Published in final edited form as:

Bioorg Med Chem Lett. 2008 November 15; 18(22): 5842–5846. doi:10.1016/j.bmcl.2008.06.084.

Exploration of a fundamental substituent effect of α -ketoheterocycle enzyme inhibitors: potent and selective inhibitors of fatty acid amide hydrolase

Jessica K. DeMartino, Joie Garfinkle, Dustin G. Hochstatter, Benjamin F. Cravatt, and Dale L. Boger*

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

Abstract

A series of C4 substituted α -ketooxazoles were examined as inhibitors of the serine hydrolase fatty acid amide hydrolase in efforts that further define and generalize a fundamental substituent effect on enzyme inhibitory potency. Thus, a plot of the Hammett σ_m versus $-\log K_i$ provided a linear correlation ($R^2 = 0.90$) with a slope of 3.37 ($\rho = 3.37$) that is of a magnitude that indicates the electron-withdrawing character of the substituent dominates its effects (a one unit change in σ_m provides a >1000-fold change in K_i).

Fatty acid amide hydrolase (FAAH)^{1,2} is the enzyme that serves to hydrolyze endogenous lipid amides^{3,4} including anandamide (**1a**)⁵ and oleamide (**1b**),⁶ Figure 1. Its distribution is consistent with its role in degrading and regulating such signaling fatty acid amides at their sites of action.³ Although it is a member of the amidase signature family of serine hydrolases, for which there a number of prokaryotic enzymes, it is currently the only characterized mammalian enzyme bearing the family's unusual Ser-Ser-Lys catalytic triad.^{7,8}

Due to the therapeutic potential of inhibiting FAAH⁹ especially for the treatment of pain,¹⁰ inflammation,¹¹ or sleep disorders,¹² there has been increasing interest in the development of selective and potent inhibitors of the enzyme.⁹ Early studies shortly following the initial discovery and characterization of FAAH led to the demonstration that the endogenous sleep-inducing molecule 2-octyl α -bromoacetoacetate is an effective FAAH inhibitor,¹³ the disclosure of a series of nonselective, reversible inhibitors bearing an electrophilic ketone (e.g., trifluoromethyl ketone-based inhibitors),^{14,15} and the reports of a set of irreversible inhibitors¹⁶ (e.g., fluorophosphonates and sulfonyl fluorides). To date, two classes of inhibitors have been disclosed that provide opportunities for the development of inhibitors with therapeutic potential. One class is the reactive aryl carbamates and ureas^{17–24} that irreversibly acylate a FAAH active site serine.²⁵ A second class is the α -ketoheterocycle-based inhibitors^{26–29} that bind to FAAH via reversible hemiketal formation with an active site serine. Many of these latter competitive inhibitors are not only potent and extraordinarily selective for FAAH versus other mammalian serine hydrolases, but members of this class have been shown to be efficacious analgesics in vivo.²⁸

In the course of these latter studies, we disclosed a fundamental substituent effect in which a well-defined correlation between the electronic character of a para substituent (Hammett σ_p) and the inhibitor potency ($-\log K_i$) was observed.²⁷ Thus, the inhibitor potency was found to

*Corresponding author: email boger@scripps.edu.

smoothly increase as the electron-withdrawing character of the substituent increased and the magnitude of the effect was remarkably large ($\rho = 2.7\text{--}3.0$)^{26,27} indicating that a unit change in σ_p leads to nearly a 1000-fold increase in K_i . Presumably this reflects the electronic effect of the substituent on activating the electrophilic carbonyl toward nucleophilic attack by the FAAH active site catalytic Ser. Herein, we further generalize this fundamental effect to include meta substituents on the α -ketoheterocycle.

Whereas the former para substituents are directly conjugated with the electrophilic carbonyl, the meta substituents would exert their effects through their inductive electron-withdrawing properties. Moreover and although intuitive expectations might suggest that such a non-conjugated substituent effect might be small, a comparison of Hammett σ_p and σ_m constants suggests the magnitude of the effects may be surprisingly similar and, in some instances, even enhanced (e.g., F, Cl, Br, and I).

