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Perspective

Discovery and Development of Fatty Acid Amide Hydrolase (FAAH) Inhibitors

Mark Seierstad and J. Guy Breitenbucher*

Johnson & Johnson Pharmaceutical Research and Development, L.L.C., 3210 Merryfield Row, San Diego, California 92121

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Introduction

The medicinal use of cannabis dates back to early recorded history. As early as 2600 B.C., ancient Chinese texts record the use of cannabis for the relief of numerous conditions including rheumatic and menstrual pain.[1] Modern scientific investigations into the biological mechanisms surrounding the effects of cannabis have resulted in the discovery of its principle active components cannabidiol and Δ^9-tetrahydrocannabinol (THC[a]),[2,3] as well as the receptors at which they act (CB1 and CB2).[4,5] More recently, cannabis-derived extracts or synthetic CB1 agonists have been approved by regulatory agencies for refractory chemotherapy-induced nausea and vomiting (nabilone, dronabinol), appetite stimulation in AIDS patients (dronabinol), neuropathic pain of multiple sclerosis, and adjunctive treatment of advanced cancer pain (Sativex). However, a major limitation to the utility of direct cannabinoid agonists as therapeutic agents is the undesirable profile of side effects, which includes dysphoria, dizziness, and effects on motor coordination and memory. In particular, the cognitive effects of these agents appear to be sufficiently aversive to markedly limit their use.[6,7]

The discovery of the CB1 and CB2 receptors instigated a search for endogenous agonists of these receptors and subsequently the enzymes that control their synthesis and degradation. Anandamide (AEA) was the first endocannabinoid signaling molecule to be recognized as such.[8] This discovery was followed by the identification of 2-arachidonyl glycerol (2-AG) as a second endocanabinoid.[9,10] Today it is recognized that a wide variety of fatty acid amide signaling molecules exist, and while their historic origin resulted in the term "endocannabinoids", it is now clear that the actions of these signaling lipids are much more diverse. The details of this diverse pharmacology have been recently reviewed.[11]

Further investigations revealed that the actions of these signaling lipids are terminated by the hydrolytic activity of a number of enzymes. Although the existence of an ethanolamide degrading activity was known since 1966,[12] it was only in 1996 when researchers at Scripps identified fatty acid amide hydrolase (FAAH) as the enzyme responsible for rat oleamide hydrolysis that the first enzyme degrading these signaling lipids was cloned and characterized.[13] Currently, in addition to FAAH, three enzymes have been identified that also contribute to the termination of endocannabinoid signaling. One is fatty acid amide hydrolase-2 (FAAH-2), which is found in human and primate genomes but not mice and rats.[14] *N*-Acylethanolamine acid amidase (NAAA),[15] also hydrolyzes ethanolamides at low pH values and prefers saturated fatty acid amide substrates such as palmitoylethanolamide (PEA). Finally monoacylglycerol lipase (MAGL) is the enzyme primarily responsible for the degradation of 2-arachidonoylglycerol (2-AG). The complex biochemical pathways responsible for endocannabinoid signaling and degradation have also been the subject of excellent recent reviews.[16,17]

One attractive approach to elicit the desirable effects of cannabinoid activation, while avoiding the negative effects of global CB1 stimulation, is to manipulate endogenous cannabinoid signaling through the inhibition of FAAH and related

* To whom correspondence should be addressed. Phone: 858-784-3036. Fax: 858-784-3116. E-mail: Jbreiten@prdus.jnj.com.

[a] Abbreviations: FAAH, fatty acid amide hydrolase; MGL, monoacylglycerol lipase; COX, cyclooxygenase; AEA, anandamide; SAR, structure−activity relationship; MAFP, methoxyarachidonyl fluorophosphonate; NSAID, nonsteroidal anti-inflammatory drug; CB1, cannabinoid-1 receptor; CB2, cannabinoid-2 receptor; CNS, central nervous system; HTS, high-throughput screening; PBQ, *p*-benzoquinone; PNL, partial nerve ligation; CCI, chronic constriction injury; AchE, acetylcholine esterase; OEA, oleoylethanolamide; AA5HT, arachidonylserotonin; ESI, electrospray ionization; QSAR, quantitative structure−activity relationships; CoMSIA, comparative molecular similarity indices analysis; EOFP, ethoxyoleyl fluorophosphonate; PEA, palmitoylethanolamide; PMSF, phenylmethylsulfonyl fluoride; 2-AG, 2-arachidonylglycerol; NAAA, *N*-acylethanolamine acid amidase; THC, Δ^9-tetrahydrocannabinol.

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enzymes. Resting concentrations of AEA in the CNS are very low because of its rapid hydrolysis by FAAH.[18,19] In addition, FAAH controls the levels of other lipid mediators with anti-inflammatory and analgesic properties, including PEA, which exerts its analgesic and anti-inflammatory effects via non-cannabinoid pathways. Inhibition of FAAH would be expected to elevate the endogenous concentrations of all of its substrates and consequently prolong and potentiate their biological effects. Such potentiation, acting preferentially on active pathways, might be expected to have a reduced risk of psychotropic effects compared with global activation of cannabinoid receptors by exogenously applied agonists. Direct evidence for such up-regulation of synthesis in a context relevant to nociception was shown by the demonstration that intraplantar injection of formalin led to release of AEA selectively in the periaqueductal gray region of the brain, which is associated with nociceptive processing.[20] Anandamide synthesis is also up-regulated following physiological stress, and it has been proposed that an increased endocannabinoid tone under stressful physiological situations may represent an endogenous neuroprotective mechanism.[21−23]

In addition, FAAH knockout mice have also been described.[18] These mice have elevated resting brain concentrations of AEA and manifest an analgesic phenotype in both the carrageenan model of inflammatory pain and the formalin model of spontaneous pain. Further studies with FAAH (−/−) mice show reductions in inflammatory responses in a number of models,[24−27] as well as improvements in slow wave sleep and memory acquisition.[28,29] Indeed, recent preclinical investigations with FAAH inhibitors will be reviewed here and clearly support their use for the treatment of chronic pain without the motor impairment typically associated with direct global activation of CB1 with exogenous agonists.

In addition to pain there are several additional therapeutic areas in which alterations in endocannabinoid processing may be beneficial. Specifically, changes in AEA and 2-AG concentrations have been reported in tissues related to a number of disorders including immunological diseases,[30] psychiatric conditions,[31] metabolic, and cardiovascular conditions.[32] Indeed the implications of FAAH inhibition are theoretically quite complicated given the number of potential substrates for the enzyme and the variety of targets (both cannabinoid and non-cannabinoid) with which these substrates interact. Excellent reviews of the pharmacology of FAAH inhibition have been published recently,[16,17,33] so the details of the pharmacology will only be commented upon here when pertinent to the discussion of specific medicinal chemistry efforts. This review will focus on the medicinal chemistry and SAR of FAAH inhibitors discovered to date, as well as discuss the catalytic mechanisms and structural biology that is critical to the design of novel FAAH inhibitors.

Structural Biology of FAAH

A mammalian enzyme that hydrolyzes *N*-acylethanolamines was known as early as 1984,[34] when it was reported that dog brain homogenates contained an amidase activity that hydrolyzed PEA at an optimal pH of 10.0. Soon after the discovery of AEA came the report of an enzyme activity in neuroblastoma and glioma cells that converted AEA to arachidonic acid.[35] This enzyme activity was found primarily in membrane fractions and could be inhibited by phenylmethylsulfonyl fluoride (PMSF). FAAH was later purified using mechanism-based affinity chromatography and sequenced by Scripps researchers in 1996.[13] Although identified as a member of the amidase signature family of enzymes, FAAH was unique in two ways: it was the first known mammalian member of this family, and FAAH contained a predicted transmembrane binding domain not present in other members of the family. Here, we briefly discuss each of the various structural/chemical features of FAAH most relevant to drug discovery.

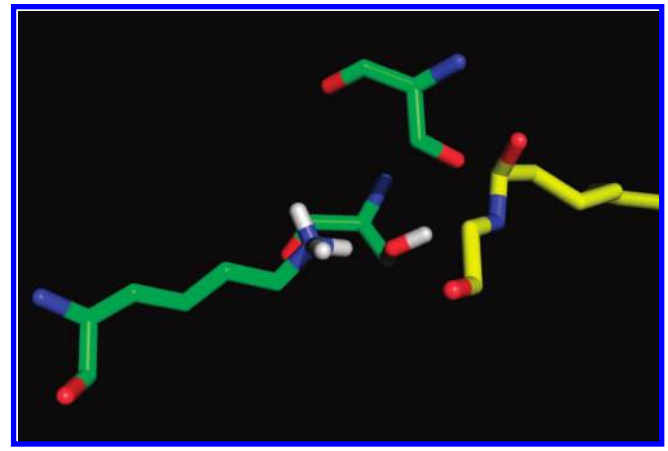


Figure 1. FAAH catalytic triad is shown in green: Lys142 (left), Ser217 (center), and Ser241 (top, deprotonated). AEA (yellow) is modeled into the FAAH crystal structure, covalently attached to Ser241 (bond not shown).

FAAH Catalytic Machinery. The first reports of inhibitors of FAAH could not unambiguously identify the nucleophilic residue as either a cysteine or serine. The amidase signature sequence contains highly conserved serine residues (including a GXSXG motif) and a moderately conserved cysteine residue.[36] Also present is a highly conserved aspartic acid residue, a common component in other hydrolase classes. In an early site-directed mutagenesis study, Kobayashi and co-workers[37] examined a *Rhodococcus* amidase from the amidase signature family. Cys203, Asp191, and Ser195 (corresponding to Cys249, Asp237, and Ser241 in FAAH) were selected for mutation, with only Asp191 and Ser195 being identified as essential for activity. Two later mutagenesis studies with FAAH gave conflicting accounts of the C249A variant; one[38] reported no hydrolysis activity, and another[39] reported wild-type-like ability to hydrolyze substrates.

