Overview of the Chemical Families of Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase Inhibitors

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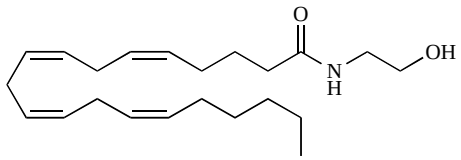
Abstract: The family of the endogenous agonists of the cannabinoid receptors – i.e., the endocannabinoids - includes several polyunsaturated fatty acid amides and esters. Arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are, respectively, the leads of these chemical families. So far, two enzymes responsible for the metabolism of AEA and 2-AG have been described: Fatty Acid Amide Hydrolase (FAAH) which hydrolyzes AEA and in some cells 2-AG, and Monoacylglycerol Lipase (MAGL) which hydrolyzes 2-AG. In spite of the early characterisation of MAGL and the nearly simultaneous clonings of the two enzymes, most of the efforts were dedicated to the study of FAAH and consequentially, the range of FAAH inhibitors available nowadays exceeds the number of compounds active upon MAGL. FAAH inhibitors can be divided in two major groups, the first one includes the inhibitors inspired by the chemical structures of FAAH substrates, which carry an arachidonoyl-, oleoyl- or palmitoyl-carbon chain that mimic the fatty acid chains of anandamide, oleamide and palmitoylethanolamide. The second group involves compounds that do not share similarities with the endocannabinoids, such as the carbamates, oxazolopyridins, 2-thioxoimidazolidin-4-ones, imidazolidine-2,4-diones and the non-steroidal anti-inflammatory drugs. However, the family of MAGL inhibitors contains few members and most of them exhibit a lack of selectivity.

The purpose of this review is to give an overview of the families of synthetic inhibitors of FAAH and MAGL. The synthetic pathways, the chemical features, potencies, selectivities and modes of inhibition are listed and discussed in order to facilitate their comparison.

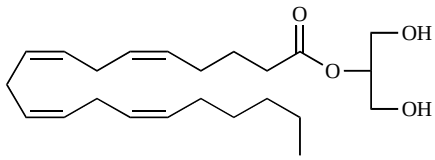
Key Words: Endocannabinoids, Fatty Acid Amide Hydrolase, Monoacylglycerol Lipase, Anandamide, 2-Arachidonoylglycerol.

I. INTRODUCTION

The multiple medicinal properties of cannabis are known for centuries. However, the deep understanding of the biological mechanisms responsible for the effects of Δ^9-tetrahydrocannabinol, its main psychoactive component, only began sixteen years ago with the cloning of the cannabinoid receptors CB_1 [1] and CB_2 [2]. Both receptors are G protein coupled. CB_1 receptors are mainly expressed in the central nervous system, while CB_2 receptors are restricted to peripheral tissues in healthy adults [3]. In addition to these two cannabinoid receptors, GPR55 was recently identified as a third G protein coupled receptor that is activated by different cannabinoids ligands [4]. The discovery of the first cannabinoid receptors launched the quest for their endogenous ligands and brought to light a family of polyunsaturated fatty acid amides or esters derived from arachidonic acid. Arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) (Fig. **1**) are, respectively, the leads of these chemical families and share many, but not all, of the pharmacological properties of Δ^9-tetrahydrocannabinol. Endogenous occurrences of AEA and 2-AG were reported in the brain, liver, spleen, lung, kidney and plasma [5-7]. Both AEA and 2-AG are agonists of CB_1 and CB_2 cannabinoid receptors. During the last years, other fatty acid derivatives were isolated and assigned as members of the endocannabinoid family. The CB_2 agonist virod-hamine (*O*-arachidonoylethanolamine) was found in rat brain [8], the selective CB_1 agonist *N*-arachidonoyldopamine (NADA) was isolated from rat brain and bovine dorsal root ganglions [9-10] and finally, oleamide was found as a CB_1 agonist *in vitro* [11]. Nevertheless the weak potency of oleamide called into question its effect *in vivo* and therefore, its "membership" in the family of the endogenous cannabinoids [12] (Fig. **2**).



Anandamide



2-arachidonoylglycerol (2-AG)

Fig. (1). Chemical structures of anandamide (AEA) and 2-arachidonoylglycerol (2-AG).

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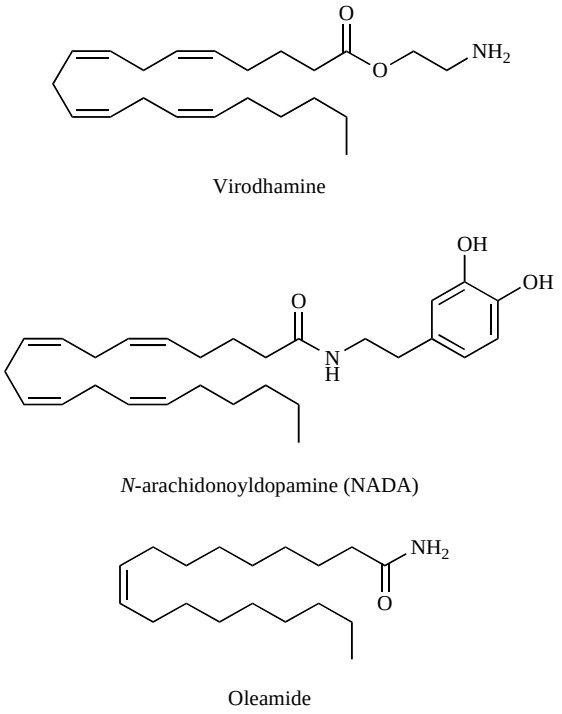


Fig. (2). Chemical structures of virodhamine, arachidonoyldopamine (NADA) and oleamide.

Since the discoveries of AEA and 2-AG, there have been several investigations of their therapeutic potential. The first studies reported the pharmacological effects of *exogenous* administration of AEA or 2-AG, leading to analgesia [13-18], inhibition of the electrically evoked twitch response of the mouse isolated vas deferens [19], cardiovascular effects [20], effect on lung function [21] and antiproliferation [22-25]. Further evidences of the therapeutic potential of AEA and 2-AG were allowed by the setting up of different methods of extraction and dosage of the *endogenous* content of AEA in tissues, which demonstrated that the endocannabinoid tone is modulated during pathological processes. Increases in the endogenous contents of AEA and 2-AG were observed within the spinal cord of chronic relapsing experimental autoimmune encephalomyelitis (CREAE) mice. The beneficial actions of both endocannabinoids in this model of multiple sclerosis were confirmed by exogenous administrations which were followed by an improvement of spasticity [26]. A significant increase in endogenous 2-AG level was reported after closed head injury (CHI) in mice and exogenous administration of 2-AG was shown to induce reduction of brain oedema, reduced hippocampal cell death and better clinical recovery [27]. Human adenomas and colorectal carcinomas were also shown to be associated with elevations of endogenous levels of AEA and 2-AG, likely to counteract cellular proliferation, since both compounds were reported to inhibit CaCo-2 cell proliferation with potencies consistent with their relative potencies at CB_1 receptors [28]. Elevation of endocannabinoid levels was also reported in other disorders including schizophrenia [29], human pituitary adenomas [30], Huntington's disease [31], obesity [32-33] and cardiogenic shock [34] (for a review on the therapeutic potential of drugs that target cannabinoid receptors, see Pertwee [35]).

The therapeutic potential of endocannabinoids, particularly AEA and 2-AG, is therefore clearly established. Nevertheless, their use as therapeutic agents would imply that sufficient concentrations could be reached at the level of their biological targets. Since both AEA and 2-AG are quickly metabolised *in vivo*, a promising way to promote their therapeutic potential might be found in the discovery of compounds that increase their half-life *in vivo*. Nowadays, the main actors responsible for the metabolism of endocannabinoids are known. Three hydrolyzing enzymes, namely Fatty Acid Amide Hydrolase (FAAH) [36-38], Mono-acylglycerol Lipase (MAGL) [39] and *N*-Palmitoylethanolamine-hydrolyzing Acid Amidase (NPAA [40-41], which was recently renamed as *N*-Acylethanolamine-hydrolyzing Acid Amidase (NAAA) [42]) have been cloned. In addition to these hydrolyzing pathways, endocannabinoids are subject to oxidative metabolism by cyclo-oxygenase (COX) [43-44] and lipoxygenase (LOX) pathways [45-46]. An uptake protein "AEA transporter", responsible for the cellular transport of endocannabinoids to the catalytic sites of the hydrolyzing enzymes, is exhaustively characterised in a large number of articles but it is still not yet cloned [47-52] (for a review on these four actors of endocannabinoid metabolism, see Vandevoorde and Lambert [53]). Each of these biological components of the endocannabinoid system is targeted by researchers who want to promote the therapeutic potential of the endocannabinoids. The validity of this strategy was strengthened by the generation of $FAAH^{(-/-)}$ mice by Cravatt and coworkers [54], in which genetic inactivation of FAAH induces reduced pain sensitivity correlated with an increase in the endogenous brain levels of anandamide, palmitoylethanolamide and oleoylethanolamide. Another way to disrupt FAAH activity is to inactivate chemically FAAH by potent and/or selective inhibitors. The purpose of this review is to give an overview of the families of synthetic inhibitors of FAAH and MAGL. The synthetic pathways, the chemical features, potencies, selectivities and modes of inhibition are listed and compared in order to facilitate the comparison of the chemical families of the inhibitors of these two enzymes involved in endocannabinoid metabolism.

II. FAAH INHIBITORS

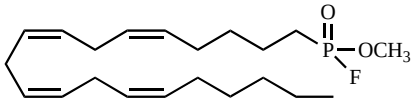
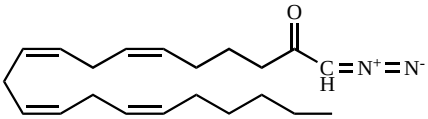
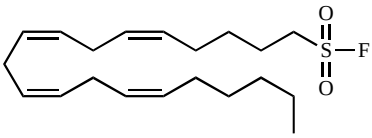
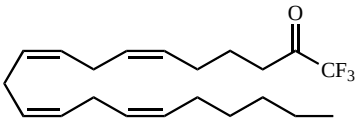
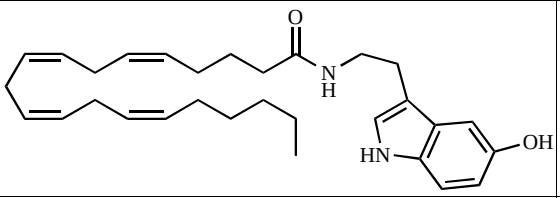
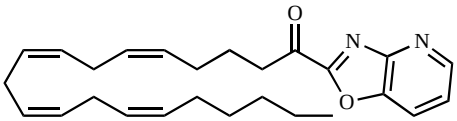
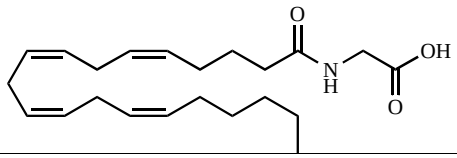
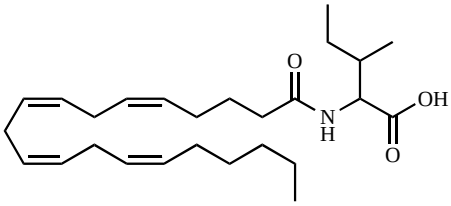
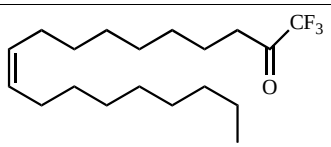
II.a Inhibitors Inspired by the Chemical Structures of FAAH Substrates

The first FAAH inhibitors designed were analogues of the substrates of this enzyme. The endogenous anandamide, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (Fig. **3**) were the inspirations of new active inhibitory compounds which shared, respectively, their unsaturated arachidonoyl-, oleoyl- or saturated palmitoyl-carbon chains. Predominant examples of these compounds are presented in Table **1**.