The candidate inhibitors bearing a varied C4 oxazole substituent were accessed from the readily available **2**,²⁶ enlisting its in situ conversion to the isomeric 4-bromooxazole via a halogen dance rearrangement, Scheme 1.³⁰ Thus, C4-lithiation of **2** followed by its in situ rearrangement to the more stable 5-lithio-4-bromooxazole³¹ and its quench with water provided **3b**, the requisite precursor for the series of derivatives to be examined. These were accessed by metallation of **3b** with *n*-BuLi or *t*-BuLi followed by reaction with appropriate electrophiles (NCS, I₂, CH₃I, (MeS)₂, DMF, CH₃CONMe₂, CF₃CO₂Et, NCCO₂Me). Similarly, the C4 pyridine (**3k**, **3l**, and **3m**) and phenyl (**3n**) derivatives were prepared from bromide **3b** using a Stille coupling reaction with the respective pyridyl or phenyl tributylstannanes. Many of these derivatives served as precursors to additional candidate inhibitors bearing further modified C4 substituents. Methyl ester **4j** was directly converted to its corresponding carboxylic acid **4o** and carboxamide **4p** using LiOH and methanolic ammonia, respectively. Carboxylic acid **4o** was also coupled with methylamine and dimethylamine to give the substituted carboxamides **4q** and **4r**. Carboxamide **4p** was dehydrated with TFAA and pyridine to provide nitrile **4s**. The trifluoromethyl derivative **3t** was prepared from iodide **3d** using the method developed by Chen et al.³² and iodide **3d** also served as the precursor to methyl ether **3u**.³³ In each case, deprotection of the TBS ether followed by Dess–Martin periodinane oxidation³⁴ of the liberated alcohol yielded the corresponding α -ketooxazole (**4b–u**).

The FAAH inhibition derived from the examination of the series of inhibitors and the correlation derived from a plot of $-\log K_i$ versus σ_m ($\rho = 3.37$, $R^2 = 0.90$ excluding **4e**, **4m**, **4p**, **4u**) is provided in Figure 2. The magnitude of the effect defined by the correlation is large ($\rho = 3.37$) and satisfyingly comparable to the observed with σ_p ($\rho = 3.0$)²⁷ where a unit change in σ_m leads to an increase in the K_i of more than 1000-fold. As such and although the substituents may engage in additional, more subtle interactions at the enzyme active site, the magnitude of the electronic effect indicates it dominates the behavior of the FAAH inhibitors.

Importantly, this allows one to quantitatively predict a K_i from the correlation based on the Hammett substituent constant (σ_p and σ_m) or use the measured K_i to draw inferences about active site binding that might not be known a priori. As an example of the latter, we can confidently establish that **4o** binds the FAAH active site as its deprotonated carboxylate ($\sigma_m = 0.02$) versus carboxylic acid ($\sigma_m = 0.35$) from the measured K_i and this conclusion is reasonable given the pH of the enzyme assay conditions (pH = 9.0).²⁶ More subtly, we were able to establish that aldehyde **4g** (and trifluoromethyl ketone **4i**) exist in protic solution as gem diols (at C4, not C2; ¹H and ¹³C NMR, data in Supporting Information) and that they inhibit FAAH with potencies at a level more consistent with this C4 substituent gem diol versus carbonyl active site binding. Although the latter C(OH)₂CF₃ gem diol most likely suffers significant destabilizing steric interactions at the enzyme active site comparable to that of a *t*-

butyl substituent, the measured K_i of the hydrated aldehyde ($\text{CH}(\text{OH})_2$) is of a magnitude that suggests it may provide a good estimate of the σ_m for this substituent (0.02 for $\text{CH}(\text{OH})_2$ vs 0.35 for CHO). That is, the correlation between σ_m and K_i is sufficiently dependable that deviations from the expectations can be utilized to establish features of active site binding not a priori known.

