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Thiadiazolopiperazinyl ureas as inhibitors of fatty acid amide hydrolase

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ABSTRACT

A series of thiadiazolopiperazinyl aryl urea fatty acid amide hydrolase (FAAH) inhibitors is described. The molecules were found to inhibit the enzyme by acting as mechanism-based substrates, forming a covalent bond with Ser241. SAR and PK properties are presented.

The endogenous cannabinoid system consists of two well-defined G protein-coupled receptors, CB_1 (1990)[1] and CB_2, a family of lipid mediators, and several degradative enzymes which terminate the actions of these mediators by hydrolysis. Anandamide (AEA)[2] (Fig. 1) was the first endogenous substance discovered with activity at cannabinoid receptors, at both of which it is a weak partial agonist. Although exogenous substances active at the cannabinoid receptors (of which the prototype is the plant-derived substance Δ^9-THC, Fig. 1) have analgesic activity, their known side effects (including dysphoria, memory deficit and abuse liability) limit their therapeutic utility. Nevertheless, this observation does suggest that manipulating the endogenous system may produce analgesic benefit. In addition, the observation that endocannabinoid synthesis is selectively up-regulated in active neural pathways also suggests that this strategy may have a greater selectivity and lower side effect profile than global activation of cannabinoid receptors via exogenous agonists.

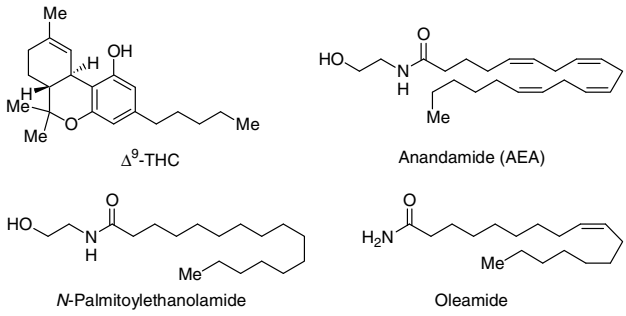

Figure 1. Δ^9–THC and substrates of FAAH.

Endogenous levels of AEA are normally very low, as it is rapidly metabolized by the enzyme Fatty Acid Amide Hydrolase (FAAH) to give ethanolamine and arachidonic acid.[3] FAAH also breaks down several other lipids including: oleoyl ethanolamide (OEA), palmitoyl ethanolamide (PEA), *O*-arachidonylethanolamine (virodamine), oleamide, and *N*-arachidonyldopamine.[4]

Although 2-AG is a full agonist at both CB_1 and CB_2 its primary route of breakdown is through the action of monoacylglycerol lipase (MAGL).[5] Of the other FAAH substrates, PEA is known to have anti-inflammatory properties and to exert analgesic effects through a non-cannabinoid pathway.[6] OEA is a known anorectic

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lipid that has effects on satiety.[7] Oleamide appears to be involved in sleep induction.[8] Less is known about the pharmacology of the other FAAH substrates.

FAAH knockout mice have elevated levels of AEA, PEA and OEA in the brain, suggesting that degradation by FAAH is the primary metabolic fate of these lipids.[9] The knockout mice are viable and healthy, but display attenuated responses in several pain assays, supporting the suggestion that a FAAH inhibitor would be an effective analgesic.

A number of groups have reported the preparation and testing of FAAH inhibitors (Fig. 2). Several series of highly potent α-keto heterocycles were described by Boger et al.[10] The ketoheterocycle OL-135 is thought to form a reversible tetrahedral intermediate derived from the interaction of the ketone to give a hemiketal. Piomelli et al.[11] disclosed carbamate-based inhibitors as did Sanofi.[12] The carbamate compounds are relatively potent and form a covalent bond with Ser 241 within the active site of FAAH.[13] URB597 and SA-47 also form a carbamate with the FAAH enzyme with the concomitant loss of a phenolic or alcoholic fragment, respectively. Both OL-135[14] and URB597[15] have been reported to be efficacious in the treatment of pain in various animal models without the motor impairment associated with direct CB_1 agonism.

We discovered the potent FAAH inhibitor (**1**) (Fig. 3) in an HTS screen of our chemical library.[16] Analogs of (**1**) could be prepared in three steps from an aryl amidine hydrochloride (Scheme 1).