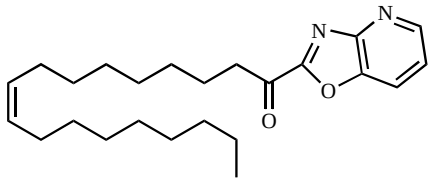
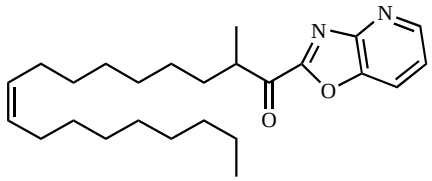
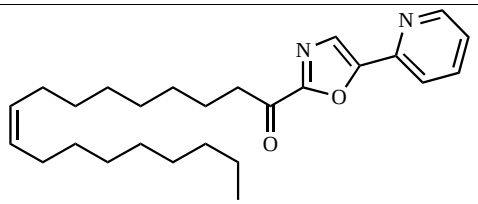
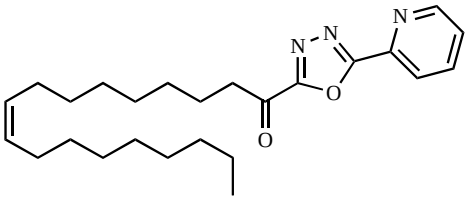
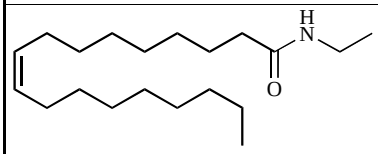
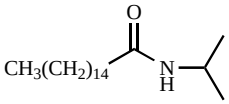
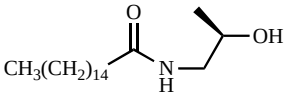
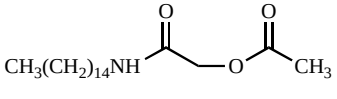
II.a.1 Arachidonoyl Analogues

Among the *arachidonoyl analogues*, methylarachidonoyl fluorophosphonate (MAFP), diazomethylarachidonoylketone (DAK) and arachidonoylsulfonyl fluoride are irreversible inhibitors of FAAH. MAFP exhibits IC_{50} values from 1 to 3 nM, depending on the source of FAAH used in the assay. Deutsch *et al.* reported IC_{50} value of 2.5 nM for MAFP incubated with rat brain homogenates [55], while De

Table 1. Inhibitors Based on the Chemical Structures of FAAH Substrates

Chemical Structure	Name	FAAH Inhibition	
		Ki or IC_{50}	**Mode of Inhibition**
Arachidonoyl-analogues			
	Methyl arachidonoyl fluorophosphonate (MAFP)	IC_{50} 1-3 nM [55-56]	Irreversible
	Diazomethylarachidonoyl ketone (DAK)	IC_{50} 520 nM-6μM [56,61]	Irreversible
	Arachidonoylsulfonyl fluoride	IC_{50} 0.11 nM [62]	Irreversible
	Arachidonoyltrifluoromethyl ketone (ATFMK)	IC_{50} 0.23-3 μM [63-64]	Transition-state inhibitor
	Arachidonoyl-serotonin	IC_{50} 5.6-12 μM [66]	Mixed
	1-oxazolo[4,5-*b*]pyridin-2-yl eicosa-*5Z,8Z,11Z,14Z*-tetraen-1-one	Ki 1nM [71]	Not yet elucidated
	*N*-arachidonoylglycine (NAGly)	IC_{50} 4.1-7 μM [72]	Competitive
	*N*-arachidonoylisoleucine (NAIle)	IC_{50} 18-34 μM [73]	Not yet elucidated
Oleoyl-analogues			
	Oleoyltrifluoromethyl ketone	Ki 1.2-82 nM [74-75]	Transition-state inhibitor

(Table 1) Contd.…

Chemical Structure	Name	FAAH Inhibition	
		Ki or IC_{50}	Mode of Inhibition
	1-oxazolo[4,5-*b*] pyridin-2-yl-*9Z*-octadecen-1-one CAY10400	Ki 2.3 nM [76]	Not yet elucidated
	2-methyl-1-oxazolo[4,5-*b*] pyridin-2-yl-*9Z*-octadecen-1-one	Ki 9.1 nM [76]	Not yet elucidated
	1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-9(*Z*)-octadecene	Ki 18 nM [78]	competitive
	1-[5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl]-octadec-9-en-1-one	Ki 3 nM [79]	Not yet elucidated
	Oleoylethylamide (OEt)	IC_{50} 5.6 μM [82]	Not yet elucidated
Palmitoyl-analogues			
	Palmitoylisopropylamide	IC_{50} 12.8 μM [83]	Mixed-type inhibitor
	*R*–palmitoyl-2-methyl ethanolamide (RP-2ME)	IC_{50} 4.07 μM [83]	Not yet elucidated
	*N*-(2-acetoxyacetyl) pentadecylamine	IC_{50} 8.3 μM [84]	Not yet elucidated

Petrocellis *et al*. used RBL-1 cells or $N_{18}TG_2$ cells and found a similar IC_{50} values of 1 nM and 3 nM, respectively [56]. MAFP is a serine hydrolase inhibitor initially designed by Huang *et al*. as an inhibitor of cytosolic phospholipase A_2 [57]. Martin and coworkers reported in 2000 a three-step synthesis of methylfluorophosphonate analogues which might be suitable for MAFP [58]. Alkylation of dimethyl phosphite by 1-iodo-(*5Z,8Z,11Z,14Z*)-eicosatetraene in presence of sodium hydride and *N,N*-dimethylformamide gives dimethyl-(*5Z,8Z,11Z,14Z*)-eicosatetraenyl phosphonate,

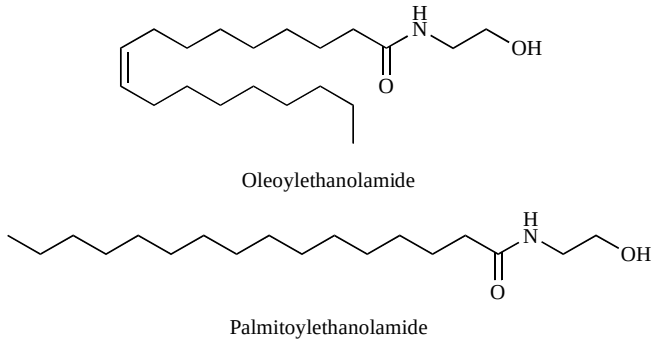


Fig. (3). Chemical structures of oleoylethanolamide and palmitoylethanolamide.

which is allowed to react with sodium iodide to afford the methyl (*5Z,8Z,11Z,14Z*)-eicosatetraenyl sodium phosphonate. This last compound is treated with (diethylamino)sulfur trifluoride to give the final MAFP (Fig. **4**). Very recently, Glaser and coworkers reported that *in vivo* administration of MAFP (i.p.) effectively inhibits FAAH in specific brain areas. At the dose of 1 mg/kg, MAFP inhibits FAAH activity in the somatosensory and visual cortices, while higher doses (4-8 mg/kg) were required to inhibit FAAH in the thalamus [59]. DAK was first synthesized by Huang *et al.* as a preliminary product for the synthesis of arachidonoyl bromomethyl ketone. They used arachidonoyl chloride (obtained from arachidonic acid that had been allowed to react with oxalyl chloride) and ethereal diazomethane [60] to synthesize DAK. DAK was characterized as an irreversible inhibitor of FAAH, with IC_{50} values from 0.52 to 6 μM, depending on the source of FAAH used [56,61]. Segall *et al.* reported in 2003 a chemical synthesis of arachidonoylsulfonyl fluoride involving the Grignard reagent arachidonoylmagnesium bromide that was allowed to react with sulfuryl chloride in order to get arachidonoylsulfonyl chloride. This last compound was added to $Bu_4N^+F^-$ to afford the final arachidonoylsulfonyl fluoride [62] (Fig. **5**). Arachidonoylsulfonyl fluoride was reported to be an irreversible inhibitor of FAAH, characterized by an IC_{50} value of 0.11 nM.

Several arachidonoyl analogues differ from the previous ones by their mode of inhibition. Arachidonoyltrifluoromethylketone (ATFMK) is an example of a transition-state inhibitor of FAAH, characterized by IC_{50} values from 0.23 to 3 μM [63-64]. It was synthesized by reaction of arachidonoyl chloride (obtained from arachidonic acid and CH_3OCHCl_2) and trifluoroacetic anhydride in presence of pyridine [63]. Arachidonoylserotonin (AA-5-HT) was first reported by Bezuglov *et al.* in 1997 [65]. One year later, Bisogno *et al.* reported the inhibitory activity of AA-5-HT upon FAAH, characterized by IC_{50} values of 12 μM and 5.6 μM when neuroblastoma N18TG2 and rat basophilic leukaemia RBL-2H3 cells were, respectively, used as a source for FAAH [66]. AA-5-HT is a mixed FAAH inhibitor, stable to FAAH-catalyzed hydrolysis. AA-5-HT is inactive at CB_1 receptors but its hypolocomotor effect in dopamine transporter knockout mice (which exhibit spontaneous hyperlocomotion) was attenuated by the TRPV-1 antagonist capsazepine [67]. Subchronic treatment with AA-5-HT (i.p.) was reported to induce an increase in both AEA and 2-AG levels in rat brain [68]. The antiproliferative effect of AA-5-HT was reported *in vitro* [25] and *in vivo* in a model of tumor xenografts induced by the subcutaneous injection of rat thyroid transformed cells [69]. This last effect was accompanied by an increase in AEA, 2-AG and PEA levels in the tumors. AA-5-HT was also reported to induce stress-induced analgesia, likely through a CB_1-indirect mechanism activated by an increase in accumulation and release of AEA [70]. In 2000, Boger and coworkers reported the synthesis of 1-(oxazolo[4,5-*b*]pyridin-2-yl)eicosa-*5Z,8Z,11Z,14Z*-tetraen-1-one (compound #38 of [71]), obtained by Dess-Martin oxidation of the corresponding alcohol. This compound inhibits FAAH from rat liver plasma membrane extracts with a Ki of 1 nM [71]. In 2001, *N*-arachidonoylglycine (NAGly), a member of the family of arachidonyl amino acids, was extracted from a variety of rat tissues, such as spinal cord, small intestine, kidney and brain [72]. NAGly competitively inhibits FAAH

