Synthesis and Evaluation of Benzothiazole-Based Analogues as Novel, Potent, and Selective Fatty Acid Amide Hydrolase Inhibitors

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Received August 19, 2008

High-throughput screening (HTS) identified benzothiazole analogue **3** as a potent fatty acid amide hydrolase (FAAH) inhibitor. Structure−activity relationship (SAR) studies indicated that the sulfonyl group, the piperidine ring and benzothiazole were the key components to their activity, with **16j** being the most potent analogue in this series. Time-dependent preincubation study of compound **3** was consistent with it being a reversible inhibitor. Activity-based protein-profiling (ABPP) evaluation of **3** in rat tissues revealed that it had exceptional selectivity and no off-target activity with respect to other serine hydrolases. Molecular shape overlay of **3** with a known FAAH inhibitor indicated that these compounds might act as transition-state analogues, forming putative hydrogen bonds with catalytic residues and mimicking the charge distribution of the tetrahedral transition state. The modeling study also indicated that hydrophobic interactions of the benzothiazole ring with the enzyme contributed to its extraordinary potency. These compounds may provide useful tools for the study of FAAH and the endocannabinoid system.

Introduction

Arachidonyl ethanolamide (AEA[a], anandamide, **1**), is a known endogenous cannabinoid ligand and signaling lipid messenger. It is believed to bind and activate cannabinoid (CB_1 and CB_2) receptors, as well as vanilloid receptor (TRPV1),[1,2] through which it exhibits different biological actions. Its effects on cell proliferation and apoptosis in various cell lines,[3] on reproduction,[4,5] memory processes,[6] antinociception,[7,8] modulation of anxiety[9] and epilepsy[10] have been reported. The signaling function of anandamide, as well as some other related fatty acid amides such as sleep-inducing agent oleamide,[11] anorexigenic agent *N*-oleoyl ethanolamide (OEA)[12] and anti-inflammatory agent *N*-palmitoyl ethanolamide (PEA),[13] are all tightly controlled by an intergral membrane enzyme, fatty acid amide hydrolase (FAAH) through rapid hydrolysis to the corresponding acids. In the rat brain, neurons that express FAAH are usually found in the proximity of axon terminals containing CB_1 receptors, providing important evidence for a role of FAAH in anandamide deactivation.[14] FAAH knockout studies indicated that mice lacking FAAH had higher levels of fatty acid amides, including AEA, and augmented behavioral response to administered AEA, and displayed CB_1 dependent analgesia phenotypes.[15,16] These findings suggest that selective FAAH inhibitors could enhance the actions of anandamide in brain regions in which this lipid mediator is physiologically important; for example, those engaged in the processing of pain[8] and emotion.[17] The increased anandamide tone produced by blocking FAAH may result in a more restricted spectrum of pharmacological effects than those produced by direct-acting CB_1 agonists and, possibly, in fewer adverse events. Therefore, intervention of this pathway via selective chemical inhibition[18] may provide an useful therapeutic tool for the treatment of anxiety, epilepsy, cancer, eating disorder, pain and sleep disorders.

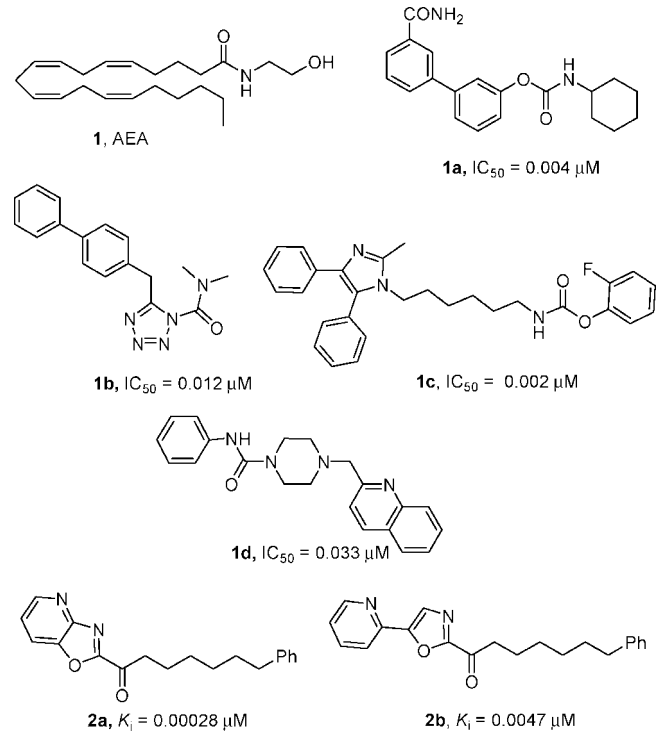

Figure 1. AEA and known FAAH inhibitors.

Despite the therapeutic potential of FAAH in various diseases, only a few structural classes of FAAH inhibitor have been identified. Some of the representative examples are shown in Figure 1. FAAH is the only characterized mammalian enzyme that is in the amidase signature family that bears an unusual catalytic Ser-Ser-Lys triad. Most of the recently reported inhibitors contain functional groups that are capable of forming a covalent bond with the enzyme catalytic serine, either

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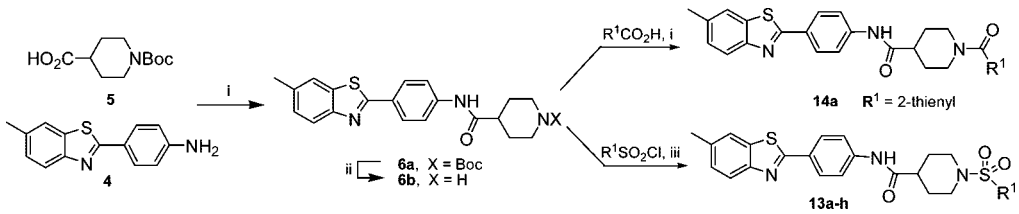
[a] Abbreviations: HTS, high-throughput screening; FAAH, fatty acid amide hydrolase; AEA, *N*-arachidonoylethanolamine; OEA, *N*-oleoyl ethanolamide; PEA, *N*-palmitoyl ethanolamide; TGH, triacylglycerol hydrolase; MAFP, methyl arachidonyl fluorophosphonate; ABPP, activity-based protein profiling; TLC, thin layer chromatograpy; HPLC, high pressure liquid chromatography.

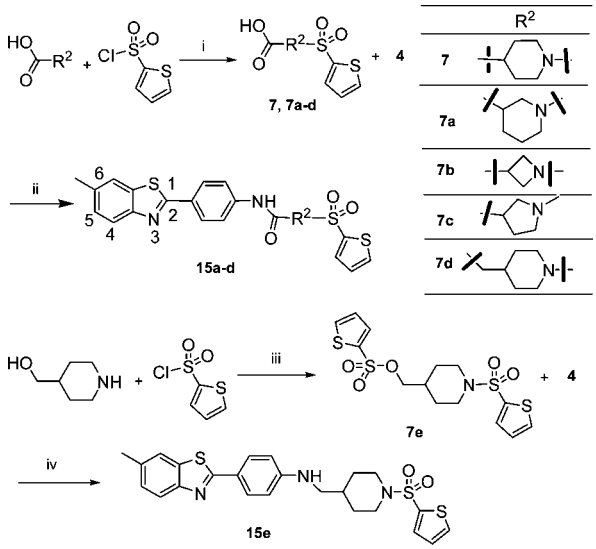
10.1021/jm801042a CCC: $40.75
Published on Web 12/10/2008

Scheme 1[a]



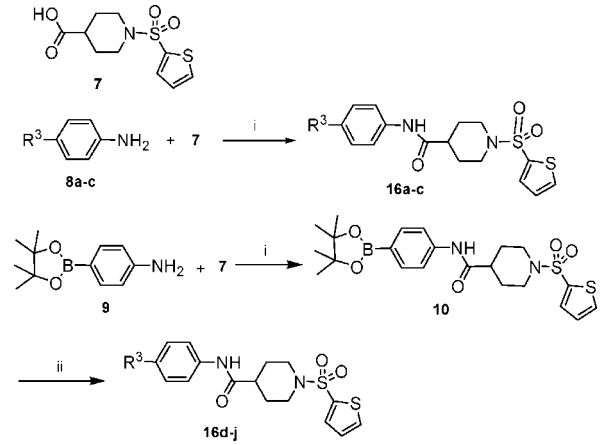
[a] Reagents and conditions: (i) DMAP, EDCI, DCM, 12 h; (ii) HCl, dioxane, MeOH, 2 h; (iii) P-BEMP, DMA, 100 °C, microwave, 90 s.

Scheme 2[a]



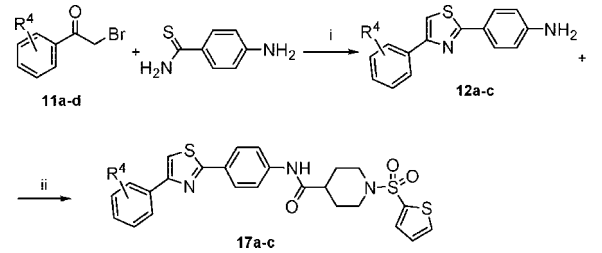
[a] Reagents and conditions: (i) $NaHCO_3$, acetone, H_2O; (ii) Step (i) in Scheme 1; (iii) pyridine, THF; (iv) NaH, THF, 80 °C.