Cravatt and co-workers[40] expressed and purified several mutants with alanine replacing conserved serine residues in the amidase signature sequence. FAAH mutants with serine residues 217 and 218 converted to alanine had significantly lower amidase activity, and S241A FAAH had no detectable amidase activity. Studies labeling FAAH with the irreversible inhibitor methoxyarachidonyl fluorophosphonate (MAFP) demonstrated covalent modification of one of the residues between 213 and 243 (FAAH has no cysteine residue in that range). Labeling with the related ethoxyoleyl fluorophosphonate (EOFP) allowed the precise assignment of Ser241 as the nucleophilic residue. Although FAAH and other amidase signature enzymes contain the GXSXG motif common in other serine hydrolases, serine residues 217 and 218 (rather than Ser241) are a part of this motif. Figure 1 shows AEA modeled into the FAAH crystal structure, bound to Ser241.

Early researchers reasonably hypothesized that the conserved aspartic acid residue was a part of a Ser/Cys-His-Asp catalytic triad; in fact, the corresponding aspartic acid in an amidase signature enzyme related to FAAH was shown by site-directed mutagenesis to be essential for amidase activity.[37] Early hypotheses of a catalytic role for histidine residues were also proposed.[41] However, mutation of the three histidine residues

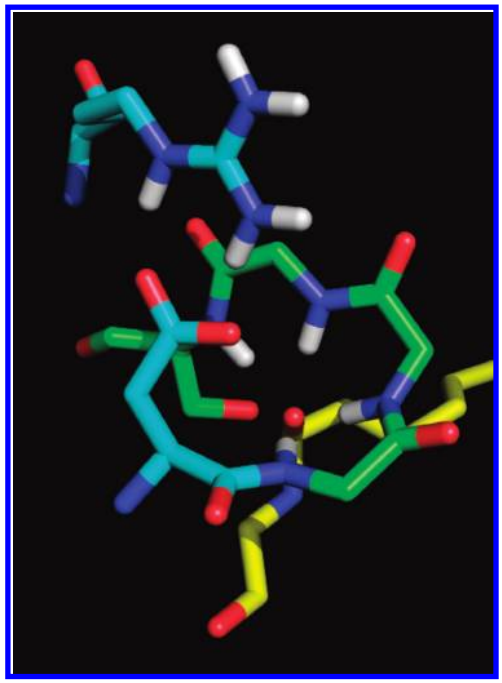


Figure 2. FAAH oxyanion hole (clockwise, Ser241, Gly240, Gly239, and Ile238 in green) is shown, along with two highly conserved residues on the face opposite the substrate: Asp237 (bottom, cyan) and Arg243 (top, cyan). AEA (yellow) is modeled into the FAAH crystal structure.

Figure 3. Proposed membrane binding regions are shown as yellow surfaces: on left are helices 18 and 19, and on right is the first resolved residue after the deleted N-terminal transmembrane domain region. Also shown in red, blue, and orange are the ends of the three internal channels (see Figure 4).

conserved among FAAH from rat, mouse, and human (His184, His358, and H449) provided FAAH mutants with significant amidase activity.[40]

One basic residue in FAAH that is highly conserved among amidase signature enzymes is Lys142. Mutation of Lys142 into alanine provided a FAAH with severely diminished amidase activity.[42] The ability of K142A FAAH to hydrolyze esters was also less than wild-type FAAH; however, the esterase activity was much less affected than the amidase activity. The role of additional serine residues was only partially clarified by mutagenesis studies. Ser217 and Ser218 were early targets for mutagenesis, both because they are highly conserved and because they are part of the GGSSGG motif found in amidase signature enzymes (and thought to correspond to the GXSXG motif common in other serine hydrolases). Various reports on the mutation of either (or both) of these residues into alanine showed significant loss of amidase activity, with S217A FAAH generally being less active than S218A FAAH.[38−40,42]

The amidase signature catalytic triad was more firmly identified in 2002 when crystal structures of three different members were reported: first malonamidase,[43] followed by peptide amidase[44] and FAAH.[45] The residues of the catalytic triad (Ser241, Ser217, and Lys142) interact through a network of hydrogen bonds that facilitates proton exchange. In a formal sense, the hydrolysis reaction involves a protonation of Lys142 via the deprotonation of Ser217, which then deprotonates the nucleophilic residue Ser241. These protons are still available to the leaving group of the substrate. In fact, it has been proposed that the ability of FAAH to protonate the leaving group is responsible for FAAH's ability to hydrolyze amides at a rate comparable to that of esters.[42]

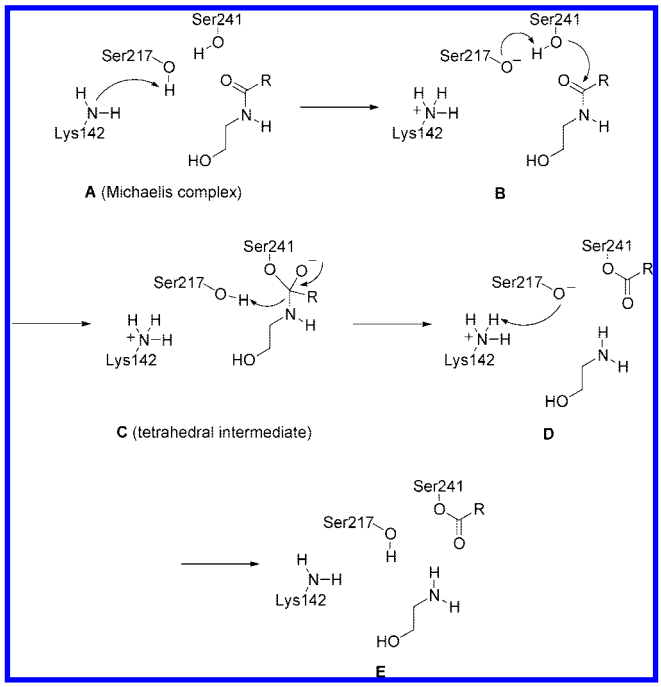
Adjacent to the nucleophilic serine side chain is a circle of four amide N−H bonds that form the oxyanion hole of FAAH (Figure 2). The carbonyl oxygen of amide or ester substrates sits in the middle of this circle, accepting a hydrogen bond from one or more of these residues. On the side of this N−H circle that is opposite the substrate sits the carboxylic acid side chain of Asp237 (this position is highly conserved among amidase signature enzymes). Site-directed mutagenesis studies of a *Rhodococcus* amidase[37] showed not only that this acidic residue is essential for catalytic activity but that even a slight change in the position of the carboxylic acid (replacing aspartic acid with glutamic acid) resulted in an enzyme with only 1% of the wild-type activity. Studies with FAAH also showed that mutation of Asp237 resulted in an inactive[38] or slightly less active[46] enzyme. Structural studies revealed that Asp237 does not directly contact the substrates,[45] yet it is highly conserved and close to the catalytic machinery.

The FAAH crystal structure shows the cationic side chain of Arg243 to be in a tight interaction with Asp237.[45] Similar to Asp237, mutation studies have shown that Arg243 is essential for high amidase activity,[46] and structural studies have suggested that Arg243 does not interact directly with substrates. This arginine residue is also highly conserved among amidase signature enzymes.[36] Recent studies have shown that in malonamidase E2 this residue can occupy a position and possibly play a role in catalysis very different from its role in FAAH.[47]

The identification of the structure of FAAH and the catalytic machinery led to a proposed mechanism of substrate hydrolysis. Quantum chemical examinations of the mechanism have been reported by Mullholland[48] and Jorgensen.[49] Scheme 1 shows a proposed mechanism consistent with these studies.

The initial proton exchange occurs when Lys142 deprotonates Ser217, resulting in zwitterionic intermediate **B**. In a concerted[49] or semiconcerted[48] process, the alkoxide of Ser217 removes the proton from Ser241, which then adds to the carbonyl of the substrate to form tetrahedral intermediate **C** (this is the compound modeled in Figures 1−4). Both of the modeling studies agree that formation of **C** from **B** is the rate-determining step, although only Jorgensen examined the process after

Scheme 1. Mechanism of Substrate Hydrolysis by FAAH



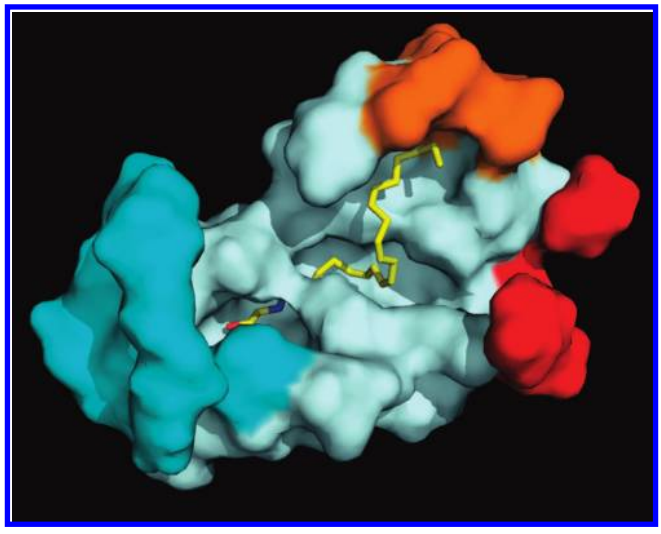


Figure 4. Interior channels of the FAAH active site, modeled with AEA (yellow). Each channel end is marked as blue, orange, or red. The top portion of the fully enclosed channels is cut away for simplified viewing.

formation of the tetrahedral intermediate. Collapse of the tetrahedral intermediate occurs along with transfer of the proton from Ser217 to the substrate's leaving group to give zwitterion **D**, leaving Ser241 covalently modified as an ester. Transfer of a proton from Lys142 to Ser217 gives **E**. The exit of ethanolamine and hydrolysis of the acylated Ser241 results in the regeneration of catalytic FAAH.