In this correlation, there are several inhibitors (**4m**, **4k**, **4p**, and **4q**) that deviate productively from expectations being more potent than predicted. All four could benefit from additional H-bonding at the active site that may increase affinity beyond that expected. Based on their relative K_i 's, the 4-pyridyl derivative **4m** and, to a lesser extent, the 2-pyridyl derivative **4k** may interact with H-bond donors including the mobile catalytic Lys142 at the FAAH active site²⁶ where such a potential H-bond may be regarded not only as a conventional H-bond stabilizing interaction, but also as a partial protonation of the pyridyl nitrogen enhancing its electron-withdrawing properties. Similarly, the primary carboxamide **4p** and, to a lesser extent, the secondary carboxamide **4q** productively deviate from correlation expectations, whereas the tertiary carboxamide **4r** falls below extrapolated³⁵ expectations. Attractive explanations for this behavior include questions on the accuracy of the carboxamide σ_m , a productive H-bonding interaction of RCONHR at the FAAH active site for **4p** and **4q** (but not **4r**) that further increase affinity, and/or destabilizing steric interactions that emerge only with the tertiary amide **4r**. Two substituents ($-\text{Me}$, $-\text{OMe}$) display substantial nonproductive deviations from the correlation. Although we do not yet have attractive explanations for their behavior, both represent electron-donating and electron-rich substituents whose activity is predicted to be among the poorest. Thus, while additional substituent features can and will modulate the binding affinity of the candidate inhibitors (e.g., H-bonding, hydrophobic or steric interactions), the magnitude of the electronic effect of the substituent ($\rho = 3.37$) indicates that the latter will typically dominate, especially for small and simple substituents.

Finally, the oxazole substituents in such inhibitors not only influence the FAAH inhibitor potency, but they can have an equally remarkable impact on the FAAH inhibition selectivity.²⁶ Although there are no other characterized mammalian members of the serine hydrolase family that bear the amidase signature sequence and its unusual Ser-Ser-Lys catalytic triad and no resulting close family of enzymes against which to counter screen the candidate inhibitors, a close collaboration with Professor Cravatt led to the implementation of a proteome-wide assay capable of simultaneously interrogating all mammalian serine hydrolases applicable to assessing the selectivity of reversible enzyme inhibitors.³⁵ This assay, which requires no modification of the inhibitor, no purified protein for conventional substrate assay, no knowledge of candidate off-site targets or even the function or substrate of the enzymes, can globally detect, identify, and quantitate all potential competitive enzyme targets in the human proteome for such inhibitors.³⁶ To date, two enzymes have emerged at potential competitive targets for inhibitors in this class: triacylglycerol hydrolase (TGH) and a previously uncharacterized membrane-associated hydrolase that lacked known substrates or function at the time (KIAA1363), but has since been characterized by Cravatt and coworkers.³⁷ Enlisting this proteome selectivity assay, we have been able to simultaneously optimize inhibitors for both FAAH potency and selectivity, identifying key features of candidate inhibitors that can increase binding at the FAAH active site while simultaneously disrupting KIAA1363 and TGH affinity. This multidimensional SAR optimization is highlighted beautifully with the inhibitors **4t**, **4s**, **4k**, **4m** and **4o** with the results summarized in Figure 3. Like observations made with the 5-substituted oxazoles,²⁶ the addition of a 4-substituent to **4a** enhances the FAAH versus KIAA1363 selectivity (>25-fold selective vs 8-fold for **4a**), but not always as substantially as the 5-substituted oxazoles (>100-fold) where such inhibitors typically fail to inhibit KIAA1363. Similarly, the addition of a 4-substituent converts the TGH selective inhibitor **4a** (>100-fold selective for TGH vs FAAH) into inhibitors that are modestly selective for FAAH (up to 5-fold selective). The exception to this is **4k** which like OL-135 (**5c**) was found

to be >300-fold selective for FAAH versus TGH. This enhancement in FAAH selectivity, while remarkable (typically >100-fold, but >40,000 for **4k**), is generally not as great as that observed in the 5-substituted oxazole series.

