Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1423–1428
Bioorganic & Medicinal Chemistry Letters

Discovery of an exceptionally potent and selective class of fatty acid amide hydrolase inhibitors enlisting proteome-wide selectivity screening: concurrent optimization of enzyme inhibitor potency and selectivity

Donmienne Leung, Wu Du, Christophe Hardouin, Heng Cheng, Inkyu Hwang, Benjamin F. Cravatt and Dale L. Boger*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

Received 9 December 2004; revised 29 December 2004; accepted 30 December 2004

Abstract—The concurrent implementation of a proteome-wide serine hydrolase selectivity screen with traditional efforts to optimize fatty acid amide hydrolase (FAAH) inhibition potency led to the expedited discovery of a new class of exceptionally potent (K_i < 300 pM) and unusually selective (>100-fold selective) inhibitors. The iterative inhibitor design and evaluation with assistance of the selectivity screen served to differentiate otherwise indistinguishable inhibitors permitting the simultaneous optimization of potency and selectivity. Significantly, the simultaneous assessment of all potential competitive enzymes with the selectivity screen does not require the use of expressed or purified enzymes or a competitive substrate, no modification of the inhibitors is required, and the relative potency for competitive enzymes can be quantified (IC_{50}'s) including those that lack known substrates or function.

Anandamide[1] and oleamide[2] have emerged as the prototypical members of a class of endogenous fatty acid amides that serve as chemical messengers.[3] Anandamide, which is the most recognized member of the endogenous fatty acid ethanolamides, binds and activates the central (CB1) and peripheral (CB2) cannabinoid receptors as well as the vanilloid receptor (VR1) through which it is thought to exert its analgesic and cannabinoid effects.[4,5] Oleamide was found to accumulate in the cerebrospinal fluid under conditions of sleep deprivation and to induce physiological sleep where it reduced mobility, shortened the sleep induction period, and lengthened the time spent in slow wave sleep two at the expense of wakening.[2] Fatty acid amide hydrolase (FAAH) is a membrane-bound enzyme that hydrolyzes fatty acid primary amides and ethanolamides including anandamide and oleamide regulating signaling at their sites of action (Fig. 1).[6–8] It constitutes the only characterized mammalian member of a unique class of serine hydrolases (amidase signature family) that bear an unusual catalytic mechanism (Ser-Ser-Lys triad).[9] This unique mammalian distribution, its selectively targetable catalytic mechanism, and the ramifications of its inactivation (increased endogenous levels of bioactive fatty acid amides) have defined FAAH as a potential new therapeutic target for pain management and sleep disorders.[10]

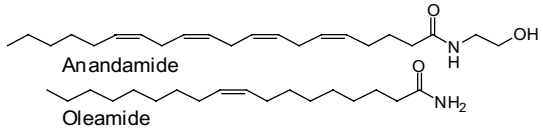


Figure 1. FAAH substrates.

Serine hydrolases represent one of the largest classes of enzymes including serine proteases, lipases, esterases, amidases, and transacetylases. Although initial efforts to develop FAAH inhibitors produced useful tools to probe enzyme function,[10] their design was expected to result in a low FAAH selectivity. However, the assessment of such inhibitors developed in our efforts[11]

* Corresponding author. Tel.: +1 858 784 7522; fax: +1 858 784 7550; e-mail: boger@scripps.edu

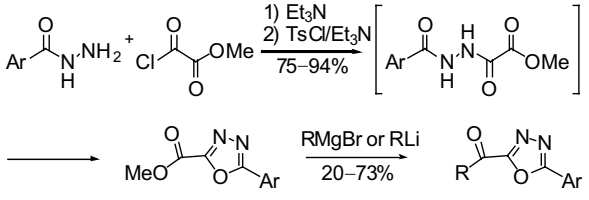
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.12.085

against candidate competitive enzymes (e.g., phospholipase A2, ceramidase) revealed no inhibition. In the absence of identifiable competitive enzymes and without a class of related mammalian enzymes to examine, the traditional approach of counter-screening the inhibitors against a panel of related enzymes to define their selectivity was not possible. Herein, we report the use of a powerful, proteome-wide serine hydrolase screen[12] adapted to define FAAH selectivity[13] that was conducted in parallel with traditional efforts to optimize FAAH inhibition resulting in the expedited discovery of a class of exceptionally potent (K_i < 300 pM) and unusually selective (>100-fold) inhibitors, 2-keto-5-(2-pyridyl)-1,3,4-oxadiazoles. To our knowledge, this represents the first report of the implementation of such a proteome-wide selectivity screen[14] conducted in tandem with traditional target optimization efforts to guide the discovery process.

