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Fatty acid amide hydrolase inhibitors. Surprising selectivity of chiral azetidine ureas

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ABSTRACT

We report the discovery of a novel, chiral azetidine urea inhibitor of Fatty Acid Amide Hydrolase (FAAH,) and describe the surprising species selectivity of VER-156084 versus rat and human FAAH and also hCB1.

Fatty Acid Amide Hydrolase (FAAH) is the serine hydrolase that is thought to be responsible for the hydrolysis of anandamide (AEA), an endogenous agonist of the cannabinoid receptor 1 (CB_1).[1] Neither FAAH knockout mice, nor rodents treated with FAAH inhibitors, elicit behaviours associated with cannabinoid-like central side effects. Furthermore, FAAH knockout mice are viable, showing increased brain levels of AEA with attenuated responses in a variety of inflammatory and neuropathic pain models.[2] A separation of the different CB_1-mediated effects is supported by the observation that cannabinoid-like side effects may be mediated by 2-arachidonoylglycerol (2-AG) and not AEA.[3] Thus, orally bioavailable FAAH inhibitors may be new treatments not just for acute and chronic pain, but also for anxiety and depression. Both SanofiAventis (SSR411298 (Phase II)) and Pfizer (PF-4457845 (Phase I)) have recently advanced FAAH inhibitors into the clinic, primarily for these respective indications. A variety of FAAH inhibitors, including keto-heterocycles (e.g., OL-135), carbamates (e.g., URB597 and SA47), and ureas (e.g., PF-750, PF-622 and JNJ-1661010) (Fig. 1) have now been reported.[4] To date all these carbamate and urea-containing FAAH inhibitors tend to form time-dependent, covalent adducts by reaction of the catalytic Ser-241 residue within the FAAH enzyme.

Irreversible inhibitors need to be selective and, based upon their serine hydrolase selectivity profiles in Activity-Based Protein Profiling (ABPP) assays,[5] most of the ureas and carbamates shown in Figure 1, apart from URB597, are highly selective as both rat and human FAAH inhibitors. Because of the substrate and agonist connection for AEA between FAAH and CB1, we decided to screen our hCB1 antagonist collection of substituted ureas for rFAAH activity, and discovered that the racemic, azetidine VER-24052, was a reasonably potent inhibitor of recombinant rat FAAH (Table 1). This appears to be the first example of FAAH inhibitor that contains a tetrasubstituted urea. However, VER-24052 was originally designed as a potent CB1 antagonist and since this activity would negate much of the benefits from inhibiting FAAH, we decided to characterize biologically both enantiomers of VER-24052 before embarking on a lead optimisation programme. We separated the enantiomers of VER-24052 (Fig. 1), as shown in Scheme 1, using chiral preparative HPLC (AD-H: iso-Hexane:EtOH (70:30)) to produce two enantiomers of unknown stereochemistry, designated, VER-156084 (>99% ee) and VER-156085 (>99% ee), respectively.[11] Re-profiling against FAAH and human CB_1 and CB_2

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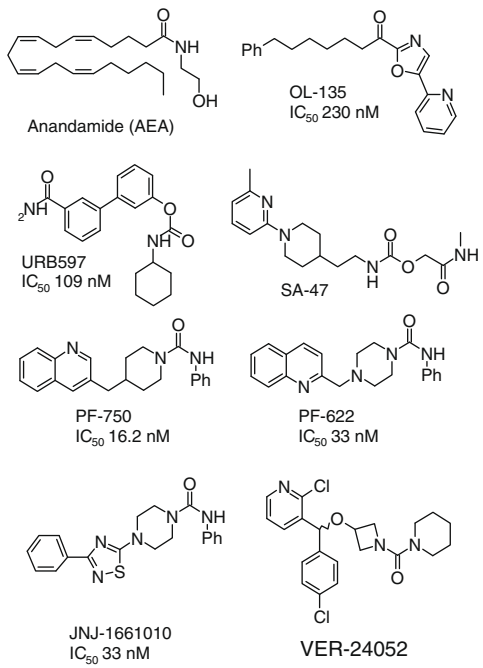


Figure 1. Substrates and inhibitors of FAAH.

receptors showed that both the rat and human FAAH activity was due to VER-156084, whereas the hCB_1 and hCB_2 activity resided mainly with its enantiomer, VER-156085 (Table 1). VER-156084 also showed a time-dependent inhibition of rat FAAH which is consistent with other urea inhibitors, indicating that it also probably inhibits FAAH by reaction with the Ser-241 residue of the active site. However, most surprisingly to us, and perhaps unknown in this area, VER-156084 was significantly much weaker as an inhibitor of human FAAH (hFAAH) than rat FAAH (rFAAH).

