Discovery of Boronic Acids as Novel and Potent Inhibitors of Fatty Acid Amide Hydrolase

Anna Minkkilä,* Susanna M. Saario, Heikki Käsnänen, Jukka Leppänen, Antti Poso, and Tapio Nevalainen

Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, FI-70211 Kuopio, Finland

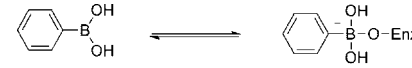
Received August 21, 2008

Abstract: A series of commercial phenyl-, heteroaryl-, alkyl-, and alkenylboronic acids were evaluated for their FAAH and MGL inhibitory activities. The compounds were generally selective for FAAH, with IC_{50} in the nanomolar or low-micromolar range. Eight of these compounds inhibited MGL with IC_{50} in the micromolar range. The most potent compound, phenylboronic acid with para-nonyl substituent (**13**), inhibited FAAH and MGL with IC_{50} of 0.0091 and 7.9 μM, respectively.

Fatty acid amide hydrolase (FAAH[a]),[1,2] a membrane-bound enzyme part of the endocannabinoid system, has been considered as a potential target for therapeutic agents in the treatment of various medical conditions including inflammation and pain. FAAH has been found to be the primary enzyme responsible for the hydrolysis of endocannabinoid *N*-arachidonoyl ethanolamide (anandamide, AEA),[3] an important lipid messenger in the brain and periphery. AEA and a second endocannabinoid 2-arachidonoylglycerol (2-AG)[4,5] are found in most mammalian tissues, and they act at CB_1 and CB_2 receptors,[6−10] modulating several physiological responses, including pain sensation, anxiety, and depression.[11] However, after release from the postsynaptic neurones, AEA and 2-AG are rapidly eliminated by uptake into cells followed by intracellular hydrolysis by FAAH and monoglyceride lipase (MGL),[12,13] respectively, resulting in weak and transient in vivo effects of the CB_1 and CB_2 receptor activation. By blockage of the FAAH and/or MGL activity with potent inhibitors, the AEA and/or 2-AG levels can be elevated, and the desired cannabimimetic effects are achieved.[14,15]

Many different classes of FAAH inhibitors have been reported in the past decade, including various fatty acid derivatives[16−20] and non-lipid inhibitors such as α-keto heterocycles,[21,22] carbamate derivatives,[14,23,24] and most recently, piperidine/piperazine ureas.[25] The most studied, in vitro and in vivo, FAAH inhibitors are 7-phenyl-1-(5-(pyridin-2-yl)oxazol-2-yl)heptan-1-one (OL-135)[22] and 3′-carbamoylbiphenyl-3-yl-cyclohexylcarbamate (URB597).[14] They have been shown to elevate the AEA levels and potentiate its actions in the brain. OL-135 has been found to display in vivo activity, producing analgesic effects in various pain models.[22,26] Furthermore, several reports have indicated that URB597 exerts analgesic,[14,15,27−29] anxiolytic,[14] and antidepressant effects.[30]

MGL has been found to be inhibited by some nonspecific inhibitors, such as hexadecylsulfonyl fluoride (HDSF), methyl arachidonylfluorophosphonate (MAFP),[12,31,32] and sulfhydryl-reactive compounds like *N*-arachidonylmaleimide (NAM).[33]



1

Figure 1. Structure of phenylboronic acid (**1**) and corresponding sp^3 hybridized transition stage analogue.

Boronic acids have been used extensively as inhibitors of various hydrolytic enzymes such as peptidases and lipases, as well as employed as other pharmaceutical agents.[34] However, boronic acids have not been described to have FAAH inhibitory activity until very recently, in a patent application[35] published during the preparation of this manuscript. As boronic acids are well described as inhibitors of other serine hydrolases and because of the fact that FAAH bears a catalytic Ser241-Ser217-Lys142 triad, wherein Ser241 is determined to act as a nucleophile,[36−38] we hypothesized that boronic acids could serve a new concept for the development of novel inhibitors of FAAH.

Herein, we present FAAH inhibitory activity data and a preliminary structure−activity relationship for various commercial boronic acids. Results of preliminary binding orientation analysis for FAAH, explored by molecular docking, are also described briefly. Moreover, a screening of the compounds against MGL is presented.

