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α-Keto Heterocycle Inhibitors of Fatty Acid Amide Hydrolase: Carbonyl Group Modification and α-Substitution

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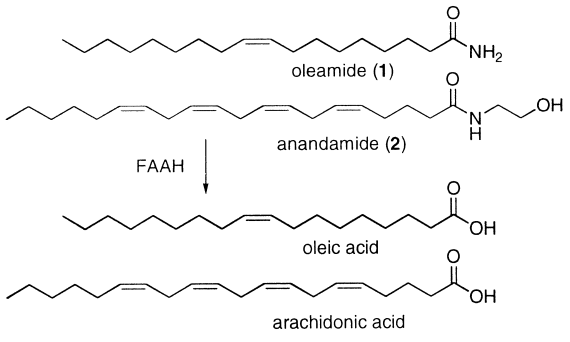
Abstract—Two sets of novel analogues of the recently disclosed α-keto heterocycle inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for regulation of endogenous oleamide and anandamide, were synthesized and evaluated in order to clarify a role of the electrophilic carbonyl group and structural features important for their activity. Both the electrophilic carbonyl and the degree of α-substitution markedly affect inhibitor potency.

Oleamide[1] (**1**) and anandamide[2] (**2**) are the archetypal members of a growing class of fatty acid amides that function as chemical messengers. Oleamide induces physiological sleep in animals[3,4] and analgesic and cannabinoid-like behavioral responses in mice, albeit without binding to cannabinoid receptors.[5,6] Moreover, it has been reported to modulate serotonin receptors,[5,7–10] benzodiazepine-sensitive $GABA_A$ receptors,[11,12] inhibit presynaptic Na^+ channels,[13] and antagonize glial gap junction cell communication.[14] Anandamide is not only an endogenous ligand for the cannabinoid CB1 receptor[2] but also activates the vanilloid (capsaicin) VR1 receptor[15,16] and it has been implicated as an endogenous analgesic.[17] The regulation of oleamide and anandamide is therefore of important therapeutic potential in the management of sleep disorders, anxiety, and pain.



Endogenous oleamide and anandamide are hydrolyzed and inactivated by the enzyme fatty acid amide hydrolase (FAAH),[18,19] a serine hydrolase distributed in the central nervous system.[20] Considering the important role of this enzyme, we have described a series of FAAH inhibitors.[21–24] Building on these studies, we recently disclosed the development of a class of exceptionally potent α-keto heterocycle inhibitors of FAAH.[24] Herein, we describe studies that examine some common carbonyl replacements and the effect of α-substitution on the inhibitory activity of the α-keto heterocycle inhibitors.

Inhibitor Synthesis

Two sets of analogues of the α-keto heterocycle inhibitors were prepared. The first set includes compounds **4**–**8** containing a modified carbonyl group as derivatives of **3**, one of the most potent inhibitors currently reported.[24] Alcohol **4** and the deoxo derivative **5** were

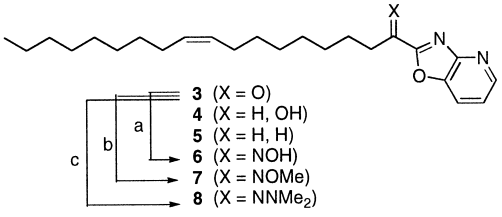


Figure 1. Reagents and conditions: (a) NH_2OH–HCl, NaOAc, MeOH, 25 °C, 30 min, quantitative; (b) NH_2OMe–HCl, Et_3N, C_6H_6, 25 °C, 2 days, 86%; (c) H_2N–NMe_2, TsOH, C_6H_6, 25 °C, 3 h, 63%.

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previously reported and included herein for comparison purposes.[24] Compounds **6–8** were prepared in a single operation from ketone **3** as shown in Figure 1.[25] Each of the three compounds was obtained as a single (*E*)-isomer.