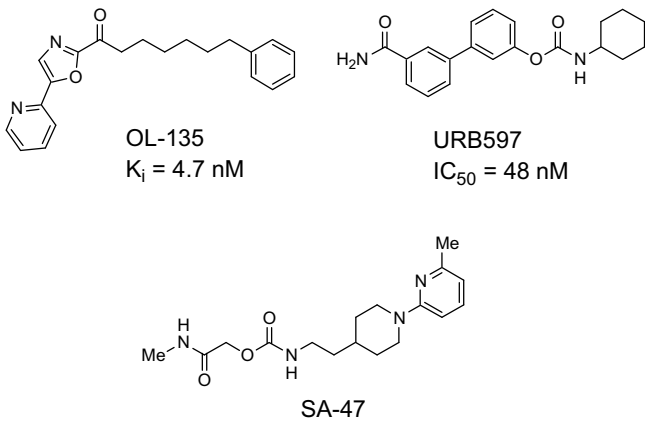


Figure 2. Known FAAH inhibitors.

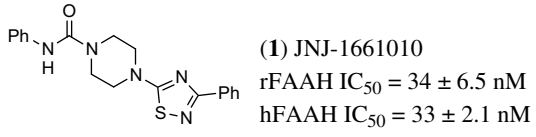
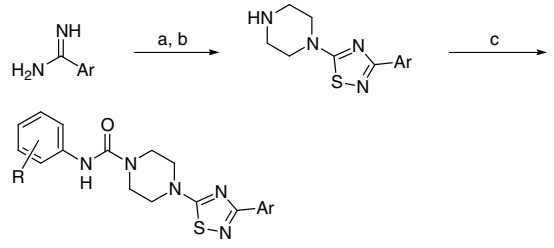


Figure 3. Initial HTS hit: JNJ-1661010. Values measured at 20 min ($N \geqslant 3$).



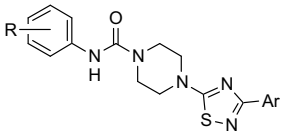
Scheme 1. Reagents and conditions: (a) CCl_3SCl, 6 N NaOH, CH_2Cl_2, 0 °C, 30 min; (b) piperazine, rt, 18 h, ≈50% for two steps; (c) R-PhNCO, CH_2Cl_2, 80%-quant. yield.

The amidine salts were treated with perchloromethyl mercaptan in strongly alkaline solution for 30 min at low temperature and then given a typical workup.[17] The crude products were treated with piperazine and stirred overnight. Treatment of the resultant piperazines with aryl isocyanates gave ureas in good yields (Table 1). Alternatively, the piperazines could be treated with phenyl carbamates of heteroaryl amines. The phenyl carbamates were prepared by treating an excess of the heteroaryl amine with phenyl chloroformate, either at room temperature or at reflux (Scheme 2). The yields of the phenyl carbamates varied considerably (Table 2).

The data presented in Table 1 indicate very tight SAR[18] on both of the pendant aryl rings. The phenyl ring attached to the urea nitrogen appears to be best if either unsubstituted (**1**) or with a chloro (**10**) or a fluoro (**13**) group in the 2-position. Other substituents yielded weakly active compounds.

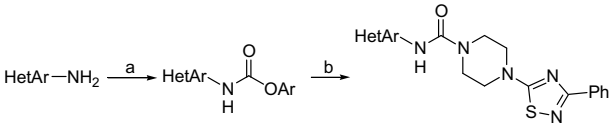
Despite the tight SAR observed for substituted phenyl groups on the urea nitrogen, many heteroaryl replacements for phenyl gave moderate to highly potent compounds (Table 2). In general, 6-membered ring heterocycles were more potent than 5-membered ring analogs. The most potent compound prepared, however, was

