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Identification of potent, noncovalent fatty acid amide hydrolase (FAAH) inhibitors

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ABSTRACT

Starting from a series of ureas that were determined to be mechanism-based inhibitors of FAAH, several spirocyclic ureas and lactams were designed and synthesized. These efforts identified a series of novel, noncovalent FAAH inhibitors with in vitro potency comparable to known covalent FAAH inhibitors. The mechanism of action for these compounds was determined through a combination of SAR and co-crystallography with rat FAAH.

Fatty acid amide hydrolase is an integral membrane enzyme that degrades the fatty acid amide family of signaling lipids, including anandamide (*N*-arachidonylethanolamine, AEA). AEA was identified as an endogenous agonist for the cannabinoid receptors CB1 and CB2 in 1992.[1] The anti-nociceptive benefit of stimulating the CB1/CB2 receptors has long been recognized and for some time it has been postulated that inhibiting FAAH would show similar therapeutic benefits through increasing lifetime of AEA in various tissues.[2,3] Evidence supporting this hypothesis includes the fact that FAAH knock-out mice show increased levels of AEA in the brain accompanied with decreased susceptibility to neuropathic and inflammatory pain.[4] Likewise, inhibition of FAAH with small molecule inhibitors (e.g., OL-135[5] and URB-597[6]) in vivo leads to similar increases in AEA levels and decreased sensitivities in pain models.[7] Except for the recent report by Wang,[8] most FAAH inhibitors reported to date appear to rely on covalent modification of the active site serine 241. Herein we describe the identification of a novel, potent and noncovalent series of FAAH inhibitors with in vitro potencies comparable to covalent FAAH inhibitors.

Compounds **1a** and **2** were identified during the course of a high-throughput screen (HTS) of the Amgen compound collection. As indicated in Table 1, **1a** and **2** inhibited rat and human FAAH with potencies comparable to that of OL-135 and URB-597 in our assays.[9] Subsequent studies revealed these ureas to be suicide substrates.[10,11] Incubation of **1b** or **2** with FAAH followed by crystallization resulted in complexes where the urea carbonyl acylated S241 with expulsion of the aniline fragment as indicated equation 1.[12] The complete loss of FAAH inhibitory activity seen with amide **1d** is consistent with mechanism-based inactivation suggested by co-crystallization studies. Interestingly, simple N-methylation of **1b** also resulted in a complete loss of FAAH activity (cf. **1b**, **1c** and **1d**, Table 1).

We were interested in developing reversible (and preferably noncovalent) FAAH inhibitors with the expectation that they might have a different selectivity[13]/safety[14] profile compared to suicide substrates like **1** or **2**. We imagined that it might be possible to create reversible FAAH inhibitors based on spirolactams or cyclic ureas related to **1**. Starting from a urea exemplified by **3** (Fig. 1), we reasoned that by installing steric hindrance adjacent to the carbonyl group would reduce its susceptibility to nucleophilic attack. In addition, tethering the aniline to the opposite side of the scissile bond would prevent it from leaving the active site after initial attack by S241 thus creating the possibility of reversible inhibition through re-cyclization of the postulated enzyme-acyl intermediate. Our strategy (summarized in Fig. 1 for a spirolactam) called

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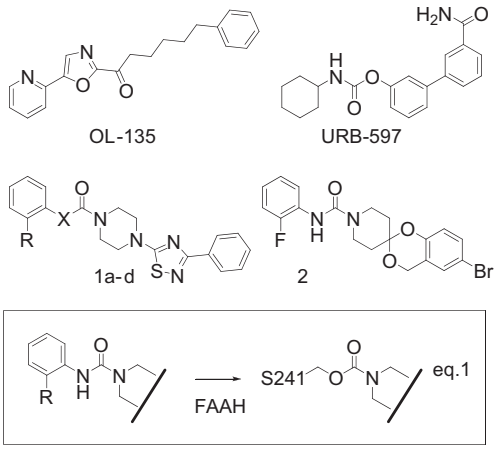
Table 1
FAAH activity of high-throughput screening hits, reference compounds and selected SAR data



Cmpd	R	X	Rat FAAH IC_{50}, μM[a]	Human FAAH IC_{50}, μM[a]
OL-135	—	—	0.025	0.016
URB-597	—	—	0.003	0.004
1a	H	NH	0.010	—
1b	F	NH	0.003	0.001
1c	F	N-Me	>10	—
1d	F	CH_2	>10	>10
2	—	—	0.036	0.005

[a] Values are means of at least two experiments. All SAR data reported herein was obtained using the assays described in Ref. 9.

