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13 July 2024

## **Fatty acid amide hydrolase (FAAH) inhibitors: a patent review (2009-2014)**

**Importance of the field:** Fatty acid amide hydrolase (FAAH) is a key enzyme responsible for the degradation of the endocannabinoid anandamide. FAAH inactivation is emerging as a strategy to treat several CNS and peripheral diseases, including inflammation and pain. The search for effective FAAH inhibitors has thus become a key focus in present drug discovery.

**Areas covered:** Patents and patent applications published from 2009 to 2014 in which novel chemical classes are claimed to inhibit FAAH.

**Expert Opinion:** FAAH is a promising target for treating many disease conditions including pain, inflammation and mood disorders. In the last few years, remarkable efforts have been made to develop new FAAH inhibitors (either reversible and irreversible) characterized by excellent potency and selectivity, to complete the arsenal of tools for modulating FAAH activity. The failure of PF-04457845 in a phase II study on osteoarthritis pain has not flattened the interest in FAAH inhibitors. New clinical trials on “classical” FAAH inhibitors are now ongoing, and new strategies based on compounds with peculiar *in vivo* distribution (e.g. peripheral) or with multiple pharmacological activities (e.g., FAAH and COX) are under investigation and could boost the therapeutic potential of this class in the next future.

Keywords: endocannabinoid system, FAAH, fatty acid amide hydrolase, fatty acid ethanolamides, PF-04457845, URB597.

## 1. Anandamide and other neuromodulatory fatty acid ethanolamides

Arachidonylethanolamide (anandamide, AEA, Figure 1) is an endogenous cannabinoid that exerts most of its action by binding and activating two G-protein-coupled-receptors known as CB1 and CB2 receptors.<sup>1,2</sup> Along with 2-arachidonoylglycerol (2-AG, Figure 1),<sup>3</sup> AEA is the most comprehensively investigated endocannabinoid. AEA is also member of the fatty acid ethanolamide (FAE) family,<sup>4</sup> a group of endogenous lipid neuromodulators that includes the palmitoylethanolamide (PEA, Figure 1), involved in a variety of biological functions related to pain and inflammation<sup>5,6</sup> and the oleoylethanolamide (OEA, Figure 1) which controls feeding and body weight in mammals.<sup>7</sup>

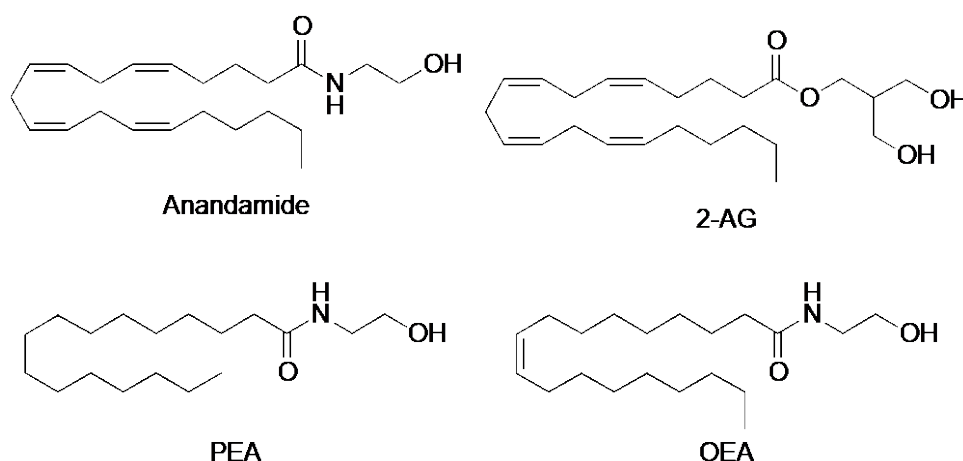


Figure 1. Structures of neuromodulatory FAEs and of 2-AG.

These FAEs are present throughout the body and their levels are finely regulated by a complex system of enzymes involved in their synthesis and inactivation.<sup>8,9</sup> Anandamide and the other FAEs are not stored in vesicles like classical neurotransmitters, rather they are synthesized on demand<sup>10</sup> from the membrane by a *N*-arachidonoyl phosphatidylethanolamine precursor in a process catalyzed by selective *N*-acyl phosphatidylethanolamine-phospholipase D (NAPE-PLD).<sup>11,12</sup> Following their synthesis and release, these FAEs are removed from their sites of action by cellular uptake, also mediated by a specific process

involving specific molecular transporters<sup>13,14,15</sup> and degraded by at least two enzymes, fatty acid amide hydrolase (FAAH),<sup>16,17</sup> mainly responsible for the hydrolysis of AEA<sup>18,19</sup> and N-acylamino acid amidase (NAAA),<sup>20</sup> mainly responsible of the hydrolysis of PEA.<sup>21</sup>

The interest in the enzymes responsible for the degradation of FAE, particularly of AEA, comes from the observation that the reduction in the tissue levels of this endocannabinoid can lead to the insurgence of several pathological conditions such as neurological disorders, inflammatory states and chronic pain, at least in animal models.<sup>22</sup> Consistently with the central role of anandamide in these pathological states, genetic (with FAAH<sup>-/-</sup> mice)<sup>23</sup> or chemical inactivation of FAAH leads to elevated endogenous levels of AEA and concomitant analgesic, anxiolytic, anti-depressant, and anti-inflammatory phenotypes.<sup>24</sup> Crucially, these phenotypes are not accompanied by the classical signs of an indiscriminate CB1 activation, obtained with the administration of an exogenous CB1 agonist, such as hypomotility, hypothermia and catalepsy and, in particular, FAAH inhibition lacks reinforcing effects.<sup>25</sup> Moreover, FAAH inhibitors have been found to counteract addiction-related effects of nicotine in different animal models, including primates.<sup>26</sup> These observations promoted FAAH as a potential therapeutic target for a range of nervous system and peripheral disorders.<sup>27</sup>

## 2. FAAH: structure and function

FAAH (EC: 3.5.1.99) is the main responsible for the inactivation of FAEs<sup>28</sup> and, in particular, it terminates the signal brought by AEA catalysing its hydrolysis to arachidonic acid and ethanolamine (Figure 2).

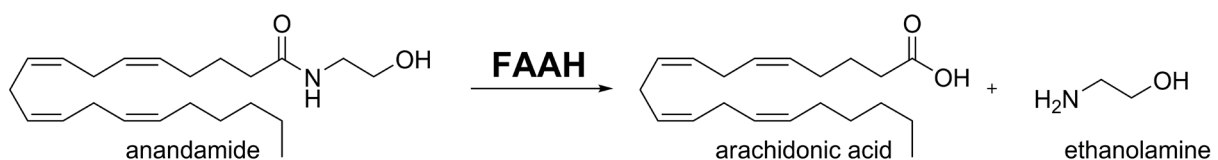


Figure 2. Reaction catalysed by FAAH.

FAAH has a distinctive catalytic triad consisting of two serines (Ser217 and Ser241) and one lysine (Lys142), rather than the typical serine-histidine-aspartate triad of other serine hydrolases. The catalytic mechanism of FAAH has been widely investigated with both experimental<sup>28</sup> and computational methods.<sup>29</sup> These studies indicate that Lys142 acts as a key base and acid in distinct steps of the catalytic process. In the early phase of the catalysis, neutral Lys142 activates Ser241 nucleophile for attack on the substrate carbonyl, an event that leads to the formation of a tetrahedral intermediate. In the final step of the reaction, the positively charged Lys142 protonates the substrate leaving group, leading to its expulsion and to the formation of an acylenzyme. The impact of Lys142 on Ser241 nucleophile strength and on leaving group protonation occurs indirectly, *via* the bridging Ser217 of the triad, which acts as a proton shuttle. The catalytic cycle of FAAH terminates with the hydrolysis of the acylenzyme, which restores a functional FAAH catalytic core. Typically, serine hydrolases cleave ester substrates at higher rates compared with structurally similar amides, reflecting the relative intrinsic reactivity of these compounds. FAAH represents a noteworthy exception to this principle, as it hydrolyses amides faster than esters. *In vivo*, FAAH catalyses the hydrolysis of FAEs in a microenvironment rich of endogenous esters, therefore if FAAH acted as a conventional serine hydrolase, it would rapidly be saturated by esters and failed to work as an amidase.<sup>28</sup>

FAAH has been crystallized in the presence of several covalent and non-covalent inhibitors. The first resolved structure has been the covalent adduct between FAAH and the inhibitor methyl arachidonylfluorophosphonate (MAFP).<sup>30</sup> Visual inspection of this structure reveals that FAAH has a series of channels and cavities that are crucial for its biological function (Figure 3). These include: i. the membrane access channel (MAC), connecting the

active site to a hole located at the membrane-anchoring face of the enzyme; ii. the cytosolic access (CA) channel, allowing the exit of hydrophilic products from the active site; iii. the acyl chain-binding pocket (ABP) that accommodates the arachidonoyl chain of co-crystallized inhibitor. Identification of multiple pockets in the proximity of the catalytic core of FAAH has offered a unique opportunity to design inhibitors featured by a wide array of binding mechanisms. Indeed, beside the well-established classes of active site-directed inhibitors of FAAH that covalently bind its Ser241, a number of non-covalent inhibitors targeting the membrane access channel (MAC) of FAAH has been reported in the recent literature.<sup>31</sup>

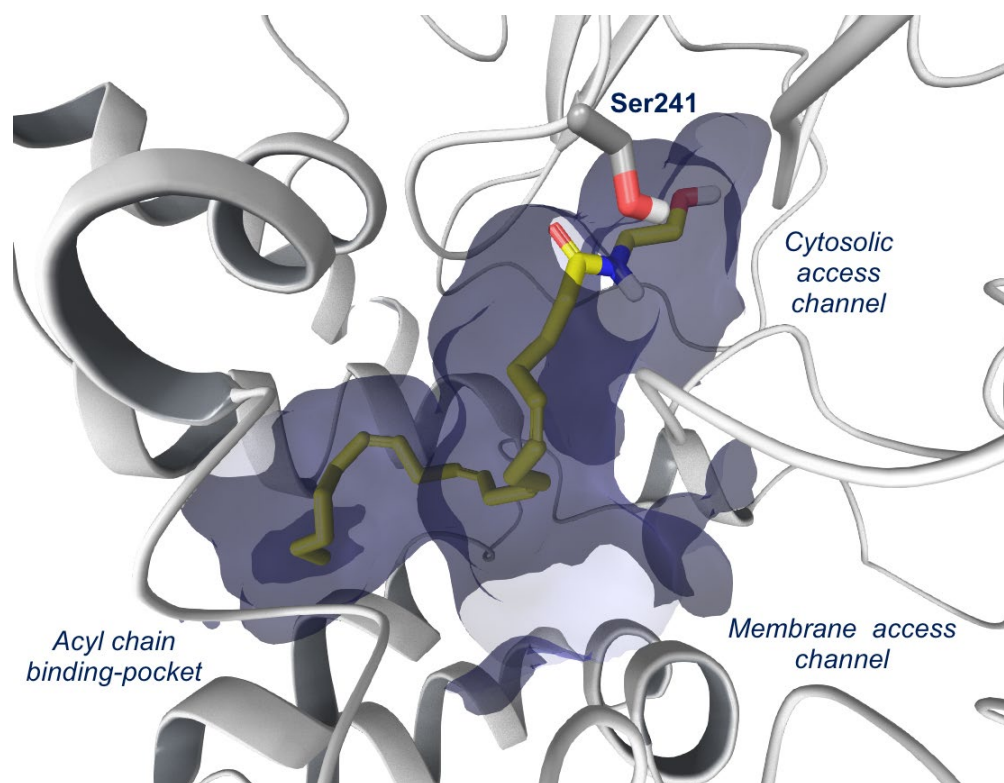


Figure 3. Substrate binding pocket for FAAH, modelled with anandamide (AEA, yellow carbon atoms).

