Bioorganic & Medicinal Chemistry Letters 21 (2011) 6538–6544

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel spirocyclic inhibitors of fatty acid amide hydrolase (FAAH). Part 1: Identification of 7-azaspiro[3.5]nonane and 1-oxa-8-azaspiro[4.5]decane as lead scaffolds

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ARTICLE INFO

Article history:
Available online 22 August 2011

Keywords:
Fatty acid amide hydrolase, FAAH
Pain
Endocannabinoid

ABSTRACT

Herein we report the identification of two new fatty acid amide hydrolase (FAAH) inhibitor lead series with FAAH k_{inact}/K_i potency values greater than 1500 M^{-1} s^{-1}. The two novel spirocyclic cores, 7-azaspiro[3.5]nonane and 1-oxa-8-azaspiro[4.5]decane, clearly distinguished themselves from the other spirocyclic cores on the basis of their superior potency for FAAH. Lead compounds from these two series have suitable FAAH potency and selectivity for additional medicinal chemistry optimization.

Fatty acid amide hydrolase (FAAH) is a serine hydrolase responsible for the degradation of fatty acid amide signaling molecules including the endocannabinoid anandamide (AEA).[1] It has been shown that inhibition of FAAH leads to elevated levels of AEA and analgesic effects in rodent models of pain without evidence of side effects commonly seen with cannabinoid receptor agonists. These findings suggest that inhibition of FAAH may provide an important class of analgesic agents.[2]

There have been several reports of both reversible and covalent inhibitors of FAAH and the subject has been recently reviewed.[3] Reversible inhibitors include α-ketooxazole **2**[4] and sulfonamide **4** (Chart 1).[5] Examples of covalent inhibitors include carbamate **3**[6] and urea **5**.[7] The FAAH program at Pfizer has reported on several covalent inhibitors, including ureas **6–9**.[8–10] The efforts of Johnson et al. at Pfizer led to the successful identification of urea PF-04457845 (**8**), a covalent inhibitor with exquisite potency and selectivity for FAAH.[10] Urea **8** was found to be orally efficacious at 0.1 mpk in a rat model of pain and is being evaluated in human clinical trials.

Due to the success of clinical candidate **8**, we sought to identify a novel core to replace the piperidine core common to many FAAH inhibitors. Once a novel core was identified, it could be optimized with the goal of discovering a new series of potent FAAH inhibitors with a divergent biological profile. For example, we have replaced the piperidine ring with azetidine (e.g., **9**).[9] However, successful replacement was achievable only by optimizing the length of the aliphatic linker between the core azetidine ring and the biaryl ether. The work culminated in azetidine **9** which required a three carbon linker for optimal potency. This was postulated to be due to the requirement to optimally position the biaryl ether tail in the lipophilic acyl chain binding channel. Failure to align this biaryl ether group properly likely results in a suboptimal alignment of the urea moiety for covalent binding to FAAH.

In the current manuscript, we report the identification of two new FAAH inhibitor lead series with FAAH k_{inact}/K_i potency values greater than 1500 M^{-1} s^{-1} (vide infra). Once suitable cores were identified, the goal of our medicinal chemistry optimization program targeted FAAH k_{inact}/K_i potency values greater than 2500 M^{-1} s^{-1}, low in vivo clearance (CL <15), and oral efficacy of 1 mpk or less in rodent models of pain. This latter optimization effort is the subject of the subsequent manuscript in this journal.[11]

FAAH inhibitors can be broken down into the following modular units: a tail group, an optional hydrocarbon linker, a piperidine-like urea core, and a urea head group which acts as a leaving group upon covalent binding to FAAH (Chart 2).[12] With this knowledge in hand and using molecular modeling and docking studies, we designed a collection of spirocyclic cores (**i–xiv**) as replacements for the

Abbreviations: FAAH, fatty acid amide hydrolase; AEA, anandamide; ABPP, activity-based protein profiling; CFA, complete Freund's adjuvant.