Membrane Binding Region. Most of the members of the amidase signature family are soluble enzymes; FAAH is an exception, as it is an integral membrane protein. Analysis of the primary sequence predicted a transmembrane domain including residues 9−29 at the N-terminus (Figure 3, yellow surface on right).[50] Deletion of residues 1−30 resulted in an enzyme with normal amidase activity but also still strongly membrane associated. The crystal structure of FAAH[45] suggested an explanation for this. Two α helices (18 and 19, yellow surface on left in Figure 3) on the same face as the N-terminus were rich in hydrophobic residues; this feature is lacking in the two soluble amidase signature enzymes for which crystal structures have been determined.[51]

Substrate Access to the Active Site. The catalytic residues of FAAH are buried deep within the enzyme and are accessible by two narrow channels. The left-side channel (as shown in Figure 4) is more hydrophilic, being defined not only by Ser217 and Lys142 but other polar residues such as Thr236 (usually a threonine, serine, or asparagine in amidase signature enzymes).[36] This channel is also wider than the other and leads to openings depicted in blue in both figures. This channel is likely the path for water molecules to access the acylated nucleophilic Ser241 and also the path for polar leaving groups from the substrate to exit FAAH.

The right-side channel is narrower, longer, and more hydrophobic. Leading away from the catalytic machinery, this channel splits into two subchannels, with the fatty chain of the ligand (MAFP in the crystal structures, AEA in Figures 1, 2, and 4) continuing down one. The path *not* filled by the ligand leads to what has been proposed to be the entrance site for substrates.[45] This opening is shown in red in Figures 3 and 4 and is very close to the location of the proposed membrane binding helices. This entrance is defined by residues that are both hydrophobic (Ile407 and Ile530) and charged (Arg486 and Asp403). It has been suggested that these residues facilitate the entrance of substrates' polar "head groups" into FAAH from the membrane.

The second of these two subchannels is shown by the crystal structure to contain the fatty chain of MAFP, and the modeled pose of AEA shown in the figures assumes that the fatty chain of AEA occupies the same space. The end of this subchannel is capped by polar residues that are at the surface of FAAH: Tyr335, Glu373, and Arg428 (which is surrounded by aromatic residues: Phe527 and Trp531, in addition to Tyr335). The location of these residues is shown in orange in Figures 3 and 4.

If the substrate entrance is that depicted in red, it is not clear how the fatty chain comes to be in the second subchannel. One possibility is that the substrates enter FAAH through the "red" opening, and the headgroup travels *beyond* the catalytic machinery until the end of the fatty tail is at the intersection of the two subchannels. The substrate then reverses course until the second subchannel is filled, and the headgroup reacts with the catalytic machinery. This seems unlikely, given that FAAH can bind substrates similar to MAFP/AEA and yet with large appendages at the end of the fatty chain, such as FP-biotin[42] and a trifluoromethylketone inhibitor attached to Sepharose beads (used for mechanism-based affinity chromatography).[13] The portion of the FAAH surface marked in orange could serve as a substrate entrance only if some of these residues could move their side chains in such a way as to create an opening; it is not known if this can happen. This question of whether FAAH and its substrates/inhibitors can undergo significant movements (relative to the crystal structure) is an important one, especially for efforts in structure-based drug discovery. There is relatively little in the published literature that addresses this question. One notable exception is a recent report on FAAH conformational changes induced by substrate binding, including a discussion on the possible role of Trp531, Trp445, and the membrane binding region.[52]

Unfortunately, to date, only the single X-ray structure of FAAH bound to MAFP has been published. While this is a critical tool for the design and optimization of FAAH inhibitors, further reports of additional ligands bound to FAAH are eagerly awaited. Questions of enzyme flexibility and substrate/inhibitor

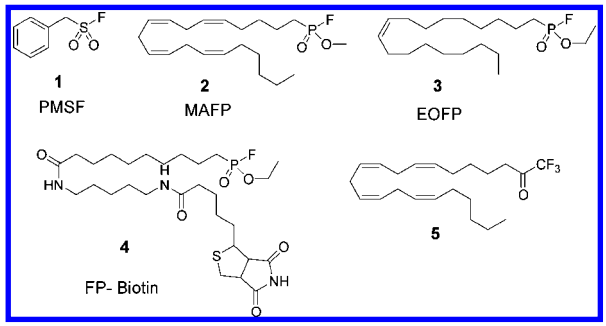


Figure 5. Representative early covalent inhibitors of FAAH.

access to the FAAH active site should be made much clearer when these data become available.

Medicinal Chemistry

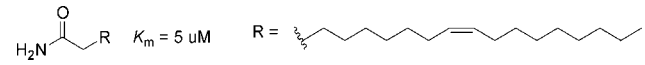
Early Inhibitors. Like many proteases and hydrolases, the first identified inhibitors of FAAH were potent electrophiles. In the case of FAAH these reactive functional groups form covalent interactions with the active site Ser241 (although this was not always recognized at the time of their discovery). The first of these was PMSF (Figure 5). Interestingly, this compound was identified inadvertently when it was added to a rat brain homogenate to inhibit protease activity and was found to inhibit AEA hydrolysis.[35] A limited amount of SAR has been done on fluorophosphonate[53,54] and fluorosulfonate[55] inhibitors and has been reviewed previously.[16] These efforts resulted in substrate inspired inhibitors such as laurylsulfonyl fluoride, MAFP, and EOFP.

While the reactivity and lack of selectivity of such agents make them poor drug candidates, they have been valuable tools for evaluating the biochemistry of FAAH and to some extent the pharmacology. For example, as discussed previously, EOFP was a critical tool in the identification of Ser241 as the active site nucleophile of FAAH, and the crystallization of FAAH was accomplished as a complex with MAFP.[45] These structures have also been used to define the resynthesis rate of FAAH in vivo ($T_{1/2}$ about 52 h in mouse brain)[56] which could be important in defining the duration of therapeutic effect of irreversible inhibitors of FAAH. The relative lack of selectivity has also made fluorophosphonates valuable tools for determining the proteomic selectivity of FAAH inhibitors. Cravatt, Boger, and others have made extensive use of labeled probes such as FP-biotin and FP-Rh to determine the selectivity of a variety of FAAH inhibitors with respect to off-target serine hydrolases.[57,58]

The first truly designed inhibitors of FAAH were described in 1994 by Deutsch.[59] These studies were conducted before the enzyme was fully characterized or the catalytic mechanism clearly understood. The design of these inhibitors was based upon the use of activated ketones as putative active site traps. 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. The most active of these was arachidonyl trifluoromethyl ketone **5** (Figure 5).

Shortly after these studies were reported, the Boger group published[60] a similar result with respect to the inhibition of oleamide hydrolysis (A previous report of the partial characterization and purification of anandamide hydrolase had suggested that AEA and oleamide were degraded by the same enzyme[61] that was later revealed to be FAAH. A later survey of substrate specificity revealed that primary amides were hydrolyzed by FAAH more rapidly than ethanolamides).[62]

Table 1. Representative Activated Carbonyl Inhibitors of Oleamide Hydrolysis



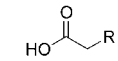
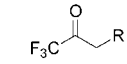
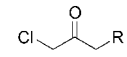
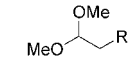
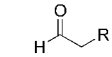
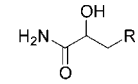
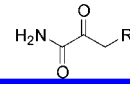
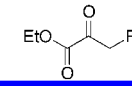
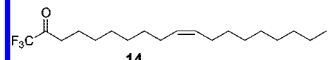
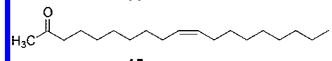
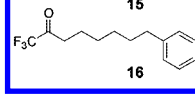
#	inhibitor	K_i (μM)	#	inhibitor	K_i (μM)
6		6.0	10		0.0012
7		0.7	11		>300
8		0.19	12		>100
9		0.017	13		0.009

Table 2. Representative Trifluoromethyl Ketone Inhibitors of FAAH

	K_i (μM)
 **14**	0.082
 **15**	>100
 **16**	0.10

		K_i (μM)
17	R = $(CH_2)_{16}CH_3$	0.24
18	R = $(CH_2)_{12}CH_3$	0.49
19	R = $(CH_2)_{10}CH_3$	0.14
20	R = $(CH_2)_8CH_3$	0.13
21	R = $(CH_2)_6CH_3$	1.2
22	R = $(CH_2)_5CH_3$	10.7
23	R = $(CH_2)_4CH_3$	26.9

Boger's work was a comprehensive survey of potential electrophilic analogs of oleamide (Table 1). These included α-chloroketones, aldehydes, α-ketoamides, α-ketoesters, trifluoromethyl ketones, and diazoketones. This work also described the first detailed kinetic studies of FAAH inhibitors. Thus, trifluoromethyl ketone **10** was found to be a competitive reversible FAAH inhibitor, while the α-chloro derivative **7** was irreversible. Boger also determined the importance of the electrophile in inhibition, showing the alcohol analogue **12** had a >1000-fold decrease in inhibitory activity vs the ketone analogue **9**. The relative potency of the inhibitors was generally in line with the electrophilicity of the reacting carbonyl. This work also suggested the possibility of additional important interactions with the enzyme not previously uncovered. The authors suggested that the inhibitors containing a H-bond acceptor (such as the α-ketoester or α-ketoamide) formed a favorable interaction with the enzyme compared to the more electrophilic aldehydes and trifluoromethyl ketones. The significance of a H-bond acceptor on the ligand was later discussed in a modeling study by Boger and Jorgensen with ketoheterocycle inhibitors.[63]

With the realization that FAAH hydrolyzed a variety of fatty acid amide substrates, the Boger group evaluated trifluoromethyl ketone inhibitors with a wide range of lipophilic groups exploring the steric and conformational requirements of inhibitors (Table 2).[64] In this study, FAAH inhibition was relatively unaffected by the nature of the lipophilic group employed, with the caveat that a sufficiently long alkyl chain was required. For instance, activity dropped off significantly for 1,1,1-trifluoro-2-octaneone **22** compared to the three-carbon longer homologue **20**. An important finding used in later FAAH inhibitors was the identification of the phenhexyl group (**16**) as a less lipophilic alternative to long chain fatty acids found in endogenous

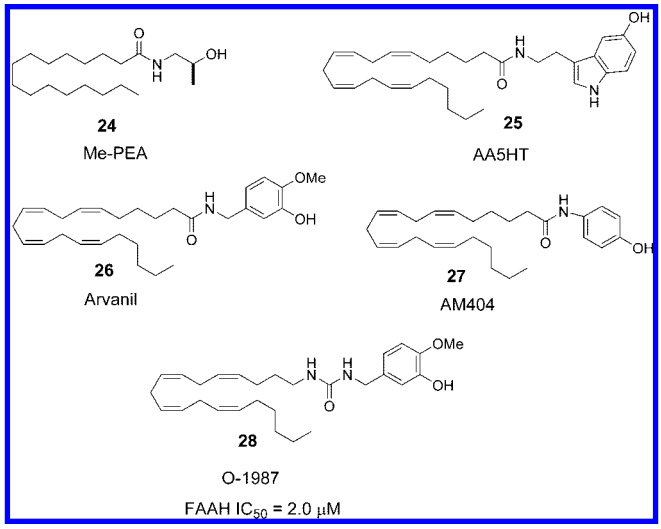
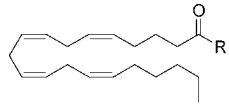
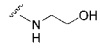


Figure 6. Representative substrate inspired inhibitors of FAAH.

