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Design, synthesis and biological evaluation of potent FAAH inhibitors

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ABSTRACT

A new series of 3-carboxamido-5-aryl-isoxazoles was designed, synthesized and evaluated for their biological activity. Different pharmacomodulations have been explored and the lipophilicity of these compounds was assessed. Investigation of the in vitro biological activity led to the identification of 5 compounds as potent FAAH inhibitors, their good FAAH inhibition capacity is probably correlated with their suitable lipophilicity. Specifically, compound **25** showed similar inhibition potency against FAAH in comparison with URB597, one of the most potent FAAH inhibitor known to date.

Fatty acid ethanolamines (FAEs), belong to a class of lipid-derived mediators and include: saturated FAEs such as palmitoylethanolamine (PEA), monounsaturated FAEs such as oleoylethanolamine (OEA), and polyunsaturated FAEs such as anandamide (AEA). Over the last decade, FAEs have been identified to induce a wide range of biological responses through several endogenous receptors. Typically, AEA relieves inflammation and pain by stimulating cannabinoid receptors CB_1/CB_2[1] and PEA exerts anti-inflammatory and analgesic activities via the peroxisome proliferator-activated receptor α (PPAR-α).[2,3] Hence, during recent decades, regulation of the AEA/PEA signaling through the inhibition of relevant biodegradation enzymes has been considered as a potent therapeutic approach to exert anti-nociceptive and anti-inflammatory effects.

Fatty acid amide hydrolase (FAAH), a membrane-bound serine hydrolase, is responsible for the principal degradation of AEA.[4,5] Its binding site comprises three functional channels: a membrane access channel, an acyl-chain binding channel, and a cytosolic port channel, which are relevant to the transport of the substrate to the catalytic site, the accommodation of the acyl chain during catalysis, and the removal of the leaving group after hydrolysis, respectively.[6] Especially, the substrate is surrounded by the catalytic triad (Ser241-Ser217-Lys142)[7,8] and the oxyanion hole residues (including Ser241, Gly240, Gly239, and Ile238)[6] during the hydrolysis. The nucleophilic hydroxyl group of the Ser241 interacts with the electrophilic carbonyl group of the substrate, which is accompanied by the formation of an acyl-enzyme adduct and the release of ethanolamine.[7,8] The inhibition of FAAH may result in the decrease of AEA hydrolysis and therefore the elevation of AEA levels, associated with analgesic and anti-inflammatory activities.[9–12]

Trifluoromethyl ketones and fluorophosphates were the first generation FAAH inhibitors identified. They showed good in vitro FAAH inhibition potency, but most of them exhibited poor selectivity for FAAH over other serine hydrolases (such as the triacylglycerol hydrolase and the membrane-associated hydrolase KIAA1363).[9–12] Over the past decade, research on FAAH inhibitors has been significantly developed. Numerous selective FAAH inhibitors have been identified. Generally, according to the different interaction mechanisms, FAAH inhibitors can be classified as covalent reversible inhibitors, such as OL-135[13,14] (Fig. 1), covalent irreversible inhibitors, such as URB597[15,16] (Fig. 1), or non-covalent reversible inhibitors, such as compound **1** disclosed by Abbott[17] (Fig. 1) and compound **2** synthesized by Amgen[18] (Fig. 1). OL-135, an α-ketoheterocycle, has been discovered in 2004 as a covalent, selective, and reversible FAAH inhibitor with an IC_{50} of 2 nM.[13] Especially, its carbonyl group has been demonstrated to interact with the Ser241 of the catalytic triad accompanied by the formation of an enzyme–substrate tetrahedral intermediate.[13] Additionally, the pyridine group of OL-135 has been identified to form H-bonds with the Lys142 and the Thr236, which is favorable for the interaction between OL-135 and FAAH.[19,20] URB597, a representative carbamate-based selective FAAH inhibitor, was identified to irreversible bind to FAAH accompanied by the generation of