To understand better how VER-156084 fits into the hFAAH catalytic pocket, we decided to determine unambiguously the absolute configuration of VER-156084. However, because neither VER-156084 nor VER-156085 provided crystals of sufficient quality for detailed X-ray analysis, we needed to separate the amine enantiomers of the precursor racemic azetidine, **6**, and then convert each of these separately to VER-156084 and VER-156085, respectively.[6] During this process we showed that neither the intermediates nor the products had racemised by chiral HPLC. Repeating both the rat and human FAAH bioassays in tandem with chiral HPLC monitoring allowed us to connect the chiral amine precursor, **7** to the end product, **8** (VER-156084) and, hence, amine **9** to **10** (VER-156085).

Conversion of chloronicotinoyl chloride **1** to the Weinreb amide, **2**, followed by Grignard reagent addition and $NaBH_4$ reduction furnished the racemic alcohol **5** (Scheme 1). Reaction of **5** with 1-benzhydrylazetidin-3-ol (PTSA, PhMe, reflux, Dean-Stark), followed by deprotection under standard conditions (1-chloroethyl chloroformate, followed by MeOH) gave the racemic azetidine ether, **6**, as the hydrochloride salt. Resolution of the azetidine **6** was achieved using the same chiral HPLC system as previously used on VER-24052, while re-crystallisation from MeCN of the D-dibenzoyl tartrate salt (mpt 140 °C) of the enantiomer, **7**, gave crystals suitable for X-ray crystallography, and confirmed the absolute stereochemistry of chiral amine, **7**, (Fig. 2).[11]

Examples of low energy conformers for VER-156084 and VER-156085 are shown in Figure 2. These molecular geometries were obtained from a conformational sampling study and optimized using B3LYP/6-311G** in Jaguar.[7] A large number of conformers were first produced by molecular mechanics with MOE's stochastic method.[8] Low energy conformers were then used as the initial structures for relaxed dihedral scans of the pyridyl ring using quantum mechanics. VER-156084 and VER-156085 have populations of stable conformers that differ in the spatial arrangement of the substituents in the aromatic rings and results in the lower activity of the R isomer. Ring rotations are hindered by repulsion between the 2-Cl and the ether lone pairs or the electron cloud of the adjacent ring giving a degree of rigidity to the molecule. The crystal of **7**, a fragment of VER-156084, underscored the preference of the 2-Cl to point away from the lone pairs of the ether oxygen. Common to both isomers is the puckering of the urea nitrogens and an out of plane twist between the saturated rings. The twist away from the carbonyl plane in the azetidine (θ–60°) is greater than that of the piperidine (θ∼40°).