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doi:10.1016/j.bmcl.2011.08.055

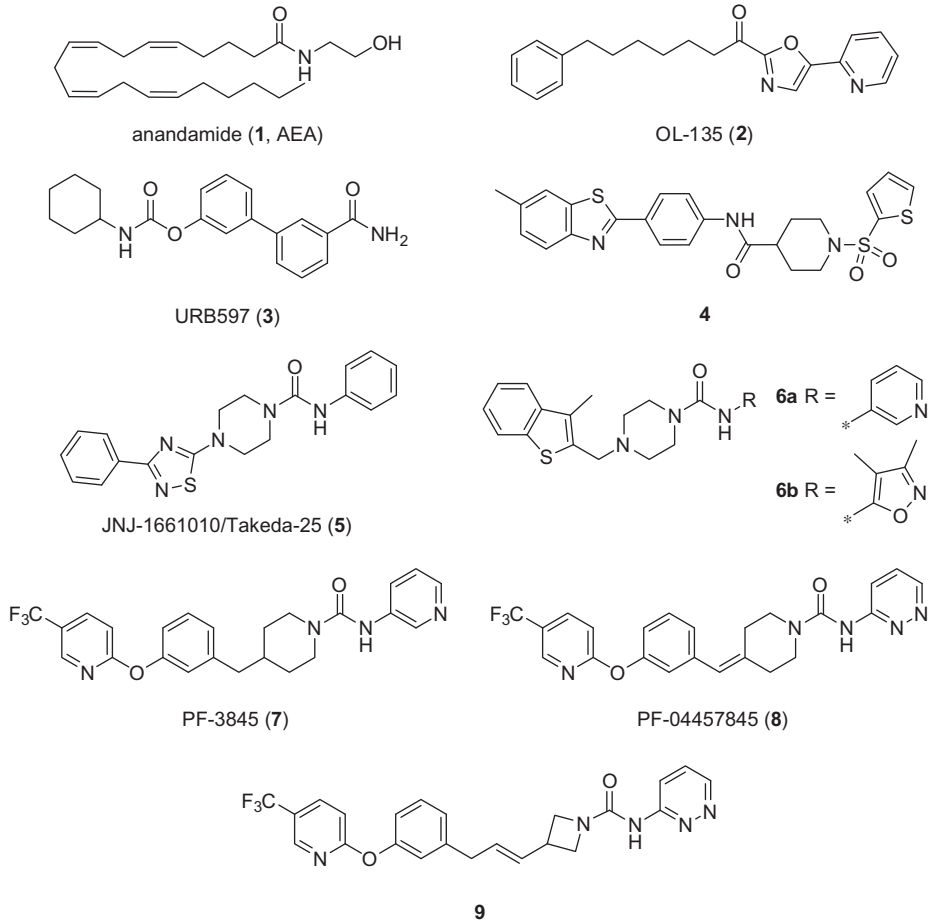


Chart 1. Substrate and inhibitors of FAAH.

methylenepiperidine core of compound **8**. An evaluation of the SAR of piperazine containing FAAH inhibitors such as **5** and **6** suggested to us that a benzothiophene or phenylthiadiazole tail group would be appropriate for evaluating diazospirocyclic cores (X = N) for FAAH activity. Similarly, the biaryl ether tail of compounds **7** and **8** was identified as being suitable for azaspirocyclic cores (X = C). Given our experience with azetidines such as **9**, we evaluated each core using variable linker lengths. Finally, three representative urea head groups were selected: 3-pyridyl, 3-pyridazinyl, and 3,4-dimethylisoxazol-5-yl. Thus, for each spirocyclic core we evaluated a compound matrix comprising of 1–2 tail groups, a linker of 0–2 carbons and three head groups.

The FAAH binding site requires a flattened U-shaped compound profile in order to align with the acyl chain binding channel (Fig. 1). Using a docking protocol we have previously described,[9] prototype inhibitors were covalently attached to the FAAH crystal structure and minimized in the binding channel. This ligand conformation was then compared to low energy conformers generated from a Monte Carlo style conformational search in the absence of protein. Furthermore, hydrocarbon linker chains of 0–2 carbons were evaluated to allow the tail group opportunity to align appropriately. If the minimized unbound conformer was of similar shape to the bound conformer, we postulated that the energy penalty for binding to FAAH was minimal and gave the spirocyclic core higher priority for synthesis. For example, Figure 1 shows an overlay of both free and bound low energy conformers of the 7-azaspiro[3.5]nonane core **vii** onto the X-ray crystal structure of FAAH inhibitor **7**.[12] Since this core could attain a similar overall shape to bound **7**, this scaffold was given high priority.