The preparation of the 2-keto-1,3,4-oxadiazoles examined herein is summarized in Scheme 1 and full details are provided in the Supplementary data. Notably, the alkyl lithium or Grignard additions to the heterocyclic ester cleanly occurred once presumably benefiting from an oxadiazole chelation with and stabilization of the addition tetrahedral intermediate.

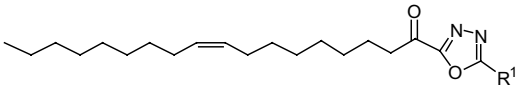
The trends observed with the inhibitors mirror those previously disclosed in our studies (Table 1). Introduction of a C5 substituent on the 2-keto-1,3,4-oxadiazole does not adversely impact activity (Ph vs H), incorporation of a H-bond acceptor heterocycle at this C5 position significantly enhances activity (2-pyridyl vs Ph) and follows trends predicted by the H-bond acceptor capabilities (2-pyridyl > 2-furyl > Ph), this enhancement by the H-bond acceptor is greatest when located immediately adjacent to the 1,3,4-oxadiazole (2-pyridyl > 3- or 4-pyridyl), an alkylphenyl side-chain series is more potent than the corresponding oleyl-based inhibitors and exhibits a well-defined optimal chain length ($Ph(CH_2)_n$: $n = 5 < 6 > 7 > 8$), and the 2-keto group is essential for potent inhibition (10^4-fold enhancement).

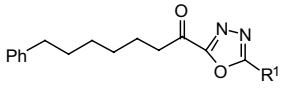
Biotinylated or fluorescently-tagged (rhodamine, Rh) fluorophosphonate (FP-biotin or FP-Rh) have been used to isolate and identify (FP-biotin) or quantitate (FP-Rh) proteome serine hydrolases through active site labeling.[12] Extending this to the assessment of inhibitor selectivity[13] requires incubation of the proteome with FP-Rh in the presence of systematically varied concentrations of inhibitor followed by SDS-PAGE to detect serine hydrolases sensitive to the inhibitor. A resulting inhibition of FP-Rh active site labeling correlates

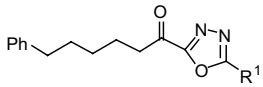


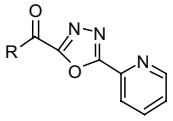
Scheme 1.

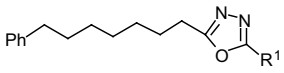
Table 1. α-Keto oxadiazole inhibitors of fatty acid amide hydrolase (FAAH)



R^1	K_i, μM
H	0.09 + 0.2
	0.016 ± 0.003
	0.003 ± 0.0002
	

R^1	K_i, μM
	0.002 ± 0.0003
	0.00029 ± 0.00003
	0.001 ± 0.0002
	0.001 ± 0.0001
	0.00056 + 0.00013
	

R^1	K_i, μM
	0.00085 ± 0.00009
	0.0032 ± 0.00012
	

R	K_i, μM
$Ph(CH_2)_5$	0.00085 ± 0.00009
$Ph(CH_2)_6$	0.00029 ± 0.00003
$Ph(CH_2)_7$	0.00077 ± 0.00016
$Ph(CH_2)_8$	0.00083 ± 0.00021
	

R^1	K_i, μM
	60 ± 2
	18 ± 0.5

with inhibitor affinity for the target and permits the simultaneous assessment of all potential competitive enzymes (Fig. 2). Moreover, the parallel profiling of such reversible inhibitors against all proteome serine hydro-

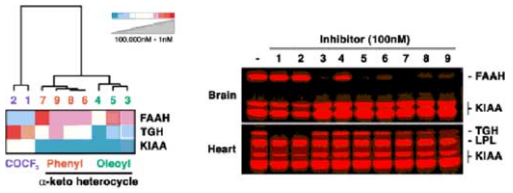


Figure 2. Proteome screening for FAAH selectivity. (Left) Comparison of the potencies and relative selectivities of FAAH inhibitors. IC_{50} values are clustered into three classes: highly potent and highly selective α-ketoheterocycle inhibitors with phenyl side-chain (red), moderately potent α-ketoheterocycle inhibitors with oleoyl side-chain (green) and trifluoromethyl ketone inhibitors selective for TGH (purple). (Right) Competitive profiling of FAAH inhibitors (100 nM) with FP-Rh (100 nM) in brain and heart membrane proteome. Enzyme targets such as FAAH, KIAA1363, TGH, and LPL are highlighted.

lases under kinetically controlled conditions can quantitatively establish relative potency and selectivity factors for each inhibitor.[13] Significantly, the selectivity screening does not require the use of a specific substrate, no modification of the inhibitor is required, and the relative selectivity factors can be rapidly and quantitatively established (IC_{50}'s).