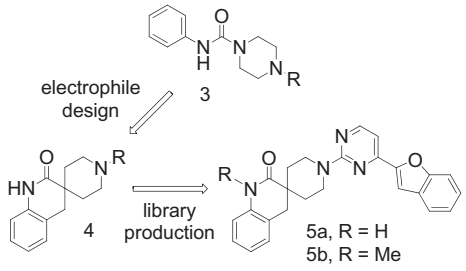


Figure 1. FAAH inhibitors design and strategy.

for designing a serine electrophile[15] that carried a substituent to target the acyl-chain binding pocket (ABP)[11] of FAAH (i.e., R in **4**). Once suitable scaffolds were identified we intended to optimize inhibitor affinity for the ABP and eventually remove the electrophile.

Toward this end, we designed a number of bicyclic and spirocyclic ureas and lactams as potential FAAH inhibitor electrophiles. The designs were prioritized through their theoretical binding to the active site of FAAH using the program Glide[16] and in-house X-ray crystallographic data.[11] Selected designs were synthesized and used to produce libraries. Early on in this effort, we identified 4-[2-benzofuran]-2-yl-pyrimidine as a highly potent piperidine nitrogen substituent. For example, spirolactam **5a** showed inhibition of rFAAH with an IC_{50} of 1.2 μM. Interestingly, the *N*-methyl spirolactam **5b** showed slightly improved FAAH inhibition (IC_{50} = 0.50 μM) in contrast to the complete loss of activity seen upon N-methylating **1b**, which suggested two distinct binding modes for these two series of compounds. Encouraged by initial results we used 4-[2-benzofuran]2-yl-pyrimidine as a key fragment to evaluate additional serine-electrophile designs, that were selected from our molecular modeling efforts.

Table 2 summarizes FAAH inhibition data for a series of 4-[2-benzofuran]2-yl-pyrimidines substituted with a pyrrolidine, piperidine or homopiperidine bearing a methyl-ketobenzimidazole unit. As indicated, the 3-(keto-benzimidazole-3-yl)piperidine **7** displayed potent inhibition of hFAAH and moderate inhibition of the rat enzyme. However, the pyrrolidine **6**, the 4-(ketobenzimidazole-3-yl) piperidine **12** and the homopiperidine **13** were significantly less potent. The potency of **7** could be improved upon by replacing the $N-1$ methyl group with an ethyl group (cf. **7** and **8**). Further improvement in potency is seen through replacement of the ketobenzimidazole with the nitrogen bearing imidazo[4,5-*b*]pyridin-2(3H)-one in **9**. Examination of each enantiomer of **9** revealed that only the (*S*) isomer **11** inhibited human and rat FAAH. With **11** in hand we set out to re-examine the SAR of the aliphatic binding pocket by further modifying the piperidine N-substituent.

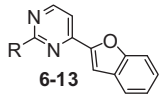
Representative syntheses of our FAAH inhibitors are shown in Scheme 1.[17] 2-Chloro-4-[2-benzofuran]2-yl-pyrimidine **14** is available from the cross-coupling of 2,4-dichloropyrimidine and benzofuran-2-yl-boronic acid.[18] The (*S*)-1-(piperidin-3-yl)-1H-benzo[*d*]imidazol-2(3H)-one subunit was prepared by the S_NAr reaction of 2-chloronitrobenzene and commercially available (*S*)-3-amino-1-Boc-piperidine which gave **15**.[19] Reduction of **15** followed by treatment with 1,1-carbonyldiimidazole (CDI) and alkylation of the ketobenzimidazole nitrogen gave **16**. Removal of the Boc protecting group provided **17**.[20] Finally, coupling of **17** and **14** in the presence of Hünig's base gave the *S*-isomer of **8**. Alternatively, S_NAr reaction of **17** with dichloro-heterocycles gave **18**.[21] Cross-coupling of **18** with aryl-boronic acids[15] afforded **19–22** and allowed us to rapidly explore the SAR of the aryl-pyrimidine fragment.