One additional feature of this study merits highlighting. In earlier studies,²⁶ we found that either a 4-substituent or 5-substituent on the oxazole can be utilized to enhance FAAH inhibitor potency, but candidate inhibitors bearing both were significantly less active. Although not extensively investigated, analogous observations were made in the course of these studies.³⁸ This suggests that the two classes of oxazole-based inhibitors may bind at the FAAH active site in a manner that places the substituent in a comparable location. This simply requires a flipped orientation of the oxazole at the active site reversing the location of the N and O of the heterocycle (Figure 4). Consistent with this suggestion, related inhibitors²⁶ bearing a C5-substituent have been shown to exhibit a significant sensitivity to steric hindrance surrounding the analogous oxazole C4 center (potency: N > O > CH) indicating that there may be ample room for one, but not two such substituents. Provocatively and while this flipped orientation of the oxazole between the two series has little impact on the FAAH inhibitor potency, it may have a more significant impact on the FAAH selectivity which often, but not always, appears to erode with the 4-substituted series disclosed herein.

A series of C4 substituted α -ketooxazoles were examined as inhibitors of the serine hydrolase fatty acid amide hydrolase in efforts that further define and generalize a fundamental substituent effect on enzyme inhibitory potency. A plot of the Hammett σ_m versus $-\log K_i$ provided a linear correlation ($R^2 = 0.90$) with a slope of 3.37 ($\rho = 3.37$) that is of a magnitude that indicates the electron-withdrawing character of the substituent dominates its effects (a one unit change in σ_m provides a >1000-fold change in K_i). Moreover, this meta substituent effect is comparable, essentially identical, to that we previously defined for para substituents ($\rho = 2.7\text{--}3.0$, $R^2 = 0.91\text{--}0.97$)^{26,29} confirming both its generality and magnitude independent of the site of substitution. Importantly, the correlation provides a useful and predictive design principle for enzyme inhibitors and is of a sufficient accuracy that subtleties of active site binding that are not known a priori may be established from a measured K_i . These observations may prove useful not only to extend to other enzyme classes, but have provided herein an additional and useful class of potent and selective FAAH inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (DA15648), and the Skaggs Institute for Chemical Biology. We are especially grateful to Professor B. F. Cravatt for the supply of rat and recombinant human FAAH and for stimulating collaborations.

References and Notes

1. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. *Nature* 1996;384:83. [PubMed: 8900284]
2. Giang DK, Cravatt BF. *Proc Natl Acad Sci USA* 1997;94:2238. [PubMed: 9122178]
3. Patricelli MP, Cravatt BF. *Vit Hormones* 2001;62:95.
4. Boger DL, Fecik RA, Patterson JE, Miyauchi H, Patricelli MP, Cravatt BF. *Bioorg Med Chem Lett* 2000;10:2613. [PubMed: 11128635]
5. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandlebaum A, Etinger A, Mechoulam R. *Science* 1992;258:1946. [PubMed: 1470919]