### 3. FAAH inhibitors: from substrate-derived inhibitors to carbamoylating agents with drug-like properties

The non-selective serine hydrolase inhibitor, phenylmethylsulfonyl fluoride (**1**, PMSF, Figure 4) was probably the first compound employed to block FAAH activity. Other inhibitors of FAAH, such as methyl arachidonyl fluorophosphonate (**2**, MAFP, Figure 4) and palmitylsulfonyl fluoride (**3** AM374, Figure 4) were also used, but these compounds were still too reactive for *in vivo* investigation.<sup>32</sup> Looking for more drug-like inhibitors, less reactive compounds were thus taken into consideration, leading to a class of  $\alpha$ -ketoheterocycles, which includes potent and selective inhibitors such as **4** (OL-92) and **5** (OL-135), that inhibit recombinant human FAAH with  $K_i$  of 2.0 and 4.7 nM, respectively.<sup>33</sup> Compounds from this class reversibly inhibit FAAH through the formation of a hemiketal species involving the nucleophile Ser241, as confirmed by X-ray crystallography.<sup>34</sup> Although these first  $\alpha$ -ketoheterocycles were potent *in vitro*, they enhanced endocannabinoid signaling *in vivo* only at high doses and only for a brief period of time,<sup>35</sup> likely due to rapid metabolism in rodents (*vide infra*).

A breakthrough in the search for FAAH inhibitors able to significantly sustain AEA levels in rodents was obtained with a class of *N*-alkylcarbamic acid *O*-aryl esters, exemplified by the structure of **6** (URB524, Figure 4) and co-developed by three universities (University of California at Irvine, Università degli Studi di Parma and Università degli Studi di Urbino, “Carlo Bo”) and Kadmus Pharmaceuticals.<sup>36</sup> This class includes the potent inhibitor **7** (URB597, rat FAAH  $IC_{50}$  = 4.6 nM; human FAAH  $k_{inact}/K_i$  = 1,590  $M^{-1} s^{-1}$ ),<sup>24,37</sup> able to block FAAH activity through irreversible carbamylation of the catalytic nucleophile Ser241,<sup>38</sup> with its biphenyl moiety serving as the leaving group. Recent QM/MM simulations suggested that stabilization of the cyclohexyl carbamic ester structure by hydrogen bonds taken with the FAAH active site reduces the speed of the hydrolysis reaction, leading to prolonged inhibition.<sup>39</sup> Investigation of FAAH function and relevance was greatly advanced by the use of URB597 *in vivo*, a consequence of its favorable pharmacological properties.<sup>40</sup> Intraperitoneal

injections of URB597 produced a profound dose-dependent inhibition of rat brain FAAH activity, with half-maximal effect at the dose of 0.15 mg/kg. FAAH inhibition onset was rapid (<15 min), persistent (>12 h) and accompanied by significant elevations in the brain content of anandamide and other fatty-acid ethanolamides that are substrates of FAAH. Similar changes in FAAH activity and FAE levels were observed in peripheral tissues. Furthermore, URB597 did not alter the brain content of the endocannabinoid 2-AG, the key substrate of monoglyceride lipase (MGL).

Another class of *N*-alkylcarbamic acid *O*-aryl ester inhibitors having a bis-arylalkylimidazole substituent at the nitrogen atom as in the case of compound **8** (BMS-1, Figure 4), was disclosed by Bristol-Myers Squibb. Compound **8** inhibited rat FAAH *in vitro* with an IC<sub>50</sub> of 2 nM and dose-dependently (0.1–10 mg/kg, iv) potentiates the effects of exogenous anandamide (1 mg/kg, iv) in a rat thermal escape test, and showed antinociceptive activity in animal models of neuropathic pain.<sup>41</sup>

A series of *O*-alkylcarbamate inhibitors was reported by Sanofi-Aventis in a number of patent applications filed in 2005. Despite the paucity of information available, these compounds have been claimed to enter clinical trials “for what appears to be anxiety and depression”.<sup>42</sup> However, no further pharmacological data have been disclosed so far.

The ability of carbamate inhibitors to produce prolonged inhibition of FAAH *in vivo* has prompted research efforts toward the development of other classes of irreversible inhibitors. This led to the discovery of piperidine/piperazine urea inhibitors (e.g., **11**, PF-750, Figure 4) which, despite lacking a reactive fragment in their structure, resulted to be able to carbamoylate the nucleophile Ser241, taking advantage of FAAH’s special aptitude to function as a C(O)-N bond hydrolase.<sup>43</sup> The urease activity of FAAH stems from its ability to deplanarize the aniline nitrogen atom of urea inhibitors, enhancing their reactivity versus nucleophiles.<sup>44</sup> Optimization of PF-750 ( $k_{\text{inact}}/K_i = 791 \text{ M}^{-1} \text{ s}^{-1}$  on human FAAH) by Pfizer led to



**12** (PF-3845), a highly potent and selective FAAH inhibitor (human FAAH  $k_{\text{inact}}/K_i = 14,310 \text{ M}^{-1} \text{ s}^{-1}$ ) endowed with anti-hyperalgesic effects in models of inflammatory pain.<sup>45</sup> At the same time Johnson&Johnson and Takeda developed new classes of piperazine-urea inhibitors, exemplified by compound **13** (JNJ-1661010)<sup>46</sup> that potently blocks rat FAAH and attenuates tactile allodynia in the rat mild thermal injury model of acute tissue damage and in the rat spinal nerve ligation model of neuropathic pain.<sup>47</sup> Further optimization of PF-3845 structure by Pfizer eventually led to the clinical candidate **14** (PF-04457845, Figure 4) which contains a pyridazinyl moiety instead of the 3-aminopyridyl within the leaving group. PF-04457845 exhibited excellent potency (human FAAH  $k_{\text{inact}}/K_i = 40,300 \text{ M}^{-1} \text{ s}^{-1}$ ) and a favourable pharmacokinetic profile, but unfortunately, it failed to induce effective analgesia in patients with pain due to osteoarthritis of the knee.<sup>48</sup>

#### 4. From covalent to non-covalent FAAH inhibitors

Non covalent inhibitors of FAAH firstly appeared in a few patent applications filed by Renovis in 2009 (*vide infra*) and, more recently, in the scientific literature. Medicinal chemistry efforts allowed Min et al.<sup>49</sup> to reduce the reactivity of the arylurea inhibitor **15** (Amgen-1, Figure 4), identifying a novel class of non-covalent inhibitors characterized by a ketobenzimidazole scaffold (**16**, Amgen-2, Figure 4). The X-ray structure of FAAH in complex with **16** showed that this compound is accommodated far apart from catalytic Ser241, occupying a portion of the ABP, proximal to the oxyanion hole, and the membrane access channel (MAC) of FAAH. Compound **16** is reported to inhibit recombinant human FAAH with high potency ( $\text{IC}_{50}$  of 28 nM), due to shape complementarity with the active site and strong hydrophobic interactions, and to possess excellent selectivity and pharmacokinetic properties.

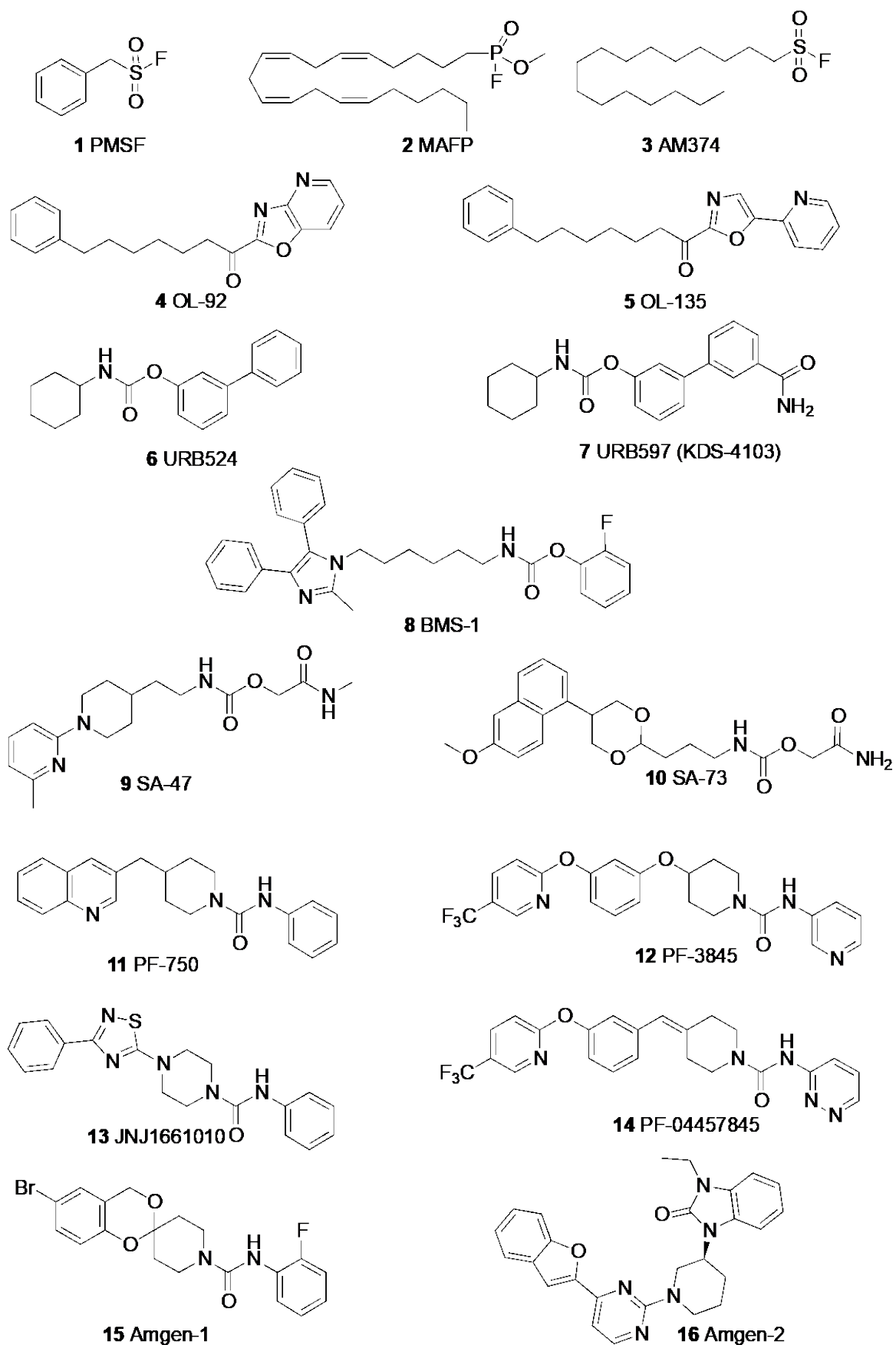


Figure 4. Reference FAAH inhibitors reported in the literature.