Table 3. Representative Anandamide Analogues as FAAH Inhibitors



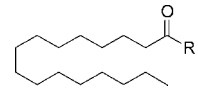
#	R	FAAH IC_{50} (μM)	#	R	FAAH IC_{50} (μM)
29		3.4	32		0.9
30		1.4	33	-OH	>100
31		37.4	34		1.2

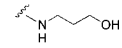
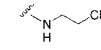
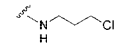
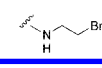
substrates. Once again the electrophilicity of the carbonyl group proved critical to activity, comparing the potent trifluoromethyl ketone **14** with the inactive methyl ketone **15**.

A popular and logical approach to FAAH inhibition is the modification of substrates to create inhibitors (Figure 6). Early work in this area identified a number of FAAH inhibitors including arachidonylserotonin **25** (AA5HT),[65] **27** (AM404),[66] and **26** (arvanil).[67] An extensive study designed to discover AEA transport inhibitors through the manipulation of the ethanolamine portion of AEA resulted in useful early inhibitors of FAAH (Table 3). The most potent of these inhibitors was the alkyl chloride derivative **32** (IC_{50} = 0.9 μM). This work suggested that small aliphatic and aromatic groups were well tolerated but larger lipophilic amides were not. Although it was speculated that most of these analogues were probably substrates for FAAH, turnover rates were not determined. A recent pharmacological investigation of AA5HT showed analgesic effects in the mouse formalin test. The analgesic effects were blocked by CB1 but not CB2 antagonists.[68]

Fowler conducted a similar study, decreasing the length of the fatty acid chain and monitoring the ability to inhibit AEA degradation. In this study, ethanolamides of aliphatic straight chain fatty acids were evaluated and there was a clear trend to decreasing FAAH inhibition with chain lengths below C-12.[69] In 2001 DiMarzo reported an SAR study on arvanil looking at a combination of VR1 and CB1 agonism, AEA transport, and FAAH inhibition. Of these analogues the most potent FAAH inhibitor was the urea analogue **28** (O-1987) (Figure 6).[70] Intravenous administration of **28** to mice produced effects in all of the typical cannabinoid tetrad tests: decreased spontaneous activity, decreased rectal temperature, and antinociception in the tail flick test. However, the lack of selectivity in these molecules makes it difficult to verify that the observed pharmacology is a result of FAAH inhibition.

Table 4. Representative Palmitoyl Amide FAAH Inhibitors



#	R	FAAH pIC_{50}	#	R	FAAH pIC_{50}
35		5.42	38		5.58
36		5.47	39		5.60
37		5.45	40		5.45

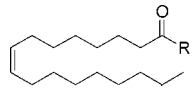
Fowler conducted an additional study of palmitoylamide analogues similar to the one previously described with arachidonic acid amides and identified some weak inhibitors. The most active analogues are shown in Table 4.[71] More recently an in vivo evaluation of compound **36** was published. The compound was effective in reversing thermal and tactile hypersensitivity in a number of neuropathic pain models in rats.[72] Importantly this paper reported that **36** reverses the reduction of spontaneous exploratory behavior induced by partial sciatic nerve injury and shows activity in varicella zoster virus induced neuropathy. Both of these models are thought to have a high degree of clinical relevance for the treatment of human neuropathic pain.

Ketoheterocycles. Boger reported a major breakthrough in the identification of highly potent and selective FAAH inhibitors in 2000.[73,74] This work describes starting with trifluoromethyl oleyl ketone and replacing the trifluoromethyl group with various heterocycles designed to activate the ketone to nucleophilic attack (Table 5). Peptidic ketoheterocycles had been previously described by others as mechanism based protease inhibitors, but this was the first example of this strategy applied to a hydrolase target.[75,76]

As with previous investigations of ketoheterocycle inhibitors of proteases, the nature of the activating heterocycle was critical to activity. Oxazoles were found to be superior to thiazoles or imidazoles in activating the adjacent ketone to nucleophilic attack. In fact, oxazole ketones significantly improved activity over the trifluoromethyl ketone **10**. Benzoxazole **57** was a modestly potent FAAH inhibitor (K_i = 370 nM); however, conversion to the pyridyloxazole afforded a significant improvement in activity (**53**, K_i = 2.3 nM). The authors commented that the significant increase in potency was relatively insensitive to the position of the pyridyl nitrogen and suggested that two or more residues of FAAH were involved in hydrogen-bond interactions. An alternative explanation for the similar activity of the pyridyloxazole isomers is that the increase in potency is due to the increased electrophilicity of the ketone. Regardless, the >100-fold increase in potency between oxazolopyridine and benzoxazole is quite remarkable. A survey of methyl-substituted benzoxazoles proved interesting as well. The difference between the activity of methyl analogues **49** and **52** suggests that a specific orientation of the oxazole oxygen and nitrogen may be required for activity. However, it should be noted that compound **52** is significantly less active than other ketoheterocycles.

Boger was also able to combine the ketoheterocycle concept with the less lipophilic phenhexyl group from previous work

Table 5. Representative Heterocyclic Ketone Inhibitors of FAAH



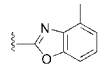
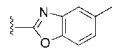
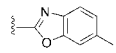
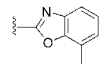
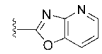
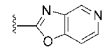
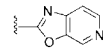
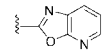
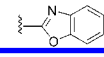
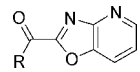
#	R	FAAH K_i (μM)	#	R	FAAH K_i (μM)
10	CF_3	0.082	**49**		> 100
41		0.017	**50**		> 100
42		> 100	**51**		> 100
43		> 100	**52**		13
44		0.065	**53**		0.0023
45		> 100	**54**		0.0072
46		> 100	**55**		0.0037
47		0.11	**56**		0.011
48		0.13	**57**		0.37

Table 6. Representative Alkylphenyl Pyridyloxazole Ketone FAAH Inhibitors



#	R	FAAH K_i (μM)
58	$Ph(CH_2)_3$	0.0069
59	$Ph(CH_2)_4$	0.0003
60	$Ph(CH_2)_5$	0.0002
61	$Ph(CH_2)_6$	0.00028
62	$Ph(CH_2)_7$	0.00039
63	$Ph(CH_2)_8$	0.00052

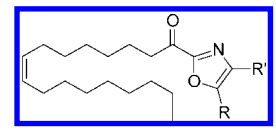
to give the very potent inhibitor **60** (OL-92) (K_i = 0.20 nM), one of the most potent and selective FAAH inhibitors known (Table 6). However, in animal studies this compound failed to demonstrate FAAH inhibition or analgesia in vivo, possibly because of poor pharmacokinetics.[77]

In 2005 Boger reported further optimization of the oxazole ketone inhibitors.[78] Interestingly, like the benzoxazole SAR discussed previously, the phenyloxazole **64** was less potent than the pyridine and other nitrogen-containing heterocycle analogues (Table 7). Also, like the benzoxazoles, the position of the nitrogen did not play nearly as significant a role in binding as its presence or absence (**65**−**67**). Alternatively, in the case of the furan substituted oxazole, there was a significant difference in activity based upon the position of the furan oxygen (**70**, **71**). In this report Boger also combined the new, more active, substituted oxazoles with the less lipophilic phenhexyl group to produce compound **73** (OL-135). Compound **73** is one of the best characterized FAAH inhibitors to date (Figure 7).

In an elegant modeling paper by Jorgensen and Boger, Monte Carlo/free energy perturbation simulations were used to propose specific H-bonding interactions that account for the significant increase in activity of the previously discussed nitrogen containing heterocycles.[63] Ketoheterocycle **65** is proposed to be more potent than **64** because it can accept a hydrogen bond from Thr236, one of the polar residues in FAAH's more hydrophilic channel. Alternatively, as discussed with compounds **53**−**57**, the observation that nitrogen-containing aromatic rings bring about improved FAAH activity could also be explained by an increase in the electrophilicity of the ketone. The authors do not report results of simulations with other pyridine isomers that are less able (**66**) or unable (**67**) to accept a hydrogen bond from Thr236. The improved activity of oxadiazole **74** over oxazole **65** was proposed to result from the smaller size of the oxadiazole N compared to the oxazole CH (both as an effect on biaryl conformation and steric clashes with FAAH).[79] These specific FAAH−inhibitor interactions were also suggested to underlie the selectivity of compound **73** for FAAH vs other hydrolases.

Cravatt has conducted extensive proteomic analysis demonstrating that **73** is highly selective for FAAH.[77] The high selectivity and potency of **73** have made it a valuable and reliable tool for the investigation of FAAH pharmacology. Compound **73** was found to increase endogenous endocannab-

Table 7. Representative Oxazole Ketone Inhibitors of FAAH



#	R	R'	FAAH K_i (μM)
41	H	H	0.10
64		H	0.32
65		H	0.018
66		H	0.061
67		H	0.056
68	H		0.031
69			> 100
70		H	0.054
71		H	0.61
72		H	13.2

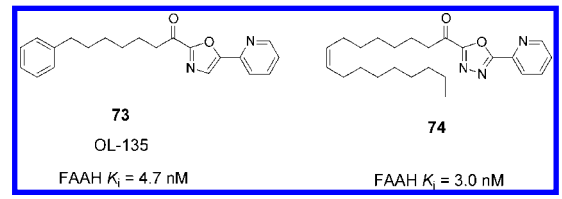


Figure 7. Representative activated ketone inhibitors of FAAH.

