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# A new group of oxime carbamates as reversible inhibitors of fatty acid amide hydrolase

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ABSTRACT

A series of oxime carbamates have been identified as potent inhibitors of fatty acid amide hydrolase (FAAH), an important regulatory enzyme of the endocannabinoid signaling system. Kinetic analysis indicates that they behave as non-competitive, reversible inhibitors, and show remarkable selectivity for FAAH over the other components of the endocannabinoid system.



Fatty acid amide hydrolase (FAAH) is an enzyme bound to intracellular membranes, mainly of the endoplasmic reticulum,[1] that catalyzes the degradation of *N*-arachidonoylethanolamine (anandamide,[2] AEA), the intestinal satiety factor *N*-oleoylethanolamine (OEA)[3,4] the peripheral analgesic and anti-inflammatory factor *N*-palmitoylethanolamine (PEA)[5] and the sleep-inducing substance oleoylamide (OA).[6] AEA, other congeners termed 'endocannabinoids' and the 'endocannabinoid-like' compounds OEA, PEA and OA, are a growing family of lipid signals that exert several biological activities, including anti-cancer, anti-ischemic, anti-inflammatory, anti-depressant, analgesic, anxiolytic, anorectic and bone-stimulant actions (to name just a few).[7] Some of the effects of endocannabinoids are mediated via the type-1 (CB1R) or type-2 (CB2R) cannabinoid receptors, while others may involve additional targets like the transient receptor potential vanilloid-1 (TRPV1)[7] and peroxisome proliferator-activated receptors.[6] Among different metabolic pathways that can modulate the endogenous tone of endocannabinoids, and hence their biological actions,[8] FAAH has emerged as a key player. In fact, FAAH inactivation by either genetic ablation of the *faah* gene[9] or chemical inhibition[10] increases the level of fatty acid amides, and consequently their central and peripheral activities.

FAAH belongs to an unusual class of serine hydrolases, that utilizes a serine–serine–lysine catalytic triad, firmly identified in 2002 when crystal structure of FAAH was reported.[11] The residues of the catalytic triad (Ser241, Ser217 and Lys142, where Ser241 is the nucleophilic residue) interact through a network of hydrogen bonds that facilitates proton exchange and the subsequent hydrolysis of the amide bond.

In the last decade these findings have boosted the search for potent, selective FAAH inhibitors, as potential therapeutics for the treatment of pain, inflammation, anxiety, depression, and other central nervous system disorders.[12,13] Important classes of FAAH inhibitors that have been recently disclosed are α-ketoheterocycles,[14,15] ureas,[16] (thio)hydantoins,[17] *O*-alkyl[18] or *O*-aryl[19] carbamates, and carbamoyl tetrazoles.[20]

A study based on the X-ray structure of FAAH bound to methoxyarachidonoyl-fluorophosphonate (MAFP)[11] and molecular modeling of carbamate-based inhibitors, led Kathuria et al. to URB597 (**1**), a phenol carbamate endowed with potent FAAH inhibition and analgesic and anti-inflammatory activity.[21] Mass spectral studies

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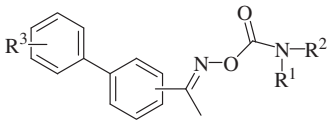
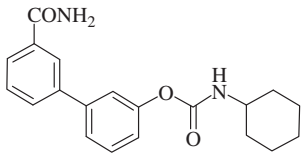


**Chart 1.**

indicated that the SAR in this series might be driven by the ability of the phenol group to leave the molecule.[22]



URB597

Other carbamates of alcohols were reported by Sanofi-Aventis[23–27] and carbamates of oximes in patents[28,29] and in a most recent paper by Bristol Myers Squibb.[30] The latter publication appeared during the preparation of this Letter, and described FAAH inhibition by a series of oxime carbamates that differ from those reported here. In fact, in this study we report the synthesis and the FAAH inhibiting activity of oxime carbamates characterized by an *N*-aryl or alkyl carbamic acid *O*-iminobiphenyl or *O*-iminoheteroaryl ester moiety (Chart 1).

These compounds were designed on the hypothesis that the carbamate of an oxime, a good leaving group, would be rapidly hydrolysed by FAAH, and that the biphenyl group would be easily accommodated in the enzyme active site, by analogy with the URB class of compounds.