6. Cravatt BF, Prospero-Garcia O, Suizdak F, Gilula NB, Henriksen SJ, Boger DL, Lerner RA. *Science* 1995;268:1506. [PubMed: 7770779] Cravatt BF, Boger DL, Lerner RA. *J Am Chem Soc* 1996;118:580. Boger DL, Henriksen SJ, Cravatt BF. *Curr Pharm Des* 1998;4:303. [PubMed: 10197045]
7. Patricelli MP, Cravatt BF. *J Biol Chem* 2000;275:19177. [PubMed: 10764768]
8. Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF. *Science* 2002;298:1793. [PubMed: 12459591]
9. Cravatt BF, Lichtman AH. *Curr Opin Chem Biol* 2003;7:469. [PubMed: 12941421] Lambert DM, Fowler CJ. *J Med Chem* 2005;48:5059. [PubMed: 16078824]
10. Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. *Proc Natl Acad Sci USA* 2001;98:9371. [PubMed: 11470906] Lichtman AH, Shelton CC, Advani T, Cravatt BF. *Pain* 2004;109:319. [PubMed: 15157693] Cravatt BF, Saghatelian A, Hawkins EG, Clement AB, Bracey MH, Lichtman AH. *Proc Natl Acad Sci USA* 2004;101:10821. [PubMed: 15247426]
11. Karsak M, Gaffal E, Date R, Wang-Eckhardt L, Rehnelt J, Petrosino S, Starowicz K, Steuder R, Schlicker E, Cravatt BF, Mechoulam R, Buettner R, Werner S, Di Marzo V, Tuetting T, Zimmer A. *Science* 2007;316:1494. [PubMed: 17556587]
12. Huitrón-Reséndiz S, Sanchez-Alavez M, Wills DN, Cravatt BF, Henriksen SJ. *Sleep* 2004;27:857. [PubMed: 15453543]
13. Patricelli MP, Patterson JP, Boger DL, Cravatt BF. *Bioorg Med Chem Lett* 1998;8:613. [PubMed: 9871570]
14. Koutek B, Prestwich GD, Howlett AC, Chin SA, Salehani D, Akhavan N, Deutsch DG. *J Biol Chem* 1994;269:22937. [PubMed: 8083191]
15. Patterson JE, Ollmann IR, Cravatt BF, Boger DL, Wong CH, Lerner RA. *J Am Chem Soc* 1996;118:5938. Boger DL, Sato H, Lerner AE, Austin BJ, Patterson JE, Patricelli MP, Cravatt BF. *Bioorg Med Chem Lett* 1999;9:265. [PubMed: 10021942]
16. Deutsch DG, Omeir R, Arreaza G, Salehani D, Prestwich GD, Huang Z, Howlett A. *Biochem Pharmacol* 1997;53:255. [PubMed: 9065728] Deutsch DG, Lin S, Hill WAG, Morse KL, Salehani D, Arreaza G, Omeir RL, Makriyannis A. *Biochem Biophys Res Commun* 1997;231:217. [PubMed: 9070252] Edgemond WS, Greenberg MJ, McGinley PJ, Muthians S, Campbell WB, Hillard CJ. *J Pharmacol Exp Ther* 1998;286:184. [PubMed: 9655859]
17. 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. *Nat Med* 2003;9:76. [PubMed: 12461523] Jayamanne A, Greenwood R, Mitchell VA, Aslan S, Piomelli D, Vaughan CW. *Br J Pharmacol* 2006;147:281. [PubMed: 16331291] Mor M, Rivara S, Lodola A, Plazzi PV, Tarzia G, Duranti A, Tontini A, Piersanti G, Kathuria S, Piomelli D. *J Med Chem* 2004;47:4998. [PubMed: 15456244] Tarzia G, Duranti A, Tontini A, Piersanti G, Mor M, Rivara S, Plazzi PV, Park C, Kathuria S, Piomelli D. *J Med Chem* 2003;46:2352. [PubMed: 12773040] Tarzia G, Duranti A, Gatti G, Piersanti G, Tontini A, Rivara S, Lodola A, Plazzi PV, Mor M, Kathuria S, Piomelli D. *ChemMedChem* 2006;1:130. [PubMed: 16892344]
18. Ahn K, Johnson DS, Fitzgerald LR, Liimatta M, Arendse A, Stevenson T, Lund ET, Nugent RA, Normanbhoj T, Alexander JP, Cravatt BF. *Biochemistry* 2007;46:13019. [PubMed: 17949010]
19. Abouab-Dellah, A.; Burnier, P.; Hoornaert, C.; Jeunesse, J.; Puech, F. *WO* 2004/099176. Abouab-Dellah, A.; Almario, G. A.; Froissant, J.; Hoornaert, C. *WO* 2005/077898. Abouab-Dellah, A.; Almario, G. A.; Hoornaert, C.; Li, A. T. *WO* 2005/070910. Abouab-Dellah, A.; Almario, G. A.; Hoornaert, C.; Li, A. T. *WO* 2007/027141.
20. Sit, S.-Y.; Xie, K.; Deng, H. *WO* 2003/06589. Sit, S.-Y.; Xie, K. *WO* 2002/087569. Sit SY, Conway C, Bertekap R, Xie K, Bourin C, Burris K, Deng H. *Bioorg Med Chem Lett* 2007;17:3287. [PubMed: 17459705]
21. Apodaca, R.; Breitenbucher, J. G.; Pattabiraman, K.; Seierstad, M.; Xiao, W. *US* 2006/0173184. Apodaca, R.; Breitenbucher, J. G.; Pattabiraman, K.; Seierstad, M.; Xiao, W. *US* 2007/004741.
22. Matsumoto, T.; Kori, M.; Miyazaki, J.; Kiyota, Y. *WO* 2006/054652. Matsumoto, T.; Kori, M.; Kouno, M. *WO* 2007/020888.
23. Ishii, T.; Sugane, T.; Maeda, J.; Narazaki, F.; Kakefuda, A.; Sato, K.; Takahashi, T.; Kanayama, T.; Saitoh, C.; Suzuki, J.; Kanai, C. *WO* 2006/088075.