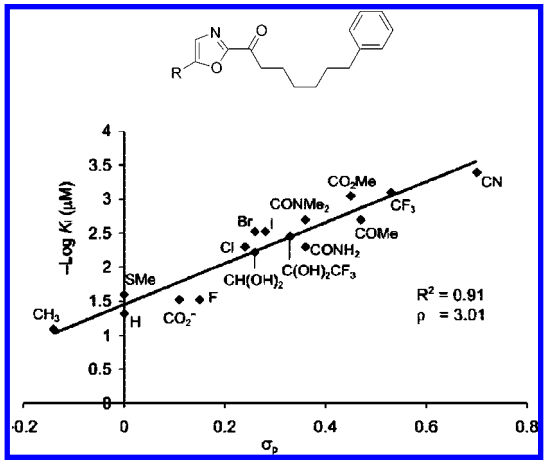


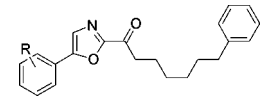
Figure 8. Plot of FAAH activity vs σ_p of the substituent on oxazole ketones.

inoid levels in the brain and spinal cord of mice. In addition, **73** produced significant analgesic effects in tail immersion, hot plate, and formalin test in mice without the associated motor impairment typical of CB1 agonism. However, the analgesia was completely reversed by the selective CB1 antagonist SR-141716 (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide, rimonabant), suggesting these effects are mediated through CB1 receptor activation.[77] Subsequently **73** 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.[80] The compound effectively blocked the hydrolysis of endocannabinoids in vivo, as previously discovered. However, the analgesic effects in these later models were not reversed by rimonabant, suggesting CB1 receptor activation does not play a role in these models. However, a selective CB2 receptor antagonist did reverse the effects of **73** in the spinal nerve ligation model but not the mild thermal injury model. These intriguing results suggest the possibility that FAAH inhibition evokes multiple analgesic mechanisms that may be model dependent.

Recent work on oxazole ketones provides quite a compelling case for the importance of the electrophilicity of the ketone in driving the SAR of FAAH inhibition. Striking correlations were demonstrated between the Hammett σ_p constant and FAAH inhibition in simple substituted oxazoles.[81,82] Using a variety of substituents on the oxazole ketone (Figure 8), the strong correlation suggests that the electrophilicity of the ketone clearly drives the SAR. Despite a range of lipophilicities and sterics embodied in the substituents, the stronger the electron withdrawing nature of the substituent on the oxazole, the more active the molecule was at FAAH. The correlation was compelling enough to suggest the protonation or hydration state of certain analogues based on FAAH activity and the back-extraction of the Hammett constant. Spectroscopic evidence confirmed that the aldehyde and trifluoromethyl ketone analogues examined in this study do exist in the hydrated state. Boger noted that some aromatic substituents on the oxazole do deviate slightly from this correlation in ways that were consistent with negative steric and positive H-bonding interactions. These substituents included phenyl and 2-pyridyl; other pyridine isomers (e.g., corresponding to **66** and **67**) were not discussed.

Follow-up SAR was reported recently that demonstrates the scope of substitution possible on the aryloxazole of **64** (Table 8).[82] In general, ortho substituted aryl oxazoles were not well tolerated. Alternatively, meta or para substituents on the pendent

Table 8. Effects of Substitution on FAAH Activity



	K_i (μM)		
R	ortho	meta	para
H		0.08	
NO_2	0.13	0.028	0.05
NH_2	0.75	0.019	0.09
CO_2CH_3	0.06	0.012	0.04
CO_2H	6.0	0.005	0.06
F	0.11	0.05	0.062
OCH_3	0.4	0.04	0.1
OH	0.17	0.05	0.14
SO_2NH_2	1.5	0.002	0.01
$CONH_2$	0.3	0.006	0.01
CN	0.13	0.015	0.04

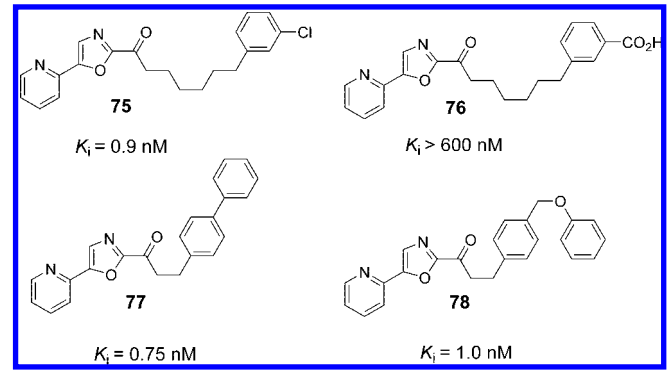
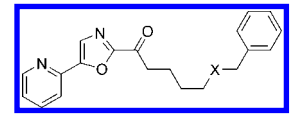


Figure 9. Representative oxazole ketone FAAH inhibitors with modifications to lipophilic side chain.

Table 9. Compound **73** Linker Replacements



#	X	K_i (nM)
73	CH_2	4.7
79	O	55
80	NCH_3	200
81	S	25
82	SO	2500
83	SO_2	>10000

aryl ring improved FAAH inhibition in almost all cases, regardless of the nature of the substituent. In light of the previous studies with simple oxazoles this might be anticipated, since substitution on the distal aryl ring would not be expected to have a significant influence on the electrophilicity of the ketone. One point of interest is that this work offers the possibility of improving the poor solubility of **73** without sacrificing activity at FAAH.

Additionally, Boger established SAR around the lipophilic portion of **73** (Figure 9). Interestingly, this region is quite tolerant of a wide variety of different groups.[83] A survey of heteroatom replacements for methylenes in the hexyl chain of **73** was also reported. Oxygen (**79**) and sulfur (**81**) were reasonably well tolerated in the alkyl linker, but nitrogen (**80**), sulfoxide (**82**), and sulfone (**83**) were not (Table 9). This finding seems reasonable given the lipophilic nature of the binding pocket of FAAH. In addition, conformational constraints to the alkyl linker such as phenyl and alkyne were evaluated and were well tolerated in this portion of the inhibitors (**77**). Lastly a

Figure 10. Recent patent applications covering piperidine oxazole ketones.

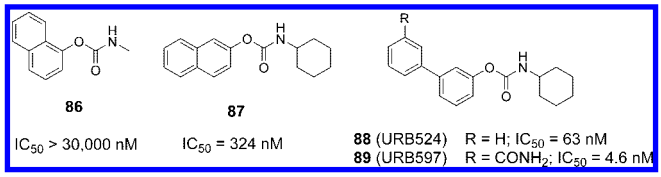


Figure 11. Representative phenyl carbamate inhibitors of FAAH.

survey of substituents on the terminal aryl of **73** was conducted showing a wide tolerability of substituents on this ring. In general, this part of the molecule was insensitive to nature and position of the substituent, with the exception that very polar moieties such as carboxylate, which were not well tolerated (**75** and **76**).

Two recent Janssen patent applications were published that describe oxazole ketone inhibitors of FAAH. These applications describe compounds in which the phenhexyl group of **73** is replaced with propylpiperidines[84] or piperidines.[85] The most potent of these (**84** and **85**) are shown in Figure 10.

In conclusion, the activated ketone series of FAAH inhibitors has been widely explored in terms of SAR, and valuable pharmacological tool compounds have come out of this effort. Compound **73** is clearly a functionally active FAAH inhibitor in vivo and produces analgesia in multiple preclinical pain models. However, as yet, there has been no data published on the pharmacokinetics of these molecules. With the known metabolic liabilities of activated ketones, this will be an important factor in evaluating their suitability as clinical candidates.[86]

Carbamates. Another well-studied class of FAAH inhibitors is the carbamate-based inhibitors. Mor and co-workers were the first to publish this type of FAAH inhibitor designed through modification of the known AChE inhibitor **86** (Figure 11).[87] Although **86** is not a FAAH inhibitor, simple modifications to this structure led to the potent inhibitor **88** (URB524) and later **89** (URB597).[88] The key to obtaining useful FAAH inhibition appeared to be the introduction of the more lipophilic cyclohexylamine in place of a methylamine. These early SAR studies clearly demonstrated the need for an activated carbonyl for FAAH inhibition. Other, less electrophilic carbamate isosteres were not effective FAAH inhibitors. Additionally, kinetic studies clearly showed the compounds were noncompetitive and nondialyzable, strongly suggesting that these compounds covalently modify FAAH (a finding later confirmed with MS analysis). An interesting follow-up study of the mass spectral properties of these carbamates demonstrated a good correlation between FAAH inhibition and the propensity of the carbamate to fragment upon ESI, suggesting the SAR might be driven by the leaving group ability of the phenol.[89] One side note that should be considered when interpreting the SAR around such compounds is that apparent IC_{50} values of these types of compounds may be highly dependent upon assay conditions. For instance, many covalent enzyme inhibitors show significant time dependence, which makes study-to-study comparisons difficult.

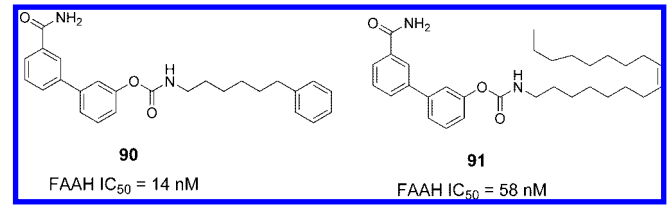


Figure 12. Representative carbamate hybrids.

A CoMSIA model was used to rationalize the SAR of phenyl carbamate based FAAH inhibitors. Initially the authors proposed a binding mode that placed the biphenyl region of these inhibitors in the lipophilic channel of the enzyme occupied by the fatty acid chain of AEA. However, it was recognized at this time that the model put the leaving group in a position opposite that proposed for FAAH substrates.