All the oximes (**3**, **9** and **12**) were synthesized by reaction of the corresponding ketones with hydroxylamine in ethanol. Oxime **9** was prepared by a Suzuki coupling between bromobenzene or 2-bromothiophene and 3-acetylbenzeneboronic acid, followed by reaction with hydroxylamine (Scheme 2), whereas oxime **12** was obtained by a Suzuki coupling between 3-bromobenzonitrile and 3-acetylbenzeneboronic acid, followed by hydrolysis with sodium perborate and condensation with hydroxylamine (Scheme 3).

The configuration of the ketoximes was assigned as *E* (which anyway is the preferred one in the synthesis[30]) on the basis of the chemical shift of the methyl group (>2.28 ppm)[31] in the $^{1}$H NMR, and in $^{13}$C NMR (12.6 ppm), which reflects its *syn* relationship to the OH function.[32] Similar findings have been reported by Buchanan[33] and Fraser.[34]

Treatment of the oximes with a suitable carbamoyl chloride yielded compounds **4a**, **10g–h**, **10k** (70–94% yield) (Schemes 1 and 2), whereas treatment with an appropriate isocyanate gave compounds **4b–f**, **5**, **6**, **10a–f**, **10i–j**, **13a–b** (50–96% yield) (Schemes 1–3, Chart 2) (see Supplementary data for details).

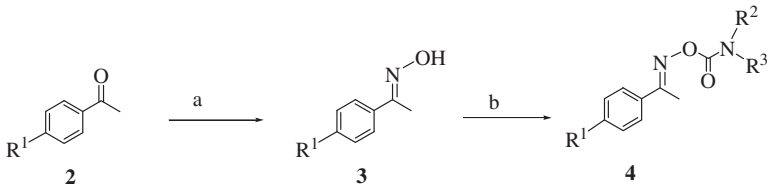
Compound **6** was synthesized by addition of phenyl isocyanate to 4-phenylcyclohexanone oxime (96%) (Chart 2).

In the first set of experiments the ability of all compounds to inhibit FAAH was tested (see Supplementary data for details). The $IC_{50}$ values of FAAH inhibition are reported in Table 1.

Firstly, a small set of biphenyl-4-yl derivatives (**4a–f**) was examined. The results showed that the *N*,*N*′-dimethyl substituted derivative (**4a**) did not exhibit any significant activity, differently from what happens for the oximes reported by Sit,[30] whereas the introduction of a phenyl or a naphthyl group on the carbamate nitrogen gave compounds with submicromolar $IC_{50}$'s (**4b** and **4c**).

Substitution of the *para*-biphenyl system with different rings (**4d–f**, **5** and **6**) was detrimental to the activity, leading to inactive compounds, with the only exception of **4d**.

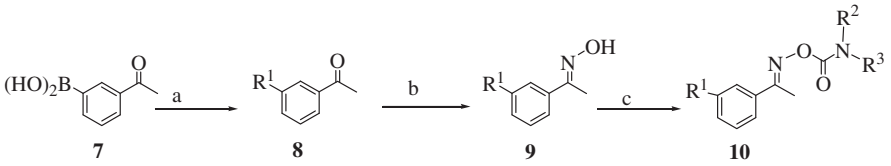
Secondly, the introduction of a biphenyl-3-yl system with a *meta* distal aromatic ring in place of the *para* phenyl ring was explored (compds **10**). The results indicated that the *meta*-substituted compounds were significantly more active than the corresponding *para* isomers (**10a** vs **4b**, **10b** vs **4c**), compound **10b** being ~40-fold more potent than **4c**. Compound **13a**, with a polar group in the *meta*-position of the distal phenyl ring, showed inhibitory activity comparable to that of the corresponding unsubstituted derivative **10a**. Compounds **10c** and **10d**, possessing a *p*-fluoro and *p*-methoxy substituted *N*-phenyl ring, maintained a good inhibitory potency, while the introduction of bulky groups on the carbamate nitrogen (**10h**, **10e**) was again detrimental to activity. Substitution with a piperidine ring (**10g**) or with a more extended fragment (**10f**) restored a certain degree of inhibitory action. The most potent compound of the series was derivative **10i** ($IC_{50}$ 8 nM), with a thiophene ring in place of the distal phenyl ring. In keeping with the other results on biphenyl-4-yl derivatives, the corresponding *para* isomer (**4d**) was found to be much less effective on FAAH activity. This preliminary SAR study clearly indicates the preference for a 'bent' (compds **10** vs **4**) group of two aromatic or heteroaromatic rings, whereas a lipophilic group (piperidino, but also phenyl, or a hydrocarbon chain) attached to the nitrogen of the carbamate is important for activity. As for the binding mode of our compounds to FAAH, their structural similarity with URB597 would suggest the occupation of the cytosolic access (CA) channel by the biphenylyl moiety, with the N-terminal moiety positioned in the acyl-chain-binding (ACB) channel of the enzyme. This hypothesis would indicate an interaction with the enzyme site