Scheme 3[a]



[a] Reagents and conditions: (i) Step (i) in Scheme 1; (ii) R^3X, $Pd(PPh_3)_4$, K_2CO_3, THF, reflux.

Scheme 4[a]



[a] Reagents and conditions: (i) *i*-PrOH, 60 °C; (ii) Step (i) in Scheme 1.

irreversibly or reversibly. Various reactive carbamate or urea-containing inhibitors have been identified, including **1a** (URB-597),[9] **1b** (LY2183240),[19] **1c** (BMS-1),[20] and **1d** (PF-622)[21] and are believed to irreversibly acylate active site Ser241, thereby inactivating the enzyme. Compound **1a** had been shown to exhibit anxiolytic-like,[9] antidepressant-like[22] and analgesic activities,[23] and has undergone extensive preclinical characterizations. However, inhibitors with such structures tend to be nonselective toward off-target serine hydrolases and may result in unwanted side effects.[24,25] Another reported class of FAAH inhibitors contains the α-keto-heterocylcles such as **2a** (OL-92) and **2b** (OL-135).[26] It was proposed that the electrophilic carbonyl in these molecules forms a reversible hemiacetal bond with the catalytic serine, conferring extraordinary potency. Extensive structure−activity relationship studies have been performed and the correlation between the electrophilicity of the carbonyl and the potency of the inhibitors further supported the covalent nature of the enzyme−inhibitor interactions.[27] This class of inhibitors was shown to have much improved selectivity over common off-targets such as triacylglycerol hydrolase (TGH) and various carboxypeptidases.[24,25] Compound **2b** was shown to elevate AEA levels in the brain and spinal cord, exhibit augmented behavioral response to anandamide,[24] and demonstrate efficacy in various pain models,[28] further validating chemical intervention of FAAH as a viable therapeutic approach.

The therapeutic potential of FAAH inhibition clearly calls for other novel, potent, and selective inhibitors. Toward this goal, a high-throughput screening (HTS) assay was conducted on in-house compound selections.[29] Benzothiazole analogue **3** was identified as a potent inhibitor with no obvious serine-reacting group. The synthesis and SAR studies of these compounds are presented herein.

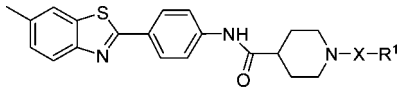
Chemistry

Preparations of these benzothiazole analogues are shown in Scheme 1−4. Different substitutions on the piperidine nitrogen could be incorporated by sulfonylation or acylation of piperidine analogue **6b** (Scheme 1), which was obtained via acid-catalyzed deprotection of **6a** following coupling of acid **5** with aniline **4**. Analogues with different R^2 azacycles (**15a−d**) were prepared by acylation of aniline **4** with corresponding sulfonylated acids **7a−d** (Scheme 2). Aniline analogue **15e** was obtained by displacement of sulfonate **7e** with aniline **4**. R^3s were introduced either by acylation of the appropriately substituted anilines **8a−c** or Suzuki coupling of the boronic ester intermediate **10** with halogen-substituted heterocycles (Scheme 3). Phenylthiazole analogues **17a−c** (Scheme 4) were generated by condensation of substituted 2-bromoacetophenones with 4-amino thiobenzamide, followed by acylation of the resulting aniline with acid **7**.

Enzymatic Assay

Crude rat FAAH enzyme was isolated from rat cortex. The human full-length enzyme was isolated from an HEK293 stable

Table 1. IC_{50} Values for Compounds of General Structures **13a**−**h**, **14a** To Inhibit Rat FAAH



13a-h, 14a

compd	X	R^1	IC_{50} (nM)[a]
3	SO_2	thiophene-2-yl	18 ± 8
13a	SO_2	-CH_2CH_3	109 ± 37
13b	SO_2	-$CH(CH_3)_2$	63 ± 31
13c	SO_2	-$N(CH_3)_2$	48 ± 24
13d	SO_2	-C_6H_5	13 ± 7
13e	SO_2	2-F-C_6H_5	29 ± 4
13f	SO_2	2-Cl-C_6H_5	263 ± 52
13g	SO_2	3-Cl-C_6H_5	1780 ± 1200
13h	SO_2	4-Cl-C_6H_5	>10000
14a	CO	thiophene-2-yl	2370 ± 620

[a] IC_{50} = 50% inhibitory concentration against FAAH prepared from rat cortex from 9 concentrations (data are expressed as means ± SD from at least two experiments).

cell line. A tritiated-anandamide carbon filtration assay for FAAH activity was adapted from Wilson et al.[30] Upon incubation of the enzyme, the compound and tritiated anandamide, the reaction was quenched with 0.7 M HCl. Hydrolysis of anandamide by FAAH was assessed by filtering the incubation mixture through activated carbon and determining the amount of radiolabeled tritiated ethanolamine product. Eleven different concentrations (over a range of 4.5 logs) of each compound were assayed in duplicate and IC_{50} values were derived.

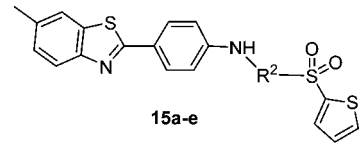
Results and Discussion

Different groups on the piperidine nitrogen were first investigated (Table 1). While small sulfonyl groups were tolerated (**13a**−**c**), it was the phenyl group, a thiophene bioisostere (**13d**), that maintained most of the potency. Small *ortho*-fluoro and -chloro substitutions on the phenyl ring were tolerated (**13e**,**f**), while the meta- and para-substituted analogues, however, saw gradual loss of potencies (**13g** and **h**), indicating potential unfavorable interaction of the para-substituent with the enzyme. Therefore, 2-thiophenesulfonyl group was kept at this site for further SAR studies. Carbonyl replacement (**14a**) of the sulfonyl led to significant drop in potency, which implies the possibility of the sulfonyl group mimicking the charge distribution of the tetrahedral intermediate in, for example, the reversible hemiketal formed by α-keto-heterocylcle analogue **2b** shown in Figure 1.

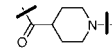
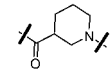
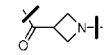
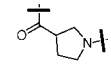
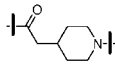
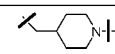
Next, ring sizes and connecting patterns of the linking azacycles (Table 2) were evaluated. Compound with 1,3-substituted piperidine ring (**15a**) was completely inactive, and aztidine analogue **15b** was much weaker. The 1,3-substituted pyrrolidine (**15c**), nevertheless, was only 3 times weaker. Introduction of an extra methylene linker between piperidine and the amide group, such as compound **15d**, completely abolished the binding potency, highlighting the importance of having the right length and shape of the linking group between two parts of the molecule.

A notable observation was that the des-carbonyl aniline analogue **15e** had almost identical potency as that of **3**, indicating that the carbonyl is not critical for the inhibitory potency and is in sharp contrast to compounds like **1a** or **2a**. In those two cases, the carbonyl was essential for the covalent bond formation and high inhibitory potency. A recent analysis by Kimball et al.[31] shows that loss of the electrophilic carbonyl in α-keto oxazole analogues (of which **2b** is a member) results in >1000-fold loss in potency. Shown in Figure 2a is the postulated binding mode for an α-keto oxazole analogue **2c**, a reversible inhibitor similar to **2b**, proposed by Boger et al.,[26] (green carbons), which is superimposed onto the X-ray crystallographic structure of the irreversible inhibitor **1e** (methyl arachidonyl fluorophosphonate,[32] MAFP; cyan carbons). The α-keto oxazole forms a reversible hemiketal with a catalytic serine (Ser-241) in the FAAH active site. In Figure 2b, we show an overlay of **2c** (green carbons) and the binding mode proposed for **3** (orange carbons). It can be seen that the sulfone in **3** mimics the tetrahedral arrangement at the covalent attachment site that forms the tetrahedral intermediate in the α-keto oxazole **2c**. This proposition would certainly indicate that the amide carbonyl is shifted comparing to that of **2c** and unable to interact with Ser-241. The significance of sulfonyl group to the binding potency and the noncritical role played by the carbonyl group in **3** suggest that the class of compounds presented here may represent a novel mechanism of inhibition of the FAAH enzyme, distinct from acting either as an acylating agent or an electrophilic carbonyl ready for hemiketal formation. This putative binding mode will be further discussed in the molecular shape overlay study.

Table 2. IC_{50} Values for Compounds **15a**−**e**



15a-e

Compd	R^2	IC_{50}[a] (nM)
3		18 ± 8
15a		>10000[b]
15b		201 ± 80
15c		65 ± 7[b]
15d		>10000
15e		16 ± 2

[a] IC_{50} = 50% inhibitory concentration against FAAH prepared from rat cortex (data are expressed as means ± SD from at least two experiments). [b] Racemic mixtures.