## 5. FAAH inhibitors: advances in the recent patent literature (2009-2014)

Encouraged by the therapeutic potential of FAAH inhibitors belonging to the carbamate and piperidine/piperazine urea classes, numerous pharmaceutical industries and university research groups have developed several structurally different FAAH inhibitors.<sup>50</sup> Electrophilic functions with different degrees of reactivity have been used to target the nucleophile Ser241, investigating structure-activity relationships which proved to be of great value for the optimization of potency, selectivity and pharmacokinetic properties of the new classes of FAAH inhibitors.<sup>51</sup> Moreover, FAAH inhibitors devoid of reactive functions were also reported, especially in more recent years. These compounds act as competitive and reversible inhibitors of FAAH and show *in vitro* potencies comparable to those of covalent FAAH inhibitors.<sup>50</sup>

Herein, we summarize the results disclosed in the patent literature in the 2009-2014 period. Two main tracks were followed by industrial and academic groups active in the field: i) chemical expansion of previously known classes of FAAH inhibitors, with the aim to overcome open pharmacological or pharmacokinetic issues; ii) disclosure of novel chemotypes characterized by new mechanisms of inhibition for FAAH. The FAAH inhibitors described in this review are grouped into the following categories: (1)  $\alpha$ -ketoheterocycles; (2) carbamates; (3) arylureas; (4) boronic acids; (5)azole derivatives; (6) ethylaminopyrimidines; (7) tetrahydronaphthyridines; (8) miscellaneous classes.

### 5.1 $\alpha$ -ketoheterocycles

Starting from the lead compound **5** (OL-135), the Boger group at the Scripps Research Institute recently investigated the influence of the heterocycle on inhibitor potency. Several compounds characterized by an oxadiazole ring were synthesized, which allowed the identification of the 1,3,4- (**17**, Figure 5) and the 1,2,4-oxadiazole (**18**) analogs of OL-135 with

subnanomolar  $K_i$  vs human FAAH.<sup>52</sup> A class of conformationally constrained inhibitors, characterized by a tetrahydronaphthalene or an indane scaffold directly linked to the oxazolyl ketone, was also described in another patent application.<sup>53</sup> The tetrahydronaphthalene derivative **19** was one of the most stereoselective inhibitors, with the (*S*)-enantiomer **20** displaying a  $K_i$  of 4 nM vs human FAAH, nearly 60-fold lower than that of the (*R*)-enantiomer **21**. In the X-ray crystal structure, compound **20** was found to form a hemiketal adduct with Ser241, similarly to what had been observed for other  $\alpha$ -ketoheterocycles.<sup>54</sup> Administration of **20** to rats (50 mg/kg, po) caused significant accumulation of AEA, PEA and OEA in the brain and their high levels were maintained for several hours, similarly to what had been observed with the carbamate inhibitor URB597. The prolonged activity of the inhibitor **20**, compared to the reversible inhibition of FAAH observed *in vitro* for  $\alpha$ -ketoheterocycles, was attributed to the presence of steric hindrance close to the keto group protecting it from *in vivo* reductive metabolism. Compound **20** was reported to have analgesic effects *in vivo* (50 mg/kg, po) significantly attenuating mechanical and cold allodynia.<sup>54</sup>

Janssen Pharmaceutica also reported oxazolyl-ketones as FAAH inhibitors. A key element in the series was the replacement of the phenylhexyl group of OL-135 with a propylpiperidine<sup>55</sup> or a piperidine ring.<sup>56</sup> This led to new  $\alpha$ -ketoheterocycle inhibitors displaying subnanomolar potency, as in the case of compounds **22** or **23**, with  $IC_{50}$  values of 0.4 nM and 2 nM on human FAAH, respectively.

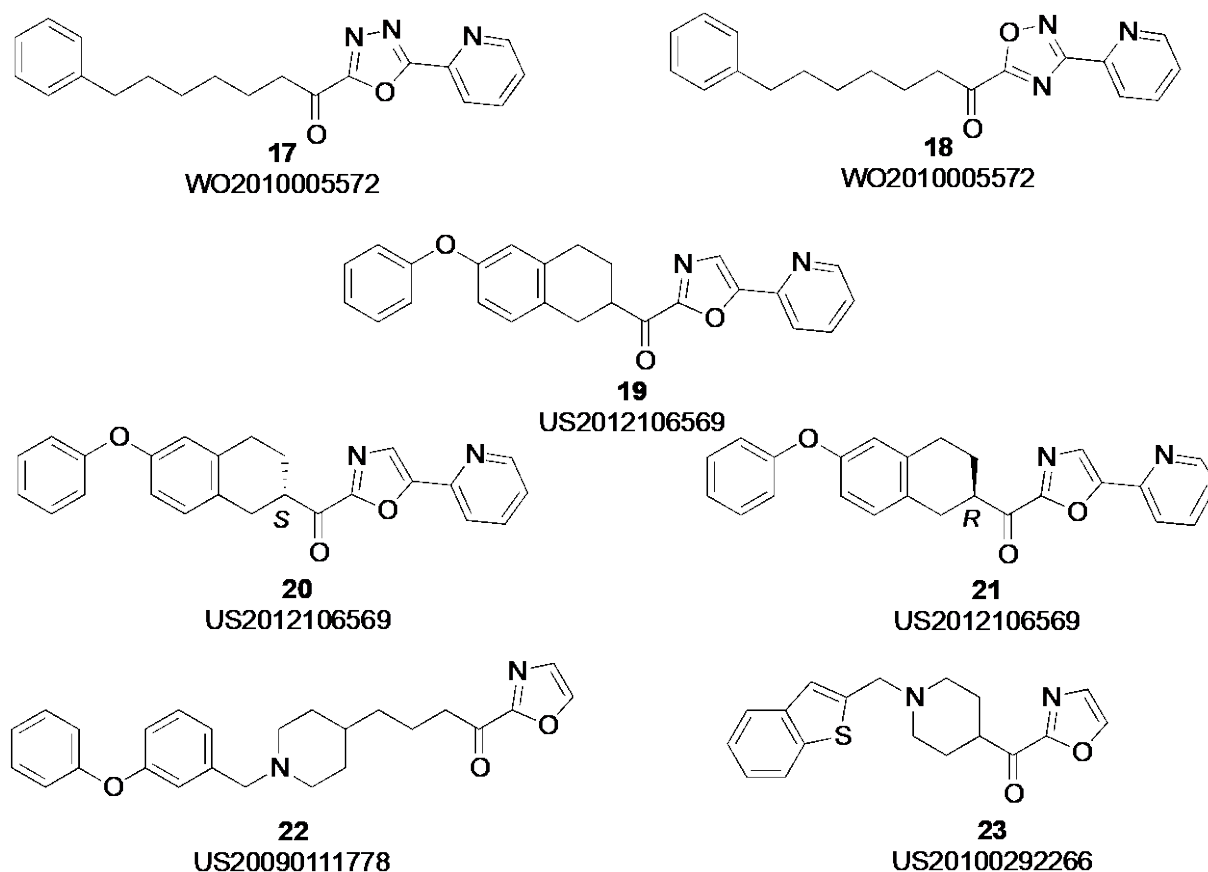


Figure 5.  $\alpha$ -Ketoheterocycle derivatives disclosed as FAAH inhibitors.

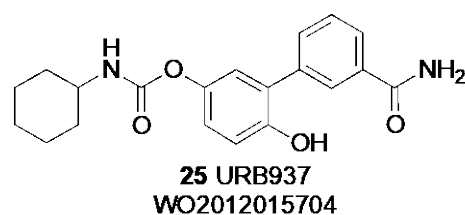
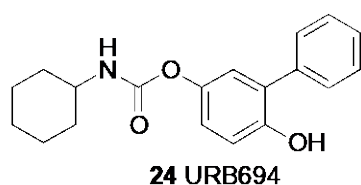
## 5.2 Carbamate inhibitors

URB597 has become the reference compound for pharmacological studies on FAAH, as well as a benchmark for the design and development of new inhibitors. However, URB597 is characterized by two main drawbacks: inhibition of plasma and liver carboxylesterases, and short *in vivo* half-life, which limits its use in chronic dosing studies.<sup>22</sup> To overcome these issues, a second-generation of carbamate FAAH inhibitors has been devised, by the introduction of electron-donating polar groups at the phenyl ring attached to the carbamate oxygen.<sup>57</sup> These include the para-hydroxyphenyl inhibitor **24** (URB694, Figure 6) that displayed decreased activity toward carboxylesterases and increased *in vivo* half-life compared to URB597, while preserving comparable *in vivo* potency in rats ( $ID_{50} = 0.19$  mg/kg for URB597 and 0.16 mg/kg for URB694).<sup>58</sup> More recently, starting from URB597 and

URB694 structures, a series of FAAH inhibitors with restricted access to the central nervous system (CNS) was developed. Compound **25** (URB937, Figure 6) is able to inhibit peripheral FAAH, increase peripheral AEA levels and attenuate behavioral responses indicative of persistent pain in rodent models of inflammation and peripheral nerve injury, although it does not inhibit FAAH activity in the CNS.<sup>59</sup> This indicates that a high degree of analgesia can be obtained by heightening the activity of AEA-based signalling involved in the regulation of nociceptive homeostasis outside of the CNS. URB937 was the subject of a patent application claiming its effectiveness in the treatment of pain, inflammation and immunity disorders.<sup>60</sup>

The existence of structural commonalities between the *N*-cyclohexylcarbamic acid *O*-biphenyl-3-yl ester class of FAAH inhibitors and the 2-arylpropionic acid class of non-steroidal anti-inflammatory drugs (NSAIDs) was exploited by the Drug Discovery and Development group at the Italian Institute of Technology to design compounds able to simultaneously target FAAH, COX-1, and COX-2. This research led to a patent application covering carbamate derivatives incorporating the scaffold of flurbiprofen (**26**, (*RS*)-2-(2-fluorobiphenyl-4-yl)propanoic acid) in their structure, exemplified by compound **27** (ARN2508, Figure 6).<sup>61</sup> This compound, initially tested as a racemic mixture, was reported to inhibit rat FAAH with an IC<sub>50</sub> of 31 nM, and COX-1 and COX-2 with IC<sub>50</sub> values of 12 and 430 nM, respectively. In the patent application, the activity of the pure enantiomers is also reported. While the two enantiomers inhibit FAAH with the same potency, the (+)-isomer inhibits COX-1 and COX-2 with IC<sub>50</sub> of 0.01 and 100 nM, respectively, and the (-)-isomer inhibits these two enzymes only at high micromolar concentrations. In the follow-up publication, ARN2508 resulted effective in a model of intestinal inflammation where a pure FAAH inhibitor was weakly active and the COX inhibitor flurbiprofen aggravated inflammation. This indicates that the simultaneous blockade of FAAH and COX-1/COX-2 results in a combination of profound anti-inflammatory and tissue-protective actions.<sup>62</sup>

**University of California, Urbino and Parma**



**Italian Institute of Technology, University of California and Bologna**

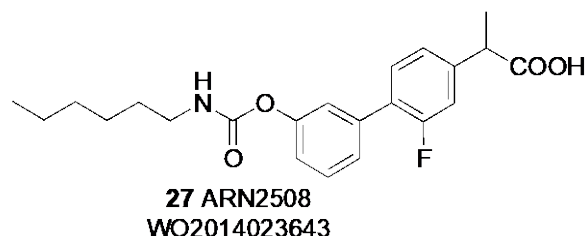
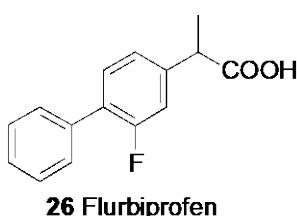


Figure 6. Carbamate-type FAAH inhibitors described by academic groups.