Table 1
SAR on pendant aryl groups



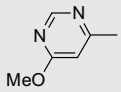
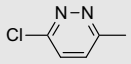
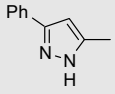
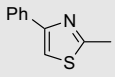
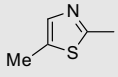
Compound	R	Ar	Apparent rFAAH IC_{50}[a] (nM)[b]	Apparent hFAAH IC_{50}[a] (nM)[b]
1	H	Ph	34 ± 6.5	33 ± 2.1
2	4-MeO	Ph	84 ± 15	152 ± 15
3	3-MeO	Ph	280 ± 145	1860 ± 856
4	2-MeO	Ph	47 ± 9.8	173 ± 18
5	4-Me	Ph	1270 ± 452	1300 ± 430
6	3-Me	Ph	148 ± 21	193 ± 8
7	2-Me	Ph	1666 ± 408	2000 ± 0
8	4-Cl	Ph	350 ± 19	347 ± 36
9	3-Cl	Ph	163 ± 22	257 ± 15
10	2-Cl	Ph	18.3 ± 5.0	31.7 ± 4.7
11	4-F	Ph	91 ± 43	96 ± 36
12	3-F	Ph	152 ± 97	143 ± 93
13	2-F	Ph	6.2 ± 1.5	19 ± 2.1
14	4-OCF_3	Ph	8330 ± 1080	5000 ± 3000
15	4-SCF_3	Ph	>10000	>10000
16	4-NO_2	Ph	>10000	10000
17	2-CF_3	Ph	>10000	>10000
18	2-OCF_3	Ph	>10000	>10000
19	2-SMe	Ph	350 ± 21	1066 ± 82
20	H	4-F-Ph	117 ± 35	833 ± 204
21	H	4-Cl-Ph	248 ± 58	>10000
22	H	4-Me-Ph	147 ± 8	>10000
23	H	3-Me-Ph	122 ± 36	69 ± 26
24	H	4-MeO-Ph	300 ± 131	>10000

Range is the standard error of the mean. A reference for this ubiquitous method of reporting error ranges may be excessive.
[a] IC_{50}s were determined with a 20 min preincubation with the enzyme.
[b] All values are with $N \geqslant 3$.



Scheme 2. Reagents and conditions: (a) 1/3 equiv $ClCO_2Ph$, THF or ACN, rt—reflux; (b) 0.9 equiv 1-(3-phenyl-[1,2,4]thiadiazol-5-yl)-piperazine, DMSO, microwave 100 °C, 30 min or 50 °C 18 h.

Table 2
Heteroaryl ureas

Compound	Yield (%) step (a)	Yield (%) step (b)	HetAr	Apparent rFAAH IC_{50}[a] (nM)[b]	Apparent hFAAH IC_{50}[a] (nM)[b]
25	—	46[c]		26 ± 2.5	26.7 ± 2.2
26	80	97		8.7 ± 2.7	9.8 ± 1.5
27	26	68		57 ± 20	266 ± 60
28	32	98		6.3 ± 0.41	10.3 ± 1.5
29	10	53		28.8 ± 7	13 ± 3.4
30	4	18		188 ± 35	253 ± 33
31	71	97		54 ± 9	50 ± 15
32	18	14		1317 ± 419	2667 ± 408
33	14	24		10.8 ± 4.4	23.0 ± 9.9
34	38	85		1.35 ± 0.79	2.02 ± 0.48
35	57	37		125 ± 23	1167 ± 204
36	78	80		638 ± 140	1037 ± 105
37	11	Quant.		113 ± 17	320 ± 12
38	30	82		>10000	>10000
39	24	84		25.7 ± 1.5	70 ± 2.9
40	74	57		41 ± 5.6	320 ± 13

Range is the standard error of the mean. A reference for this ubiquitous method of reporting error ranges may be excessive.
[a] IC_{50}s were measured with 10 min preincubation.
[b] Values are with $N \geqslant 3$.
[c] Prepared from the isocyanate.

the bicyclic benzothiadiazole derivative (**34**). Interestingly, despite the mechanism of FAAH inhibition, having more electron deficient heteroaryl substituents, and thus better leaving groups, on the urea does not translate to more potent/rapid inhibition of the enzyme. This suggests that interactions between the heteroaryl amine and the enzyme may be an important factor in determining the rate (and therefore apparent IC_{50}) of inhibition of the FAAH enzyme.