Subsequently, studies were conducted on the bicyclic heteroaryl portion of **3**. Benzothiazole was much more potent than the thiazole analogue (**16a** vs **16d**) as shown in Table 3, suggesting the interactions of this moiety with the enzyme are mainly hydrophobic in nature. This was confirmed by surveying several benzo-fused heterocycle analogues. Quinoline **16e**, a bioisostere of benzothiazole, remained as potent. As the heterocycles shifted to more polar quinoxaline (**16f**), benzooxazole (**16b**) and benzimidazole (**16c**), the potency dropped progressively.

The substituents on the benzothiazole ring were also probed (Table 3). Even though 6-methoxy analogue **16h** was slightly weaker than **3**, other substituents such as 6-choro (**16g**), the unsubstituted (**16a**), and 4-methyl (**16i**) analogues were all equally potent as **3**. This is in qualitative agreement with the

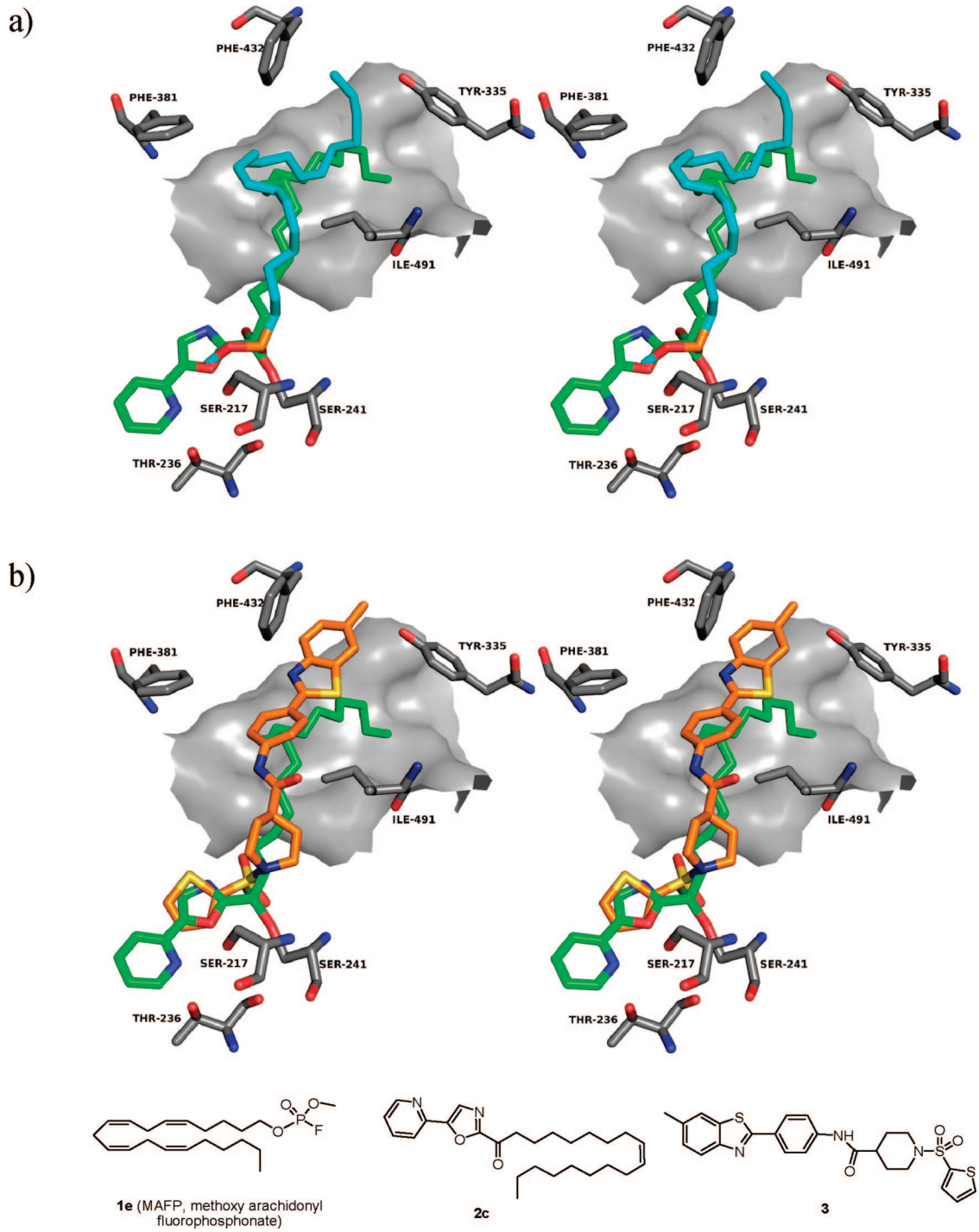
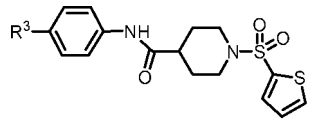


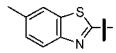
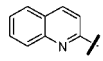
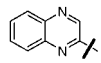
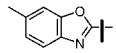
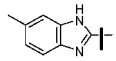
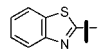
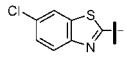
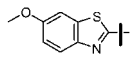
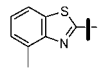
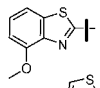
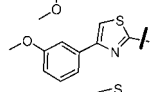
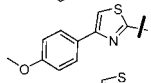
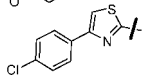
Figure 2. Stereo views of inhibitor binding modes for FAAH. (a) Superimposed on the crystallographic bound conformation of fatty acid inhibitor MAFP (cyan carbons) is the reversible α-keto oxazole inhibitor **2c** (green carbons) with a bound conformation analogous to that proposed by Boger et al.[26] (b) The α-keto oxazole inhibitor **2c** shown with putative binding mode for compound **3** (orange carbons). The sulfonyl group in **3** mimics the charge distribution of the α-keto tetrahedral intermediate created by the covalent bond with SER-241. The surface shown in gray indicates the posterior region of the pocket, which is predominantly hydrophobic in nature.

SAR findings of the insensitivity of substitution on the distal phenyl ring of **2b** by Hardouin et al.,[33] where according to our modeling study, occupies the same hydrophobic pocket as the benzothiazole of **3**. The 4-methoxy analog **16j**, however, saw much boosted potency, resulting in the most active analogue in this series. The improvement of potency of **16j** over **16h** may indicate that larger groups at this site can be accommodated. This hypothesis was put to test by preparing the 4-phenyl substituted thiazole analogues. These analogues were also shown to be very potent FAAH inhibitors (Table 3, **17a**−**c**), in the same range as their benzothiazole parent compounds. This indicates that there is probably a large binding pocket in this region and the potency can potentially be further optimized.

Human FAAH Time-Dependent Preincubation Study. To determine the likely mechanism of the inhibition, a time-dependent inhibition study was performed using human FAAH. The homology between human and rat FAAH is very high and little species differences[26] were observed for active ketone-based inhibitors. Our data (not shown) also indicated that this class of compounds exhibited similar potency against human and rat

Table 3. IC_{50} Values for Compounds with General Structures **16** and **17**



Compd	R^3	IC_{50}[a] (nM)
3		18 ± 8
16d		270 ± 58
16e		21 ± 6
16f		91 ± 18
16b		235 ± 75
16c		> 10000
16a		15 ± 2
16g		14 ± 4
16h		71 ± 32
16i		14 ± 4
16j		1.7[b]
17a		19 ± 7
17b		25 ± 11
17c		44 ± 15

[a] IC_{50} = 50% inhibitory concentration against FAAH prepared from rat cortex (data are expressed as means ± SD from at least two experiments). [b] $n = 1$.

FAAH. A study in which the preincubation times of the inhibitor and the enzyme were varied was carried out. In principle, if a compound inhibits the enzyme via an irreversible mechanism, upon prolonged preincubation the potency should become greater; a constant IC_{50}, conversely, supports a reversible mechanism. This type of time-dependent preincubation study had been used to characterize mechanism of inhibition of both FAAH inhibitors[34] and other enzyme inhibitors.[35]

Time-dependent increase of potency was observed for **1a** (Table 4), from micromolar to nanomolar within 10 min of preincubation. Compound **2a**, to the contrary, exhibited no increase of potency upon prolonged incubation, supporting literature characterization of it being a reversible inhibitor.[26] Benzothiazole compound **3** showed no time-dependent improvement of potency, suggesting it might also be a reversible inhibitor. However, the mechanisms of action are most likely

Table 4. IC_{50} (nM) Values of FAAH Inhibitor at Different Preincubation Times

compound	0[a]	5 min	30 min	60 min
1a	2187 ± 228[b]	47 ± 7	16 ± 2	11 ± 2
2a	31 ± 8	17 ± 4	16 ± 2	12 ± 2
3	11 ± 2	20 ± 6	34 ± 12	19 ± 6

[a] PreIncubation time of the inhibitor with human FAAH prior to addition of substrate. [b] IC_{50} = 50% inhibitory concentration against human FAAH prepared from a HEK-293-based stable cell line expressing recombinant human FAAH (data are expressed as means ± SD from at least three experiments).

