent the mean of three independent experiments performed in duplicate with 95% confidence intervals shown in parentheses or enzyme inhibition (%) at 10 μM compound concentration (n=2).
[b] Enzyme inhibition (%) at 100 μM compound concentration (n=2).
[c] For **8a**, **8b**; No inh. at 100 μM (n=4).
[d] Enzyme inhibition (%) at 1.0 mM compound concentration.[12]

Synthesis

Compounds **61**, **156** and **157** were prepared applying known procedures[124]: (*R*)-2-Methyl-*cis*-octadecenoic acid **162b** and (*S*)-2-methyl-*cis*-octadecenoic acid **162c** were prepared in house via Evans auxiliary based enantioselective synthesis (data not shown). Reduction of acids **162a-c** to alcohols **163a-c** was performed with lithium aluminium hydride in THF. The alcohols were then oxidized to aldehydes

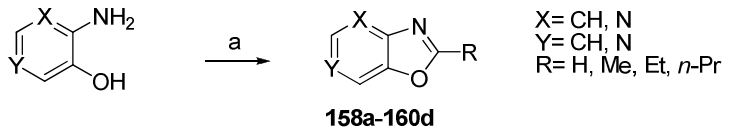
164a-c by iodoxybenzoic acid (IBX)[190] or by pyridinium chlorochromate (PCC).[191,192]

Formation of cyanohydrins **165a-c** occurred by treatment of aldehydes with potassium cyanide in THF/H_2O. Cyanohydrins were then condensed with 2-amino-3-hydroxypyridine via imidate-intermediate **166a-c**. In this method cyanohydrins were first treated with HCl in $CHCl_3$. HCl was formed *in situ* with ethanol and acetyl chloride. The product alcohols **167a-c** from the coupling were formed with low yields (4-25%). These alcohols were then oxidized to end products **61**, **156** and **157** using IBX in DMSO with moderate to good yields (62-91%). **Scheme 2.**



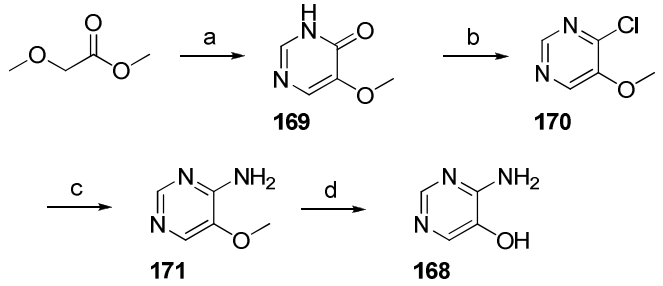
Scheme 2. Reagents and conditions; a) $LiAlH_4$, THF, rt; b) IBX, DMSO, rt, or PCC, CH_2Cl_2, rt; c) KCN, THF/H_2O, rt; d) AcCl, EtOH, $CHCl_3$, rt; e) 2-Amino-3-hydroxypyridine, 2-ethoxyethanol (EtOH for **b**, **c**), rfx; f) IBX, DMSO, rt.

Compounds **158a-160d** were prepared via condensation of 2-aminophenol, 2-aminopyridin-3-ol or 4-aminopyrimidin-5-ol (**168**) with orthoesters[193]. *p*-TsOH was used as acid catalyst (0.5 mol-%). In small scale reactions (0.2 mmol) the amount of catalyst used was higher (up to 8 mol-%), which did not negatively affect the reactions. The yields varied quite a lot (16-77 %) which could be due to the purifications rather than the actual reactions. All compounds were purified by recrystallization or vacuum distillation. In small scale cases flash chromatography (FC) was sometimes needed to purify the compounds. **Scheme 3**.



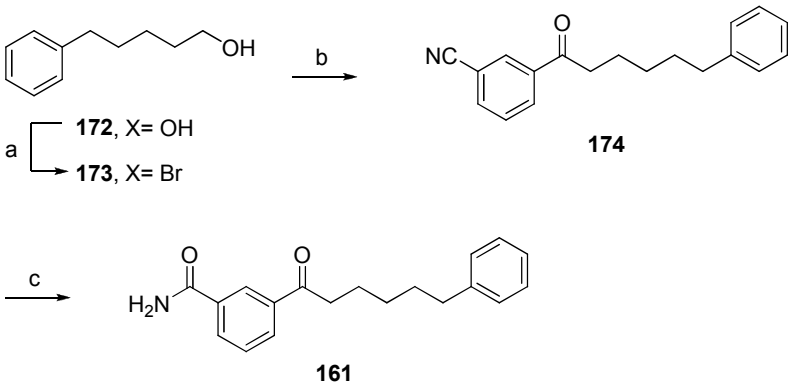
Scheme 3. a) R-Orthoester, *p*-TsOH, 100-190 °C.

Literature procedures[194,195,196] were followed to prepare compound **168** in four steps (**Scheme 4**).



Scheme 4. a) i. Methyl formate, NaH, THF, 20 °C, 20 h; ii. formamidine acetate, EtOH, rt, 14 h, reflux, 24 h, 31%; b) $POCl_3$, reflux, 2.5 h, 86%; c) NH_3, EtOH, autoclave, 130 °C, 18 h, 75%; d) *n*-BuSH, NaH, DMF, 110 °C, 20 h, 74%.

Commercially available 5-phenylpentanol **172** was converted to alkyl bromide **173,** which in turn was treated with magnesium to obtain the Grignard reagent for reaction with 3-cyanobenzaldehyde. Unfortunately the Grignard reaction did not work as expected, and a mixture of compounds was detected. Eventually after separation by flash chromatography, compound **174** was obtained in 6% yield, and further oxidized to compound **161** in good yield. **Scheme 5**.



Scheme 5. Reagents and conditions: a) PPh_3, CBr_4, CH_2Cl_2, rt; b) Mg, I_2, Et_2O, 3-cyanobenzaldehyde, rfx; c) H_2O_2, NaOH, EtOH, 50 °C.

Discussion

As can be seen from **Table 6,** compound **61** and its methylated analogues **156** and **157** were the only active FAAH inhibitors of this series within detection limits (10 μM for FAAH and 100 μM for MGL). The activity difference between (*S*)- and (*R*)-enantioriched samples was small, (*S*)-enantiomer (**156**) giving slightly better inhibition than (*R*) (**157**). Enantiomers were not pure, though. Both compounds were determined by chiral HPLC, having 37 ee-%. This was quite surprising, but since this 2:1 ratio already gave such small difference in activity, the compounds were not prepared again and the reason for partial racemization was not determined. This decision was supported also by the 20-fold decrease in activity of both enantioriched compounds **156**-**157** compared to unmethylated **61**.

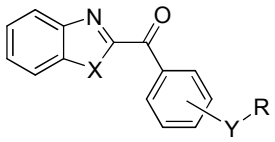
As expected, small fused heterocycles **158a**-**160d** failed to give any inhibitory activity against either of enzymes. Compound **161** was also inactive, indicating that aryl amide and proper alkyl tail are not moieties that alone raise the FAAH activity. Apparently ketone carbonyls need to be activated by electron withdrawing groups to react with active site Ser241. As Saario *et al.* have reported previously, carbamate compound URB597 (**101**) was found to inhibit AEA hydrolysis with an IC_{50} value of 3.8 nM in our method, which is comparable to the previously reported 4.6 nM[16], and only 30% inhibition of 2-AG hydrolysis in rat brain membranes was evident at 1 mM[152].

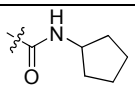
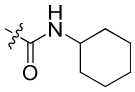
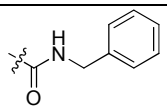
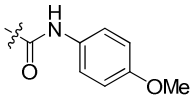
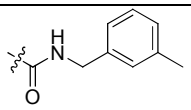
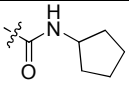
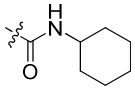
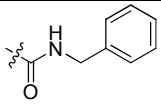
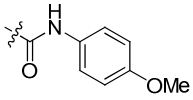
4.2.2. Carbamate derivatives of 2-benzoxazolyl- and 2-benzothiazolyl (3-hydroxyphenyl)methanones[197]

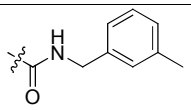
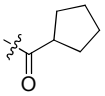
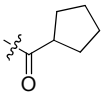
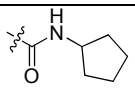
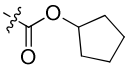
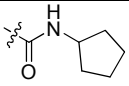
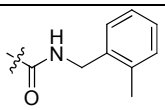
Next the attention was turned to “hybrids” series. Compounds **175**-**200** were prepared after finding out that the first compound in this series, *N*-cyclohexylcarbamate derivative **179** gave such a high FAAH-inhibition activity (IC_{50} 47 nM). The various *N*-alkyl carbamoyl phenols having either 2-benzoxazolylmethanone (**175**-**182**) or 2-benzothiazolylmethanone (**183**-**190**) as *meta*-substituent were prepared to screen the most suitable alkyl group for next modifications. The importance of the carbamate moiety was studied by preparing compounds **191-196d,** as well as was the difference of *meta*- and *para*-substitution

by preparing compounds **197** and **198.** In addition, benzyl ether intermediates **199** and **200** were tested against both enzymes. (**Table 7**).

Table 7. IC_{50} values or the percentage of inhibition of FAAH and MGL by compounds **175**-**200**.[197]



Compd	X	Y	R	FAAH (nM)[a]	MGL (μM)[a]
175	O	3-O	$CONHC_2H_5$	379 (303-474)	25%
176	O	3-O	CONH-*n*-C_3H_7	109 (90-132)	43%
177	O	3-O	CONH-*n*-C_4H_9	54 (46-64)	41%
178	O	3-O		28 (23-34)	46%
179	O	3-O		47 (36-62)	17%
180	O	3-O		152 (122-189)	43%
181	O	3-O		nd[b]	nd[b]
182	O	3-O		32 (26-40)	16 (14-18)[c]
183	S	3-O	$CONHC_2H_5$	238 (177-321)	20%
184	S	3-O	CONH-*n*-C_3H_7	143 (113-180)	30%
185	S	3-O	CONH-*n*-C_4H_9	103 (81-131)	24%
186	S	3-O		47 (38-57)	29%
187	S	3-O		56 (41-77)	21%
188	S	3-O		121 (105-140)	25%
189	S	3-O		nd[b]	nd[b]

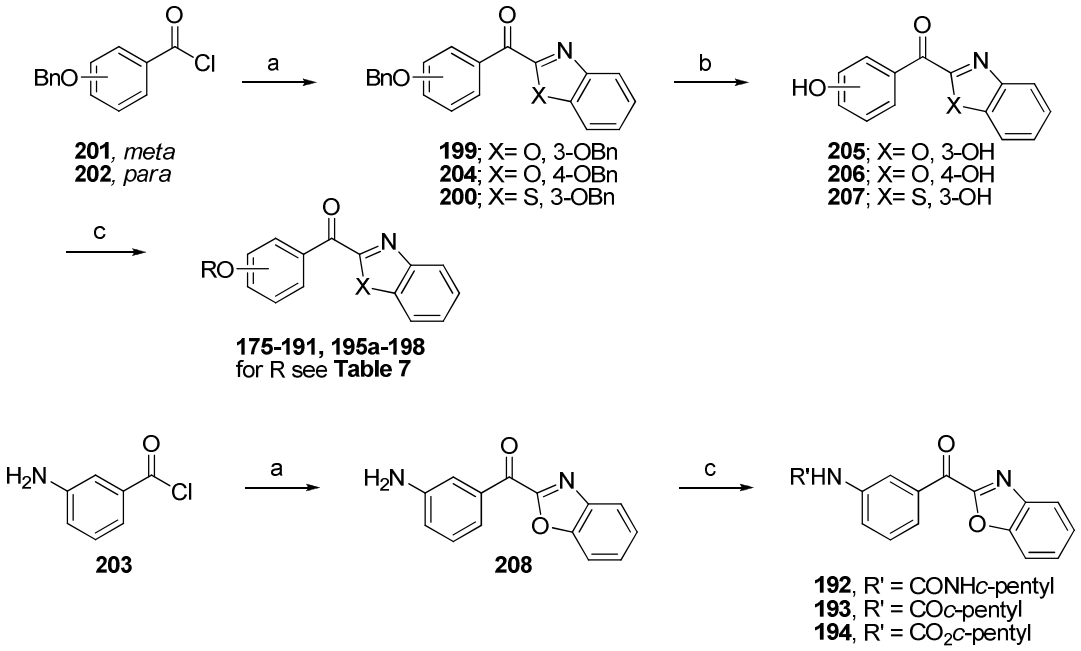
190	S	3-O		38 (33-43)	23 (20-27)[c]
191	O	3-O		12%	0%
192	O	3-NH		5%	0%
193	O	3-NH		6%	0%
194	O	3-NH		5%	0%
195a-d	O	3-O	**a**; Et **b**; *n*-Pr **c**; *n*-Bu **d**; Bn	nd[b]	nd[b]
196a-d	S	3-O	**a**; Et **b**; *n*-Pr **c**; *n*-Bu **d**; Bn	nd[b]	nd[b]
197	O	4-O		288 (237-349)	14 (12-17)
198	O	4-O		137 (112-169)	25 (21-29)[c]
199	O	3-O	Bn	0%	0%
200	S	3-O	Bn	0%	0%

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 10 μM (for FAAH) and 100 μM (for MGL) compound concentrations (n= 2).
[b] Not stable in used assay conditions.
[c] Remaining enzyme activity at 1 mM was ~11-24%.

Synthesis

The synthesis of the series was started from 3- or 4-hydroxybenzoic acid or 3-aminobenzoic acid (**Scheme 6**). The phenol functionality was protected as benzyl ether, and the acids were then converted to acyl chlorides **201**-**203** according to literature procedures.[198,199,200] 3-Aminobenzoic acid did not need protection. Acyl chlorides were then coupled with benzoxazole or benzothiazole in order to prepare

compounds **199**, **200** and **204** following the method described by Harn *et al.*[201] Deprotection of the phenolic hydroxyl was first attempted with hydrogenation catalyzed by Pd/C. Unfortunately under these conditions the methanone was reduced to alcohol before the cleavage of benzyl group. Although the alcohols were easily oxidized back to ketone with IBX[190] or DDQ,[202,203] another method was needed to avoid the extra step. Finally the removal of the benzyl protecting group without reducing the ketone was carried out using BF_3•Et_2O and Me_2S.[204] Ionic liquid based microwave-assisted cleavage of benzyl ether also proved to be as efficient a method.[205] Deprotected phenols **205-207** and aniline **208** were then coupled with electrophiles (isocyanates, chloroformates and acyl chlorides).



Scheme 6. Reagents and conditions: a) Benzoxazole or benzothiazole, *n*-BuLi, 1 M $ZnCl_2/Et_2O$, CuI, THF, -75 °C -> 0 °C, 54-55%; b) BF_3•Et_2O, Me_2S, CH_2Cl_2, rt, 84-91% or *N*-1-BuPyrBr, MW, 30 s, 90%; c) Et_3N or pyridine, RNCO, *c*-pentylNCO, *c*-pentylCOCl or *c*-pentylOCOCl, toluene or CH_2Cl_2, rt or heating up to 93 °C, 41-99%.

Although the preparation of 2-benzoxazolylketone and 2-benzothiazolylketone intermediates **199-200**, **204** and **208** was straightforward, it turned out not to be possible to prepare the corresponding oxazolopyridine- or oxazolopyrimidines

(general structure **153**, X= N, Y= CH or X= N, Y= N) this way. An attempt to prepare these compounds via imidate-method also failed.

Discussion

The alkyl moiety of the most potent compound of **Table 7** was selected for testing the other carbonyl derivatives. The most potent FAAH inhibitor in this series was the cyclopentylcarbamate **178** (IC_{50} 28 nM). Compounds within the same range of lipophilicity inhibit FAAH with IC_{50} values almost equal (32-56 nM) to that for compound **178.** The inhibition of FAAH activity was dependent on the carbamate group, as the compounds lacking this functionality were unable to inhibit FAAH even at 10 μM. This supports the postulated mechanism by which carbamates inhibit FAAH by carbamoylation of the enzyme's nucleophilic serine. Compounds **175**-**177,** as well as the corresponding sulphur analogues (**183**-**185**) were found to have a trend for increasing potency for FAAH with increasing length of the alkyl group (= lipophilicity). Interestingly, compounds **182** and **190** containing 3-methylbenzylcarbamate had clearly higher inhibition activity against MGL enzyme activity compared to the other compounds in this series. Thus, the introduction of the methyl group in the 3-position of the benzyl ring increased FAAH as well as MGL enzyme inhibition. However, none of these compounds could inhibit MGL activity at the nanomolar concentration range. Furthermore, there were no significant differences in FAAH or MGL enzyme inhibition between the benzoxazoles and the benzothiazoles. Compounds **181** and **189** were not stable enough to give reliable inhibition activity. Apparently the carbamate was rapidly hydrolyzed in the test conditions.

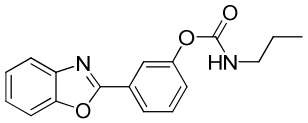
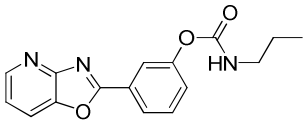
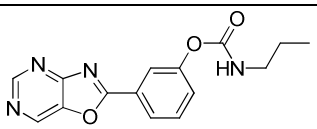
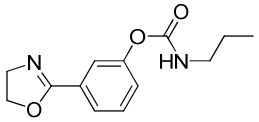
To clarify the importance of the carbamate group we prepared some other carbonyl derivatives for comparison. Carbonyl compounds **191** (ester), **192** (amide), **193** (urea), **194** (reverse carbamate) and **195a**-**196d** (carbonates) were not effective FAAH inhibitors. The fact that **194** did not inhibit FAAH or MGL supports the proposed carbamoylation mechanism, where the phenol part is the leaving group[144]. Carbonates **195a-196d** as well as **181** and **189** were not stable in the assay conditions and their decomposition was detected by TLC (data not shown). 4-Substituted compounds **197**-**198** were also synthesized. The activity of these compounds against FAAH was still in nanomolar range (IC_{50} 288 and 137 nM).

It was noteworthy that a 10-fold difference was found between *meta*- (**178,** IC_{50} 28 nM) and *para*-substituted (**197**, IC_{50} 288 nM) *c*-pentylcarbamates. In addition compound **197** was found clearly more active against MGL than **178**.

4.2.3. Importance of the ketone linker[206]

Since it was found from the previous series that Ser241 in FAAH-active site was likely not interacting with the methanone carbonyl but with the carbamate carbonyl, it was decided to explore this further and prepare compounds **209**-**212** (**Table 8**). *n*-Propyl was chosen as initial *N*-alkyl group, and *N*-pentyl analogue of the most active compound of this series was planned to prepare later since the supply of cyclopentylisocyanate was delayed during that time.

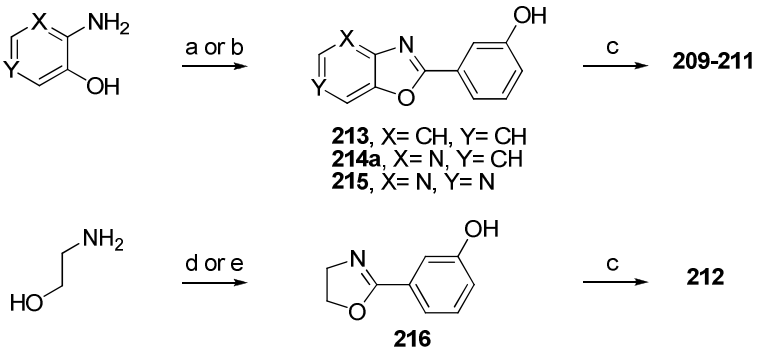
Table 8.

Compd	Structure	FAAH (nM)[a]	MGL[b]
209		3000 (2600-3600)	26%
210		680 (590-780)	29%
211		4500 (4000-5200)	18%[c]
212		33 (28-38)	32%

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 10 μM compound concentration (n= 2).
[b] Enzyme inhibition (%) at 100 μM compound concentration (n= 2).
[c] Inhibition of human recombinant MGL.

Synthesis

The preparation of phenolic intermediates of **209**-**212** is presented in **Scheme 7**. In the course of preparation of **213**-**215** it was found that microwave assisted direct condensation of 3-hydroxybenzoic acid and 2-amino-3-hydroxypyridine is a novel method and thus worth of a separate report.[207] This study is presented in the next chapter. In preparation of phenol **216** the MW-assisted method was attempted – unsuccessfully. This compound was then prepared via method described by Vorbrügger and later by coupling of imidate salt with aminoalcohol.[208,209]



Scheme 7. a) 3-Hydroxybenzoic acid, boric acid, Na_2SO_4, *m*-xylene, autoclave, 200 ^oC, 16 h, 21-83%; b) 3-Hydroxybenzoic acid, MW, 250 ^oC, 2-6 min, 71-77%; c) *n*-PrNCO, Et_3N, toluene, rt or 90 ^oC, 20 h, 50-95%; d) 3-Hydroxybenzoic acid, PPh_3, CCl_4, MeCN, pyridine, rt, 21 h, 29%; e) Methyl 3-hydroxybenzimidate hydrochloride, TEA, CH_2Cl_2, rfx, 24 h, 90%.

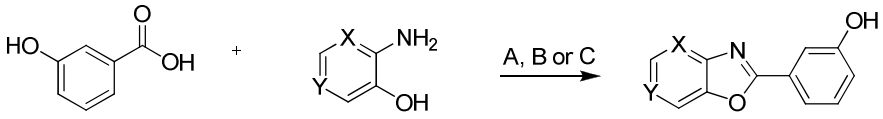
Discussion

The results of the inhibition studies showed that the absence of a ketone group within these fused bicyclic aromatic compounds (e.g. **176** vs. **209**) decreases the FAAH-inhibition activity. Introducing a nitrogen atom into the fused oxazole-containing bicycle (**210**) increased the inhibitory activity compared to compound **209** (IC_{50} of 0.68 μM vs. 3 μM). Addition of a second nitrogen did not enhance the activity further: pyrimidineoxazole-containing **211** was clearly less active than **210**. Additionally compound **212**, which only contained the unsubstituted 2-oxazoline ring, showed good inhibitory activity against FAAH with an IC_{50} value of 33 nM. Design of the next series was therefore focused on derivatives of **212**.

4.2.4. The preparation of 2-substituted oxazolo[4,5-*b*]pyridines[207]

Traditional condensations

Oxazoles are rigid and stable structures often found in natural products and widely used in medicinal chemistry. 2-Phenyl benzoxazoles have traditionally been synthesized by heating aminoalcohols with benzoic acids in the presence of polyphosphoric acid[210] or trimethylsilyl polyphosphate[211] as dehydrating reagents. Activated acids such as orthoesters and acid anhydrides have been used in the preparation of 2-phenyl-pyrido- and pyrimidino oxazoles.[212] In addition, Zhuravlev presented a convenient method to prepare these compounds using palladium-catalyzed C-2 arylation of oxazolo[4,5-*b*]pyridine.[213] In order to prepare the phenols needed for the carbamates, the method published by Terashima and Ishii[214] was explored. Refluxing 3-hydroxybenzoic acid **217a** and 2-aminophenol **218** with 100 mol% of boric acid in *m*-xylene for 18 h with a Dean-Stark apparatus for water removal gave only a 13% yield of compound **213**. Addition of Na_2SO_4 to the reaction mixture to chemically scavenge the water produced in the reaction increased the yield to 37%. Eventually, carrying out the reaction at higher temperature (200 ºC) and under pressure (50 psi) in an autoclave overnight gave a satisfactory yield (83%). The same method was then applied for 2-amino-3-hydroxypyridine **219** and 2-amino-3-hydroxypyrimidine **158,** giving compounds **214a** and **215** in 58% and 23% yield. **Scheme 8.**



217a

218, X= CH, Y=CH
219, X= N, Y= CH
158, X= N, Y= N

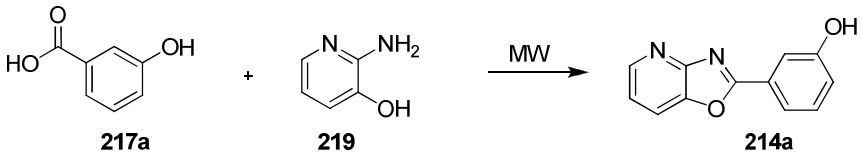
213, X= CH, Y= CH
214a, X= N, Y= CH
215, X= N, Y= N

Scheme 8. Reagents and conditions: **217a** (415 mg, 3 mmol, 100 mol-%), **218/219/158** (330 mg, 3 mmol, 100 mol-%), boric acid (185 mg, 3 mmol, 100 mol-%). Methods and yields: **A**, reflux with a Dean-Stark trap, 18 h, 13% for **213**; **B**, Na_2SO_4, reflux 18 h, 37% for **213**; **C**, autoclave, 200 ºC, 50 psi, 18 h, 83% for **213**, 58% for **214a** and 23% for **215**.