**2** **3** **4**

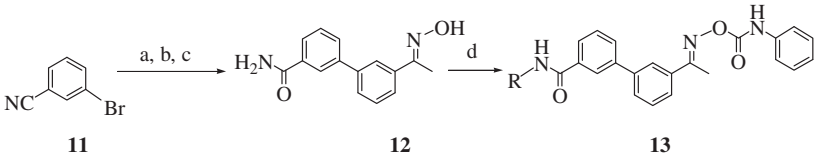
**4a** $R^1$ = Ph; $R^2$ = Me; $R^3$ = Me
**4b** $R^1$ = Ph; $R^2$ = H; $R^3$ = Ph
**4c** $R^1$ = Ph; $R^2$ = H; $R^3$ = 1-naphthyl
**4d** $R^1$ = 2-thiophenyl; $R^2$ = H; $R^3$ = Ph
**4e** $R^1$ = cyclohexyl; $R^2$ = H; $R^3$ = 1-naphthyl
**4f** $R^1$ = 2,5-dimethyl-pyrrol-1-yl; $R^2$ = H; $R^3$ = 1-naphthyl

**Scheme 1.** Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, EtOH, 2 N HCl, reflux; (b) for **4b–f**: aryl isocyanate, toluene, reflux (50–96%); for **4a**: *N*,*N*′-dimethylcarbamoyl chloride, reflux, 30 min, 70%.



**10a** $R^1$ = Ph; $R^2$ = H; $R^3$ = Ph
**10b** $R^1$ = Ph; $R^2$ = H; $R^3$ = 1-naphthyl
**10c** $R^1$ = Ph; $R^2$ = H; $R^3$ = 4-fluorophenyl
**10d** $R^1$ = Ph; $R^2$ = H; $R^3$ = 4-methoxyphenyl
**10e** $R^1$ = Ph; $R^2$ = H; $R^3$ = 5-benzo[*b*]thiophenyl
**10f** $R^1$ = Ph; $R^2$ = H; $R^3$ = -$(CH_2)_5$-Ph
**10g** $R^1$ = Ph; $R^2$, $R^3$ = -$(CH_2)_5$-
**10h** $R^1$ = Ph; $R^2$ = Ph; $R^3$ = -Ph
**10i** $R^1$ = 2-thiophenyl; $R^2$ = H; $R^3$ = Ph
**10j** $R^1$ = 2-thiophenyl; $R^2$ = H; $R^3$ = $C_{12}H_{25}$
**10k** $R^1$ = 2-thiophenyl; $R^2$, $R^3$ = -$(CH_2)_5$-

**Scheme 2.** Reagents and conditions: (a) Bromobenzene or 2-bromothiophene, $Pd(PPh_3)_4$, 1 M $Na_2CO_3$, dioxane, $N_2$, reflux, 3–8 h; (b) $NH_2OH{\cdot}HCl$, 2 N HCl, EtOH, reflux; (c) aryl isocyanate, toluene, reflux (50–90%) or carbamoylchloride, reflux, 30 min (70–94%).



**13a** R = H
**13b** R = PhNHCO

**Scheme 3.** Reagents and conditions: (a) $ArB(OH)_2$, $Pd(PPh_3)_4$, 1 M $Na_2CO_3$, dioxane, $N_2$, reflux (90%); (b) $NaBO_3{\cdot}4H_2O$, $H_2O$, dioxane, reflux (51%); (c) $NH_2OH{\cdot}HCl$, EtOH, $N_2$, HCl, reflux (64%); (d) phenyl isocyanate, toluene, reflux.

**Chart 2.**

analogous to that supposed by Sit et al.[30] for their compounds with the same key functional group.