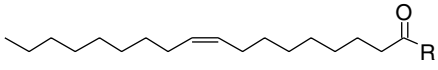
Two enzymes emerged in the screen as competitive targets for the inhibitors detailed herein: triacylglycerol hydrolase (TGH) and an uncharacterized membrane-bound hydrolase that lacks known substrates and function (KIAA1363).[13] Tables 2–4 summarize key results of the selectivity screening that highlight the simultaneous optimization of selectivity for FAAH over these two competitive enzymes, each of which display distinct SAR (structure–activity relationship) profiles, which was conducted concurrent with FAAH inhibition optimization. Pertinent to the off-site targets of the second generation FAAH inhibitors, simple trifluoromethyl ketone inhibitors (e.g., **1** and **2**) exhibited an intrinsic selectivity that typically favored TGH by >1000-fold and KIAA1363 by 10- to 100-fold (Table 2). Despite this unfavorable intrinsic selectivity, the iterative identification of inhibitors selective for FAAH proved straightforward with the benefit of the selectivity screen. Representative results of the iterative development of the FAAH inhibitors are summarized in Table 3 and Figure 2. The inhibitor affinity for FAAH versus KIAA1363 typically increased as the side-chain size (length) increased thereby improving selectivity (e.g., Tables 2 and 4),[15] and the binding to KIAA1363 is

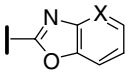
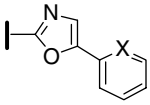
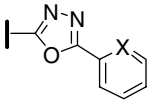
Table 2. Selectivity screening: IC_{50}, μM (selectivity)

n	K_i (FAAH, μM)[a]	FAAH	KIAA	TGH
$CH_3(CH_2)_nCOCF_3$				
6	1.2	30	1.5 (0.05)	0.002 (0.00007)
8	0.13 (**1**)	10	0.4 (0.04)	0.01 (0.001)
10	0.14	10	0.5 (0.05)	0.07 (0.007)
16	0.24	6.4	6.6 (1)	4.6 (0.7)
$Ph(CH_2)_nCOCF_3$				
5	0.17	5	0.8 (0.16)	0.0005 (0.0001)
6	0.10 (**2**)	5	0.2 (0.04)	0.001 (0.0002)
7	0.025	2	0.2 (0.1)	0.005 (0.002)

[a] FAAH K_i, provided for comparison purposes.

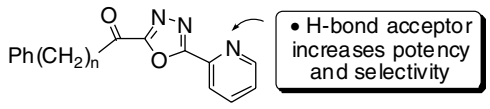
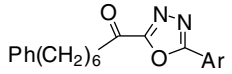
Table 3. Selectivity screening: IC_{50}, μM (selectivity)



R	K_i (FAAH, μM)[a]	FAAH	KIAA	TGH
$-CF_3$	0.080	5	1 (0.20)	5 (1)
	0.10	2	>100 (>50)	9 (4)
 X = CH	0.37	10	>100 (>10)	10 (1)
X = N (**3**)	0.0023	0.04	60 (1500)	1 (25)
 X = CH	0.32	5	>100 (>20)	50 (10)
X = N (**4**)	0.018	0.2	>100 (>500)	100 (500)
 X = CH	0.016	0.2	>100 (>500)	20 (100)
X = N (**5**)	0.003	0.004	>100 (>10^4)	20 (5000)

[a] FAAH K_i, provided for comparison purposes.

Table 4. Selectivity screening: IC_{50}, μM (selectivity)

	K_i (FAAH, μM)[a]	FAAH	KIAA	TGH
				
n				
5	0.00085	0.004	50 (10^4)	0.03 (8)
6	0.00029 (**7**)	0.001	90 (10^5)	0.14 (140)
7	0.00077	0.0006	60 (10^5)	0.2 (330)
8	0.00083	0.0008	3 (>10^3)	0.5 (625)
• Selectivity: $n = 8 > 7 > 6 > 5$				
• Potency: $n = 8 < 7 < 6 > 5$				
				
Ar				
Ph	0.002 (**6**)	0.025	>100 (>4000)	0.25 (10)
2-Pyr	0.00029 (**7**)	0.001	90 (10^5)	0.14 (140)
3-Pyr	0.001 (**8**)	0.02	>100 (>5000)	0.1 (5)
4-Pyr	0.001 (**9**)	0.02	>100 (>5000)	0.03 (2)
2-Furyl	0.00056	0.001	>100 (>10^5)	0.08 (80)

[a] FAAH K_i, provided for comparison purposes.