MW-assisted condensations

Microwave irradiation has previously been applied in condensation reactions by Bougrin *et al.*[215] and this method was applied for the preparation of **214a**. The reactions were carried out in a CEM Discover© microwave reactor with varying parameters (**Table 9**). Reaction times were shortened dramatically, and the best results were obtained without solvent, catalyst or water scavenger with 2 min hold time at 250 ºC, giving **214a** in 77% isolated yield (entry 7).

Table 9. Optimization of the condensation reaction under MW irradiation.



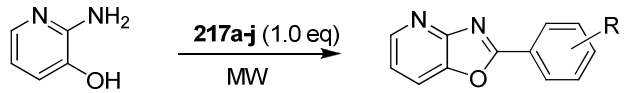
217a **219** **214a**

Entry	Solvent	Temp. and power	Other reagents	Time[a] (min)	Yield[b] (%)
1	*m*-xylene	200 ºC / 300 W	H_3BO_3, Na_2SO_4	15	25
2	toluene	160 ºC / 1st 150 W, 2nd 300 W	H_3BO_3, Na_2SO_4	2x 15	0[c]
3	-	200 ºC /150 W	H_3BO_3, Na_2SO_4	15	13
4	-	200 ºC /300 W	-	15	67
5	-	200 ºC /200 W	-	15	40
6	-	250 ºC /300 W	-	1	69
7	-	250 ºC /300 W	-	2	77
8	-	250 ºC /300 W	-	10	12

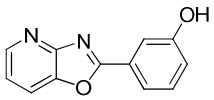
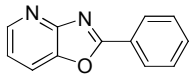
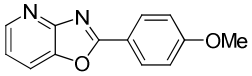
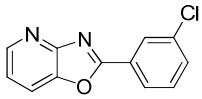
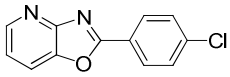
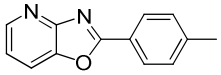
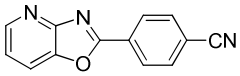
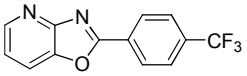
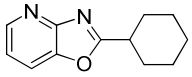
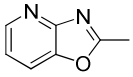
[a] Hold time is the time at the specific temperature. Ramp time varied from 3-10 min.
[b] Isolated yields.
[c] Only the starting materials were detected.

Next the optimized reaction conditions were applied to different aromatic and aliphatic acids, and the results are shown in **Table 10**.

Table 10. The condensation reactions of **219** with different acids **217a-j**.



219 **214a-j**

Entry	Time (min)	T (ºC)	Product	Nr	Yield (%)[a]
1	2	250		**214a**	77
2	2	250		**214b**	60 (60)
3	4x2	170		**214c**	trace (44)
4	3	170		**214d**	67
5	3	250		**214e**	56
6	2	170		**214f**	82
7	2	170		**214g**	trace (18)
8	4	170		**214h**	58 (40)
9	12	250		**214i**	85 (51)
10	2	250		**214j =160b**	34 (20)

[a] Isolated yields. Yields in parentheses are for reactions using 100 mol-% of boric acid.

Discussion

For some acids the selected temperature (250 ºC) was too high and resulted in low yields. These reactions were tested at lower temperature, improving the yield in several cases (entries 3-4 and 6-8). Some low-yielding reactions were also repeated in the presence of boric acid, since it has been reported to catalyze the condensation under normal heating reactions. Surprisingly, boric acid was found both to increase the yield in some cases (entries 3 and 7) but also to decrease it in others (entries 8-10).

When 2- and 4-hydroxy-, 4-dimethylamino-, 2-chloro-, 4-acetyl-, and 2-, 3- and 4-nitrobenzoic acids were subjected to the same reaction conditions, none of the desired products were obtained. The main products isolated from reactions of **219** with 2-, and 4-hydroxy- and 4-dimethylaminobenzoic acid were the decarboxylated products (phenol or *N*,*N*-dimethylaniline, ^{1}H NMR). This implies that the presence of a strong electron donor in the 2- or 4-position of the carboxylic acid results in decarboxylation rather than condensation. Usually aromatic acids containing *ortho-* or *para*-electron donating groups undergo decarboxylation in acidic conditions via arenium ion mechanism, but there is also reports of rapid heat induced decarboxylation of *ortho-* and *para*-hydroxybenzoic acids in neutral conditions.[216] With the remaining acids there was no clear main product, only a tarlike material or recovered starting materials.

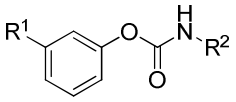
In summary, 2-substituted oxazolo[4,5-*b*]pyridines are easily produced by microwave-assisted direct condensation. The reactions are fast and operationally simple (solvent-free conditions, easy work-up).

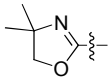
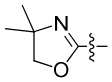
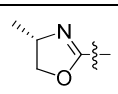
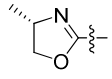
4.2.5. 3-(2-Dihydrooxazolyl)phenyl *N*-alkyl carbamates[206]

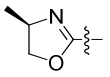
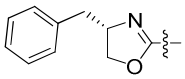
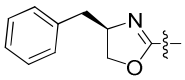
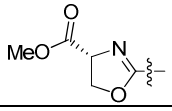
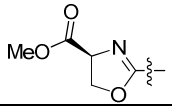
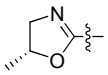
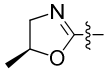
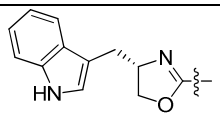
The optimization of the heterocyclic portion by compounds **209**-**212** (**Table 8**) revealed that 3-(2-(4,5-dihydro)-oxazolyl)-phenyl *N*-propylcarbamate **212** was a suitable lead for further modifications. The results of the *in vitro* experiments are summarized in **Table 11** (compounds **220**-**234**). 2-(4,5-Dihydro)-oxazolyl moiety has been widely utilized in ligands of stereoselective catalysts, proving their high chemical stability in various chemical environments.[217] Thus the potential importance of stereochemistry in FAAH inhibition was investigated by preparing

chiral 2-oxazoline derivatives of **221** (compounds **225**–**234**). The enantiopurity of chiral compounds was determined using chiral normal phase HPLC. The modification of **212** was started by changing the *N*-alkyl group from *n*-propyl to cyclopentyl. As found earlier, the cyclopentyl derivative **178** (IC_{50} = 28 nM) was more active than the *n*-propyl derivative **176** (IC_{50} = 109 nM). Similar enhancement in activity was observed for compound **220** in comparison to **212** (IC_{50} 12 nM vs. 33 nM). The next modification was done in 2-oxazoline part by adding 4-(*S*)-methyl and 4-dimethyl substituents to it. Later it was noted that changing the carbamate *N*-alkyl group from cyclopentyl to cyclohexyl gave a further increase in activity (**221** vs. **220**), and hence cyclohexyl was used as the *N*-alkyl group for the rest of this series.

Table 11. The structures and inhibition activities of the derivatives of **212**.



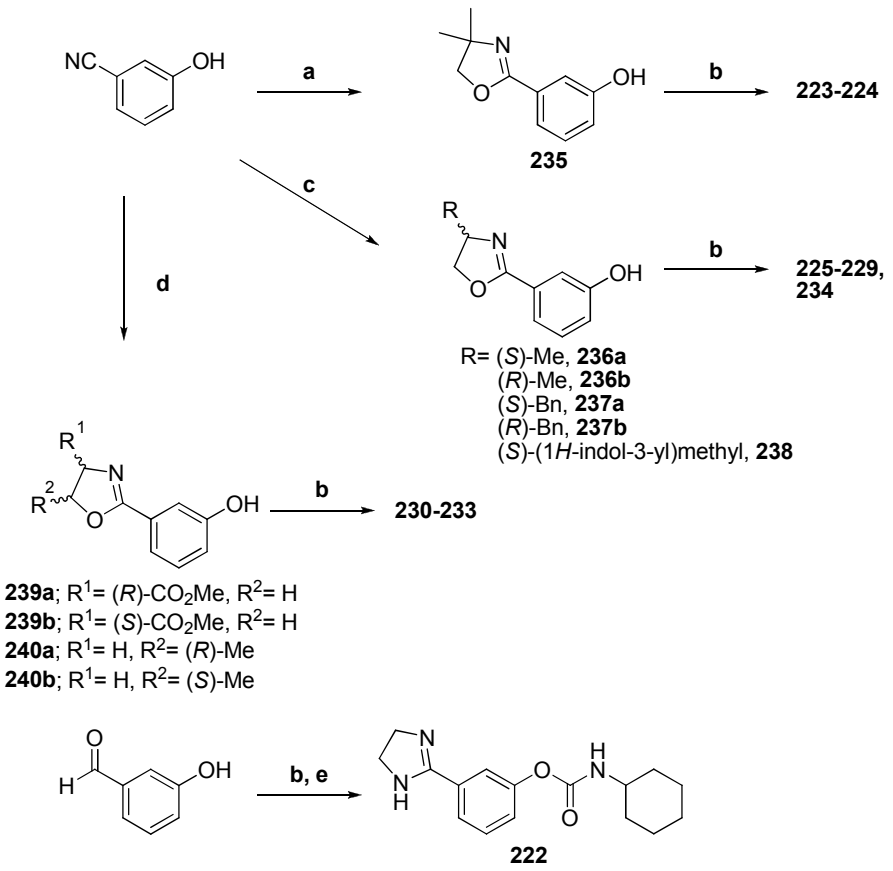
Compound	R^1	R^2	FAAH (nM)[a]	MGL[b]
220		*c*-pentyl	13 (11-14)	10%[c]
221		*c*-hexyl	1.2 (0.98-1.4)	22%
222		*c*-hexyl	215 (184-251)	37%
223		*c*-pentyl	65 (53-81)	28%
224		*c*-hexyl	46 (37-57)	20%
225		*c*-pentyl	110 (97-130)	28%
226		*c*-hexyl	51 (45-58)	28%

227		*c*-hexyl	16 (14-18)	16%
228		*c*-hexyl	2100 (1700-2700)	17%
229		*c*-hexyl	590 (510-690)	14%
230		*c*-hexyl	90 (77-110)	35%
231		*c*-hexyl	9.4 (7.8-11)	25%
232		*c*-hexyl	73 (58-93)	13%[d]
233		*c*-hexyl	6.8 (5.6-8.3)	14%[d]
234		*c*-hexyl	1900 (1600-2400)	25%

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 10 μM compound concentration (n= 2).
[b] Enzyme inhibition (%) at 100 μM compound concentration (n= 2).
[c] Inhibition of enzymatic activity (% of control) at 1 μM (n=2).
[d] Inhibition of human recombinant MGL.

Synthesis

Compounds **220** and **221** were prepared as described for **212**. **Scheme 9** illustrates the synthesis of compounds **222-234**. Imidazoline derivative **222** was prepared from the corresponding carbamoylated aldehyde and ethane-1,2-diamine by *N*-bromosuccinimide (NBS) cyclication.[218] Compound **235** was prepared by condensing 3-cyanophenol with 2-amino-2-methylpropanol using bismuth triflate catalysis under microwave irradiation.[219] Unfortunately this method gave poor yields. Thus, for the preparation of intermediates **236a-238**, the method by Witte and Seeliger[220] was applied. Compounds **239a-240b** were prepared in high yields via acidic Pinner-imidates[209].



Scheme 9. a) 2-Amino-2-methylpropanol, $Bi(OTf)_3$, MW / reflux, 3 min / 4 h, 29%; b) RNCO (R= *c*-pentyl or *c*-hexyl), Et_3N, toluene, rt or 90 °C, 4-24 h, 52-95%; c) Aminoalcohol, $ZnCl_2$, PhCl, reflux, 22 h, 33-90%; d) i. HCl-gas, MeOH, CH_2Cl_2, 2 °C, 3 d, 92%; ii. Aminoalcohol, (Et_3N in case of serine hydrochlorides), CH_2Cl_2, reflux, 2-18 h, 68-95%; e) Ethane-1,2-diamine, NBS, CH_2Cl_2, -2 °C-rt, 85%.

Discussion

2-Imidazoline (**222**, 215 nM) gave clearly lower FAAH inhibition than the corresponding 2-oxazoline derivative (**221**, 1.2 nM). Thus 2-oxazolyl was chosen as the heterocyclic part of compounds in the enantiomeric pair study. Substitution in the 4-position of the oxazoline ring decreased inhibition. A clear relationship between the activity against FAAH and stereochemistry of the 4-position of oxazoline was revealed by the data of compounds **226-233**. With 4-methyl (**226** vs. **227**) and 4-benzyl (**228** vs. **229**) substituents, the difference in activity between enantiomers was only 3-fold, but with methyl carboxylate (**230** vs. **231**) already 10-fold. This could be explained by the methyl ester's additional hydrogen bonding site or by the optimal size of the substituent. The lower activity of benzyl substituent

analogues in general indicates that the enantiomeric differences arise from a rather hindered region. It might be that interactions between FAAH active site and benzyl group lead to a suboptimal positioning of the carbamate functionality in the vicinity of the catalytic serine. Thus the effect of a substituent in 5-position of oxazoline was studied with 5-methyl analogs **232-233**. A bulky *S*-indolyl group (**234**) decreased the activity, and the other enantiomer was not prepared. None of the compounds tested showed good activity against MGL at 100 µM compound concentration.

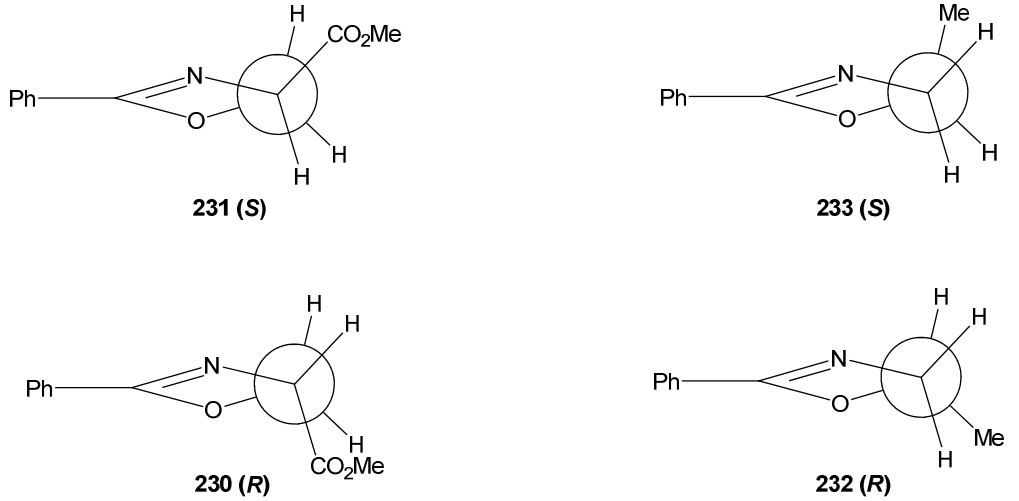


Figure 24. Compounds **231** and **233** are 10-fold more active than their enantiomeric pairs.

The stereochemistry of the compounds is illustrated in **Figure 24**. In these Newman projections the more active compounds **231** and **233** are the ones with their chiral carbon substituent “up” from the plane of the ring. This trend was also present in other enantiomeric pairs. These examples suggest that the stereochemistry in the oxazoline is more important than the regiochemistry of substitution (C4 or C5). This implies that the oxazoline ring conformation is locked within the enzyme's active site. This would explain the activity difference between the enantiomeric pairs since the substituent of the chiral carbon is pointing to a specific direction and thus filling the possible hydrophobic pocket or conversely causing steric hindrance.

4.2.6. 5-Membered aromatic heterocycles as *O*-phenyl carbamate substituents

During the study of chiral 2-oxazoline derivatives (previous chapter) other heterocycles were also evaluated as possible FAAH-inhibitors. At this point *N*-cyclopentylcarbamate was kept as the *N*-alkyl moiety of the lead (**Figure 23**). Later cyclopentyl was replaced with cyclohexyl.

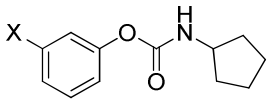
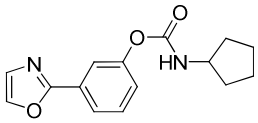
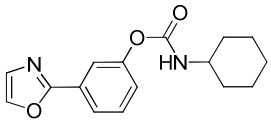
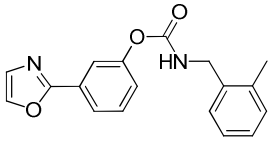


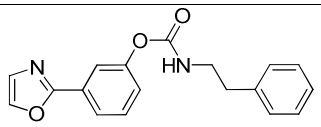
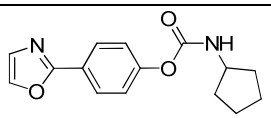
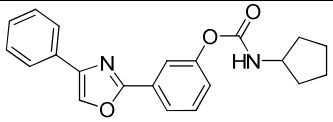
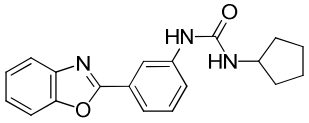
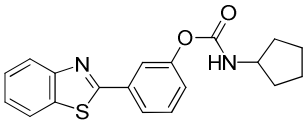
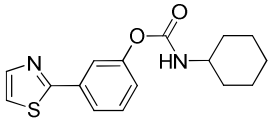
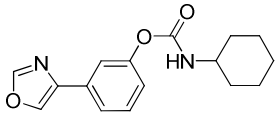
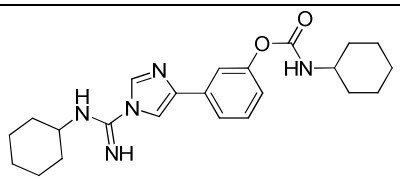
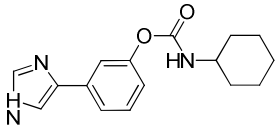
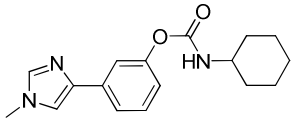
Figure 23. General structure of the lead for the optimization of the X-substituent.

Oxazoles, imidazoles and thiazole

The first modification was to prepare the aromatic derivative of **212,** and compounds **241-253** were prepared for this purpose (**Table 12**). Compound **241** appeared to be such a potent FAAH inhibitor (IC_{50} 5.2 nM) that a small series of *N*-alkyl carbamates (**242**-**245**) and a *para*-substituted (**246**) compound were also prepared. Screening concentrations for both enzyme inhibition measurements were lowered from 10 to 0.1 μM for FAAH, and from 100 to 1 μM for MGL. This was done since majority of screened carbamate compounds were active in earlier concentrations leading to increasing number of laborous IC_{50} determinations.

Table 12.

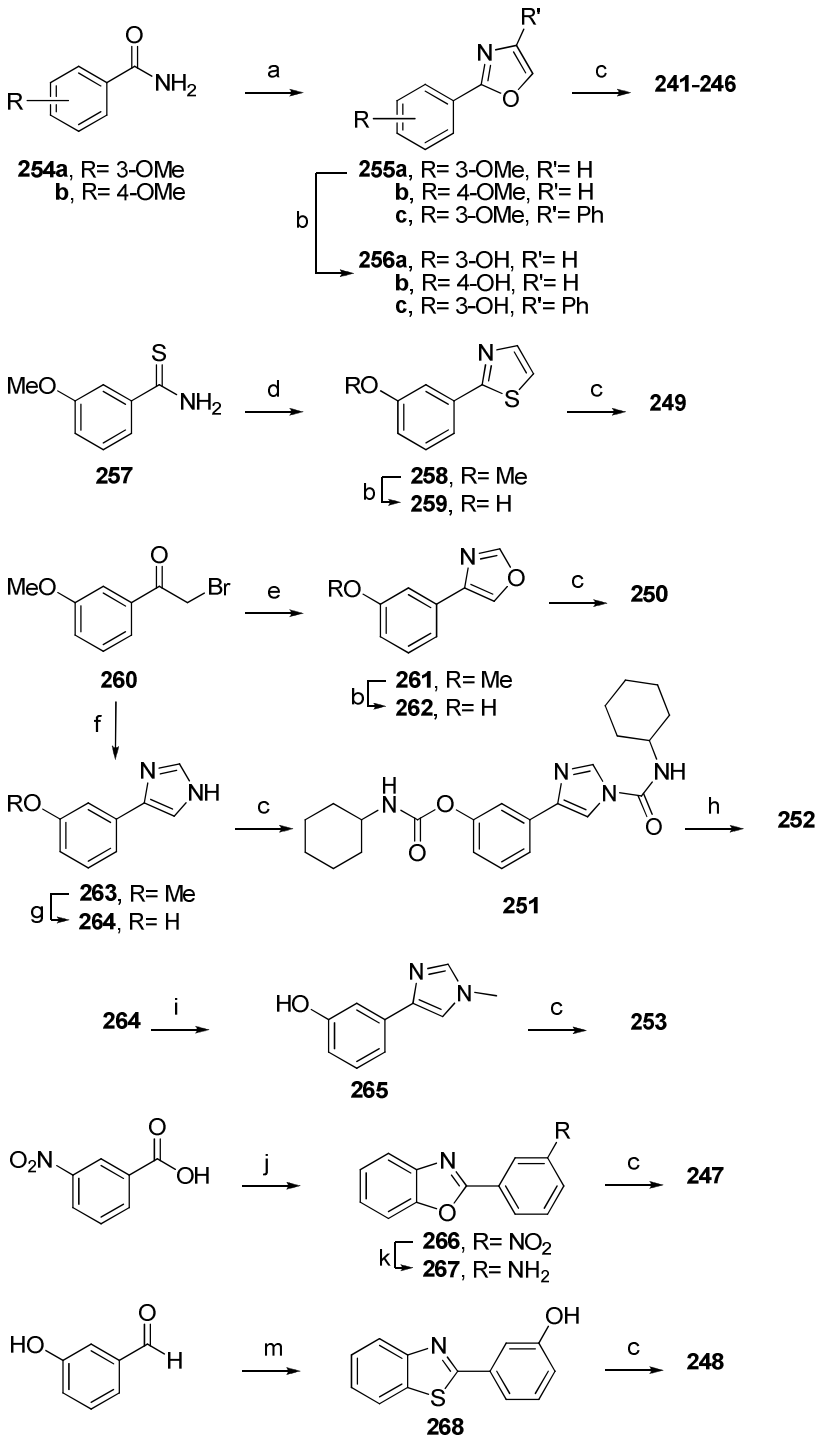
Compd	**Structure**	**FAAH (nM)**[a]	**MGL**[b]
241		5.2 (4.6-5.9)	11%[c]
242		0.74 (0.59-0.92)	26%
243		6.6 (5.0-8.7)	9%[c]

244		11.9 (9.5-15.0)	6%[c]
245		91 (74-113)	8%[c]
246		0%[d]	10%
247		0%[d]	0%[c]
248		0%[d]	8%[c]
249		5.7 (5.0-6.6)	22%
250		11 (10-13)	14%
251		43 (37-48)	13%
252		3.3 (2.8-3.8)	14%[c]
253		6.9 (5.5-8.7)	8%[c]

[a] IC_{50} values represent the mean of three independent experiments (n= 3) performed in duplicate (95% confidence intervals (95% CI) are given in parentheses) or enzyme inhibition (%) at 10 µM compound concentration (n= 2).
[b] Enzyme inhibition (%) at 100 µM compound concentration (n= 2).
[c] Inhibition of enzymatic activity (% of control) at 1 µM (n=2).
[d] Enzyme inhibition (%) at 100 nM compound concentration (n= 2).