After it was established that phenylboronic acid (**1**) (Figure 1) could inhibit FAAH (but not MGL at 100 μM) with an IC_{50} of 2.6 μM, a series of meta-, para-, and ortho-substituted phenylboronic acids and a range of heteroaryl-, alkyl-, and alkenylboronic acids were purchased and studied to establish their initial structure−activity relationships for the inhibition of FAAH. Altogether, 22 commercial compounds were tested for their inhibitory potencies against FAAH in the assay described previously.[39] The screening against MGL activity was determined using 2-AG as a substrate and human recombinant MGL (hrMGL) as an enzyme source (see Supporting Information).

The utility of boronic acids as reversible enzyme inhibitors is based on the conversion between the trigonal planar sp^2 and the tetrahedral sp^3 boron, which makes these compounds ideal transition state analogues (Figure 1) to the carbonyl containing substrates.[34] Because of the boron open electron shell, boronic acids are described as strong Lewis acids. Among the substituted phenylboronic acids the pK_a values are dependent on the electronic nature and the position of substitution, varying in the range of 4.5−9.3 and thus making a part of them suitable for ready conversion to the sp^3 hybridized form under physiological conditions.[34,40] To study the dependence of FAAH inhibition potencies on calculated pK_a values (see Supporting Information) and the steric contributors, we selected and tested compounds with various electronic and steric properties.

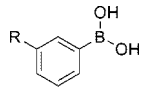
As evident from the results presented in Table 1 for the meta-substituted phenylboronic acids, the electron-withdrawing ethoxycarbonyl (**2**) and trifluoromethyl (**3**) moiety in the meta-position of the phenyl ring positively correlated with the FAAH IC_{50} values. This is presumably due to lower pK_a values (pK_a of 8.1 and 7.9, respectively) compared to that of **1** (pK_a of 8.9) and increased strength of the presumed covalent bond formed between the catalytic hydroxyl group of Ser241 and boronic acid, thereby enhancing the stability of formed tetrahedral adduct and lowering the IC_{50} to the nanomolar level (0.080−0.12 μM).

* To whom correspondence should be addressed. Phone: +358 40 355 3715. Fax: +358 17 162 456. E-mail: anna.minkkila@uku.fi.

[a] Abbreviations: AEA, anandamide; 2-AG, 2-arachidonoylglycerol; FAAH, fatty acid amide hydrolase; MGL, monoglyceride lipase, monoacylglycerol lipase; CB_1, cannabinoid 1; CB_2, cannabinoid 2.

10.1021/jm801051t CCC: $40.75
Published on Web 11/05/2008

Table 1. IC_{50} Values for FAAH and MGL Inhibition by Meta-Substituted Phenylboronic Acids



compd	R	IC_{50} (95% CI[a]) or % inhibition at 100 μM[b] FAAH (μM)	MGL (μM)	calcd pK_a
2	CO_2Et	0.12 (0.10−0.13)	34%	8.1
3	CF_3	0.080 (0.073−0.087)	38 (30−48)	7.9
4	CN	1.6 (1.2−2.2)	21%	7.5
5	Ph	0.13 (0.11−0.14)	71 (63−80)	8.6
6	OMe	1.9 (1.6−2.2)	7%	8.4

[a] IC_{50} values represent the mean from three independent experiments performed in duplicate with 95% confidence intervals shown in parentheses. Enzyme activity was completely abolished at the highest tested concentration. [b] The percentage of inhibition is represented as the mean from two independent experiments performed in duplicate.

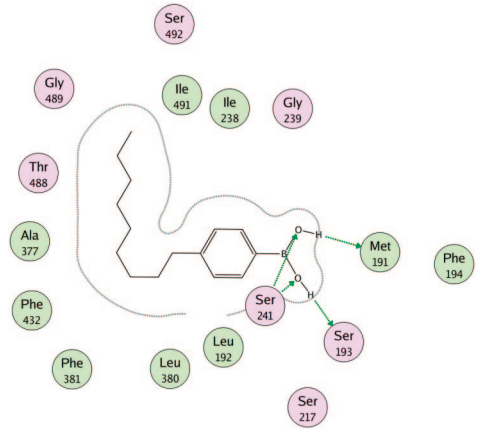
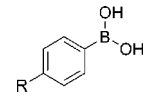


Figure 2. Schematic presentation[41] of putative FAAH binding mode of **13**. Polar and hydrophobic amino acids are colored purple and green, respectively. Green arrows depict hydrogen bonding interactions with side chains.