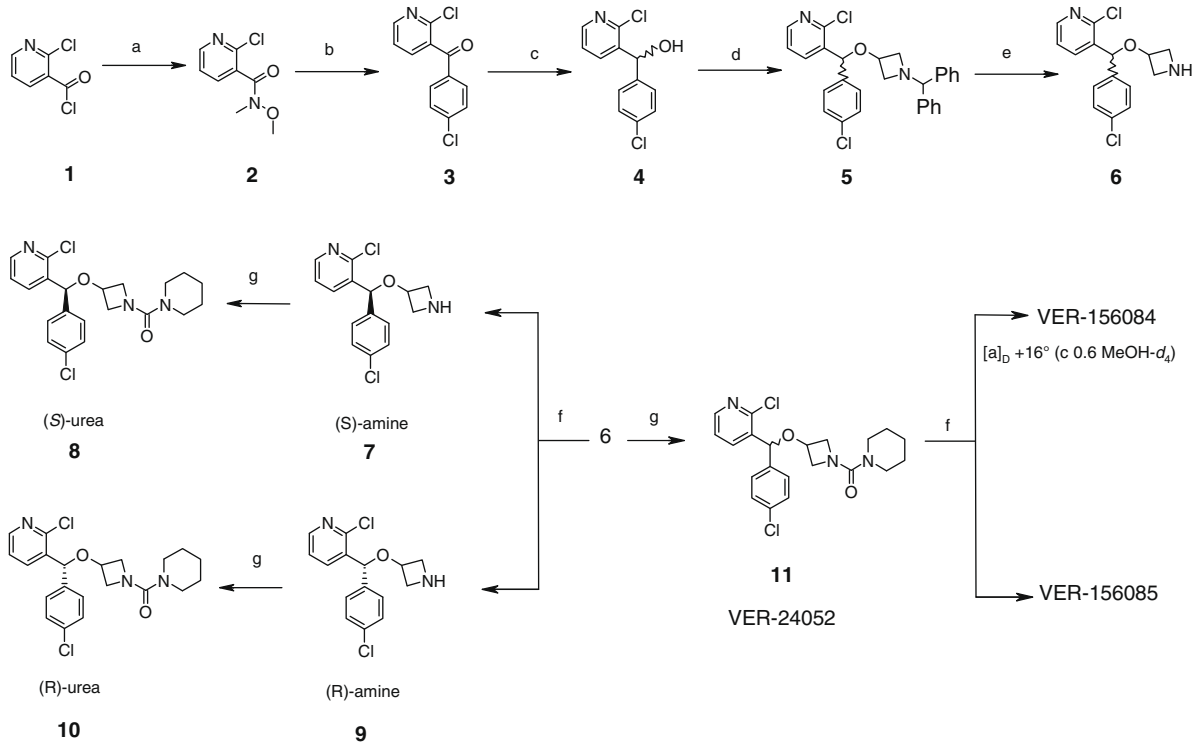
VER-156084 is less potent in hFAAH than rFAAH by ∼7-fold. Species selectivity has been reported previously for FAAH inhibitors, with PF-750 being more potent in human than rat FAAH by ∼8-fold,[9] while several other inhibitors, including JNJ-1661010,[4] are equipotent. Mileni and co-workers studied the structural basis for this selectivity by crystallizing the adduct of PF-750 bound to a humanized rat protein, in which six rat residues in the arachidonoyl binding channel (ACB) were mutated to human residues. Figure 3 highlights the differences between the rat (1MT5.pdb)[10] and hybrid (2VYA.pdb)[9] crystals and their effect on the binding site shape. Flexible alignment of VER-156084 with known inhibitors (Fig. 2C) shows the overlap between the pyridyl ring in VER-156084 and the quinoline ring in PF-750. We postulate that the aromatic ring in VER-156084 occupies a similar site in the protein as the pyridyl ring of PF-750 (Fig. 3). This would place VER-156084 in close contact with residues that differ between rat and human FAAH. The species selectivity observed for VER-156084 supports the

Table 1
Rat FAAH, human FAAH inhibition and CB receptor binding

Compds	Rat FAAH inhibition IC_{50}[a] (nM)			Human FAAH inhibition IC_{50}[a] (nM)			hCB_1 binding K_i[b] (nM)	hCB_2 binding K_i[b] (nM)
	t = 0 h	t = 1 h	t = 3 h	t = 0 h	t = 1 h	t = 3 h		
URB597	1148	78	63		22			
OL-135	191	119	112		306			
VER-24052	1205	291	188	NT	NT	NT	<10	NT
VER-156084	465	140	78	>2000	1031	531	560	7190
VER-156085	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	<10	10900

[a] Apparent IC_{50} determined with rat or human FAAH by pre-incubation of compound with FAAH for time period indicated, followed by addition of arachidonylaminomethylcoumarin amide (AAMCA) probe.

[b] K_i determined by radioligand displacement binding assay with ^{3}H-SR141716A (hCB_1) or ^{3}H-CP55940 (hCB_2).



Scheme 1. Reagents and conditions: (a) MeNHOMe·HCl, Et_3N, DCM, 10 °C, 2 h, 83%; (b) 4-chlorophenylmagnesium bromide, THF, 0 °C →rt, 19 h, 100%; (c) $NaBH_4$, MeOH, 1.5 h, 89%; (d) 1-benzhydrylazetidin-3-ol, PTSA·H_2O, PhMe, Dean-Stark conditions, 3.5 h, 35%; (e) (i) 1-chloroethyl chloroformate, DCM, rt, 2 h; (ii) MeOH, rt, 24 h, 85%; (f) chiral HPLC; (g) (i) triphosgene, pyridine DCM, rt; (ii) piperidine, DCM, 50%.