Synthesis

Microwave-assisted condensation for preparation of 2-oxazoles, 4-oxazoles and 2-thiazole moieties was developed for preparation of the most of this series. First 3- or 4-methoxybenzamides (**254a**-**b**) or 3-methoxybenzothioamide (**257**) were condensed with bromoacetaldehyde diethyl acetal to gain 2-oxazoles **255a**-**c** and 2-thiazole **259**. The reactions were fast and selective. In addition, solvents or other reagents were not needed, except for the preparation of thiazole **258** THF was used as a solvent. MW-assisted condensation was productive also for the preparation of 4-oxazole **261** from 3-methoxy-2'-bromoacetophenone **260** and formamide. Heating the same starting materials in an oil bath gave the corresponding imidazole (**263**) as product. This result correlates with the Brederecks reports about formamide reactions.[221] The demethylations of phenyl methyl ethers were carried out using an excess of ionic liquid (*N*-butylpyridinium bromide or *N,N*,-dimethylimidazolium bromide) under microwave irradiation.[205] Unfortunately the cleavage of methyl ether of the imidazole derivative **263** in similar conditions failed, giving *N*-butylated compounds. The desired product was eventually achieved by using classical BBr_3-demethylation[222] or by adding two equivalents of aq. 48% HBr in *N*-BuPyBr / MW -demethylation. 2-(3-Nitrophenyl)benzo[*d*]oxazole **266** was prepared by MW-assisted condensation (as pyridineoxazoles in chapter 4.4.6.). Reduction to amine **267** provided the desired nucleophile for carbamoylation, resulting in compound **247**. Method for the preparation of 3-(benzo[*d*]thiazol-2-yl)phenol **268** was found from literature[223], utilizing ionic liquid for (oxidative) condensation of aldehyde and 2-thiophenol. **Scheme 10**.



Scheme 10. Reagents and conditions: a) 2-Bromoacetaldehyde diethyl acetal (2-bromo-1-phenylethanone for **17c**), neat, MW-irradiation, 5 min, 45-79%; b) 1-Butyl-3-methylimidazolium bromide or *N*-butylpyridinium bromide, MW-irradiation, 4 x 20 s, 100 ^{o}C 60-65%; c) Et_3N, RNCO, toluene, reflux, 43-90%; d) 2-Bromoacetaldehyde diethyl acetal, THF, MW-irradiation, 30 min, 100 ^{o}C, 42%; e) Formamide, MW-irradiation, 100 ^{o}C, 51%; f) Formamide, 165 ^{o}C, 85%; g) *N*-

Butylpyridinium bromide, HBr, MW-irradiation, 5 x 20 s, 100 ^{o}C, 53%; or BBr_3, CH_2Cl_2, -78 ^{o}C-rt, 80%; h) NaOH, MeOH, CH_2Cl_2, rt, 72 h, 71%; i) MeI, DMF, 0 ^{o}C-rt, 48%; j) 2-Aminophenol, boric acid, MW, 250 ^{o}C, 5 min, 77%; k) H_2, Pd/C, THF:EtOH (1:9), rt, 90 min, 90%; m) 2-Aminothiophenol, 1-butyl-3-methylimidazolium bromide, MW, 200 ^{o}C, 5 min, 73%.

In some cases the carbamoylation of phenol as the last reaction step was found difficult. In the carbamoylation conditions used (isocyanate, TEA) the imidazole group of **264** was found to be more nucleophilic than the phenolic hydroxyl, and both functionalities were carbamoylated, resulting in compound **251**. Ureas are known to react with alcohols[224], and selective alcoholysis of **251** was attempted and compound **252** was achieved. This method was poorly reproducible, since when the reaction was repeated, the cleavage of both carbamate and urea took place as a main reaction, and desired compound was found as a minor product only. The better nucleophilicity of the imidazole compared to the phenol was then exploited in methylation of compound **264** by iodomethane giving compound **265** (**Scheme 10**).

Discussion

Compound **242** appeared to be the most active compound against FAAH prepared within this work so far. This correlates to the findings of Piomelli *et al.*[141,143] that in many cases cyclohexylcarbamate functionality with the phenyl having a suitable polar group in 3-position gives best activities against FAAH. Although other *N*-alkyl derivatives of **242** with similar lipophilicity (**241** and **243-244)** were also active, **242** was ten-fold more active than these. These differences can be explained by a rather hindered substrate recognition site, ACB-channel, where the *N*-alkyl group is pointing.

Adding a bulky phenyl substituent to 4-position of the oxazole (**245**) resulted in loss of nanomolar activity. Compound **246** was also clearly less active (91 nM) than its *meta*-substituted derivative **241**. This supports our previous findings concerning activity differences of *meta*- and *para*-substituted *O*-phenyl carbamates as FAAH inhibitors. Compounds **247**-**248** were tested to fill out the series. These were not able to attain nanomolar inhibition activity, as was expected. Compound **249** having a thiazole moiety instead of oxazole and compound **250** in which the oxazole is 4-substituted gave a clearly lower activity than **242**, indicating that CA-channel

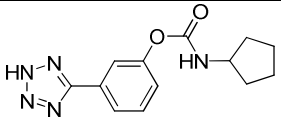
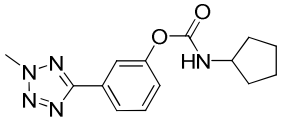
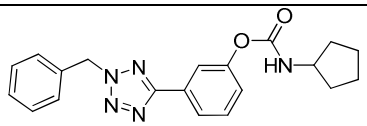
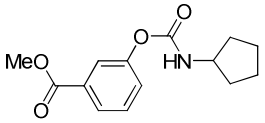
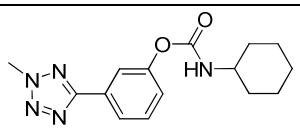
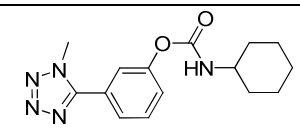
probably contains some backbone hydrogen bond donor sites serving as points of recognition.

With the imidazole series (**251**-**253**) an interesting result was found. The rather bulky "double electrophile" containing **251** was able to inhibit FAAH at 43 nM concentration, albeit being less active than the smaller derivatives **252-253** (3.3 and 6.9 nM). The slight difference in activity of **252** and **253** could be explained by steric hindrance in active site, as with chiral oxazolines earlier, but the activity of **251** does not support this idea.

5-Tetrazoles

Tetrazoles are widely used in medicinal chemistry as rigid functional groups and nitrogen bioisosteres of carboxyl group.[225] Tetrazole compounds prepared for this study are presented in **Table 13**. In addition, one methyl ester was included to this series as a bioisostere of methyltetrazole.

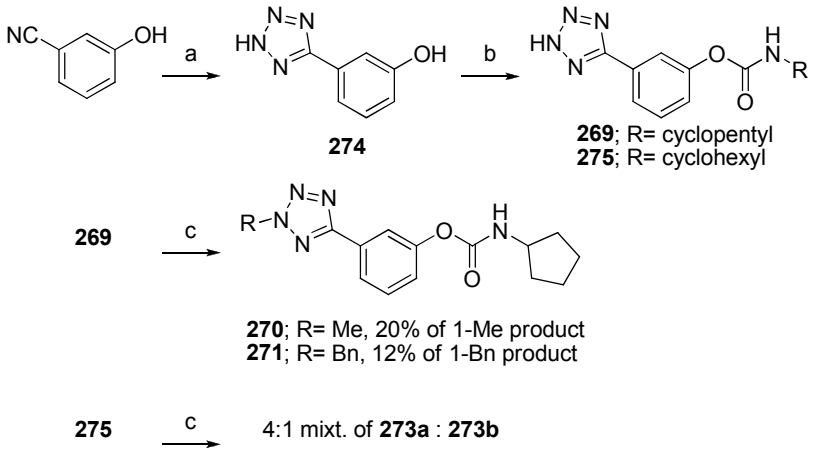
Table 13.

Compd	Structure	FAAH (nM)[a]	MGL[b]
269		21 (18-24)	16%
270		2.5 (2.0-3.1)	13%
271		39 (33-45)	17%
272		17 (13-21)	44%
273a		1.1 (0.9-1.4)	24%[c]
273b		66 (56-76)	24%[c]

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses).
[b] Enzyme inhibition (%) at 100 μM compound concentration (n= 2).
[c] Assay: human recombinant MGL. Inhibition activity in 100 μM compound concentration (n= 2).

Synthesis

5-Tetrazole moiety was constructed by cycloaddition reaction of 3-cyanophenol and sodium azide in the presence of triethylammonium hydrochloride. The resulting phenol **274** was then carbamoylated by either cyclopentyl- or cyclohexylisocyanate. Compounds **270-271** and **273a-b** were prepared from **269** and **275** by the alkylation of the tetrazole. In the methylation of tetrazole the isomeric ratio was 4:1 in favor of the less hindered 2-position (**270** and **273a**). In the benzylation reaction a similar trend in regioselectivity was observed, albeit with the ratio of 8:1 for the 2-isomer. 1-Substituted methyltetrazole **273b** was isolated from the mixture of these regioisomers by flash chromatography (**Scheme 11**). Ester **272** was prepared in the usual way.



Scheme 11. Reagents and conditions: a) NaN_3, Et_3NHCl, toluene, reflux, 85%; b) Et_3N, RNCO, toluene, reflux, 80%; c) MeI, Et_3N, acetone, 2 ^oC, 44% or BnBr, KI, Et_3N, acetone, 2 ^oC, 60%.

Discussion

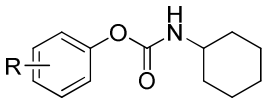
In addition to *N*-cyclohexylcarbamates, a series of *N*-cyclopentylcarbamates with 5-tetrazole and methyl ester substituents in the *O*-phenyl ring were prepared. The compound **269** exists most likely in an ionized form at physiological pH, which probably affects its inhibition activity, thus explaining the activity difference between **269** and **270**. Interestingly, the benzyl group (**271**) did not notably decrease

the FAAH inhibition. As a conclusion, the FAAH inhibition by tetrazolyl compounds elucidated that the position of the tetrazole's substituent (**273a** vs **273b**) was as important a factor as its size (**270** vs **271**). With the tetrazole compounds, the replacement of *N*-cyclopentyl with *N*-cyclohexyl group (**270** vs **273b**) resulted in only two-fold improvement of the inhibition potency against FAAH. Methyl ester **272** (17 nM) was found to be a 7-fold less potent FAAH inhibitor as its bioisostere **271** (2.5 nM), but roughly as active as the rest of the series. Compound **272** was chosen to be the lead for next modifications.

4.2.7. Small polar groups as *O*-phenyl carbamate *meta*-substituents

After discovering the inhibition potency of methyl ester **272** (IC_{50} 17 nM) some other polar groups were prepared and tested against the enzymes.

Table 14.

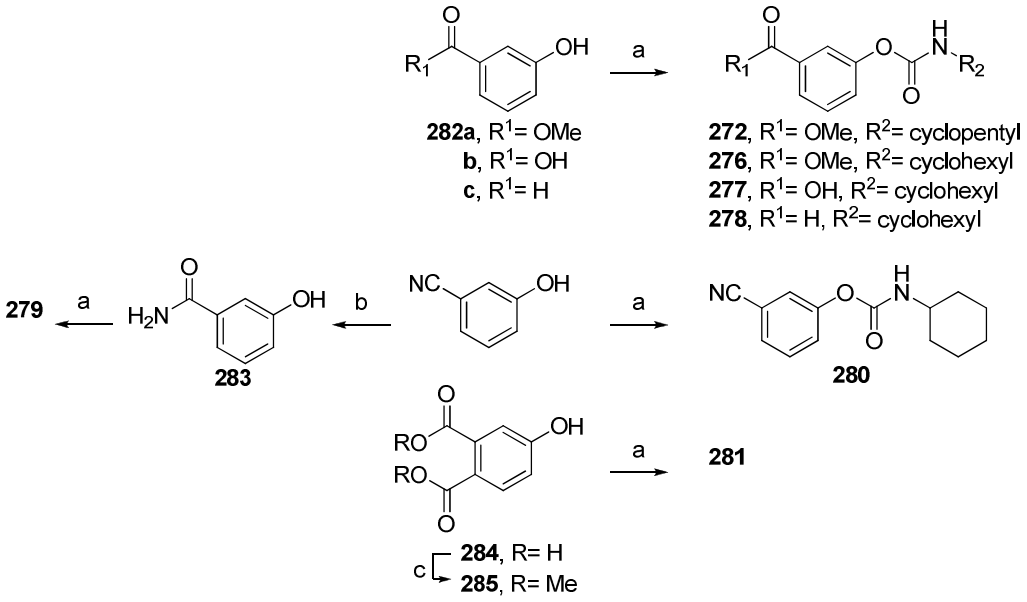


Compd	R	FAAH (nM)[a]	MGL (μM)[a]
276	3-CO_2Me	3.9 (3.3-4.7)	34%
277	3-CO_2H	9%	14%
278	3-CHO	100 (87-115)	31%
279	3-$CONH_2$	62 (54-72)	18%
280	3-CN	49 (42-58)	34 (26-45)[b]
281	3,4-di-CO_2Me	23 (19-27)	12 (9.3-15)

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 100 nM (for FAAH) and 100 μM (for MGL) compound concentrations (n= 2).
[b] Inhibition of enzymatic activity was 86% at 1 mM.

Synthesis

Esters **272**, **276** and **281** were prepared from corresponding carboxylic acids by refluxing them in methanol in the presence of catalytic amount of H_2SO_4. Carbamoylations were carried out in the usual way. The amide **283** was prepared by oxidation of the nitrile with sodium perborate[226] prior to carbamoylation. **Scheme 12**.



Scheme 12. a) Et_3N, RNCO, toluene, reflux, 80-95%; b) $NaBO_3$ • 4 H_2O, H_2O, MeOH, 54%; c) MeOH, H_2SO_4, reflux, 98%.

Discussion

The free acid **277** (9% inhibition at 100 nM) was not as potent against FAAH as the ester **276** (3.9 nM). This supports the hypothesis found with tetrazoles, that acidic (anionic) species are not tolerated. Aldehyde **278**, amide **279** and nitrile **280** were all clearly less active than the ester **276**. The diester **281** revealed to be interesting by relatively good MGL-inhibition (12 μM). This was one of the rare compounds tested in this study that inhibited MGL fully at higher concentration. Most of the carbamates inhibiting MGL are not able to fully block the enzyme and some "residual" enzymatic activity is found with even 1 mM concentration.

4.2.8. *para*-Substituted phenolic *N*-alkyl carbamates[153]

SPB01403 (**120**), found by virtual screen of compounds agains CB2 receptor,[152] was used as the lead to a series of *para*-substituted phenolic alkyl carbamates against MGL and FAAH (**Table 14**). Consequently, the structurally similar compound, SEW01169 (**286**, **Figure 25**), was purchased and tested for its FAAH and MGL inhibitory activity, and it was found to be a slightly more potent FAAH inhibitor (IC_{50} 160 nM) than **120**, albeit less potent inhibitor against 2-AG hydrolysis with

IC_{50} value of 43 μM. Being even more active than **120** (IC_{50}: FAAH, 520 nM, MGL 31 μM) against FAAH, **286** was used as a another lead (**Table 15**).

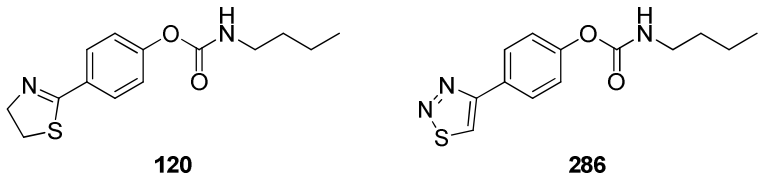
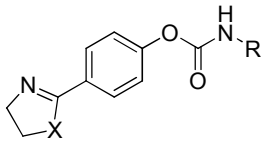


Figure 25. The lead structures for study of *para*-substituted carbamates, SPB01403 (**120**) and SEW01169 (**286**).

4-(2-Thiazolin-2-yl)- and 4-(2-oxazolin-2-yl)-phenyl N-alkyl carbamates

Table 14.

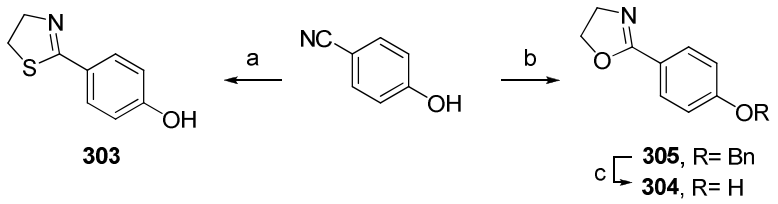


Compd	X	R	FAAH (nM)[a]	MGL (μM)[a]
287	S	ethyl	15000 (10000-22000)	102 (80-131)[b]
288	S	*n*-propyl	1700 (1300-2300)	46 (38-56)[c]
120	S	*n*-butyl	520 (390-700)	31 (25-39)
289	O	*n*-butyl	168 (145-197)	34 (28-41)
290	S	*n*-hexyl	8.2 (6.8-9.9)	8.2 (6.4-10)
291	S	dodecyl	6.3 (4.5-8.7)	2.4 (1.8-3.2)
292	S	*t*-butyl	7000 (5000-10000)	0%
293	S	*c*-pentyl	127 (109-148)	11 (9-13)[c]
294	O	*c*-pentyl	100 (77-130)	11 (9-14)[d]
295	S	*c*-hexyl	262 (198-346)	27 (21-35)[b]
296	S	benzyl	273 (236-316)	30 (24-39)[b]
297	S	2-methylbenzyl	62 (56-60)	35 (30-42)[b]
298	O	2-methylbenzyl	34 (31-38)	31 (27-36)[b]
299	S	4-methylbenzyl	281 (198-399)	67 (57-80)[b]
300	O	4-methylbenzyl	182 (136-243)	85 (70-102)[c]
301	S	4-methoxybenzyl	317 (252-340)	46 (38-55)[d]
302	S	$(CH_2)_2Ph$	38 (35-42)	28 (25–31)

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 100 μM (for MGL) compound concentrations (n= 2).
[b] Remaining enzyme activity at 1 mM was 12-19%.
[c] Remaining enzyme activity at 1 mM was 6-7%.
[d] Remaining enzyme activity at 1 mM was 21-26%.

Synthesis

4-(4,5-Dihydrothiazol-2-yl)phenol **303**[227] was prepared by simply heating a mixture of cysteamine and 4-cyanophenol (**Scheme 13**). Unfortunately this method was not suitable for the preparation of 4-(4,5-dihydrooxazol-2-yl)phenol **304**, thus it was obtained by the reaction described by Witte and Seeliger[220]. For this purpose the hydroxyl group of 4-cyanophenol was protected as the benzyl ether **305**[228].



Scheme 13. Reagents and conditions: a) 2-Aminoethanethiol, 100 °C, 10 min, 88%; b) i. BnBr, K_2CO_3, KI, acetone, reflux, 2 h, 98%; ii. 2-Aminoethanol, $ZnCl_2$, C_6H_5Cl, reflux, 48 h, 50%; c) H_2, Pd/C, ethanol, rt, 90 min, 75%.

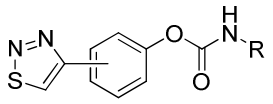
Discussion

Among the dihydrothiazoline moiety containing open chain *N*-alkyl carbamates (**120**, **387**-**391**), the chain length was the main factor affecting the inhibition potencies toward both enzymes. Compounds **390** and **391** inhibited hrMGL-mediated 2-AG hydrolysis with IC_{50} values of 8.4 μM and 2.1 μM, respectively. FAAH inhibition with these compounds was in low nanomolar range. Replacement of the open chain *N*-alkyl groups with the bulky *N*-*tert*-butyl group (**292**) reduced the inhibitory potencies toward FAAH and MGL (IC_{50}s; 7.0 μM and >100 μM, respectively). This is probably due to steric hindrance near the carbamate group, thus interfering with crucial interactions within the active sites of the enzymes. The differences in the FAAH and MGL inhibition potencies among the other dihydrothiazoline derivatives with different *N*-alkyl groups (**293**-**302**) were mainly not notable. However, with cycloalkyl and benzylic groups (**293**-**301**) complete MGL inhibition was not observed, even at the highest concentrations tested (1 mM). This remaining enzyme activity can be explained by either an additional enzyme

activity distinct from MGL or by the low aqueous solubility of these compounds. An interesting observation concerning FAAH inhibition was that the *N*-2-methylbenzyl derivatives **297**, **298** (IC_{50}: 62 and 34 nM) and phenethyl derivative **302** (IC_{50} 38 nM) were more potent FAAH inhibitors than the other derivatives with cyclic or benzylic *N*-alkyl moiety (IC_{50}s: 0.10–0.32 μM). When comparing the activities of thiazoline and oxazoline derivatives, no clear difference in the inhibition potencies toward either FAAH or MGL was seen.

Thiadiazolyl-derivatives

Table 15.



Compd	*o, m, p*	R	FAAH (nM)[a]	MGL (μM)[a]
286	*para*	*n*-butyl	160 (130-200)	43 (27–66)[b,c]
306	*para*	*n*-hexyl	19 (15-24)	11 (9.8–13)
307	*para*	dodecyl	12 (11-14)	1.9 (1.7–2.1) 1.0 (0.8–1.4)[b]
308	*para*	*c*-pentyl	20 (14-29)	44%[b]
309	*para*	*c*-hexyl	21 (18-25)	28%
310	*para*	benzyl	44 (34-56)	24 (15–39)[b,c]
311	*ortho*	*n*-butyl	0%	27%[b]
312	*ortho*	dodecyl	50 (42-58)	18 (12-26)[b,c]
313	*meta*	*n*-butyl	6.9 (5.4-8.8)	13%
314	*meta*	*n*-dodecyl	0.24 (0.2-0.3)	8.0 (5.9–11.0)[b,d]

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 100 nM (for FAAH) and 100 μM (for MGL) compound concentrations (n= 2).
[b] Inhibition of hrMGL.
[c] Remaining enzyme activity 22–26% at 1 mM.
[d] Remaining enzyme activity was 13% at 1 mM.

Discussion

Compounds presented in the **Table 15** were prepared by Ms Anna Minkkilä, thus their preparation is not described in this thesis.

Not surprisingly, in this series of 1,2,3-thiadiazol-4-yl substituted derivatives (**286**, **306**-**314**), the length and the shape of the *N*-alkyl group had a similar effect on the inhibitory potencies against both enzymes, FAAH and MGL, as it has been in the previous series. Thus, by replacing the *N*-*n*-butyl chain of **286** with *N*-*n*-hexyl (**306**) or *N*-dodecyl (**307**), the inhibitory potencies toward FAAH and MGL were improved, resulting in the most potent MGL-inhibitor in this series. Compound **307** inhibited FAAH with an IC_{50} value of 12 nM and, hydrolysis of 2-AG in rat brain membranes as well as hrMGL-mediated 2-AG hydrolysis with IC_{50} values of 1.9 µM and 1.0 µM, respectively. When comparing these results to those observed with the corresponding dihydrothiazoline derivatives **290** and **291**, it can concluded that the heterocyclic moiety in these two compounds has a minor effect on their FAAH and MGL inhibitory capacities. However, the compounds with *N*-cyclopentyl (**308**), *N*-cyclohexyl (**309**) and *N*-benzyl (**310**) moieties were ten-fold more potent FAAH inhibitors than the corresponding thiazoline and oxaxoline derivatives **293**-**296**. This effect was not seen for MGL.