Table 5. Selectivity screening: IC_{50}, μM (selectivity)

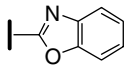
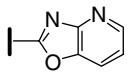
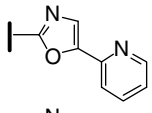
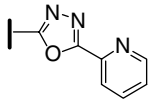
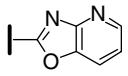
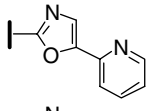
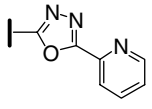
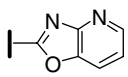
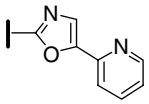
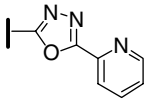
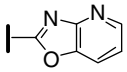
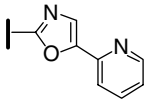
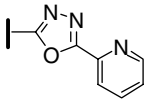
R	K_i (FAAH, μM)	FAAH	KIAA	TGH
$Ph(CH_2)_5COR$				
$-CF_3$	0.17	5	0.8 (0.16)	0.0005 (0.0001)
	0.22	10	>100 (>10)	0.020 (0.002)
	0.0002	0.001	10 (10^4)	0.001 (1)
	0.011	0.1	>100 (>10^3)	0.2 (2)
	0.00085	0.004	50 (10^4)	0.03 (8)
$Ph(CH_2)_6COR$				
$-CF_3$	0.10	5	0.2 (0.04)	0.0009 (0.0002)
	0.00028	0.0003	20 (10^4)	0.003 (10)
	0.0047	0.010	>100 (>10^4)	0.6 (60)
	0.00029	0.001	90 (10^5)	0.14 (140)
$Ph(CH_2)_7COR$				
$-CF_3$	0.025	2	0.20 (0.1)	0.005 (0.002)
	0.00039	0.0003	20 (6700)	0.005 (16)

Table 5 (*continued*)

R	K_i (FAAH, μM)	FAAH	KIAA	TGH
	0.0075	0.008	>100 (>10^4)	1 (125)
	0.00077	0.0006	60 (10^5)	0.2 (330)
Ph(CH$_2$)$_8$COR –CF$_3$	Nd	Nd	Nd	Nd
	0.00052	0.0001	10 (10^5)	0.005 (50)
	0.0078	0.03	>100 (>10^3)	6 (200)
	0.00083	0.0008	3 (3800)	0.5 (625)

completely or substantially disrupted with introduction of an electrophilic carbonyl heterocycle (Table 3) such that the achieved FAAH selectivity is superb (>1000-fold). Even more impressive given the intrinsic >1000-fold TGH selectivity, the screening revealed that the TGH potency and selectivity decreases as the side-chain size (length) increases (e.g., Tables 2 and 4),[15] and that incorporation of a second, properly positioned, weakly basic nitrogen (H-bond acceptor) into the heterocycle improves FAAH affinity and selectivity relative to that of TGH (Tables 3 and 4). Notably, the FAAH selectivity and affinity SAR's are not identical, and it is the combined optimization of properties that distinguishes otherwise comparable candidate inhibitors (multidimensional SAR).

Thus, both the selectivity and potency of the 2-keto-5-(2-pyridyl)-1,3,4-oxadiazoles (e.g., **5**) disclosed herein exceed those of the 2-keto-5-(2-pyridyl)oxazoles (e.g., **4**),[16] which in turn are more selective than the exceptionally potent 2-keto-oxazolopyridines (e.g., **3**).[17] These trends are well represented in the additional series summarized in Table 5. Each overcomes the intrinsic selectivity for TGH or KIAA1363 observed with the simpler α-ketoheterocycles or trifluoromethyl ketone inhibitors.

Importantly, the enhanced selectivity of the exceptionally potent 2-keto-5-(2-pyridyl)-1,3,4-oxadiazoles (e.g., **5**) versus that of the equipotent 2-keto-oxazolopyridines[17] (e.g., **3**) serve to differentiate the otherwise indistinguishable inhibitors. Remarkable potencies and useful selectivities (>100-fold) were obtained with use of the proteomics-wide selectivity screen. Thus, a class of α-ketoheterocycles, 2-keto-5-(2-pyridyl)-1,3,4-oxadiazoles (e.g., **7**), was discovered in an expedited manner that are not only extraordinarily potent (K_i = 300 pM, >100-fold more potent than the corresponding trifluoromethyl ketones), but that also improve the intrinsic selectivity for FAAH >10^6-fold.

