Exceptionally potent inhibitors of fatty acid amide hydrolase: The enzyme responsible for degradation of endogenous oleamide and anandamide

Dale L. Boger*[†‡], Haruhiko Sato[†‡], Aaron E. Lerner[†‡], Michael P. Hedrick[†‡], Robert A. Fecik[†‡], Hiroshi Miyauchi[†‡], Gordon D. Wilkie[†‡], Bryce J. Austin[†‡], Matthew P. Patricelli[§‡], and Benjamin F. Cravatt[§‡]

[†]Departments of Chemistry, [§]Cell Biology, and [‡]The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Communicated by Julius Rebek, Jr., The Scripps Research Institute, La Jolla, CA, February 25, 2000 (received for review January 6, 2000)

The development of exceptionally potent inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of oleamide (an endogenous sleep-inducing lipid), and anandamide (an endogenous ligand for cannabinoid receptors) is detailed. The inhibitors may serve as useful tools to clarify the role of endogenous oleamide and anandamide and may prove to be useful therapeutic agents for the treatment of sleep disorders or pain. The combination of several features—an optimal C12–C8 chain length, π-unsaturation introduction at the corresponding arachidonoyl $\Delta^{8,9}/\Delta^{11,12}$ and oleoyl $\Delta^{9,10}$ location, and an α-keto N4 oxazolopyridine with incorporation of a second weakly basic nitrogen provided FAAH inhibitors with K_is that drop below 200 pM and are 10^2–10^3 times more potent than the corresponding trifluoromethyl ketones.

Oleamide (1) and anandamide are prototypical members of a class of endogenous fatty acid amides that serve as chemical messengers. Oleamide was found to accumulate in the cerebrospinal fluid under conditions of sleep deprivation (2–4) and to induce physiological sleep in animals (2). In a structurally specific manner, it modulates serotonergic systems (5–9), benzodiazepine-sensitive $GABA_A$ receptors (10), blocks glial gap junction cell–cell communication (11, 12), and exhibits the characteristic *in vivo* analgesic and cannabinoid behavioral effects of anandamide in mice, albeit without cannabinoid receptor binding (9, 13). Most exciting of its activities are its sleep-inducing properties (2, 14) where it reduces mobility, shortens the sleep induction period (14), and lengthens the time spent in slow wave sleep 2 at the expense of wakening (2). Unlike many endogenous sleep-inducing molecules and typical sleep aids that act as central nervous system (CNS) depressants, oleamide induces sleep in a manner indistinguishable from physiological sleep (2, 14). Its endogenous concentrations and temporal associations are consistent with those required of serotonergic and GABAergic neurotransmission, which may be involved in sleep induction (1, 2, 14, 15). In addition to suggesting that oleamide may play a central role in sleep, the studies indicate the potential of developing sleep aids that lack the side effects of sedatives and hypnotics and the suicide-abuse potential of CNS depressants.

Anandamide (16) is an endogenous fatty acid ethanolamide that binds to the central CB1 and peripheral CB2 cannabinoid receptors through which it is thought to exhibit its analgesic and cannabinoid effects (17–20). It blocks glial gap junction communication (11, 12, 21, 22), differentially modulates the serotonergic system (7, 23, 24), modulates sleep and memory in rats analogous to oleamide (25), and exhibits a range of biological properties (17, 26, 27). Most exciting of these properties is the demonstration that endogenous anandamide levels increase on pain stimulation, implicating its role in suppressing pain neurotransmission and in behavioral analgesia (28). Most recently, anandamide has been shown to activate the vanilloid receptor (VR1) analogous to capsaicin and olvanil (*N*-vanillyloleamide), providing what may be a common site of action for the oleamide and anandamide analgesic effects (29).

Other endogenous fatty acid primary amides and ethanolamides have been described and their biological properties defined (30, 31). These amides include erucamide (32), an angiogenic factor stimulating new blood vessel formation, and palmitoyl ethanolamide (33), which may act as an endogenous ligand for the peripheral CB2 receptor (28, 34).