The second set of modifications includes derivatives **11–15** substituted with methyl group(s) on the 2-position, α to the carbonyl group, of the α-keto heterocycle inhibitors **9**,[24] **10**,[24] and **3**.[24] Oxazole and benzoxazole derivatives **11–14** were prepared by the reaction of zinc–copper bimetallic anion of the oxazoles with the corresponding oleoyl chlorides (Fig. 2).[26,27] The oxazolopyridine derivative **15** was synthesized from methyl oleate (**16**) by the method described for **3** (Fig. 3).[28]

Inhibition Studies

All enzyme assays were performed at 20–23 °C by using solubilized rat liver plasma membrane extracts[22] in a reaction buffer of 125 mM Tris/1 mM EDTA/0.2% glycerol/0.02% Triton X-100/0.4 mM Hepes, pH 9.0 buffer.[21] The initial rates of hydrolysis (≤10–20% reaction) were monitored by following the breakdown of ^{14}C-oleamide to oleic acid as described.[2,21] The inhibition was competitive by a Lineweaver–Burke analysis, linear least squares fits were used for all reaction progress curves, and R^2 values were consistently >0.97.[24] K_i values are the average of three determinations and were established by the Dixon method (x-intercepts of weighted linear fits of [I] vs 1/rate plots at constant substrate concentration, which were converted to K_i values by using the formula $K_i = -x_{int}/[1 + [S]/K_m]$).

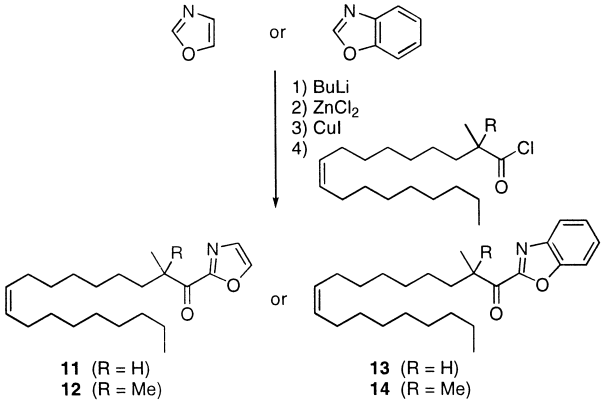


Figure 2.

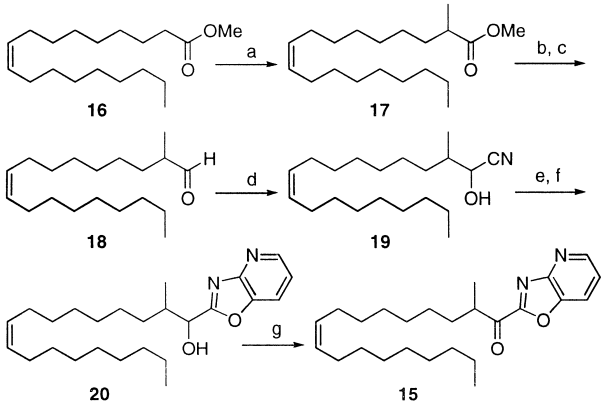
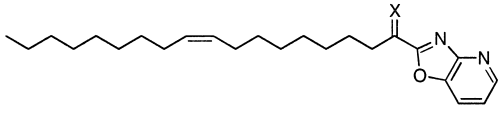


Figure 3. Reagents and conditions: (a) LDA, MeI, THF, −78 °C, 2 h, 94%; (b) DIBAL, THF, −78 °C, 3 h, quantitative; (c) Dess–Martin periodinane, CH_2Cl_2, 25 °C, 3 h, 87%; (d) KCN, THF–H_2O, 25 °C, 2 days, 88%; (e) AcCl, EtOH, $CHCl_3$, 25 °C, 20 h; (f) 2-amino-3-hydroxypyridine, 2-ethoxyethanol, 80 °C, 20 h; (g) Dess–Martin periodinane, CH_2Cl_2, 25 °C, 2 h, 81%.

Results and Discussion

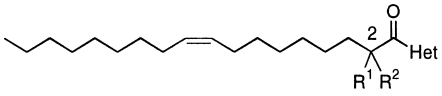
In order to examine the behavior of carbonyl replacements within α-keto heterocycle inhibitors such as **3**, the derivatives **6–8** were prepared and evaluated for their inhibitory activity against FAAH. The results are summarized in Table 1 along with the K_i's for **3–5**. As previously reported, the α-hydroxy derivative **4** retained significant inhibitory activity with a K_i of 1.8 μM; however, it was approximately 10^3 times less potent than the parent α-keto compound **3**. Deoxo compound **5** did not show any inhibition at a concentration of 100 μM. The derivatives **6–8**, in which the carbonyl was replaced with an oxime or hydrazone, also retained significant inhibitory activity with K_i's of 1.5, 4.5, and 0.55 μM, respectively, although they were 200–2000 times less potent than **3**. The derivatives **6–8** were established to be stable under the conditions of the enzyme assay. Thus, all exhibited reduced K_i's and those of **6** and **7** were approximately 10^3 times less potent than the parent α-