Follow-up studies on a larger set of compounds[88] afforded a QSAR model in which the activity of the compounds was inversely correlated with the lipophilicity of the phenol. This turned out to be a harbinger of a subsequent finding of Cravatt in which definitive mass spectral evidence was obtained on **89** bound to FAAH.[90] These studies demonstrated that the phenol was indeed the leaving group. The data also confirm that the carbamate is binding to the active site Ser241 of FAAH. Support for a binding model having the cyclohexyl ring occupying the lipophilic channel in FAAH was strengthened with the synthesis of analogues wherein the cyclohexyl group of **89** was replaced by lipophilic side chains such as phenhexyl (**90**) and oleyl (**91**) to create potent inhibitors (Figure 12). Interestingly, one QSAR model of these molecules that fits the data quite well contains an indicator variable for a H-bond acceptor on the biphenyl portion of the molecule. The currently proposed binding model would place this biphenyl group in the more hydrophilic channel, the same region occupied by the oxazole pyridine of the previously discussed heterocyclic ketones.

Pharmacologically compound **89** is probably the best documented of all FAAH inhibitors. Early studies[91] showed the compound did not inhibit related biological targets (AChE, BCh, MGL, CB1, or CB2). Administration of **89** to rats and subsequent ex vivo evaluation of brain FAAH activity showed almost complete block of the enzyme by the compound. The compound also elevated endogenous AEA levels in the brains of these rats. Encouragingly, the compound did not produce catalepsy, hypothermia, or hyperphagia, three of the typical effects of exogenous cannabinoids. The compound did, however, produce antinociceptive effects in the mouse hot-plate test, which was reversed by the CB1 antagonist rimonabant. These findings again support the notion that inhibition of FAAH produces pharmacology distinct from exogenous CB1 agonism. A more detailed study of compound **89**'s pharmacology was published later with detailed time-course data demonstrating elevations of AEA, oleoylethanolamide (OEA), and PEA for between 2 and 6 h after administration of the compound.[92] Importantly, the FAAH activity ex vivo was still approximately 70% inhibited 16 h after administration of the compound, although endocannabinoid levels had returned to normal. This suggests that an almost complete inhibition of FAAH is required to elevate endocannabinoid concentrations in rat brain. Another interesting observation is the complete recovery of central FAAH activity 24 h after administration of **89**. Given the compound's covalent mechanism of action and past results on enzyme turnover in mice ($T_{1/2}$ about 52 h in mouse brain),[56] this suggests either slow turnover of **89** in vivo or perhaps a more rapid resynthesis of FAAH in rats than is suggested by the previous mouse data.

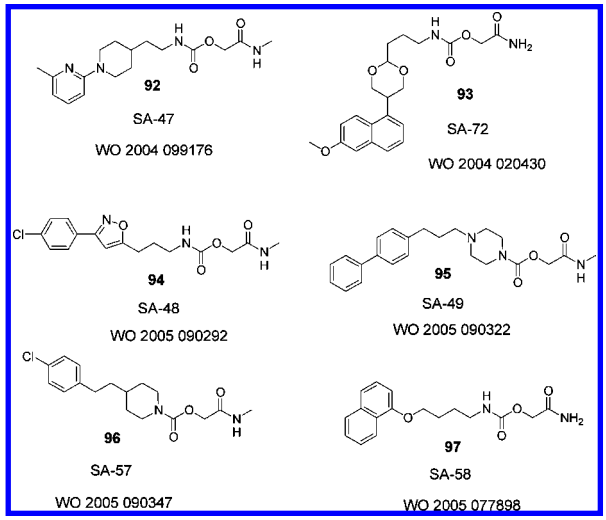


Figure 13. Representative carbamate FAAH inhibitors from Sanofi-Aventis.

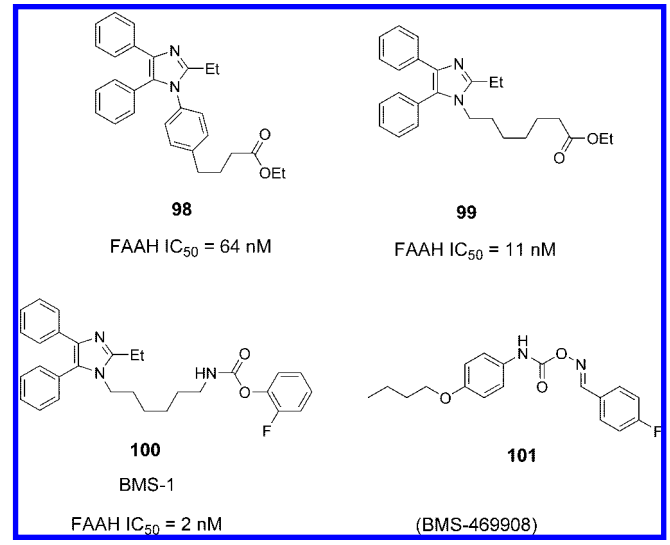


Figure 14. Representative FAAH inhibitors from Bristol-Myers Squibb.

A number of papers have reported analgesic efficacy with compound **89**. It was first reported to produce significant analgesic effects in the CFA (inflammatory) but not the PNL (neuropathic) model.[93] The effects in the CFA model were partially reversed by CB1 and CB2 specific antagonists. In a related paper **89** produced an analgesic effect in the mouse CCI model (neuropathic) when administered orally; again, these effects were reversed by both CB1 and CB2 antagonists. Compound **89** also reduced carrageenan-induced edema in a dose dependent manner. A CB2 antagonist, but not a CB1 antagonist, reversed the anti-inflammatory effects. It also demonstrated analgesic effects in PBQ induced writhing in mice, which was also blocked by a CB1 antagonist.[94] Of note is that motor impairment (rotorod) was not observed at efficacious doses of **89**.

With regards to anxiety and depression, the data on compound **89** are more controversial. Early reports with the compound demonstrated robust dose dependent effects in both the zero-maze and isolation-induced vocalization in rats.[91] However, subsequent studies in plus-maze, forced-swim, and tail suspension tests showed no effect of the compound at doses that potentiated AEA analgesia,[95] although there was a small statistically significant effect in a modified variant of the plus-maze test. One should also be cautious in the interpretation of FAAH pharmacological data using compound **89**. It is evident from recent proteomic and esterase screening that **89** interacts with a number of additional hydrolases and esterases that complicate interpretation of the compound's pharmacology.[57]

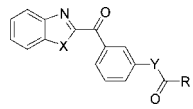
Another series of carbamate based FAAH inhibitors were reported by Sanofi-Aventis in a number of patent applications.[96−100] Representative examples of compounds are shown in Figure 13. Very little has been published on the pharmacology of these inhibitors although the patent applications report activity in reducing PBQ induced writhing (visceral pain) in mice. A recent report on the selectivity of two examples from these patents **92** (SA-47) and **93** (SA-72) demonstrated that they are highly selective for FAAH in a proteomic analysis and carboxyesterase screening.[57] In a recent poster, scientists at Vernalis[101] reported that another example, compound **96** (SA-57, VER-154403), inhibited FAAH (IC_{50} = 13 nM) and produced analgesic activity in formalin and carrageenan models of inflammatory pain when administered orally. Sanofi's research pipeline report indicated that FAAH inhibitors entered clinical trials for anxiety and depression, one in 2006 (SSR-411298) and a second compound in 2007 (SSR-101010). However, no specific structures or clinical data have been reported. Sanofi is reported to be developing these compounds for anxiety/depression, although the only preclinical data reported in the patent applications are for pain.

An early published patent application and recent paper by Bristol-Myers Squibb reported a unique class of carbamate based FAAH inhibitors (Figure 14).[102,103] The initial hit from an HTS screen was an ester (presumably a substrate) (**98**). This compound was effectively optimized to more potent inhibitors through the replacement of the phenyl linker with an alkyl chain (**99**) and incorporation of a phenyl carbamate (**100**) as the putative electrophile. Substitution of the ester or carbamate with amides or ureas rendered the compounds inactive at FAAH, supporting the hypothesis of a covalent mechanism of action. It is interesting that the size of the lipophilic portion of these compounds is substantially larger than in any other known FAAH inhibitors. If one assumes the binding mode is similar to other phenyl carbamate based inhibitors, then the lipophilic channel of FAAH must be able to accommodate groups significantly larger than what is expected given the SAR of other known FAAH inhibitors. Compound **100** (BMS-1) shows activity in both the formalin and Chung pain models. In addition, **100** was recently found to be relatively nonselective with respect to other hydrolases and esterases by proteomic and microsomal esterase profiling.[57]

Scientists at BMS also reported a series of oxime-carbamates.[104,105] Interestingly, **101** (BMS-469908) (Figure 14) was found to be competitive and reversible, making it unclear if the carbamate plays the same role of covalent inhibition that might be expected of its structure. In addition, the SAR suggests that the length of the alkoxy side chain of **101** plays a role in activity, with longer alkyl groups being more active than shorter ones. Compound **101** was reported to reduce thermal hyperalgesia and paw edema in the rat carrageenan model after iv dosing.

A report from the Helsinki University of Technology attempted to marry the oxazole ketone and carbamate chemotypes into a new class of FAAH inhibitors.[106] The most potent of these was compound **102** (Table 10). The carbamate functionality was critical to activity at FAAH as evidenced by the inactivity of the urea (**103**) and other amide replacements. In addition, the SAR was insensitive to changes in the ketoheterocycle (**102** vs **104**), indicating the ketone is not acting as

Table 10. Representative Aryl Carbamate FAAH Inhibitors



#	X	Y	R	FAAH IC_{50} (μM)
102	O	O		28
103	O	NH		>100
104	S	O		47

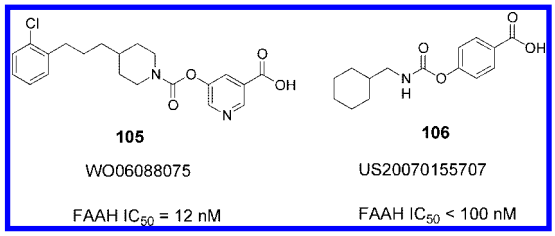


Figure 15. Recent carbamates from Astellas and Kadmus.

the serine trap in these molecules. No mechanistic or pharmacological studies were reported with these inhibitors, although the compounds were revealed to have little activity for the functionally related enzyme MGL.