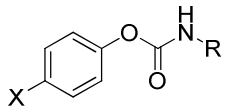
To support our previous results of the influence of the substituent's position in the phenyl ring on the FAAH and MGL inhibitory activities (i.e. *para*-substitution is more favourable to the MGL inhibitory activities)[197], *meta*- and *ortho*-analogues of **286** and its most potent derivative with *N*-dodecyl group (**307**) were prepared and tested (compounds **311**-**314**). Compound **311**, the *ortho*-substituted analogue of **286**, did not inhibit either of the enzymes; only 27% inhibition of MGL was observed at 100 mM, and no inhibition of FAAH could be detected at 0.1 mM. On the contrary, the *meta*-substituted analogue **313** inhibited FAAH with an IC_{50} value of 6.9 nM. However, only 13% inhibition of MGL by **313** was observed, which is similar to the results obtained with the *ortho*-substituted **311** and the *para*-substituted **286**. The *meta*-substituted compound **314** with a more lipophilic *N*-dodecyl group inhibited MGL with an IC_{50} value of 8.0 µM, being 8-fold less potent than the *para*-substituted analogue **307**. Moreover, **314** inhibited FAAH with an IC_{50} value of 240 pM. The *ortho*-substituted compound **312** with *N*-dodecyl group inhibited both FAAH and MGL, although with higher IC_{50} values than *para*- and *meta*-substituted analogues **307** and **314**. These data clearly support the findings that *para*-

substitution is more favourable to MGL inhibition, whereas *meta*-substitution is favourable for FAAH.[197]

The effect of para-substituents with different electronic properties

The catalytic site serine of FAAH and MGL is expected to have a strong interaction with the reactive carbonyls of the ligands. Therefore, it was decided to study whether the electronic properties of the *para*-substituent in *O*-phenyl carbamates have an effect on the FAAH and MGL inhibitory activities by preparing and testing the compounds **315**-**328** (**Table 16**). The presumption was that compounds containing electron withdrawing groups (EWG) in *para*-position on the phenyl ring would increase the electrophilicity of the carbonyl carbon, thus having higher inhibitory activity than corresponding compounds with electron donating groups (EDG).

Table 16.



Compd	X	R	FAAH (nM)[a]	MGL (μM)[a]
315	-H	*n*-hexyl	91 (75-110)	41%
316	-H	dodecyl	92 (76-112)	36%
317	-Me	*n*-hexyl	27%	0%[b]
318	-Br	*n*-hexyl	18 (16-20)	17 (11-26)[b,c]
319	-OMe	*n*-butyl	5%	0%[b]
320	-CO_2Me	*n*-butyl	35%	19 (12-29)[b]
321	-CO_2Me	*n*-hexyl	31 (23-41)	35 (30-41.5) 19 (16-24)[b]
322	-CO_2Me	dodecyl	25 (22-30)	2.8 (2.5-3.2)
323	-CO_2Me	*c*-hexyl	41%	14 (12-18)
324	-NO_2	*n*-butyl	16%	24 (18-33)[b]

325	-NO_2	*n*-hexyl	27 (21-35)	18 (12–27)[b]
326	-NO_2	*c*-hexyl	25%	19 (17-21)
327	-CN	*n*-butyl	25%	30 (20–45)[b]
328	-CN	*n*-hexyl	13 (12-14)	12 (9.0–17)[b]

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 100 nM (for FAAH) and 100 μM (for MGL) compound concentrations (n= 2).
[b] Inhibition of hrMGL.
[c] Remaining enzyme activity at 1 mM was 16%.

Discussion

The synthesis of compounds presented in the **Table 16** was carried out by Ms Anna Minkkilä, except compounds **317**, **318**, **327**, **328.** These were prepared from commercially available phenols and isocyanates in the usual way. The EWG and EDG had opposite effects on the inhibitory potencies against MGL. EWG (methyl ester, nitro, and cyano) substituted compounds **320**-**328** maintained the potencies against MGL similar to those of the corresponding *N*-alkyl heterocycle derivatives presented in **Tables 14**-**15**. EDG (methyl and methoxy) substituted compounds **317** and **319** had clearly diminished inhibition potencies. However, differences in inhibitory potencies against FAAH were hardly notable when comparing the results of EWG-substituted *N*-*n*-butyl derivatives **320**, **324**, and **327** with that of corresponding methoxy-substituted **319**. On the contrary, within *N*-*n*-hexyl derivatives EWG-substituted **318**, **321**, **325** and **328** were more potent than methyl-substituted **317**. Clear correlation was not achieved by trying to fit data values of *N*-*n*-hexyl into a plot of pIC_{50} vs. Hammet constant (σ_p). However, a rough correlation of FAAH inhibition activity to substituent σ_p-constants is presented in **Figure 26,** which illustrates the electronic effect.

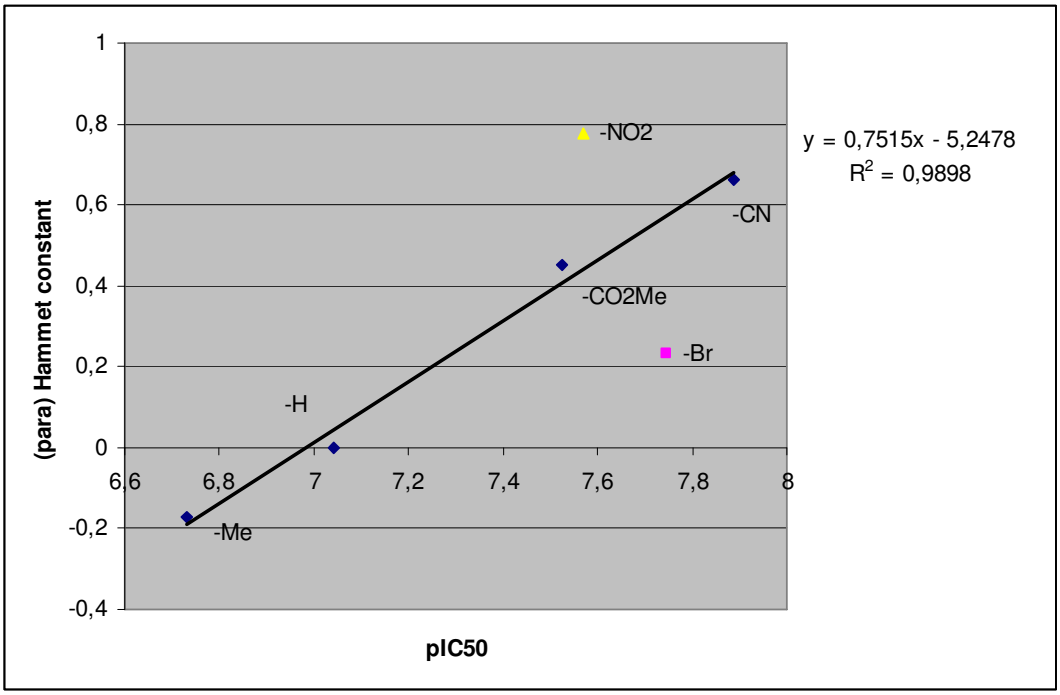


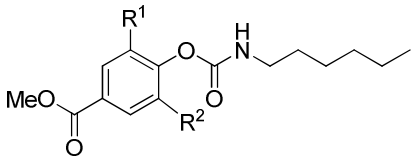
Figure 26. pIC_{50} vs. σ_p-constant[229] of **317** (-Me), **315** (-H), **318** (-Br), **320** (CO_2Me), **325** ($-NO_2$), **328** (-CN). Correlation is excellent when (-Br) and ($-NO_2$) are excluded. IC_{50} of **317** (-Me) was converted from 27% of inhibition at 100 nM to IC_{50} value of 185 nM.

Surprisingly, the nonsubstituted compounds **315** and **316** were quite potent FAAH inhibitors (IC_{50}s; 91-92 nM) in comparison with the EWG-substituted compounds, while Tarzia *et al.* have reported that phenylcarbamic acid cyclohexyl ester lacks inhibitory activity against FAAH[141]. This observed increase in FAAH inhibition potencies might be due to the more flexible and lipophilic nature of *N*-*n*-hexyl and *N*-dodecyl groups. A similar observation can be made when comparing the results of *N*-cyclohexyl (**323**, **326**) and/or *N*-*n*-butyl derivatives (**320**, **324**, and **327**) to those of corresponding *N*-*n*-hexyl (**321**, **325**, **328**) and *N*-dodecyl derivatives (**322**). Altogether, these results indicate that both FAAH and MGL inhibition potencies can be enhanced by EWG at *para*-position in the carbamates phenyl ring.

The effect of ortho-substituent

In order to study the effects of steric and electronic properties of *ortho*-substituents to MGL and FAAH inhibitory activity, four derivatives of **321** with *ortho*-substituent in the phenyl ring were prepared (**Table 17**).

Table 17.



Compd	R^1	R^2	FAAH (nM)[a]	MGL (μM)[a]
329	-OMe	H	5.5 (4.9-6.2)	4.2 (2.7–6.2)[b]
330	-Me	H	55 (38-79)	22 (18–27)[b]
331	-I	H	24 (21-25)	14 (11–16)[b]
332	-*t*-Bu	-*t*-Bu	4%	18%[b]

[a] IC_{50} values represent the mean of three independent experiments performed in duplicate (95% confidence intervals are given in parentheses) or enzyme inhibition (%) at 100 nM (for FAAH) and 100 μM (for MGL) compound concentrations (n= 2).
[b] Inhibition of hrMGL.

Discussion

Compounds presented in the **Table 17** were prepared by Ms Anna Minkkilä, thus their preparation and chemical data is not described in this thesis.

The *ortho*-methoxy substituent (**329**) improved the inhibitory potency toward both FAAH and hrMGL when compared to the results obtained with the parent compound **321**. This might be due to favourable interactions between the oxygen atom in the methoxy group and the active site residues of the enzymes. Me- or I-substitution (**331** and **330**) did not have a significant effect on the affinity toward either FAAH or MGL. As expected, a bulky di-*tert*-butyl substituent (**332**) diminished the affinity toward these enzymes notably.

5. SUMMARY

5.1. General discussion

Endocannabinoid hydrolyzing enzymes FAAH and MGL are very interesting target proteins. They are considered to be the most important enzymes in the endogenous cannabinoid system. FAAH efficiently hydrolyzes amides, both primary and secondary, and its main substrate is the endocannabinoid AEA. The role of MGL is to hydrolyze 2-AG, which is more abundant and a full agonist of cannabinoid receptors, and it seems that MGL could serve as the more important target enzyme. However, the first potent FAAH inhibitors were developed already over a decade ago, and since then numerous compounds from a variety of chemical classes have been introduced. MGL inhibitors have been presented to a much lesser extent in the literature, probably because of the speculative accuracy of homology models compared to a proper crystal structure, which is yet to be published. The current lack of selective and potent inhibitors of MGL and MGL gene-depleted animals are also disadvances in MGL studies, compared to FAAH. However, recent reports have revealed breakthroughs on selective and highly potent MGL inhibitors, which will most likely boost the scientific output on this field.

FAAH inhibitors are traditionally divided into two groups, reversible and irreversible inhibitors. The largest structural class of reversible inhibitors are α-ketoheterocycles; the most important irreversible inhibitors are carbamates and ureas. Other important irreversible inhibitors are fluorophosphonates, which have served as pharmacological tools in seminal enzyme studies (e.g. MAFP bound FAAH -crystal, FP-compounds in ABPP). The reversibility of activated ketones is based on a hemiacetal inhibitor-Ser241 intermediate, compared to irreversible inhibitors giving carbamoylated (or phosphorylated) Ser241. Obviously, the hemiacetal is more easily reverted to the active enzyme, than *N*-alkyl,*O*-Ser241-carbamate is hydrolyzed, which in turn is more easily hydrolyzed than the phosphorylated or alkylated (maleimide as Michael acceptor) species. As an example, complete recovery of central FAAH activity occurs 24 h after administration of covalent inhibitor URB597.[104] This is shorter than the enzyme

turnover in mice ($T_{1/2}$ about 52 h in mouse brain)[230], indicating either slow turnover of inhibitor *in vivo* or a more rapid resynthesis of FAAH.

Due to their reactive nature, irreversible inhibitors are often considered less selective than reversible ones. Fortunately, enzymes are constructed in a way that requires for substances to fulfill numerous properties, e.g. lipophilicity, shape, functionality, to reach the place of action. In the case of FAAH inhibitors, there are several examples indicating that reversibility/irreversibility does not correlate with selectivity.

Some of the recent FAAH inhibitors have also been well evaluated for their selectivity. This concerns at least piperidinyl and piperazidinyl ureas **125** and **126** and their analogues. Highly potent compounds of urea class have been presented, and this functional group can be considered as the next generation of carbamoylating inhibitors following carbamates. FAAH inhibitor efficacy is mainly ruled by shape and steric and lipophilic properties within the alkyl part of urea/carbamate, which is based on the structure of ACB channel and a yet unrevelealed entrance cavity in the FAAH active site. The leaving group cavity (CA channel) is wider, having possibly a better tolerance of structural differences. On the other hand, it consists of hydrophilic parts and leads to an aqueous environment, thus setting corresponding demands for leaving group properties. These have been shown to have an effect on activity of carbamoylating inhibitors. Phenol and aniline are good leaving groups providing easily tunable structures to gain more efficient inhibitors. Moreover, this study and others have shown that steric and hydrogen bond interactions in the CA channel also have a role in substrate recognition.

5.2. Conclusions

The present study includes design, preparation and characterization of 101 compounds, which were tested against FAAH and MGL enzymes. Various synthesis methods were used to achieve the desired compounds. Microwave-assisted novel or little known reactions applied in synthesis include condensation of carboxylic acids and 2-aminophenol/2-amino-3-hydroxypyridine to prepare corresponding fused 2-oxazoles, oxazole formation via condensation of bromoketones and amides, and cleavage of benzyl and methyl ethers using ionic liquids.

The compounds were almost exclusively novel (100) by the time they were prepared. FAAH inhibition of IC_{50} value below 100 nM was reached by 47 compounds and value between 0.1-1 µM by 22 compounds. The most potent FAAH inhibitor of this study was 3-(oxazol-2-yl)phenyl *N*-cyclohexylcarbamate (**242**) with an IC_{50} value of 740 pM. As a comparison, substrate-like 3-(1,2,3-thiadiazol-4-yl)phenyl dodecylcarbamate **314**, prepared by Anna Minkkilä, was only three-fold more active (IC_{50}; 240 pM).

MGL inhibition values were three orders of magnitude higher, and the most potent compounds were *para*-substituted *O*-phenyl carbamates with a *n*-hexyl, dodecyl, *c*-hexyl, or alkylphenyl as lipophilic part and an electron withdrawing group as phenyl substituent. None of the MGL inhibiting compounds were selective over FAAH. *N*-Alkyl phenyl carbamates can provide various modification sites to improve the selectivity and potency, but for the determination of selectivity it is important to have a proper method for selectivity screening.

The following conclusions can be made from the present study:

1. Activated ketones as FAAH inhibitors are very well studied by other groups, but their use as MGL-inhibitors has not yet been reported.
2. Both enantiomers of *α*-methylated derivatives of compound **61** are less potent FAAH inhibitors than the non-substituted parent compound. The steric hindrance in that position apparently reduces the affinity.
3. Within phenolic *N*-alkyl carbamates as enzyme inhibitors, FAAH prefers *meta*-substituted and MGL *para*-substituted compounds. This observation is based on studying several carbamate compounds.
4. Electron withdrawing substituent in phenolic *N*-alkyl carbamate can improve the potency of the inhibitors of both enzymes. This is apparently due to increased electrophilicity of the carbamate carbonyl, positive interactions to the active site residues, and/or improved leaving group properties of the phenol.
5. 4,5-Dihydrooxazol-2-yl, thiazolin-2-yl, oxazol-2-yl, 2-methyltetrazol-5-yl, imidazol-4-yl and 1,2,3-thiadiazol-4-yl were found to be the best heterocycle

substituents of the *O*-phenyl of carbamates. Methyl ester was the best acyclic substituent.

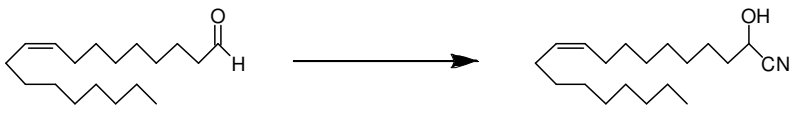
6. Chiral 4- or 5- monosubstituted 4,5-dihydrooxazol-yl compounds were less active than the unsubstituted one. Based on inhibition activity data collected from a series of enantiomeric pairs, 10:1 stereoselectivity was observed for FAAH.
7. FAAH/MGL-selectivity was high (>1000) for most of the *meta*-substituted phenolic *N*-alkyl carbamates.

6. EXPERIMENTAL

Experimental part includes all the compounds prepared in this work, in case their preparation and/or data are not fully described in the literature.

Commercially available starting materials were used without further purification. All dry reactions were performed under argon in flame-dried glassware and solvents were distilled. In microwave reactions CEM Discover -microwave reactor was used. Analytical thin-layer chromatography was carried out on Merck silica gel F254 (60 Å, 40-63 μm, 230-400 mesh) precoated aluminium sheets and detected under UV-light. Purification of reaction products was carried out by flash chromatography (FC) on J. T. Bakers silica gel for chromatography (pore size 60 Å, particle size 50 nM). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm on the δ scale from an internal standard (TMS 0.00 ppm) or residual solvent ($CDCl_3$ 7.26 and 77.0 ppm; DMSO-d_6 2.50 and 39.52 ppm). Melting points were determined in open capillaries using Stuart SMP3 and are uncorrected. Optical rotation data was recorded on Perkin Elmer 343 polarimeter using Na lamp (589 nm) and 100 mm cuvette at room temperature. HRMS-spectra were recorded on Waters Micromass LCT Premier (ESI) spectrometer. Chiral HPLC-analysis was carried out using Waters pump and UV-detector (254 nm) and Daicel Chiralcel AD analytical chiral column. Retention time (Rt) and ee-% are reported. Elemental analyses (CHN) were recorded using a Perkin Elmer 2400 CHN -elemental analyzer. Analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values.

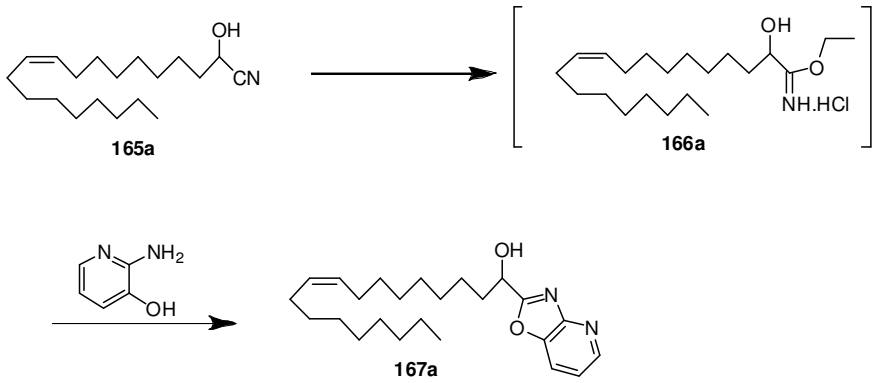
6.1. 2-Hydroxy-cis-10-nonadecenenitrile (165a)[124]



164a **165a**

Cis-9-octadecenal **164a** (320 mg, 1.2 mmol, 100 mol-%) was stirred with potassium cyanide (780 mg, 12 mmol, 1000 mol-%) in THF (4.0 ml) and water (4.5 ml) for 48 h at rt. Water (5 ml) and Et_2O (20 ml) were added and organic phase was separated. Aqueous phase was extracted with Et_2O (3 x 20 ml). Combined organic phases were washed with sat. $NaHCO_3$ (10 ml) and brine (10 ml), dried ($MgSO_4$) and evaporated giving compound **165a** (350 mg, 98%) as yellow oil; R_f (EtOAc) 0.7; ^{1}H NMR ($CDCl_3$) 5.38-5.29 (m, 2H), 4.44 (t, 1H, *J* = 6.6 Hz), 3.42 (br s, 1H), 2.10-2.00 (m, 4H), 1.85-1.79 (m, 2H), 1.60-1.20 (m, 22H), 0.89-0.85 (m, 3H); ^{13}C NMR ($CDCl_3$) 130.0, 129.6, 120.1, 61.2, 35.1, 31.1, 29.7, 29.6, 29.5 (2 C), 29.2, 27.2, 29.0, 28.9, 27.2, 27.1, 24.5, 22.6, 14.0.

6.2. 1-Oxazolo[4,5-b]pyridin-2-yl-octadec-9-en-1-ol (167a)[124]

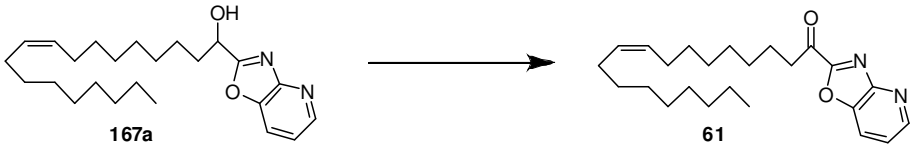


EtOH (1.4 ml, 24 mmol, 2000 mol-%) was treated with AcCl (1.3 ml, 1600 mol-%) in $CHCl_3$ (3.0 ml) at 0 ^{o}C. After 10 min 2-hydroxy-*cis*-10-nonadecenenitrile **165a** (350 mg, 1.2 mmol, 100 mol-%) was added in $CHCl_3$ (3 ml) and mixture was allowed to warm to rt for 20 h. The reaction mixture was evaporated to dryness giving compound **166a** (300 mg, 93%) as yellow solid: ^{1}H NMR ($CDCl_3$) 11.89 (s, 1H), 10.57 (s, 1H), 5.41-5.30 (m, 2H), 4.79-4.65 (m, 2H), 4.47 (dd, 1H, *J* = 8.0, 4.5 Hz), 2.05-1.98 (m, 4H), 1.80-1.75 (m, 2H), 1.51-1.40 (m, 5H), 1.38-1.23 (m, 20H), 0.89-0.85 (m, 3H); ^{13}C NMR ($CDCl_3$) 182.5, 130.0, 129.7, 71.9, 69.8, 33.7, 31.9, 29.8, 29.7, 29.5, 29.3 (3 C), 29.2, 29.1, 27.2 (2 C), 25.1, 22.7, 14.1, 13.7.

Crude imidate **166a** (100 mg, 0.29 mmol, 100 mol-%) was dissolved in 2-ethoxyethanol (1.5 ml) followed by addition of 2-amino-3-hydroxypyridine (48 mg, 0.44 mmol, 150 mol-%) under argon at rt. The reaction mixture was refluxed ($T_{oilbath}$= 130 ^{o}C) for 13 h, cooled and quenched by EtOAc:Et_2O (2:1, 30 ml) and 1

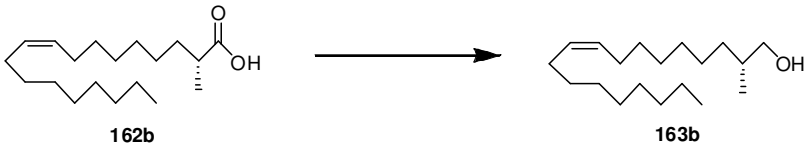
M NaOH (10 ml). Aqueous phase was extracted with EtOAc:Et_2O (2:1, 3 x 30 ml). Combined organic phases were washed with brine (3 x 20 ml), dried ($MgSO_4$), filtered and evaporated. Purification two times by FC (eluent, 50% EtOAc/hex) gave compound **167a** (5 mg, 4%) as yellow oil: R_f (10% MeOH/CH_2Cl_2) 0.6; ^{1}H NMR ($CDCl_3$) 8.59 (dd, 1H, *J* = 4.8, 1.1 Hz), 7.83 (dd, 1H, *J* = 8.2, 1.1 Hz), 7.30 (dd, 1H, *J* = 8.2, 4.8 Hz), 5.38-5.29 (m, 2H), 5.01 (dd, 1H, *J* = 7.4, 5.3 Hz), 3.01 (br s, 1H), 2.16-1.92 (m, 6H), 1.49 (quint, 2H, *J* = 7.4 Hz), 1.4-1.2 (m, 20H), 0.89-0.85 (m, 3H).