Table 1. Inhibitory activity of compounds **4–8** against FAAH



Compounds	X	K_i (μM)
3	O	0.0023±0.0001
4	OH, H	1.8±0.4
5	H, H	>100
6	NOH	1.5±0.2
7	NOMe	4.5±1.7
8	$NNMe_2$	0.55±0.14

Table 2. Inhibitory activity of compounds **11–15** against FAAH



Compounds	Het	R^1, R^2	K_i (μM)
9	Oxazol-2-yl	H, H	0.10±0.06[a]
11	Oxazol-2-yl	Me, H	1.4±0.11
12	Oxazol-2-yl	Me, Me	14.2±1.1
10	Benzoxazol-2-yl	H, H	0.15±0.03[b]
13	Benzoxazol-2-yl	Me, H	2.0±0.2
14	Benzoxazol-2-yl	Me, Me	37.1±11.2
3	Oxazolopyridin-2-yl	H, H	0.0023±0.001
15	Oxazolopyridin-2-yl	Me, H	0.0091±0.0015

[a] $K_i = 0.017 \pm 0.02$.[24]
[b] $K_i = 0.37 \pm 0.13$.[24]

keto compound **3**. This approximates that of alcohol **4** and indicates that the effect of the electrophilic carbonyl is lost with **6** and **7** and diminished with **8**.

The derivatives **11**–**15** were prepared in order to investigate the effect of substituent(s) at the 2-position of the α-keto heterocycle inhibitors. The results are summarized in Table 2. In a study of the FAAH substrate selectivity, substitution of the oleamide α-position produced a pronounced reduction in the rate of hydrolysis.[9,29] Thus, 2-methyloleamide and 2,2-dimethyloleamide were hydrolyzed more slowly than oleamide by roughly 10- and 100-fold, respectively. In a near identical fashion, the examination of **11**–**15** revealed that the monomethyl derivatives lowered the inhibitor potency roughly 10-fold, whereas the dimethyl derivatives were roughly 100-fold less potent. In the group of oxazole derivatives, **9**, its 2-methyl derivative **11**, and 2,2-dimethyl derivative **12** showed inhibitory activity with K_i's of 0.10, 1.4, and 14 μM, respectively. Similarly, the group of benzoxazole derivatives **10**, **13**, and **14** showed inhibitory activity with K_i's of 0.15, 2.0, and 37 μM, respectively. In the case of the oxazolopyridine derivatives, **15** showed reduced inhibitory activity with a K_i of 0.0091 μM. Thus, with the exception of **15** (4-fold reduction), each monomethyl derivative showed roughly a 10-fold weaker activity than the corresponding unsubstituted compounds, whereas the 2,2-dimethyl derivatives showed >100-fold weaker activity. These results parallel those made in a study of FAAH substrates and indicate that mono-substitution on the 2-position adjacent to the carbonyl group has a significant influence reducing binding affinity roughly 10-fold, whereas disubstitution is even more detrimental, presumably preventing effective active site binding an additional 10-fold.