Fatty acid amide hydrolase (FAAH), referred to as oleamide hydrolase and anandamide amidohydrolase in early studies, is an integral membrane protein that degrades fatty acid primary amides and ethanolamides including oleamide (35–37) and anandamide (Fig. 1) (35–44). The distribution of FAAH in the central nervous system suggests that it degrades neuromodulating fatty acid amides at their sites of action and is intimately involved in their regulation (45). FAAH hydrolyzes a wide range of oleoyl and arachidonoyl amides (46) and esters (47–50), illustrating a range of fatty acid substrates, and it appears to work most effectively on arachidonoyl and oleoyl substrates (35, 36).

Despite this important role, only a select set of FAAH inhibitors have been disclosed (51–58). These include the discovery of the endogenous inhibitor 2-octyl γ-bromoacetoacetate (51), which was reported earlier as an endogenous sleep-inducing compound (52). The remaining inhibitors consist of reversible electrophilic carbonyl inhibitors (53–55) (trifluoromethyl ketones, α-keto esters and amides, and aldehydes) or irreversible inhibitors (56–59) (sulfonyl fluorides and fluorophosphonates) incorporated into the fatty acid structures. A select set of the reversible electrophilic carbonyl FAAH inhibitors were shown to be sleep-inducing agents (1). These results, along with the demonstration that the endogenous sleep-inducing compound 2-octyl γ-bromoacetoacetate is a potent inhibitor of FAAH, suggest it may be a useful target for the development of sleep aids that act in part by preserving endogenous oleamide. Similarly, FAAH inhibitors may prove to be effective analgesics by virtue of preserving endogenous levels of oleamide and anandamide; the latter is released on pain stimulation (28).

Herein, we disclose the development of exceptionally potent α-keto heterocycle inhibitors of FAAH that are 10^2–10^3 times more potent than the corresponding trifluoromethyl ketones. Since Edwards' disclosure of α-keto heterocycles as effective protease inhibitors, a number of enzyme inhibitors have been described on the basis of analogous design principles (60–70). The inhibitors developed herein define additional features that may contribute independently to binding affinity which, when

Abbreviation: FAAH, fatty acid amide hydrolase.

*To whom reprint requests should be addressed. E-mail: boger@scripps.edu.

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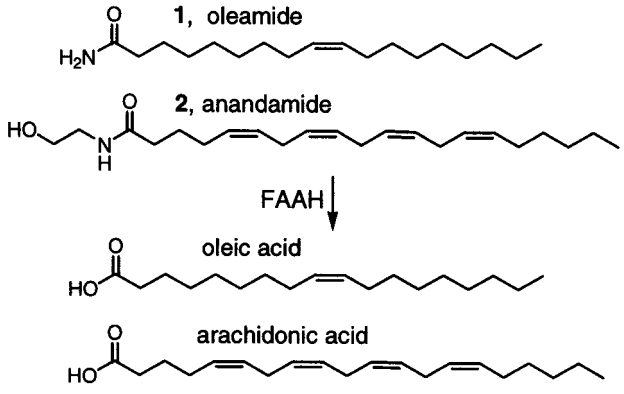
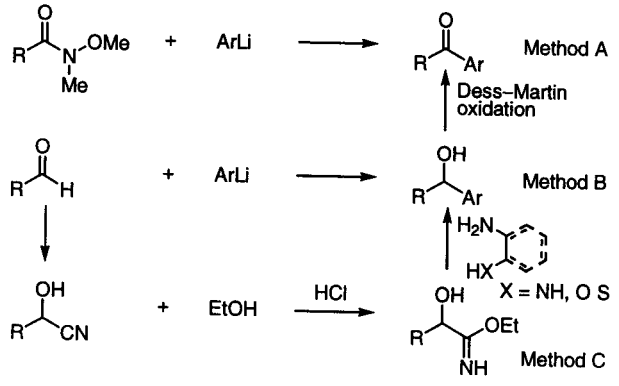


Fig. 1. FAAH substrates.

combined with the electrophilic carbonyl of the α-keto heterocycle, provides extraordinarily potent inhibitors.