An interesting class of enol carbamates was recently disclosed by Sigma-Tau as FAAH inhibitors.<sup>63</sup> The authors of a paper published in 2010 propose that hydrolysis of the carbamate group, catalyzed by FAAH, releases an enol leaving group, which would tautomerize to the more stable keto form, thus shifting the equilibrium toward formation of the products. Nonlinear regression analysis of the Michaelis-Menten curves suggested that these compounds act as non-competitive inhibitors of FAAH, i.e., they significantly reduce FAAH  $V_{\max}$  without affecting the  $K_m$  for AEA.<sup>64</sup> The most potent compound of the series (**28**, ST-4070, Figure 7) was more than 1000-fold selective for FAAH over several related proteins including CB<sub>1</sub>, CB<sub>2</sub>, MAGL, DAGL and NAPE-PLD. Compound **28** was active in several models for neuropathic pain after oral administration at 10-100 mg/kg in rodents.<sup>65</sup> Sigma-Tau also developed a carbamoyl oxime class, exemplified by compound **29** (ST-4020, Figure 7) having an IC<sub>50</sub> value in the nanomolar range (<10 nM) in the inhibition of mouse FAAH.<sup>66</sup> Sigma-Tau also reported a class of carbamates closely related to URB597. Compound **30** (ST4068, Figure 7) inhibits FAAH in the nanomolar range and is active *in vivo* at 30 and 100 mg/kg per os in a

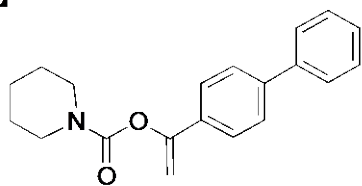
mechanical hyperalgesia assay.<sup>67</sup> Compounds of the class reported in this patent application were later disclosed in a publication by Campiani et al.<sup>68</sup> Differently from URB597, ST4068 and derivatives act as reversible inhibitors of FAAH.

Carbamate-based inhibitors were also thoroughly investigated by Sanofi-Aventis. Several compounds were reported, in which the aromatic substituent at the oxygen atom of the first generation of carbamic acid esters was replaced by an alkyl-thiazolyl<sup>69</sup> (e.g., **31**, Figure 7) or by an alkyl-isoxazolyl substituent (**32**, Figure 7).<sup>70</sup> Sanofi-Aventis also reported inhibitors in which the carbamate nitrogen atom was inserted in a ring, as in the case of compound **33**.<sup>71</sup> Compounds **31-33** were described as potent inhibitors of mouse FAAH with potencies in the nanomolar range ( $IC_{50}$  = 1, 3, and 0.46 nM, for compound **31**, **32** and **33**, respectively). Compounds belonging to these classes display analgesic activity when administered to mice by the oral route at doses within 1-30 mg/kg range.

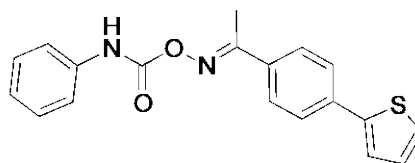
Astellas developed a class of tertiary carbamates characterized by a N-(pyridin-3-yl)oxycarbonyl-piperidin-4-yl core attached to an arylazole moiety, as in compounds **34** and **35** (Figure 7), carrying a 3-phenyl-1,2,4-oxadiazol-5-yl or a 4-phenyl-1,2,3-triazol-2-yl substituent. These two compounds were reported to inhibit human FAAH, expressed in a bladder-derived cell line (HTB-9), with  $IC_{50}$  values of 0.077 and 0.047 nM, respectively.<sup>72</sup>



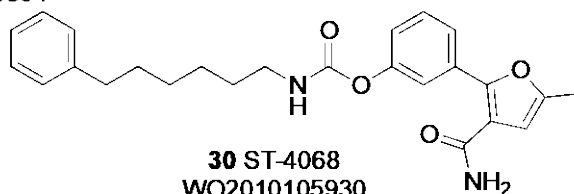
**Sigma-Tau**



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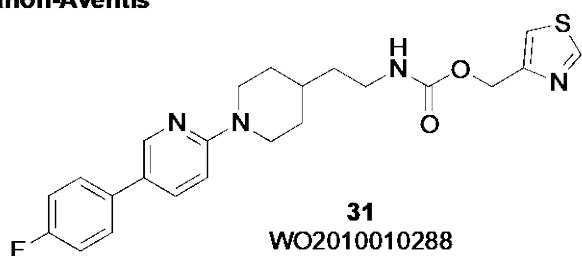


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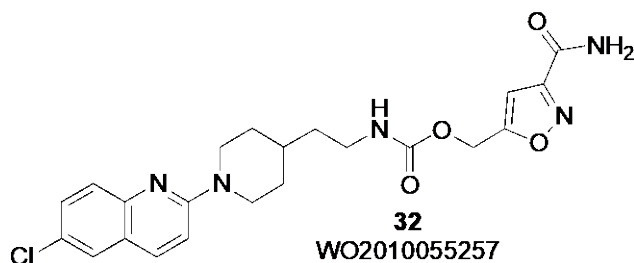


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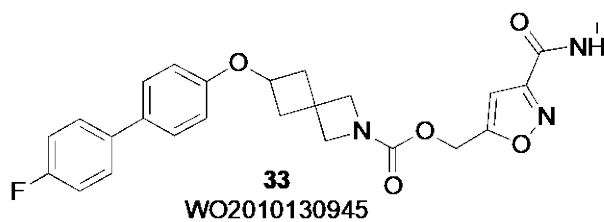
**Sanofi-Aventis**



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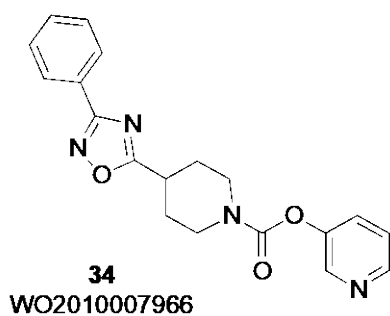


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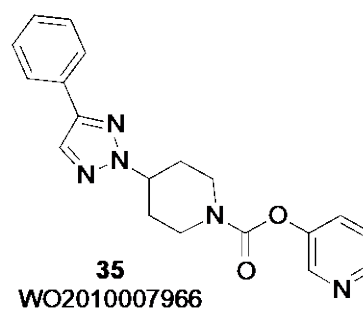


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**34**  
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**35**  
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Figure 7. Carbamate-type FAAH inhibitors described by Sigma-Tau, Sanofi-Aventis and Astellas.

**5.3 Aryl ureas**

Urea-based FAAH inhibitors were mainly investigated and advanced to clinical trials by Pfizer. Several patents appeared in literature, covering the chemical space around **14** (PF-04457845, the first FAAH inhibitor to reach clinical phase II), including ether benzylidene piperidine<sup>73</sup> and benzylidene 3-methylpiperidine derivatives.<sup>74</sup> Most of these compounds (exemplified by **36-38**, Figure 8) show inactivation efficiencies on human FAAH similar to that of PF-04457845 (i.e.  $k_{inact}/K_i$  ratio close to  $40,000 \text{ M}^{-1} \text{ s}^{-1}$ ). Pfizer also reported new classes of FAAH inhibitors characterized by the 7-azaspiro[3,5]nonane-7-carboxamide<sup>75</sup> or by the 1-oxa-8-azaspiro[4,5]decane-8-carboxamide nucleus bearing heteroaryl leaving groups different from the classical pyridin-3-yl or pyridazin-3-yl ones (Figure 8).<sup>76</sup> With few exceptions (i.e., **39**,  $k_{inact}/K_i$  ratio of  $21,700 \text{ M}^{-1} \text{ s}^{-1}$ ; and **40**,  $k_{inact}/K_i$  ratio of  $30,800 \text{ M}^{-1} \text{ s}^{-1}$ ) these compounds were generally less efficient than PF-04457845 at inhibiting FAAH. Compounds **39** and **40** were reported to have analgesic activity in rats at the dose of 3 mg/kg, when tested in the Freund's adjuvant (CFA) assay of inflammatory pain. Pfizer's aryl ureas based on the spirocyclic cores were recently reported in the scientific literature,<sup>77</sup> with the disclosure of PF-04862853 as an orally efficacious inhibitor of FAAH for the treatment of pain.<sup>78</sup> Despite this compound inhibits human FAAH less efficiently than other reported derivatives ( $k_{inact}/K_i$  ratio of  $4,190 \text{ M}^{-1} \text{ s}^{-1}$ ) it displays a favorable pharmacokinetic profile in dogs and a good efficacy in the CFA assay in rats.