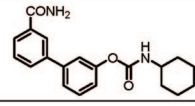
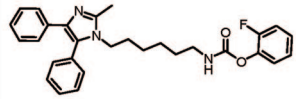
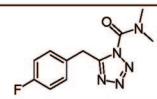
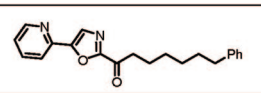
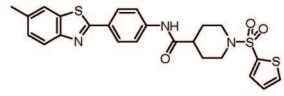
different between **3** and **2a** since, as discussed earlier, benzothiazole compounds are unlikely to form a covalent bond with the enzyme.

Activity-Based Protein Profiling Study. With the use of a modified activity-based protein profiling (ABPP) procedure that was first developed by Leung et al.,[36] we have profiled some known FAAH inhibitors in both rat and human tissues previously.[25] The same screening assay was conducted on compound **3**, along with several known FAAH inhibitors, in different rat tissues to characterize its selectivity against other serine hydrolases (Table 5). The color scheme in each box indicates how selective (red being most nonselective, and green being most selective) the inhibitor is in this tissue. Number of off-target protein bands and molecular weights are also shown in each box. Some of the known irreversible inhibitors such as **1c** and **1f** (LY2077855),[37] a close analogue of **1b**, are poorly selective and multiple protein bands were missing due to the off-target binding. Compound **1a** had significantly improved selectivity over **1c** but still not nearly as selective as the reversible ketone inhibitor **2b**. Compound **3** was exclusively specific against FAAH in rat brain and had no missing protein bands in all the other tissues that were tested. No off-targets such as triacylglycerol hydrolase (TGH), an uncharacterized membrane-associated hydrolase KIAA 1363, or carboxy esterases were observed. The potency determined via this approach was close to that obtained using the tritiated-anandamide enzymatic assay (data not shown).

Molecular Shape Overlapping Study. To explore potential interactions for the class of inhibitors in the present study we used the X-ray crystallographic bound conformation of MAFP as a guide in directing placement of plausible bound conformations for compound **3**, which served to constrain the overlays and maximize the shape and electrostatic similarity with the bound MAFP conformation.

The key features of the resulting pose are shown in Figure 2b (orange carbons), which exhibits a similar position and orientation as found in other reported docking studies.[26,38] The sulfonyl group of compound **3** (shown in yellow) occupies a similar location to the acyl moiety of bound MAFP (cyan carbons in Figure 2a), and can engage in similar hydrogen bonding to catalytic residues, for example, Ser-217 and Ser-241. The placement of the benzothiazole heterocycle occupies a similar region as the hydrocarbon tail in MAFP, which extends into the predominantly hydrophobic pocket created by residues such as Phe-381, Ile-491, Tyr-335 and Phe-432. This proposed binding mode also appears to provide rationalization for the majority of observed SAR. In particular, it explains the loss of potency observed with the more polar benzooxazole (**16b**) and benzimidazole (**16c**) analogues, and shows the general tolerance for the variations present in **16a**, **16g**−**i**, and **17a**−**c**. There is a clear indication for the potential source of the insensitivity to the loss of carbonyl in **15e**, and additionally it gives justification

Table 5. Selectivity Profiling of FAAH Inhibitors in Representative Rat and Human Tissues

Inhibitor	Structure	Rat Tissue[a,b]			
		Brain	Liver	Heart	Intestine
1a[c]		1(60)	2(60)	1(60)	1(60), 2(50), 3(40)
1c[c]		1(150), 1(70), 1(60)	2(70), 2(60), 2(50), 1(35)	1(75), 1(60), 1(55), 1(33)	1(75), 1(60), 2(50), 2(40), 1(33)
1f[c]		11(21-98)	14(21-98)	8(21-98)	20(21-98)
2b[c]		1(60)	1(60)	1(60)	1(60)
3		1(60)			

[a] The number of affected protein bands, if any, with the approximate range of molecular weight (in parentheses) is indicated for each inhibitor in a particular tissue proteome. [b] The relative selectivity of FAAH inhibitors is categorized into four classes that are represented by different color schemes: exceptionally selective (green) with no visible change in band patterns except in rat brain the only protein band with diminished labeling is the 60 kDa FAAH band, highly selective (yellow) with only 1−2 visible missing or diminished protein bands, moderately selective (orange) with 3−5 visible affected protein bands, and poorly selective (red) with five or more affected protein bands. [c] These data were reported previously[25] and were included for comparison.

for the loss of potency observed with the carbonyl isostere analogue **14a**.

Conclusions

In the current study, we investigated the structure−activity relationship of a novel series of benzothiaozle analogues as potent FAAH inhibitors that sprung from a HTS campaign. Smaller groups on the sulfonyl are tolerated but thiophene (**3**) and phenyl analogues gave the best potency. A piperidine ring seems to be optimal for activity, while the pyrrolidine analogue is only 3 times weaker. The interaction between the benzothiazole moiety with the enzyme is mainly driven by hydrophobicity, which was manifested by the weaker potency of the thiazole analogue **16d**, as well as the diminished activity of the corresponding more polar benzoxazole (**16b**) and benzimidazole (**16c**) analogues. Among the substituted benzothiazoles, 4-methoxy analogue **16j** provided the best potency (IC_{50} = 1.7 nM) and resulted in 10-fold improvement over HTS hit **3**. Similar substituted phenyl thiazole analogues are also potent FAAH inhibitors suggesting that this site could allow for further optimization.

Other two major SAR finding are that the sulfonyl group was critical and the amide linkage was not necessary, indicating that these compounds act in a distinctive manner from most of the previously reported classes of inhibitors. Molecular overlay of compound **3** with known FAAH inhibitor MAFP suggested hydrogen bonds between the sulfonyl group and catalytic serine residues, which form the tetrahedral hemiacetal bonds for the α-ketoheterocycle inhibitors such as **2a**. This putative binding mode also revealed the hydrophobic interactions of the benzothiazole moiety with the enzyme pocket that is occupied by the hydrocarbon tail in MAFP and is consistent with major SAR findings. The potencies of inhibitor **3** at different preincubation times showed no time-dependent change, was consistent with a possible reversible mechanism and provided further evidence that these compounds are not acylating agents. The selectivity of compound **3** against other serine hydrolases is exceptional, with no off-target proteins identified in multiple rat tissues. The structural novelty, exquisite potency, exceptional selectivity and unique mechanism of action for these inhibitors make them appealing compounds for the study of FAAH-related pharmacologies, and the endocannabinoid system.

Experimental Section

Chemistry. Unless otherwise noted, all solvents, including anhydrous solvents and chemicals were purchased from Aldrich Co. and/or Acros Organics, and used without further purification. Melting points were recorded on a Unimelt apparatus (Arthur H. Thomas Company, Philadelphia, PA) and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz (Varian Mercury 300) using deuterate solvent and TMS as the internal standard. Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). NMR coupling constants (*J*) in hertz are indicated parenthetically. Low-resolution mass spectra were obtained with a Finnigan SSQ7000 single quad mass spectrometer. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. Analytical thin layer chromatography (TLC) was carried out on E. Merck TLC plates coated with silica gel 60 F_{254} (0.25 mm layer thickness). TLC visualization was carried out using either a UV lamp and/or charring solution as indicated. Flash chromatography was performed on a Biotage Flash 40 chromatography system (Charlottesville, VA) using 40, 90, or 120 g cartridges at 32−63 μm, 60 Å silica gel. Solvent mixtures used for TLC and flash chromatography are reported in v/v total. Preparative HPLC was performed on a Waters Symmetry C8 column (25 mm × 100 mm, 7 μm particle size) using a gradient of 10−100%/0.1% aqueous TFA over 8 min (10 min run time) at a flow rate of 40 mL/min.

***tert*-Butyl 4-(4-(6-Methylbenzo[*d*]thiazol-2-yl)phenylcarbamoyl)piperidine-1-carboxylate (6a).** To a solution of 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (**5**, 2.29 g, 10 mmol) and 4-(6-methylbenzo[*d*]thiazol-2-yl)aniline (**4**, 2.40 g, 10 mmol)

in dichloromethane in a vial was added 4-dimethylamino pyridine (24.4 mg, 0.2 mmol) followed by addition of ethyl dimethyamino cabodiimide hydrochloride (2.87 g, 15 mmol) and the mixture was stirred overnight. The reaction was concentrated and water was added to the residue. Upon sonication, the solid was collected. It was triturated with isopropyl alcohol and filtered to give *tert*-butyl 4-(4-(6-methylbenzo[*d*]thiazol-2-yl)phenylcarbamoyl)piperidine-1-carboxylate as off-while solid (**6a**, 3.9 g, 86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.41 (s, 9 H) 1.45−1.57 (m, 2 H) 1.77−1.85 (m, 2 H) 2.45 (s, 3 H) 2.51−2.61 (m, 1 H) 2.74−2.82 (m, 2 H) 3.99−4.03 (m, 2 H) 7.27−7.40 (m, 1 H) 7.80 (d, J = 8.82 Hz, 2 H) 7.87−7.93 (m, 2 H) 7.98−8.04 (m, 2 H) 10.23 (s, 1 H); MS (DCI/NH_3) m/z 452 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[*d*]thiazol-2-yl)phenyl)piperidine-4-carboxamide (6b).** To a suspension of **6a** (3.61 g, 8 mmol) in ethyl acetate (20 mL) and methanol (5 mL) was added HCl in dioxane (4 N, 30 mL) and the reaction was stirred at room temperature overnight. The solid was filtered to give the HCl salt of **6b** (2.68 g, 87%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.81−1.96 (m, 2 H) 1.97−2.08 (m, 2 H) 2.49 (s, 3 H) 2.69−2.83 (m, 1 H) 2.88−3.02 (m, 2 H) 3.31−3.43 (m, 2 H) 7.36−7.41 (m, 1 H) 7.82−7.90 (m, 2 H) 7.91−7.96 (m, 2 H) 8.02−8.09 (m, 2 H) 8.76 (br, 1 H) 9.13 (br, 1 H) 10.58 (s, 1 H); MS (DCI/NH_3) m/z 352 $(M + H)^+$.