Under standard conditions, (**1**) has an IC_{50} of 12 nM at human FAAH (60 min assay with no preincubation). To determine the effect of preincubation time on apparent IC_{50}, the assay was short-

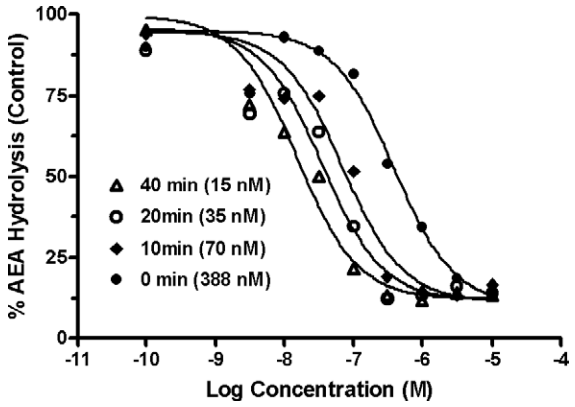


Figure 4. Apparent IC_{50} of (**1**) with differing preincubation times.

ened to 10 min and (**1**) was preincubated with enzyme for different times (Fig. 4). The apparent IC_{50} for (**1**) at human FAAH was reduced from 70 to 15 nM after a 40 min preincubation. A time dependence of apparent IC_{50} values for enzyme inhibition is usually observed when a compound is either a slow on tight binding ligand, an irreversible inhibitor, or a mechanism-based substrate.

To determine the reversibility of (**1**), we used a dialysis approach to examine the mechanism of FAAH inhibition. Test compounds (at IC_{80} concentrations) were incubated with membranes of recombinant cells expressing rat FAAH, and dialyzed against 1 L of PBS buffer at 4 or 22 °C. After 18 h dialysis, the contents of the dialysis cassette were recovered and assayed for FAAH activity (Fig. 5). The activity of enzyme preincubated with OL-135 was fully recovered[19] after dialysis at both temperatures, consistent with a full reversibility of inhibition. FAAH preincubated with URB597 failed to recover activity after 18 h of dialysis at either temperature, consistent with the formation of an irreversible covalent bond, as expected for this carbamate. Enzyme incubated with (**1**) remained substantially inhibited after dialysis at 4 °C. However, at 22 °C, significant activity was recovered, suggesting a temperature-dependent release of the compound from the active site. The recovery of enzyme activity after 18 h dialysis at 22 °C, albeit not complete, rules out irreversible inhibition of FAAH due to (**1**).

To distinguish between a tightly bound non-covalent ligand and a mechanism-based substrate inhibitor, compound (**1**) was incubated (twofold molar excess of FAAH) with partially purified rat FAAH enzyme for 3 h at 22 °C and examined by LC-ESI-MS. Accord-

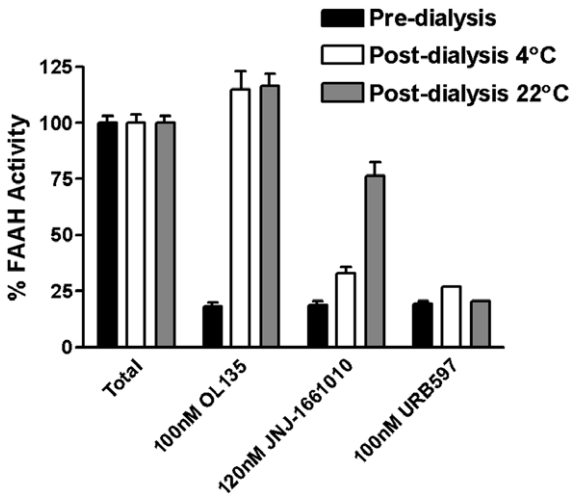


Figure 5. Residual FAAH activity pre- and post-dialysis (18 h) for JNJ-1661010, OL-135 (reversible) and URB597 (irreversible) at 4 and 22 °C ($N \geqslant 3$).