Methods

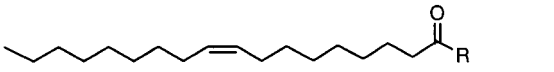
Inhibitor Synthesis. The α-keto heterocycles were prepared by addition of the heteroaryl lithium reagent to the Weinreb amide (Method A) or indirectly from the aldehyde proceeding through the α-hydroxy heterocycles followed by Dess–Martin oxidation via addition of the heteroaryl lithium reagent (Method B) or by cyanohydrin formation, acid-catalyzed conversion to the imidate (HCl–EtOH, $CHCl_3$), and condensation with a 2-aminoalcohol, 2-aminoaniline, 2-aminophenol, or *o*-amino-hydroxypyridine (Method C, Scheme 1). Full details are provided in supplemental data (see www.pnas.org).

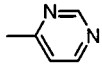
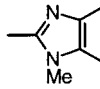
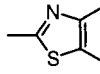


Scheme 1.

Inhibition Studies. All enzyme assays were performed at 20–23°C by using either solubilized rat liver plasma membrane extracts (54) or solubilized COS-7 membrane extracts from cells transiently transfected with human FAAH cDNA (36) 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 (51). The initial rates of hydrolysis (≤10–20% reaction) were monitored by following the breakdown of ^{14}C-oleamide to oleic acid as described (2, 51). 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. IC_{50} values were determined from the inhibition observed at three to five different inhibitor concentrations (from three or more trials at each inhibitor concentration) by using the formula $IC_{50} = [I]/[(\upsilon_0/\upsilon_i)-1]$ (71), where υ_0 is the control reaction rate without inhibitor and υ_i is the rate with inhibitor at concentration [I]. K_i values were determined 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 $K_i = -x_{int}/[1 + [S]/K_m]$).

Table 1. α-Keto heterocycle inhibitors of FAAH



Compound	R	K_i, μM	Compound	R	K_i, μM
3	CF_3	0.082 ±0.012		CH_3	> 100
4	H	8.5 ±2.5	**5**	CH_2Br	1.0 ±0.2
6	CO_2Et	0.50 ±0.02	**7**	$CONH_2$	0.90 ±0.1
8		> 100	**15**		> 100
9		> 100	**16**		> 100
10		0.017 ±0.002	**17**		0.13 ±0.02
11		4.5 ±1.5			
12		9.8 ±2.8	**18**		0.11 ±0.02
13		3.7 ±0.9	**19**		0.54 ±0.14
14		0.065 ±0.02	**20**		2.5 ±0.2
21		> 100	**22**		> 100
23		0.37 ±0.13	**24**		> 100

- Potency approaches that of trifluoromethyl ketone.
- Potency increases with additional basic nitrogen.

Results

Nature of the Heterocycle. A range of five- and six-membered monocyclic heterocycles and the three most prevalent bicyclic heterocycles (benzthiazole, benzimidazole, and benzoxazole) were incorporated into the oleoyl α-keto heterocycles **8–24**. The results of their examination are summarized in Table 1 along with the comparison data for the trifluoromethyl ketone **3** and the related inhibitors **4–7** (54). The inhibitors contain the oleyl chain possessing a 9-*Z* double bond and a carbonyl at the site of the oleamide carboxamide and adjacent to the electron-deficient heterocycle. Although many of the inhibitors were more potent than oleyl aldehyde (**4**) and comparable to the α-keto ester **6** and carboxamide **7**, only two (**14** and **10**) matched the potency of the trifluoromethyl ketone **3**. Many of the observations made by Edwards on the relative potencies of α-keto heterocycles against elastase were also observed with FAAH. These observations include the unique potency of the benzoxazole vs. benzthiazole and benzimidazole, the more potent activity of the oxazole **10** vs. the thiazole or imidazole, and the substantially more potent behavior of the 2-methyl vs. 1-methyl tetrazoles **14** and **13**. In contrast to the observations of Edwards and unique to the studies with FAAH, the oxazole **10** proved substantially more

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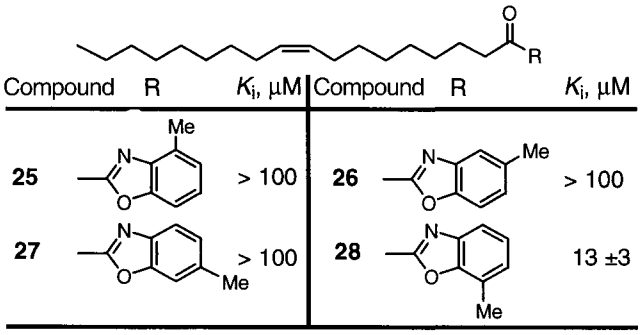
Table 2. Substituted α-keto benzoxazole inhibitors of FAAH