1-(Thiophen-2-ylsulfonyl)piperidine-4-carboxylic Acid (7). To a solution of isonipecotic acid (1.29 g, 10 mmol) and sodium bicarbonate (920 mg, 11 mmol) in water/acetone (1:1, 15 mL) was added a solution of thiophene-2-sulfonyl chloride (1.82 g, 10 mmol) in acetone (8 mL). After 1 h, the solid was collected, dissolved in dichloromethane and triturated with hexane. The solid was filtered and dried in vacuo to give the title compound (**7**, 1.35 g, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.52−1.65 (m, 2 H) 1.86−1.97 (m, 2 H) 2.26−2.36 (m, 1 H) 2.43−2.55 (m, 2 H) 3.42−3.50 (m, 2 H) 7.25−7.34 (m, 1 H) 7.62 (dd, J = 3.73, 1.36 Hz, 1 H) 7.97−8.06 (m, 1 H) 12.28 (br, 1H); MS (DCI/NH_3) m/z 276 $(M+H)^+$, 293 $(M + NH_4)^+$.

1-(Thiophen-2-ylsulfonyl)piperidine-3-carboxylic Acid (7a). Compound **7a** was prepared in a similar manner to the synthesis of compound **7**, substituting nipecotic acid for isonipecotic acid in 50% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.31−1.45 (m, 1 H) 1.51−1.57 (m, 1 H) 1.70−1.86 (m, 2 H) 2.47−2.60 (m, 3 H) 3.29−3.37 (m, 1 H) 3.51−3.53 (m, 1 H) 7.26−7.31 (m, 1 H) 7.64 (dd, J = 3.73, 1.36 Hz, 1 H) 8.05 (dd, J = 5.09, 1.36 Hz, 1 H); MS (DCI/NH_3) m/z 276 $(M + H)^+$, 293 $(M + NH_4)^+$.

1-(Thiophen-2-ylsulfonyl)azetidine-3-carboxylic Acid (7b). Compound **7b** was prepared in a similar manner to the synthesis of compound **7**, substituting azitidine-3-carboxylic acid for isonipecotic acid in 81% yield. ^{1}H NMR (300 MHz, DMSO-D_6) δ ppm 3.70−3.81 (m, 3 H) 3.96 (t, J = 8.82 Hz, 2 H) 7.32−7.38 (m,1 H) 7.77 (dd, J = 3.73, 1.36 Hz, 1 H) 8.14 (dd, J = 5.09, 1.36 Hz, 1 H)12.68 (s, 1 H); MS (DCI/NH_3) m/z 248 $(M + H)^+$, 265 $(M + NH_4)^+$.

1-(Thiophen-2-ylsulfonyl)pyrrolidine-3-carboxylic Acid (7c). Compound **7c** was prepared in a similar manner to the synthesis of compound **7**, substituting pyrrolidine-3-carboxylic acid for isonipecotic acid in 72% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80−2.05 (m, 2 H) 2.91−3.02 (m, 1 H) 3.17−3.30 (m, 2 H) 3.34−3.49 (m, 2 H) 7.28 (dd, J = 5.09, 3.73 Hz, 1 H) 7.70 (dd, J = 3.73, 1.36 Hz, 1 H) 8.04 (dd, J = 5.09, 1.36 Hz, 1 H) 12.54 (s, 1 H); MS (DCI/NH_3) m/z 262 $(M + H)^+$, 279 $(M + NH_4)^+$.

2-(1-(Thiophen-2-ylsulfonyl)piperidin-4-yl)acetic Acid (7d). Compound **7d** was prepared in a similar manner to the synthesis of compound **7**, substituting piperidin-4-ylacetic acid for isonipecotic acid in 47% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.20−1.31 (m, 2 H) 1.58 − 1.66 (m, 1 H) 1.70−1.79 (m, 2 H) 2.14 (d, J = 6.78 Hz, 2 H) 2.26−2.35 (m, 2 H) 3.58−3.62 (m, 2 H) 7.28 (dd, J = 5.09, 3.73 Hz, 1 H) 7.61 (dd, J = 3.73, 1.36 Hz, 1 H) 8.04 (dd, J = 5.09, 1.36 Hz, 1 H) 12.02 (s, 1 H); MS (DCI/NH_3) m/z 290 $(M + H)^+$, 307 $(M + NH_4)^+$.

(1-(Thiophen-2-ylsulfonyl)piperidin-4-yl)methyl Thiophene-2-sulfonate (7e). To a solution of 4-piperidinemethanol (920 mg, 8 mmol) in pyridine (5 mL) was added a solution of thiophene-2-sulfonyl chloride (3.64 g, 20 mmol) in pyridine (15 mL) at −15 °C. The reaction was stirred for 1 h before it was placed in a refrigerator (−5.5 °C). The cold solution of the reaction mixture was poured in crushed ice (30 g) and stirred for 1 h. The solid was filtered and dried to afford the title compound as an off-white solid (2.75 g, 84%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.13−1.28 (m, 2 H) 1.62−1.72 (m, 3 H) 2.24−2.33 (m, 2 H) 3.59 (d, J = 11.87 Hz, 2 H) 3.98 (d, J = 5.76 Hz, 2 H) 7.25 − 7.30 (m, 2 H) 7.60 (dd, J = 3.73, 1.36 Hz, 1 H) 7.86 (dd, J = 3.73, 1.36 Hz, 1 H) 8.03 (dd, J = 5.09, 1.36 Hz, 1 H) 8.16 (dd, J = 5.09, 1.36 Hz, 1 H); MS (DCI/NH_3) m/z 425 $(M + NH_4)^+$.

***N*-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (10).** Compound **10** was prepared in a similar manner to the synthesis of **6a**, substituting 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**9**) for compound **4** in 55% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.27 (s, 12 H) 1.61−1.75 (m, 2 H) 1.88−1.94 (m, 2 H) 2.33−2.47 (m, 3 H) 3.60−3.70 (m, 2 H) 7.28−7.32 (m, 1 H) 7.58 (s, 4 H) 7.66 (dd, J = 3.73, 1.36 Hz, 1 H) 8.06 (dd, J = 5.09, 1.36 Hz, 1 H) 9.94 (s, 1 H); MS (DCI/NH_3) m/z 477 $(M + H)^+$.

***N*-(4-(4-(3-Methoxyphenyl)thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (12a).** A solution of 4-aminobenzothioamide (268 mg, 1.76 mmol) and 2-bromo-1-(3-methoxyphenyl)ethanone (404 mg, 1.76 mmol) in isopropyl alcohol (15 mL) was heated at 60 °C for 2 h. The reaction was cooled to 0 °C and the solid was collected and washed with cold isopropyl alcohol to obtain the title compound (336 mg, 69%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.84 (s, 3 H) 6.92−6.96 (m, 3 H) 7.38 (t, J = 7.97 Hz, 1 H) 7.54−7.63 (m, 2 H) 7.83−7.87 (m, 2 H) 8.05 (s, 1 H); MS (DCI/NH_3) m/z 283 $(M + H)^+$.

***N*-(4-(4-(4-Methoxyphenyl)thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (12b).** Compound **12b** was prepared in a similar manner to the synthesis of compound **12a**, substituting 2-bromo-1-(4-methoxyphenyl)ethanone for 2-bromo-1-(3-methoxyphenyl)ethanone in 70% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.81 (s, 3 H) 6.95−6.98 (m, 2 H) 6.99−7.05 (m, 2 H) 7.83−7.88 (m, 3 H) 7.93−7.98 (m, 2 H); MS (DCI/NH_3) m/z 283 $(M + H)^+$.

***N*-(4-(4-(4-Chlorophenyl)thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (12c).** Compound **12c** was prepared in a similar manner to the synthesis of compound **12a**, substituting 2-bromo-1-(4-chlorophenyl)ethanone for 2-bromo-1-(3-methoxyphenyl)ethanone in 61% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.00−7.02 (m, 2 H) 7.51−7.56 (m, 2 H) 7.87−7.91 (m, 2 H) 8.03−8.08 (m, 2 H) 8.12 (s, 1 H); MS (DCI/NH_3) m/z 287 $(M + H)^+$.