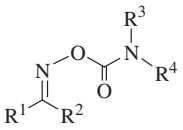
We decided to further characterize FAAH inhibition by the most potent compound, **10i**. First of all, this substance was found to be chemically stable at pH 1.5 (yielding a recovery of 69 ± 1% after 3 h at 37 °C) and pH 8.4 (recovery of 98 ± 1% after 6 h at 37 °C), that mimicked stomach and intestine environment, respectively. Then, we found that compound **10i** inhibited FAAH reversibly, because ~80% of enzyme activity was recovered after 18 h dialysis of enzyme-inhibitor complexes in the presence of an excess (10 μM) of inhibitor (Fig. 1a). On the contrary, under similar dialysis conditions of FAAH–URB597 complexes, Karbarz et al. demonstrated that this substance is an irreversible inhibitor of enzyme activity.[35] We also performed time-dependent inhibition experiments, by pre-incubating FAAH with compound **10i** at 10 μM concentration for different time periods, and then measuring substrate turnover.[36] The results demonstrated that inhibition by compound **10i** was not time-dependent (Fig. 1b), further suggesting that it hits as a reversible inhibitor of FAAH. Under the same experimental conditions, URB597 inhibited FAAH in a time-dependent manner,[37] confirming that URB597 is a covalent and irreversible inhibitor of enzyme activity.[35,38]

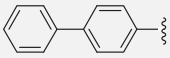
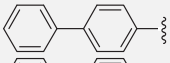
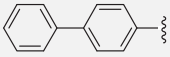
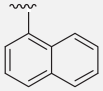
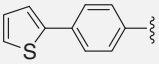
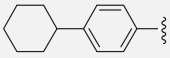
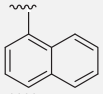
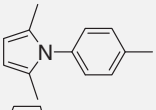
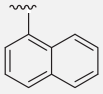
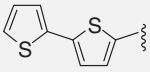
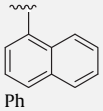
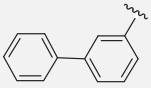
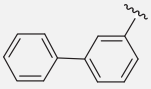
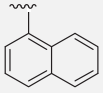
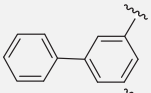
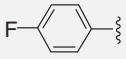
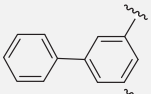
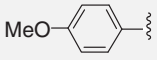
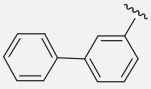
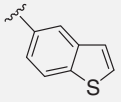
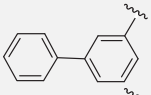
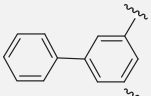
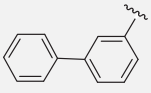
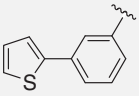
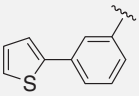
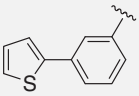
Next, we ascertained by standard substrate-velocity plots that compound **10i** was a non-competitive inhibitor of FAAH with respect to the natural substrate AEA (Fig. 2a). In fact, compound **10i** markedly reduced the maximal velocity ($V_{max}$) without affecting the Michaelis–Menten constant ($K_m$) of the reaction, a typical effect of canonical non-competitive inhibitors.[37] The decrease in $V_{max}$ without effect on $K_m$ could be visualized also by Lineweaver–Burk analysis of double-reciprocal plots of the kinetic data (Fig. 2b). Interestingly, also Sit et al. found that the most active oxime carbamate of their series, compound **50**, was a reversible inhibitor of FAAH.[29] However, this compound acted as a competitive rather than non-competitive inhibitor, likely because of the different structure.

The apparent $K_i$ of compound **10i** towards FAAH, calculated from Michaelis–Menten plots, is shown in Table 2.

Unlike direct agonists of CB1R, FAAH inhibitors do not evoke classical effects triggered by this receptor (e.g., catalepsy, hypothermia, and hyperphagia), nor are they coupled by unwanted side

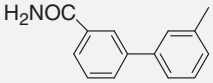
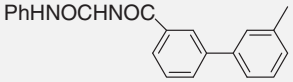
**Table 1**
Inhibition of FAAH by oxime carbamates