Evaluation of these spirocyclic cores required the preparation of Boc protected spirocycles with functionality enabling orthogonal incorporation of a linker of varying lengths to tether the terminal lipophilic tail. The Boc protected spirocyclic synthetic intermediates that were selected synthesis or purchase are shown in Chart 3.[13–19] With spirocyclic synthetic intermediates **11–22** in hand, a series of prototype compounds with varying length hydrocarbon linkers were prepared. These analogs were prepared in a highly divergent manner with representative examples shown in Schemes 1 and 2. The synthesis of N-linked diazaspirocycles is illustrated in Scheme 1 involving either an N-arylation or reductive amination as the key bond disconnections. The synthesis of C-linked azaspirocycles is illustrated in Scheme 2. The shortest linked compounds (e.g., **29**) were prepared by addition of a Grignard reagent followed by deoxygenation/debenzylation and nucleophilic displacement to give the biaryl ether. The 1 or 2 atom linked analogs (e.g., **32** and **35**) differed by the requirement of homologation followed by a Wittig olefination. In all instances, the final assembly of the analogs included a Boc deprotection followed by urea formation. The synthesis of 6-azaspiro[2.5]octane containing compounds required preparation from intermediate **36**[10] utilizing a Pd-catalyzed diazomethane cyclopropanation as depicted in Scheme 3.

The array of compounds with varying spirocyclic cores were assayed in an enzyme-coupled human FAAH assay as previously described.[8b] Since these compounds covalently bind to the FAAH enzyme, potency was measured using the second order rate constant k_{inact}/K_i. As these values are independent of preincubation times and substrate concentrations, k_{inact}/K_i values are, in this case, preferred over IC_{50} values for assessing compound potency. A plot