A recent patent application by Astellas reports a series of pyridine-carbamate FAAH inhibitors (Figure 15). The most potent compound described is **105** (IC_{50} = 12 nM).[107] The SAR in the patent suggests a wide variety of piperidine replacements are tolerated, with a large number of compounds reported to have IC_{50} < 1 μM. A recent patent by Kadmus also provided examples of carbamates with carboxylates appended to the phenol portion of the inhibitor.[108] Compound **106** was reported to have good oral exposure but poor CNS penetration.

Arylureas. An early example of an electrophilic, urea-like FAAH inhibitor was reported by Lilly (Figure 16).[109] Compound **110** (LY-2183240) was originally described as an AEA transport inhibitor but was later revealed to be a highly potent FAAH inhibitor.[110] Mass spectral studies verified the inhibition was due to covalent modification of Ser241 of FAAH. Proteomic analysis suggested that this compound was one of the least selective FAAH inhibitors known, and activity at a variety of off targets was verified in independent laboratories.[57] Despite these shortcomings, the compound did show dose dependent elevations of AEA in rat brain and reduced pain behaviors in the formalin test without reduction in rotorod performance.

In 2006 a new class of urea-based FAAH inhibitors was published. Takeda and Janssen both published patent applications on piperazine arylurea FAAH inhibitors (Figure 16).[111−113] Compound **107** in the Takeda patent application was reported to produce 100% block of rat FAAH activity at 1 μM and significantly reduce infarct volume in a rat stroke model. We recently reported **108** (JNJ-1661010) at a London pain meeting.[114] The compound is a potent, time-dependent inhibitor of FAAH. Mass spectral data on the compound bound to rat FAAH suggested that the carbonyl of the urea was acting as an electrophile with the aniline fragment functioning as the leaving group upon binding to the enzyme. The compound had activity in both the mild thermal injury model of acute tissue injury

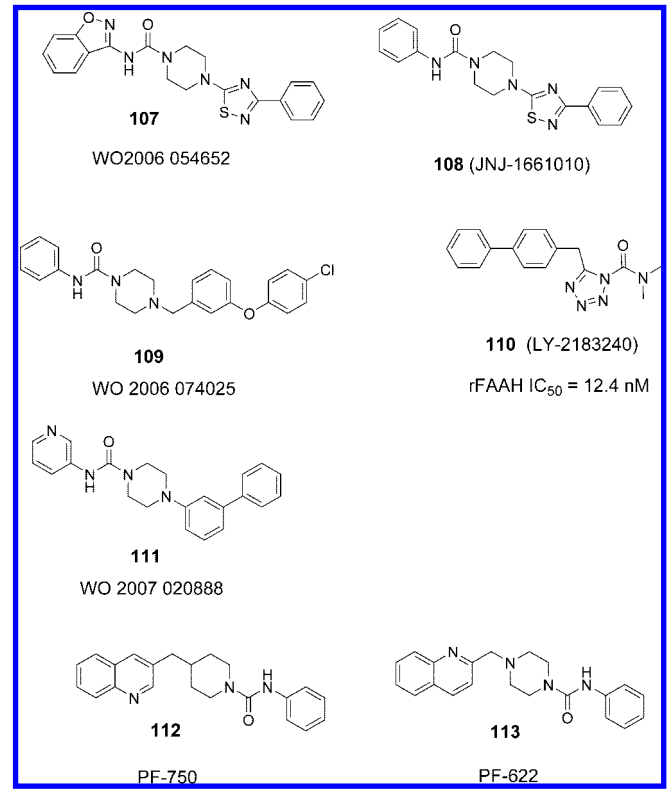


Figure 16. Representative urea based FAAH inhibitors.

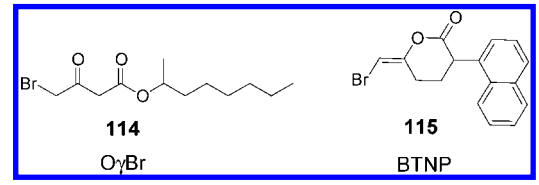


Figure 17. Early irreversible FAAH inhibitors.

pain and the Chung model of neuropathic pain, with no associated motor impairment.

Pfizer and Scripps reported data on two related urea based FAAH inhibitors that also appeared in a Janssen patent application (Figure 16).[115,116] Both mass spectral data and radiolabeled studies on **112** (PF-750) and **113** (PF-622) show a covalent binding mode similar to the previously discussed carbamates. These findings, along with those of compound **108**, were remarkable given that ureas (as opposed to aryl carbamates) are generally stable to hydrolysis. In fact, this class of FAAH inhibitors is the first that we are aware of in which a stable urea acts as a mechanism based covalent inhibitor of any enzyme. The authors propose a binding-induced conformational change in the urea that decreases the urea's hydrolytic stability when bound to the enzyme to account for this unique mode of action. In addition, **112** and **113** were highly selective for FAAH based upon proteomic profiling data. These recent findings suggest that stable covalent modification of FAAH is possible without significant cross-reactivity against related enzymes.

Miscellaneous Inhibitors. There are a number of FAAH inhibitors reported that do not fall into the broad categories described above (Figure 17). One of the first discovered FAAH inhibitors was 2-octyl γ-bromoacetoacetate (OγBr, **114**), which was discovered in the 1970s[117] as an endogenous sleep promoting substance isolated from rat cortex. It was later discovered by Cravatt and Boger to be a potent FAAH inhibitor (K_i = 0.82 μM).[118] Interestingly this compound

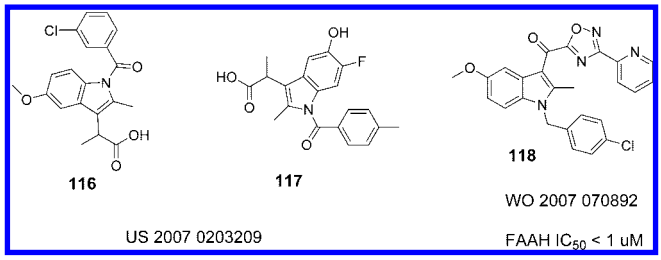


Figure 18. Representative dual FAAH/COX inhibitors from Microbia.

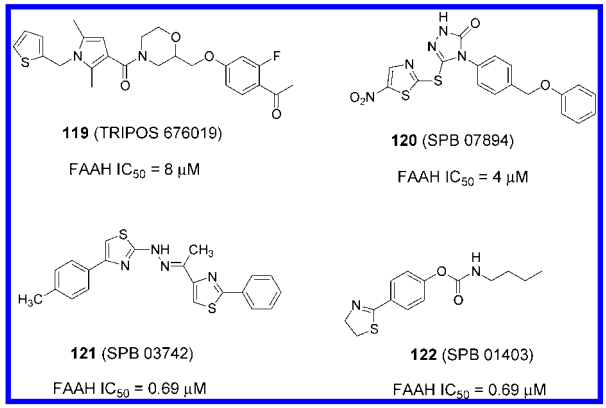


Figure 19. Representative FAAH inhibitors identified by virtual screening of MGL.

shows reversible competitive binding on short incubation times but is irreversible upon long incubations, suggesting a change in binding mode over time. Analoguing suggested that both the ketone and the bromine were essential to inhibition but the ester was not essential to FAAH activity. Another early inhibitor of FAAH was (*E*)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one (BTNP, **115**) (rat brain AEA hydrolysis $IC_{50} = 0.8\ \mu M$). Kinetic and dialysis results suggest the compound was a covalent inhibitor of anandamide hydrolysis.[119] The structural similarities of these two molecules are intriguing when one considers the potential of hydrolysis of BTNP to the keto acid.

Microbia has published a number of patent applications covering dual COX/FAAH inhibitors as potential analgesics. Some FAAH inhibition data are reported, and representative examples of inhibitors having $IC_{50} < 1\ \mu M$ are shown in Figure 18.[120,121] No SAR or pharmacology has been published on this class of inhibitors.

A publication from the University of Kuopio in Finland reported a virtual screen for MGL ligands. While the screen failed to identify MGL inhibitors, cross-screening for FAAH activity uncovered inhibitors of FAAH with the novel structures shown below (Figure 19). The most potent of these was compound **122** (SPB-01403), which contains a phenyl carbamate similar to compound **89**. This might account for its activity, although no kinetic or other mechanistic information was provided. It should be noted that the compounds were purchased from a commercial library, and data on structural characterization and purity of these compounds were not reported in the paper.[122]

The Lambert group reported identification of a unique series of thiohydantoin based FAAH inhibitors (Figure 20) in a screen of CB1 ligands.[123] The most potent of these was compound **123** ($pIC_{50} = 5.94$). The group established a link between the length of the aliphatic chain and activity at FAAH with chains of 10−14 carbons being optimal. Kinetic analysis confirmed

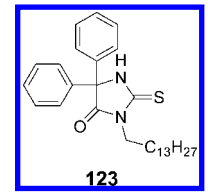


Figure 20. Representative thiohydantoin based FAAH inhibitor.

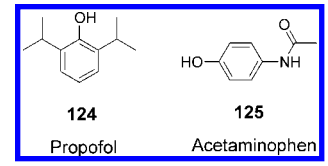


Figure 21. Known drugs speculated to derive efficacy from FAAH inhibition.

that the compounds are competitive inhibitors of AEA hydrolysis, and the inhibition is not time dependent. A subsequent modeling study[124] hypothesizes that the entire hydantoin structure can be accommodated in the lipophilic binding tunnel of FAAH with no proposed interaction with the catalytic machinery of FAAH.