| Compd | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **4a** |  | $CH_3$ | $CH_3$ | $CH_3$ | >50,000 |
| **4b** |  | $CH_3$ | Ph | H | 760 ± 100 |
| **4c** |  | $CH_3$ |  | H | 401 ± 60 |
| **4d** |  | $CH_3$ | Ph | H | 200 ± 20 |
| **4e** |  | $CH_3$ |  | H | >1000 |
| **4f** |  | $CH_3$ |  | H | >1000 |
| **5** |  | $CH_3$ |  | H | >1000 |
| **6** | $-(CH_2)_2-CH(Ph)-(CH_2)_2-$ | | Ph | H | >50,000 |
| **10a** |  | $CH_3$ | Ph | H | 69 ± 6 |
| **10b** |  | $CH_3$ |  | H | 10 ± 1 |
| **10c** |  | $CH_3$ |  | H | 17 ± 2 |
| **10d** |  | $CH_3$ |  | H | 28 ± 3 |
| **10e** |  | $CH_3$ |  | H | 232 ± 35 |
| **10f** |  | $CH_3$ | $-(CH_2)_5-Ph$ | H | 100 ± 10 |
| **10g** |  | $CH_3$ | $-(CH_2)_5-$ | | 220 ± 30 |
| **10h** |  | $CH_3$ | Ph | Ph | >50,000 |
| **10i** |  | $CH_3$ | Ph | H | 8 ± 1 |
| **10j** |  | $CH_3$ | $C_{12}H_{25}$ | H | 116 ± 12 |
| **10k** |  | $CH_3$ | $-(CH_2)_5-$ | | 112 ± 17 |

(*continued on next page*)

**Table 1** (*continued*)

| Compd | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **13a** |  | $CH_3$ | Ph | H | 84 ± 8 |
| **13b** |  | $CH_3$ | Ph | H | >25,000 |

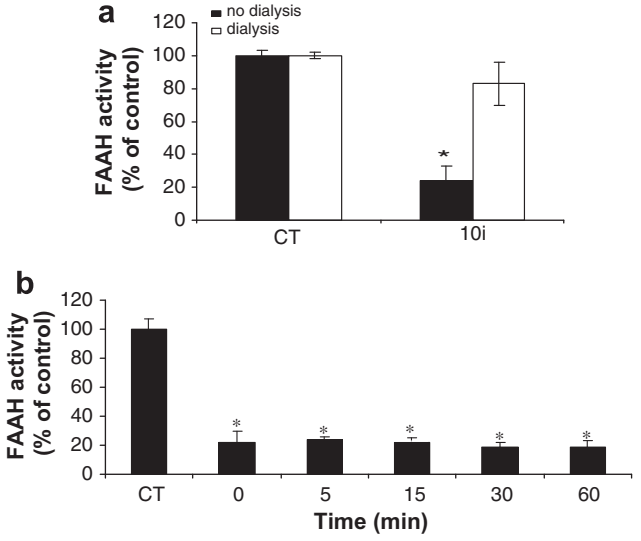



**Figure 1.** (a) Residual FAAH activity pre- and post-dialysis (18 h) at 4 °C. FAAH was pre-incubated for 20 min with compound **10i** at an excess concentration of 10 μM, and then was dialyzed against 2 L of 20 mM Tris–HCl buffer (pH 7.0). Residual FAAH activity was assayed as described in Supplementary data. Control values (CT) before and after dialysis were 929 ± 29 pmol/min per mg of protein, and 292 ± 5 pmol/min per mg of protein, respectively. Results are the means ± S.D. of three independent experiments. (b) FAAH activity was assayed as described in Supplementary data, after pre-incubation with 10 μM **10i** at room temperature. Activity values were expressed as pmol/min per mg of protein, and as percentage of controls in brackets (100% = 942 ± 66 pmol/min per mg of protein). Results are the means ± S.D. of three independent experiments. $^*p$ <0.001 versus CT.