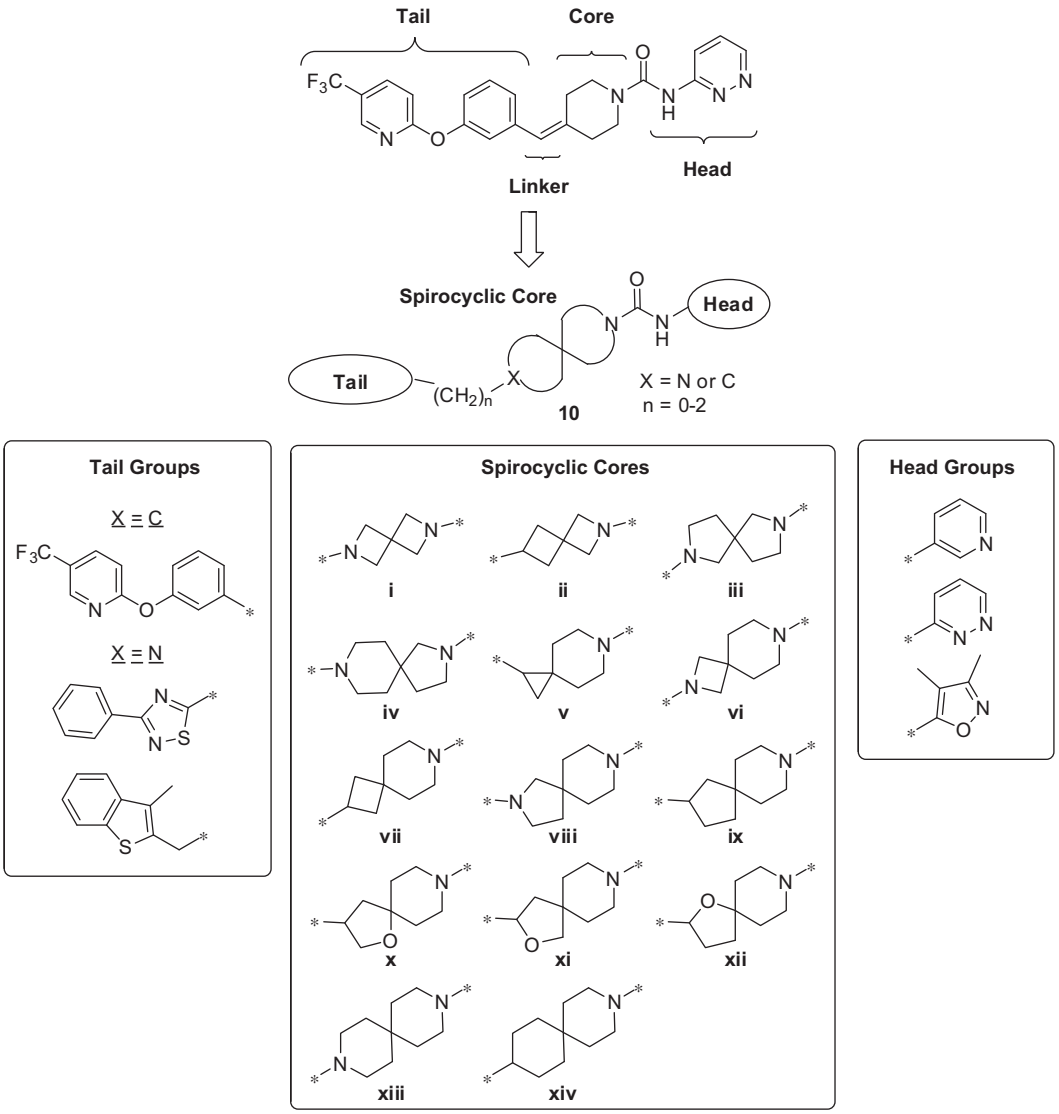


Chart 2. Strategy to replace piperidine core with a spirocycle core.

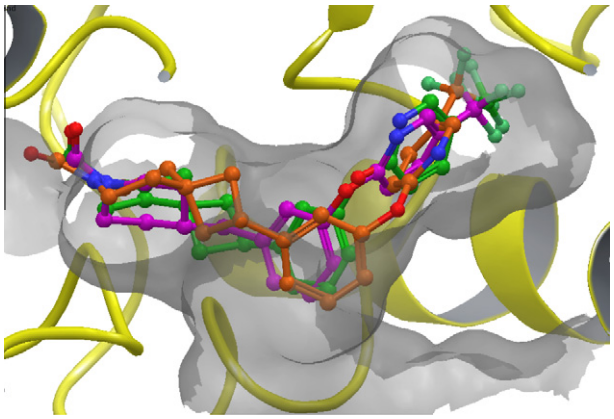


Figure 1. Overlay of (i) a minimized prototype from 7-azaspiro[3.5]nonane core **vii** (green) covalently bound to FAAH, (ii) a minimized prototype from 7-azaspiro[3.5]nonane core **vii** using a MacroModel conformer search (no protein; orange), and (iii) a X-ray co-crystal structure (2WAP) of FAAH (yellow) and inhibitor **7** (purple).[12]

of the FAAH k_{inact}/K_i potency values as a function of spirocyclic core is shown in Figure 2. Two spirocyclic cores, 7-azaspiro[3.5]nonane (core **vii**) and 1-oxa-8-azaspiro[4.5]decane (core **x**), clearly distinguished themselves from the other cores on the basis of potency for FAAH, being the only series with member compounds having FAAH k_{inact}/K_i values greater than 1000 M^{-1} s^{-1}.

A closer look at the SAR for specific examples from the most potent cores 7-azaspiro[3.5]nonane (**vii**) and 1-oxa-8-azaspiro[4.5]decane (**x**) is presented in Table 1. For both cores **vii** and **x**, linkers of one to two carbons (entries 2–3, and 8) diminished FAAH activity by four to fivefold relative to the no linker analogs (**29** and **41**) which had FAAH potencies of 1570 and 3130 M^{-1} s^{-1} (entries 1 and 7).