6.3. Oxazolo[4,5-b]pyridin-2-yl-octadec-9-en-1-one (61)[124]



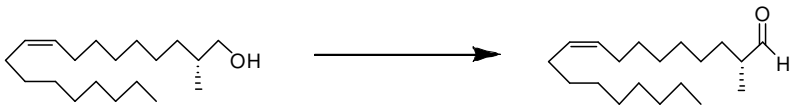
Iodoxybenzoic acid (160 mg, 0.57 mmol, 1000 mol-%) was stirred in DMSO (2 ml) at rt until all solids dissolved. Compound **167a** (22 mg, 0.057 mmol, 100 mol-%) in DMSO (4 ml) was added and the mixture was stirred at rt for 2 h and quenched with water (10 ml) which caused formation of white precipitate. The suspension was filtered through cotton (flushed with water and EtOAc) and the filtrate was extracted with CH_2Cl_2 (4 x 25 ml). Combined organic phases were washed with brine (20 ml), dried ($MgSO_4$), filtered and evaporated. Purification by FC (eluent, 10% EtOAc/hex) gave compound **61** (20 mg, 92%) as yellow oil: R_f (50% EtOAc/hex) 0.47; ^{1}H NMR ($CDCl_3$) 8.76 (dd, 1H, *J* = 4.8, 1.4 Hz), 8.00 (dd, 1H, *J* = 8.3, 1.4 Hz), 7.49 (dd, 1H, *J* = 8.3, 4.8 Hz), 5.39-5.30 (m, 2H), 3.27 (t, 1H, *J* = 7.4 Hz), 2.05-1.98 (m, 4H), 1.82 (quint, 2H, *J* = 7.4 Hz), 1.44-1.25 (m, 20H), 0.87 (t, 3H, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$) 190.4, 158.3, 154.2, 148.7, 143.6, 130.0, 129.7, 123.1, 120.2, 39.8, 31.9, 29.8, 29.7, 29.5, 29.3 (2 C), 29.2, 29.1 (2 C), 27.2 (2 C), 23.8, 22.7, 14.1.

6.4. (R)-2-Methyl-cis-9-octadecenol (163b)[231]



$LiAlH_4$ (0.7 g, 18 mmol, 280 mol-%) was stirred in THF (60 ml) for 15 min at 0 °C. Carboxylic acid **162b** (2.0 g, 6.6 mmol, 100 mol-%) was added dropwise during 10 min and the mixture was allowed to warm to rt and stirred additional 4.5 h. The mixture was cooled on ice bath and quenched with water (0.5 ml), 10% NaOH (1.0 ml) and water (1.5 ml) in this order. Filtration through a pad of Celite, drying over anhydrous Na_2SO_4, evaporation and purification by FC (eluent, CH_2Cl_2) gave compound **163b** (1.7 g, 92%) as colourless oil; R_f (CH_2Cl_2) 0.4; ^{1}H NMR ($CDCl_3$) 5.38-5.29 (m, 2H), 3.51-3.38 (m, 2H), 2.07-1.98 (m, 4H), 1.64-1.54 (m, 1H), 1.4-1.2 (m, 22H), 0.91-0,87 (m, 6H); ^{13}C NMR ($CDCl_3$) 129.9, 129.8, 68.4, 35.8, 33.1, 31.9, 29.8, 29.7 (2 C), 29.6, 29.5, 29.3 (2 C), 27.2 (2 C), 26.9, 22.7, 16.6, 14.1; HRMS (ESI) calcd for $[M+H]^+$ $C_{19}H_{39}O$: 283.3001, found 283.3013.

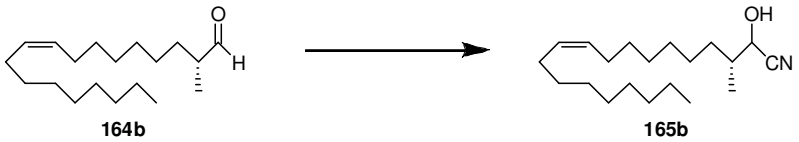
6.5. (R)-2-Methyl-cis-9-octadecenal (164b)[231]



163b **164b**

Iodoxybenzoic acid (2.3 g, 8.3 mmol, 150 mol-%) was stirred in DMSO (10 ml) at rt until all solids dissolved. (*R*)-2-methyl-*cis*-9-octadecenol **163b** (1.6 g, 5.5 mmol, 100 mol-%) was added in DMSO (4 ml) and the mixture was stirred at rt for 3 h and quenched with water (10 ml) which caused formation of white precipitate. The suspension was filtered through cotton (flushed with Et_2O) and the filtrate was extracted with Et_2O (3 x 100 ml). Combined organic phases were washed with brine (2 x 50 ml), dried ($MgSO_4$) and evaporated. Purification by FC (eluent, 5% EtOAc/hex) gave compound **164b** (1.4 g, 88%) as yellow oil; R_f (50% EtOAc/hex) 0.7; ^{1}H NMR ($CDCl_3$) 9.60 (d, 1H, *J*= 2.0 Hz), 5.39-5.29 (m, 2H), 2.32 (d of sext, 1H, *J* = 2.0, 6.8 Hz), 2.07-1.98 (m, 4H), 1.72-1.62 (m, 1H, *J* = 7.4 Hz), 1.4-1.2 (m, 21H), 1.08 (d, 3H, *J*= 6.8 Hz), 0.89-0.85 (m, 3H); ^{13}C NMR ($CDCl_3$) 205.2, 130.0, 129.7, 46.3, 31.9, 30.5, 29.7, 29.6 (2 C), 29.5, 29.4, 29.3, 29.1, 27.2, 27.1, 26.9, 22.7, 14.1, 13.3; HRMS (ESI) calcd for $[M+Na]^+$ $C_{19}H_{36}ONa$: 303.2664, found 303.2666.

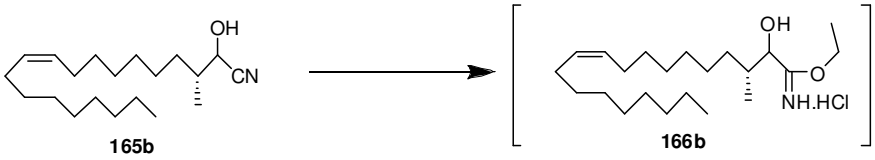
6.6. (R)-3-Methyl-2-hydroxy-cis-10-nonadecenenitrile (165b)[231]



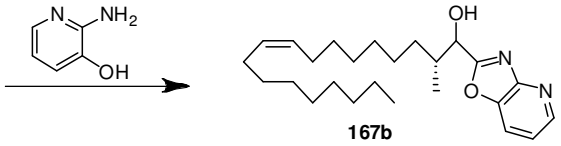
164b 165b

This compound was prepared as described for **165a**. FC (eluent, CH_2Cl_2) gave compound **165b** (1.2 g, 87%) as yellow oil: R_f (EtOAc) 0.8; ^{1}H NMR ($CDCl_3$) 5.39-5.30 (m, 2H), 4.37 (dd, 1H, *J* = 12.3, 6.5 Hz), 2.35 (dd, 1H, *J* = 17.1, 6.5 Hz), 2.08-2.01 (m, 4H), 1.92-1.82 (m, 1H), 1.40-1.20 (m, 22H), 1.08 (dd, 3H, *J* = 7.5, 7.0 Hz), 0.90-0.86 (m, 3H); ^{13}C NMR ($CDCl_3$) two signals were found for carbons next to chiral centers; 130.1, 129.7, 119.3, 118.9, 66.3, 66.0, 37.9 (2 C), 32.0, 31.9, 31.5, 29.7 (2 C), 29.5, 29.3, 29.1 (2 C), 27.2, 27.1, 26.8, 26.7, 22.7, 14.7, 14.5, 14.1; HRMS (ESI) calcd for $[M+Na]^+$ $C_{20}H_{37}NONa$: 330.2773, found 330.2780.

6.7. (R)-2-Methyl-1-oxazolo[4,5-b]pyridin-2-yl-cis-octadec-9-en-1-ol (167b)[231]



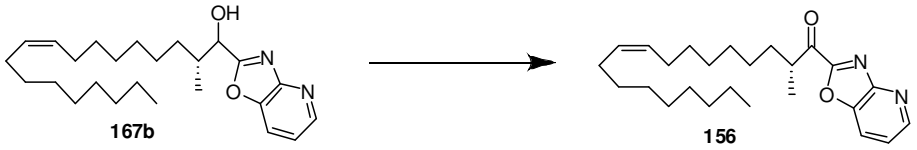
165b 166b



167b

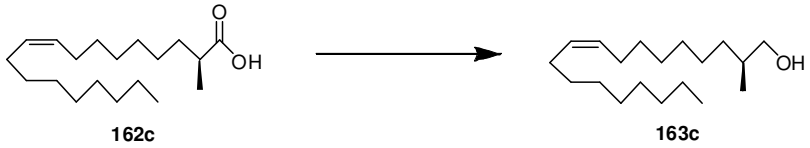
This compound was prepared as described for **167a**. FC (eluent 25% EtOAc/cyclohexane) gave compound **167b** (43 mg, 17%) as yellow oil: R_f (EtOAc) 0.6; ^{1}H NMR ($CDCl_3$) 8.54 (d, 1H, *J* = 4.5 Hz), 7.81 (d, 1H, *J* = 8.1 Hz), 7.49 (dd, 1H, *J* = 8.1, 4.5 Hz), 5.40-5.27 (m, 2H), 4.94 (d, ½H, *J* = 4.2 Hz), 4.86 (d, ½H, *J* = 5.5 Hz), 3.66 (br s, 1H), 2.20-2.10 (m, 1H), 2.05-1.90 (m, 4H), 1.4-1.2 (m, 22H), 0.97 (d, 1½H, *J* = 7.0 Hz), 0.94 (d, 1½H, *J* = 7.0 Hz), 0.88-0.84 (m, 3H). ^{13}C NMR ($CDCl_3$) two signals were found for carbons next to chiral centers; 171.1, 170.7, 154.8, 146.4, 129.9, 129.7, 120.1, 118.6, 118.5, 72.6, 71.6, 38.5, 38.2, 32.8, 31.9, 31.4, 29.7 (2 C), 29.6 (2 C), 29.5, 29.3, 29.2, 27.2, 27.1, 26.9, 22.6, 15.4, 14.1, 14.0; HRMS (ESI) calcd for $[M+Na]^+$ $C_{25}H_{40}N_2O_2Na$: 423.2987, found 423.2991.

6.8. (R)-2-Methyl-1-oxazolo[4,5-b]pyridin-2-yl-cis-octadec-9-en-1-one (156)[231]



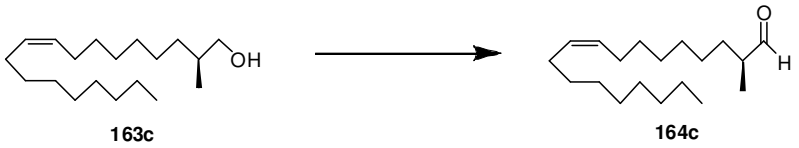
This compound was prepared as described for **61**. FC (eluent, 10% EtOAc/hex) gave compound **156** (23 mg, 58%, ee% = 37%) as yellow oil: R_f (EtOAc) 0.6; $[\alpha]^{20}$ = -2 (CH_2Cl_2, c = 1); Rt 19.33 min (Chiralcel AD, 0.5% 2-propanol in hexane, 1.0 ml/min, 254 nm); ^{1}H NMR ($CDCl_3$) 8.75 (dd, 1H, *J* = 4.8, 1.5 Hz), 8.00 (dd, 1H, *J* = 8.3, 1.5 Hz), 7.49 (dd, 1H, *J* = 8.3, 4.8 Hz), 5.40-5.28 (m, 2H), 3.84 (sext, 1H, *J* = 6.8 Hz), 2.06-1.88 (m, 5H), 1.60-1.51 (m, 1H), 1.4-1.2 (m, 24H), 0.88-0.85 (m, 3H). ^{13}C NMR ($CDCl_3$) 190.4, 158.3, 154.3, 148.7, 143.6, 130.0, 129.7, 123.1, 120.2, 39.8, 31.9, 29.8, 29.7, 29.5, 29.3 (2 C), 29.2, 29.1 (2 C), 27.2 (2 C), 23.8, 22.7, 16.4, 14.1; HRMS (ESI) calcd for $[M+Na]^+$ $C_{25}H_{38}N_2O_2Na$: 421.2831, found 421.2840.

6.9. (S)-2-Methyl-cis-9-octadecenol (163c)[231]



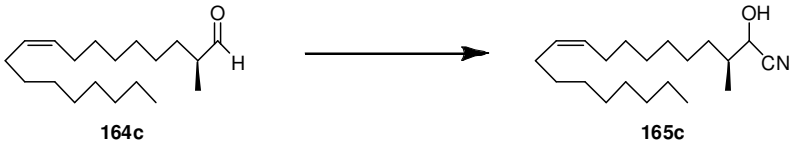
This compound was prepared as described for **163b.** Yellow oil (2.3 g, 88%): R_f (CH_2Cl_2) 0.4; ^{1}H NMR and ^{13}C NMR ($CDCl_3$) match to those of **163b**; HRMS (ESI) calcd for $[M+H]^+$ $C_{19}H_{39}O$: 283.3001, found 283.3000.

6.10. (S)-2-methyl-cis-9-octadecenal (164c)[231]



This compound was prepared as described for **164b**. Yellow oil (1.8 g, 92%): R_f (10% EtOAc/hex) 0.6; ^{1}H NMR and ^{13}C NMR ($CDCl_3$) match to those of **164b**; HRMS (ESI) calcd for $[M+Na]^+$ $C_{19}H_{36}ONa$: 303.2664, found 303.2692.

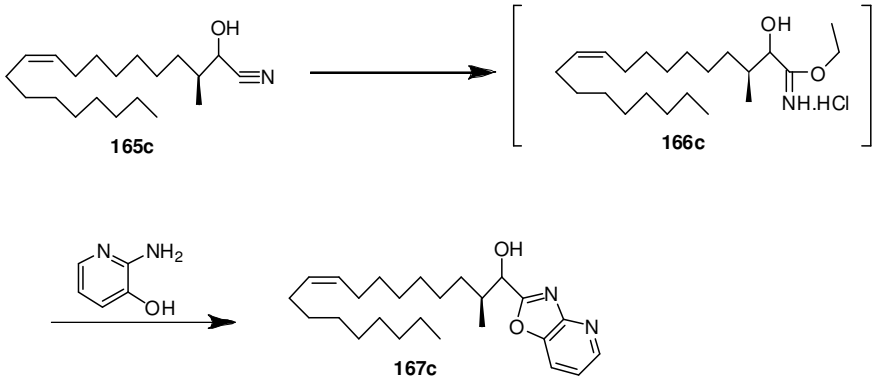
6.11. (S)-3-Methyl-2-hydroxy-cis-10-nonadecenenitrile (165c)[231]



164c 165c

This compound was prepared as described for **165a.** Colourless oil: (1.4 g, 85%): R_f (50% EtOAc/hex) 0.7; ^{1}H NMR and ^{13}C NMR ($CDCl_3$) match to those of **165b**; HRMS (ESI) calcd for $[M+Na]^+$ $C_{20}H_{37}NONa$: 330.2773, found 330.2796.

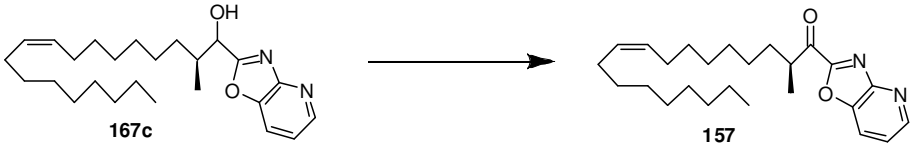
6.12. (S)-2-Methyl-1-oxazolo[4,5-b]pyridin-2-yl-cis-octadec-9-en-1-ol (167c)[231]



165c 166c 167c

This compound was prepared as described for **167a**. Yellow oil: R_f (EtOAc) 0.6; ^{1}H NMR and ^{13}C NMR ($CDCl_3$) match to those of **167b**; HRMS (ESI) calcd for $[M+Na]^+$ $C_{25}H_{40}N_2O_2Na$: 423.2987, found 423.2986.

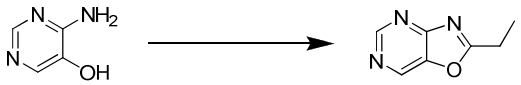
6.13. (S)-2-Methyl-1-oxazolo[4,5-b]pyridin-2-yl-octadec-9-en-1-one (157)[231]



167c 157

This compound was synthesized as described for **61**. FC (eluent, 10% EtOAc/cyclohexane) gave compound **157** (47 mg, 79%, ee%= 37%) as yellow oil: R_f (EtOAc) 0.6; $[\alpha]^{20}$ = -2 (CH_2Cl_2, c = 1); Rt 25.41 min (Chiralcel AD; 0.5 % 2-propanol in hexane, 1.0 ml/min, 254 nm); ^{1}H NMR and ^{13}C NMR ($CDCl_3$) match to those of **156**; HRMS (ESI) calcd for $[M+Na]^+$ $C_{25}H_{38}N_2O_2Na$: 421.2831, found 421.2839.

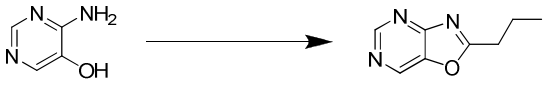
6.14. 2-Ethyl-oxazolo[4,5-d]pyrimidine (159c)



168 **159c**

4-Aminopyrimidin-5-ol (30 mg, 0.2 mmol, 100 mol-%), triethylortho propionate (350 mg, 2.0 mmol, 1000 mol-%) and *p*-TsOH (2 mg, 0.02 mmol, 8 mol-%) were heated progressively during 2 h untill 165 °C and kept at this temperature for 1 h. The mixture was cooled, evaporated, eluted (EtOAc) through a pad silica gel and recrystallized from petroleum ether giving compound **159c** (23 mg, 77%) as red crystals: R_f (EtOAc) 0.25; mp. 49 °C; ^{1}H NMR ($CDCl_3$) 9.10 (s, 1H), 8.88 (s, 1H), 3.06 (q, 2H, *J* = 7.6 Hz), 1.48 (t, 3H, *J* = 7.6 Hz). ^{13}C NMR ($CDCl_3$) 174.6, 162.0, 155.0, 142.2, 138.3, 22.7, 10.4; HRMS (ESI) calcd for $[M+H]^+$ $C_7H_8N_3O$: 150.0667, found 150.0630.

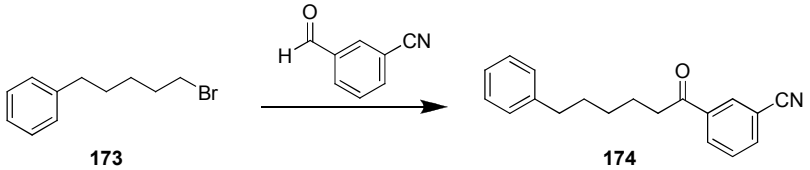
6.15. 2-Propyl-oxazolo[4,5-d]pyrimidine (159d)



168 **159d**

This compound was prepared as **158c**. Light red crystals (20 mg, 61%): R_f (EtOAc) 0.25; mp. 51-52 °C; ^{1}H NMR ($CDCl_3$) 9.11 (s, 1H), 8.90 (s, 1H), 3.01 (t, 2H, *J* = 7.5 Hz), 1.96 (sext, 2H, *J* = 7.5 Hz), 1.06 (t, 3H, *J* = 7.5 Hz). ^{13}C NMR ($CDCl_3$) 173.8, 162.0, 155.0, 142.1, 138.3, 30.9, 19.9, 13.6, HRMS (ESI) calcd for $[M+H]^+$ $C_8H_{10}N_3O$ 164.0824, found 164.0810.

6.16. 1-(3'-Cyanophenyl)6-phenylhexan-1-one (174)

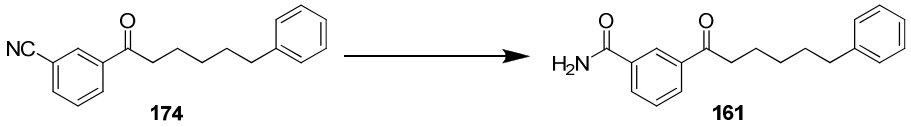


173 **174**

5-Phenyl-1-bromopentane **173** (360 mg, 1.6 mmol, 100 mol-%) in THF (2.0 ml) was cannulated to a mixture of Mg-chips (42 mg, 1.7 mmol, 110 mol-%) in THF (3.0 ml) at rt. Mixture was heated up to reflux and I_2-crystal was added. After the mixture had cleared it was cooled to rt and cannulated to a mixture of 2-cyanobenzaldehyde (210 mg, 1.6 mmol, 100 mol-%) in THF (2.0 ml). The mixture was refluxed for 17 h

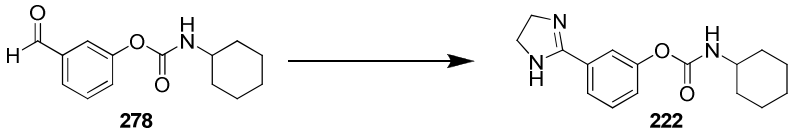
and cooled to rt followed by addition of ice (10 ml) and 2 drops of conc. H_2SO_4. The mixture was extracted with EtOAc (3 x 50 ml) and combined organic phases were washed with water (50 ml) and brine (50 ml), dried (Na_2SO_4) and evaporated. Purification by FC (5% EtOAc/hex) gave compound **174** (27 mg, 6%) as yellow oil: R_f (20% EtOAc/hex) 0.4; ^{1}H NMR ($CDCl_3$) 8.22-8.21 (m, 1H), 8.16 (ddd, 1H, *J* = 7.9, 1.6, 1.4 Hz), 7.82 (dt, 1H; *J* = 7.8, 1.4 Hz), 7.59 (td, 1H, *J* = 7.9, 0.4 Hz), 7.29-7.25 (m, 2H), 7.19-7.16 (m, 3H), 2.96 (t, 2H, *J* = 7.3 Hz), 2.63 (t, 2H, *J* = 7.8 Hz), 1.78 (qui, 2H, *J* = 7.6 Hz), 1.68 (qui, 2H, *J* = 7.6 Hz), 1.47-1.39 (m, 2H). ^{13}C NMR ($CDCl_3$) 198.1, 142.4, 137.7, 135.8, 131.9, 131.7, 129.6, 128.4, 128.3, 125.7, 117.9, 113.1, 38.5, 35.7, 31.2, 28.8, 23.8; HRMS (ESI) calcd for $[M+Na]^+$ $C_{19}H_{19}NONa$: 300.1364, found 300.1364.

6.17. 3-(6'-Phenylhexan-1-onyl)benzamide (161)



Compound **174** (24 mg, 0.087 mmol, 100 mol-%) was treated with 0.5 M aq. NaOH (170 µl, 0.087 mmol, 100 mol-%) and 30% aq. H_2O_2 (100 µl, 0.87 mmol, 1000 mol-%) in EtOH (1 ml) at rt The mixture was heated to 50 ^{o}C for 3 h and then neutralized with 0.2 M aq. HCl. The mixture was diluted with H_2O (10 ml), extracted with CH_2Cl_2 (3 x 20 ml) and the combined organic phases were washed with brine (2 x 10 ml), dried (Na_2SO_4) and evaporated resulting in white solid. Purification by FC (hex) gave compound **161** (20 mg, 80%) as white solid: mp. 128 ^{o}C, R_f (5% MeOH in CH_2Cl_2) 0.3; ^{1}H NMR ($CDCl_3$) 8.38 (t, 1H, *J* = 1.6 Hz), 8.10 (dt, 1H, *J* = 7.7, 1.4 Hz), 8.04 (ddd, 1H, *J* = 7.7, 1.1, 0.6 Hz), 7.56 (t, 1H, *J* = 7.8 Hz), 7.29-7.25 (m, 2H), 7.19-7.16 (m, 3H), 6.29 (br s, 1H), 5.94 (br s, 1H), 3.00 (t, 2H, *J* = 7.4 Hz), 2.63 (t, 3H, *J* = 7.8 Hz), 1.78 (qui, 2H, *J* = 7.6 Hz), 1.68 (qui, 2H, *J* = 7.6 Hz), 1.47-1.39 (m, 2H); ^{13}C NMR ($CDCl_3$) 199.6, 168.3, 142.5, 137.3, 133.8, 131.7, 131.3, 129.0, 128.4, 128.3, 126.7, 125.7, 38.6, 35.7, 31.2, 28.9, 24.0; HRMS (ESI) calcd for $[M+Na]^+$ $C_{19}H_{21}NO_2Na$: 318.1470, found 318.1455.