Pfizer

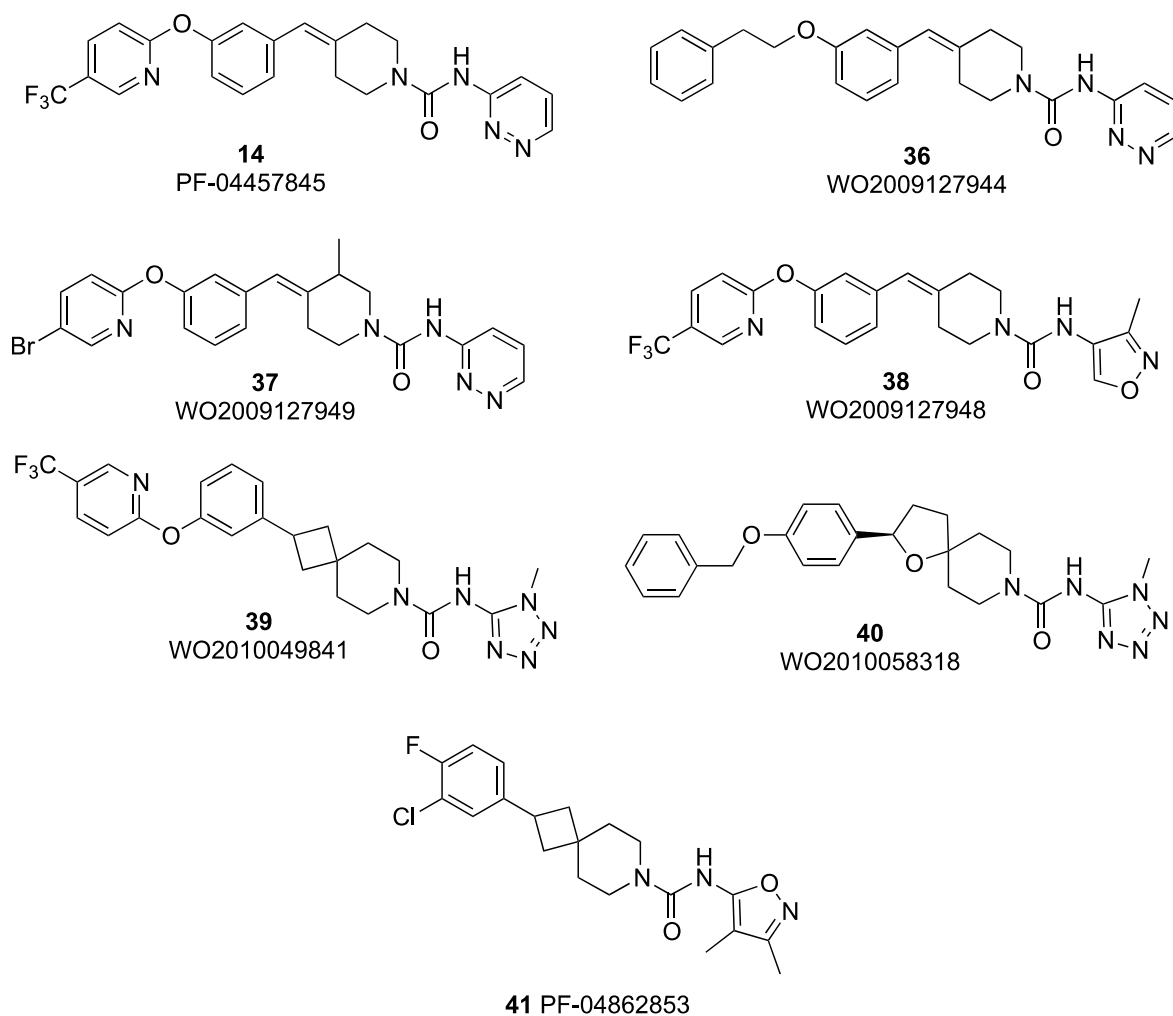


Figure 8. Aryl ureas covered by Pfizer.

Other companies also developed their own urea classes.<sup>79</sup> Vernalis disclosed the structure of different series of azetidione derivatives (Figure 9). A first series of azetidione-1-yl(piperidin-1-yl)methanone derivatives, exemplified by compound **42** (VER-156084), was also described in the scientific literature.<sup>80</sup> This compound was reported to be a time-dependent inhibitor of human FAAH, displaying an IC<sub>50</sub> value of 1031 nM, after 1 h of pre-incubation. Vernalis attempted to improve the *in vitro* potency on human FAAH, as well as to reduce microsomal metabolism of azetidione-1-yl(piperidin-1-yl)methanone analogues of VER-156084, but without success interrupted the development of VER-156084 in favor of

alternative chemotypes.<sup>81</sup> The second series developed by Vernalis was characterized by a *N*-(pyridazin-3-yl)azetidione-1-carboxamide core, as exemplified by compound **43**, which inhibits human FAAH with an IC<sub>50</sub> of 38 nM after 1 h of pre-incubation. Compound **43** exhibits a dose-dependent analgesic activity in a rat model of thermal pain sensitivity after oral administration of the compound at the dose of 1, 3 or 10 mg/kg.<sup>82</sup>

Vernalis

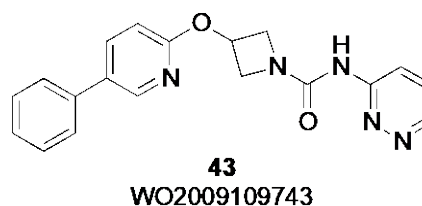
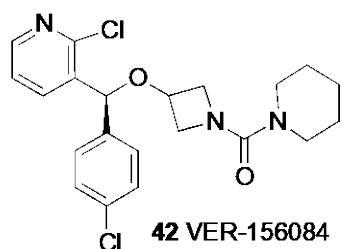


Figure 9. Azetidione urea derivatives described by Vernalis.

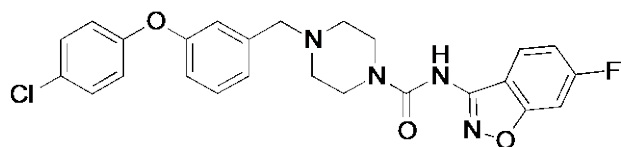
Janssen Pharmaceutica reported several patents covering heteroaryl-substituted ureas<sup>8384</sup> exemplified by compounds **44** and **45** (Figure 10), which inhibit human and rat FAAH in the nanomolar range and are effective in a mild effect thermal injury model when administered orally to rats at 10 or 20 mg/kg.<sup>85</sup> Janssen also reported a class of spirocyclic diamine ureas as single digit nanomolar inhibitors of human and rat FAAH, exemplified by compounds **46** and **47** (JNJ-42119779, Figure 10).<sup>86</sup> The chemical synthesis and the pharmacological characterization of JNJ-42119779 have been very recently reported in the literature, where this compound has been shown to be effective in the spinal nerve ligation (Chung) model of neuropathic pain at 20 and 60 mg/kg, po.<sup>87</sup>

Piperazine ureas were reported to inhibit rat FAAH. In particular, compound **48**, which possesses an IC<sub>50</sub> of 18 nM, controls ventricular pressure in rats and is not toxic at 300 mg/kg, after daily administration per os for 4 consecutive days.<sup>88</sup> More recently, Janssen also reported a patent application specifically covering compound **49** (4-(2,2-difluoro-

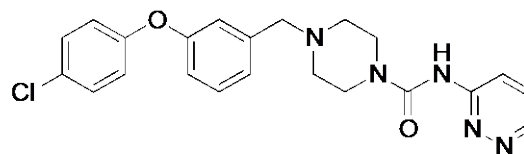
benzo[1,3]dioxol-5-ylmethyl)-piperazine-1-carboxylic acid (4-chloro-pyridin-3-yl)-amide, Figure 10). This compound, which inhibits human and rat FAAH with IC<sub>50</sub> values of 75 and 320 nM, respectively, was described to be less prone to inhibit CYP2D6 liver cytochrome and to overcome behavioral side-effects in rats described for the corresponding dechlorinated analogue.<sup>89</sup>

Azetidine ureas were also covered by Janssen Pharmaceutica.<sup>90</sup> A recent patent application reported several potent FAAH inhibitors possessing a variety of heteroaryl substituents (other than the classical pyridin-3-yl one) at the primary nitrogen of the urea functionality, including the imidazo[1,2-b]pyridazin-3-yl fragment of compound **50** and the pyrrole[2,3-b]pyridin-5-yl one of compound **51**. They were reported to inhibit human FAAH with IC<sub>50</sub> of 5 and 3 nM, respectively. In the same patent application, Janssen also describes pyrrolidinyl urea derivatives of the kind of compound **52**. These pyrrolidinyl ureas were in general less potent than azetidiny ones, inhibiting FAAH with IC<sub>50</sub> values ranging from 50 to 5,000 nM.

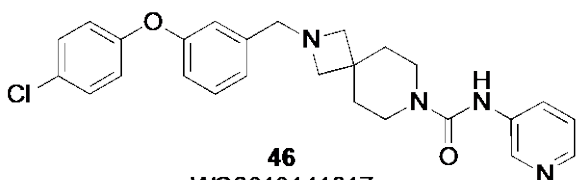
**Janssen**



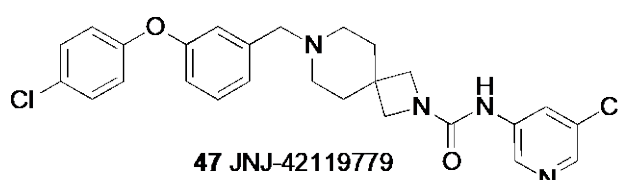
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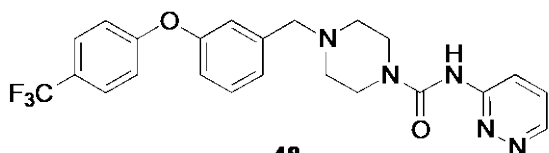
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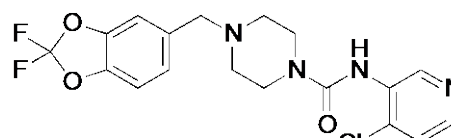
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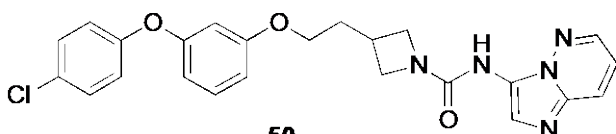
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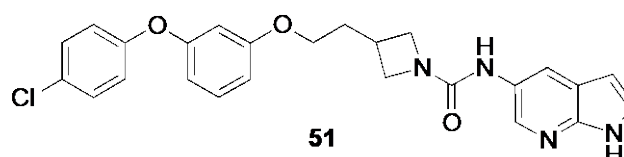
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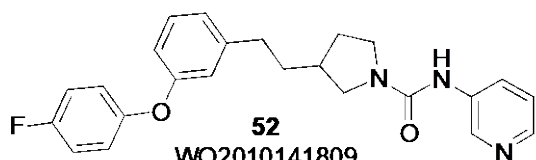
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**50**  
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**51**  
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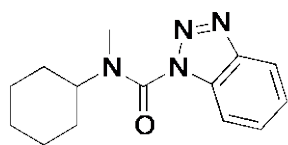


**52**  
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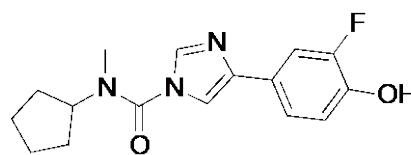
Figure 10. Urea-type FAAH inhibitors covered by Janssen Pharmaceutica.