One can anticipate that the inadvertent overestimation of enzyme inhibitor selectivity typical of counter-screening against a panel of related enzymes can be avoided using comparable strategies of proteome-wide selectivity screening. This strategy does not require that the enzymes be recombinantly expressed or purified, no modification of the inhibitors is required, and the assay is conducted under 'substrate free' conditions permitting simultaneous identification of inhibitors for enzymes with diverse substrate preferences (e.g., FAAH and TGH) and even novel enzymes that lack known substrates (e.g., KIAA1363).

Acknowledgments

We gratefully acknowledge the financial support of the NIH (DA15648, D.L.B.; DA15197 and 17259, B.F.C.) and The Skaggs Institute for Chemical Biology.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.12.085. Experimental details for establishing FAAH inhibition (K_i's), FAAH selectivity (IC_{50}'s), and for the preparation of the 2-keto-5-(2-pyridyl)-1,3,4-oxadiazoles disclosed herein, and an expanded Figure 2 (Figure S1).

References and notes

1. Dervane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946.

2. Cravatt, B. F.; Prospero-Garcia, O.; Suizdak, G.; Gilula, N. B.; Henriksen, S. J.; Boger, D. L.; Lerner, R. A. *Science* **1995**, *268*, 1506.
3. Reviews: Boger, D. L.; Henriksen, S. J.; Cravatt, B. F. *Curr. Pharm. Des.* **1998**, *4*, 303; Schmid, H. H. O.; Schmid, P. C.; Natarajan, V. *Prog. Lipid Res.* **1990**, *29*, 1.
4. Martin, B. R.; Mechoulam, R.; Razdan, R. K. *Life Sci.* **1999**, *65*, 573.
5. Axelrod, J.; Felder, C. C. *Neurochem. Res.* **1998**, *23*, 575; Zygmunt, P. M.; Petersson, J.; Andersson, D. A.; Chuang, H.; Sorgard, M.; Di Marzo, V.; Julius, D.; Hogestatt, E. D. *Nature* **1999**, *400*, 452; Smart, D.; Gunthorpe, M. J.; Jerman, J. C.; Nasir, S.; Gray, J.; Muir, A. I.; Chambers, J. K.; Randall, A. D.; Davis, J. B. *Br. J. Pharmacol.* **2000**, *129*, 227.
6. Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature* **1996**, *384*, 83; Giang, D. K.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2238.
7. Boger, D. L.; Fecik, R. A.; Patterson, J. E.; Miyauchi, H.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2613.
8. Patricelli, M. P.; Cravatt, B. F. *Vitam. Horm.* **2001**, *62*, 95.
9. Bracey, M. H.; Hansen, M.; Masuda, K.; Stevens, R. C.; Cravatt, B. F. *Science* **2002**, *298*, 1793; McKinney, M. K.; Cravatt, B. F. *J. Biol. Chem.* **2003**, *278*, 37393.
10. Cravatt, B. F.; Lichtman, A. H. *Curr. Opin. Chem. Biol.* **2003**, *7*, 469.
11. Patterson, J. E.; Ollmann, I. R.; Cravatt, B. F.; Boger, D. L.; Wong, C.-H.; Lerner, R. A. *J. Am. Chem. Soc.* **1996**, *118*, 5938; Boger, D. L.; Sato, H.; Lerner, A. E.; Austin, B. J.; Patterson, J. E.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 265.
12. Kidd, D.; Lui, Y.; Cravatt, B. F. *Biochemistry* **2001**, *40*, 4005; Liu, Y.; Patricelli, M. P.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14694.
13. Leung, D.; Hardouin, C.; Boger, D. L.; Cravatt, B. F. *Nat. Biotechnol.* **2003**, *21*, 687.
14. Jessani, N.; Cravatt, B. F. *Curr. Opin. Chem. Biol.* **2004**, *8*, 54.
15. An additional example of this trend may be found in Table 5.
16. Boger, D. L.; Miyauchi, H.; Du, W.; Hardouin, C.; Fecik, R. A.; Cheng, H.; Hwang, I.; Hedrick, M. P.; Leung, D.; Aceveda, O.; Guimaraes, C. R. W.; Jorgensen, W. L.; Cravatt, B. F. *J. Med. Chem.* **2005**, *48*, in press doi:10.1021/jm049585o; Lichtman, A. H.; Leung, D.; Shelton, C. C.; Saghatelian, A.; Hardouin, C.; Boger, D. L.; Cravatt, B. F. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 441.
17. Boger, D. L.; Sato, H.; Lerner, A. E.; Hedrick, M. P.; Fecik, R. A.; Miyauchi, H.; Wilkie, G. D.; Austin, B. J.; Patricelli, M. P.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5049.