6.18. 3-(4,5-Dihydro-1H-imidazol-2-yl)phenyl cyclohexylcarbamate (222)



278 222

To a mixture of 3-formylphenyl cyclohexylcarbamate (***278****, see 6.57.* 3-Formylphenyl cyclohexylcarbamate (278), 300 mg, 1.2 mmol, 100 mol-%) in CH_2Cl_2 (10 ml) was added ethane-1,2-diamine (85 µL, 1.3 mmol, 105 mol-%) at -2 ^oC (T_{bath}). The mixture was stirred for 30 min and *N*-bromosuccinimide (230 mg, 1.3 mmol, 105 mol-%) was added. The mixture was allowed to warm to rt during 1 h and evaporated to dryness. Resulting solid was dissolved to 10% TEA in EtOAc, filtered through a pad of silica, solvents were evaporated and residue recrystallized from EtOAc/CH_2Cl_2 (6:5) giving **222** (290 mg, 83%) as white crystals: mp. 139-141 ^oC; R_f (10% TEA in EtOAc) 0.3; ^1H NMR ($CDCl_3$) 7.60 (dt, 1H, *J* = 7.7, 1.3 Hz), 7.53 (s, 1H) 7.37 (t, 1H, *J* = 7.9 Hz), 7.20 (dd, 1 H, *J* = 8.2, 1.5 Hz), 5.07 (br d, 1H, *J* = 7.5 Hz), 3.77 (br s, 4H), 3.60-3.48 (m, 1H), 2.05-1.96 (m, 2H), 1.79-1.69 (m, 2H), 1.67-1.58 (m, 1H), 1.43-1.31 (m, 2H), 1.28-1.13 (m, 3H); ^{13}C NMR (MeOD) 166.7, 156.0, 152.8, 132.5, 130.4, 125.4, 125.0, 121.8, 51.7, 50.4 (2 C), 34.0, 26.6, 26.1; Anal. calcd for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62; Found C, 66.54; H, 7.33; N, 14.27.

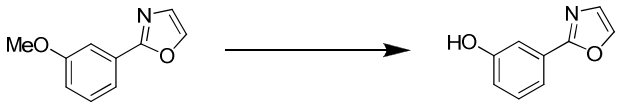
6.19. 2-(3-Methoxyphenyl)oxazole (255a)[232]

254a 255a

3-Methoxybenzamide (**254a**, 2.0 g, 13 mmol, 100 mol-%) and 2-bromoacetaldehyde diethyl acetal (2.5 ml, 16 mmol, 120 mol-%) were placed to a round bottomed flask and MW-irradiated for 5 min (power 300 W for 30 s, then 50 W, air cooling, T_{max}= 100 ^oC). The reaction mixture was dissolved-in a mixture of EtOAc (150 ml), MeOH (10 ml) and water (50 ml). The organic phase was washed with $NaHCO_3$, water and brine, dried (Na_2SO_4), and evaporated. The tan oil was Kugelrohr-distilled to yield 2.05 g of clear oil. The oil was diluted with hex (5 ml) and put to freezer overnight, which caused crystallization. The solid was filtrated, washed with ice-cold hex (20

ml) and dried under reduced pressure giving **255a** (1.0 g, 45%) as a white crystals: mp. 23-24 °C; R_f (EtOAc) 0.62; ^{1}H NMR ($CDCl_3$) 7.69 (d, 1H, *J* = 0.7 Hz), 7.65-7.62 (ddd, 1H, *J* = 7.7, 1.4, 0.9 Hz), 7.59 (dd, 1H, *J* = 1.4 Hz), 7.36 (t, 1H, *J* = 7.5 Hz) , 1H, 7.23 (d, 1H, *J* = 0.7 Hz), 6.99, (ddd, *J* = 8.3, 2.6, 0.9 Hz, 1H), 3.86 (s, 3H), ^{13}C NMR ($CDCl_3$) 161.8, 159.8, 138.5, 129.8, 128.6, 128.3, 118.7, 116.9, 110.9, 55.3; Anal. calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00; Found C, 68.43; H, 4.84; N, 7.86.

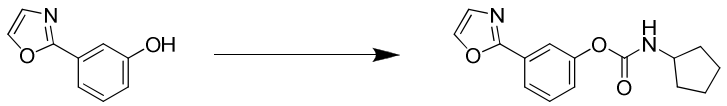
6.20. 2-(3-Hydroxyphenyl)oxazole (256a)[232,233]



255a **256a**

2-(3-Methoxyphenyl)oxazole (**255a**, 0.9 g, 5.1 mmol, 100 mol-%) and 1-butyl-3-methylimidazolium bromide (2.2 g, 10 mmol, 200 mol-%) were MW-irradiated in a round bottomed flask for 4 x 20 s (power 300 W, air cooling, T_{max} 200 °C). The reaction mixture was dissolved in EtOAc (200 ml) and water (50 ml). The organic phase was washed with water and brine, dried (Na_2SO_4), filtered and evaporated. FC (25% EtOAc/hex) gave **256a** (0.5 g, 60%) as a white solid: mp. 130-131 °C; R_f (40% EtOAc/hex) 0.5; ^{1}H NMR ($CDCl_3$) 9.81 (s, 1H), 8.19 (d, 1H, *J* = 0.8 Hz), 7.43-7.38 (m, 2H), 7.36 (d, 1H, *J* = 0.8 Hz), 7.33 (t, 1H, *J* = 7.9 Hz), 6.91 (ddd, 1H, *J* = 1.1, 2.5, 8.1 Hz), 5.05 (br s, 1H), 4.43 (app t, 2H, *J* = 9.5 Hz), 4.06 (app t, 2H, *J* = 9.5 Hz), 3.24 (q, 2H, *J* = 6.7 Hz), 1.60 (sext, 2H, *J* = 7.3 Hz), 0.97 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$) 160.8, 157.8, 139.9, 130.3, 128.4, 128.1, 117.7, 116.6, 112.4.

6.21. General procedure for the preparation of phenolic carbamates; 3-(Oxazol-2-yl)phenyl cyclopentylcarbamate (241)[232]

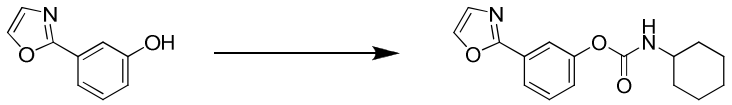


256a **241**

To a solution of 2-(3-hydroxyphenyl)oxazole **256a** (64 mg, 0.40 mmol, 100 mol-%) in dry toluene (4 ml) were added triethylamine (29 mg, 0.28 mmol, 100 mol-%) and cyclopentyl isocyanate (180 mg, 1.6 mmol, 400 mol-%). After stirring at rt for 12 h, the reaction was complete as judged by TLC (10% Et_2O/CH_2Cl_2), and the mixture

was diluted with EtOAc (8 ml), filtered through a pad of silica gel and evaporated to dryness. Recrystallization from EtOAc/hex (1:10 ml) gave compound **241** (103 mg, 94%) as white crystals: mp. 120-122 °C; R_f (10% Et_2O in CH_2Cl_2) 0.50; ^{1}H NMR (DMSO-d_6) 8.25 (s, 1H), 7.90 (d, 1H, *J* = 7.1 Hz), 7.81 (d, 1H, *J* = 7.8 Hz), 7.66 (s, 1H), 7.54 (t, 1H, *J* = 8.0 Hz), 7.41 (s, 1H), 7.27 (dd, 1H, *J* = 1.6, 8.1 Hz), 3.90-3.82 (m, 1H), 1.80-1.90 (m, 2H), 1.61-1.72 (m, 2H); 1.56-1.45 (m, 4H); ^{13}C NMR (DMSO-d_6) 160.1, 153.4, 151.5, 140.4, 130.3, 128.6, 128.0, 123.9, 122.3, 119.0, 52.4, 32.2, 23.3; Anal. calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29; Found C, 65.99; H, 5.81; N, 10.20.

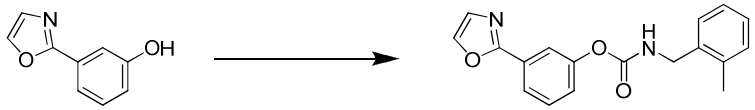
6.22. 3-(Oxazol-2-yl)phenyl cyclohexylcarbamate (242)[232]



256a **242**

Recrystallization from EtOAc/hex gave **242** (115 mg, 80%) as white crystals: mp. 142-143 °C; R_f (10% Et_2O/CH_2Cl_2) 0.30; ^{1}H NMR ($CDCl_3$) 7.88 (d, 1H, *J* = 7.8 Hz), 7.82-7.80 (m, 1H), 7.70-7.69 (m, 1H), 7.44 (t, 1H, *J* = 8.0 Hz), 7.25-7.21 (m, 2H), 5.06 (br d, 1H, *J* = 7.5 Hz), 3.66-3.51 (m, 1H) 2.08-1.97 (m, 2H), 1.79-1.69 (m, 2H), 1.66-1.58 (m, 1H), 1.43-1.31 (m, 2H), 1.29-1.13 (m, 3H); ^{13}C NMR ($CDCl_3$) 161.2, 153.3, 151.4, 138.7, 129.7, 128.6, 128.5, 123.6, 123.0, 119.7, 50.2, 33.2, 25.4, 24.7; Anal. calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78; Found C, 66.72; H, 6.44; N, 9.74.

6.23. 3-(Oxazol-2-yl)phenyl 2-methylbenzylcarbamate (243) [232]

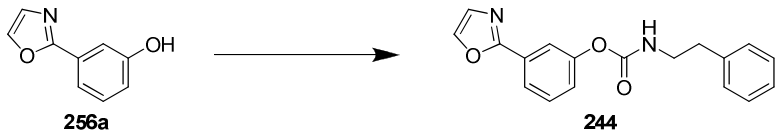


256a **243**

Recrystallization from EtOAc/hex gave **243** (154 mg, 89%) as white crystals: mp. 120-121 °C; R_f (5% Et_2O/CH_2Cl_2) 0.5; ^{1}H NMR (DMSO-d_6) 8.35 (t, 1H, *J* = 5.9 Hz), 8.24 (s, 1H), 7.85-7.81 (m, 1H), 7.70 (t, 1H, *J* = 1.8 Hz), 7.56 (t, 1H, *J* = 8.0 Hz), 7.41 (s, 1H), 7.33-7.27 (m, 2H), 7.23-7.17 (m, 3H), 4.29 (d, 1H, *J* = 5.9 Hz), 2.32 (s, 3H); ^{13}C NMR (DMSO-d_6) 160.1, 154.2, 151.5, 140.4, 136.7, 135.5, 130.4,

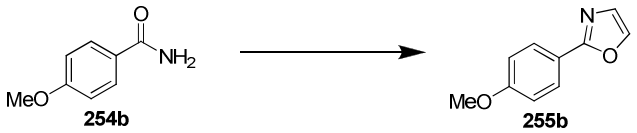
130.0, 128.6, 128.0, 127.4, 127.0, 125.9, 123.9, 122.5, 119.0, 42.1, 18.6; Anal. calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09; Found C, 69.78; H, 5.14; N, 9.02.

6.24. 3-(Oxazol-2-yl)phenyl phenethylcarbamate (244) [232]



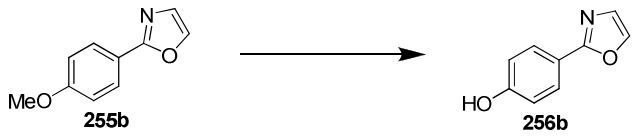
Recrystallization from EtOAc/hex gave compound **244** (83 mg, 73%) as white crystals: mp. 112-113 °C; R_f (5% Et_2O/CH_2Cl_2) 0.50; ^{1}H NMR (DMSO-d_6) 8.25 (d, 1H, *J* = 0.6 Hz), 7.96 (t, 1H, *J* = 5.6 Hz), 7.83-7.79 (m, 1H), 7.64 (t, 1H, *J* = 1.9 Hz), 7.54 (t, 1H, *J*= 8.0 Hz), 7.41 (d, 1H, *J* = 0.9 Hz), 7.35-7.20 (m, 6H), 3.36-3.29 (m, 2H), 2.82 (t, 2H, *J* = 7.4 Hz); ^{13}C NMR (DMSO-d_6) 160.1, 154.0, 151.5, 140.4, 139.1, 130.3, 128.7, 128.6, 128.3, 128.0, 126.2, 123.8, 122.4, 119.0, 42.0, 35.2; Anal. calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09; Found C, 70.04; H, 5.09; N, 9.02.

6.25. 2-(4-Methoxyphenyl)oxazole (255b)[234]



This compound was synthesized and worked up as described for **255a** using 4-methoxybenzamide (2.0 g, 13 mmol, 100 mol-%) as starting material. Purification by FC (Eluent 33% EtOAc/hex) gave **255b** (2.0 g, 90%) as a yellow oil: R_f (EtOAc) 0.50; ^{1}H NMR ($CDCl_3$) 7.98 (d, 2H, *J* = 9.0 Hz), 7.66 (s, 1H), 7.19 (s, 1H), 6.97 (d, 2H, *J* = 8.8 Hz), 3.86 (s, 3H).

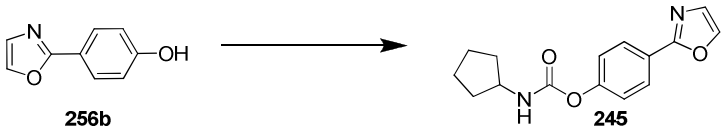
6.26. 2-(4-Hydroxyphenyl)oxazole (256b)[232]



This compound was synthesized and worked up as described for **256a** using **255b** (1.5 g, 9.3 mmol, 100 mol-%) as starting material. Purification by FC (Eluent 33% EtOAc/hex) and recrystallization (MeOH/CH_2Cl_2/hex) gave **256b** (0.97 g, 65%) as a

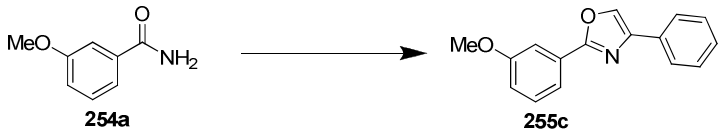
white solid: mp. 173-175 °C; R_f (50% EtOAc/hex) 0.3; ^{1}H NMR (DMSO-d_6) 7.87-7.83 (m, 3H), 7.21 (d, 1H, *J* = 0.8 Hz), 6.92-6.88 (m, 2H), 4.93 (br s, 1H); ^{13}C NMR (MeOD) 164.0, 161.4, 140.0, 129.2, 128.4, 119.6, 116.8.

6.27. 4-(Oxazol-2-yl)phenyl cyclopentylcarbamate (245) [232]



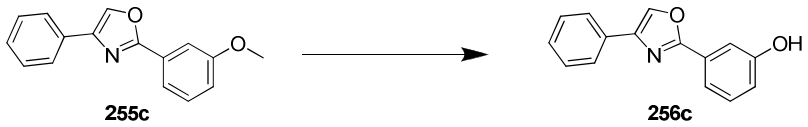
A white solid (168 mg, 95%): mp. 168-169 °C; R_f (5% Et_2O/CH_2Cl_2) 0.20; ^{1}H NMR (DMSO-d_6) 8.21 (s, 1H), 7.98 (d, 2H, *J* = 8.7 Hz), 7.90 (d, 1H, *J* = 7.2 Hz), 7.37 (s, 1H), 7.28 (d, 2H, *J* = 8.6 Hz), 3.92-3.81 (m, 1H), 1.92-1.80 (m, 2H), 1.72-1.60 (m, 2H), 1.58-1.45 (m, 4H); ^{13}C NMR (DMSO-d_6) 160.4, 153.2, 152.8, 140.0, 128.5, 127.1, 123.6, 122.4, 52.4, 32.2, 23.3; Anal. calcd for $C_{15}H_{16}N_2O_3$ C, 66.16; H, 5.92; N, 10.29; Found C, 66.29; H, 5.66; N, 10.32.

6.28. 2-(3-Methoxyphenyl)4-phenyloxazole (255c)



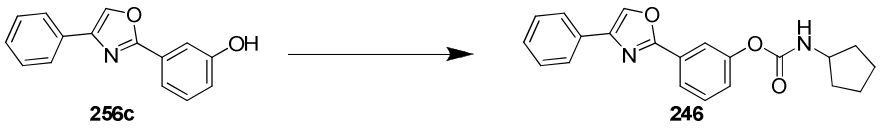
3-Methoxybenzamide (**254a**, 1.5 g, 10 mmol, 100 mol-%) and 2-bromo-1-phenylethanone (2.4 g, 12 mmol, 120 mol-%) were added to round bottomed flask and MW-irradiated for 5 min (power 200 W for 30 s, then 50 W, air cooling, T_{max}= 125 °C). The reaction mixture was dissolved to EtOAc (150 ml), MeOH (10 ml) and water (50 ml). The organic phase was washed with sat. $NaHCO_3$, water and brine, dried (Na_2SO_4), and evaporated. Purification by FC (5% EtOAc/hex) gave **255c** (2.0 g, 79%) as a red oil (impure): R_f (50% EtOAc/hex) 0.58; ^{1}H NMR ($CDCl_3$) 7.96 (s, 1H), 7.84-7.81 (m, 2H), 7.72-7.70 (ddd, 1H), 7.66 (dd, 1H, *J* = 2.6, 1.6 Hz), 7.46-7.41 (m, 2H), 7.39 (t, 1H, *J* = 8.0 Hz), 7.36-7.30 (m, 1H), 7.01 (ddd, 1H, *J* = 8.3, 2.6, 1.0 Hz), 3.90 (s, 3H); HRMS (ESI) calcd for $[M+Na]^+$ $C_{16}H_{13}NO_2$: 274.0844; Found 274.0839.

6.29. 3-(4-Phenyloxazol-2-yl)phenol (256c)



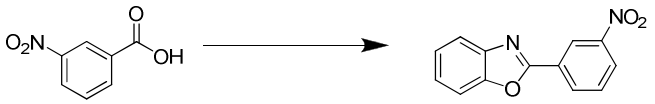
Compound **255c** (150 mg, 0.6 mmol, 100 mol-%) and *N*-butylpyridinium bromide (320 mg, 1.5 mmol, 250 mol-%) were MW-irradiated in a sealed tube for 3 x 30 s (power 125 W, T_{max}= 100 °C). The reaction mixture was dissolved to EtOAc (50 ml) and the organic phase was washed with water and brine, dried (Na_2SO_4), and evaporated. Purification by FC (CH_2Cl_2) gave **256c** (84 mg, 60%) as a yellow wax: R_f (EtOAc) 0.50; ^{1}H NMR (MeOD-d_4) 8.29 (s, 1H), 7.85-7.81 (m, 2H), 7.57 (ddd, 1H, *J* = 7.7, 1.5, 1.0 Hz), 7.51 (dd, 1H, *J* = 2.3, 1.7 Hz), 7.45-7.40 (m, 2H), 7.36-7.31 (m, 2H), 6.9 (ddd, 1H, *J* = 8.2, 2.5, 1.0 Hz), 4.17-4.03 (m, 1H), 2.10-1.99 (m, 2H), 1.79-1.59 (m, 4H), 1.57-1.46 (m, 2H); ^{13}C NMR (MeOD-d_4) 163.6, 159.2, 143.2, 135.6, 132.4, 131.2, 129.8, 129.6, 129.2, 126.7, 118.9, 118.7, 114.1; HRMS (ESI) calcd for $[M-H]^+$ $C_{15}H_{10}NO_2$: 236.0712, found 236.0717.

6.30. 3-(4-Phenyloxazol-2-yl)phenyl cyclopentylcarbamate (246)



Recrystallization from EtOAc/hex gave **246** (60 mg, 66%) as white crystals: mp. 159-161 °C; R_f (10% Et_2O in CH_2Cl_2) 0.50; ^{1}H NMR ($CDCl_3$) 7.97-7.93 (m, 2H), 7.91-7.88 (m, 1H), 7.84-7.79 (m, 2H), 7.49-7.40 (m, 3H), 7.36-7.30 (m, 1H), 7.27-7.23 (m, 1H), 5.03 (br d, 1H, *J* = 6.8 Hz), 4.17-4.03 (m, 1H), 2.10-1.99 (m, 2H), 1.79-1.59 (m, 4H), 1.57-1.46 (m, 2H); ^{13}C NMR ($CDCl_3$) 161.6, 154.1, 151.8, 142.5, 134.0, 131.4, 130.1, 129.2, 129.1, 128.6, 126.1, 124.2, 123.7, 120.2, 53.5, 33.6, 24.0; Anal. calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04; Found C, 72.05; H, 5.55; N, 7.99.

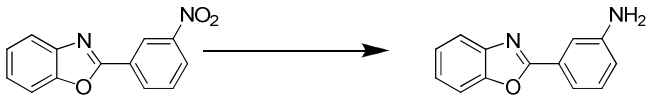
6.31. 2-(3-Nitrophenyl)benzo[d]oxazole (266)[235]



266

3-Nitrobenzoic acid (170 mg, 1.0 mmol, 100 mol-%), 2-aminophenol (110 mg, 1.0 mmol, 100 mol-%) and boric acid (62 mg, 1.0 mmol, 100 mol-%) were irradiated by microwave reactor (300 W, 4 min ramp to 250 °C, hold 1 min). The tan solid was collected with EtOAc (150 mL) washed with sat. aq. $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL), dried with Na_2SO_4, filtered and evaporated. Recrystallization (EtOAc/hex) gave compound **266** (170 mg, 71 %) as crystals: mp 209-210 °C, R_f (50% EtOAc/hex) 0.8; ^{1}H NMR ($CDCl_3$), 9.10 (t, 1H, *J* = 1.8 Hz), 8.59 (ddd, 1H, *J* = 7.8, 1.5, 1.1 Hz), 8.39 (ddd, 1H, *J* = 8.2, 2.3, 1.1 Hz), 7.83-7.80 (m, 1H), 7.73 (t, 1H, *J* = 8.0 Hz), 7.65-7.60 (m, 1H), 7.45-7.39 (m, 2H).

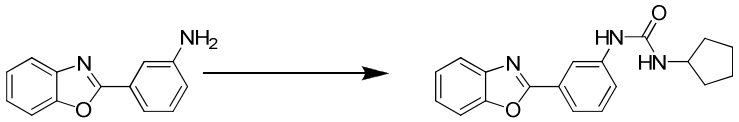
6.32. 3-(Benzo[d]oxazol-2-yl)aniline (267)[235]



266 **267**

Compound **266** (210 mg, 0.88 mmol, 100 mol-%) was dissolved in THF (5 mL) and EtOH (50 mL) and 10% Pd/C (64 mg, 0.06 mmol, 7 mol-%) was added. The mixture was stirred under H_2-gas for rt for 90 min and the mixture was eluted through a pad of silica with EtOAc and evaporated to dryness. Recrystallization (EtOH) gave compound **267** (170 mg, 90%) as red crystals: mp 177-178 °C; R_f (50% EtOAc/hex) 0.5; ^{1}H NMR ($CDCl_3$) 7.78-7.74 (m, 1H), 7.64 (ddd, 1H, *J* = 1.0, 1.5, 7.7 Hz), 7.58-7.54 (m, 2H), 7.36-7.28 (m, 3H); 6.84 (ddd, 1H, *J* = 0.9, 2.4, 8.0 Hz), 3.85 (s, 2H).

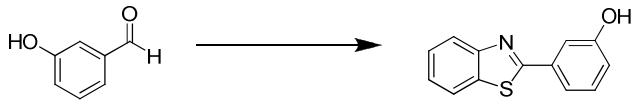
6.33. 1-(3-(Benzo[d]oxazol-2-yl)phenyl)3-cyclopentylurea (247)



267 **247**

Recrystallization (MeOH) gave compound **247** (44 mg, 65%) as a light brown crystals: mp. 244-246 °C, R_f (20% Et_2O/CH_2Cl_2) 0.67; ^1H NMR (DMSO-d_6) 8.90 (s, 1H), 8.49 (s, 1H), 7.82-7.80 (m, 2H), 7.75-7.72 (m, 1H), 7.46-7.39 (m, 4H), 6.25 (d, 1H, J = 7.1 Hz), 4.02-3.93 (m, 1H), 1.91-1.83 (m, 2H), 1.70-1.51 (m, 4H), 1.44-1.36 (m, 2H); ^{13}C NMR (DMSO-d_6) 162.5, 154.7, 150.2, 141.5, 141.4, 129.7, 126.8, 125.5, 124.8, 120.7, 119.8 (2 C), 115.9, 110.9, 51.0, 32.8, 23.2; Anal. calcd for $C_{19}H_{19}N_3O_2$; C, 71.01; H, 5.96; N, 13.08; Found C, 70.20; H, 6.25; N, 12.78; HRMS (ES+) *m/z* 322.1564 (MH^+, calc. for $C_{19}H_{20}N_3O_2$ 322.1556).