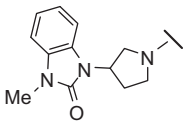
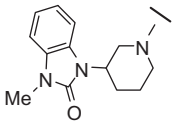
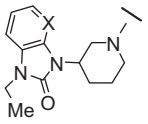
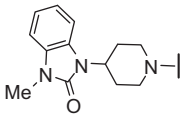
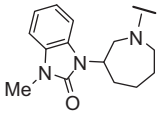
Additional SAR and whole cell assay data for this series of FAAH inhibitors is summarized in Table 3. Moving the benzofuran subunit from the 4-position to the 5-position on the pyrimidine ring (**19**) resulted in a complete loss of potency against rat and human FAAH. In contrast, both alternate pyrimidine isomers (**20** and **21**) were well tolerated as long as a 1,3-relationship between the piperidine and benzofuran units was maintained.

Finally, we examined the SAR of the aryl ring attached to the central pyrimidine (compounds **23**–**35**, Table 3). Replacement of the benzofuran subunit with a simple phenyl ring (cf. compounds **9** and **23**) resulted in a significant loss of potency against both the rat and human enzymes. In general, substituents in either the *ortho* or *meta* position were unable to restore inhibitory activity, particularly on the rat enzyme. On the other hand, small *para* substituents were generally well tolerated with fluorine being preferred (cf. compounds **20** to **24–28** and **23**–**30**). Finally, changing the benzofuran subunit to a 5-chloro-2-thiophene resulted in a significant improvement in potency when combined with aza-ketobenzimidazoles (cf. **11**, Table 2 to **32** and **33**, Table 3). Gratifyingly, compounds **32** and **33** showed inhibition of rat and human FAAH in both biochemical and whole cell assays with potencies comparable to that observed with benchmark FAAH inhibitors.

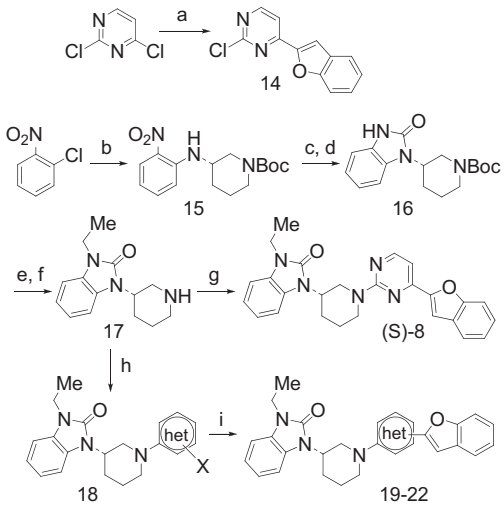
While our compounds were originally envisioned as electrophiles for S241 of FAAH, we were skeptical that the ketobenzimidazole identified in these studies was sufficiently reactive to be attacked by S241. Thus, we set out to determine the mechanism of inhibition for these FAAH inhibitors in greater detail. First, we examined the reversibility of FAAH inhibition through evaluation of progress curves.[22] In the experiment, rFAAH and a test compound were incubated at a concentration 100 fold greater than the usual assay conditions. After 1 h, the mixtures were diluted by a factor of 100 with buffer containing anandamidoaminomethylcumarin (AAMCA). The FAAH dependant hydrolysis of AAMCA

Table 2
Rat and human FAAH SAR data for a series of 2-substituted 4-[2-benzofuran]2-yl-pyrimidines (**6–13**)



Cmpd	R	Rat FAAH IC_{50},[a] μM	Human FAAH IC_{50},[a] μM
6		>10	>10
7		0.78	0.03
8, X = CH **9**, X = N **10**, (*R*), X = N **11**, (*S*), X = N		0.06 0.03 >10 0.02	0.01 0.009 >10 0.006
12		>10	>10
13		>10	0.35

[a] Values are means of at least two experiments. All SAR data reported herein was obtained using the assays described in Ref. 9.