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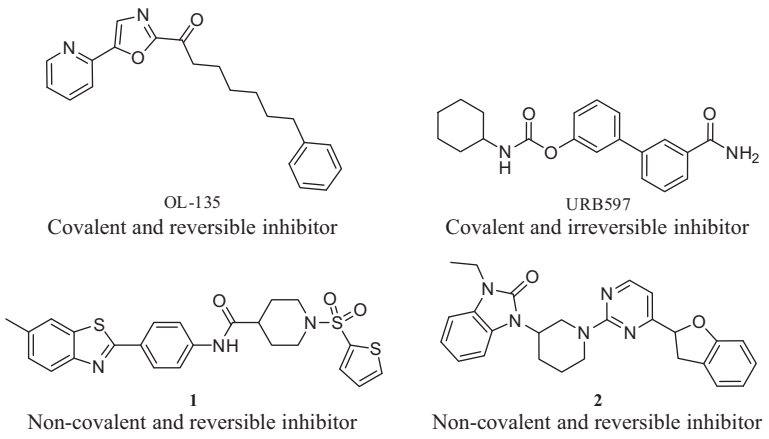


Figure 1. Structures of representative FAAH inhibitors.

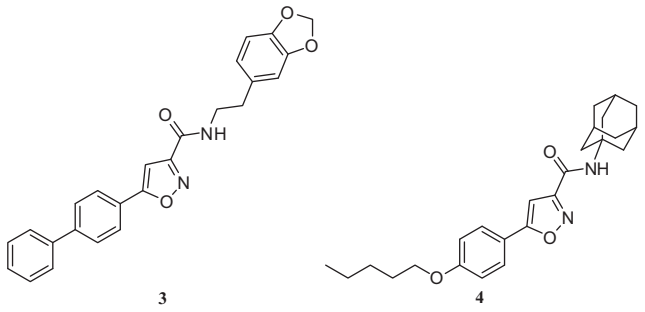


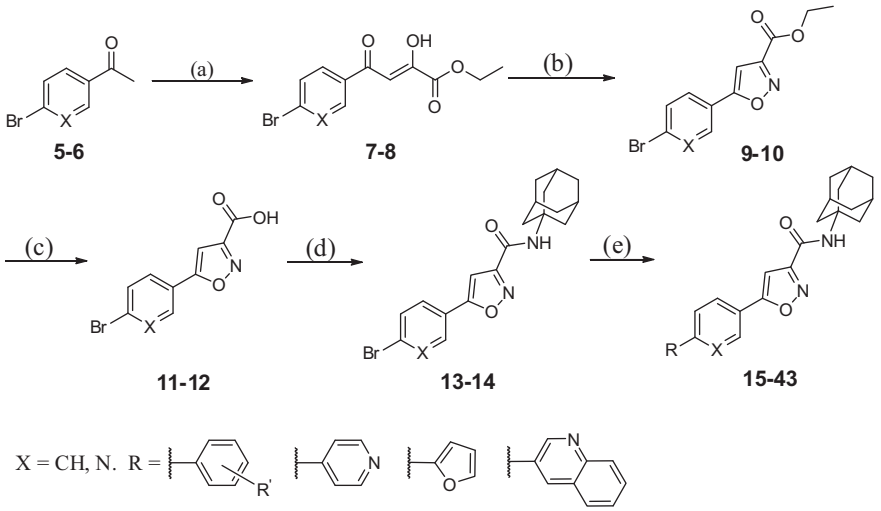
Figure 2. Structures of previously synthesized FAAH inhibitors **3** and **4**.

a carbamylated-enzyme adduct. To date, URB597 is considered as one of the most potent FAAH inhibitors with an IC_{50} of 4.6 nM.[15,16]