Known Drugs That May Inhibit FAAH. An intriguing aspect of FAAH inhibition which has arisen in the literature, is that currently marketed analgesics and anesthetics may already derive some of their efficacy from FAAH inhibition (Figure 21). The anesthetic agent propofol (**124**) is reported to competitively inhibit FAAH with an IC_{50} of 14 μM in rat brain membranes; mice anesthetized with 100 mg/kg propofol showed significant elevations in brain AEA compared to mice anesthetized with 60 mg/kg thiopental, a much weaker FAAH inhibitor ($IC_{50} = 2$ mM).[125] Citing this finding, Schelling and co-workers studied a mixed population of patients undergoing orthopedic surgery and sampled plasma AEA over a 40 min time period in etomidate-sevoflurane anesthetized vs propofol anesthetized subjects.[126] They found a significant difference in plasma AEA levels in the propofol group (1.5× to 2×) compared with the comparator group.

Some NSAIDs have also been shown to inhibit FAAH; the concentrations required are possibly similar to those achieved in clinical use.[127] The contribution of FAAH inhibition to acetaminophen analgesia has been a subject of particular speculation. Biotransformation of acetaminophen (**125**) may produce a metabolite that both inhibits FAAH and has agonist activity at TRPV1.[128] However, no studies record measurements of plasma concentrations of endocannabinoids in NSAID or acetaminophen treated humans or preclinical species.

Conclusion

In conclusion, the past 10 years have seen an explosion in the number and quality of FAAH inhibitors available for pharmacological evaluation. The predominance of evidence from animal models is that there is clear analgesic pharmacology associated with FAAH inhibition with compounds of varying structure and mechanism in a variety of pain models. Furthermore, it is clear from the evidence that it is possible to produce analgesia at doses that do not produce sedation/catalepsy typically associated with analgesic doses of CB1 agonists. There is still much discussion regarding other potential indications for FAAH inhibitors, with mixed results in anxiety/depression models and some limited investigations in other areas. Medicinal chemistry efforts on FAAH inhibitors appear to be accelerating, with a large number of companies now reporting activity in

drug discovery. Debate is still ongoing regarding the most advantageous means of inhibiting FAAH. It appears from the work on compound **89** that to elicit pharmacological effects of FAAH inhibition, substantial block of the enzyme in vivo is necessary, making covalent modification a potentially attractive strategy. However, many of these inhibitors (such as the phenyl carbamates) are not selective, raising serious potential safety concerns. Recently, the issues surrounding the selectivity of covalent inhibitors are beginning to be addressed. Results on selectivity of the alkyl carbamate inhibitors of Sanofi-Aventis and arylureas are quite promising with respect to target selectivity and provide some reason for excitement around this strategy. The heterocyclic ketones have proven to be highly potent and selective FAAH inhibitors and clearly elicit the desired pharmacology in vivo. However, potential pharmacokinetic issues inherent with the activated ketone functionallity have not yet been vetted in the literature. This will be an important issue to address in advancing this class of inhibitors. Alternatively, there are very few examples of compounds that inhibit FAAH through nonmechanism based inhibition, and the ones that are known are relatively weak inhibitors. Continued exploration in this area may ultimately turn out to be the most promising avenue. Excitingly, the past 2 years have seen Sanofi-Aventis enter two FAAH inhibitors into the clinic. Results of these studies are anxiously awaited and will shed light on the potential for FAAH inhibition in treating human disease.

Addendum

During review of this manuscript a number of important papers describing FAAH inhibitor SAR have been published. Brief highlights of these results are presented here. The most important of the recent disclosures is a paper by Scripps and Pfizer describing a second FAAH crystal structure.[129] Because of the difficulties in expressing and purifying human FAAH, the researchers created a partially humanized rat FAAH construct using site-directed mutagenesis of certain active site residues. The construct was catalytically viable and was inhibited by urea FAAH inhibitors, such as **112**, with potencies similar to that observed with wild-type human FAAH. The structure of compound **112** bound to this construct confirms the previously suggested binding of the urea carbonyl to active site Ser241. The structure also reveals an absence of the aniline portion of the inhibitor; this is in full agreement with previous mass spectral data suggesting the aniline acts as a leaving group when arylureas bind to FAAH. The paper also highlights significant structural changes in the protein bound to the urea inhibitor when compared to the previously reported structure of rat FAAH bound to MAFP. In particular, the dramatic movement of Phe432 resulted in a significant increase in the size of the acyl chain binding pocket (Figure 4). Such conformational flexibility in the binding site could have important implications to the access of small molecules into the active site of FAAH and perhaps explain the binding of certain very large FAAH inhibitors such as compound **100**.

With respect to α-ketoheterocycle SAR there have been three recent publications. Boger has reported more SAR on ketooxazoles in which a complete matrix of substitutions on the oxazole and lipophilic potions of **73** were explored.[130] This work provided additional support for the strong correlation between the σ constant of the oxazole substituent and potency. Additionally, replacements of the phenhexyl group of **73** with other, more conformationally restricted groups, such as biphenylethyl and 4-phenoxyphenethyl, provided highly potent FAAH inhibitors. The most active of these was compound **126** (Figure 22). The

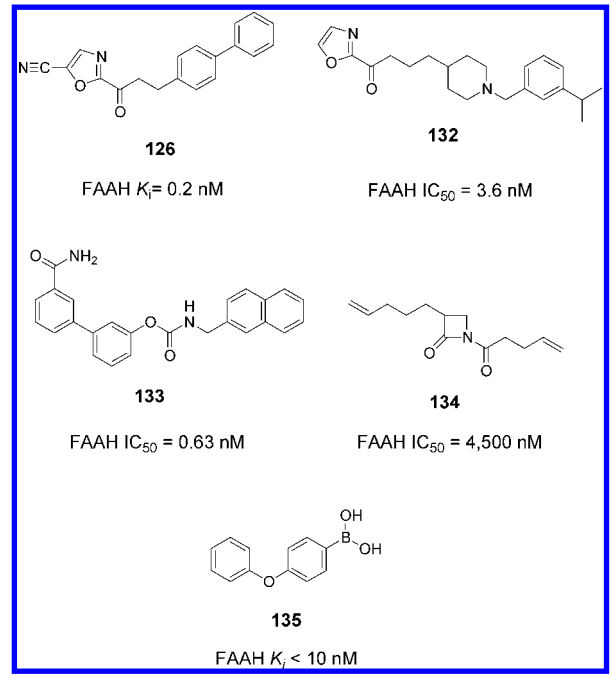
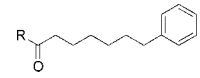
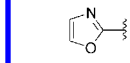
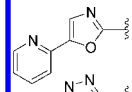
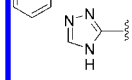
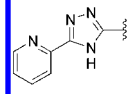
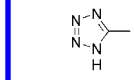
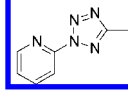


Figure 22. Recently disclosed FAAH inhibitors.

Table 11. Recent α-Ketoheterocycle FAAH Inhibitors



R	#	K_i (nM)
	**127**	48
	**73**	4.7
	**128**	>1000
	**129**	>10,000
	**130**	>1000
	**131**	1.1

impact of these changes on SAR for two related hydrolases was also discussed. A more recent paper by Boger described the replacement of the oxazole of **73** with other aryl substituted heterocycles.[131] The SAR generated suggests that subtle changes in the nature of the heterocycle have dramatic effects upon the activity of these analogues (Table 11). The most interesting SAR disclosed was the triazoles (**128**, **129**) and tetrazoles (**130**, **131**). These data suggest that deprotonation of these acidic heterocycles has a significant negative impact on activity. In contrast, when the tetrazole is substituted with a pyridine so that it cannot be deprotonated (**131**), the activity is superior to that of **73**. A third paper on the SAR of propylpiperidine based ketooxazoles was published by our group.[132] The most active compound in this paper was compound **132**, which showed activity in the Chung model of neuropathic pain in rats (Figure 22).

In the urea and carbamate classes of FAAH inhibitors there were two recent papers describing SAR. The first describes the SAR of various analogues of compound **89**.[133] Using modeling studies where the binding mode places the cyclohexyl group of **89** in the more hydrophobic channel, the researchers designed more lipophilic carbamates, which significantly increased the activity of these compounds. The most potent example was compound **133** (Figure 22). A paper from our group describing the SAR around compound **108** was also published recently.[134] Interestingly, dialysis studies with this compound demonstrated slow on and slow off inhibition of the enzyme. These data suggest that although these compounds covalently modify FAAH at the urea, the resulting piperazine−FAAH complex slowly hydrolyzes to regenerate active enzyme over time.

With respect to novel classes of FAAH inhibitors, one paper reported the design of β-lactam FAAH inhibitors. The most potent example was compound **134** (Figure 22).[135] Although no kinetic studies were done to determine the mechanism of binding, this paper does open up the possibility that additional types of "serine traps" might be used to inhibit FAAH. Related to this concept, a recent patent application by Infinity Pharmaceuticals describes boronic acid inhibitors of FAAH represented by compound **135** (Figure 22).[136] Because boronic acids are well-known covalent modifiers of serine proteases, these compounds presumably bind covalently to FAAH through an interaction of boron with Ser241.

Biographies

Mark Seierstad received his Ph.D. in Organic Chemistry in 2000 from the University of Illinois at Urbana−Champaign, studying stereochemical aspects of tandem nitroalkene cycloadditions under the direction of Prof. Scott Denmark. He studied computational organic chemistry at the University of Minnesota as a postdoctoral associate with Prof. Christopher Cramer. In 2002 he joined the Computer Aided Drug Discovery group at Johnson & Johnson Pharmaceutical Research & Development in San Diego, CA.

J. Guy Breitenbucher received his Ph.D. in Organic Chemistry in 1993 from the University of California at Riverside, studying the methodology and total synthesis of plant alkaloids with Prof. Steven Angle. He then went on to a postdoctoral appointment in the laboratories of Prof. Clayton Heathcock at University of California at Berkeley, studying polypropionate natural product synthesis. After postdoctoral studies Dr. Breitenbucher went on to positions in medicinal chemistry at Bristol-Myers Squibb PRI and Axys Pharmaceuticals. In 1999 Dr. Breitenbucher accepted a position in medicinal chemistry at Johnson & Johnson PRD in San Diego, CA, where he is currently Team Leader of the pain therapeutics team. Dr. Breitenbucher is the coauthor/coinventor of over 50 scientific papers and published patent applications.

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