Scheme 1. Representative synthesis of FAAH inhibitors. Reagents and conditions: (a) $Pd(Ph_3P)_4$, benzofuran-2-yl boronic acid, Na_2CO_3, H_2O, EtOH, toluene 40–66%; (b) (*S*)-*t*-butyl 3-aminopiperidine-1-carboxylate, *i*-Pr_2NEt, DMF 60 °C, 75%; (c) H_2 (1 atm), 10% Pd/C, THF; (d) CDI, THF, 60 °C 73% from **15**; (e) NaH, EtI, DMF, 0 °C, 94%; (f) HCl, dioxane, 98%; (g) **14**, *i*-Pr_2NEt, DMF 60 °C, 72%; (h) *i*-Pr_2NEt, THF, 25 °C; 5-bromo-2-chloropyrimidine 46%, or 2,4-dichloropyrimidine, 73%, or 4,6-dichloropyrimidine, 94%; 4,6-dichloropyrimidine-2-amine, 68% or 2,6-dichloropyrazine, 8%; (i) $Pd(Ph_3P)_2Cl_2$, benzofuran-2-yl boronic acid, Na_2CO_3 (2 equiv), dioxane/water, 120 °C, 46–77% (for **19–21**) or $Pd(Ph_3P)_4$, aryl boronic acid, 2:2:1 benzene/MeOH/2 M Na_2CO_3, 85 °C, 41–52% (for **22**).

was then monitored by UV. Under these conditions compounds **7**, **33**, and **35** were shown to be reversible inhibitors of FAAH while URB-597 was shown to inhibit the enzyme irreversibly, as expected.[23]

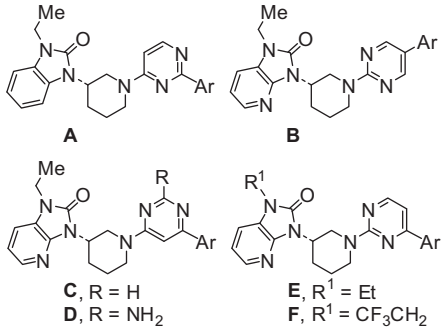
Next, we were interested in replacing the ketobenzimidazole fragment of our inhibitors with a noncarbonyl bearing heterocycle. We reasoned that if we could replace the carbonyl while maintaining comparable FAAH inhibition, it would provide evidence for a noncovalent mechanism of action. Thus, we evaluated a number of simple 5,6-heteroycles. We realized success with the identification of 3-pyrrolo[2,3-*b*]pyridine as a replacement for the ketobenzimidazole (see **41–48**, Table 4). The synthesis of pyrrolopyridines **41–48** is summarized in Scheme 2. Condensation of *N*-Boc-3-piperidinone with **36** under basic conditions yielded **37**.[24] Hydrogenation[25] and subsequent Boc removal gave **39**. Finally, coupling **39** with 2-Cl-4-[2-benzothiophene]pyrimidine followed by alkylation of the pyrrolopyridine nitrogen provided compounds **41** and **42**. Similarly, protection of **37** as its *N*-phenylsulfonyl-derivative, followed by methylation provided the 2-methylpyrrolopyridine **40**.[26] De-protection and elaboration as described above provided compounds **41–46**. As indicated by the SAR data in Table 4, the pyrrolopyridine-based FAAH inhibitors exhibited potencies comparable to the ketobenzimidazole-based inhibitors (cf. **7** and **8** with 41–**48**), demonstrating that the carbonyl was not required for potent FAAH inhibition. Likewise, the potencies of the enantiomers of **46** (**47** and **48**) showed that the activity resided in the *S*-series, suggesting a binding mode similar to that of the ketobenzimidazoles.

The X-ray co-crystal data obtained for compounds **8** and **45** bound in the rFAAH active site revealed an unexpected and unique binding mode. As shown in Figure 2, both inhibitors adopted a binding mode in which the pyrimidine bridges F432 and M436 thus positioning the 4-aryl substituent in the solvent exposed portion of the membrane access channel (MAC) while the piperidine was positioned in the ABP. The aryl-pyrimidine makes van der Waals contact with several hydrophobic residues including F432, M436, L433, W531, T488, and I491. Both the ketobenzimidazole (compound **8**) and pyrrolopyridine (compound **45**) fragments are perpendicular to the plane of the piperidine ring and directed down the cytosolic port. The ketobenzimidazole and pyrrolopyridine fragments make contact with the I238/G239 side of the oxy-anion hole, without engaging in a covalent interaction with S241.

In fact, no specific polar interaction is apparent for either inhibitor. Overall, the binding mode displayed by both **8** and **45** is consistent with our SAR, noncovalent inhibition, and suggested that shape was the primary source of recognition of these inhibitors by FAAH.