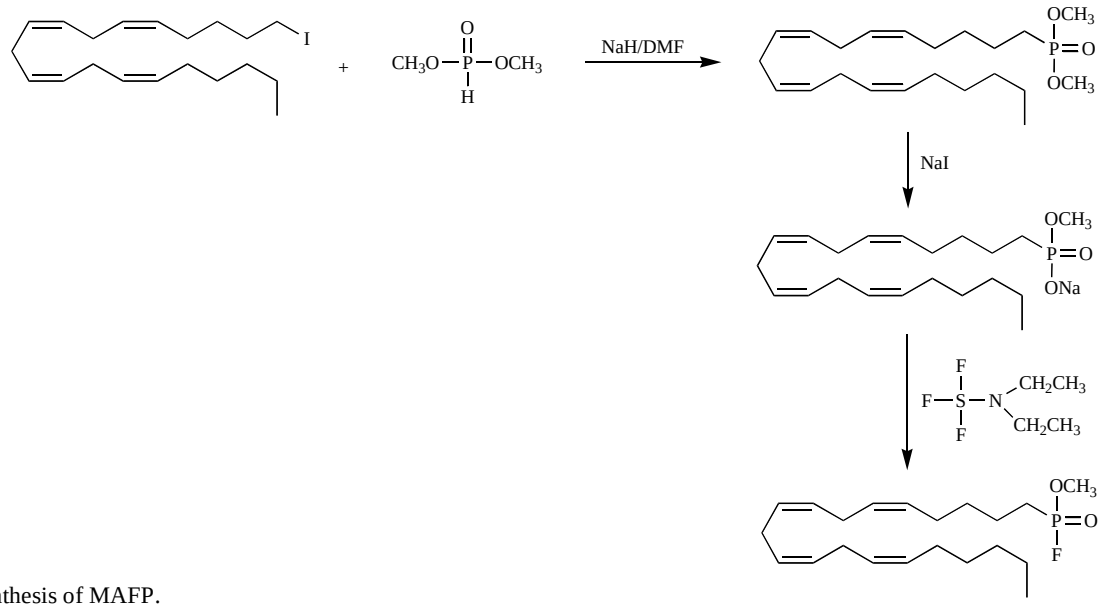


Fig. (4). Synthesis of MAFP.

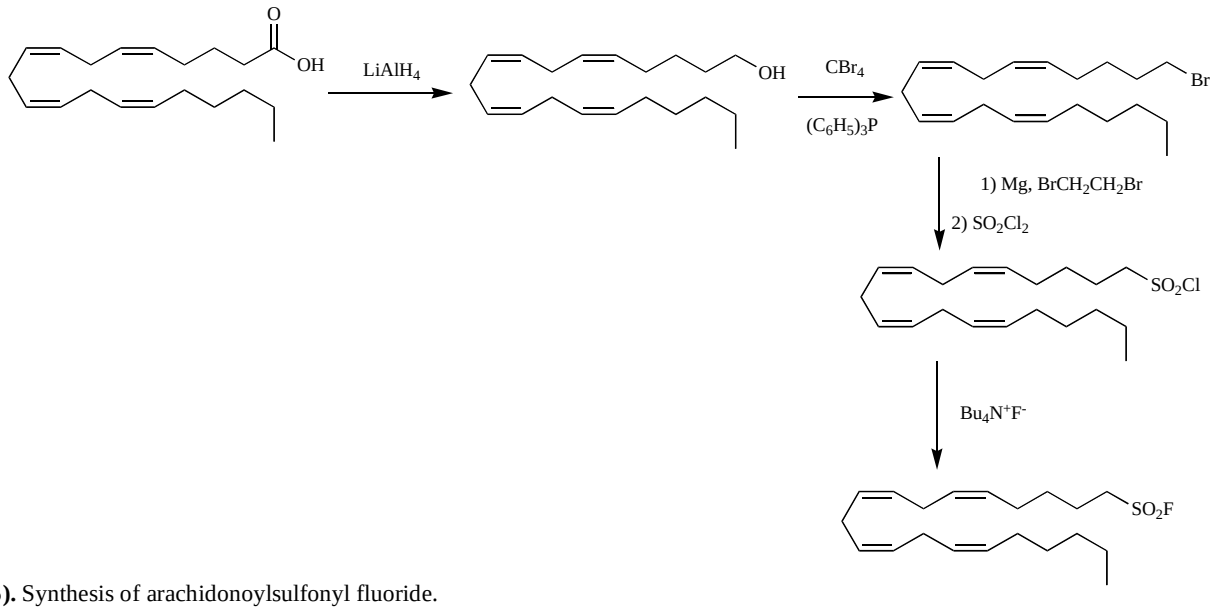


Fig. (5). Synthesis of arachidonoylsulfonyl fluoride.

in N18TG2 and RBL-2H3 cells with IC_{50} values of 7 and 4.1 μM, respectively, and induces antino-ciceptive actions in the formalin test [72]. In view of these promising results, NAGly became the starting point of a structure-activity relationship study on *N*-arachidonoyl-amino acids ran by Cascio and coworkers [73]. In this study, the *N*-arachidonoyl-amino acids were synthesized by acyla-tion of arachidonic acid with amino acid *tert*-butyl esters according to the DEPC protocol (diethyl cyanophosphonate/triethylamine), followed by a de-protection with TFA (trifluoroacetic acid). Interestingly, NAGly was the most potent inhibitor of the rat and mouse FAAH, and *N*-arachidonoylisoleucine (NAIle) exhibits inhibitory activity only upon human FAAH with IC_{50} values of 18 μM (human EFM-19 cells) and 34 μM (human MCF-7 cells).

II.a.2 Oleoyl Analogues

As it is shown above, the development of arachidonoyl analogues of endocannabinoids afforded FAAH inhibitors with interesting potencies. Unfortunately, these compounds also exhibit a good affinity for the cannabinoid receptor 1. MAFP and arachidonoylsulfonyl fluoride exhibit IC_{50} values of 20 nM and 304 nM upon CB_1 receptors, whilst ATFMK and DAK have Ki values of 650 nM and 1.3 μM. In order to avoid this high affinity for CB_1 receptors, analogues of endogenous compounds like oleoylethanolamide and palmitoylethanolamide were designed, since these two fatty acid amides, although substrates for FAAH, are inactive at cannabinoid receptor 1.

Among the *oleoyl analogues*, oleoyltrifluoromethyl ketone was first synthesized by Patterson *et al*. by reaction of trifluoroacetic anhydride and oleoyl chloride, previously obtained by activation of oleic acid by oxalyl chloride [74]. Oleoyltrifluoromethyl ketone is a potent inhibitor of FAAH, characterized by Ki values that differ slightly in respect with the pH of the assay: its Ki was 1.2 nM at pH 10 [74], and 82 nM when tested at pH 9 [75]. Boger and coworkers have also synthesized oxazolopyridinyl derivatives of oleic acid. 1-oxazolo[4,5-*b*]pyridin-2-yl-*9Z*-octadecen-1-one (commercially available as an experimental research compound under the name of CAY10400) and its α-methyl homologue 2-methyl-1-oxazolo[4,5-*b*]pyridin-2-yl-*9Z*-octadecen-1-one exhibit Ki of 2.3 and 9.1 nM, respectively [76]. CAY10400 was synthesized by Dess-Martin oxidation of the corresponding alcohol [71]. 2-methyl-1-oxazolo[4,5-*b*]pyridin-2-yl-*9Z*-octadecen-1-one was synthesized in a 5-step reaction starting from methyl oleate that had been alkylated by iodomethane. The alkylated product was oxidized to the corresponding aldehyde by Dess-Martin periodinane, which was afterwards converted into 2-hydroxy-3-methyl-10-nonadecenitrile by action of KCN. The product was allowed to react with acetyl chloride and 2-amino-3-hydroxypyridine to give the corresponding oxazolopyridinyl derivative. This last compound was finally oxidized to the corresponding ketone by Dess-Martin periodinane [76] (Fig. **6**).

Boger and coworkers extended the series of oleoyl analogues with the discovery of potent α-keto oxazoles and α-keto oxadiazoles. 1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-9(*Z*)-octadecene was synthesized by reaction of oleoyl chloride and 5-(2-pyridyl)oxazole that had been previously treated with n-BuLi [77]. 1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-9(*Z*)-octadecene competitively inhibits FAAH with a Ki value of 18 nM, and affects neither triacylglycerol hydrolase (TGH) nor the membrane-bound hydrolase KIAA1363 [78], suggesting the selectivity of this inhibitor for FAAH. 1-[5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl]-octadec-9-en-1-one is an example of α-keto oxadiazole compounds designed by Boger and coworkers. It was synthesized by reaction of oleoyl lithium and methyl 5-(pyridin-2-yl)-1,3,4-oxadiazole-2-carboxylate. Oleoyl lithium was obtained by reaction of 1-bromo-heptadec-8-ene and *t*-BuLi. Methyl 5-(pyridin-2-yl)-1,3,4-oxadiazole-2-carboxylate was synthesized by reaction of 2-picolinyl hydrazide and methyl oxalyl chloride, followed by treatment of the reaction mixture by TsCl [79] (Fig. **7**). 1-[5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl]-octadec-9-en-1-one exhibits selective inhibition of FAAH (Ki 3 nM) compared to KIAA1363 and TGH. Monte Carlo simulations of α-keto oxazoles and α-keto oxadiazoles have confirmed