24. Moore SA, Nomikos GG, Dickason–Chesterfield AK, Sohober DA, Schaus JM, Ying BP, Xu YC, Phebus L, Simmons RM, Li D, Iyengar S, Felder CC. *Proc Natl Acad Sci USA* 2005;102:17852. [PubMed: 16314570] Alexander JP, Cravatt BF. *J Am Chem Soc* 2006;128:9699. [PubMed: 16866524]
25. Alexander JP, Cravatt BF. *Chem Biol* 2005;12:1179. [PubMed: 16298297]
26. Boger DL, Sato H, Lerner AE, Hedrick MP, Fecik RA, Miyauchi H, Wilkie GD, Austin BJ, Patricelli MP, Cravatt BF. *Proc Natl Acad Sci USA* 2000;97:5044. [PubMed: 10805767] Boger DL, Miyauchi H, Hedrick MP. *Bioorg Med Chem Lett* 2001;11:1517. [PubMed: 11412972] Boger DL, Miyauchi H, Du W, Hardouin C, Fecik RA, Cheng H, Hwang I, Hedrick MP, Leung D, Acevedo O, Guimarães CRW, Jorgensen WL, Cravatt BF. *J Med Chem* 2005;48:1849. [PubMed: 15771430] Guimarães CRW, Boger DL, Jorgensen WL. *J Am Chem Soc* 2005;127:17377. [PubMed: 16332087] Leung D, Du W, Hardouin C, Cheng H, Hwang I, Cravatt BF, Boger DL. *Bioorg Med Chem Lett* 2005;15:1423. [PubMed: 15713400] Romero FA, Du W, Hwang I, Rayl TJ, Kimball FS, Leung D, Hoover HS, Apodaca RL, Breitenbucher BJ, Cravatt BF, Boger DL. *J Med Chem* 2007;50:1058. [PubMed: 17279740] Hardouin C, Kelso MJ, Romero FA, Rayl TJ, Leung D, Hwang I, Cravatt BF, Boger DL. *J Med Chem* 2007;50:3359. [PubMed: 17559203] Kimball FS, Romero FA, Ezzili C, Garfinkle J, Rayl TJ, Hochstatter DG, Hwang I, Boger DL. *J Med Chem* 2008;51:937. [PubMed: 18247553] Garfinkle J, Ezzili C, Rayl TJ, Hochstatter DG, Hwang I, Boger DL. *J Med Chem* 2008;51 in press. For a review of α -ketoheterocycle enzyme inhibitors, see Maryanoff BE, Costanzo MJ. *Bioorg Med Chem* 2008;16:1562. [PubMed: 18053726]
27. Romero FA, Hwang I, Boger DL. *J Am Chem Soc* 2006;68:14004. [PubMed: 17061864] Although we are unaware of reports that enlist Hammett σ_p or σ_m values on oxazoles, studies on heterocycles including furan and thiophene exhibit near perfect Hammett σ_p or σ_m correlations with those established for phenyl as we have used them, see: Freeman F. *J Chem Edu* 1970;47:140.
28. Lichtman AH, Leung D, Shelton CC, Saghatelian A, Hardouin C, Boger DL, Cravatt BF. *J Pharmacol Exp Ther* 2004;311:441. [PubMed: 15229230] Chang L, Luo L, Palmer JA, Sutton S, Wilson SJ, Barbier AJ, Breitenbucher JG, Chaplan SR, Webb M. *Br J Pharmacol* 2006;148:102. [PubMed: 16501580]
29. For additional studies, see: Du W, Hardouin C, Cheng H, Hwang I, Boger DL. *Bioorg Med Chem Lett* 2005;15:103. [PubMed: 15582420] Muccioli GG, Fazio N, Scriba GKE, Poppitz W, Cannata F, Poupaert JH, Wouters J, Lambert DM. *J Med Chem* 2006;49:417. [PubMed: 16392827] Saario SM, Poso A, Juvonen RO, Jarvinen T, Salo–Ahen OMH. *J Med Chem* 2006;49:4650. [PubMed: 16854070] Myllymaki MJ, Saario SM, Kataja AO, Castillo–Melendez JA, Navalainen T, Juvonen RO, Jarvinen T, Koskinen AMP. *J Med Chem* 2007;50:4236. [PubMed: 17665899]
30. Stanetty P, Spina M, Mihovilovic MD. *Synlett* 2005;9:1433.
31. Schnürch M, Spina M, Khan AF, Mihovilovic MD, Stanetty P. *Chem Soc Rev* 2007;36:1046. [PubMed: 17576473]
32. Chen Q-Y, Wu S-W. *J Chem Soc, Chem Commun* 1989:705. Qing FL, Fan J, Sun HB, Yue XJ. *J Chem Soc Perkin Trans 1* 1997:3053.
33. Hui Z, Ma D, Cao W. *Synlett* 2007;2:243.
34. Dess DB, Martin JC. *J Am Chem Soc* 1991;113:7277.
35. We could not locate a σ_m for –CONMe₂, but would expect it to approach that reported for –CONH₂ (0.28) or –CONHMe (0.35).
36. Leung D, Hardouin C, Boger DL, Cravatt BF. *Nature Biotech* 2003;21:687.
37. Kidd D, Liu Y, Cravatt BF. *Biochemistry* 2001;40:4005. [PubMed: 11300781] Liu Y, Patricelli MP, Cravatt BF. *Proc Natl Acad Sci USA* 1999;96:14694. [PubMed: 10611275]
38. Chiang KP, Niessen S, Saghatelian A, Cravatt BF. *Chem Biol* 2006;13:1041. [PubMed: 17052608]
39. For example, the 4,5-dibromo derivative, 1-(4,5-dibromooxazol-2-yl)-7-phenylheptan-1-one, exhibited a K_i of 47 nM. This is 16-fold less potent than the 5-bromo derivative (K_i = 3 nM), 16-fold less potent than 4b, and >100-fold less active than estimates based on the combined substituent effects.