As previously reported by our research group, 3-carboxamido-5-arylisoxazole has been regarded as an interesting scaffold for developing FAAH inhibitors.[21,22] Typically, compound **3** (IC_{50} = 88 nM, Fig. 2) displayed a potent FAAH inhibitory capacity in vitro and produced protective efficacy on a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis mouse model.[21,22] Recently, different pharmacomodulations were carried out on this series.[22] The replacement of the benzodioxol ethyl group appended to the carboxamide function by an adamant-1-yl group and the substitution of the terminal phenyl ring by a pentoxy group led to a substantial increase of FAAH inhibitory efficacy (**4**, IC_{50} = 8 nM, Fig. 2). Compound **4** produced anti-inflammatory effect in a dextran sulfate sodium (DSS)-induced acute colitis model in mice.[22]

In this context, a novel series of compounds based on this interesting scaffold was designed. The replacement of the phenyl moiety of compound **4** by a pyridine group or the introduction of different aromatic substituents instead of the pentoxy group at the *para* position of the phenyl ring is supposed to optimize physicochemical parameters (such as Log*P*).

The target compounds were obtained in five steps. The Claisen condensation of aromatic ketones **5** and **6** with diethyl oxalate in the presence of sodium ethanolate afforded β-diketoesters **7** (47%) and **8** (39%), respectively. It has been demonstrated that these β-diketoesters are in their enol form, which is probably due to the formation of an internal hydrogen bond.[23–25] Then, cyclization reaction was performed to convert compounds **7** and **8** into 5-aryl isoxazole-3-carboxylates **9** (78%) and **10** (71%) by addition of hydroxylamine hydrochloride in ethanol at reflux. Furthermore,



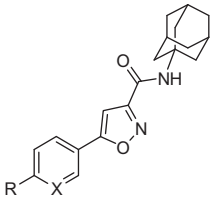
Scheme 1. Synthesis of *N*-(adamantan-1-yl)-5-aryl isoxazole-3-carboxamides **15**–**43**. Reagents and conditions: (a) Diethyl oxalate, sodium ethanolate, ethanol, reflux 6 h, 39–47%. (b) Hydroxylamine hydrochloride, ethanol, reflux, 2 h, 71–78%. (c) Sodium hydroxide, 95% ethanol, rt, overnight, 95–98%. (d) HBTU, HOBt, DIEA, 1-adamantanamine hydrochloride, DCM, rt, 12 h, 53–67%. (e) Aryl boronic acid or boronic acid pinacol ester, potassium carbonate, tetrakis (triphenylphosphine) palladium(0), DMF, H_2O, MW, 100 °C, 30 min, 76–89%.

saponification into 5-aryl isoxazole-3-carboxylate carboxylic acids **11** (98%) and **12** (95%) was carried out, followed by amidification with 1-adamantanamine hydrochloride to achieve to precursor compounds **13** (67%) and **14** (53%). Finally, a tetrakis (triphenylphosphine)palladium(0)-catalyzed Suzuki reaction with different aromatic boronic acids or boronic acid pinacol esters afforded *N*-(adamantan-1-yl)-5-aryl isoxazole-3-carboxamide derivatives **15**–**43** with good yields (76–89%) (Scheme 1).

The *N*-(adamantan-1-yl)-5-aryl isoxazole-3-carboxamides **15**–**43** and reference compounds (**4** and URB597) were assessed for their ability to inhibit the hydrolysis of 7-amino-4-methyl coumarin-arachidonamide (AMC-AA), a fluorescent FAAH substrate, by human recombinant FAAH (expressed in SF21 cells) after a 15 min preincubation with 4 U/mL of enzyme at 37 °C in Tris buffer pH 9.[26] All the compounds were firstly screened at the concentration of 10 μM. The half maximal inhibitory concentrations (IC_{50}) were determined for compounds exhibiting a specific inhibition superior to 50% for FAAH.