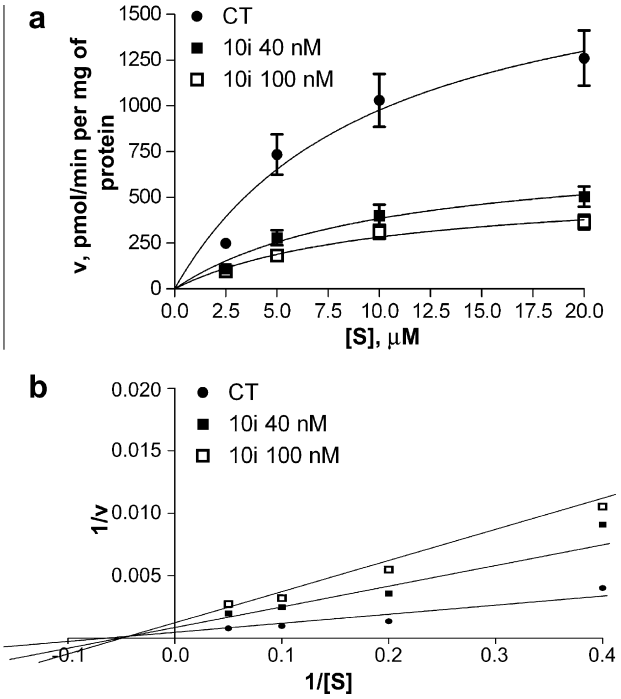



**Figure 2.** (a) Michaelis–Menten curves of FAAH activity, determined by using different concentrations of substrate ([$^3$H-ethanolamine]AEA, 0–20 μM range) and inhibitor (40 and 100 nM). Results are the means ± S.D. of three independent experiments. Non-linear regression analysis of the kinetic data allowed to calculate the following $K_m$ (μM) and $V_{max}$ (pmol/min per mg of protein) values for [$^3$H]anandamide hydrolysis: CT = 9.8 ± 1.2 and 1938 ± 213; **10i** 40 nM = 10 ± 1 and 779 ± 78; **10i** 100 nM = 10 ± 2 and 568 ± 57. The type of inhibition was non-competitive, because the difference between $V_{max}$ values of control and compound **10i** was statistically significant ($p$ <0.01), whereas that between $K_m$ values was not ($p$ >0.05). (b) Lineweaver–Burk analysis of the same Michaelis–Menten curves shown in panel A, further showing that the type of inhibition of compound **10i** was non-competitive. It should be recalled that in double-reciprocal plots (1/v vs 1/[S]) the intercept on the y axis corresponds to $1/V_{max}$, whereas that on the $x$ axis corresponds to $-1/K_m$.

effects typically due to CB1R activation. Thus, chemical inhibition of FAAH is considered a desirable alternative to CB1R agonists for therapeutic exploitation.[39,40] Of course, it is all the more important that the beneficial effects of increasing the endogenous tone of AEA through FAAH inhibition are not thwarted by unwanted modulation of other biological targets that recognize this endocannabinoid, like cannabinoid and vanilloid receptors, or the proteins that synthesize (*N*-acylphosphatidylethanolamide-phospholipase D, NAPE-PLD) and purportedly transport it (anandamide membrane transporter, AMT). These proteins form the so-called 'endocannabinoid system (ECS)', that includes other well-characterized elements like the enzymes responsible for synthesis (diacylglycerol lipase, DAGL) or degradation (monoacylglycerol lipase, MAGL) of 2-arachidonoylglycerol.[41] In this context, it is also noteworthy that AEA can have opposite biological effects when acting at different receptors (e.g., CBRs vs TRPV1), and a different activity compared to that of 2-arachidonoylglycerol.[42] Therefore, it is not surprising that interference of a drug directed towards a certain element of the ECS with any other element of the same system might prevent an effective therapeutic exploitation, for instance in the treatment of cancer,[39] or of neurodegenerative/neuroinflammatory diseases.[42] Overall, the promiscuity of drugs targeted towards a single element of the ECS, but able to interact with others, has raised concerns about their true efficacy as novel ther-

**Table 2**
Apparent $K_i$ values towards FAAH (nM), and inhibition ($IC_{50}$, nM) of the activity of the ECS elements by compound **10i**

| Compd | $K_i$ | CB1R | CB2R | TRPV1 | AMT | NAPE-PLD | MAGL | DAGL |
|---|---|---|---|---|---|---|---|---|
| **10i** | 38 ± 14 | >10,000 [1250] | 10,000 [1250] | >10,000 [1250] | 100 [12] | >10,000 [1250] | >10,000 [1250] | >10,000 [1250] |

Selectivity index values are reported in square brackets.

apeutics. On this basis, a careful evaluation of the selectivity of FAAH inhibitors towards other targets of the ECS seems a pre-requisite for effective drug development. For this reason, in a second set of experiments we analysed the interaction of compound **10i** with the other well-characterized components of the ECS, in order to calculate selectivity index values compared to FAAH (see Supplementary data for details). The results are summarized in Table 2.