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References and Notes

1. Boger, D. L.; Henriksen, S. J.; Cravatt, B. F. *Curr. Pharm. Des.* **1998**, *4*, 303.
2. Devane, 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.
3. Cravatt, B. F.; Prospero-Garcia, O.; Siuzdak, G.; Gilula, N. B.; Henriksen, S. J.; Boger, D. L.; Lerner, R. A. *Science* **1995**, *268*, 1506. Cravatt, B. F.; Lerner, R. A.; Boger, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 580.
4. Basile, A. S.; Hanus, L.; Mendelson, W. B. *NeuroReport* **1999**, *10*, 947.
5. Cheer, J. F.; Cadogan, A.-K.; Marsden, C. A.; Fone, K. C. F.; Kendall, D. A. *Neuropharmacology* **1999**, *38*, 533.
6. Mechoulam, R.; Fride, E.; Hanus, L.; Sheskin, T.; Bisogno, T.; Di Marzo, V.; Bayewitch, M.; Vogel, Z. *Nature (London)* **1997**, *389*, 25.
7. Huidobro-Toro, J. P.; Harris, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8078.
8. Thomas, E. A.; Carson, M. J.; Neal, M. J.; Sutcliffe, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14115.
9. Boger, D. L.; Patterson, J. E.; Jin, Q. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4102.
10. Thomas, E. A.; Cravatt, B. F.; Sutcliffe, J. G. *J. Neurochem.* **1999**, *72*, 2370.
11. Yost, C. S.; Hampson, A. J.; Leonoudakis, D.; Koblin, D. D.; Bornheim, L. M.; Gray, A. T. *Anesth. Analg.* **1998**, *86*, 1294.
12. Verdon, B.; Zheng, J.; Nicholson, R. A.; Ganellin, C. R.; Lees, G. *Br. J. Pharmacol.* **2000**, *129*, 283.
13. Nicholson, R. A.; Zheng, J.; Ganellin, C. R.; Verdon, B.; Less, G. *Anesth.* **2001**, *94*, 120.
14. Guan, X.; Cravatt, B. F.; Ehring, G. R.; Hall, J. E.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *J. Cell. Biol.* **1997**, *139*, 1785. Boger, D. L.; Patterson, J. E.; Guan, X.; Cravatt, B. F.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4810.
15. Zygmunt, P. M.; Petersson, J.; Andersson, D. A.; Chuang, H.; Sorgard, M.; Di Marzo, V.; Julius, D.; Hogestatt, E. D. *Nature (London)* **1999**, *400*, 452.
16. 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.
17. Walker, J. M.; Huang, S. M.; Strangman, N. M.; Tsou, K.; Sanudo-Pena, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12198.
18. Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature (London)* **1996**, *384*, 83.
19. Giang, D. K.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2238.
20. Thomas, E. A.; Cravatt, B. F.; Danielson, P. E.; Gilula, N. B.; Sutcliffe, J. G. *J. Neurosci. Res.* **1997**, *50*, 1047.
21. Patricelli, M. P.; Patterson, J. E.; Boger, D. L.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 613.
22. 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.
23. 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.
24. Boger, D. L.; Sato, H.; Lerner, A. E.; Hedrick, M. P.; Facik, 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*, 5044.
25. Spectroscopic data for **6**–**8**. **6**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.62 (dd, 1H, J=4.9 and 1.3 Hz), 7.86 (dd, 1H, J=8.2 and 1.3 Hz), 7.36 (dd, 1H, J=8.2 and 4.9 Hz), 5.43–5.26 (m, 2H), 3.01 (t, 2H, J=7.9 Hz), 2.08–1.92 (m, 4H), 1.79–1.62 (m, 2H), 1.50–1.17 (m, 21H), 0.87 (t, 3H, J=6.6 Hz); IR (film) ν_{max} 2922, 2852, 1546, 1466, 1416, 1262, 1142, 1060, 1002, 921, 809 cm^{-1}; FABHRMS (NBA–NaI) m/z 400.2970 ($C_{24}H_{37}N_3O_2+H^+$ requires 400.2959). **7**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.60 (dd, 1H, J=4.8 and 1.4 Hz), 7.88 (dd, 1H, J=8.2 and 1.4 Hz), 7.33 (dd, 1H, J=8.2 and 4.8 Hz), 5.43–5.26 (m, 2H), 4.15 (s, 3H), 2.94 (t, 2H, J=7.7 Hz), 2.08–1.93 (m, 4H), 1.76–1.60 (m, 2H), 1.48–1.17 (m, 20H), 0.87 (t, 3H, J=6.9 Hz); IR (film) ν_{max} 3003, 2921, 2854, 1605, 1551, 1537, 1463, 1407, 1260, 1049, 947, 901, 785 cm^{-1}; FABHRMS (NBA–NaI) m/z 414.3118 ($C_{25}H_{39}N_3O_2+H^+$ requires

414.3115). **8**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.54 (dd, 1H, J = 4.7 and 1.4 Hz), 7.82 (dd, 1H, J = 8.2 and 1.4 Hz), 7.26 (dd, 1H, J = 8.2 and 4.7 Hz), 5.45–5.26 (m, 2H), 3.01 (s, 6H), 2.97 (t, 2H, J = 8.0 Hz), 2.10–1.92 (m, 4H), 1.81–1.63 (m, 2H), 1.50–1.16 (m, 20H), 0.87 (t, 3H, J = 6.6 Hz); IR (film) ν_{max} 2921, 2854, 1532, 1455, 1404, 1260, 1127, 1091, 1029, 925, 783 cm^{-1}; FABHRMS (NBA–NaI) m/z 427.3427 ($C_{26}H_{42}N_4O + H^+$ requires 427.3431).