However, an interesting observation was made with the phenylboronic acid with an electron-withdrawing meta-cyano group (**4**) and the lowest pK_a value (7.5) in the series; the IC_{50} value was as high as 1.6 μM, close to that determined for **1** (IC_{50} = 2.6 μM). The compounds with slightly electron-donating phenyl (**5**) and strongly electron-donating methoxy (**6**) groups inhibited FAAH with IC_{50} of 0.13 and 1.9 μM, respectively. Presumably, the rather high pK_a for **6** (8.4) suggests that this compound might not be as capable of forming the supposed covalent bond with the catalytic serine as **2** and **3**. The low IC_{50} of **5** is expected to be due to favorable lipophilic interactions between the distant phenyl group and enzyme's active site residues (see Figure 2 for plausible amino acids).

Concerning the influence of pK_a values on the inhibition potencies, similar observations were made in the series of para-substituted phenylboronic acids to those of meta-substituted compounds (Table 2). The electron-withdrawing CF_3 (**8**), NO_2 (**9**), and CN (**10**) groups (pK_a = 7.4−8.1) at the para-position decreased the IC_{50} to the nanomolar range (IC_{50} = 0.036−0.29 μM), while the electron-donating methoxy group (**12**) (pK_a = 9.3) did not have any effect on the IC_{50} (IC_{50} = 2.0) compared to that achieved with compound **1**. As expected, on the basis of the relatively high pK_a (9.0) of **7**, the fluoride group at para-position did not enhance the inhibitory potency (IC_{50} = 1.5 μM). The most potent para-substituted compounds were lipophilic phenyl (**11**) and nonyl (**13**) substituted phenylboronic acids, albeit with rather high pK_a (8.9 and 9.1, respectively). These

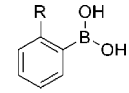
Table 2. IC_{50} Values for FAAH and MGL Inhibition by Para-Substituted Phenylboronic Acids



compd	R	IC_{50} (95% CI[a]) or % inhibition at 100 μM[b] FAAH (μM)	MGL (μM)	calcd pK_a
7	F	1.5 (1.4−1.7)	3%	9.0
8	CF_3	0.036 (0.029−0.046)	20 (17−24)	8.1
9	NO_2	0.21 (0.18−0.26)	43%	7.4
10	CN	0.29 (0.26−0.31)	36%	7.7
11	Ph	0.021 (0.018−0.023)	19 (16−23)	8.9
12	OMe	2.0 (1.8−2.2)	11%	9.3
13	C_9H_{19}	0.0091 (0.0074−0.011)	7.9 (6.7−9.3)	9.1

[a] IC_{50} values represent the mean from three independent experiments performed in duplicate with 95% confidence intervals shown in parentheses. Enzyme activity was completely abolished at the highest tested concentration. [b] The percentage of inhibition is represented as the mean from two independent experiments performed in duplicate.

Table 3. IC_{50} Values for FAAH and MGL Inhibition by Ortho-Substituted Phenylboronic Acids



compd	R	IC_{50} (95% CI[a]) or % inhibition at 100 μM[b] FAAH (μM)	MGL (%)	calcd pK_a
14	F	0.71 (0.64−0.79)	13	8.7
15	CF_3	68 (55−85)	20	8.5
16	Ph	110 (89−130)	2	8.9
17	OMe	13 (11−15)	11	9.0

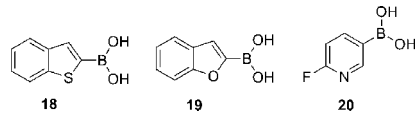
[a] IC_{50} values represent the mean from three independent experiments performed in duplicate with 95% confidence intervals shown in parentheses. Enzyme activity was completely abolished at the highest tested concentration. [b] The percentage of inhibition is represented as the mean from two independent experiments performed in duplicate.

compounds inhibited FAAH with IC_{50} of 0.021 and 0.0091 μM, respectively. The para-substituted compounds, albeit with slightly higher pK_a (7.4−9.4), were generally more potent FAAH inhibitors than the meta-substituted compounds (pK_a = 7.5−8.6). This is evident when comparing the inhibition potencies of meta-substituted compounds **3**-**5** (IC_{50} = 0.080−1.6 μM) with those of para-substituted **8**, **10**, and **11** (IC_{50} = 0.021−0.29 μM). These observations indicate that there is more steric tolerance near the enzyme's catalytic site for the para-subtitution than for the meta-substitution.