General Procedure for the Preparation of Sulfonamide Analogues 13a−13h. A Smith Porcess vial (0−2 mL) was charged with a stir bar and PS-BEMP resin (5 equiv). To the vessel was added the sulfonyl chloride monomer (1.2 equiv) in dimethylacetamide (1 mL) and the amine **6b** (1 equiv) in dimethylacetamide (0.3 mL). The reaction vessel was sealed and heated to 100 °C for 900 s in an Ermys Optimizer. After cooling, the reaction mixture was filtered through a prepacked column of celite. The reaction products were collected and concentrated to dryness. The residues were dissolved in 1:1 DMSO/MeOH and purified by reverse phase HPLC.

1-(Ethylsulfonyl)-*N*-(4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)piperidine-4-carboxamide (13a). The title compound was obtained in 48% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.18−1.28 (m, 3 H) 1.58−1.73 (m, 2 H) 1.92 (dd, J = 13.39, 2.88 Hz, 2 H) 2.46 (s, 3 H) 2.52−2.59 (m, 1 H) 2.80−2.94 (m, 2 H) 3.01−3.12 (m, 2 H) 3.59−3.72 (m, 2 H) 7.34 (dd, J = 8.14, 1.36 Hz, 1 H) 7.80 (d, J = 8.82 Hz, 2 H) 7.87−7.92 (m, 2 H) 8.01 (d, J = 8.82 Hz, 2 H) 10.24 (s, 1 H); MS (DCI/NH_3) m/z 444 $(M + H)^+$.

1-(Isopropylsulfonyl)-*N*-(4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)piperidine-4-carboxamide (13b). The title compound was obtained in 54% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23 (d, J = 6.78 Hz, 6 H) 1.65 (td, J = 12.12, 3.56 Hz, 2 H) 1.89 (dd, J = 13.22, 2.71 Hz, 2 H) 2.46 (s, 3 H) 2.53−2.62 (m, 1 H) 2.94 (td, J = 12.29, 2.20 Hz, 2 H) 3.32−3.39 (m, 1 H) 3.65−3.69

(m, 2 H) 7.34 (dd, J = 7.97, 1.53 Hz, 1 H) 7.79−7.81 (m, 2 H) 7.87−7.92 (m, 2 H) 7.98−8.04 (m, 2 H) 10.24 (s, 1 H); MS (DCI/NH_3) m/z 458 $(M + H)^+$.

1-(*N*,*N*-Dimethylsulfamoyl)-*N*-(4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)piperidine-4-carboxamide (13c). The title compound was obtained in 43% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.72 (m, 2 H) 1.84−1.94 (m, 2 H) 2.46 (s, 3 H) 2.52−2.59 (m, 1 H) 2.76 (s, 6 H) 2.83−2.90 (m, 2 H) 3.57−3.67 (m, 2 H) 7.34 (dd, J = 8.31, 1.86 Hz, 1 H) 7.75−7.84 (m, 2 H) 7.87−7.92 (m, 2 H) 7.99−8.03 (m, 2 H) 10.26 (s, 1 H); MS (DCI/NH_3) m/z 459 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[d]thiazol-2-yl)phenyl)-1-(phenylsulfonyl)piperidine-4-carboxamide (13d).** The title compound was obtained in 45% yield. ^{1}H NMR (300 MHz, DMSO-D_6) δ ppm 1.59−1.73 (m, 2 H) 1.87−1.93 (m, 2 H) 2.31−2.41 (m, 3 H) 2.45 (s, 3 H) 3.59−3.73 (m, 2 H) 7.34 (dd, J = 7.97, 1.53 Hz, 1 H) 7.63−7.70 (m, 2 H) 7.71−7.80 (m, 5 H) 7.87−7.92 (m, 2 H) 7.96−8.03 (m, 2 H) 10.13 (s, 1 H); MS (DCI/NH_3) m/z 492 $(M + H)^+$.

1-(2-Fluorophenylsulfonyl)-*N*-(4-(6-methylbenzo[d]thiazol-2-yl)phenyl)piperidine-4-carboxamide (13e). The title compound was obtained in 35% yield. ^{1}H NMR (500 MHz, DMSO-d_6-D_2O) δ ppm 1.59−1.68 (m, 2 H) 1.87−1.94 (m, 2 H) 2.44−2.51 (m, 4 H) 2.62−2.67 (m, 2 H) 3.74−3.78 (m, 2 H) 7.36 (d, J = 8.54 Hz, 1 H) 7.45−7.54 (m, 2 H) 7.75−7.84 (m, 4 H) 7.88−7.92 (m, 2 H) 8.00−8.01 (m, 2 H); MS (ESI) m/z 510 $(M + H)^+$.

1-(2-Chlorophenylsulfonyl)-*N*-(4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)piperidine-4-carboxamide (13f). The title compound was obtained in 31% yield. ^{1}H NMR (500 MHz, DMSO-d_6-D_2O) δ ppm 1.57−1.66 (m, 2 H) 1.85−1.92 (m, 2 H) 2.46 (s, 3 H) 2.51−2.57 (m, 1 H) 2.81−2.87 (m, 2 H) 3.76−3.80 (m, 2 H) 7.36 (d, J = 8.24 Hz, 1 H) 7.57−7.62 (m, 1 H) 7.68−7.78 (m, 4 H) 7.87−7.92 (m, 2 H) 7.99−8.03 (m, 3 H); MS (ESI) m/z 526 $(M + H)^+$.

1-(3-Chlorophenylsulfonyl)-*N*-(4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)piperidine-4-carboxamide (13g). The title compound was obtained in 27% yield. ^{1}H NMR (500 MHz, DMSO-d_6-D_2O) δ ppm 1.62−1.71 (m, 2 H) 1.89 (m, 2 H) 2.31−2.39 (m, 3 H) 2.46 (s, 3 H) 3.69 (m, 2 H) 7.36 (d, J = 8.54 Hz, 1 H) 7.55−7.59 (m, 4 H) 7.73−7.77 (m, 2 H) 7.88−7.91 (m, 2 H) 8.00 (m, J = 8.85 Hz, 2 H); MS (ESI) m/z 526 $(M + H)^+$.

1-(4-Chlorophenylsulfonyl)-*N*-(4-(6-methylbenzo[*d*]thiazol-2-yl)phenyl)piperidine-4-carboxamide (13h). The title compound was obtained in 45% yield. ^{1}H NMR (500 MHz, DMSO-d_6-D_2O) δ ppm 1.67 (s, 2 H) 1.88 (s, 2 H) 2.29−2.37 (m, 3 H) 2.46 (s, 3 H) 3.70 (m, 2 H) 7.37 (s, 1 H) 7.49 (d, J = 7.93 Hz, 2 H) 7.66 (d, J = 8.24 Hz, 2 H) 7.75 (d, J = 8.85 Hz, 2 H) 7.90 (d, J = 8.24 Hz, 2 H) 8.00 (d, J = 8.85 Hz, 2 H); MS (DCI/NH_3) m/z 506 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[*d*]thiazol-2-yl)phenyl)-1-(thiophene-2-carbonyl)piperidine-4-carboxamide (14a).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting thiophene-2-carboxylic acid for **5** and **6b** for **4** in 41% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.58−1.72 (m, 2 H) 1.86−1.97 (m, 2 H) 2.46 (s, 3 H) 2.64−2.77 (m, 1 H) 3.09 (m, 2 H) 4.30 (m, 2 H) 7.13 (dd, J = 5.09, 3.73 Hz, 1 H) 7.32−7.37 (m, 1 H) 7.42 (d, J = 3.73 Hz, 1 H) 7.76 (d, J = 4.41 Hz, 1 H) 7.80 (d, J = 8.82 Hz, 2 H) 7.87−7.92 (m, 2 H) 8.02 (d, J = 8.82 Hz, 2 H) 10.27 (s, 1 H); MS (DCI/NH_3) m/z 462 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[*d*]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-3-carboxamide (15a).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting **7a** for **5** in 29% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.36 − 1.63 (m, 2 H) 1.83−1.95 (m, 2 H) 2.28−2.42 (m, 2 H) 2.46 (s, 3 H) 2.68−2.77 (m, 1 H) 3.60−3.64 (m, 1 H) 3.77−3.80 (m, 1 H) 7.31−7.36 (m, 2 H) 7.67−7.68 (m, 1 H) 7.76−7.79 (m, 2 H) 7.88−7.91 (m, 2 H) 8.00−8.06 (m, 3 H) 10.38 (s, 1 H); MS (DCI/NH_3) m/z 498 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[*d*]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)azetidine-3-carboxamide (15b).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting **7c** for **5** in 72% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.45 (s, 3 H) 3.41−3.56 (m, 1 H) 3.87−4.01 (m, 4 H) 7.32−7.41 (m, 2 H) 7.69 (m, 2 H) 7.77−7.81 (m, 1 H) 7.85−7.95 (m, 2 H) 8.00−8.02 (m, 2 H) 8.14−8.18 (m, 1 H) 10.28 (s, 1 H); MS (DCI/NH_3) m/z 470 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[*d*]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)pyrrolidine-3-carboxamide (15c).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting **7b** for **5** in 76% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.85−1.99 (m, 1 H) 2.03−2.15 (m, 1 H) 2.45 (s, 3 H) 3.12 (m, 1 H) 3.25−3.38 (m, 3 H) 3.56 − 3.60 (m, 1 H) 7.27−7.37 (m, 2 H) 7.71−7.75 (m, 3 H) 7.87−7.92 (m, 2 H) 7.98−8.07 (m, 3 H) 10.32 (s, 1 H); MS (DCI/NH_3) m/z 484 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[*d*]thiazol-2-yl)phenyl)-2-(1-(thiophen-2-ylsulfonyl)piperidin-4-yl)acetamide (15d).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting **7d** for **5** in 40% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23−1.38 (m, 2 H) 1.73−1.85 (m, 3 H) 2.27−2.43 (m, 4 H) 2.45 (s, 3 H) 3.60−3.64 (m, 2 H) 7.26−7.31 (m, 1 H) 7.32−7.35 (m 1 H) 7.62 (dd, J = 3.73, 1.36 Hz, 1 H) 7.75−7.78 (m, 2 H) 7.87−7.92 (m, 2 H) 7.97−8.06 (m, 3 H) 10.19 (s, 1 H); MS (DCI/NH_3) m/z 512 $(M + H)^+$.