Bial-Portela reported tetrasubstituted ureas characterized by a carbamoylbenzotriazole structure, exemplified by compound **53** (Figure 11), which fully inhibits brain FAAH in mice 1 h after its oral administration at the dose of 30 mg/kg, or by a carbamoyl-imidazole core, exemplified by compound **54**, which fully inhibits mouse FAAH *in vitro* at 10,000 nM.<sup>91</sup> Also Makriyannis et al. at the Northwestern University reported tetrazolyl and imidazolyl-ureas as FAAH inhibitors, exemplified by compounds **55** and **56** (Figure 11).<sup>92</sup>

#### Bial Portela

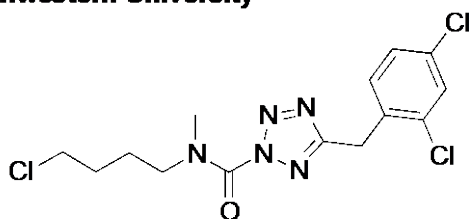


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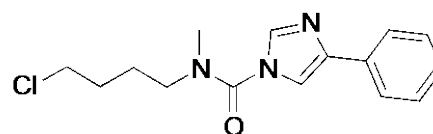


**54**  
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#### Northwestern University



**55**  
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**56**  
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Figure 11. Tetrasubstituted ureas reported by Bial Portela and Northwestern University.

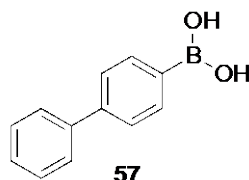
### 5.4 Boronic acids

Infinity Pharmaceuticals filed the first patent application of boronic acids as FAAH inhibitors in 2008.<sup>93</sup> In the same year, a series of commercially available arylboronic acids, exemplified by **57** (biphenyl-4-ylboronic acid,  $IC_{50} = 21$  nM vs rat FAAH), was reported by Minkkila et al<sup>94</sup> to potently inhibit FAAH (Figure 12). In the same article Minkkila and collaborators proposed that these compounds form a reversible covalent complex with FAAH. In fact, the presence of the boron atom, able to easily change its hybridization from  $sp^2$  to  $sp^3$ , allows these compounds to form a tetrahedral adduct with Ser241, similar to the intermediate formed by FAAH and AEA during the mechanism of hydrolysis.

A follow-up patent application filed by Infinity Pharmaceuticals reported an optimized synthetic procedure based on the Suzuki reaction to obtain **58**, a difluoro-substituted derivative of 1,1'-biphenyl-4-ylboronic acid (Figure 12). Infinity Pharmaceuticals included other heteroaryl boronic acids, exemplified by compounds **59** and **60** which were reported to

inhibit human FAAH with  $K_i < 10$  nM.<sup>95</sup> Boronic acids characterized by a 6,6 or a 6,5 bicyclic system (i.e., compounds **61** or **62**, having  $K_i < 10$  nM)<sup>96</sup> or by a cycloaliphatic ring (i.e. piperidine **63**, having  $K_i < 100$  nM)<sup>97</sup> were also covered by Infinity Pharmaceuticals as human FAAH inhibitors.

#### University of Kuopio



#### Infinity

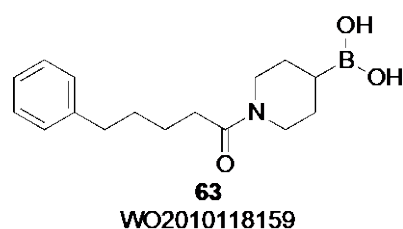
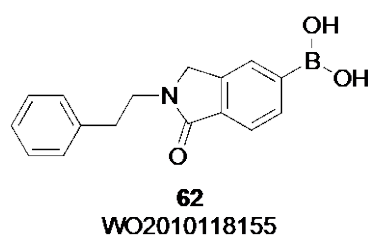
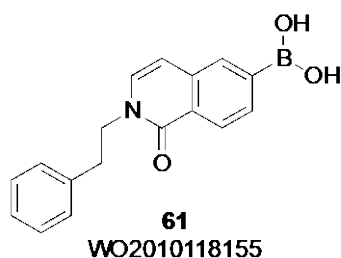
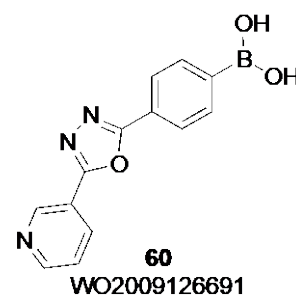
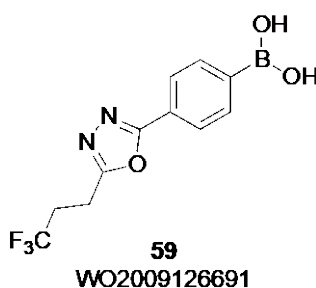
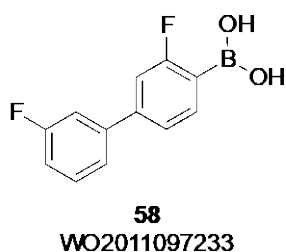


Figure 12. Examples of boronic acids described as FAAH inhibitors.

## 5.5 Azole derivatives

Merck designed various heterocycle-based FAAH inhibitors with an imidazole,<sup>98</sup> pyrazole,<sup>99</sup> oxazole<sup>100</sup> or azaindole<sup>101</sup> core, exemplified by compounds **64**, **65**, **66** and **67** which inhibit human FAAH with single digit IC<sub>50</sub> values (Figure 13). Merck also reported a class of 2-(4-(1*H*-imidazol-4-yl)phenyl)cyclopropanecarboxamide derivatives, potentially useful as FAAH imaging agents, exemplified by the tritiated inhibitor **68**.<sup>102</sup> The non-tritiated



analog was reported to have an  $IC_{50}$  of 1.1 nM on human FAAH. Merck also published a scientific paper describing the identification of a 2-(4-(1*H*-imidazol-4-yl)phenyl)cyclopropanecarboxamide derivative as a promising FAAH PET tracer.<sup>103</sup> As a first step, a FAAH inhibitor from this new class was identified: compound **69** (Figure 13) exhibits good potency vs human and rhesus monkey FAAH ( $IC_{50}$  of 1.0 nM and 5.5 nM, respectively) and excellent selectivity, having only 2 moderate off-targets (i.e., AChE,  $IC_{50}$  = 1.63  $\mu$ M and PDE4,  $IC_{50}$  = 9.75  $\mu$ M) out of a panel of 168. Moreover, it shows rapid and significant brain penetration in rats (brain-to-plasma concentration ratio of 7:1 at 2 h following administration at 2 mg/kg, po). As a next step, a radiolabeled derivative of compound **69** was synthesized and investigated as potential <sup>11</sup>C PET tracer. The resulting compound **70** (MK-3168) exhibits good brain uptake and FAAH-specific signal in PET studies on rhesus monkey, with accumulation in the frontal cortex, striatum, and hippocampus regions all of which are FAAH-enriched areas. **70** was therefore proposed to be a good PET tracer for imaging FAAH in the brain, suitable for clinical application.<sup>103</sup>

Very recently, Merck also described in the scientific literature researches focused on the discovery and development of a class of oxazole inhibitors as possible clinical candidates for inflammatory diseases.<sup>104</sup> They reported the identification of a first pyrazole hit (**71**, Figure 13) by high throughput screening (HTS) and the following medicinal chemistry efforts aimed at optimizing both *in vitro* potency on FAAH and pharmacokinetic properties. This led to the discovery of the oxazole **72** (MK-4409, Figure 13), a potent and reversible FAAH inhibitor ( $IC_{50}$  = 11 nM) devoid of functionalities able to form covalent bonds with the FAAH catalytic site. Compound **72** is effective in animal models of inflammatory (i.e., CFA assay at 10 mg/kg, po) and neuropathic pain (i.e., spinal nerve ligation assay at 3 mg/kg, po) without leading to loss of cognition or motor skill impairment.<sup>104</sup>

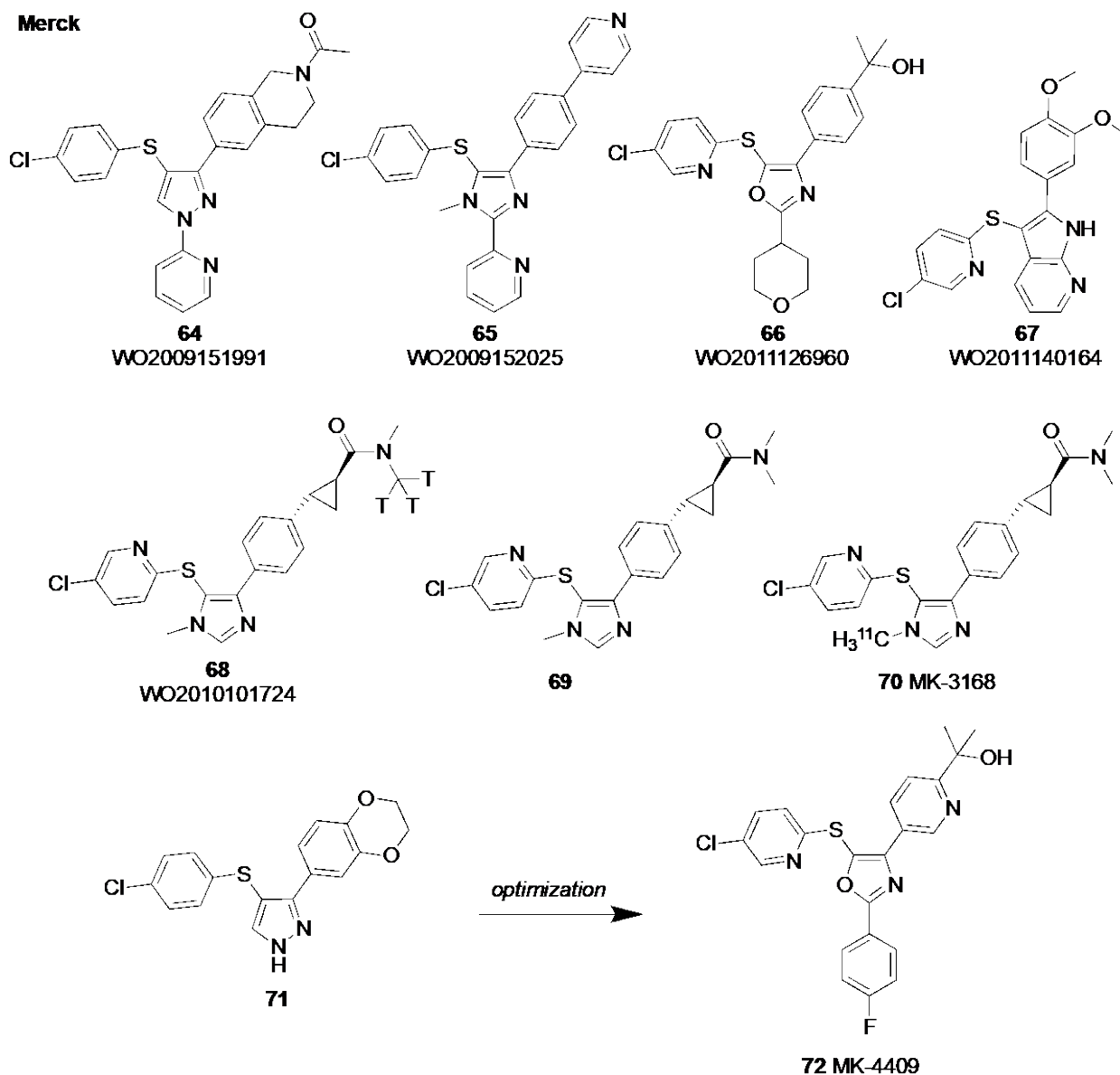


Figure 13. Examples of azole derivatives reported as FAAH inhibitors.