As illustrated in Table 1, among all synthesized compounds, biphenyl derivatives **15**, **21**, **25**, **26**, and **28** with substitutions at the 3-position possessed the best FAAH inhibitory capacity. These 5 compounds demonstrated a good activity against FAAH with IC_{50} values in a submicromolar range. Nevertheless, when replacing the phenyl ring appended to the isoxazole by a pyridinyl ring (**37**–**43**), a decrease of activity was observed. Indeed, these compounds manifested less than 25% FAAH inhibition. Moreover, compounds with a pyridinyl group (**19**), a quinolinyl group (**34**) or a furanyl group (**35**) on the phenyl ring manifested slight inhibition activity against FAAH from 17% to 31% inhibition. When the substituent on the terminal phenyl ring is bearing at the 2-position (**20**, 47% inhibition), a slight decrease of inhibitory activity was observed in comparison with the corresponding 3-substituted derivative **21** (58% inhibition). The FAAH inhibitory capacity of the molecules was almost completely lost when the substituent is bearing at the 4-position. For instance, compounds **16**, **22**, **27**, and **31** were determined to exert less than 25% inhibition of FAAH. Additionally, biphenyl derivatives bearing a carbamoyl substituent either at 3-position (17) or at 4-position (18) were determined to show no obvious activity towards FAAH (<15%). Furthermore, the introduction of an additional methoxy group at the 4-position of compound **21** led to a sharp decrease of inhibitory capacity from 58% to 14% (**23**). Surprisingly, the introduction of a third methoxy

Table 1
Percentage of FAAH inhibition at 10 μM, half-maximum inhibitory concentration (IC_{50}) towards human recombinant FAAH and cytotoxicity on HT29 and HEK293 cells at 10 μM of compounds **15**–**43**, and reference compounds **4** and URB597



Compounds	X	R	FAAH % inhibition at 10 μM	FAAH IC_{50}[a] (μM)	HT29 % inhibition at 10 μM	HEK293 % inhibition at 10 μM
4	CH	Pentoxy	79	0.46 (0.008)[b]	N.D.[c]	N.D.[c]
15	CH	3-Acetylphenyl	56	0.59 ± 0.02	1	16
16	CH	4-Acetylphenyl	18		4	5
17	CH	3-Carbamoylphenyl	11		0	3
18	CH	4-Carbamoylphenyl	10		0	4
19	CH	Pyridin-4-yl	17		1	9
20	CH	2-Methoxyphenyl	47		2	25
21	CH	3-Methoxyphenyl	58	0.72 ± 0.04	0	20
22	CH	4-Methoxyphenyl	15		0	2
23	CH	3,4-Dimethoxyphenyl	14		7	29
24	CH	3,4,5-Trimethoxyphenyl	42		6	32
25	CH	3-Cyanophenyl	64	0.24 ± 0.07	0	0
26	CH	3-(Morpholinomethyl)Phenyl	69	0.83 ± 0.08	0	38
27	CH	4-(Morpholinomethyl)phenyl	16		0	20
28	CH	3-(4-Methyl-1-piperazinylmethyl)phenyl	63	1.13 ± 0.06	97	93
29	CH	4-(4-Methyl-1-piperazinylmethyl)phenyl	41		90	89
30	CH	3-(Morpholine-4-carbonyl)phenyl	49		10	54
31	CH	4-(Morpholine-4-carbonyl)phenyl	24		0	23
32	CH	3-(4-Methyl-1-piperazinylcarbonyl)phenyl	44		55	47
33	CH	4-(4-Methyl-1-piperazinylcarbonyl)phenyl	23		24	37
34	CH	Quinolin-3-yl	22		0	24
35	CH	Furan-2-yl	31		0	12
36	CH	3,4-(Methylenedioxy)phenyl	18		0	9
37	N	phenyl	23		0	14
38	N	3-Acetylphenyl	22		0	18
39	N	4-Acetylphenyl	13		0	5
40	N	3-Carbamoylphenyl	20		14	1
41	N	Pyridin-4-yl	23		0	1
42	N	4-(Morpholine-4-carbonyl)phenyl	12		0	10
43	N	4-(4-Methyl-1-piperazinylcarbonyl)phenyl	10		0	3
URB597			98	0.26 ± 0.07	0	0