4-(6-Methylbenzo[*d*]thiazol-2-yl)-*N*-((1-(thiophen-2-ylsulfonyl)piperidin-4-yl)methyl)aniline (15e). To a solution of 4-(6-methylbenzo[*d*]thiazol-2-yl)aniline (120 mg, 0.5 mmol) and the **7e** (203 mg, 0.5 mmol) in tetrahydrofuran (10 mL) was added sodium hydride (60%, 60 mg, 1.5 mmol) and heated at 80 °C overnight. The reaction mixture was concentrated, taken up in water (20 mL) and extracted with ethyl acetate (3 × 10 mL). The organics were combined, dried and concentrated and the residue was purified by flash chromatography (silica gel, 4:1 hexane/ethyl acetate) to obtain a yellow solid (106 mg, 44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.21−1.36 (m, 2 H) 1.74−1.79 (m, 1 H) 1.82−1.86 (m, 2 H) 2.25−2.37 (m, 2 H) 2.42 (s, 3 H) 2.95−3.04 (m, 2 H) 3.62−3.71 (m, 2 H) 6.46 (t, J = 5.59 Hz, 1 H) 6.66 (d, J = 8.82 Hz, 2 H) 7.24−7.29 (m, 2 H) 7.60−7.65 (m, 1 H) 7.72−7.81 (m, 4 H) 8.01−8.05 (m, 1 H); MS (DCI/NH_3) m/z 484 $(M + H)^+$.

***N*-(4-(Benzo[*d*]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16a).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting 4-(1,3-benzothiazol-2-yl)phenylamine for **4** and **7** for **5** in 77% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.74 (m, 2 H) 1.95 (m, 2 H) 2.36−2.48 (m, 3 H) 3.64−3.71 (m, 2 H) 7.31 (dd, J = 5.09, 3.73 Hz, 1 H) 7.41−7.56 (m, 2 H) 7.67 (dd, J = 3.73, 1.36 Hz, 1 H) 7.78 (d, J = 8.82 Hz, 2 H) 8.00−8.14 (m, 5 H) 10.17 (s, 1 H); MS (DCI/NH_3) m/z 484 $(M + H)^+$.

***N*-(4-(6-Methylbenzo[*d*]oxazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16b).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting 4-(6-methyl-benzooxazol-2-yl)-phenylamine for **4** and **7** for **5** in 30% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.78 (m, 2 H) 1.88−2.01 (m, 2 H) 2.37−2.48 (m, 6 H) 3.63−3.71 (m, 2 H) 7.19−7.22 (m, 1 H) 7.29−7.32 (m, 1 H) 7.55−7.70 (m, 3 H) 7.78−7.84 (m, 2 H) 8.05−8.14 (m, 3 H) 10.19 (s, 1 H); MS (DCI/NH_3) m/z 482 $(M + H)^+$.

***N*-(4-(6-Methyl-1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16c).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting 4-(5-methyl-1*H*-benzoimidazol-2-yl)-phenylamine for **4** and **7** for **5** in 57% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.78 (m, 2 H) 1.94 (m, 2 H) 2.34−2.47 (m, 6 H) 3.59−3.74 (m, 2 H) 7.00 (t, J = 7.80 Hz, 1 H) 7.26−7.50 (m, 3 H) 7.66−7.78 (m, 3 H) 8.01−8.11 (m, 3 H) 10.04 (s, 1 H) 12.59 (d, J = 10.51 Hz, 1 H); MS (DCI/NH_3) m/z 481 $(M + H)^+$.

***N*-(4-(Thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16d).** To a solution of **10** (144 mg, 0.3 mmol) and 2-bromothiazole (97 mg, 0.6 mmol) in tetrahydrofuran was added potassium carbonate (103 mg, 0.75 mmol) and palladium tetrakistriphenylphosphine (17 mg, 0.015 mmol). The reaction was heated to reflux overnight, cooled and concentrated. The residue was partitioned between dichloromethane:isopropyl alcohol (10:1,

3 × 15 mL) and water (10 mL). The organic layers were combined, dried, concentrated and the residue was purified by HPLC to afford the title compound in 40% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.62−1.77 (m, 2 H) 1.93 (m, 2 H) 2.34−2.48 (m, 3 H) 3.62−3.71 (m, 2 H) 7.28−7.33 (m, 1 H) 7.66−7.73 (m, 4 H) 7.85−7.91 (m, 3 H) 8.06 (dd, J = 5.09, 1.36 Hz, 1 H) 10.08 (s, 1H); MS (DCI/NH_3) m/z 434 $(M + H)^+$.

***N*-(4-(Quinolin-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16e).** The title compound was prepared in a similar manner to the synthesis of **16d**, substituting 2-chloroquinoline for 2-bromothiazole in 40% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.64−1.79 (m, 2 H) 1.95 (m, 2 H) 2.35−2.48 (m, 3 H) 3.64−3.73 (m, 2 H) 7.31 (dd, J = 5.09, 3.73 Hz, 1 H) 7.53−7.62 (m, 1 H) 7.65−7.70 (m, 1 H) 7.73−7.79 (m, 3 H) 7.97 (m, 1 H) 8.01−8.13 (m, 3 H) 8.24 (d, J = 8.82 Hz, 2 H) 8.41 (d, J = 8.82 Hz, 1 H) 10.05 (s, 1 H); MS (DCI/NH_3) m/z 478 $(M + H)^+$.

***N*-(4-(Quinoxalin-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16f).** The title compound was prepared in a similar manner to the synthesis of **16d**, substituting 2-chloroquinoxaline for 2-bromothiazole in 58% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.65−1.79 (m, 2 H) 1.91−2.00 (m, 2 H) 2.37−2.48 (m, 3 H) 3.65−3.73 (m, 2 H) 7.31 (dd, J = 5.09, 3.73 Hz, 1 H) 7.68 (dd, J = 3.73, 1.36 Hz, 1 H) 7.78−7.90 (m, 4 H) 8.06−8.12 (m, 3 H) 8.30−8.33 (m, 2 H) 9.55 (s, 1 H) 10.13 (s, 1 H); MS (DCI/NH_3) m/z 479 $(M + H)^+$.

***N*-(4-(6-Chlorobenzo[*d*]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16g).** The title compound was prepared in a similar manner to the synthesis of **16d**, substituting 2,6-dichlorobenzothiazole for 2-bromothiazole in 25% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.72 (m, 2 H) 1.90−1.99 (m, 2 H) 2.38−2.49 (m, 3 H) 3.65−3.69 (m, 2 H) 7.28−7.37 (m, 1 H) 7.55 (dd, J = 8.82, 2.03 Hz, 1 H) 7.65−7.70 (m, 1 H) 7.78 (d, J = 8.48 Hz, 2 H) 7.98−8.09 (m, 4 H) 8.29 (d, J = 2.03 Hz, 1 H) 10.19 (s, 1 H); MS (DCI/NH_3) m/z 518 $(M + H)^+$.

N-(4-(6-Methoxybenzo[d]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16h). The title compound was prepared in a similar manner to the synthesis of 1**6d**, substituting 2-chloro-6-methoxybenzothiazole for 2-bromothiazole in 31% yield. ^{1}H NMR (300 MHz, DMSO-D_6) δ ppm 1.63−1.77 (m, 2 H) 1.91 − 1.97 (m, 2 H) 2.36−2.48 (m, 3 H) 3.63−3.72 (m, 2 H) 3.85 (s, 3 H) 7.11 (dd, J = 8.99, 2.54 Hz, 1 H) 7.31 (dd, J = 5.09, 3.73 Hz, 1 H) 7.66−7.71 (m, 2 H) 7.73 −7.76 (m, 2 H) 7.89 (d, J = 8.82 Hz, 1 H) 7.91 − 7.98 (m, 2 H) 8.07 (dd, J = 4.92, 1.19 Hz, 1 H) 10.14 (s, 1 H); MS (DCI/NH_3) m/z 514 $(M+H)^+$.