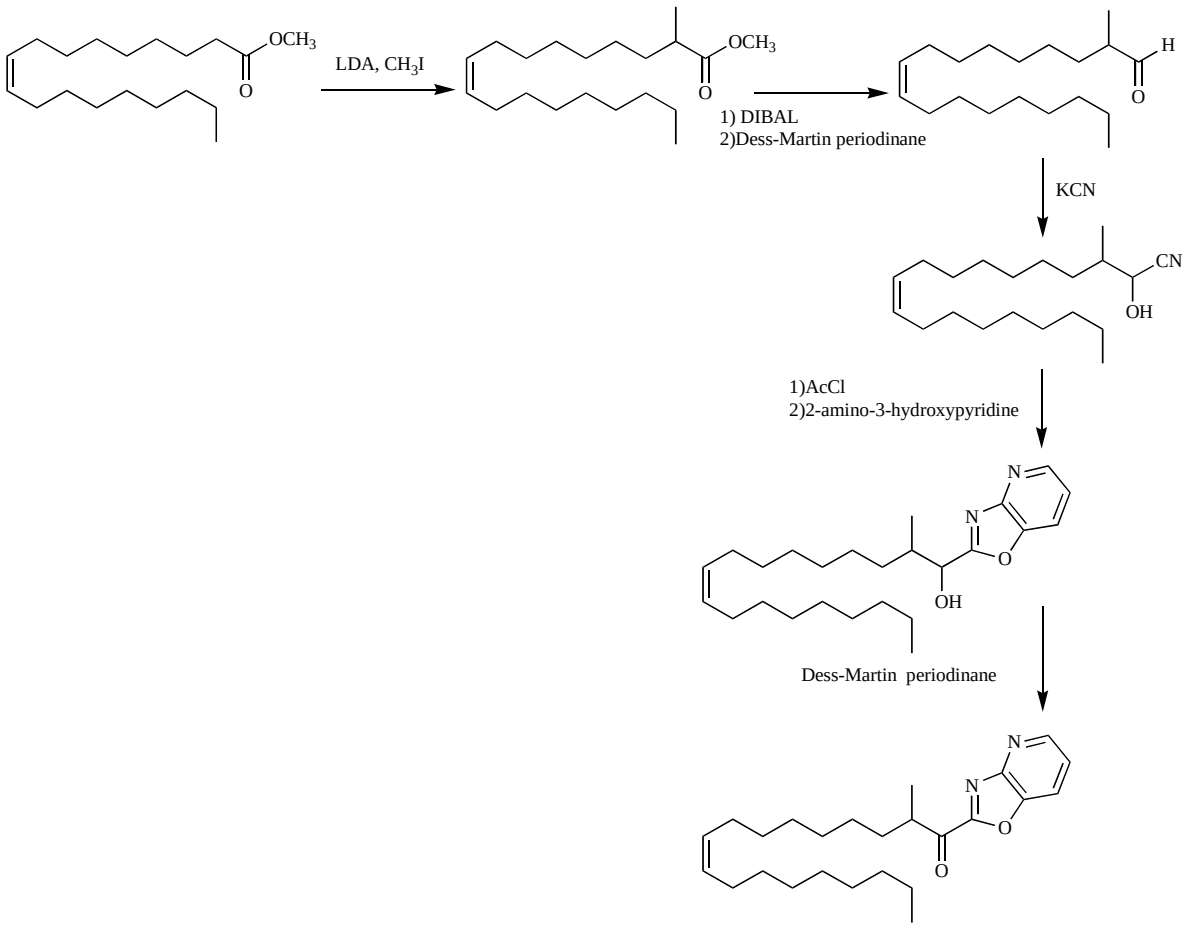


Fig. (6). Synthesis of 2-methyl-1-oxazolo[4,5-*b*]pyridin-2-yl-*9Z*-octadecen-1-one.

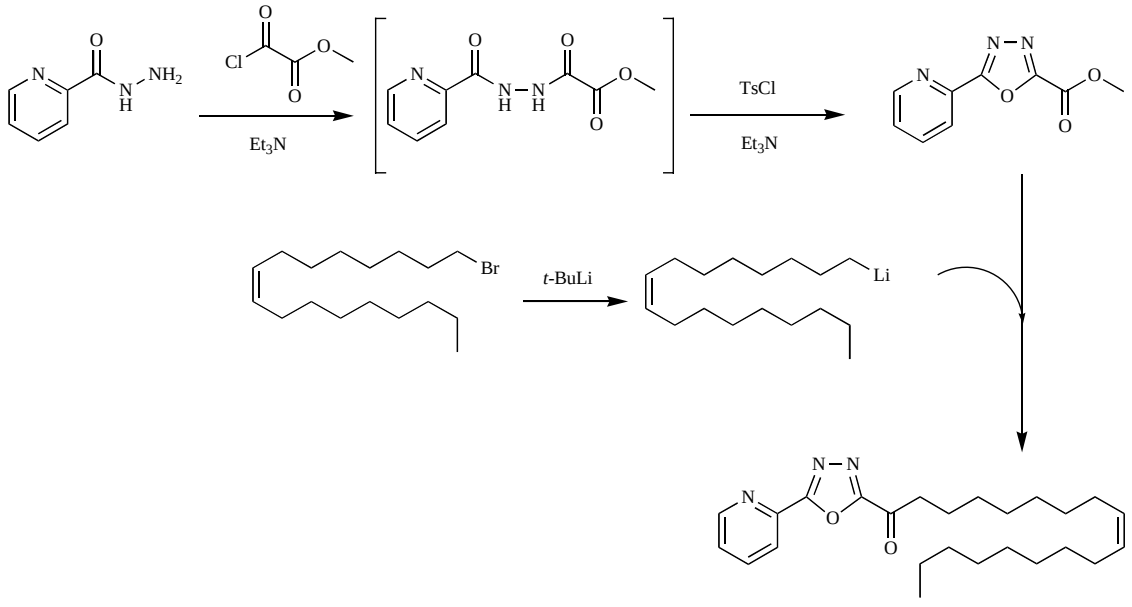


Fig. (7). Synthesis of 1-[5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl]-octadec-9-en-1-one.

that incorporation of a pyridine at the C5 position of the 2-keto-oxa(dia)zoles increases binding affinity and therefore FAAH inhibition, by formation of hydrogen bonds between the basic pyridyl nitrogen proximal to the oxa(dia)zole ring and the hydroxyl group of Thr236. The higher potencies of oxadiazole compounds, compared to the corresponding oxazoles was also explained by a decrease of steric interactions and lower torsional-energy penalty upon binding [80].

Oleoylethylamide (OEt), a closely related analogue of OEA was firstly reported in 2003 as a FAAH inhibitor of moderate potency (IC_{50} 5.6 μM) and capable of potentiating the TRPV-1 (transient receptor potential vanilloid) -mediated effects of anandamide in human-TRPV-1 HEK transfected cells [81]. OEt was synthesized by reaction of oleoyl chloride and ethylamine. Despite its moderate potency upon FAAH, OEt was shown to induce antinociception in the neuropathic pain model where a partial ligature of the sciatic nerve was induced to the rat [82].

II.a.3 Palmitoyl Analogues

The saturated endogenous anti-inflammatory PEA was also chosen as a template for new FAAH inhibitors that do not exhibit affinity for the cannabinoid receptors. Palmitoylisopropylamide and *R*–palmitoyl-2-methyl ethanolamide (RP-2ME) were synthesized by reaction of palmitoyl chloride and the corresponding amines. They interfere with FAAH with IC_{50} values of 12.8 and 4.07 μM, respectively [83]. *N*-(2-acetoxyacetyl)pentadecylamine is a "retro" analogue of PEA in which the amide bond has been inversed. This compound was synthesized in a 3-step reaction starting from pentadecanol that was allowed to react with MsCl and subsequently with sodium azide to give pentadecyl azide. This azide was then reduced to the corresponding amine with $LiAlH_4$. This amine was finally allowed to react with acetoxyacetyl chloride to afford *N*-(2-acetoxyacetyl)pen-tadecylamine (Fig. **8**). This compound is characterized by an IC_{50} value of 8.3 μM upon FAAH [84].

II.b FAAH Inhibitors that do not Share Similarities with the Endocannabinoids

The growing knowledge of the protein structure [38] and the catalytic mechanisms [85-87] of FAAH has increased the understanding of the inhibition mechanisms of the available inhibitors as well as facilitated the discovery of more potent and selective inhibitors of this enzyme. Most of these novel compounds have a completely distinct scaffold from the endocannabinoids (Table **2**).

II.b.1 Oxazolo-Pyridins and α-Keto Oxazoles

Some inhibitors are related to the endocannabinoids only by the presence of a long carbon chain. Examples of such are CAY10401 (1-oxazolo[4,5-*b*]pyridin-2-yl-9-octadecyn-1-one) [71] and CAY10435 (1-oxazolo[4,5-*b*]pyridin-2-yl-1-dodecanone) [69], which were designed by Boger and coworkers and contain an octadecyne or a dodecanyl chain, respectively. CAY10401 and CAY10435 were synthesized from the corresponding alcohols by the procedure described in Fig. **6**. The alkyne CAY10401 inhibits FAAH with a Ki value of 0.14 nM, while the octadecyl analogue CAY10435 exhibits IC_{50} values of 0.81 nM, 83 nM and 50 μM for FAAH, TGH and KIAA1363, respectively. Boger and coworkers have optimized their oxazolo-pyridins and substitution of the fatty acyl chain by phenylpentyl chain led to the discovery of PHOP (1-oxazolo[4,5-b]pyridin-2-yl-6-phenyl-1-hexanone, CAY10402), a potent FAAH inhibitor characterized by a Ki value of 0.094 nM upon human FAAH [71]. Like the other oxazolo-pyridins, PHOP was synthesized starting from the corresponding alcohol by a procedure similar to that presented in Fig. **6**. Boger *et al*. have also extended their pool of α-keto oxazole compounds by the synthesis of phenylalkyl derivatives. They synthesized 1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-7-phenylheptane (OL-135) by reaction of 7-phenylheptanoyl chloride (obtained from the corresponding acid and oxalyl chloride) and 5-(2-pyridyl) oxazole that was previously treated with n-BuLi. This compound exhibits a Ki value of 4.7 nM upon FAAH [77].

II.b.2 Carbamates

The family of the carbamate FAAH inhibitors was reported by Piomelli and coworkers in 2003. A first structure-activity relationship study permitted the discovery of URB524 (*N*-cyclohexylcarbamic acid biphenyl-3-yl ester). URB524 was synthesized by reaction of 3-phenylphenol and isocyanatocyclohexane [88]. URB524 inhibits FAAH with an IC_{50} value of 63 nM, likely in an irreversible way, since other carbamate related inhibitors also interact irreversibly with FAAH (see below). URB524 was later shown to be devoid of effect upon MAGL [89]. Simultaneously to URB524, Piomelli and coworkers highlighted two other carbamates, namely URB532 and URB597, which became subjects to an extensive pharmacological characterization. URB532 (*n*-butylcarbamic acid 4-benzyloxyphenyl ester) was synthesized by reaction of 4-benzyloxyphenol and *n*-butylisocyanate [89]. URB532 irreversibly inhibits FAAH with an IC_{50} value of 396 nM. URB532 is highly selective for FAAH since this compound was reported not to interact with a panel of varied biological targets including serine hydrolases, MAGL, CB_1 and CB_2 receptors, anandamide