From these data it can be concluded that **10i**, besides acting as a powerful FAAH inhibitor, also shows a good degree of selectivity towards the other elements of ECS, leading to $IC_{50}$'s ~1000-fold higher than those shown towards FAAH. Incidentally, it should be stressed that the ability of compound **10i** to inhibit the AEA membrane transporter AMT (Table 2) could simply reflect the contribution of FAAH to the transport process. In fact, hydrolysis by FAAH drives AEA uptake by creating and maintaining a concentration gradient across the plasma membrane.[40,43,44]

Finally, the analysis of the interference of compound **10i** with different off-targets revealed that the $IC_{50}$'s values towards 45 receptors, five ion channels and two transporters were always >10,000 nM, with the exception of seven targets that showed $IC_{50}$'s between 1000 and 10,000 nM (Table 1 in Supplementary data). Also these data speak in favor of a high degree of specificity of compound **10i** for FAAH over other potential unrelated targets.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.050.

## References and notes


1. Fezza, F.; De Simone, C.; Amadio, D.; Maccarrone, M. *Subcell. Biochem.* **2008**, *49*, 101.
2. Ahn, K.; McKinney, M. K.; Cravatt, B. F. *Chem. Rev.* **2008**, *108*, 1687.
3. Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946.
4. Lambert, D. M.; Vandevoorde, S.; Jonsson, K. O.; Fowler, C. J. *Curr. Med. Chem.* **2002**, *9*, 663.
5. Cravatt, B. F.; Prospero-Garcia, O.; Siuzdak, G.; Gilula, N. B.; Henriksen, S. J.; Boger, D. L.; Lerner, R. A. *Science* **1995**, *268*, 1506.
6. Fu, J.; Gaetani, S.; Oveisi, F.; Lo Verme, J.; Serrano, A.; Rodríguez De Fonseca, F.; Rosengarth, A.; Luecke, H.; Di Giacomo, B.; Tarzia, G.; Piomelli, D. *Nature* **2003**, *425*, 90.
7. Di Marzo, V. *Nat. Rev. Drug Disc.* **2008**, *7*, 438.
8. Okamoto, Y.; Tsuboi, K.; Ueda, N. *Vitam. Horm.* **2009**, *81*, 1.
9. Rodriguez de Fonseca, F.; Navarro, M.; Gomez, R.; Escuredo, L.; Nava, F.; Fu, J.; Murillo-Rodriguez, E.; Giuffrida, A.; LoVerme, J.; Gaetani, S.; Kathuria, S.; Gall, C.; Piomelli, D. *Nature* **2001**, *414*, 209.
10. Cravatt, B. F.; Demarest, K.; Patricelli, M. P.; Bracey, M. H.; Giang, D. K.; Martin, B. R.; Lichtman, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9371.
11. Bracey, M. H.; Hanson, M. A.; Masuda, K. R.; Stevens, R. C.; Cravatt, B. F. *Science* **2002**, *298*, 1793.
12. Maccarrone, M. *Curr. Pharm. Des.* **2006**, *12*, 759.
13. Petrosino, S.; Di Marzo, V. *Curr. Opin. Invest. Drugs* **2010**, *11*, 51.
14. Boger, D. L.; Miyauchi, H.; Du, W.; Hardouin, C.; Fecik, R. A.; Cheng, H.; Hwang, I.; Hedrick, M. P.; Leung, D.; Acevedo, O.; Guimaraes, C. R. W.; Jorgensen, W. L.; Cravatt, B. F. *J. Med. Chem.* **2005**, *48*, 1849.
15. De Martino, J. K.; Garfunkle, J.; Hochstatter, D. G.; Cravatt, B. F.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5842.
16. Ahn, K.; Johnson, D. S.; Mileni, M.; Beidler, D.; Long, D. J.; McKinney, M. K.; Weerapana, E.; Sadagopan, N.; Liimatta, M.; Smith, S. E.; Lazerwith, S.; Stiff, C.; Kamtekar, S.; Bhattacharya, K.; Zhang, Y.; Swaney, S.; Van Becelaere, K.; Stevens, R. C.; Cravatt, B. F. *Chem. Biol.* **2009**, *16*, 411.