***N*-(4-(4-Methylbenzo[*d*]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16i).** The title compound was prepared in a similar manner to the synthesis of 1**6d**, substituting 2-chloro-4-methylbenzothiaozole for 2-bromothiazole in 61% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.78 (m, 2 H) 1.92−1.98 (m, 2 H) 2.36−2.48 (m, 3 H) 2.71 (s, 3 H) 3.64−3.72 (m, 2 H) 7.29−7.35 (m, 3 H) 7.67 (dd, J = 3.73, 1.36 Hz, 1 H) 7.75−7.80 (m, 2 H) 7.88−7.94 (m, 1 H) 8.01−8.08 (m, 3 H) 10.17 (s, 1 H); MS (DCI/NH_3) m/z 498 $(M + H)^+$.

***N*-(4-(4-Methoxybenzo[d]thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (16j).** The title compound was prepared in a similar manner to the synthesis of 1**6d**, substituting 2-chloro-4-methoxybenzothiaozole for 2-bromothiazole in 67% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.78 (m, 2 H) 1.92−1.97 (m, 2 H) 2.34−2.48 (m, 3 H) 3.62−3.75 (m, 2 H) 3.98 (s, 3 H) 7.05−7.08 (m, 1 H) 7.28−7.33 (m, 1 H) 7.38 (t, J = 7.97 Hz, 1 H) 7.62−7.65 (m, 1 H) 7.67 (dd, J = 3.73, 1.36 Hz, 1 H) 7.73−7.80 (m, 2 H) 7.97−8.04 (m, 2 H) 8.07 (dd, J = 5.09, 1.36 Hz, 1 H) 10.16 (s, 1 H); MS (DCI/NH_3) m/z 514 $(M + H)^+$.

***N*-(4-(4-(3-Methoxyphenyl)thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (17a).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting **12a** for **5** in 47% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.78 (m, 2 H) 1.92−1.97 (m, 2 H) 2.35−2.48 (m, 3 H) 3.63−3.73 (m, 2 H) 3.84 (s, 3 H) 6.95 (m, 1 H) 7.29−7.41 (m, 1 H) 7.38 (t, J = 7.97 Hz, 1 H) 7.58−7.64 (m, 2 H) 7.67 (dd, J = 3.73, 1.36 Hz, 1 H) 7.71 − 7.76 (m, 2 H) 7.94 − 7.99 (m, 2 H) 8.07 (dd, J = 4.75, 1.36 Hz, 1 H) 8.13 (s, 1 H) 10.10 (s, 1 H); MS (DCI/NH_3) m/z 540 $(M + H)^+$.

***N*-(4-(4-(4-Methoxyphenyl)thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (17b).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting **12b** for **5** in 91% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.77 (m, 2 H) 1.92 − 1.97 (m, 2 H) 2.35−2.49 (m, 3 H) 3.63−3.72 (m, 2 H) 3.81 (s, 3 H) 6.99−7.05 (m, 2 H) 7.29−7.32 (m, 1 H) 7.66−7.75 (m, 3 H) 7.92−7.99 (m, 5 H) 8.07 (dd, J = 5.09, 1.36 Hz, 1 H) 10.10 (s, 1 H); MS (DCI/NH_3) m/z 540 $(M + H)^+$.

***N*-(4-(4-(4-Chlorophenyl)thiazol-2-yl)phenyl)-1-(thiophen-2-ylsulfonyl)piperidine-4-carboxamide (17c).** The title compound was prepared in a similar manner to the synthesis of **6a**, substituting **12c** for **5** in 88% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.63−1.77 (m, 2 H) 1.92 − 1.97 (m, 2 H) 2.35−2.47 (m, 3 H) 3.62−3.72 (m, 2 H) 7.31 (dd, J = 5.09, 3.73 Hz, 1 H) 7.50−7.59 (m, 2 H) 7.66−7.76 (m, 3 H) 7.96 (m, 2 H) 8.04−8.09 (m, 3 H) 8.17 (s, 1 H) 10.10 (s, 1 H); MS (DCI/NH_3) m/z 540 $(M + H)^+$.

In Vitro Assay for FAAH Activity. Crude rat FAAH enzyme was isolated from rat cortex as follows:

Rat cortex (Pel-freeze Catalog number 56006−2) was homogenized using a motorized homogenizer in 50 mM Tris, pH 8.0, buffer containing 320 mM sucrose and the protease inhibitors benzamidine 1 mM, leupeptin 0.5 μg/mL, pepstatin 0.7 μg/mL, and aprotinin 2 μg/mL (Sigma) at 1:5 (w/v). The smooth homogenate was centrifuged at 1100g for 5 min at 4 °C, and the supernatant carefully taken. This centrifugation was repeated twice, leaving the pellet each time. The supernatant was then centrifuged at 22 000g for 30 min at 4 °C, and the resulting P2 pellet containing crude rat FAAH was resuspended in the same Tris buffer at a 1:0.5 ratio, original w/v, and stored at −80 °C.

Human FAAH Cloning, Expression and Purification. The cloning, expression and purification of the human enzyme is described in Kage et al.[29] Briefly, full length human FAAH (accession number U82535) was amplified from cerebellum cDNA. The full-length product was subcloned into pcDNA3.1 hygro(−) and used for stable cell line generation by transfection into HEK293 cells using Lipofectamine 2000 (Invitrogen) as per the manufacturer's directions. Crude lysates were made from individual clones by dislodging adherent cells, spinning down the cell suspension and resuspending the cell pellet in a small volume of Tris-EDTA (pH 8) containing protease inhibitors. This suspension was then sonicated five times for 10 s, aliquoted and stored at −80 °C. The resulting lysates were screened for FAAH activity using a [^{14}C]-anandamide assay, as described in Omeir et al.,[39] and a single clone with significant FAAH activity was selected for scale up.

^{3}H-anandamide carbon filtration assay for FAAH activity was adapted from Wilson et al.[30] Tritiated anandamide was obtained from American Radiolabeled Chemicals (Catalog number ART626, 60 Ci/mmol, 1 mCi/mL) and unlabeled anandamide was obtained from Sigma (Catalog number A0580). Prior to the assay, a 10× stock of substrate was made in ethanol and consisted of 100 μM anandamide and 0.25 μCi of tritiated anandamide.

A 5× reaction buffer containing 625 mM Tris, pH 8.0 (Invitrogen 15568−025), 5 mM EDTA, and 0.5% BSA, fatty acid free (Calbiochem 126609) was used for all experiments, and experiments were carried out in 96 well plates. For preincubation experiments, 20 μL of 5× buffer, 10 μL of enzyme and water were combined for an 80 μL reaction mix. Ten microliters of 10× compound was added to the reaction mixture and allowed to preincubate for 15 min, or as indicated. The reaction was started by addition of 10 μL of 10× substrate and allowed to proceed for 14 min, then stopped by addition of 10 μL of 0.7 M HCl. For other experiments, 20 μL of 5× buffer, 10 μL of 10× substrate and water were combined for an 80 μL reaction mix. Ten microliters of 10× compound was added to the reaction mix, and the reaction was started by addition of 10 μL of enzyme. After 14 min, the reaction was stopped by addition of 10 μL of 0.7 M HCl.

Isolation of ^{3}H-Ethanolamine Product by Phase Separation. Twenty-five microliters of activated carbon was loaded into 96 well filter plates (Millipore MultiScreen plates Catalog number MAFCNOB10). To prewet the carbon, 100 μL of methanol was pipetted into each well and the plate was then centrifuged into a "waste" OptiPlate at 755*g* for 5 min.

Sixty microliters of the above reaction was placed onto the wetted carbon filtered plate and placed on top of a 96 well OptiPlate containing 150 μL of MicroScint 40, followed by centrifugation at 755*g* for 5 min to collect the radiolabeled ^{3}H-ethanolamine product. The OptiPlate was then read on a Packard TopCount after incubation for 1 h at room temperature.

Selectivity Screening. The selectivity screening was conducted as detailed.[25]

Molecular Overlay Study. The ligand pose from the cocrystallized X-ray structure of methyl arachidonyl fluorophosphonate (MAFP) in complex with FAAH (PDB ID 1MT5) was extracted. An exhaustive set of conformers for **3** was then generated using OMEGA (OpenEye Scientific Software, Santa Fe, NM; http://www.eyesopen.com/). This set of conformers was then triaged based on similarity to the three-dimensional shape and electrostatics of the known MAFP bound conformation using the program ROCS (OpenEye Scientific Software, Santa Fe, NM; http://www.eyesopen.com/), from which we selected the top 10 most similar poses. These poses where then scored by FRED (OpenEye Scientific Software, Santa Fe, NM; http://www.eyesopen.com/) for complementarity against the FAAH active site. The resulting pose from the top of this list was retained and is the pose shown in Figure 2b.

Acknowledgment. We thank the Abbott high-throughput screening group and Victoria Scott for contributing to the development of the screening method for fatty acid amide hydrolase.

Supporting Information Available: Elemental analysis data for all novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM801042A