In moving forward we evaluated the in vivo pharmacokinetic (PK) parameters and CNS uptake of selected compounds (Table 5). As shown, compounds **8**, **33** and **35** exhibited low clearance, displayed good absorption and reasonable mean residence times. Unfortunately, both **8** and **33** had very limited CNS exposure. We reasoned that the poor CNS penetration might be due in part to the highly lipophilic nature of these compounds ($c\log P$ = 4.9–5.4). We had already demonstrated that we could decrease lipophilicity and improve potency by adding polarity to the ketobenzimidazole fragment, so next we turned to the aryl-pyrimidine subunit. Based on crystallographic data available at the time we suspected that adding a small polar group to the pyrimidine ring of compound **21** would be well tolerated and was calculated to significantly reduce the compound's lipophilicity. A NH_2 (compound **22**, $c\log P$ = 4.3) group was found to be beneficial while larger groups were generally poorly tolerated. This modification, in combination with a p-fluorophenyl substituent on the pyrimidine ring led to the much less lipophilic **35** ($c\log P$ = 3.7). Compound **35** was potent against rat and human FAAH in both our biochemical and whole cell

Table 3
Rat and human FAAH biochemical and whole cell assay SAR



Cmpd	Core	Ar	Rat FAAH IC_{50},[a] μM	RBL cells EC_{50}	Human FAAH IC_{50},[a] μM	T84 cells EC_{50}
OL-135			0.025	0.17	0.016	3.0
URB597			0.003	0.004	0.004	0.003
19	B	2-Benzofuran	>10	—	>10	—
20	A	2-Benzofuran	0.30	—	0.03	—
21[b]	C	2-Benzofuran	0.57	—	0.27	—
22	D	2-Benzofuran	0.20	—	0.01	—
23	E	Ph	0.22	—	0.05	—
24	A	2F-Ph	3.0	—	0.13	—
25	A	3F-Ph	1.2	—	0.05	—
26	A	4F-Ph	0.12	—	0.01	—
27	A	4Cl-Ph	0.16	—	0.03	—
28	A	4Me-Ph	0.28	—	0.04	—
29	A	5-Cl-2-thiophene	0.34	—	0.06	—
30	E	4F-Ph	0.03	—	0.01	—
31	E	2-Thiophene	0.19	1.8	0.03	0.35
32	E	5-Cl-2-thiophene	0.004	0.28	0.001	0.003
33	F	5-Cl-2-thiophene	0.008	0.10	0.002	0.002
34	E	2-Benzothiophene	0.026	0.08	0.002	—
35	D	4F-Ph	0.043	0.10	0.014	0.02

[a] Values are means of at least two experiments. All biochemical SAR data reported herein was obtained using the assays described in Ref. 9.
[b] Data reported for racemic compound.

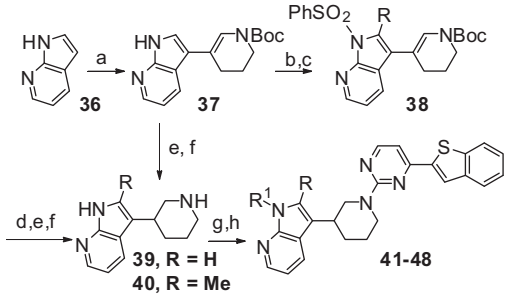
Table 4
Rat and human FAAH inhibition data for pyrrolopyridines

Cmpd	R	R^1	Rat FAAH IC_{50}, μM[a]	Human FAAH IC_{50}, μM[a]
41	H	Me	0.12	0.009
42	H	CH_2CN	0.083	0.009
43	Me	H	0.075	0.005
44	Me	Et	0.075	0.011
45	Me	CH_2CN	0.018	0.002
46-(rac)	Me	CH_2CH_2OH	0.053	0.003
47-(*S*)	Me	CH_2CH_2OH	0.039	0.002
48-(*R*)	Me	CH_2CH_2OH	>10	>10

[a] Values are means of at least two experiments. All biochemical SAR data reported herein was obtained using the assays described in Ref. 9.

assays and additionally had good permeability (35 nM/s)[27] and was not a substrate for P-gp (efflux ratio = 1.1) resulting in improved CNS penetration. Correcting the total exposure of **35** for the fraction unbound in rat plasma and brain tissue (Table 5), one calculates a brain/plasma ratio of 0.41 for compound **35**, compared to a ratio of <0.05 for compound **8** using the same formula.[28]

In summary, we have identified a series of highly potent, novel, noncovalent FAAH inhibitors.