Subtle changes in the makeup of the spirocycle resulted in dramatic losses in potency. For example, the diazaspiro analog of core **vii** (**25**) was more than 100-fold less potent than the lead compound **29** (entries 4 and 1). This may be due to required geometry of the sp^3 protonated nitrogen, presenting an undesirable cationic core in a rather lipophilic binding site.[8b,12] For core **x**, replacement of the oxygen atom with carbon (**44**) or replacement with an isomeric furan (**43**) resulted in 10-fold reductions in FAAH activity (entries 9 and 10). The enantiomers of racemic **41** were separated by supercritical fluid chromatography using a chiral stationary phase. Both enantiomers retained activity against FAAH with the

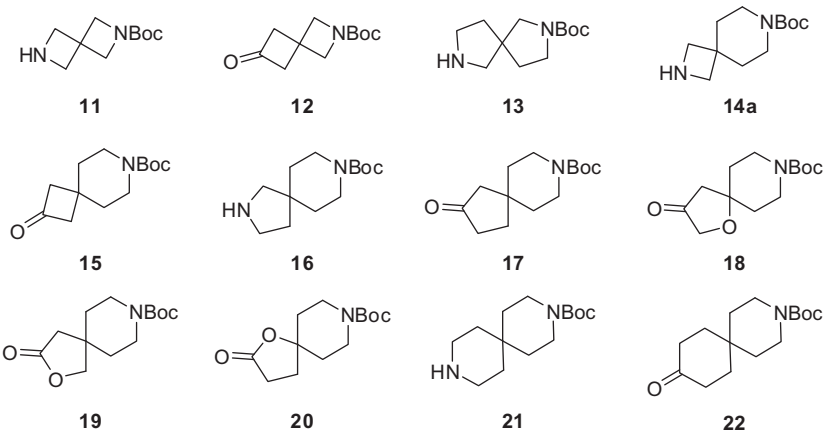
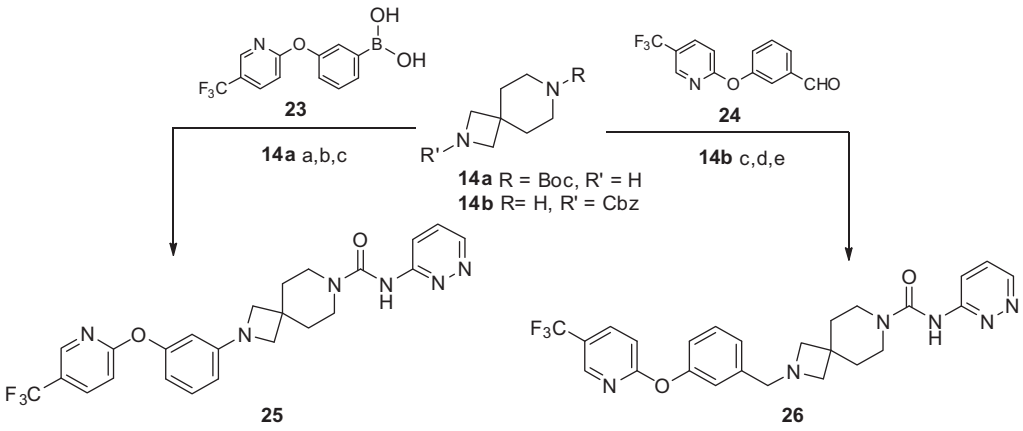
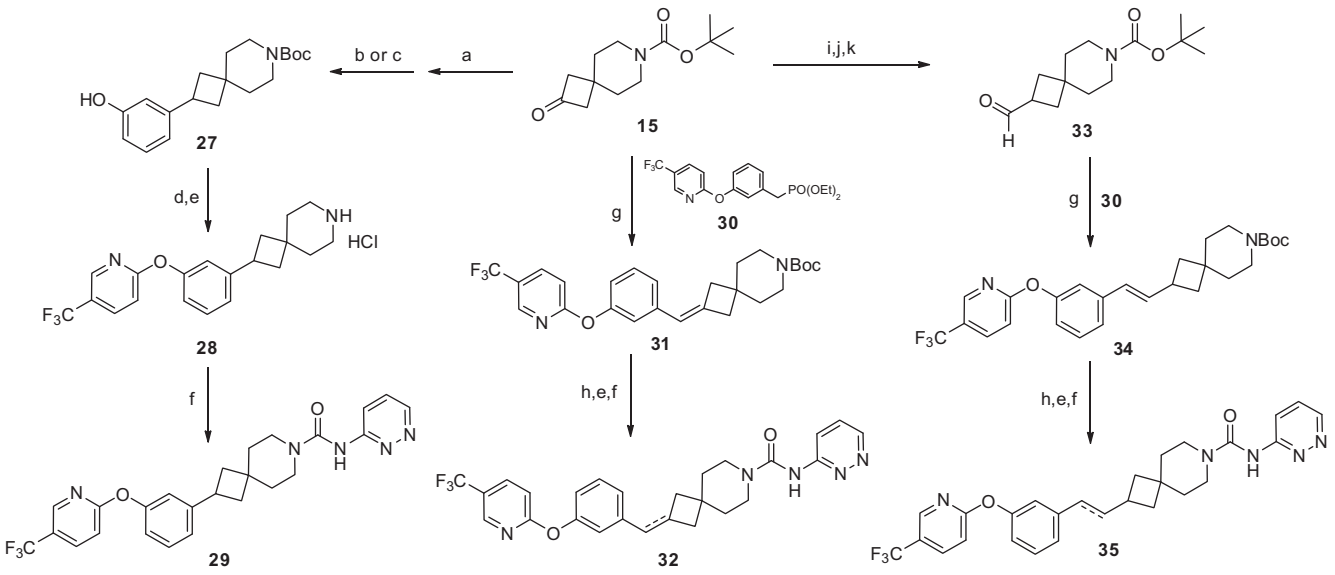