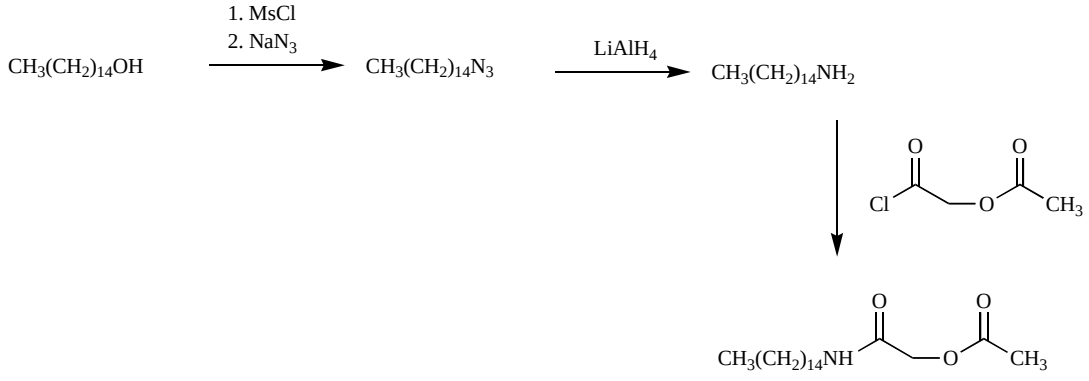
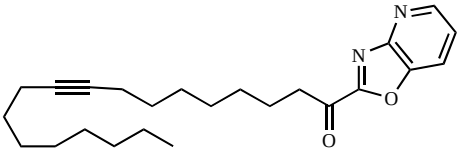
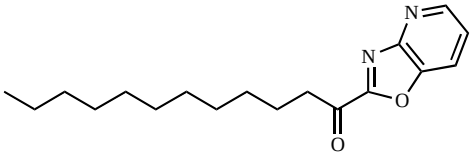
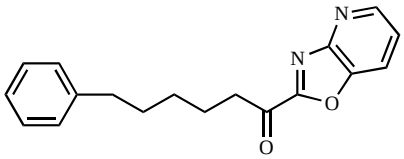
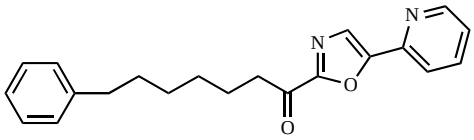
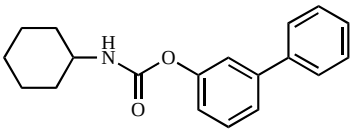
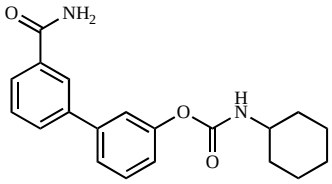
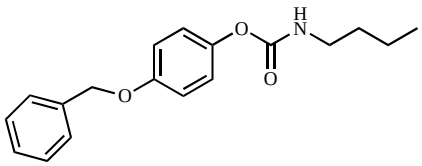
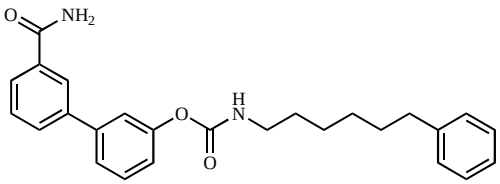
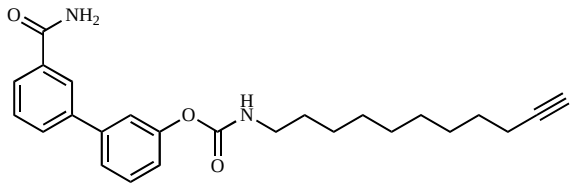
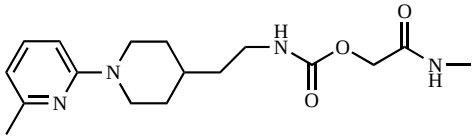
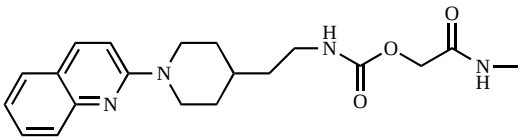
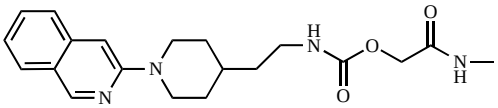
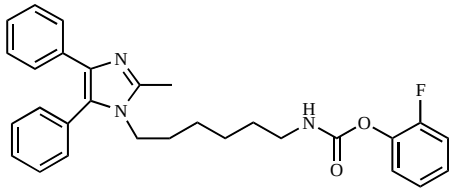
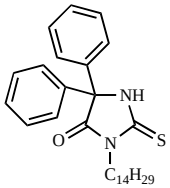
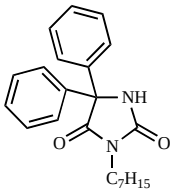
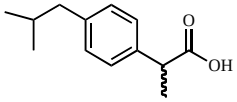


Fig. (8). Synthesis of *N*-(2-acetoxyacetyl)pentadecylamine.

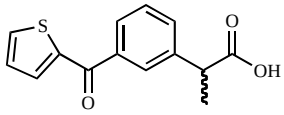
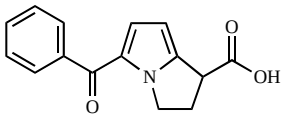
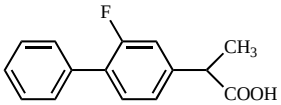
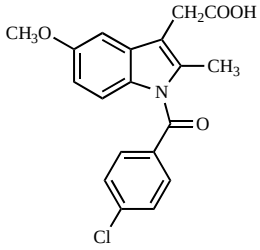
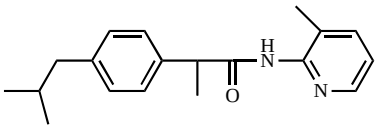
Table 2. FAAH Inhibitors That do not Share Similarities with the Endocannabinoids

Chemical Structure	Name	FAAH Inhibition	
		Ki or IC_{50}	**Mode of Inhibition**
Oxazolo-pyridins and α-keto oxazoles			
	CAY10401 (1-oxazolo[4,5-*b*]pyridin-2-yl-9-octadecyn-1-one)	Ki 0.14 nM [71]	Not yet elucidated
	CAY10435 (1-oxazolo[4,5-*b*]pyridin-2-yl-1-dodecanone)	IC_{50} 0.81 nM [78]	Not yet elucidated
	PHOP (1-oxazolo[4,5-*b*]pyridin-2-yl-6-phenyl-1-hexanone, CAY10402	Ki 0.094 nM (human) Ki 0.2 nM (rat) [71]	Not yet elucidated
	1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-7-phenylheptane (OL-135)	Ki 9 nM (human) Ki 4.7 nM (rat) [77]	Competitive
Carbamates			
	URB524 (*N*-cyclohexylcarbamic acid biphenyl-3-yl ester)	IC_{50} 63 nM [88]	Likely irreversible
	URB597 (cyclohexylcarbamic acid 3'-carbamoyl-biphenyl-3-yl ester)	IC_{50} 4.6 nM (brain membranes) IC_{50} 0.5 nM (intact neurons)[89]	Irreversible
	URB532 (*n*-butylcarbamic acid 4-benzyloxyphenyl ester)	IC_{50} 396 nM [89]	Irreversible
	JP83	IC_{50} 14 nM [93]	Irreversible

(Table 2) Contd.…

Chemical Structure	Name	FAAH Inhibition	
		Ki or IC_{50}	**Mode of Inhibition**
	JP104	IC_{50} 7.3 nM [93]	Irreversible
	Compound 47 of [95]	IC_{50} 85 μM [95]	Likely irreversible
	Compound 126 of [95]	IC_{50} 113 μM [95]	Likely irreversible
	Compound 166 of [95]	IC_{50} 87 μM [95]	Likely irreversible
	Compound 1 of [96]	IC_{50} < 10 nM [96]	Likely irreversible
2-thioxoimidazolidin-4-ones and the imidazolidine-2,4-diones			
	5,5'-diphenyl-3-tetradecyl-2-thioxo-imidazolidin-4-one (compound 46 of [97])	IC_{50} 1.148 μM [97]	Competitive
	3-heptyl-5,5'-diphenylimidazolidine-2,4-dione (compound 14 of [97])	IC_{50} 7.58 μM [97]	Not yet elucidated
NSAIDs			
	Ibuprofen	*R*: IC_{50} 230 μM *S*: IC_{50} 750 μM [99]	Mixed-type

(Table 2) Contd….

Chemical Structure	Name	FAAH Inhibition	
		Ki or IC_{50}	**Mode of Inhibition**
	Suprofen	IC_{50} 170 μM [98]	Not yet elucidated
	Ketorolac	*R*: IC_{50} 50 μM *S*: IC_{50} 440 μM [99]	Not yet elucidated
	Flurbiprofen	*R*: IC_{50} 60 μM *S*: IC_{50} 50 μM [99]	Not yet elucidated
	Indomethacin	Ki 120 μM [100]	Competitive
	α-methyl-4-(2-methylpropyl)-*N*-(3-methyl-2-pyridinyl) benzeneacetamide (IbuAM5)	IC_{50} 2.47 μM (pH 8) IC_{50} 4.71 μM (pH 6) [107]	Mixed

transporter, ion channels, neurotransmitter transporters and receptors (including opiate μ, muscarinic M_2, brain nicotinic receptors etc.) [89]. Consistent with its lack of affinity for CB_1 receptors, URB532 does not induce *in vivo* the side effects produced by exogenous cannabinoids (i.e., catalepsy, hypothermia, hyperphagia). *In vivo* effects of URB532 were also investigated in an animal model of analgesy (hot plate test), and in two animal models of anxiety: isolation-induced ultrasonic emission test, and elevated zero-maze test. URB532 was shown to exert anxiolytic and analgesic actions which are prevented by the CB_1 antagonist rimonabant and correlated with an increase in brain levels of AEA, PEA and OEA, but not 2-AG [89]. Similar effects were reported for URB597 [cyclohexylcarbamic acid 3'-carbamoyl-biphenyl-3-yl ester], which is even more potent since it inhibits FAAH with an IC_{50} value of 4.6 nM in brain membranes and 0.5 nM in intact neurons. URB597 was synthesized by reaction of cyclohexylisocyanate and 3'-hydroxylbiphenyl-3-carboxylic acid amide [89-90]. Besides its anxiolytic and analgesic effects assessed in the hot plate test, URB597 was assessed in neuropathic and inflammatory pain models. Systemic administration of URB597 was shown to exert a CB_1- and CB_2-mediated antinociceptive effect in the CFA model of inflammatory pain. Nevertheless, URB597 was devoid of any effect in the partial sciatic nerve-ligation model of neuropathic pain [91].

The mechanism by which carbamates inhibit FAAH is becoming clearer. Mor and coworkers presented 3D-QSAR analyses suggesting that the biphenyl moiety of carbamates mimicks the folded conformation that is adopted by the first 10 carbons (components of the first two *cis* double bonds) of arachidonic acid when this fatty acid is bound to various proteins, such as adipocyte lipid-binding protein or cyclooxygenase 1 or 2. In order to prove that the biphenyl group of the carbamate and the fatty acyl chain of arachidonoyl endocannabinoids really bind to the same pocket within the FAAH active site, the authors turned to docking studies using the crystal structure of FAAH that is covalently bound to MAFP. After deletion of the MAFP moiety from the binding pocket of FAAH and refinement of the protein model, they proved the conformational similarity between

the biphenyl fragment of URB524 and the first 10 carbons of the arachidonoyl chain of MAFP, suggesting that similar results would be obtained with the arachidonoyl endocannabinoids such as anandamide [92]. This hypothesis was, however, questioned by Alexander and Cravatt. They reported that the orientation proposed by Mor and coworkers would place the *N*-alkyl substituents of the inhibitors in the cytoplasmic access channel of FAAH, which would imply that these substituents serve as leaving groups and thus, move away after carbonylation of the enzyme's serine nucleophile S241 [93]. Alexander and Cravatt provided evidence that carbamates inactivated FAAH rather by carbamylation of the serine S241. To that end, they incubated purified recombinant FAAH and URB532 or URB597. After tryptic digestion and MALDI-TOF mass spectrometry, they recorded fragments consistent with the tryptic peptide amino acids 217-243, containing the serine S241 modified by the CO-N-alkyl subsituent of the carbamate (Fig. **9**), proving that the O-biaryl group served as a leaving group. These observations confirmed the mass spectrometry results reported one year earlier by Basso *et al.*, who protonated several carbamates, including URB524, by electrospray ionization. They found a correlation between the lability of the C(O)-O bond of the carbamates and their FAAH inhibitory potencies, suggesting the role of the phenolic fragment as a leaving group [94].