Ortho-substituted phenylboronic acids were also tested (Table 3). The bulky ortho-substituents, CF_3 (**15**) and phenyl (**16**), proximal to the boronyl group were suggested to decrease the inhibition potencies because of steric inhibition in the formation of the tetrahedral adduct and therefore to give relative high IC_{50} values (IC_{50} of 68 and 110 μM, respectively). Furthermore, the poor inhibition of FAAH by **17** can be due to the electron-donating property of methoxy group and/or the formation of internal coordination between the methoxy oxygen and the boronyl group. Interestingly, the compound with fluoride substituent (**14**) inhibited FAAH at the nanomolar level (IC_{50} = 0.71 μM). This is presumably due to the rather small size and the electron-withdrawing effect of the fluoride. In the series of ortho-substituted boronic acids, the steric effects were considered to affect the IC_{50} more likely than the pK_a.

After the substitution effects of various substituted phenylboronic acids were established, a range of aromatic heterocyclic and alkyl- and alkenylboronic acids were tested against FAAH and MGL. The results for heterocyclic and alkyl derivatives

Table 4. IC_{50} Values for FAAH and MGL Inhibition by Heteroarylboronic Acids

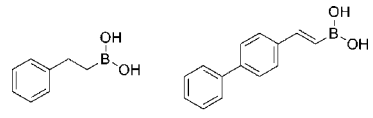


18 19 20

	IC_{50} (95% CI[a]) or % inhibition at 100 μM[b]		
compd	FAAH (μM)	MGL (μM)	calcd pK_a
18	0.15 (0.13−0.17)	32 (25−41)	7.3
19	0.33 (0.29−0.37)	100 (93−110)[c]	6.5
20	10 (9.3−11)	2%	6.2

[a] IC_{50} values represent the mean from three independent experiments performed in duplicate with 95% confidence intervals shown in parentheses. Enzyme activity was completely abolished at the highest tested concentration. [b] The percentage of inhibition is represented as the mean from two independent experiments performed in duplicate. [c] Remaining enzyme activity 8.4% at 1 mM.

Table 5. IC_{50} Values for FAAH and MGL Inhibition by Alkyl- And Alkenylboronic Acids



21 22

	IC_{50} (95% CI[a]) or % inhibition at 100 μM[b]		
compd	FAAH (μM)	MGL (μM)	calcd pK_a
21	1.3 (1.0−1.6)	4%	10.4
22	0.014 (0.013−0.015)	31 (25−39)[c]	10.0

[a] IC_{50} values represent the mean from three independent experiments performed in duplicate with 95% confidence intervals shown in parentheses. Enzyme activity was completely abolished at the highest tested concentration. [b] The percentage of inhibition is represented as the mean from two independent experiments performed in duplicate. [c] Remaining enzyme activity 16% at 1 mM.

are presented in Tables 4 and 5, respectively. The heterocyclic benzothiophene (**18**) and benzofuran (**19**) derivatives were shown to be potent FAAH inhibitors, with IC_{50} of 0.15 and 0.33 μM, respectively. In contrast, the 6-fluoropyridine-3-boronic acid (**20**) inhibited FAAH in the micromolar range (IC_{50} = 10 μM).

On the basis of reported pK_a values for different types of boronic acids, the relative order of the formation of tetrahedral boron is aryl > alkyl.[40] Consequently, the calculated pK_a values of the alkyl (**21**) and alkenyl (**22**) derivatives (10.4 and 10.0, respectively) were expected to have a negative influence on inhibition potencies. However, **21** was shown to be as potent an FAAH inhibitor as **1**, with an IC_{50} of 1.3 μM. Moreover, **22** inhibited FAAH with an IC_{50} of 0.014 μM (Table 5).