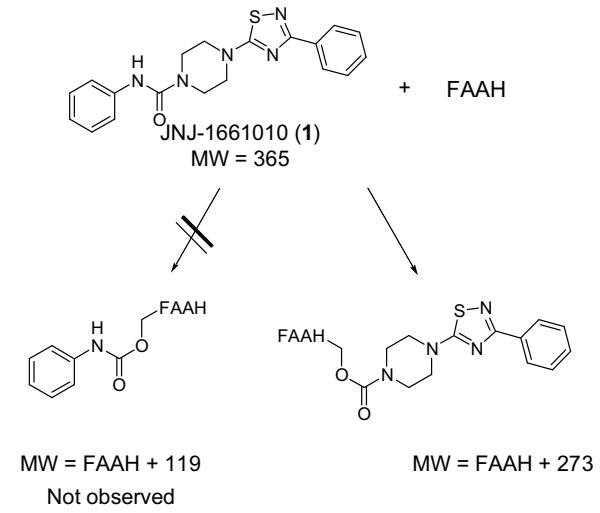


Figure 6. Results of LC-ESI-MS analysis of partially purified rat FAAH incubated with (**1**).

ingly, the measured MW increased by 274 Da to that of FAAH protein itself, suggesting a covalent modification of the enzyme with the piperazinyl fragment and concomitant loss of aniline (Fig. 6). This increase in mass, along with a return of FAAH activity in the dialysis experiments, suggests that (**1**) must be a covalent, mechanism-based substrate inhibitor.

To confirm the formation of a covalently linked piperazinyl fragment, we incubated partially purified soluble rat FAAH lacking the transmembrane domain with (**1**) tritiated on the piperazinyl group. Ten micrograms of this preparation was incubated with 400 nM ^{3}H (**1**) for 60 min, and then unbound ligand was separated from bound ligand by gel filtration. Enzyme activity of an unlabeled preparation run on a parallel column confirmed co-elution

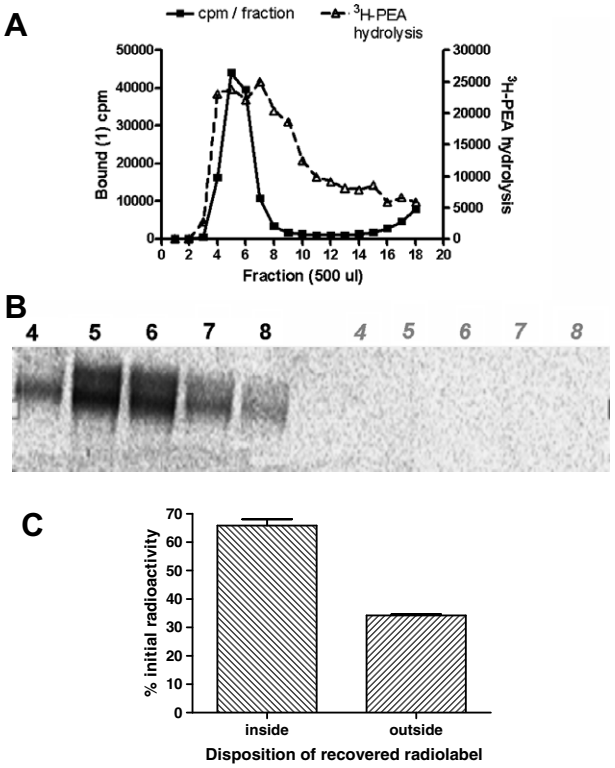


Figure 7. (A) Separation of radiolabeled FAAH after incubation with ^{3}H (**1**); (B) autoradiograph of sds gel of column fractions 4-8 after denaturation (grey, labeling carried out in excess unlabeled (**1**); (C) dialysis of FAAH ^{3}H (**1**) complex.

of the labeled protein peak with enzyme activity (Fig. 7A). The radiolabeled preparation was then denatured in 8 M urea and subjected to SDS gel electrophoresis and autoradiography. A single radiolabeled band (Fig. 7B) was found, suggesting that the complex between enzyme and the piperazinyl fragment was covalent and stable to protein denaturation. The peak radiolabeled fractions were subjected to dialysis as described, and after 18 h at room temperature, about 35% of the total radioactivity was recovered in the outer compartment, confirming that the bond formed between the enzyme and piperazinyl fragment is reversible (Fig. 7c).