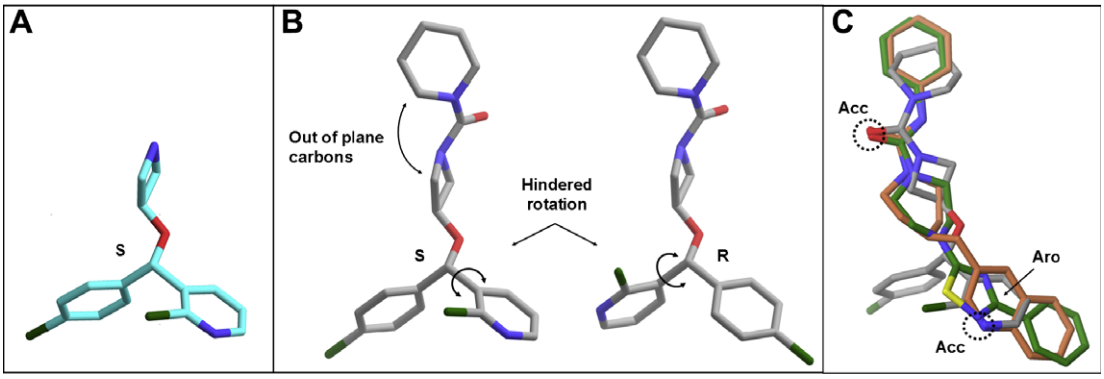


Figure 2. (A) X-ray crystal structure of **7**, dibenzoyl tartrate salt has been removed for clarity. (B) Ab initio optimized structures of VER-156084 (S) and VER-156085 (R). (C) Flexible alignment of VER-156084 (grey carbons) with inhibitors JNJ-1661010 (green carbons) and PF-750 (brown carbons).

placement of this ligand in a site where there are mutations between rat and human. Leu192 in rFAAH is replaced in hFAAH by a Phe which can form an aromatic CH–π interaction with the ligands. rFAAH Ile491 is replaced in hFAAH by a smaller Val residue. Both mutations have an impact on the binding cavity shape and volume, which should affect bulky rigid ligands like VER-156084. Additional protein–ligand interactions and a better fit could explain the enhanced binding of VER-156084 to hFAAH.

Further investigative profiling of VER-156084 showed that it suffered from off-target serine hydrolase activity, as shown by ABPP, was prone to high plasma protein binding across species and high microsomal turnover. The surprisingly lower inhibitory potency of these molecules for human over rat FAAH also represents a key drug design and development issue regarding species selectivity for workers in this area.

In conclusion, we have identified a new series of tetra substituted urea-based FAAH inhibitors and believe that this is also the first example of two enantiomers having different activity versus both human and rat FAAH, which can best be explained by the nature of the residues in the ACB channel. In future publications we shall describe how we used this modeling information to design highly selective, potent, orally active hFAAH inhibitors with excellent in vivo activity.

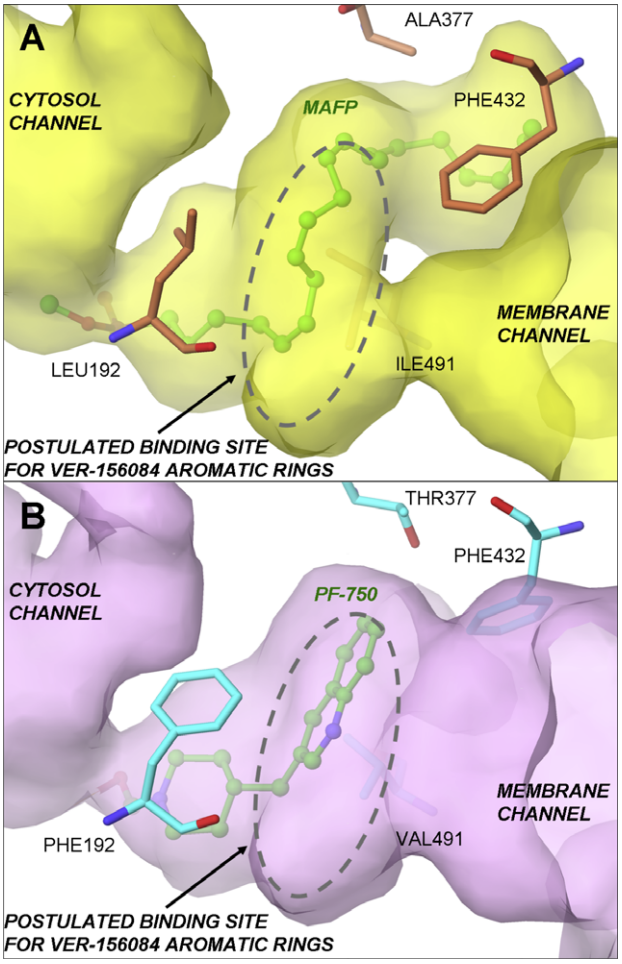


Figure 3. Binding sites of rFAAH (A, 1MT5) and hybrid r/hFAAH (B, 2VYA). Residues shown include three that differ between both species and a conserved flexible phenylalanine.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.097.

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