Compounds **1**−**22** were shown to be less effective as inhibitors of MGL. However, eight compounds showed over 50% inhibition at 100 μM in the screening against MGL activity and were further determined for their IC_{50} values. The most potent MGL inhibitor was **13** with an IC_{50} of 7.9 μM. Compounds **8** and **11**, with less lipophilic para-substituents in the phenyl ring (CF_3 and Ph, respectively), inhibited MGL with IC_{50} of 20 and 19 μM, respectively (Table 2). The corresponding meta-substituted phenylboronic acids **3** and **5** (IC_{50} of 38 and 71 μM, respectively) (Table 1) were less potent MGL inhibitors than **8** and **11**. Within the heteroarylboronic acids, **18** (IC_{50} = 32 μM) was a more potent MGL inhibitor than **19** (IC_{50} = 100 μM) (Table 4). Compound **22** inhibited MGL with an IC_{50} of 31 μM (Table 5). On the basis of the obtained results, the MGL inhibition was suggested to be more dependent on the size and lipophilicity than the pK_a values of the compounds.

Molecular docking was utilized to gain insight on the FAAH binding mode of the boronic acids presented here. A putative binding mode of **13** is shown in Figure 2. Additional details are presented in Supporting Information.

In conclusion, we discovered that boronic acids are potent inhibitors of FAAH, with IC_{50} values in the nanomolar or low-micromolar range. Furthermore, some of these compounds were found to inhibit MGL with IC_{50} values in the micromolar range, demonstrating that boronic acids could offer a new approach for designing novel MGL inhibitors. The present results indicate that the lipophilic and/or electron-withdrawing para-substituents in phenylboronic acids are beneficial for both FAAH and MGL inhibitory activity. Moreover, some correlation was observed between the calculated pK_a values and inhibition potencies against FAAH within each series of substituted phenylboronic acids. Besides the phenylboronic acids, the benzothiophene- and benzofuran- and lipophilic alkenylboronic acids were able to inhibit FAAH and MGL activity. In order to elucidate the FAAH binding mechanism and orientation of boronic acid derivatives in more detail, mixed quantum and molecular mechanics calculations (QM/MM) will be carried out.[42,43] In addition, the IC_{50} data of the present and future compounds will be exploited in quantitative structure−activity relationship (QSAR) modeling. As boronic acids are known to inhibit other hydrolytic enzymes, the selectivity of these compounds for FAAH over other hydrolases and cannabinoid receptors should also be studied carefully. Moreover, although boronic acids have previously been reported to form covalent bond with catalytic serine residues of the most serine hydrolases,[34] this is not necessarily true in the case of the inhibition of FAAH, and therefore, the inhibition mechanism of FAAH by boronic acids would be an important issue to study in more detail in the future. These results can be helpful in the further design of a compound with potent inhibitory activities toward the hydrolysis of AEA and/or 2-AG.

Acknowledgment. We thank Minna Glad, Tiina Koivunen, Miia Reponen, Helly Rissanen, and Sari Ukkonen for technical help. The Graduate School for Organic Chemistry and Chemical Biology (GSOCCB), The Academy of Finland (Grant 110277), and The National Graduate School in Informational and Structural Biology (ISB) have provided financial support for this study.

Supporting Information Available: Purities, ^{1}H NMR spectra, and CofAs of the compounds, biological testing methods, and details of molecular docking. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **1996**, *384*, 83–87.

(2) Giang, D. K.; Cravatt, B. F. Molecular characterization of human and mouse fatty ccid amide hydrolases. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *4*, 2238–2242.

(3) Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **1992**, *258*, 1946–1949.

(4) Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gobher, A.; Almog, S.; Martin, B. R.; Compton, D. R.; Pertwee, R. G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **1995**, *50*, 83–90.

(5) Sugiura, T.; Kondo, S.; Sukawaga, A.; Nakane, S.; Shinoda, A; Itoh, K.; Yamasita, A.; Waku, K. 2-Arachidonoylglycerol: a possible

endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89–97.
(6) Devane, W. A., 3rd; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **1988**, *34*, 605–613.
(7) Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **1990**, *346*, 561–564.
(8) Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993**, *365*, 61–65.
(9) Stella, N.; Schweitzer, P.; Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **1997**, *388*, 773–778.
(10) Sugiura, T.; Kodaka, T.; Nakane, S.; Miyahita, T.; Kondo, S.; Suhara, Y.; Takayama, H.; Waku, K.; Seki, C.; Baba, N.; Ishima, Y. Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure−activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. *J. Biol. Chem.* **1999**, *274*, 2794–2801.
(11) Lambert, D. M.; Fowler, C. J. The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. *J. Med. Chem.* **2005**, *48*, 5059–5087.
(12) Dinh, T. P.; Carpenter, D.; Leslie, F. M.; Freund, T. F.; Katona, I.; Sensi, S. L.; Kathuria, S.; Piomelli, D. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10819–10824.
(13) Blankman, J. L.; Simon, G. M.; Cravatt, B. F. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* **2007**, *14*, 1309–1311.
(14) Kathuria, S.; Gaetani, S.; Fegley, D.; Valino, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La Rana, G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* **2003**, *9*, 76–81.
(15) Jaymanne, A.; Greenwood, R.; Mitchell, V. A.; Aslan, S.; Piomelli, D.; Vaughan, C. W. Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. *Br. J. Pharmacol.* **2006**, *147*, 281–288.
(16) Deutch, D. G.; Lin, S.; Hill, W. A.; Morse, K. L.; Salehani, D.; Arreaza, G.; Omeir, R. L.; Makriyannis, A. Fatty acid sulfonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid Receptor. *Biochem. Biophys. Res. Commun.* **1997**, *231*, 217–221.
(17) Segall, Y.; Quistad, G. B.; Nomura, D. K.; Casida, J. E. Arachidonylsulfonyl derivatives as cannabinoid CB1 receptor and fatty Acid amide hydrolase inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3301–3303.
(18) Deutch, D. G.; Omeir, R.; Arreaza, G.; Salehani, D.; Prestwich, G. D.; Huang, Z.; Howlett, A. Methyl arachidonyl fluorophosphonate: a potent irreversible inhibitor of anandamide amidase. *Biochem. Pharmacol.* **1997**, *53*, 255–260.
(19) Koutek, B.; Prestwich, G. D.; Howlett, A. C.; Chin, S. A.; Salehani, D.; Akhavan, N.; Deutch, D. G. Inhibitors of arachidonoyl ethanolamide hydrolysis. *J. Biol. Chem.* **1994**, *269*, 22937–22940.
(20) De Petrocellis, L.; Melck, D.; Ueda, N.; Maurelli, S.; Kurahashi, Y.; Yamamoto, S.; Marino, G.; Di Marzo, V. Novel inhibitors of brain, neuronal, and basophilic anandamide amidohydrolase. *Biochem. Biophys. Res. Commun.* **1997**, *231*, 82–88.
(21) 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. Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5044–5049.
(22) Lichtman, A. H.; Leung, D.; Shelton, C.; Saghatelian, A.; Hardouin, C.; Boger, D. L.; Cravatt, B. F. Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: evidence for an unprecented combination of potency and selectivity. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 441–448.
(23) Myllymäki, M. J.; Saario, S. M.; Kataja, A.; Castillo-Melendez, J. A.; Nevalainen, T.; Juvonen, R. O.; Järvinen, T.; Koskinen, A. M. P. Design, synthesis, and in vitro evaluation of carbamate derivatives of 2-benzoxazolyl- and 2-benzothiazolyl-(3-hydroxyphenyl)-methanones as novel fatty acid amide hydrolase inhibitors. *J. Med. Chem.* **2007**, *50*, 4236–4242.
(24) Sit, S.-Y.; Xie, K. WO Patent 087569, 2002.
(25) Ahn, K.; Johnson, D. S.; Fitzgerald, L. R.; Liimatta, M.; Arendse, A.; Stevenson, T.; Lund, E. T.; Nugent, R. A.; Nomanbhoy, T. K.; Alexander, J. P.; Cravatt, B. F. Novel mechanistic class of fatty acid amide hydrolase inhibitors with remarkable selectivity. *Biochemistry* **2007**, *46*, 13019–13030.