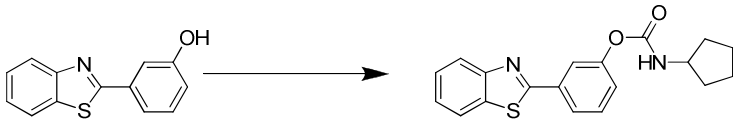
6.34. 3-(Benzo[d]thiazol-2-yl)phenol (268)[223]



268

3-Hydroxybenzaldehyde (120 mg, 1.0 mmol, 100 mol-%), 2-aminothiophenol (130 mg, 1.0 mmol, 100 mol-%) and 1-butyl-3-methylimidazolium bromide (90 mg, 0.4 mmol, 40 mol-%) were irradiated with microwaves (50 W, 2 min ramp to 200 °C, hold 3 min). The tan solid was collected with EtOAc (50 ml) and washed with sat. aq. $NaHCO_3$ (30 mL) and brine (30 ml), dried with Na_2SO_4, filtered and evaporated. Recrystallization (EtOAc/hex) gave compound **268** (167 mg, 73 %) as a yellow solid: mp. 168-169 °C; R_f (50% EtOAc/hex) 0.53; ^1H NMR ($CDCl_3$) 8.07 (d, 1H, J = 8.2 Hz), 7.89 (dd, 1H, J = 8.0, 0.6 Hz), 7.67 (dd, 1H, J = 2.3, 1.8 Hz), 7.58 (ddd, 1H, J = 7.7, 1.5, 0.9 Hz), 7.49 (ddd, 1H, J = 8.3, 7.3, 1.3 Hz), 7.39 (ddd, 1H, J = 8.2, 7.3, 1.1 Hz), 7.33 (t, 1H, J = 7.9 Hz), 6.98 (ddd, 1H, J = 8.1, 2.5, 0.9 Hz), 6.51 (s, 1H).

6.35. 3-(Benzo[d]thiazol-2-yl)phenyl cyclopentylcarbamate (248)

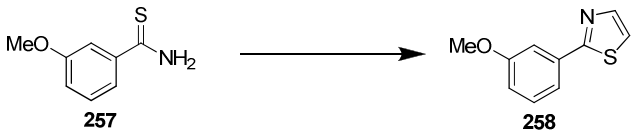


268 **248**

Recrystallization (EtOAc/hex) gave compound **248** (70 mg, 91%) as white crystals: mp. 178-179 °C; R_f (10% Et_2O/CH_2Cl_2) 0.50; ^1H NMR (DMSO-d_6) 8.17 (d, 1H, J = 7.6 Hz), 8.09 (d, 1H, J = 8.0 Hz), 7.92 (t, 2H, J = 7.8 Hz), 7.84-7.82 (m, 1H), 7.60-7.55 (m, 2H), 7.51-7.47 (m, 1H), 7.33 (dd, 1H, J = 1.6, 8.1 Hz), 3.93-3.84 (m, 1H),

1.92-1.81 (m, 2H), 1.72-1.64 (m, 2H), 1.58-1.42 (m, 4H); ^{13}C NMR (DMSO-d_6) 166.4, 153.4 (2 C), 151.7, 134.5, 133.9, 130.5, 126.8, 125.7, 124.7, 123.8, 123.0, 122.4, 120.0, 52.4, 32.2, 23.3; Anal. calcd for $C_{19}H_{18}N_2O_2S$; C, 67.43; H, 5.36; N, 8.28; Found C, 67.18; H, 5.65; N, 8.22.

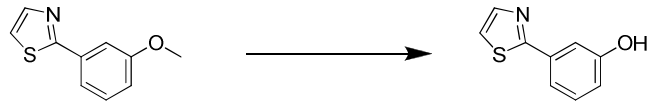
6.36. 2-(3-Methoxyphenyl)thiazole (258)[236]



257 258

To a mixture of 3-methoxybenzothioamide (420 mg, 2.5 mmol, 100 mol-%) in THF (2.5 ml) was added 2-bromoacetaldehyde diethyl acetal (0.5 ml, 3.3 mmol, 130 mol-%), and the mixture was MW-irradiated in a sealed tube for 30 min (power 300 W, air cooling, T_{max}= 115 ^{o}C). Purification of the resulting mixture by FC (Eluent 1% EtOAc in toluene) gave **258** (200 mg, 42%) as an oil: R_f (10% EtOAc/toluene) 0.50; ^{1}H NMR ($CDCl_3$) 7.85 (d, 1H, *J* = 3.3 Hz), 7.55 (dd, 1H, *J* = 2.4, 1.7 Hz) 7.51 (ddd, 1H, *J* = 7.7, 1.5, 1.0 Hz) 7.33 (t, 1H, *J* = 8.1 Hz), 7.30 (d, 1H, *J* = 3.3 Hz), 6.96 (ddd, 1H, *J* = 8.2, 2.6, 0.9 Hz), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$) 168.2, 159.9, 143.5, 134.7, 129.9, 119.1, 118.8, 116.2, 111.0, 55.3.

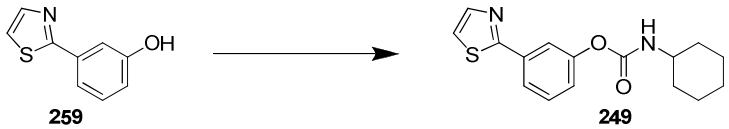
6.37. 2-(3-Hydroxyphenyl)thiazole (259)[236]



258 259

Compound **258** (170 mg, 0.9 mmol, 100 mol-%) and 1-butyl-3-methylimidazolium bromide (700 mg, 3.2 mmol, 350 mol-%) were MW-irradiated for 2 x 30 s (power 300 W, air cooling, T_{max} 100 ^{o}C). The reaction mixture was partitioned between EtOAc (30 ml) and water (30 ml). The organic phase was dried (Na_2SO_4), and evaporated. Resulting crude product was purified by FC (Eluent 25% EtOAc/hex) and recrystallized from EtOAc/hex giving **259** (70 mg, 44%) as a white solid: mp. 141-143 ^{o}C, R_f (35% EtOAc/hex) 0.50; ^{1}H NMR ($CDCl_3$) 7.87 (d, 1H, *J* = 3.2 Hz), 7.52 (ddd, 1H, *J* = 2.5, 1.6, 0.4 Hz), 7.49 (ddd, 1H, *J* = 7.7, 1.6, 1.0 Hz), 7.34 (d, 1H, *J* = 3.3 Hz), 7.31 (ddd, 1H, *J* = 8.1, 7.7, 0.4 Hz), 6.92 (ddd, 1H, *J* = 8.1, 1.6, 1.0 Hz), 5.68 (br s, 1H).

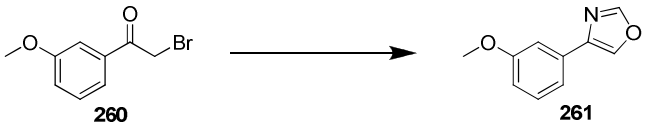
6.38. 3-(Thiazol-2-yl)phenyl cyclohexylcarbamate (249)[232]



259 **249**

Purification by FC (Eluent 1-2% MeOH/CH_2Cl_2) and recrystallization from EtOAc/hex gave **249** (35 mg, 50%) as white crystals; mp. 147 °C; R_f (EtOAc) 0.80; ^{1}H NMR ($CDCl_3$) 7.86 (d, 1H, *J* = 3.2 Hz), 7.81-7.74 (m, 2H), 7.42 (t, 1H, *J* = 7.9 Hz), 7.34 (d, 1H, *J* = 3.2 Hz), 7.21 (dd, 1H, *J* = 8.1, 1.6 Hz) 4.98, (br d, 1H, *J* = 7.3 Hz), 3.67-3.52 (m, 1H), 2.10-1.98 (m, 2H), 1.80-1.70 (m, 2H), 1.68-1.58 (m, 2H), 1.45-1.32 (m 2H), 1.30-1.14 (m, 3H); ^{13}C NMR ($CDCl_3$) 167.4, 153.3, 151.6, 143.7, 134.8, 129.8, 123.3, 123.2, 119.8, 119.1, 50.2, 33.2, 25.4, 24.7; Anal. calcd for $C_{16}H_{18}N_2O_2S$: C, 63.55; H, 6.00; N, 9.26; Found C, 63.38; H, 5.78; 9.14.

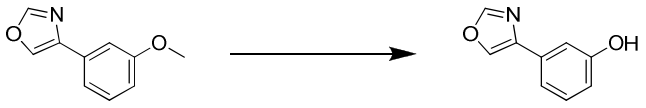
6.39. 4-(3-Methoxyphenyl)oxazole (261)[232]



260 **261**

2-Bromo-1-(3-methoxyphenyl)-ethanone (**260**, 230 mg, 1.0 mmol, 100 mol-%) and formamide (0.4 ml, 10 mmol, 1000 mol-%) were MW-irradiated (300 W) in a sealed tube until the temperature reached 100 °C (30 s) and kept at that temperature for 30 s. The irradiation was repeated and the mixture was partitioned between EtOAc (30 ml) and water (30 ml). The organic phase was dried (Na_2SO_4), and evaporated. Purification by FC (Eluent 10% EtOAc/hex) and recrystallization from CH_2Cl_2/hex gave **261** (90 mg, 51%) as light yellow crystals; mp. 81-82 °C; R_f (EtOAc) 0.60; ^{1}H NMR ($CDCl_3$) 7.93-7.92 (m, 2H), 7.34-7.32 (m, 1H), 7.32-7.30 (m, 2H), 6.90-6.85 (m, 1H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$) 160.0, 151.2, 140.3, 133.9, 132.0, 129.8, 117.9, 114.1, 110.8, 55.2; Anal. calcd for $C_{10}H_9NO_2$ C, 68.56; H, 5.18; N, 8.00; Found C, 68.04; H, 5.10; N, 7.74; HRMS (ESI) calcd for $[M+Na]^+$ $C_{10}H_9NO_2$: 198.0531, found 198.0524.

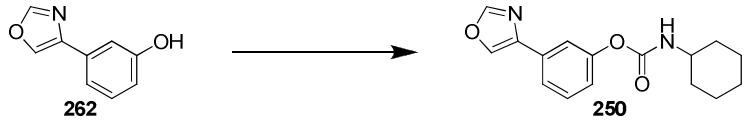
6.40. 4-(3-Hydroxyphenyl)oxazole (262)[232]



261 **262**

Compound **261** (200 mg, 1.1 mmol, 100 mol-%) and 1-butylpyridinimium bromide (490 mg, 2.2 mmol, 200 mol-%) were MW-irradiated (300 W) in a sealed tube until the temperature reached 100 °C (took 30 s) and kept at that temperature for 30 s. Another portion of ionic liquid (430 mg, 2.0 mmol, 180 mol-%) was added and irradiation was repeated for five times. The mixture was diluted with CH_2Cl_2 (10 ml) and purified by FC (Eluent CH_2Cl_2). Recrystallization from MeOH/CH_2Cl_2/hex gave **262** (100 mg, 56%) as light yellow crystals: mp. 123-124 °C; R_f (10% MeOH/CH_2Cl_2) 0.50; ^{1}H NMR (DMSO-d_6) 9.53 (s, 1H), 8.56 (d, 1H, J = 0.9 Hz), 8.43 (d, 1H, J = 0.9 Hz), 7.23-7.20 (m, 3H), 6.76-6.70 (m, 1H); ^{13}C NMR (DMSO-d_6) 157.7, 152.5, 139.3, 135.0, 131.9, 129.8, 116.1, 115.0, 112.0; Anal. calcd for $C_9H_7NO_2$: C, 67.07; H, 4.38; N, 8.69; Found C, 66.89; H, 4.30; N, 8.32.

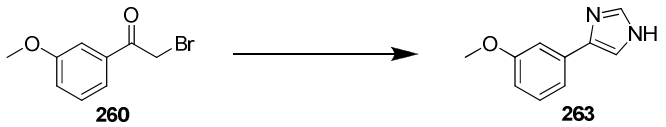
6.41. 3-(Oxazol-4-yl)phenyl cyclohexylcarbamate (250)[232]



262 **250**

Purification by FC (Eluent 1% MeOH/CH_2Cl_2) and recrystallization from EtOAc/hex gave **250** (160 mg, 90%) as white crystals; mp. 139.5-140.5 °C; R_f (10% MeOH/CH_2Cl_2) 0.40; ^{1}H NMR ($CDCl_3$) 7.93 (d, 2H, J = 6.9 Hz), 7.58 (d, 1H, J = 7.8 Hz), 7.53 (s, 1H), 7.39 (t, 1H, J = 7.9 Hz), 7.10 (dd, 1H, J = 8.1, 1.6 Hz), 4.95 (br d, 1H, J = 6.8 Hz), 3.64-3.52 (m, 1H), 2.08-1.97 (m, 1H), 1.80-1.70 (m, 2H), 1.67-1.58 (m, 1H), 1.44-1.32 (m, 2H), 1.30-1.14 (m, 3H); ^{13}C NMR ($CDCl_3$) 153.5, 151.5, 151.2, 139.8, 134.0, 132.0, 129.6, 122.3, 121.4, 118.9, 50.1, 33.2, 25.4, 24.7; Anal. calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78; Found C, 67.05; H, 6.61; N, 9.71.

6.42. 4-(3-Methoxyphenyl)-1H-imidazole (263)[232,237]



260 263

2-Bromo-1-(3-methoxyphenyl)-ethanone (**260**, 530 mg, 2.3 mmol, 100 mol-%) and formamide (2.0 ml, 50 mmol, 2200 mol-%) were heated up until the temperature (T_{oil}) reached 165 °C, during 90 min. The mixture was cooled to rt and partitioned between EtOAc (30 ml) and water (50 ml). The pH of aqueous layer was adjusted to 12 with 35% NaOH, and extracted with EtOAc. The organic phase was dried (Na_2SO_4), and evaporated to dryness. Recrystallization from H_2O/MeOH) gave **263** (340 mg, 85%) as light yellow crystals: mp. 121-122 °C; R_f (EtOAc) 0.1; ^{1}H NMR ($CDCl_3$) 11.16 (br s, 1H), 7.68 (d, 1H, *J* = 1.0 Hz), 7.34 (d, 1H, *J* = 1.0 Hz), 7.31-7.23 (m, 3H), 6.80-6.76 (m, 1H), 3.74 (s, 3H); ^{13}C NMR ($CDCl_3$) 159.9, 138.4, 135.7, 134.3, 129.8, 117.4, 115.9, 112.7, 110.2, 55.1; Anal. calcd for $C_{10}H_{10}N_2O$: C, 68.95; H, 5.79; N, 16.08; Found C, 68.83; H, 5.43; N, 16.32.

6.43. 4-(3-Hydroxyphenyl)1H-imidazole (264)[232,238]

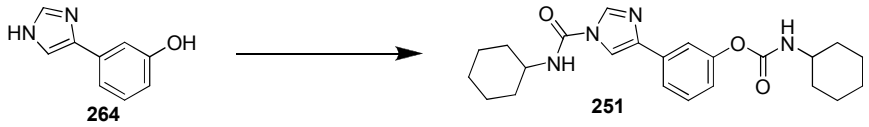
263 264

Method A: Compound **263** (1.5 g, 8.6 mmol, 100 mol-%) was dissolved to dry CH_2Cl_2 (90 ml) and cooled to –78 °C. BBr_3 (1 M in CH_2Cl_2, 25 ml, 300 mol-%) was added dropwise during 5 min. The mixture was allowed to warm to rt and stirred overnight, quenched by addition of water (150 ml). The organic phase was separated and extracted with H_2O (90 ml). Combined aqueous phases were neutralized with 35% NaOH (pH adjusted to 6) and extracted with EtOAc. The combined organic phases were dried (Na_2SO_4), evaporated and purified by FC giving **264** (1.1 g, 80%) as a white solid: mp. 213-215 °C; R_f (20% MeOH/CH_2Cl_2) 0.1; ^{1}H NMR ($CDCl_3$) 9.35 (br s, 1H), 7.81 (br s, 1H), 7.53 (br s, 1H), 7.19-7.11 (m, 3H), 6.62 (dt, *J* = 7.1, 2.1 Hz, 1H); ^{13}C NMR (DMSO-d_6) 157.7, 138.2, 136.0, 135.2, 129.6, 115.4 (2 C), 113.4, 111.4.

Method B[222]: Compound **263** (400 mg, 2.3 mmol, 100 mol-%), *N*-butylpyridinium bromide (990 mg, 4.6 mmol, 200 mol-%) and 48% aq. HBr (780 mg, 4.6 mmol, 200

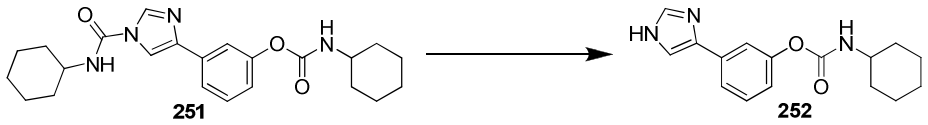
mol-%) were placed in a 10 mL tube and MW-irradiated for 5 x 20 s (300 W, T_{max} 100 °C). Temperature of the reaction raised to 148 °C and pressure to 250 psi. The mixture was worked up and purified as described in Method A resulting in title compound **264** (240 mg, 53%).

6.44. 3-[1-(Cyclohexylcarbamoyl)1H-imidazol-4-yl]phenyl cyclohexylcarba-mate (251)[232]



Purification by FC (Eluent 25-70% EtOAc/hex) and recrystallization from EtOAc/hex gave **251** (154 mg, 43%) as a white solid; mp. 158-165 °C; R_f (EtOAc) 0.55; ^{1}H NMR ($CDCl_3$) 8.06 (d, 1H, *J* = 1.2 Hz), 7.54-7.44 (m, 3H), 7.28 (t, 1H, *J* = 7.8 Hz), 6.97 (dd, 1H, *J* = 8.0, 1.6 Hz), 6.22 (d, 1H, *J* = 7.6 Hz), 5.12 (d, 1H, *J* = 8.1 Hz), 3.82-3.71 (m, 1H), 3.63-3.51 (m, 1H), 2.10-1.95 (m, 4H), 1.82-1.71 (m, 4H), 1.71-1.59 (m, 2H), 1.45-1.31 (m, 4H), 1.31-1.14 (m, 6H); ^{13}C NMR ($CDCl_3$) 154.0, 151.2, 148.0, 141.9, 136.2, 134.5, 129.4, 122.0, 120.6, 118.4, 111.4, 50.4, 50.3, 33.2, 32.9, 25.4, 25.3, 24.9, 24.7; Anal. calcd for $C_{23}H_{30}N_4O_3$: C, 67.29; H, 7.37; N, 13.65; Found C, 67.30; H, 7.37; 13.63.

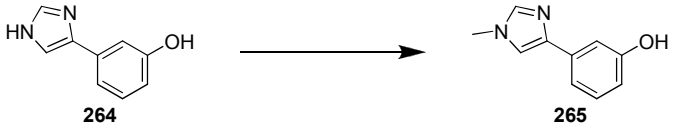
6.45. 3-(1H-Imidazol-4-yl)phenyl cyclohexylcarbamate (252)[232]



Compound **251** (240 mg, 0.58 mmol, 100 mol-%) was suspended in CH_2Cl_2 (10 ml) at rt followed by addition of MeOH (0.048 ml, 1.5 mmol, 260 mol-%) and grinded NaOH (23 mg, 0.58 mmol, 100 mol-%). The mixture was stirred at rt for 72 h and then directly purified by FC (Eluent 0-100% EtOAc/hex) and recrystallized from toluene giving **252** (120 mg, 71%) as a white solid: mp. 163-164.5 °C; R_f (EtOAc) 0.2; ^{1}H NMR (DMSO-d_6) 12.19 (s, 1H), 7.73-7.54 (m, 4H), 7.46 (s, 1H), 7.32 (t, 1H, *J* = 7.9 Hz), 6.89 (d, 1H, *J* = 7.9 Hz), 3.36-3.28 (m, 1H), 1.87-1.79 (m, 2H), 1.75-1.68 (m, 2H), 1.60-1.53 (m, 1H), 1.33-1.20 (m, 4H), 1.18-1.01 (m, 1H); ^{13}C NMR (DMSO-d_6) 153.6, 151.5, 139.3, 136.3, 136.0, 129.3, 120.7, 119.4, 117.6, 113.2,

49.8, 32.6, 25.2, 24.6; Anal. calcd for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73; Found C, 66.88; H, 6.61; 14.28; HRMS (ESI) calcd $[M+Na]^+$ for $C_{16}H_{19}N_3O_2Na$, 308.1375, found 308.1366.

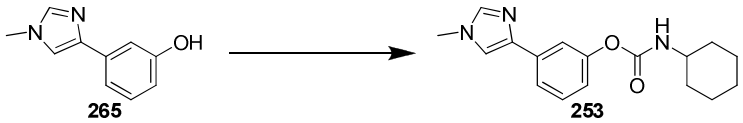
6.46. 3-(1-Methyl-1H-imidazol-4-yl)phenol (265)[232]



264 265

To a mixture of **264** (250 mg, 1.6 mmol, 100 mol-%) in *N,N*-dimethylformamide (10 ml) was added MeI (110 µL, 1.7 mmol, 110 mol-%) and stirred at 0 °C for 90 min. The mixture was allowed to warm to rt and another portion of MeI (110 µL, 1.7 mmol, 110 mol-%) was added, and stirring continued for 12 h at rt. Solvent was evaporated and residue purified by FC (Eluent 0-5% MeOH/CH_2Cl_2) giving **265** (131 mg, 48%) as an off-white solid: R_f (10% MeOH/CH_2Cl_2) 0.47; mp. 160-166 °C; ^{1}H NMR (MeOD-d_4) 7.61 (s, 1H), 7.35 (s, 1H), 7.18-7.11 (m, 3H), 6.68-6.62 (m, 1H), 3.74 (s, 3H).

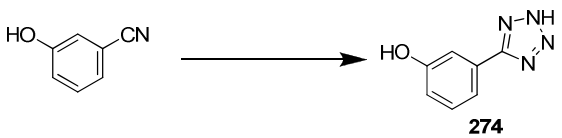
6.47. 3-(1-Methyl-1H-imidazol-4-yl)phenyl cyclohexylcarbamate (253)[232]



265 253

Recrystallization from EtOAc/ Et_2O/hex gave **253** (125 mg, 66%) as a red solid: mp. 139.5-140.5 °C; R_f (10% MeOH/CH_2Cl_2) 0.58; ^{1}H NMR (DMSO-d_6) 7.69 (d, 1H, J = 7.7 Hz), 7.65-7.62 (m, 2H), 7.55 (d, 1H, J = 7.8 Hz), 7.43 (t, 1H, J = 1.9 Hz); 7.31 (t, 1H, J = 7.9 Hz), 6.89 (ddd, 1H, J = 8.0, 2.1, 0.8 Hz), 3.68 (s, 3H), 3.37-3.29 (m, 1H), 1.88-1.79 (m, 2H), 1.77-1.68 (m, 2H), 1.66-1.51 (m, 1H), 1.35-1.01 (m, 5H); ^{13}C NMR (DMSO-d_6) 154.4, 152.4, 140.7, 139.4, 136.9, 130.1, 121.4, 120.2, 118.3, 118.2, 50.6, 33.9, 33.4, 26.0, 25.4; Anal. calcd for $C_{17}H_{21}N_3O_2$: C, 68.20; H, 7.07; N, 14.04; Found C, 68.47; H, 7.47; N, 13.82.

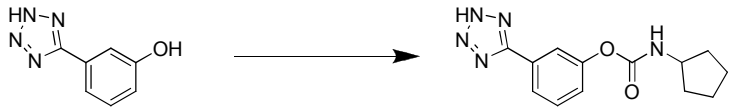
6.66. 5-(3-Hydroxyphenyl)1H-tetrazole (274)[239]



274

3-Hydroxybenzonitrile (1.43 g, 12 mmol, 100 mol-%), Et_3NHCl (3.3 g, 24 mmol, 200 mol-%) and NaN_3 (1.6 g, 8 mmol, 200 mol-%) were refluxed in dry toluene (120 ml) for 20 h. The mixture was cooled to rt, diluted with H_2O (90 ml) and EtOAc (20 ml), and extracted with H_2O (20ml). Water phase was acidified by a dropwise addition of 32% HCl until white solid precipitated. The precipitate was filtrated, washed with water and dried to give **269** (2.2 g, 90%) as a white solid: mp. 220-222 °C; R_f (8% MeOH and 2% AcOH in $CHCl_3$) 0.25; ^{1}H NMR (DMSO-d_6) 9.94 (s, 1H), 7.47-7.38 (m, 2H), 7.40 (t, 1H, J = 8.0 Hz), 6.98 (ddd, 1H, J = 8.0, 2.0, 1.0 Hz); ^{13}C NMR (DMSO-d_6) 158.0, 155.3 (br), 130.6, 125.1, 118.3, 117.6, 113.6.