[a] IC_{50} values were obtained after a 15 min preincubation with the enzyme by a fluorogenic assay. Data represent the mean ± SEM of three experiments performed in duplicate.
[b] Data from Ref. 22. Evaluated for the ability to inhibit the hydrolysis of [^{3}H]-AEA by a recombinant human FAAH (expressed in *Escherichia coli*).
[c] N.D. means not determined.

Table 2
Calculated *C*Log*P* values of 10 representative compounds bearing a 3-substituted phenyl ring

Compounds	**15**	**17**	**21**	**25**	**26**	**28**	**30**	**32**	**38**	**40**
*C*Log*P*[a]	5.73	4.80	6.21	5.72	6.04	6.60	4.92	5.48	4.58	3.71

[a] *C*Log*P* was calculated by SYBYL-X 2.0 (Tripos).

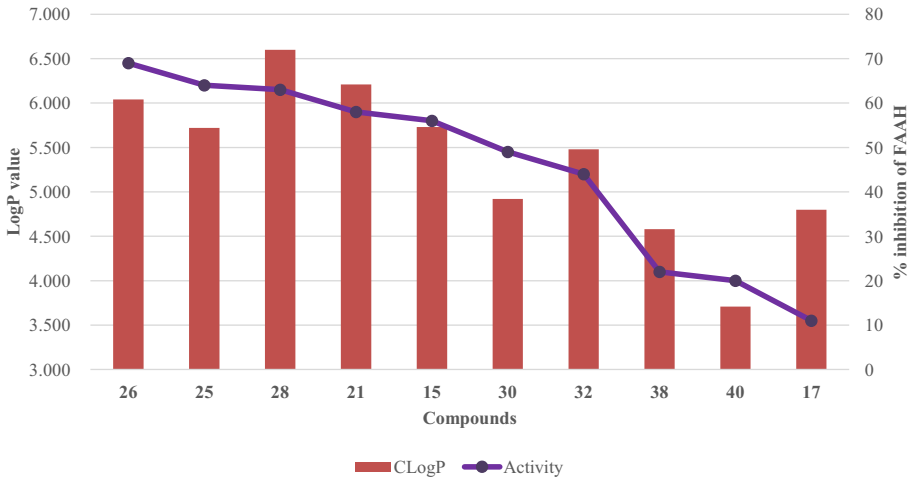


Figure 3. Lipophilicity–activity relationships of 10 representative compounds bearing a 3-substituted phenyl ring.

group at the 5-position of the terminal phenyl ring of compound **23** brought about an obvious recovery of inhibitory potency to 42% (**24**). Moreover, the replacement of the methylene function appended to the terminal phenyl ring of compounds **26** (69% inhibition) and **28** (63% inhibition) by a carbonyl function led to a substantial attenuation of the inhibitory activity to 49% (**30**) and 44% (**32**), respectively. Overall, a phenyl group is more favorable than other aromatic groups (such as pyridinyl, quinolinyl, and furanyl groups) for the design of FAAH inhibitors. Substituents at position 3 are more favorable than substituents at position 4. Specifically, acetyl, morpholinomethyl, cyano, methoxy, and 4-methyl-1-piperazinylmethyl substituents are preferred rather than a carbamoyl group. Notably, compound **15** (IC_{50} = 0.59 μM) possessed similar FAAH inhibitory capacity in comparison with compound **4** (IC_{50} = 0.46 μM), a previously identified potent FAAH inhibitor in our group. The replacement of the acetyl group of compound **15** by a cyano group (**25**) brought about an impressive improvement of inhibitory potency against FAAH. Compound **25** is approximately 2 times more potent against FAAH than compounds **4** and **15**. This compound manifests a moderate efficacy (maximum effect that a drug can produce, 64% inhibition at 10 μM concentrations) but a good potency (amount required to produce a given effect, IC_{50} = 0.24 μM). It shows a similar inhibitory potency against FAAH than the positive control URB597 (IC_{50} = 0.26 μM), one of the most potent FAAH inhibitors to date.