A published crystal structure of rFAAH bound to MAFP[20] reveals the presence of a long lipophilic tunnel and a relatively large hydrophilic area containing Ser241 and other polar residues. As it was not immediately clear how compounds such as (**1**) bound to the active site, we docked (**1**) in two ways[21]: with the phenyl urea portion of the molecule in the hydrophobic tunnel and the phenylthiadiazole in the hydrophilic pocket (Fig. 8); and, with the phenylthiadiazole in the hydrophobic tunnel and the phenylurea in the hydrophilic pocket (Fig. 9). Molecular modeling suggested, on the basis of size, that both configurations of (**1**) within the enzyme active site were possible, however several factors suggested binding as shown in Figure 9. First, the crystal structure with FAAH bound to MAFP showed the long lipophilic tail of MAFP in the hydrophobic pocket, and the phenylthiadiazole is lipophilic. Second, the loss of aniline is consistent with the urea portion of the molecule in the more solvent accessible hydrophilic pocket, and would be consistent with work published by others.[22] Finally, the observed SAR for this class of molecules is consistent with the phenythiadiazole being in the hydrophobic pocket.

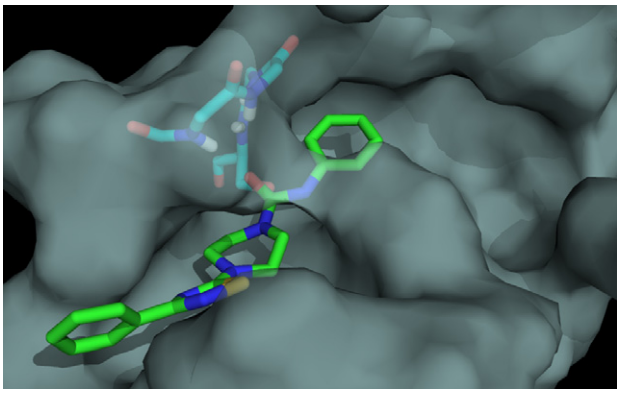


Figure 8. Compound (**1**) docked with the phenyl urea portion of the molecule in the hydrophobic tunnel and the phenylthiadiazole in the hydrophilic pocket.

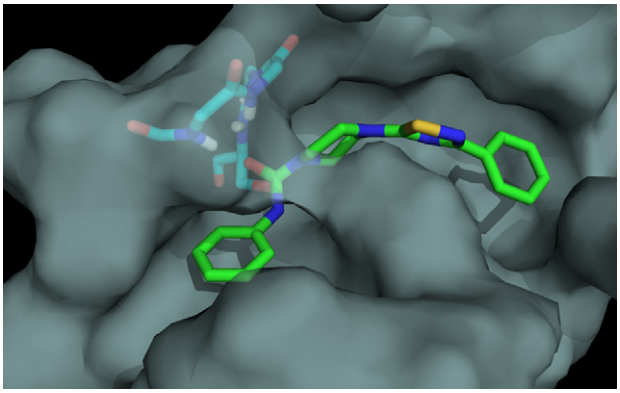


Figure 9. Compound (**1**) docked with the phenylthiadiazole in the hydrophobic tunnel and the phenylurea in the hydrophilic pocket.

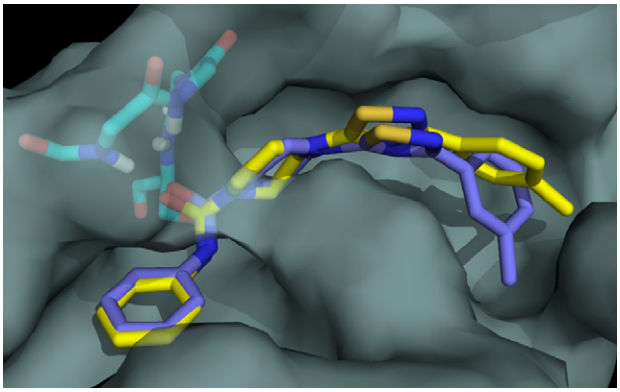


Figure 10. Overlay of 3- and 4-substituted phenyl compounds (**5**) and (**6**).

Substitution was not tolerated in the 4-position of the phenyl pendant to the thiadiazole by the human enzyme, but 3-substitution gave compounds equipotent to (**1**). A rationale for the preceding observation can be found in Figure 10, which shows an overlay of compounds (**5**) and (**6**) docked within the active site of rFAAH. The 4-methyl group of (**5**) is proposed to be in a sterically crowded space whereas the methyl group in (**6**) resides within a small lipophilic pocket.