## 5.6 Ethylaminopyrimidines

Janssen Pharmaceutica developed a class of inhibitors characterized by an arylhydroxyethylaminopyrimidine scaffold (Figure 14).<sup>105</sup> The most potent compound of the series is compound **73** with  $IC_{50}$  values of 1 and 3 nM on human and rat FAAH, respectively.

The discovery and optimization of arylhydroxyethylaminopyrimidines as FAAH inhibitors have also been reported very recently in the scientific literature.<sup>106</sup> In brief, an HTS

campaign led to the discovery of a racemic hit (**74**) as a competitive FAAH inhibitor (IC<sub>50</sub> values of 30 and 591 nM on human and rat FAAH, respectively) lacking reactive functionalities. The synthesis and testing of the isolated optical antipodes revealed that the (*R*)-isomer (**75**) is significantly more potent than the (*S*)-isomer (**76**) due to a specific, stereochemically dependent interaction with FAAH, confirmed by the X-ray crystal structure of humanized rat FAAH bound to an inhibitor from the (*R*) series, compound **77**. This compound displays a binding mode similar to that of compound **16** (the ketobenzimidazole inhibitor Amgen-2): it occupies both the ABP and the MAC of FAAH, while its hydroxyl group is well positioned to establish a hydrogen bond with Thr488 (*cf.* Figure 3). For the corresponding (*S*) isomer, such an interaction seems not to be readily accessible, accounting for the high stereoselectivity of this class.<sup>106</sup> Further expansion of the series led to **78** (JNJ-40413269) which inhibits human FAAH with an IC<sub>50</sub> of 5.3 nM and was found to have excellent pharmacokinetic properties, as well as to be orally efficacious in the rat spinal nerve ligation (Chung) model of neuropathic pain at doses of 10-100 mg/kg.<sup>106</sup>

A class of rather similar compounds, in which the hydroxyl group is replaced by an amino group, was also disclosed by Janssen Pharmaceutica. The resulting pyrimidinylethylenediamines (e.g., compound **79**) are in general less potent than hydroxyethylaminopyrimidine derivatives. Compound **79**, one of the most potent FAAH inhibitors from this series has IC<sub>50</sub> values of 20 and 60 nM vs human and rat FAAH, respectively.<sup>107</sup>

## Janssen

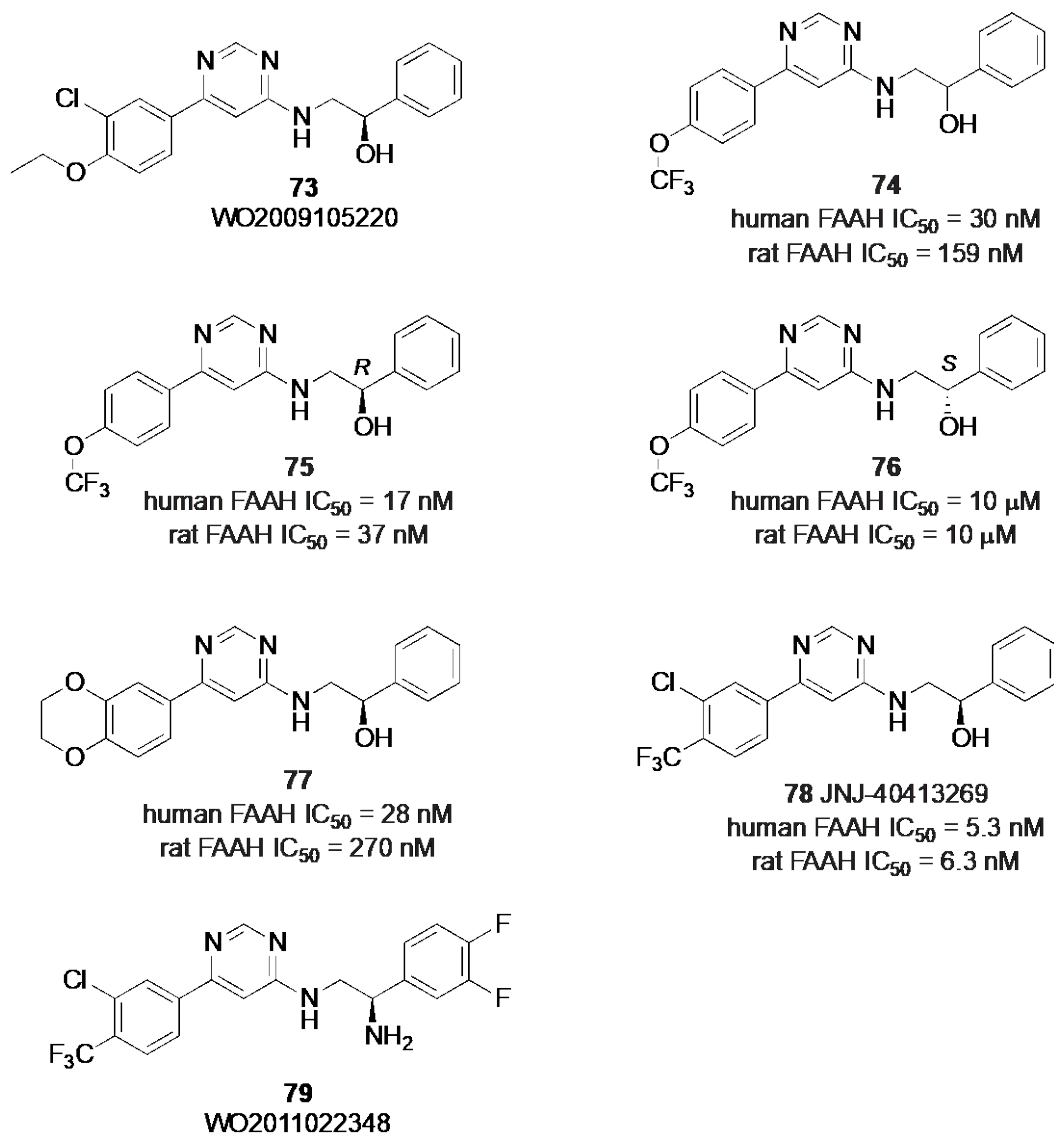


Figure 14. Examples of ethylaminopyrimidines reported as FAAH inhibitors.

## 5.7 Tetrahydronaphthyridine derivatives

Renovis claimed a series of substituted 1,2,3,4-tetrahydro-2,6-naphthyridines, exemplified by compound **80**, which inhibits microsomal human FAAH with an  $IC_{50}$  value lower than 100 nM (Figure 15).<sup>108</sup> This class of compounds was identified through a HTS campaign on human FAAH using a microsomal protein preparation and a fluorescent assay readout.<sup>109</sup> The first hit (the tetrahydropyridopyrimidine **81**) was the subject of an intensive

optimization program that led to the tetrahydronaphthyridine **82** (RN-450), able to inhibit human and rat FAAH with IC<sub>50</sub> values of 13 and 25 nM, respectively. The mechanism of action of RN-450 was thoroughly investigated and it was shown that this compound acts as a competitive and reversible FAAH inhibitor. Indeed, when increasing concentrations of RN-450 are incubated in the presence of human FAAH, the enzyme V<sub>max</sub> remains unchanged while significant variation in the K<sub>m</sub> is observed. The inhibitory potency of RN-450 was found to be time-independent and a rapid dilution experiment allowed to restore FAAH activity almost completely, demonstrating the reversible nature of FAAH inhibition by RN-450.<sup>109</sup>

Renovis also claimed a class of benzoxazole derivatives as FAAH inhibitors exemplified by compound **83** which inhibits human FAAH with an IC<sub>50</sub> value of 1.3 nM.<sup>110</sup>

#### Renovis

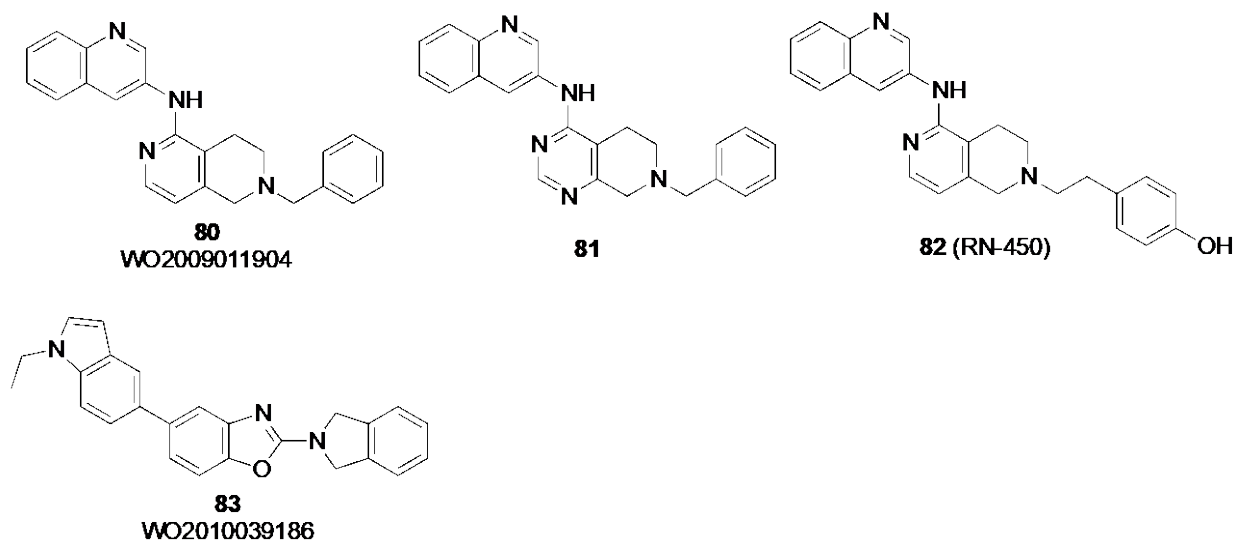


Figure 15. Examples of compounds claimed as FAAH inhibitors by Renovis.

### 5.8 Miscellaneous classes

A patent application by Bial-Portela reports several compounds characterized by a 5-*O*-substituted-3-*N*-phenyl-1,3,4-oxadiazolone scaffold, exemplified by 3-(4-amino-3-(2-

methoxyethoxy)phenyl)-5-(2,4-difluorophenoxy)-1,3,4-oxadiazol-2(3H)-one **84** (Figure 16).<sup>111</sup> Compounds of this class inhibit rat FAAH *in vitro* ( $IC_{50} < 100$  nM) and mouse FAAH *in vivo* ( $ED_{50} < 30$  mg/kg, po), both in the liver and in the brain. Bial-Portela further covered this class of FAAH inhibitors reporting two patent applications in 2010.<sup>112,113</sup> Compounds having a 3-aryl-5-methoxy-1,3,4-oxadiazol-2(3H)-one ring had been reported to inhibit hormone-sensitive lipase (HSL), targeting the catalytic serine by their 2-carbonyl fragment and leading to the formation of a carbazate adduct.<sup>114</sup> While no details are reported about FAAH-inhibition mechanism, a similar reaction with nucleophilic serine of FAAH can be hypothesized.