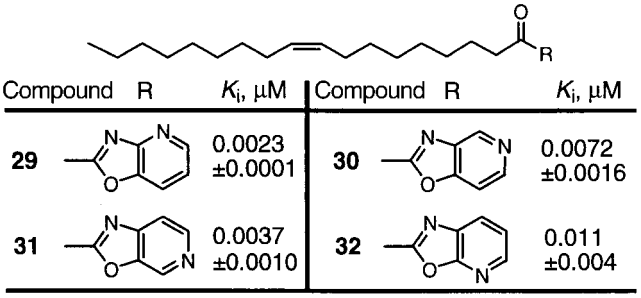
- Sensitive to steric interactions surrounding active site.
- Defines limits to depth and width of FAAH active site.

potent than the oxazoline **11**, and the six-membered heterocycles containing two nitrogen atoms, one of which remains weakly basic (**17–19** vs. **20**), were unusually potent, exceeding the activity of the α-keto ester and carboxamide **6** and **7** and approaching that of trifluoromethyl ketone **3.** Although there are many potential explanations for this behavior, one that proved consistent with subsequent observations is the enhancement of the inhibitor potency by incorporation of a weakly basic nitrogen.

Steric Requirements Surrounding the Benzoxazole. The benzoxazole **23** was chosen for further examination because it provided the greatest opportunity for functionalization. The 4-, 5-, 6-, and 7-methylbenzoxazoles were prepared to define sites available for functionalization without adversely affecting the inhibitor potency (Table 2). Substitution of any available position on the benzoxazole results in a greatly diminished (**28**) or complete loss of activity (**25–27**). This behavior defines precise limits to the size and depth of the FAAH active site, which in turn has implications for its substrate specificity or selectivity.

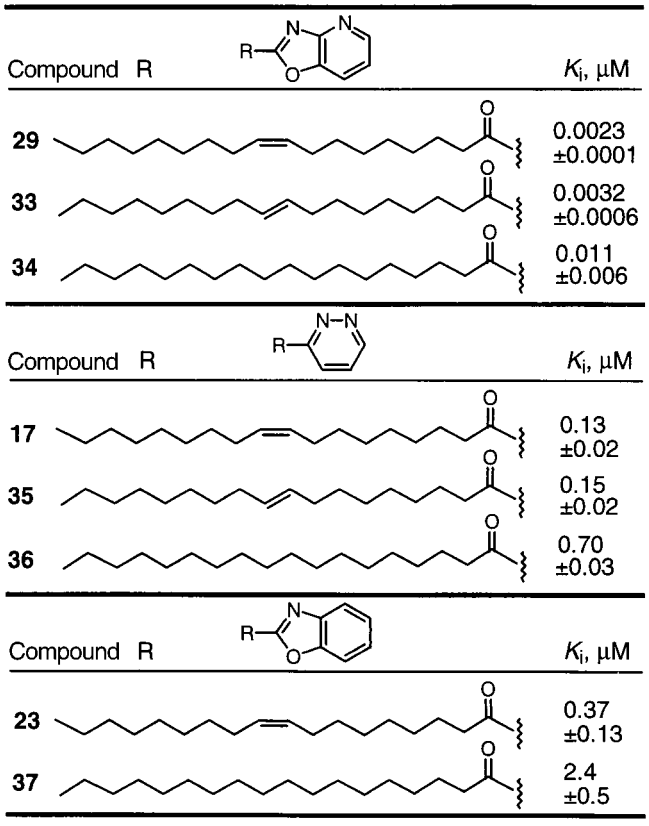
Oxazolopyridines: Incorporation of Nitrogen into the Benzoxazole. On the basis of the observation that incorporation of an additional basic nitrogen seemed to correlate with enhanced inhibitor potency, the four oxazolopyridines **29–32** were examined and were found to be more potent inhibitors (Table 3). The introduction of a nitrogen into the benzoxazole enhanced the potency 50–200 times, providing inhibitors that are 10–50 times more potent than the trifluoromethyl ketone **3**. Although N4 incorporation provided the most potent inhibitor **29**, N5–N7 incorporation also provided effective inhibitors (N4 > N6 > N5 > N7), and there is only a 4- to 5-fold difference in the most and least potent agent in the series. Although it is tempting to invoke