Scheme 2. Synthesis of indole based FAAH inhibitors. Reagents and conditions: (a) *N*-Boc-3-piperidione, NaOMe, MeOH 80 °C, 45%; (b) $PhSO_2Cl$ (2 equiv), NaH, THF 0 °C 95%; (c). LDA −78 °C, then MeI 0 °C, THF 60%; (d) KOH, MeOH, 23 °C, 95%; (e) $Pd(OH)_2$/C 50 PSI H_2, AcOH/EtOH (6:1) 60%; (f) TFA, CH_2Cl_2 (1:1), 95%; (g) 2-Cl-4-[2-benzothiophene]pyrimidine Na_2CO_3, DMF, 85 °C 55%; (h) R-I, NaH, DMF, 18–82%.

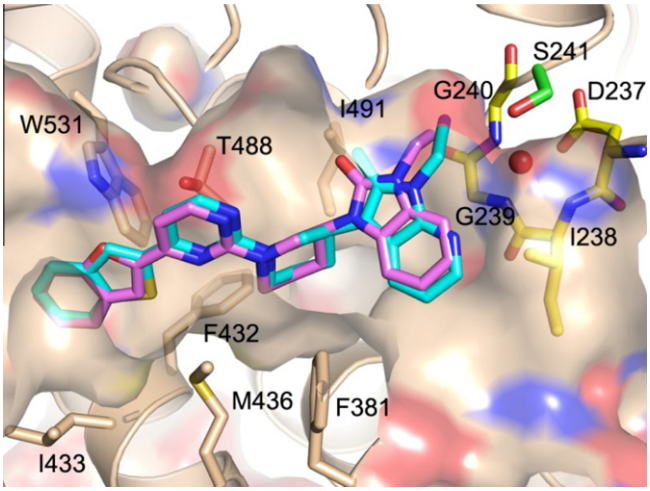


Figure 2. Overlay of the X-ray co-crystal structures of compounds **8** (2.3 Å; PDB code: **3QJ9**) and **45** (2.1 Å; PDB code: **3QK5**) with rat FAAH Δ-TM 30. Residues making up the oxy-anion hole are shown in yellow while S241 is shown in green.

Table 5
Pharmacokinetic parameters in rat for compounds **8**, **33** and **35**[a]

	8	**33**	**35**
CL (L/h/Kg)[b]	0.40	0.45	0.24
V_{dss} (L/Kg)[b]	1.5	0.87	0.69
C_{max} (μM)^	4.4	1.4	3.4
M_{RT} (h)^	5.5	3.7	5.2
F (%)	92	49	99
CNS uptake[b]			
Total concentration (μM) Plasma/blood/brain			
0.5 h	2.9/2.3/0.15	1.0/0.41/0.05	2.2/0.77/0.38
2 h	1.3/1.0/0.07	0.17/0.05/0.02	0.70/0.21/0.13
Fub (plasma/brain)[c]	0.0016/0.001	—	0.001/0.0024
c log *P*	5.4	4.9	3.7
Permeability (nM/s)[d]	—	—	35
P-gp efflux ratio[d]	—	—	1.1

[a] Values are means of at least two individual test subjects.
[b] Following 0.5 mg/Kg dose i.v.
[c] Determined by ultra centrifugation in rat tissues as described in Ref. 27b.
[d] Determined in LLC-PK1 cell monolayers as described in Ref. 27a.
^ Following 2.0 mg/Kg dose p.o.

Overall, compounds of this series displayed good PK parameters being of low clearance, high bioavailability and reasonable residence times. Compound **35** showed modest but reasonable CNS penetration and shows that physico-chemical properties of this

series of FAAH inhibitors can be optimized to target the CNS. We have determined these compounds to have a unique binding mode by X-ray crystallography. We expect that these compounds will be useful in further studying the pharmacology of FAAH inhibition. Additionally, the novel binding mode displayed by **8** and **45** should provide a useful starting point for the design of future FAAH inhibitors.

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