(26) Chang, L.; Luo, L.; Palmer, J. A.; Sutton, S.; Wilson, S. J.; Barbier, A. J.; Breitenbucher, J. G.; Chaplan, S. R.; Webb, M. Inhibition of fatty acid amide hydrolase produces analgesia by multible mechanisms. *Br. J. Pharmacol.* **2006**, *148*, 102–113.
(27) Holt, S.; Comelli, F.; Costa, B.; Fowler, C. J. Inhibitors of fatty acid amide hydrolase reduce carrageenan-induced hind paw inflammation in pentobarbital-treated mice: comparison with indomethacin and possible involvement of cannabinoid receptors. *Br. J. Pharmacol.* **2005**, *146*, 467–476.
(28) Russo, R.; Loverne, J.; La Rana, G.; Compton, T. R.; Parrot, J.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; Calignano, A.; Piomelli, D. The fatty acid amide hydrolase inhibitor URB597 (cyclohexylcarbamic acid 3′-carbamoylbiphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 236–242.
(29) Jhaveri, M. D.; Richardson, D.; Kendall, D. A.; Barret, D. A.; Chapman, V. Analgesic effects of fatty acid amide hydrolase inhibition in a rat model of neuropathic pain. *J. Neurosci.* **2006**, *26*, 13318–13327.
(30) Gobbi, G.; Bambico, F. R.; Mangieri, R.; Bortolato, M.; Campolongo, P.; Solinas, M.; Cassano, T.; Morgese, M. G.; Debonnel, G.; Duranti, A.; Tontini, A.; Tarzia, G.; Mor, M.; Trezza, V.; Goldberg, S. R.; Cuomo, V.; Piomelli, D. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 18620–18625.
(31) Saario, S. M.; Savinainen, J. R.; Laitinen, J. T.; Järvinen, T.; Niemi, R. Monoglyceride lipase-like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. *Biochem. Pharmacol.* **2004**, *67*, 1381–1387.
(32) Tornqvist, H.; Belfrage, P. Purification and some properties of a monoglycerol-hydrolyzing enzyme of rat adipose tissue. *J. Biol. Chem.* **1976**, *251*, 813–819.
(33) Saario, S. M.; Salo, O. M. H.; Nevalainen, T.; Poso, A.; Laitinen, J. T.; Järvinen, T.; Niemi, R. Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. *Chem. Biol.* **2005**, *12*, 649–659.
(34) Yang, W.; Gao, X.; Wang, B. Boronic acid compounds as potential pharmaceutical agents. *Med. Res. Rev.* **2003**, *23*, 346–368.
(35) Adams, J.; Behnke, M. L.; Castro, A. C.; Evans, C. A.; Crenier, L.; Grogan, M. J.; Liu, T.; Snyder, D. A.; Tibbits, T. T. WO Patent Appl. 063300, 2008.
(36) Patricelli, M. P.; Cravatt, B. F. Fatty acid hydrolase competitively degrades bioactive amides and esters through a nonconventinal catalytic mechanism. *Biochemistry* **1999**, *38*, 14125–14130.
(37) Patricelli, M. P.; Lovato, M. A.; Cravatt, B. F. Chemical and mutagenic investigations of fatty acid amide hydrolase: evidence for a family of serine hydrolases with distinct catalytic properties. *Biochemistry* **1999**, *38*, 9804–9812.
(38) Bracey, M. H.; Hanson, M. A.; Masuda, K. R.; Stevens, R. C.; Cravatt, B. F. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* **2002**, *298*, 1793–1796.
(39) Saario, S. M.; Poso, A.; Juvonen, R. O.; Järvinen, T.; Salo, O. M. H. Fatty acid amide inhibitors from virtual screening of the endocannabinoid system. *J. Med. Chem.* **2006**, *49*, 4650–4656.
(40) Hall, D. G. Structure, Properties, and Preparation of Boronic Acid Derivatives. Overview of Their Reactions and Applications. In *Boronic Acids*, 1st ed.; Hall, D. G., Eds.; Wiley-VCH: Weinheim, Germany, 1995; pp 1−99.
(41) Clark, A. M.; Labute, P.; Santavy, M. 2D structure depiction. *J. Chem. Inf. Model.* **2006**, *46*, 1107–1123.
(42) Tubert-Brohman, I.; Acevedo, O.; Jorgensen, W. L. Elucidation of hydrolysis mechanisms for fatty acid amide hydrolase and its Lys142Ala variant via QM/MM simulations. *J. Am. Chem. Soc.* **2006**, *128*, 16904–16913.
(43) Lodola, A.; Mor, M.; Rivara, S.; Christov, C.; Tarzia, G.; Piomelli, D.; Mulholland, A. J. Identification of productive inhibitor binding orientation in fatty acid amide hydrolase (FAAH) by QM/MM mechanistic modelling. *Chem. Commun.* **2008**, *2*, 214–216.

JM801051T