Alexander and Cravatt reported also the synthesis of new carbamates in which the *N*-alkyl substituent was replaced by a *N*-(6-phenyl)hexyl or a *N*-undec-10-ynyl group. JP83 (6-phenyl-hexyl)-carbamic acid 3'-carbamoyl-biphenyl-3-yl ester) was synthesized in a 4-step reaction starting from the synthesis of 2-(6-phenyl-hexyl)-isoindole-1,3-dione by reaction of 6-phenyl-1-hexanol and isoindole-1,3-dione in presence of triphenylphosphine. The product was then converted into 6-phenyl-1-hexylamine by hydrazine monohydrate, and afterwards to (6-isocyanato-hexyl)-benzene by phosgene. The final product JP83 is then obtained by reaction of (6-isocyanato-hexyl)-benzene and 3'-hydroxy-biphenyl-3-carboxylic amide (Fig. **10**). JP104 (undec-10-ynyl-carbamic acid 3'-carbamoyl-biphenyl-3-yl ester) was synthesized in a similar way, starting from 10-undecyn-1-ol instead of 6-phenyl-1-hexanol. JP83 and JP104 were shown to inhibit FAAH with IC_{50} values of 14 and 7.3 nM, respectively [93].

The synthesis of carbamates as inhibitors of FAAH is also the subject of several patents. Among others, piperidinyl- and piperazinyl-alkylcarbamates were patented by Sanofi-Aventis. Carbamic acids [2-[1-(6-methyl-2-pyridinyl) -4-piperidinyl]ethyl]-, 2-(methylamino)-2-oxoethyl ester (compound 47 of the patent [95]), [2-[1-(2-quinolinyl)-4-piperidinyl]ethyl]-, 2-(methylamino)-2-oxoethyl ester (compound 126 of the patent [95]), and [2-[1-(3-isoquinolinyl)-4-piperidinyl]ethyl]-, 2-(methylamino)-2-oxoethyl ester (compound 166 of the patent [95]) inhibit FAAH with IC_{50} values of 85, 113 and 87 μM, respectively. These compo-

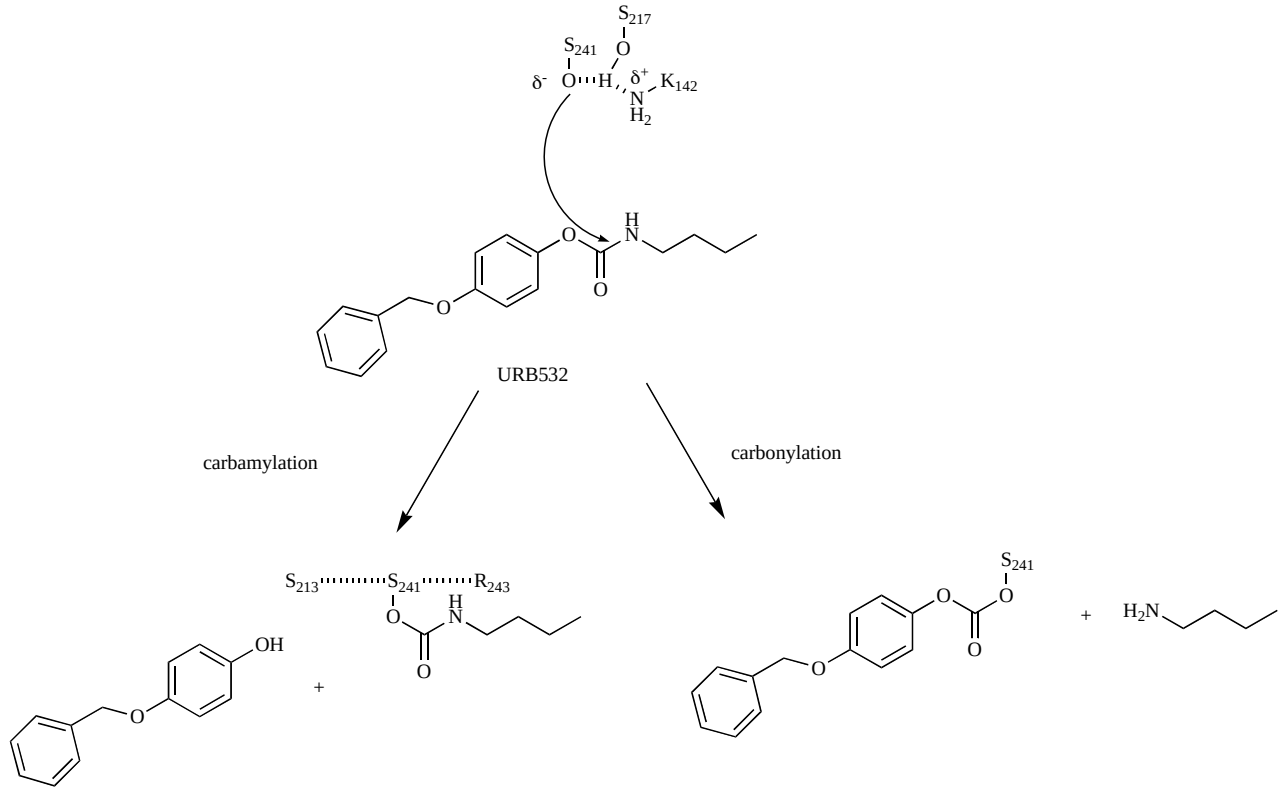


Fig. (9). Mechanism of FAAH inhibition by carbamates. Adapted from Fig. 2A of Alexander and Cravatt, 2005 [93]. Right scheme: Carbonylation, *N*-butyl group serves as a leaving group, consistent with the orientation proposed by Mor *et al.* [92]. Left scheme: Carbamylation, O-biaryl group serves as a leaving group, consistent with the model of Basso *et al.* [94] and Alexander and Cravatt [93], supported by MS data.

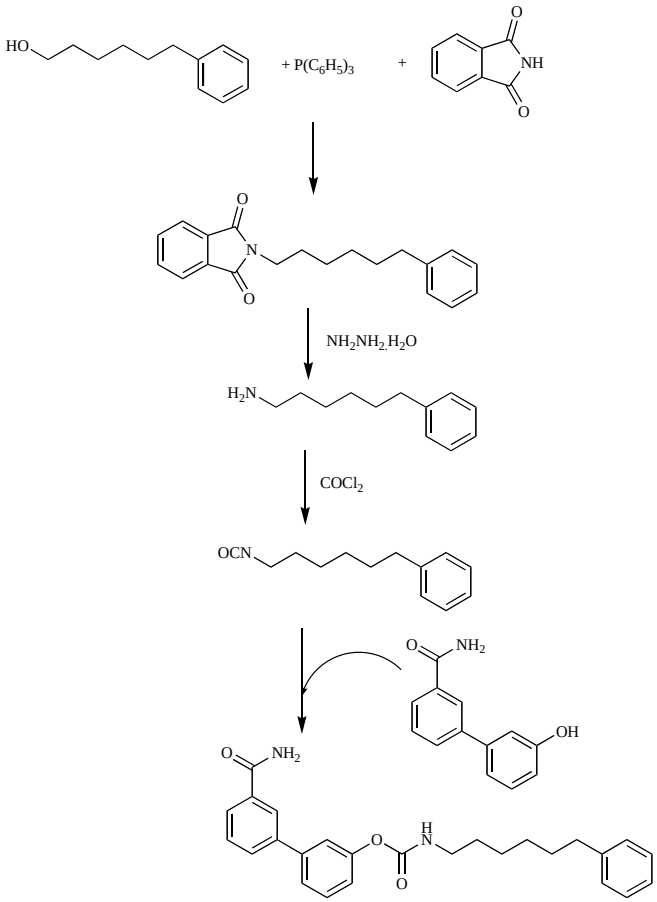


Fig. (10). Synthesis of JP83 (6-phenyl-hexyl)-carbamic acid 3'-carbamoyl-biphenyl-3-yl ester.

unds were also shown to elicit analgesic activity by suppressing the abdominal stretches induced by i.p. administration of phenylbenzoquinone [95]. Carbamic acid [6-(2-methyl4,5-diphenyl-1H-imidazol-1-yl)hexyl]-, 2-fluorophenyl ester (compound II of the patent) was patented by Bristol-Myers Squibb. This compound inhibits recombinant human FAAH with IC_{50} < 10 nM, and exerts analgesia in several pain models (chronic formalin-induced pain model, acute thermal Hargreaves pain model and Chung neuropathic pain model) [96].

II.b.3 2-Thioxoimidazolidin-4-Ones and Imidazolidine-2,4-Diones

Very recently, two new classes of FAAH inhibitors were reported by Muccioli *et al.*, namely the 2-thioxoimidazolidin-4-ones and the imidazolidine-2,4-diones [97]. 5,5'-diphenyl-3-tetradecyl-2-thioxo-imidazolidin-4-one (compound 46 of [97]) and 3-heptyl-5,5'-diphenylimidazolidine-2,4-dione (compound 14 of [97]) exhibited the highest potencies with IC_{50} values of 1.15 and 7.58 μM, respectively. The compounds were synthesized in presence of KOH in a very quick and simple method using micro-wave-assisted condensation of benzile and 1-tetradecyl-2-thiourea or heptylurea, respectively. These inhibitors were shown to be insensitive to the pH, since IC_{50} values upon FAAH were similar when the assays were conducted at pH 7.6 and pH 9.0. The mode of inhibition was also investigated for one member of the 2-thioxoimidazolidin-4-one family, 3-octyl-5,5'-diphenyl-2-thioxoimidazolidin-4-one. It was reported as a competitive inhibitor of FAAH, suggesting that the other 5,5'-diphenyl-2-thioxoimidazolidin-4-ones act in the same way.