Figure 1.
FAAH Substrates.

compd	R	K_i , nM	compd	R	K_i , nM
4a	H	48	4k	2-Pyr	1.9
4b	Br	3.0	4l	3-pyr	18
4c	Cl	4.0	4m	4-pyr	1.6
4d	I	6.5	4n	Ph	65
4e	CH ₃	520	4o	CO ₂ H	53
4f	SCH ₃	29	4p	CONH ₂	1.6
4g	CHO	55	4q	CONHMe	1.8
4h	COCH ₃	2.0	4r	CONMe ₂	35
4i	CF ₃ CO	470	4s	CN	0.5
4j	CO ₂ CH ₃	3.4	4t	CF ₃	3.7
			4u	OMe	740

Figure 2.
Rat FAAH inhibition (K_i , nM) and plot of σ_m versus $-\log K_i$.

Cmpd	FAAH K_i , nM	FAAH IC_{50} , μ M	KIAA1363 IC_{50} , μ M	TGH IC_{50} , μ M

4a (H)	48	2.5	20 (8)	0.02 (0.008)

4t (CF ₃)	3.7	0.4	10 (25)	0.06 (0.15)
4s (CN)	0.6	0.02	30 (1500)	0.03 (1.5)
4k (2-pyr)	1.9	0.03	>100 (>3000)	10 (330)
4m (4-pyr)	1.7	0.14	>100 (>700)	0.7 (5)
4o (CO ₂ H)	53	0.5	>100 (>200)	2.5 (5)

5a (CF ₃)	0.8	0.07	>100 (>1400)	0.2 (3)
5b (CN)	0.4	0.007	>100 (>14000)	0.02 (3)
5c (2-pyr)	4.7	0.002	>100 (>50000)	0.6 (300)
5d (CO ₂ H)	30	0.9	>100 (>100)	>100 (>100)

Figure 3.
Selectivity Screening, IC_{50} , μ M (selectivity).

Figure 4.

Scheme 1.