Cytotoxicity of these compounds was determined at 10 μM using a cell proliferation assay on human colorectal adenocarcinoma cells HT29 and human embryonic kidney cells HEK293. This test is based on a colorimetric method, which measures the activity of cellular enzymes that reduce the tetrazolium dye (MTS, uncolored) to its insoluble formazan giving a purple color. This assay measures cellular metabolic activity via NADPH-dependent cellular oxidoreductase enzymes and reflects, under defined conditions, the number of viable cells. No cytotoxicity was observed for our new compounds on HT29 cells except for compounds with a piperazinyl moiety (**28**, **29**, **32**, and **33**). These 4 compounds inhibited also proliferation of HEK293 cells.

An investigation of the lipophilicity–activity relationships of compounds with substituents at the 3-position on the terminal phenyl ring was performed. Lipophilicity is an important property in drug discovery and the prediction of bioavailability.[27] The logarithm of the octanol/water partition coefficient (Log*P*) is widely used to assess the lipophilicity of molecules. In terms of different calculation rules, Log*P* values can be calculated by atomic methods (such as *A*Log*P* and *X*Log*P*), which consider the contribution of each atom; fragmental methods (such as *C*Log*P*, ACD/Log*P* and KowWIN), which consider the contribution of each small fragment in a molecule; or property-based methods (such as *M*Log*P*), which is based on the three-dimensional (3D) structures of the molecules and topological approaches.[27] Actually, *C*Log*P* was identified to display more accurate prediction of Log*P* in a wide range of 108 compounds, in comparison with ACD/Log*P* and KowWIN,[28] Hence, Log*P* values of our compounds were calculated by *C*Log*P* method (Table 2). As represented in Figure 3, our 5 most potent FAAH inhibitors (**15**, **21**, **25**, **26**, and **28**) possessed high *C*Log*P* values ranging from 5.5 to 6.6. Conversely, compounds with a low FAAH inhibitory capacity (**17**, **30**, **32**, **38**, and **40**) possessed *C*Log*P* values below 5.5. This result indicates that compounds with substituents at the 3-position of the terminal phenyl ring conferring higher Log*P* values (such as acetyl, methoxy, and cyano group) showed better FAAH inhibition potency. Hence, the low inhibitory capacity of compound **17** (*C*Log*P* = 4.80) with a carbamoyl group might be attributed to its weak lipophilicity. The replacement of the methylene linker of compounds **26** (*C*Log*P* = 6.04) and **28** (*C*Log*P* = 6.60) with a carbonyl linker (**30**, *C*Log*P* = 4.92, **32**, *C*Log*P* = 5.48) and the replacement of the phenyl ring of compound **15** (*C*Log*P* = 5.73) by a pyridine ring (**38**, *C*Log*P* = 4.58) led to a substantial attenuation of the FAAH inhibitory activity probably because of the decrease of lipophilicity.

In conclusion, a series of new 3-carboxamido-5-aryl-isoxazoles was designed, synthesized and screened for their inhibitory potency against FAAH. Their cytotoxicity was determined and their lipophilicity was calculated. Compounds bearing a 3-substituted biphenyl moiety with high Log*P* values displayed a good inhibitory

percentage against FAAH. Compound **25** was identified as a potent FAAH inhibitor devoid of cytotoxicity on HT29 and HEK293 cells. Its good biological activity is probably due to its suitable lipophilicity. This compound possesses a FAAH inhibitory potency comparable to URB597, one of the most potent FAAH inhibitor identified to date.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.004.

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