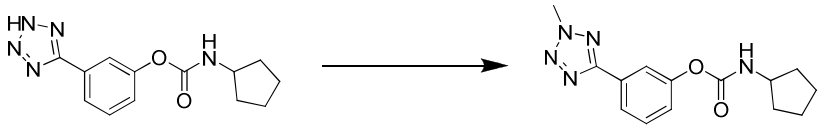
6.48. 3-(1H-Tetrazol-5-yl)phenyl cyclopentylcarbamate (269)[232]



274 269

After a typical carbamoylation reaction, the reaction mixture was extracted with H_2O (3 x 20 ml). The aqueous phase was acidified with 32% HCl (0.8 ml) until white precipitate formed. The precipitate was filtered and recrystallized from H_2O/MeOH giving **269** (48 mg, 65%) as white crystals: mp. 174-177 °C; R_f (8% MeOH, 2% AcOH in $CHCl_3$) 0.40; ^{1}H NMR (DMSO-d_6) 7.94 (d, 1H, J = 7.2 Hz), 7.89 (d, 1H, J = 7.8 Hz), 7.77 (s, 1H), 7.62 (t, 1H, J = 8.0 Hz), 7.34 (dd, 1H, J = 8.1, 1.6 Hz), 3.92-3.83 (m, 1H), 1.91-1.80 (m, 2H), 1.73-1.63 (m, 2H), 1.59-1.45 (m, 4H); ^{13}C NMR (DMSO-d_6) 155.0, 153.4, 151.7, 130.6, 125.2, 124.7, 123.4, 120.3, 52.4, 32.2, 23.3; Anal. calcd for $C_{13}H_{15}N_5O_2$: C, 57.13; H, 5.53; N, 25.63; Found C, 56.90; H, 5.30; N, 25.23.

6.49. 3-(2-Methyl-2H-tetrazol-5-yl)phenyl cyclopentylcarbamate (270)[232]

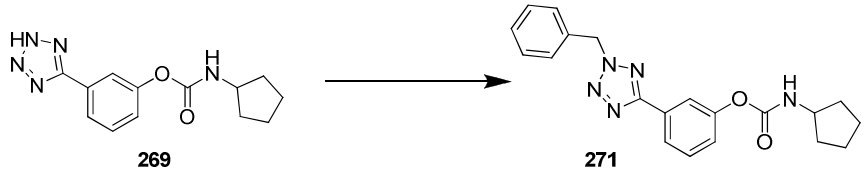


269 270

Compound **269** (120 mg, 0.45 mmol, 100 mol-%) in acetone (2.5 ml) was cooled to 2 °C (T_{bath}). Triethylamine (70 µL, 0.50 mmol, 110 mol-%) was added followed by addition of MeI (160 mg, 1.1 mmol, 250 mol-%). After 2 h another portion of MeI (450 mg, 3.2 mmol, 700 mol-%) was added. The mixture was stirred for another 1 h

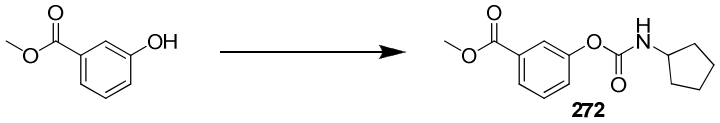
at 2 °C and diluted with EtOAc (35 ml). The organic phase was washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4) and evaporated giving 100 mg (74%) of 4:1 mixture of 2-methylated and 1-methylated tetrazoles. The isomers were separated by FC (Eluent 0.5-1% MeOH/CH_2Cl_2) and major product was recrystallized from EtOAc/hex giving **270** (60 mg, 46%) as a white solid: mp. 145-147 °C; R_f (3% MeOH/CH_2Cl_2) 0.7; ^{1}H NMR ($CDCl_3$) 7.97 (d, 1H, *J* = 7.7 Hz), 7.91 (s, 1H), 7.47 (t, 1H, *J* = 7.9 Hz), 7.28-7.22 (m, 1H), 5.05 (d, 1H, *J* = 5.8 Hz), 4.39 (s, 3H), 4.14-4.01 (m, 1H), 2.10-1.98 (m, 2H), 1.79-1.58 (m, 4H), 1.56-1.46 (m, 2H); ^{13}C NMR ($CDCl_3$) 164.6, 153.7, 151.5, 129.8, 128.5, 123.6, 123.5, 120.1, 53.0, 39.5, 33.1, 23.5; Anal. calcd for $C_{14}H_{17}N_5O_2$: C, 58.52; H, 5.96; N, 24.37; Found C, 58.52; H, 5.85; N, 24.35.

6.50. 3-(2-Benzyl-2H-tetrazol-5-yl)phenyl cyclopentylcarbamate (271)[232]



Compound **269** (100 mg, 0.37 mmol, 100 mol-%) in acetone (2.5 ml) was cooled to 2 °C (T_{bath}). Triethylamine (60 µL, 0.41 mmol, 110 mol-%) was added followed by addition of BnBr (50 µL, 0.41 mmol, 110 mol-%) and KI (30 mg, 0.19 mmol, 50 mol-%). After 90 min the mixture was diluted with EtOAc (40 ml). The organic phase was washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4) and evaporated giving 105 mg (78%) of 9:1 mixture of 2-benzylated and 1-benzylated tetrazoles respectively. The isomers were separated by FC (Eluent CH_2Cl_2) and major product was recrystallized from EtOAc/hex giving compound **271** (81 mg, 60%) as a white solid: mp. 129-130 °C; R_f (3% MeOH/CH_2Cl_2) 0.9; ^{1}H NMR ($CDCl_3$) 7.97 (d, 1H, *J* = 7.2 Hz), 7.90 (s, 1H), 7.48-7.34 (m, 6H), 7.26-7.20 (m, 1H), 5.79 (s, 2H), 5.02 (br s, 1H), 4.11-4.00 (m, 1H), 2.08-1.96 (m, 2H), 1.76-1.56 (m, 4H), 1.54-1.46 (m, 2H); ^{13}C NMR ($CDCl_3$) 164.8, 153.7, 151.4, 133.3, 129.8, 129.0, 128.9, 128.6, 128.3, 123.7, 123.6, 120.2, 56.8, 53.0, 33.1, 23.5; Anal. calcd for $C_{20}H_{21}N_5O_2$: C, 66.10; H, 5.82; N, 19.27; Found C, 65.71; H, 5.85; N, 19.28.

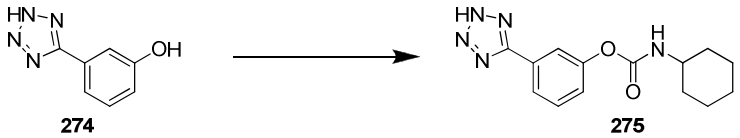
6.51. Methyl 3-(cyclopentylcarbamoyloxy)benzoate (272)[232]



272

Recrystallization from EtOAc/hex gave **272** (1.36 g, 79%) as colourless crystals: mp. 112-114 °C; R_f (10% Et_2O/CH_2Cl_2) 0.5; ^{1}H NMR ($CDCl_3$) 7.85 (d, 1H, *J* = 7.6 Hz), 7.78 (s, 1H), 7.39 (t, 1H, *J* = 7.9 Hz) 7.34-7.29 (m, 1H), 5.53 (br d, 1H, *J* = 7.0 Hz), 4.08-3.97 (m, 1H), 3.89 (s, 3H), 2.01-1.91 (m, 2H), 1.72-1.40 (m, 6H); ^{13}C NMR ($CDCl_3$) 166.2, 153.6, 150.9, 131.1, 128.9, 126.2, 126.0, 122.6, 52.8, 52.0, 32.7, 23.3; Anal. calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32; Found C, 63.96; H, 6.30; N, 5.39.

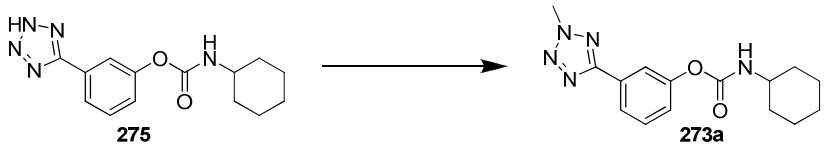
6.52. 3-(1H-Tetrazol-5-yl)phenyl cyclohexylcarbamate (275)



274 **275**

This compound isolated as described for **269** and recrystallized from H_2O/MeOH giving **275** (1.1 g, 62%) as white crystals: mp. 180-182 °C; R_f (8% MeOH, 2% AcOH in $CHCl_3$) 0.25; ^{1}H NMR (DMSO-d_6) 7.89 (t, 1H, *J* = 8.0 Hz), 7.78 (s, 1H), 7.62 (t, 1H, *J* = 7.9 Hz), 7.35 (d, 1H, *J* = 7.8 Hz), 3.42-3.29 (m, 1H), 1.91-1.81 (m, 2H), 1.77-1.66 (m, 2H), 1.62-1.53 (m, 1H), 1.36-1.20 (m, 4H), 1.19-1.06 (m, 1H); ^{13}C NMR (DMSO-d_6) 155.0, 153.1, 151.7, 130.6, 125.3, 124.6, 123.4, 120.2, 49.9, 32.5, 25.1, 24.6; HRMS (ESI) calcd for $[M+Na]^+$ $C_{14}H_{17}N_5O_2Na$: 310.1280, found 310.1291.

6.53. 3-(2-Methyl-2H-tetrazol-5-yl)phenyl cyclohexylcarbamate (273a)

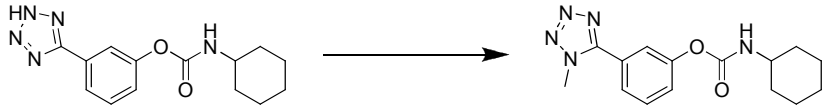


275 **273a**

This compound was prepared and worked up as described for **270**. The isomers were separated by FC (Eluent 0.5-1% MeOH/CH_2Cl_2) and major product was recrystallized from EtOAc/hex giving **273a** (270 mg, 35%) as a white solid: mp.

158-160 °C; R_f (3% MeOH/CH_2Cl_2) 0.5; ^{1}H NMR ($CDCl_3$) 7.97(d, 1H, *J* = 7.8 Hz), 7.93-7.90 (m, 1H), 7.47 (t, 1H, *J* = 7.9 Hz), 7.28-7.23 (m, 1H), 4.39 (s, 3H), 3.65-3.52 (m, 1H), 2.07-1.98 (m, 2H), 1.80-1.70 (m, 2H), 1.68-1.59 (m, 1H), 1.45-1.33 (m, 2H), 1.30-1.17 (m, 3H); ^{13}C NMR ($CDCl_3$) 164.7, 153.3, 151.5, 129.8, 128.5, 123.6, 123.5, 120.1, 50.2, 39.5, 33.2, 25.4, 24.7; Anal. calcd for $C_{15}H_{19}N_5O_2$: C, 59.79; H, 6.36; N, 23.24; Found C, 59.49; H, 6.20; N, 23.13.

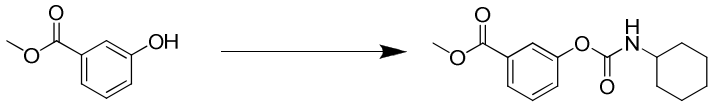
6.54. 3-(1-Methyl-1H-tetrazol-5-yl)phenyl cyclohexylcarbamate (273b)



275 273b

The minor product from preparation of **273a** was recrystallized from EtOAc/hex giving **273b** (26 mg, 3%) as white crystals: mp. 157-161 °C; R_f (3% MeOH/CH_2Cl_2) 0.30; ^{1}H NMR ($CDCl_3$) 7.61-7.51 (m, 3H), 7.40-7.32 (m, 1H), 5.08-4.97 (br d, 1H), 4.19 (s, 3H), 3.64-3.51 (m, 1H), 2.10-1.97 (m, 2H), 1.82-1.70 (m, 2H), 1.69-1.57 (m, 1H), 1.46-1.33 (m, 2H), 1.32-1.14 (m, 3H); ^{13}C NMR ($CDCl_3$) 153.8, 153.0, 151.5, 130.3, 125.2, 124.6, 124.4, 122.1, 50.3, 35.1, 33.2, 25.4, 24.7; Anal. calcd for $C_{15}H_{19}N_5O_2$: C, 59.79; H, 6.36; N, 23.24; Found C, 59.80; H, 6.30; N, 23.07.

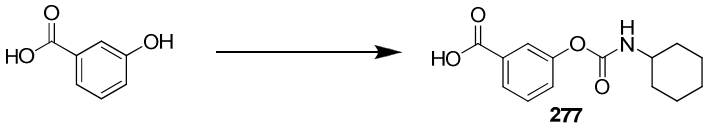
6.55. Methyl 3-(cyclohexylcarbamoyloxy)benzoate (276)[232]



276

Recrystallization from EtOAc/hex gave **276** (400 mg, quant.) as white crystals: mp. 131-132 °C; R_f (10% Et_2O/CH_2Cl_2) 0.5; ^{1}H NMR ($CDCl_3$) 7.87 (d, 1H, *J* = 7.7 Hz), 7.80 (t, 1H, *J* = 1.9 Hz), 7.42 (t, 1H, *J* = 7.9 Hz), 7.36-7.31 (m, 1H), 4.95 (d, 1H, *J* = 7.0 Hz), 3.91 (s, 3H), 3.64-3.51 (m, 1H), 2.07-1.98 (m, 2H), 1.80-1.70 (m, 2H), 1.67-1.60 (m, 1H), 1.45-1.32 (m, 2H), 1.29-1.17 (m, 3H); ^{13}C NMR ($CDCl_3$) 166.3, 153.3, 151.0, 131.3, 129.1, 126.3, 126.2, 122.7, 52.1, 50.2, 33.1, 25.3, 24.7; Anal. calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05; Found C, 65.32; H, 6.96; N, 5.19.

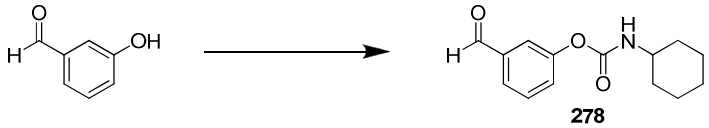
6.56. 3-(Cyclohexylcarbamoyloxy)benzoic acid (277)



277

Recrystallization from EtOAc/hex gave **277** (280 mg, 73%) as a white solid: mp. 205-206 °C; R_f (10% MeOH/CH_2Cl_2) 0.3; ^{1}H NMR (DMSO-d_6) 13.13 (s, 1H), 7.83-7.75 (m, 2H), 7.61-7.58 (m, 1H), 7.50 (t, 1H, J = 7.9 Hz), 7.38-7.34 (m, 1H), 3.40-3.27 (m, 1H), 1.89-1.79 (m, 2H), 1.76-1.66 (m, 2H), 1.61-1.52 (m, 1H), 1.34-1.05 (m, 5H); ^{13}C NMR (DMSO-d_6) 166.6, 153.2, 151.1, 132.0, 129.6, 126.2, 125.7, 122.3, 49.8, 32.5, 25.1, 24.5; Anal. calcd for $C_{14}H_{17}NO_4$ C, 63.87; H, 6.51; N, 5.32; Found C, 63.95; H, 6.60; N, 5.61.

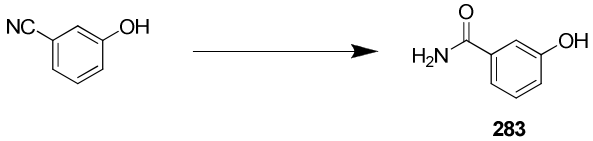
6.57. 3-Formylphenyl cyclohexylcarbamate (278)



278

Recrystallization from EtOAc/hex gave **278** (2.9 g, 69%) as white crystals; mp. 120-122 °C; R_f (2% MeOH/CH_2Cl_2) 0.8; ^{1}H NMR ($CDCl_3$); 9.92 (s, 1H), 7.71 (d, 1H, J = 7.6 Hz), 7.67-7.65 (m, 1H), 7.52 (t, 1H, J = 7.8 Hz), 7.43-7.39 (m, 1H), 5.01 (br d, 1H, J = 6.8 Hz), 3.63-3.51 (m, 1H), 2.09-1.98 (m, 2H), 1.81-1.71 (m, 2H), 1.68-1.59 (m, 1H), 1.45-1.32 (m, 2H), 1.30-1.14 (m, 3H); ^{13}C NMR ($CDCl_3$) 191.4, 153.1, 151.7, 137.6, 129.9, 127.8, 126.4, 122.4, 50.3, 33.2, 25.4, 24.7; Anal. calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66; Found C, 67.83; H, 6.86; N, 5.65.

6.58. 3-Hydroxybenzamide (283)[226]

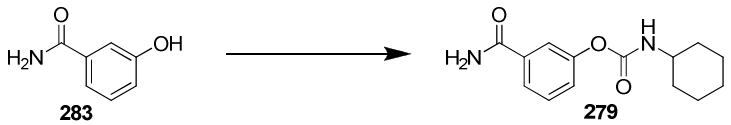


283

3-Cyanophenol (295 mg, 2.48 mmol, 100 mol-%) and $NaBO_3 \cdot 4H_2O$ (1.15 g, 7.45 mmol, 300 mol-%) in H_2O (8 ml) were heated to 50 °C and MeOH (14 ml) was added until mixture was clear. The mixture was stirred at 50 °C for 70 h and excess MeOH was evaporated, and the pH of remaining mixture was adjusted to 5 with conc. HCl (aq). Mixture was extracted with CH_2Cl_2 and EtOAc (5 x 15 ml). Organic

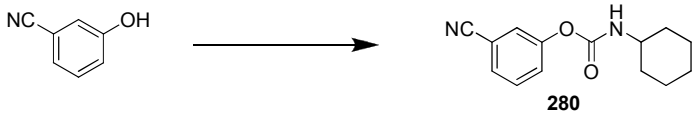
phases were combined, washed with brine and dried (Na_2SO_4). Evaporation of solvent gave **283** (183 mg, 54%) as white solid: mp. 165-168 ^oC; R_f (50% EtOAc/hex) 0.10; ^1H NMR (DMSO-d_6) 9.58 (s, 1H), 7.84 (br s, 1H), 7.29-7.20 (m, 4H), 6.89 (ddd, 1H, J = 7.9, 2.5, 1.0 Hz).

6.59. 3-Carbamoylphenyl cyclohexylcarbamate (279)[232]



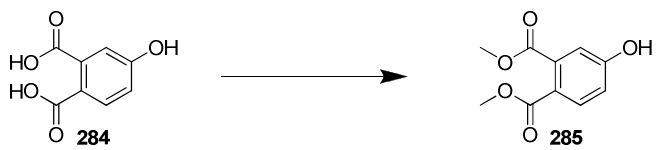
Purification by FC (Eluent 10% MeOH/CH_2Cl_2) and recrystallization (EtOAc) gave **279** (40 mg, 15%) as white crystals; mp. 169-179 ^oC; R_f (15% MeOH/CH_2Cl_2) 0.36; ^1H NMR (DMSO-d_6) 8.01 (s, 1H), 7.78 (d, 1H, J = 7.9 Hz), 7.71 (d, 1H, J = 7.8 Hz), 7.58 (s, 1H), 7.44 (m, 2H), 7.26-7.24 (m, 1H), 3.36-3.28 (m, 1H), 1.86-1.80 (m, 2H), 1.74-1.68 (m, 2H), 1.59-1.53 (m, 1H), 1.30-1.20 (m, 4H), 1.17-1.08 (m, 1H); ^{13}C NMR (DMSO-d_6) 167.8, 154.2, 151.9, 136.3, 130.0, 125.6, 124.8, 121.7, 50.7, 33.4, 26.0, 25.4; Anal. calcd for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68; Found C, 64.32; H, 6.97; N, 10.47.

6.60. 3-Cyanophenyl cyclohexylcarbamate (280) [232]



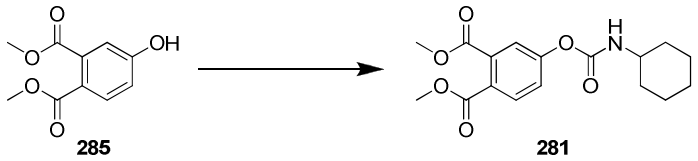
Recrystallization from EtOAc/hex gave compound **280** (1.47 g, 68%) as white needles: mp. 117-120 ^oC; R_f (50% EtOAc/hex) 0.56; ^1H NMR ($CDCl_3$) 7.50-7.38 (m, 4H), 4.98 (d, 1H, J = 7.3 Hz), 3.61-3.51 (m, 1H), 2.05-1.98 (m, 2H), 1.80-1.71 (m, 2H), 1.68-1.60 (m, 1H), 1.44-1.33 (m, 2H), 1.29-1.15 (m, 3H); ^{13}C NMR ($CDCl_3$) 152.6, 151.2, 130.1, 128.8, 126.5, 125.3, 118.0, 113.2, 50.3, 33.1, 25.3, 24.7; Anal. calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47; Found C, 68.89; H, 6.55; N, 11.36.

6.61. Dimethyl 4-hydroxyphthalate (285)[240]



3-Hydroxyphthalic acid (**284**, 360 mg, 2.0 mmol, 100 mol-%) was dissolved in MeOH (20 ml), and conc. H_2SO_4 (11 µL, 0.2 mmol, 10 mol-%) was added to the mixture. The mixture was refluxed for 24 h, cooled to rt, poured to sat. $NaHCO_3$ (30 ml), and most of the MeOH was evaporated to dryness. The mixture was extracted with EtOAc and combined organic phases were dried (Na_2SO_4), and evaporated giving **285** (400 mg, 95%) as a white solid: mp. 110-111 °C; R_f (EtOAc) 0.6; ^{1}H NMR ($CDCl_3$) 7.74 (d, 1H, *J* = 8.6 Hz), 7.24 (br s, 1H), 7.01 (d, 1H, *J* = 2.6 Hz), 6.92 (dd, 1H, *J* = 8.4, 2.6 Hz), 3.90 (s, 3H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$) 169.6, 167.2, 159.4, 135.6, 131.9, 121.5, 117.3, 115.3, 53.0, 52.5.

6.62. Dimethyl 4-(cyclohexylcarbamoyloxy)phthalate (281) [232]



Recrystallization from EtOAc/hex gave **281** (190 mg, 80%) as white crystals: mp. 112-112.5 °C; R_f (EtOAc) 0.5; ^{1}H NMR ($CDCl_3$) 7.75 (d, 1H, *J* = 8.5 Hz), 7.46 (d, 1H, *J* = 2.1 Hz) 7.32 (dd, 1H, *J* = 8.5, 2.1 Hz), 5.05 (d, 1H, *J* = 7.4 Hz) 3.90 (s, 3H), 3.89 (s, 3H), 3.62-3.49 (m, 1H), 2.06-1.95 (m, 2H), 1.80-1.70 (m, 2H), 1.67-1.59 (m, 1H), 1.44-1.30 (m, 2H), 1.28-1.16 (m, 3H); ^{13}C NMR ($CDCl_3$) 167.5, 167.1, 153.2, 152.4, 133.9, 130.5, 127.8, 123.7, 121.8, 52.7, 52.6, 50.3, 33.1, 25.3, 24.7; Anal. calcd for $C_{17}H_{21}NO_6$: C, 60.89; H, 6.31; N, 4.18; Found C, 60.98; H, 6.38; N, 4.27.

Biological testing protocols

Enzyme inhibition assays were organized and carried out by Ph. D. Susanna M. Saario and co-workers in the Department of Pharmaceutical Chemistry, University of Kuopio.[12,49,152,179,241,242]

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ISBN 978-952-248-240-2
ISBN 978-952-248-241-9 (PDF)
ISSN 1795-2239
ISSN 1795-4584 (PDF)