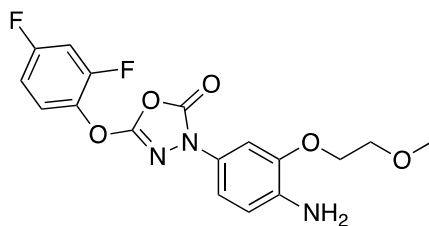
Infinity claimed a class of substituted isoxazolines as FAAH inhibitors (Figure 16), characterized by a bromine atom or an aryloxy substituent in position 3 and exemplified by compounds **85-88**.<sup>115</sup> These compounds inhibit FAAH with  $K_i < 100$  nM. It was hypothesized that these compounds may undergo a nucleophilic attack by Ser241, resulting in the elimination of the bromo or aryloxy substituent at position 3 on the isoxazoline nucleus and the subsequent formation of a FAAH-isoxazoline adduct. This irreversible mechanism of action was supported by a rapid dilution assay showing that no enzyme activity was recovered 2 h after removal of the inhibitor.<sup>115</sup>

Ironwood reported a class of benzylpyrrolyloxoacetamide derivatives for use as FAAH inhibitors,<sup>116,117</sup> exemplified by **89** (Figure 16). These compounds inhibit FAAH, extracted by human brain, with an  $IC_{50}$  value of 4 nM. The mechanism of action of this class of compound was not described in the patent application. However,  $\alpha$ -keto amides of this kind have been reported to inhibit serine proteases (i.e., HCV NS3 protease) by forming a reversible hemiketal structure with the catalytic serine.<sup>118</sup> It is conceivable that these oxoacetamides may form an hemiketal adduct with FAAH Ser241, similarly to what had been observed for the class of  $\alpha$ -ketoacetamides. A compound of the same class (**90**) was also claimed to be

useful for the treatment or prevention of neuronal injury or neurodegeneration,<sup>119</sup> as well as for the treatment of the restless legs syndrome.<sup>120</sup> Recently, the pharmacokinetic profile of the benzylpyrrolyloxoacetamide **91** (MM-433593) was reported in the literature, showing low bioavailability in male and female monkeys (18%).<sup>121</sup> MM-433593 undergoes phase I and phase II biotransformations, giving at least 18 metabolites in monkeys, with the major biotransformation pathway involving oxidation of the methyl group at 5-position of the indole ring, followed by conjugation with glucuronide, sulfate, or glutathione.

Recently, Allergan claimed a class of *N*-alkyl-4-oxazolecarboxamide derivatives, exemplified by compounds **92** and **93** (Figure 16), which inhibit rat brain FAAH with IC<sub>50</sub> values of 180 and 20 nM, respectively. No information on the mechanism of action is reported in the patent application for these inhibitors.<sup>122</sup>

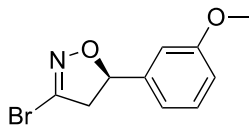
**Bial Portela**



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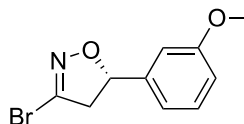
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**Infinity**



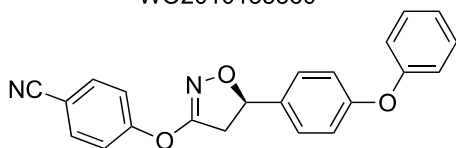
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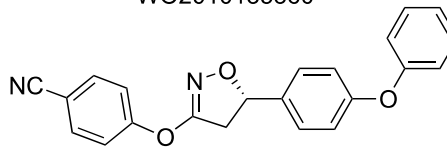
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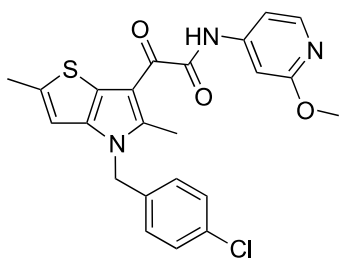
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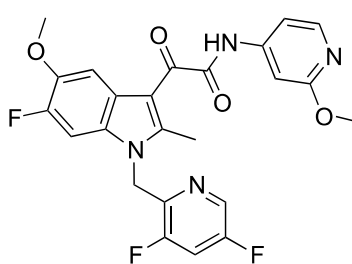
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**Ironwood**



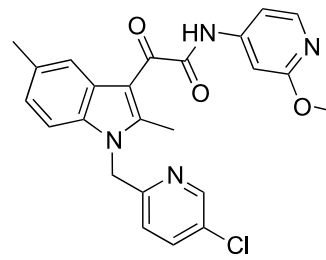
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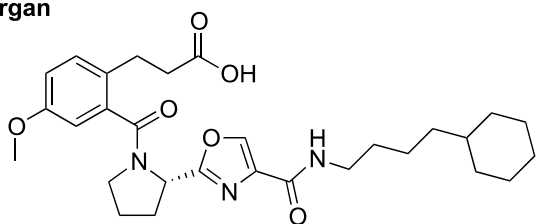
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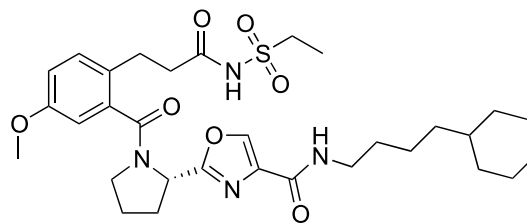
**91** MM-433593

**Allergan**



**92**

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**93**

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Figure 16. Examples from classes of compounds claimed as FAAH inhibitors.



## 6. Expert Opinion

The availability of potent and selective FAAH inhibitors endowed with good *in vivo* activity has allowed significant advancement in the understanding of FAAH functions and in the definition of its potential role as a drug target. The levels of FAEs in the CNS are mainly controlled by FAAH and their modulation through FAAH inhibition can be exploited to obtain beneficial effects, at least in animal models. In fact, while inactivation of FAAH by small-molecule inhibitors provokes significant elevation in the CNS levels of AEA, PEA and OEA, producing analgesic and anti-inflammatory effects, these occur in the absence of cognitive alterations typically associated to CB<sub>1</sub> receptor stimulation with exogenous agonists. As compelling evidence from animal experiments is showing that FAAH inhibitors can be effective at ameliorating signs of acute, inflammatory, visceral and neuropathic pain, the search for FAAH inhibitors suitable for clinical investigation is a strong focus in current drug discovery. In this scenario, even if the failure of PF-04457845 (**14**) in a phase II study evaluating its ability to control osteoarthritis pain has not flatted the interest in FAAH inhibitors, particular effort is needed to find new strategies that could allow the exploitation of this pharmacological class for effective therapeutic applications. From the point of view of medicinal chemistry, this means that new patentable chemical classes of FAAH inhibitors with proven *in vivo* activity are welcome, as this could provide new opportunities to develop drugs with different pharmacokinetic or pharmacodynamic profiles. On the other hand, new candidates should present some advantages, compared to traditional classes of carbamates and ureas. In fact, according to the information reported in the public database [clinicaltrials.gov](http://clinicaltrials.gov), clinical trials on FAAH inhibitors are still based on the use of PF-04457845 and URB597. Ongoing or completed studies with PF-04457845 evaluate the potential for treating cannabinoid dependence, acute and chronic pain, Tourette syndrome and fear

conditioning. An ongoing study with URB597 evaluates the potential of this compound for the treatment of schizophrenia.

The interest in this area is proven by the number of new and attractive FAAH inhibitors that have been described both in patent applications and in scientific literature. Many of these are carbamate and urea-based inhibitors, developed in academic and industrial settings, complementing the work performed in the early 2000. Compounds characterized by peripheral *in vivo* distribution or by the ability to inhibit multiple targets (i.e., FAAH and COX) were reported and may perhaps be useful in those pathological conditions where a single target inhibitor or a FAAH inhibitor with non-restricted distribution could fail.<sup>48</sup> Another example of multitarget inhibitors is represented by 1-indol-1-yl-propan-2-ones which simultaneously inhibit FAAH and the cytosolic phospholipase A2 and could exert improved analgesic and anti-inflammatory properties *in vivo* compared to selective FAAH inhibitors.<sup>123</sup> On the other hand, a significant change in the exploration of the chemical space of FAAH inhibitors occurred, with the relevant introduction of compounds devoid of an electrophilic function in their structure, as in the cases of ethylaminopyrimidines, azoles and tetrahydronaphthyridines. Compounds from these classes potently inhibit FAAH *in vitro* and *in vivo* and achieve anti-inflammatory effects in animal models similarly to the covalent FAAH inhibitors URB597 (**7**) and-PF-04457845 (**14**). The interest in developing non-covalent inhibitors could be attributed to the reduction of idiosyncratic risk that, in compounds bearing reactive groups, might eventually lead to failure in clinical trials. Additional advantages linked to their non-covalent mechanism of action should be further investigated and described in the scientific literature. Regarding the issue of inhibition kinetics, both in the sense of inactivation onset and reversibility, its pharmacological significance still needs to be assessed. In particular, it could significantly affect behavioral effects, which represent potential applications for FAAH inhibitors. Moreover, covalent mechanisms do not necessarily

result in irreversible inhibition, as shown by different classes of compounds (e.g.,  $\alpha$ -keto heterocycles), and even for carbamoylating agents it has been shown that chemical modulation can afford different degrees of enzyme recovery.

In conclusion, even if more than ten years have passed since the emergence of FAAH inhibitors as new possible therapeutic agents, the deep research activity in this field, as well as the huge number of patent applications filed in the last five years characterize this field as one of the most promising in medicinal chemistry.

### **Highlights**

1. FAAH is a key component of the endocannabinoid system and it is the main responsible for the termination of anandamide signaling *in vivo*. FAAH also concurs to the deactivating hydrolysis of other neuromodulatory amides, such as palmitoylethanolamide and oleoylethanolamide.
2. Selective inhibition of FAAH enhances the endocannabinoid tone at local levels exerting beneficial effects in animal models of pain, anxiety and rewarding effects from substances of abuse, while avoiding the classical drawbacks of generalized CB<sub>1</sub>-receptor stimulation.
3. Remarkable efforts have been made both by academia and pharmaceutical companies to optimize the pharmacodynamic and pharmacokinetic properties of known classes of FAAH inhibitors, such as carbamates and arylureas, which have led to the identification of promising clinical candidates.
4. Despite the failure of PF-04457845 phase II study on osteoarthritis pain, the potential clinical applications of FAAH inhibitors still continue to be investigated in drug dependence, Tourette syndrome, fear conditioning and schizophrenia.

5. The ongoing search for novel chemical entities targeting FAAH has culminated in the development of potent competitive and non-covalent inhibitors, the therapeutic potential of which still needs to be fully assessed.

### **Declaration of interest**

M.M. is inventor in the patent applications referred in 40 and 64. Except for this, the authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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