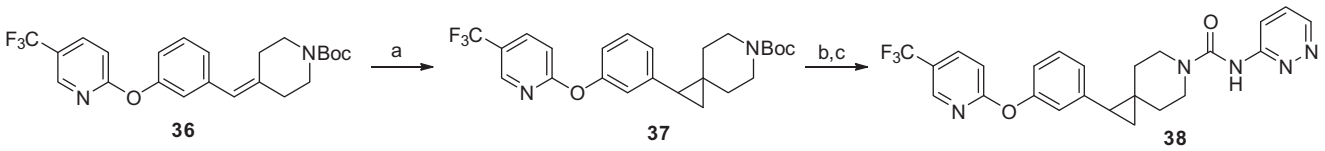
Chart 3. Spirocyclic synthetic intermediates.



Scheme 1. Representative synthesis of diazaspirocycle prototypes. Reagents and conditions: (a) Compound **23**, $Cu(OAc)_2$, NEt_3, DCM (23%); (b) TFA, DCM (quant.); (c) phenyl pyridazin-3-ylcarbamate, DIEA, CH_3CN, room temp (39–81%); (d) H_2, Pd/C (quant.); (e) compound **24**, $NaBH(OAc)_3$ (50%).



Scheme 2. Representative synthesis of azaspirocycle prototypes. Reagents and conditions: (a) 3-Benzyloxyphenylmagnesium bromide, 2-MeTHF, 0 °C; (b) Raney Ni, EtOH, reflux; (c) (i) Et_3SiH, TFA, BF_3OEt_2, DCM, 0 °C, (ii) H_2, 10% Pd/C, MeOH, 45 psi (36–38% from **15**); (d) 2-chloro-5-(trifluoromethyl)pyridine, cesium carbonate, DMF, 90 °C (85%); (e) 4 N HCl/dioxane, DCM; (f) phenyl pyridazin-3-ylcarbamate, DIEA, CH_3CN, room temp (36–96% over two steps); (g) KO*t*Bu, THF (50–60%); (h) H_2, 10% Pd/C, MeOH, 20 psi, 1 h (86–90%); (i) Ph_3PCH_3Br, KO*t*Bu, THF (39%); (j) 9-BBN, H_2O_2 (16%); (k) DMP, DCM (92%).