II.b.4 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

In 1997, Fowler and coworkers reported the inhibitory actions of non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen and suprofen, upon FAAH [98]. Unlike the above presented compounds which were deliberately designed as FAAH inhibitors, NSAIDs, that have been known several years before the characterization of the endocannabinoid system, were discovered as FAAH inhibitors by *in vitro* screening. Ibuprofen was found to produce a stereoselective mixed-type inhibition of FAAH, since its *R*-isoform (IC_{50} 230 μM) was more potent than its *S*-isoform (IC_{50} 750 μM). Such stereoselectivity was also reported for ketorolac [IC_{50}(*R*-) 50μM; IC_{50}(*S*-) 440μM)], but not for flurbiprofen [IC_{50}(*R*-) 60 μM; IC_{50}(*S*-) 50 μM)] [99]. Suprofen and indomethacin were also found to inhibit FAAH moderately, with IC_{50} values of 170 and 120 μM [100], respectively. Interestingly, the inhibitory potencies of ibuprofen, flurbiprofen and indomethacin were reported to be higher at lower pH values, which are usually consistent with inflammatory conditions [101-102]. A synergistic interaction between ibuprofen and AEA was recently reported. Combination of AEA and ibuprofen (locally administered) produced antinociceptive effect either in the acute or the inflammatory phases of the formalin test, by a mechanism that was blocked by the CB_1 antagonist AM251. However, the combination was devoid of effect upon paw oedema [103]. The role of the endocannabinoid system was also investigated in the antinociceptive effect of flurbiprofen. AM251 was reported to block the effect of flurbiprofen (administered intrathecally) on formalin-induced pain behaviour [104]. Progress in the understanding of the mecha-nism of action of flurbiprofen was made by Seidel and co-workers who reported that AM251 reverses the flurbiprofen-induced inhibition of capsaicin evoked spinal CGRP release (calcitonin gene related peptide) but has no effect on flurbiprofen-induced COX (cyclo-oxygenase) inhibition [105]. The involvement of the endocannabinoid system was also demonstrated in the antinociceptive effect of indomethacin. Gühring and coworkers reported that AM251 reverses the indomethacin-induced antinociception in the formalin and zymosan-induced heat hyperalgesia tests. In addition to its inhibitory effect upon FAAH and COX, indomethacin was shown to lower NO production, and therefore limit the anandamide transporter activation [106]. Very recently, Holt and coworkers reported the inhibitory effect upon FAAH of several ibuprofen and indomethacin analogues [107]. Indomethacin 4-methoxyphenyl ester was shown to inhibit FAAH with similar IC_{50} values of the micromolar range at pH 6.2 and pH 8.4, suggesting that the free carboxylic acid group induces pH sensitivity. The ibuprofen amide analogue α-methyl-4-(2-methylpropyl)-*N*-(3-methyl-2-pyridinyl) benzeneacetamide (ibuAM5) was found to be more potent rat brain FAAH inhibitor than ibuprofen, characterized by IC_{50} values of 2.47 μM and 4.71 μM at pH 8 and pH 6, respectively [107].

III. MAGL INHIBITORS

In spite of the early characterisation of MAGL in 1976 and the nearly simultaneous clonings of FAAH and MAGL (which was succeeded by the cloning of rat FAAH in 1996 [36] and the mouse MAGL in 1997 [108]), the interest showed in the role of MAGL in the endocannabinoid system was belated. Bioassays to investigate MAGL activity and inhibition were set up later than those dedicated to FAAH activity. Until then, most inhibitors were only tested for their ability to block FAAH, and the selectivity question was tackled only later. Among the FAAH inhibitors presented above, only carbamates were tested for their ability to block both FAAH and MAGL. For this reason, MAGL vs FAAH inhibitions are only compared for the MAGL inhibitors presented below (Tables **3** and **4**). Moreover, hydrolysis of 2-AG and its analogue 2-oleoylglycerol (2-OG) not only occurs by the well characterized cytosolic MAGL (MAGL-cy), but also by a membrane-bound enzyme responsible for MAG hydrolysis (MAGLm) [109]. Additionally, a third type of MAG hydrolysis activity was recently reported by Stella in the microglial cell line BV-2 which does not express MAGL mRNA [110]. Therefore, the source of MAGL used is mentioned in the next tables presenting the inhibition data.

III.a Inhibitors Inspired by the Chemical Structures of MAGL Substrates

Most MAGL inhibitors available at present time are derived from the structure of 1-arachidonoylglycerol (1-AG), a more stable isomer of 2-AG. α-Me-1-AG (α-methyl-1-arachidonoyl glycerol, compound O-1428) was shown to inhibit both MAGLcy and MAGLm with IC_{50} values of 10-15 μM [111] and 71 μM [112], respectively, with weak effect upon FAAH (IC_{50}> 100μM). α-Me-1-AG moderately binds to the CB_1 receptor (Ki 1.8 μM) but does not induce the behavioural effects of the exogenous cannabinoids *in vivo* (hypolocomotion, hypothermia and analgesia) [111]. Interestingly, the longer homologue O-4081 (C22:4) was found to be a more potent inhibitor, but does not distinguish FAAH from MAGL (IC_{50} values of 5.1 μM, 5.8 μM and 8.5 μM upon FAAH, MAGLcy and MAGLm, respectively) [112]. Such a lack of selectivity was also found for other homologues which differ from 1-AG and α-Me-1-AG by the level of unsaturation [112]. *N*-arachidonoylmaleimide (NAM) was synthesized by Saario and coworkers, from arachidonoyl alcohol and maleimide, following a modified Mitsunobu reaction [113]. NAM was found to inhibit MAGLm selectively in rat cerebellar membranes with a low IC_{50} value of 0.14 μM compared to its IC_{50} value of 3.3 μM upon FAAH [114]. Molecular modeling studies indicated that NAM binds likely to the sulfhydryl group of cysteine near the catalytic site. Interestingly, the ring saturated analogue (*N*-arachidonoylsuccinimide) was shown to be devoid of any effect (no inhibition at 1 mM), suggesting that NAM acts through a Michael addition reaction [113].

III.b MAGL Inhibitors that do not Share Similarities with the Endocannabinoids

Very recently, Quistad and coworkers reported the efficacy of some organophosphorus compounds as inhibitors of MAGL. The three alkylphosphonofluoridates IDFP (isopropyl dodecylfluorophosphonate), MOPF (methyl octylphosphonofluoridate) and EOPF (ethyl octylphosphonofluoridate) inhibited MAGLcy with IC_{50} values of 0.76, 3 and 3 nM, respectively. However, the compounds also affected FAAH with IC_{50} values of 2, 0.79 and 0.6 nM,

Table 3. Inhibitors Inspired by the Chemical Structures of MAGL Substrates

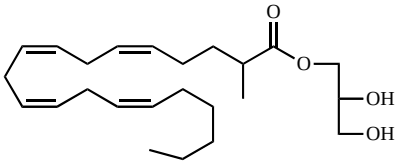
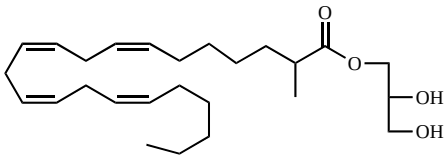
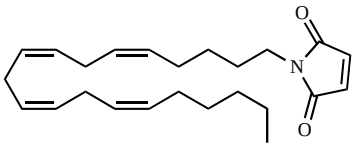
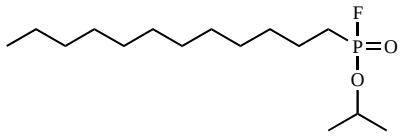
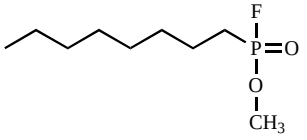
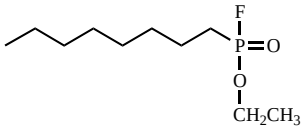
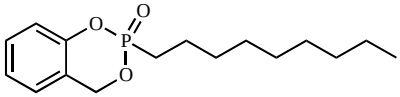
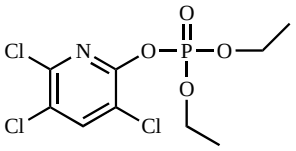
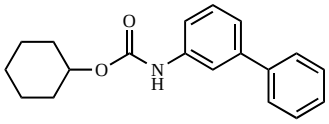
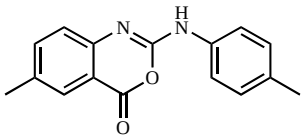
Chemical Structure	Name	MAGL Inhibition		FAAH Inhibition Ki or IC_{50}
		Ki or IC_{50}	Source of MAGL	
	α-Me-1-AG (O-1428)	IC_{50} 10-15 μM [111-112] IC_{50} 71 μM [112]	MAGLcy (rat cerebellar homogenates) MAGLm (rat cerebellar homogenates)	IC_{50} > 100 μM [111-112]
	O-4081	IC_{50} 5.8 μM [112] IC_{50} 8.5 μM [112]	MAGLcy (rat cerebellar homogenates) MAGLm (rat cerebellar homogenates)	IC_{50} 5.1 μM [112]
	NAM	IC_{50} 0.14 μM [113]	MAGLm (rat cerebellar homogenates)	IC_{50} 3.3 μM [114].

Table 4. MAGL Inhibitors That do not Share Similarities with the Endocannabinoids

Chemical Structure	Name	MAGL Inhibition		FAAH Inhibition IC_{50}
		IC_{50}	Source of MAGL	
	IDFP	0.76 nM [115]	MAGLcy (rat cerebellar homogenates)	2 nM [62]
	MOPF	3.0 nM [115]	MAGLcy (rat cerebellar homogenates)	0.79 nM [115]
	EOPF	3.0 nM [115]	MAGLcy (rat cerebellar homogenates)	0.60 nM [115]
	*S*-nonyl BDPO	0.31 nM [115]	MAGLcy (rat cerebellar homogenates)	0.15 nM [115]
	CPO	34 nM [115]	MAGLcy (rat cerebellar homogenates)	40 nM [115]
	URB602	28 μM [119] 75 μM [120]	MAGLcy (rat cerebellar homogenates) Rat brain MAGL expressed in HeLa cells	> 100 μM [119]
	URB754	200 nM [120] No inhibition [122]	Rat brain MAGL expressed in HeLa cells MAGLm	32 μM

respectively. Consistent with their ability to block MAGLcy, intraperitoneal administrations of IDFP, EOPF, and to a lower extent MOPF induced a significant increase in the brain levels of 2-AG, which was associated to moderate up to severe hypomotility for the higher doses of IDFP and EOPF used (10 mg/kg) [115]. IDFP was synthesized according to the method described by Segall *et al.*, starting from dodecyl bromide which was converted into diethyl 1-dodecylphosphonate by diethylphosphite. Diethyl 1-dodecylphosphonate was then cleaved into the corresponding phosphonic acid by trimethylbromosilane. 1-dodecylphosphonic acid was fluorinated by (dimethylamino)sulfur trifluoride, affording 1-dodecyldifluorophosphonate which was finally allowed to react with isopropanol to give the final IDFP [116] (Fig. **11**). Two other kinds of organophosphorus compounds were also reported by Quistad for their ability to inhibit MAGLcy. *S*-nonylbenzodioxaphosphorin oxide (*S*-nonyl BDPO) and the pesticide chlorpyrifos oxon (CPO) inhibited MAGLcy with IC_{50} values of 0.31 and 34 nM, respectively. Unfortunately, like the other organo-phosphorus compounds presented in the study, *S*-nonyl BDPO and CPO inhibit equally FAAH with IC_{50} values of 0.15 and 40 nM, respectively. The stereospecific synthesis of *S*-nonyl BDPO was reported by Wu and Casida, by reaction of nonyl phosphonic dichloride, *O*-hydroxy benzyl alcohol and *S*-proline methyl ester in presence of triethylamine [117]. Synthesis of CPO occurs by oxidation of chlorpyrifos treated with excess bromine in acetonitrile, as reported by Kim *et al*. [118].