Table 3. α-Keto oxazolopyridine inhibitors of FAAH



- Potency increases with introduction of basic nitrogen.
- Potency increases *ca.* × 200 and N4 > N6 > N5 > N7.
- Relatively insensitive to location of additional nitrogen.

Table 4. Impact of double bond in the C18 α-keto heterocycle inhibitors of FAAH



- C18 $\Delta^{9,10}$: *Z* (cis) > *E* (trans) > saturated.

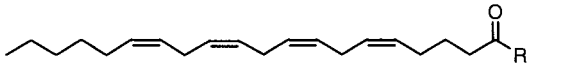
an active-site dual interaction of a single residue with N3 and N4, the comparable activity of **29–32** suggests the interaction of the second nitrogen is more flexible.

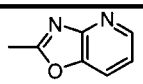
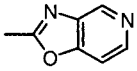
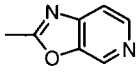
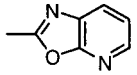
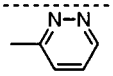
Impact of the Double Bond. The importance of the oleyl double bond was examined with three of the initial potent inhibitors (Table 4). Identical to observations made with the trifluoromethyl ketone and α-keto ester inhibitors (54, 55), **29** and **17** containing the cis double bond were more potent than **33** and **35,** respectively, containing the trans double bond, which in turn were more potent than **34** and **36** in which the double bond was removed. Similarly, **23** was more potent than **37**.

Arachidonyl-Based Inhibitors. Because two of the best substrates for FAAH are arachidonamide and oleamide (36), we examined five of the potent α-keto heterocycles incorporated into the arachidonoyl skeleton (Table 5). In each instance, the inhibitors were unstable and decomposed rapidly under typical working conditions. Several proved too unstable to purification to assess accurately their inhibitor potency, and that of **40** could only be approximated (about 50% purity). Where this could be assessed accurately, the arachidonoyl inhibitors were two to five times more potent than the oleoyl-based inhibitors. Despite this enhancement, which is consistent with the FAAH substrate preference for arachidonamide vs. oleamide (relative rate of hydrolysis, 1:0.7), their instability precludes effective utility.

In studies with conformationally restricted trifluoromethyl ketone inhibitors, a well-defined trend favoring a bound bent, but not hairpin, conformation was observed and defined the shape characteristics of the active site (55). The enhanced potency of the arachidonoyl-based inhibitors is likely to be

Table 5. Arachidonyl-based α-keto heterocycle FAAH inhibitors



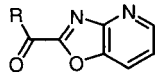
Compound	R	K_i, μM	Compound	R	K_i, μM
38		0.001 ±0.0002	**39**		0.002 ±0.001
40		ca. 0.018 unstable	-		unstable
41		0.047 ±0.017			

- Potency: arachidonyl > oleyl inhibitors (×2–5).
- Stability: oleyl >> arachidonyl inhibitors.

related to this shape characteristic of the FAAH active site and their enhanced preference for adoption of the required bound conformation.

The Fatty Acid Chain. Well-behaved trends were observed with modifications in the fatty acid chain (Table 6). A very well-defined effect of the chain length was observed, and the greatest potency was found with saturated straight chain lengths of C12–C8. This is a chain length that terminates at the location of the $\Delta^{9,10}$ double bond of oleamide, and the $\Delta^{8,9}/\Delta^{11,12}$ double bonds of arachidonamide, and corresponds to the location of the bend in the bound conformation identified in studies with

Table 6. Modifications in the fatty acid side chain of α-keto heterocycle inhibitors of FAAH