Scheme 3. Synthesis of 6-azaspiro[2.5]octane analogs. Reagents and conditions: (a) $PdCl_2(dba)_2$, CH_2N_2, DCM (14%); (b) TFA, DCM (99%); (c) phenyl pyridazin-3-ylcarbamate, DIEA, CH_3CN, room temp (5%).

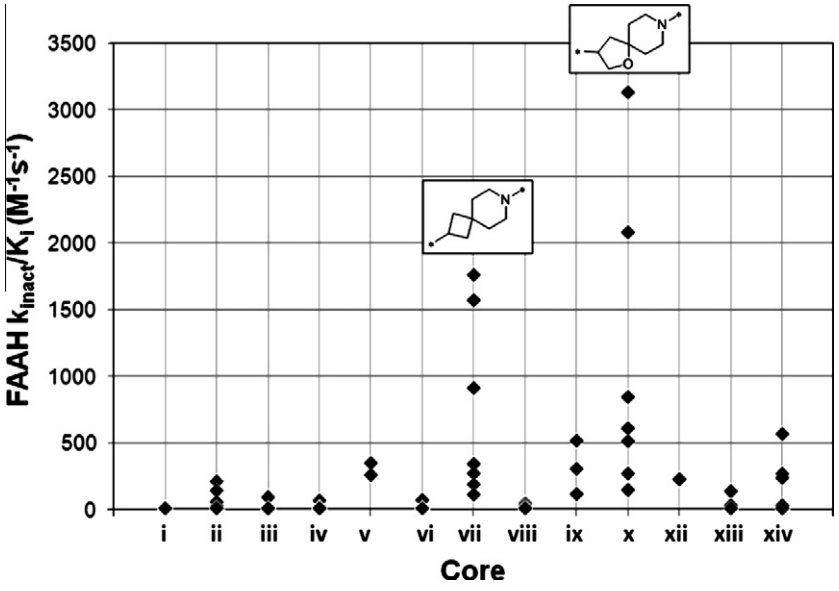
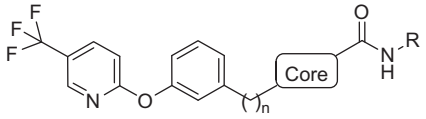


Figure 2. Plot of hFAAH k_{inact}/K_i values as a function of spirocycle core.

Table 1
FAAH potency data for representative compounds



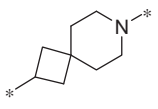
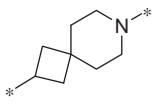
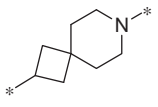
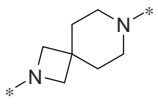
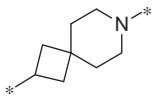
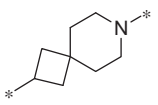
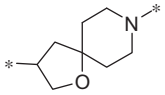
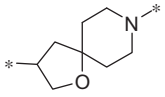
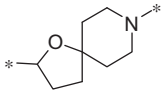
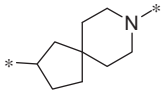
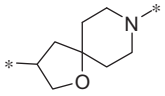
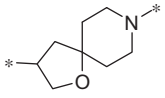
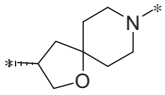
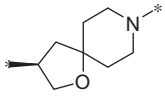
Entry	Compd	*n*	Core	R	hFAAH k_{inact}/K_i[a] (M^{-1} s^{-1})	MW	*C* log *P*
1	**29**	0			1570	483	4.5
2	**32**	1			272	497	5.0
3	**35**	2			344	511	5.6
4	**25**	0			18.3	484	2.7
5	**39**	0			1760	500	4.6

Table 1 (*continued*)

Entry	Compd	*n*	Core	R	hFAAH k_{inact}/K_i[a] (M^{-1} s^{-1})	MW	*C* log *P*
6	**40**	0			914	482	5.2
7	**41**	0			3130	499	2.9
8	**42**	1			846	512	4.1
9	**43**	0			229	499	2.9
10	**44**	0			307	497	5.1
11	**45**	0			515	516	3.1
12	**46**	0			2080	498	3.7
13	**47**	0			1340	499	2.9
14	**48**	0			3040	499	2.9

[a] Each k_{inact}/K_i value corresponds to an average of at least two independent determinations.