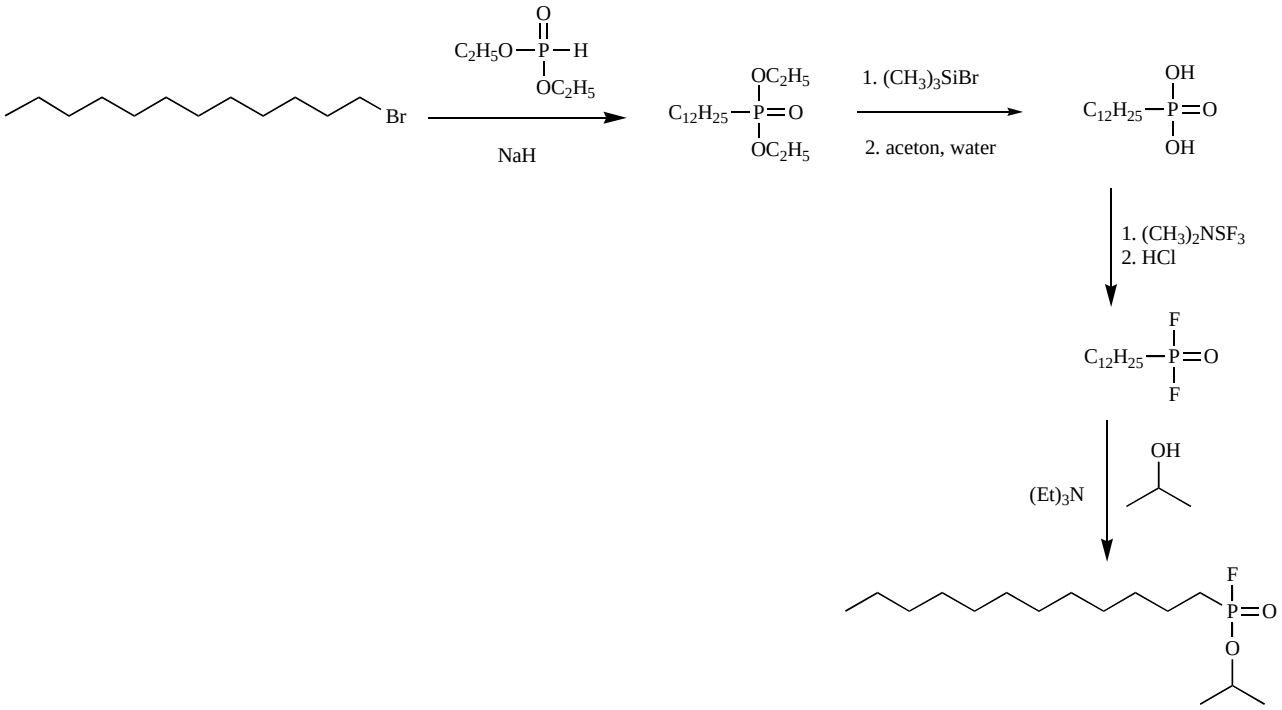


Fig. (11). Chemical synthesis of isopropyl dodecylfluorophosphonate IDFP.

As it is shown above, carbamates are a pool of potent and selective inhibitors of FAAH. However, this chemical family also includes MAGL inhibitors. Inversion of *N*- and *O*-substituents of URB524 leaded to [1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester (URB602) which was reported as a selective MAGL inhibitor by Hohmann *et al.* [119]. URB602 was reported to inhibit noncompetitively MAGLcy with an IC_{50} value of 28 μM and not to inhibit FAAH at concentrations up to 100 μM. Synthesis of URB602 was first reported by Tarzia *et al.* by reaction of biphenyl-3-ylamine and di-imidazol-1-ylmethanone in presence of DMAP, followed by cyclohexanol [88]. *In vivo*, URB602 [microinjection in dorsolateral, lateral or ventrolateral periaqueductal grey matter (PAG)] was found to enhance non-opioid stress-induced analgesia by a mechanism blocked by the CB_1 inverse agonist rimonabant. Consistent with its selective inhibition of MAGLcy, microinjection of URB602 in ventrolateral PAG induces an increase in the endogenous levels of 2-AG but does not affect AEA levels. In 2005, Makara and coworkers used URB602 in order to investigate the functions of 2-AG in the brain. They found that the increase in 2-AG levels subsequent to MAGL inhibition by URB602 enhances retrograde signaling from pyramidal neurons to GABAergic terminals in the hippocampus [120]. In this study, the authors turned to a more potent inhibitor, namely URB754 [(6-methyl-2-[(4-methylphenyl)amino]-4H-3,1-benzoxazin-4-one]. This compound was isolated from a library of serine hydrolase inhibitors, and inhibits recombinant rat MAGL with an IC_{50} value of 200 nM and more weakly FAAH with an IC_{50} of 32 μM [120]. Synthesis of URB754 was reported in the eighties by Garin *et al.* by cyclisation of potassium 5-methylanthranilate and methyl-*N*-(4-methylphenyl)dithiocarbamate in presence of HgO below room temperature [121]. However, very recently, URB754 was reported to lack inhibitory activity upon the 2-AG hydrolysis by rat cerebellar membranes, suggesting either a difference between the previously characterized MAGLcy and the membrane-bound MAGL or a difference in purity between the two sources of URB754 used by the authors of the studies [122].

IV. CONCLUSION

The pharmacological properties of endocannabinoids are now well established. AEA and 2-AG, the first endocannabinoids isolated have wide biological actions that could be beneficial in pathological conditions such as pain, inflammation, neurodegeneration, cancer, multiple sclerosis, Huntington's disease, obesity and cardiogenic shock. Unfortunately, the therapeutic use of endocannabinoids is limited by their short half-life *in vivo*, subsequent to quick enzymatic hydrolysis. FAAH and MAGL are now considered as the main causes of hydrolysis of AEA and 2-AG, respectively. The search of potent and selective agents capable of inhibiting these enzymes is therefore inherent in the promotion of medicines acting on the endocannabinoid system. As it is evident from this review, FAAH has been the subject of most interest, whereas the pharmacological characterization of MAGL is only at the beginning. Among the compounds presented in this review, some potent inhibitors are invaluable pharmacological tools which have proved that the modulation of the endocannabinoid tone is an efficient method to promote the therapeutic potential of endocannabinoids. Nevertheless, most of these inhibitors were only tested on the targeted enzyme. There is therefore a lack of information about the selectivity of these compounds, although compounds like URB597 and OL-135 have been characterized in more detail and show to have promising

effects in models of inflammation, inflammatory pain and anxiety.

Undoubtedly, future research will be devoted to the design of potent and selective agents tested at least upon the three well known enzymes responsible for the endocannabinoid metabolism (FAAH, MAGL but also NAAA which was not presented in the present review). Nevertheless, the quest for selective inhibitors can be tricky. A recent article from Saario and coworkers [114] shows that virtual screening of compounds using a model of human MAGL does not afford inhibitors of the membrane-bound rat MAGL activity among the hit molecules, but it does give FAAH inhibitors. If the study does not rule out that human MAGL might be distinct from the membrane-bound rat MAGL, it should convince us that the selectivity for a specific enzyme is not a target easy to reach. Nevertheless, let us hope that such potent and selective compounds will become some of our future medicines.

V. ACKNOWLEDGEMENTS

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ABBREVIATIONS

AA-5-HT	=	Arachidonoylserotonin
AEA	=	Arachidonoylethanolamide, anandamide
2-AG	=	2-Arachidonoylglycerol
ATFMK	=	Arachidonoyltrifluoro-methylketone
CAY10400	=	1-Oxazolo[4,5-*b*]pyridin-2-yl-9*Z*-octadecen-1-one
CAY10401	=	1-Oxazolo[4,5-*b*]pyridin-2-yl-9-octadecyn-1-one
CAY10402	=	1-Oxazolo[4,5-*b*]pyridin-2-yl-6-phenyl-1-hexanone
CAY10435	=	1-Oxazolo[4,5-*b*]pyridin-2-yl-1-dodecanone
CGRP	=	Calcitonin gene related peptide
CHI	=	Closed Head Injury
COX	=	Cyclo-oxygenase
CPO	=	Chlorpyrifos oxon
CREAE	=	Chronic Relapsing Experimental Autoimmune Encephalomyelitis
DAK	=	Diazomethylarachidonoylketone
EOPF	=	Ethyl octylphosphonofluoridate
FAAH	=	Fatty Acid Amide Hydrolase
IbuAM5	=	α-Methyl-4-(2-methylpropyl)-*N*-(3-methyl-2-pyridinyl) benzeneacetamide
IDFP	=	Isopropyl dodecylfluorophosphonate
JP83	=	6-Phenylhexyl carbamic acid 3'-carbamoyl biphenyl-3-yl ester
JP104	=	Undec-10-ynyl-carbamic acid 3'-carbamoyl biphenyl-3-yl ester
LOX	=	Lipoxygenase
MAFP	=	Methylarachidonoyl fluorophosphonate
MAGL	=	Monoacylglycerol Lipase
MAGLcy	=	Cytosolic Monoacylglycerol Lipase
MAGLm	=	Membrane-bound enzyme responsible for monoacylglycerol hydrolysis
α-Me-1-AG	=	α-Methyl-1-arachidonoyl glycerol
MOPF	=	Methyl octylphosphonofluoridate
NAAA	=	*N*-Acylethanolamine-hydrolyzing Acid Amidase
NADA	=	*N*-Arachidonoyldopamine
NAGly	=	*N*-Arachidonoylglycine
NAIle	=	*N*-Arachidonoylisoleucine
NAM	=	*N*-Arachidonoylmaleimide
S-nonyl BDPO	=	*S*-Nonylbenzodioxaphosphorin oxide
NPAA	=	*N*-Palmitoylethanolamine-hydrolyzing Acid amidase
NSAID	=	Non-steroidal anti inflammatory drug
O-1428	=	α-Methyl-1-arachidonoyl glycerol
OEA	=	Oleoylethanolamide
OEt	=	Oleoylethylamide
OL-135	=	1-Oxo-1-[5-(2-pyridyl)oxazol-2-yl]-7-phenylheptane
PAG	=	Periaqueductal grey matter
PEA	=	Palmitoylethanolamide
PHOP	=	1-Oxazolo[4,5-*b*]pyridin-2-yl-6-phenyl-1-hexanone
RBL	=	Rat Basophilic Leukemia
RP-2ME	=	*R*-palmitoyl-(2-methyl)ethanolamide
TGH	=	Triacylglycerol hydrolase
TRPV-1	=	Transient receptor potential vanilloid
URB524	=	*N*-Cyclohexylcarbamic acid biphenyl-3-yl ester
URB532	=	*n*-Butylcarbamic acid 4-benzyloxyphenyl ester

URB597	=	Cyclohexylcarbamic acid 3'-carbamoyl biphenyl-3-yl ester
URB602	=	[1,1'-Biphenyl]-3-yl carbamic acid, cyclohexyl ester
URB754	=	[6-Methyl-2-[(4-methylphenyl)-amino]-4H-3,1-benzoxazin-4-one

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