26. Harn, N. K.; Gramer, C. J.; Anderson, B. A. *Tetrahedron Lett*. **1995**, *36*, 9453.

27. Spectroscopic data for **11**–**14**. **11**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.82 (s, 1H), 7.33 (s, 1H), 5.41–5.25 (m, 2H), 3.61 (m, 1H), 2.08–1.77 (m, 6H), 1.40–1.19 (m, 23H), 0.87 (t, 3H, J = 6.6 Hz); IR (film) ν_{max} 2959, 2926, 2854, 1704, 1485, 1463, 1379, 1260, 1085, 1020, 984, 914, 798 cm^{-1}; FABHRMS (NBA–NaI) m/z 348.2909 ($C_{22}H_{37}NO_2 + H^+$ requires 348.2897). **12**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.76 (s, 1H), 7.31 (s, 1H), 5.41–5.23 (m, 2H), 2.09–1.87 (m, 6H), 1.38 (s, 6H), 1.38–1.10 (m, 20H), 0.87 (t, 3H, J = 6.6 Hz); IR (film) ν_{max} 2962, 2926, 2855, 1694, 1485, 1470, 1358, 1260, 1091, 1021, 915, 802 cm^{-1}; FABHRMS (NBA–NaI) m/z 362.3060 ($C_{23}H_{39}NO_2 + H^+$ requires 362.3054). **13**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.90 (br d, 1H, J = 7.3 Hz), 7.66 (br d, 1H, J = 7.6 Hz), 7.57–7.42 (m, 2H), 5.43–5.24 (m, 2H), 3.79 (m, 1H), 2.07–1.80 (m, 6H), 1.42–1.18 (m, 23H), 0.87 (t, 3H, J = 6.6 Hz); IR (film) ν_{max} 2923, 2854, 1704, 1605, 1531, 1463, 1448, 1379, 1277, 1002, 989, 934, 805, 746 cm^{-1}; FABHRMS (NBA–NaI) m/z 398.3055 ($C_{26}H_{39}NO_2 + H^+$ requires 398.3054). **14**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.89 (br d, 1H, J = 7.3 Hz), 7.65 (br d, 1H, J = 7.6 Hz), 7.55–7.40 (m, 2H), 5.40–5.20 (m, 2H), 2.11–1.87 (m, 6H), 1.46 (s, 6H), 1.37–1.13 (m, 20H), 0.87 (t, 3H, J = 6.6 Hz); IR (film) ν_{max} 2925, 2854, 1694, 1613, 1527, 1463, 1449, 1388, 1349, 1278, 987, 929, 804, 746 cm^{-1}; FABHRMS (NBA–NaI) m/z 412.3225 ($C_{27}H_{41}NO_2 + H^+$ requires 412.3210).

28. Spectroscopic data for **15**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.76 (dd, 1H, J = 4.8 and 1.5 Hz), 8.00 (dd, 1H, J = 8.4 and 1.5 Hz), 7.49 (dd, 1H, J = 8.4 and 4.8 Hz), 5.43–5.25 (m, 2H), 3.84 (m, 1H), 2.06–1.86 (m, 4H), 1.72–1.47 (m, 2H), 1.44–1.18 (m, 23H), 0.87 (t, 3H, J = 6.9 Hz); IR (film) ν_{max} 2920, 2854, 1732, 1615, 1599, 1557, 1463, 1409, 1258, 1124, 1096, 921, 785, 733 cm^{-1}; FABHRMS (NBA–NaI) m/z 399.3010 ($C_{25}H_{38}N_2O_2 + H^+$ requires 399.3006).

29. Boger, D. L.; Fecik, R. A.; Patterson, J. E.; Miyauchi, H.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2613.