Compound	R	K_i, μM	Compound	R	K_i, μM
34	$CH_3(CH_2)_{16}$	0.011 ±0.006	**51**	$Ph(CH_2)_3$	0.0069 ±0.0010
42	$CH_3(CH_2)_{14}$	0.0019 ±0.0002	**52**	$Ph(CH_2)_4$	0.00030 ±0.00009
43	$CH_3(CH_2)_{12}$	0.0017 ±0.0009	**53**	$Ph(CH_2)_5$	0.00020 ±0.00005
44	$CH_3(CH_2)_{10}$	0.00057 ±0.00024	**54**	$Ph(CH_2)_6$	0.00028 ±0.00020
45	$CH_3(CH_2)_8$	0.00075 ±0.00017	**55**	$Ph(CH_2)_7$	0.00039 ±0.00006
46	$CH_3(CH_2)_6$	0.00069 ±0.00015	**56**	$Ph(CH_2)_8$	0.00052 ±0.00018
47	$CH_3(CH_2)_5$	0.0021 ±0.0003			
48	$CH_3(CH_2)_4$	0.015 ±0.002			
49	$CH_3(CH_2)_3$	0.050 ±0.009			
50	CH_3	> 100			

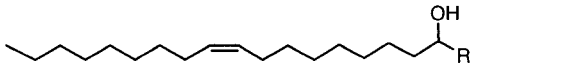
- C18 < C16 < C14 < C12–C8 > C7 > C6 > C5 > C2

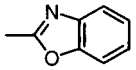
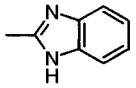
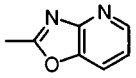
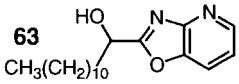
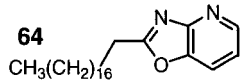
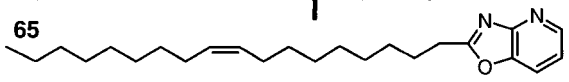
K_i = 200 pM

- $Ph(CH_2)_3 < Ph(CH_2)_4 < Ph(CH_2)_5 > Ph(CH_2)_6 > Ph(CH_2)_7 > Ph(CH_2)_8 >$ C1–C18

57	$CH_2{=}CH(CH_2)_7$	0.00015 ±0.00001	**58**	$HC{\equiv}C(CH_2)_7$	0.00018 ±0.00002
59		0.00014 ±0.00002			

Table 7. Comparison with α-hydroxy heterocycle FAAH inhibitors



Compound	R	K_i, μM	Compound	R	K_i, μM
60		> 100	**61**		> 100
62		1.8 ±0.4			
63		1.2 ±0.2	**64**		> 100
65					> 100

- Ketone >> alcohol (×10^3) >> alkane (×10^5).

trifluoromethyl ketone inhibitors (55). The inhibitor potency progressively increased as the chain length was shortened from C18 to C12 (K_i, 11 → 0.6 nM), leveled off at C12–C8 with subnanomolar K_is (0.57–0.73 nM), and subsequently diminished sharply as the chain length was shortened from C8 to C2, ultimately providing inactive inhibitors (K_i = 0.7 → >100,000 nM). This behavior indicates that each of the first C1–C8 carbons in the chain contributes significantly to inhibitor and substrate binding and that C10–C12 contribute nominally to binding. More importantly, these results indicate that the terminal carbons of the longer C14–C18 inhibitors may actually diminish inhibitor binding affinity relative to the substrate or may not be involved in substrate binding.

Incorporating unsaturation into the fatty acid chain increases inhibitor potency (Table 6). The incorporation of a benzene ring provided inhibitors with subnanomolar K_is with the most potent inhibitor **53** possessing a K_i of 200 pM. Again, the inhibitors exhibited a well defined trend, with the optimal C5 methylene spacer corresponding to incorporation of both the $\Delta^{9,10}$ and $\Delta^{8,9}/\Delta^{11,12}$ double bonds of oleamide and anandamide. This extraordinary potency was observed with the structurally simple inhibitors **51–56** amendable to further modification. These observations, like those of the straight chain inhibitors **42–50**, are analogous to those made with a series of trifluoromethyl ketones (55). The distinction is that the α-keto oxazolopyridines are 10^2–10^3 times more potent than the corresponding trifluoromethyl ketones.