17. Muccioli, G. G.; Fazio, N.; Scriba, E. K. G.; Poppitz, W.; Cannata, F.; Poupaert, H. J.; Wouters, J.; Lambert, M. D. *J. Med. Chem.* **2006**, *49*, 417.
18. Hertzog, D. L. *Expert Opin. Ther. Pat.* **2004**, *14*, 1435.
19. Tarzia, G.; Duranti, A.; Tontini, A.; Piersanti, G.; Mor, M.; Rivara, S.; Plazzi, P. V.; Park, C.; Kathuria, S.; Piomelli, D. *J. Med. Chem.* **2003**, *46*, 2352.
20. Maione, S.; Morera, E.; Marabese, I.; Ligresti, A.; Luongo, L.; Ortar, G.; Di Marzo, V. *Br. J. Pharmacol.* **2008**, *155*, 775.
21. Kathuria, S.; Gaetani, S.; Fegley, D.; Valiño, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La Rana, G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. *Nat. Med.* **2003**, *9*, 76.
22. Basso, E.; Duranti, A.; Mor, M.; Piomelli, D.; Tontini, A.; Tarzia, G.; Traldi, P. J. *Mass Spectrom.* **2004**, *39*, 1450.
23. Abouabdellah, A.; Bartsch-Li, R.; Hoornaert, C.; Ravet, A. WO 2005090292, 2005.
24. Abouabdellah, A.; Almario Garcia, A.; Hoornaert, C.; Li, A. T. WO 2005090322, 2005.
25. Abouabdellah, A.; Almario Garcia, A.; Hoornaert, C.; Lardenois, P.; Marguet, F. WO 2005090347, 2005.
26. Abouabdellah, A.; Almario Garcia, A.; Froissant, J.; Hoornaert, C. WO 2005077898, 2005.
27. Abouabdellah, A.; Bas, M.; Dargazanli, G.; Hoornaert, C.; Li, A. T.; Medaisko, F. WO 2004020430, 2004.
28. Sit, S.-Y.; Xie, K. WO 2002087569, 2002.
29. Sit, S.-Y; Kai, X.; Hongfeng, D. US 6,949,574, 2005.
30. Sit, S. Y.; Conway, C. M.; Xie, K.; Bertekap, R.; Bourin, C.; Burris, K. D. *Bioorg. Med. Chem. Lett.* **2010**, 1272.
31. Bachmann, W. E.; Barton, M. X. *J. Org. Chem.* **1938**, *3*, 300.
32. Smith, J. H.; Kaiser, E. T. *J. Org. Chem.* **1974**, *39*, 728.
33. Buchanan, G. W.; Dawson, B. A. *Can. J. Chem.* **1977**, *55*, 1437.
34. Fraser, R. R.; Capoor, R.; Bovenkamp, J. W.; Lacroix, B. V.; Pagotto, J. *Can. J. Chem.* **1983**, *61*, 2616.
35. Karbarz, M. J.; Luo, L.; Chang, L.; Tham, C. S.; Palmer, J. A.; Wilson, S. J.; Wennerholm, M. L.; Brown, S. M.; Scott, B. P.; Apodaca, R. L.; Keith, J. M.; Wu, J.; Breitenbucher, J. G.; Chaplan, S. R.; Webb, M. *Anesth. Analg.* **2009**, *108*, 316.
36. Wang, X.; Sarris, K.; Kage, K.; Zhang, D.; Brown, S. P.; Kolasa, T.; Surowy, C.; El Kouhen, O. F.; Muchmore, S. W.; Brioni, J. D.; Stewart, A. O. *J. Med. Chem.* **2009**, *52*, 170.
37. Gattinoni, S.; De Simone, C.; Dallavalle, S.; Fezza, F.; Nannei, R.; Amadio, D.; Minetti, P.; Quattrociocchi, G.; Caprioli, A.; Borsini, F.; Cabri, W.; Penco, S.; Merlini, L.; Maccarrone, M. *ChemMedChem* **2010**, *5*, 357.
38. Alexander, J. P.; Cravatt, B. F. *Chem. Biol.* **2005**, *12*, 1179.
39. Seierstad, M.; Breitenbucher, J. G. *J. Med. Chem.* **2008**, *51*, 7327.
40. Ahn, K.; Johnson, D. S.; Cravatt, B. F. *Expert Opin. Drug Discov.* **2009**, *4*, 763.
41. Maccarrone, M. In *Endocannabinoids: The Brain and Body's Marijuana*; Onaivi, E., Sugiura, T., Di Marzo, V., Eds.; CRC: Boca Raton, 2006. Chapter 20.
42. Centonze, D.; Finazzi-Agrò, A.; Bernardi, G.; Maccarrone, M. *Trends Pharmacol. Sci.* **2007**, *28*, 180.
43. McKinney, M. K.; Cravatt, B. F. *Annu. Rev. Biochem.* **2005**, *74*, 411.
44. Alexander, J. P.; Cravatt, B. F. *J. Am. Chem. Soc.* **2006**, *128*, 9699.