We assessed the possibility of mechanism-based non-specificity by assaying liver carboxylesterases. In an assay measuring changes in the rate of ester (4-nitrophenyl-acetate) hydrolysis in rat liver microsomes,[23] (**1**) did not measurably inhibit ester hydrolysis. OL-135 and URB597 significantly decreased the rate of ester hydrolysis (Fig. 11). These data suggest that (**1**) is not a general inhibitor of esterases, whereas URB597 decreases liver esterase activity by 40%.

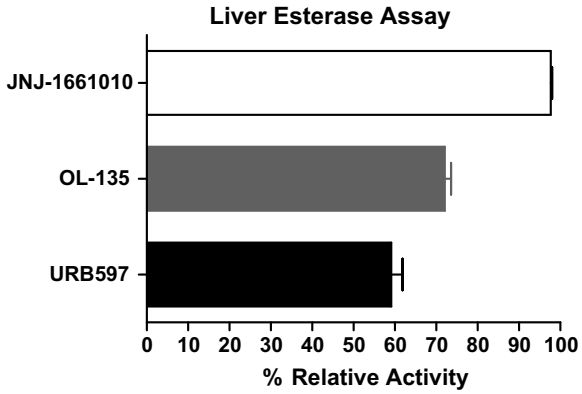


Figure 11. Inhibition of liver esterases by various FAAH inhibitors (10 μM with 30 min preincubation). DMSO control showed no inhibition.

Table 3
Summary of pharmacokinetic data for JNJ-1661010

	P.O.	I.P.	I.V.
Dose (mg/kg)	10	10	1
C_{max} (μM)	0.301 (±57%)	1.58 (±7%)	3.06 (±38%)
T_{max} (min)	110 (±103%)	35 (±66%)	—
Plasma $T_{1/2}$ (min)	264 (±66%)	94 (±5%)	34 (±13%)
Mean retention Time (min)	382 (±95%)	135 (±7%)	49 (±19%)
Cl (L/min)	0.116 (±18)	0.032 (±10%)	0.006 (±18%)
Volume of distribution (L)	45	4.6	0.57
F (%)	5	20	—

Vehicle is 1:4:15 pharmasolve: cremaphore: 5% dextrose in water.

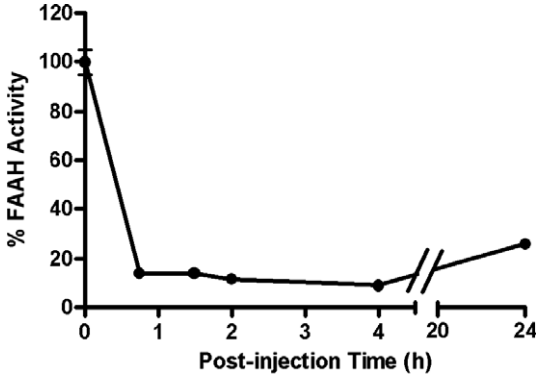


Figure 12. Inhibition of FAAH after i.p. dosing with 20 mg/kg (**1**).

Rats dosed with 20 mg/kg i.p. (**1**) had a plasma C_{max} of 26.9 μM at the T_{max} of 0.75 h and a C_{max} in the brain of 6.04 μM at the T_{max} of 2 h (Table 3). Compound (**1**) had a blood–brain barrier coefficient of 0.042.[24]

We examined the in vivo properties of (**1**) by measuring both ex vivo inhibition of brain FAAH and the elevation of AEA after dosing the compound at 20 mg/kg i.p. Brain FAAH was profoundly inhibited by a single 20 mg/kg dose of (**1**) for an extended period (Fig. 12). Even after 24 h, FAAH activity had recovered only to 25% of untreated values. In parallel, rats that had been dosed with **1** (20 mg/kg, i.p.) showed elevated levels of AEA in brain tissue. Four hours post dosing of (**1**), rat brain AEA levels increased by up to a factor of 1.4 thus suggesting in vivo inhibition of FAAH. Additional in vivo studies will be reported elsewhere.

In conclusion, we have described a series of aryl piperazinyl urea FAAH inhibitors with characteristics consistent with them being mechanism-based substrates of the enzyme. While this work was in progress, a report describing the mechanism of action of a closely related series of molecules (first disclosed in one of our patent applications)[25] appeared.

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