Even more significantly, introduction of a double or triple bond at the oleamide $\Delta^{9,10}$ position provided even more potent inhibitors, **57** and **58** with K_is of 150 and 180 pM, respectively. Thus, **57** proved to be 5 times more potent than **45**, precisely following the trends seen with **29** vs. **34** (×5), **17** vs. **36** (×5), and **23** vs. **37** (×6) (Table 4). Remarkably, the incorporation of a $\Delta^{9,10}$ triple bond into the oleoyl-based inhibitor **59** provided the most potent inhibitor observed to date, K_i 140 pM.

The Electrophilic Carbonyl. Key to the design of the inhibitors was the electrophilic carbonyl, which is required for potent enzyme inhibition. A set of the α-hydroxy precursors to the initial inhibitors were examined and typically proved inactive as FAAH inhibitors (Table 7). Significantly, the α-hydroxy precursors **62** and **63** to the potent α-keto oxazolopyridines **29** and **44** retained significant FAAH inhibition with K_is of 1.8 and 1.2 μM, respectively. Although this is 10^3 times less potent than the corresponding keto derivative, they approximate the potency of the

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Table 8. Inhibition of recombinant human FAAH

Compound	K_i, μM (human)	K_i, μM (rat)
23	0.073	0.37
29	0.0013	0.0023
53	0.000094	0.00020

• Relative and absolute potencies against rat and human FAAH not distinguishable.

initial series of α-keto heterocycles and **4–7** (Table 1). This behavior indicates that the pyridine nitrogen of the N4 oxazolopyridine, and presumably that of the N5–N7 oxazolopyridines, in conjunction with the α-hydroxy group contributes substantially to FAAH active site binding independent of the contributions of the electrophilic carbonyl. The agents **64** and **65** lacking the α-hydroxy groups were inactive, losing at least an additional 10^2-fold binding affinity with removal of the alcohol or at least 10^5-fold binding affinities with respect to removal of the keto group.

Notably, the α-keto heterocycles do not exist predominantly in the hydrated state. Thus, **16**, **17**, **21**, and **23** showed no detectable hydrate or hemiacetal formation in CD_3OD, 7% D_2O–acetone-d_6, or 5% D_2O–DMSO-d_6, **29** exhibited only 2–4% hydrate or 9% hemiacetal (CD_3OD) formation (^1H NMR), and **63** showed no hydrate formation (10% D_2O in acetone-d_6 or DMSO-d_6) and slow time-dependent hemiacetal formation (10–20%, 1–24 h, CD_3OD). Under identical conditions, the trifluoromethyl ketone **3** was completely (CD_3OD) or predominately hydrated (>90%, 7% D_2O–acetone-d_6) (54).

Inhibition of Human FAAH. Previous work demonstrated the rat (35) and human enzyme (36) are very homologous (84% sequence identity), exhibit near identical substrate specificities, and incorporate an identical amidase signature sequence and SH3-binding domain suggesting the observations made with rat FAAH will be similar to those of human FAAH. The additive design principles elucidated with rat liver FAAH were also observed for recombinant human FAAH (Table 8). Addition of a weakly basic N4 nitrogen into the benzoxazole ring led to an increase in potency of nearly 100-fold for compound **29** over **23**. Incorporation of a shortened alkyl chain with π unsaturation (**53**) increased potency by another order of magnitude and resulted in an inhibitor with a K_i of less than 100 pM. Overall, the relative and absolute potencies of these inhibitors against human FAAH were very similar to rat liver FAAH.

Discussion. A potent class of competitive inhibitors of FAAH was developed on the basis of complementary binding interactions provided by the electrophilic carbonyl of an α-keto heterocycle and that of heterocycles (oxazolopyridines) incorporating a weakly basic nitrogen. FAAH belongs to a new class of amidases that has not been extensively studied and possess a distinct combination of active site residues involved in catalysis. Mutagenesis studies have characterized FAAH as a possible serine–lysine dyad amidase that lacks a participating active site histidine (72). It utilizes a serine nucleophile (Ser-241) and incorporates two additional active site serines (Ser-217 and -218) that enhance catalysis (73). Key to its enhanced amide vs. ester bond cleavage is its enlistment of Lys-142 with an apparently perturbed p*K*a (7.8) as a base for Ser-241 deprotonation and for subsequent protonation of the amine leaving group (72). It is possible that the impact of the second weakly basic nitrogen of the oxazolopyridines is derived from hydrogen bonding to one or more of these active site residues and that the positioning of this residue is sufficiently flexible to interact with a weakly basic nitrogen in a range of locations.