R enantiomer (**48**) being somewhat more potent (entries 13 and 14). The reason for strong sensitivity to positioning of the oxygen atom in the 1-oxa-8-azaspiro[4.5]decane (**x**) core but weak sensitivity to changing the chiral center is unclear and cannot be readily explained by molecular modeling studies.

With respect to the head urea leaving groups, the 7-azaspiro[3.5]nonane (**vii**) core series tolerated both the 3-pyridazinyl and 3,4-dimethylisoxazol-5-yl groups equally well (entries 1 and 5). In contrast, the 1-oxa-8-azaspiro[4.5]decane (**x**) core series preferred the more basic 3-pyridyl and 3-pyridazinyl groups over the 3,4-dimethylisoxazol-5-yl group by roughly fivefold (entries 7,11, and 12).

On the basis of their superior FAAH potencies, lead compounds from these two series (**29**, **39**, and **48**) were selected for further profiling and assayed for selectivity versus the serine hydrolase superfamily of enzymes (>200 human enzymes, including FAAH).[8a,20] The three compounds were assayed at 100 μM in a functional proteomic screen based on competitive activity-based protein profiling (ABPP) in human brain membrane and soluble liver proteonomes using a rhodamine-tagged fluorophosphonate ABPP probe. As has been observed previously for compound **8**,[10] all three lead spirocycles **29**, **39**, and **48** were found to be exquisitely selective for FAAH. In addition, in vitro assays suggested that these series have low risk for high clearance, drug–drug interactions or inhibition of the hERG channel (data not shown). Finally, the lead compounds **29**, **39**, and **48** were evaluated in the rat CFA pain model[12,21] at 10, 25, and 10 mpk, ip, respectively. Only compound **39** was found to be active, albeit at a higher dose. Given the relatively modest potency for FAAH and marginal CNS drug-like properties (MW at or near 500), these results suggested the need for further optimization to improve in vivo efficacy.

In summary, two cores, 7-azaspiro[3.5]nonane (**vii**) and 1-oxa-8-azaspiro[4.5]decane (**x**), clearly distinguished themselves from the other spirocyclic cores on the basis of their potency for FAAH. Lead compounds from these two series (**29**, **39**, and **48**) have suitable potency and selectivity for FAAH but marginal CNS drug-like properties. The medicinal chemistry optimization of these two series is the subject of the subsequent article in this journal.[11]

Acknowledgments

We would like to thank Prof. Benjamin F. Cravatt for his advice and extensive discussions on this project, J.T. Collins for chiral resolutions, S. Yang for 2D NMR determinations, and T.K. Nomanbhoy at ActivX Biosciences for ABPP proteome profiling.

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13. Intermediates **11**–**14a**, **16**, **20**–**22** are available from a number of commercial vendors.
14. Intermediate **12** was prepared as described in Meyers, M. J.; Muizebelt, I.; van Wiltenburg, J.; Brown, D. L.; Thorarensen, A. *Org. Lett.* **2009**, *11*, 3523.
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16. Intermediate **17** was prepared using diazomethane generated in situ from the portionwise addition of 1-methyl-1-nitrosourea (Aldrich) to the biphasic mixture of 40% KOH and intermediate **15** in 10:1 2-MeTHF/MeOH (93%).
17. Intermediate **18** was prepared as described in Long, S. A.; Wang, J. L.; Meyers, M. J.; Pelc, M., Schweitzer, B. A.; Thorarensen, A. WO2010/058318, 2010.
18. Intermediate **19** was prepared from intermediate **15** by a Baeyer–Villiger reaction using 30% hydrogen peroxide and 0.5 N sodium hydroxide in THF/MeOH (47%).
19. Intermediate **20** was prepared from *tert*-butyl 1-oxo-7-azaspiro[3.5]nonane-7-carboxylate by a Baeyer–Villiger reaction using 30% hydrogen peroxide and 0.5 N sodium hydroxide in THF/MeOH (46%).
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21. The Pfizer Institutional Animal Care and Use Committee reviewed and approved the animal use in these studies. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.