Well-defined relationships were observed in the development of the inhibitors. Several oleyl α-keto heterocycles exhibit FAAH inhibition comparable to the corresponding α-keto ester and carboxamide. The more potent include six-membered heterocycles incorporating a second weakly basic nitrogen as well as benzoxazole. Substitution at any of the available sites on the α-keto benzoxazole inhibitor eliminated activity, defining limits to the depth and width of the FAAH active site. Incorporation of an additional basic nitrogen into the benzoxazole providing the four isomeric oxazolopyridines (N4–N7) afforded exceptionally potent inhibitors 50–200 times more active than the benzoxazole and 8–40 times more active than the corresponding trifluoromethyl ketone. Arachidonoyl-based inhibitors were found to be two to three times more potent than the oleoyl-based inhibitors, consistent with the relative rates of FAAH hydrolysis of arachidonamide vs. oleamide, but are sufficiently unstable so as to preclude their use. The removal of the oleoyl $\Delta^{9,10}$ cis double bond or the incorporation of a trans olefin reduced inhibitor potency consistent with prior observations (54, 55). The inhibitor potency exhibited a smooth dependency on the fatty acid chain length, C18 < C16 < C14 < C12–C8 > C7 > C6 > C5 > C2, exhibiting the maximum potency at C12–C8, which corresponds to the location of the oleoyl $\Delta^{9,10}$ cis double bond and the arachidonoyl $\Delta^{8,9}/\Delta^{11,12}$ double bonds. This optimal length appears to correspond to the location of a bend, but not hairpin conformation, in the bound conformation identified with conformationally restrained inhibitors (55). Incorporation of π-unsaturation into the medium length (C12–C8) inhibitors at the sites of oleoyl or arachidonoyl unsaturation further enhances the inhibitor potency, and this may be accomplished with incorporation of a double bond, triple bond, or phenyl ring. The combination of these features: C8–C12 chain length, π unsaturation incorporation at the arachidonoyl $\Delta^{8,9}/\Delta^{11,12}$ and oleoyl $\Delta^{9,10}$ location, and an α-keto N4-oxazolopyridine provides inhibitors with potencies that drop below K_is of 200 pM and are 10^2–10^3 times more potent than the corresponding trifluoromethyl ketones. With these inhibitors, removal of the keto group reduces potency $>10^5$ times, and its reduction to an alcohol reduces potency 10^3 times. Although the α-hydroxy oxazolopyridines are 10^3 times less potent than the corresponding ketones, they exhibit inhibition comparable to many of the initial α-keto heterocycle or related α-keto ester and carboxamide inhibitors.

It is likely that similar design principles may be explored with α-keto heterocycle protease inhibitors that will extend their potencies beyond that achieved by introduction of the electrophilic carbonyl. The *in vivo* properties of the inhibitors detailed herein and their effects on the oleamide and anandamide sites of action are under investigation and will be disclosed in due time. Such inhibitors should prove to be useful tools to probe FAAH and related amidase mechanisms of catalysis and the biological role of oleamide and anandamide and may prove useful therapeutic agents in their own right with applications as sleep aids or analgesics that act by preserving endogenous levels of oleamide and anandamide.

We gratefully acknowledge the financial support of the Skaggs Institute for Chemical Biology, National Institutes of Health (CA42056, D.L.B.; MH58542, B.F.C.), fellowships for R.A.F. (American Cancer Society 576211) and M.P.P. (National Science Foundation), and the postdoctoral sabbatical leaves of H.S. and H.M., sponsored by Chugai Pharmaceutical and Sumitomo Pharmaceutical, respectively. Supplemental data: full experimental details for the inhibitor syntheses, full characterization of all inhibitors and new intermediates, and a Lineweaver–Burke analysis of **53** are provided (see www.pnas.org).

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