Optimization of α-Ketooxazole Inhibitors of Fatty Acid Amide Hydrolase

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A series of α-ketooxazoles containing conformational constraints in the flexible C2 acyl side chain of **2** (OL-135) and representative oxazole C5 substituents were prepared and examined as inhibitors of fatty acid amide hydrolase (FAAH). Exceptionally potent and selective FAAH inhibitors emerged from the series (e.g., **6**, K_i = 200 and 260 pM for rat and rhFAAH). With simple and small C5 oxazole substituents, each series bearing a biphenylethyl, phenoxyphenethyl, or (phenoxymethyl)phenethyl C2 side chain was found to follow a well-defined linear relationship between $-\log K_i$ and Hammett σ_p of a magnitude (ρ = 2.7–3.0) that indicates that the substituent electronic effect dominates, confirming its fundamental importance to the series and further establishing its predictive value. Just as significantly, the nature of the C5 oxazole substituent substantially impacts the selectivity of the inhibitors whereas the effect of the C2 acyl chain was more subtle but still significant even in the small series examined. Combination of these independent features, which display generalized trends across a range of inhibitor series, simultaneously improves FAAH potency and selectivity and can provide exquisitely selective and potent FAAH inhibitors.

Fatty acid amide hydrolase (FAAH[a])[1,2] is an enzyme that serves as the catabolic regulator[3,4] of several endogenous lipid amides[5,6] including anandamide (**1a**)[7–10] and oleamide (**1b**)[11–13] (Figure 1). Its distribution is consistent with its role of regulating (degrading) such neuromodulating and 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 are a number of prokaryotic enzymes, it is currently the only characterized mammalian enzyme bearing the family's unusual Ser-Ser-Lys catalytic triad.[1,2,14–17]

Because of the therapeutic potential of inhibiting FAAH[3,17–20] especially for the treatment of pain,[21–23] inflammatory,[24] or sleep disorders[13,25] and with the emerging concerns surrounding alternative targets (e.g., COX-2 inhibitors), there has been an increasing interest in the development of potent inhibitors of this enzyme.[26] Early studies led to the discovery that the endogenous sleep-inducing molecule 2-octyl α-bromoacetoacetate is an effective FAAH inhibitor[27] and to the disclosure of a series of nonselective reversible inhibitors bearing an electrophilic ketone (e.g., trifluoromethyl ketone based inhibitors),[28–31] as well as a set of irreversible inhibitors[32–37] (e.g., fluorophosphonates and sulfonyl fluorides). To date, only two classes of inhibitors have been disclosed that provide significant opportunities for the development of inhibitors with therapeutic potential. One class is the reactive aryl carbamates and ureas[38–50] that irreversibly acylate a FAAH active site serine[49] and that have been shown to exhibit anxiolytic activity[38] and produce analgesic effects.[39] To date and where examined, the selectivity of such inhibitors has often been low,[31,48–50] further complicating the development of inhibitors that irreversibly and covalently modify the target enzyme. A second class is the α-ketoheterocycle-based inhibitors[51–59] that bind to FAAH via reversible hemiketal formation with an active site serine. Not only are these competitive inhibitors 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.[58,59]

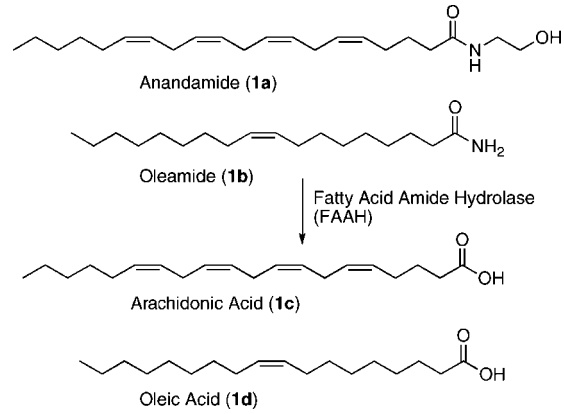

Figure 1. Substrates of fatty acid amide hydrolase (FAAH).

In these preceding studies, **2**[53] emerged as an important lead inhibitor for further study (Figure 2). It has been shown that **2** is a potent (K_i = 4.7 nM)[53] and selective (>100- to 300-fold)[31] FAAH inhibitor that induces analgesia by raising endogenous anandamide levels.[53,58,59] It has been shown to exhibit analgesic activity in the tail flick assay,[58] hot plate assay,[58] formalin test of noxious chemical pain (first and second phases),[58] the mild thermal injury (MTI) model of peripheral pain,[59] and the spinal nerve ligation (SNL) model of neuropathic pain[59] with efficacies that match or exceed those of morphine (at 1–3 mg/kg in MTI/SNL), ibuprofen (at 100 mg/kg in MTI), or gabapentin (at 500 mg/kg in SNL) and at administered doses (10–20 mg/kg, ip) that approach or exceed those of such common pain medications.[59] It was shown to lack significant off-site target activity (Cerep assay profiling), does not bind cannabinoid (CB1 or CB2) or vanilloid (TRP) receptors, and does not significantly inhibit

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[a] Abbreviations: CB1, cannabinoid 1 receptor; CB2, cannabinoid 2 receptor; EDTA, ethylenediaminetetraacetic acid; EDCI, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride; FAAH, fatty acid amide hydrolase; MTI, mild thermal injury; SAR, structure–activity relationship; SNL, spinal nerve ligation; TGH, triacylglycerol hydrolase; TRP, vanilloid receptors.

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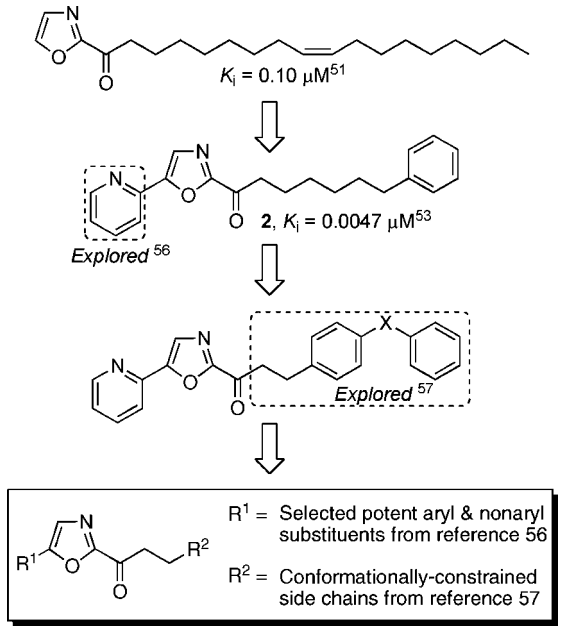


Figure 2. Progression of the inhibitor series.

common P450 metabolism enzymes (3A4, 2C9, 2D6) or the human ether-a-go-go related gene (hERG). Significantly, the inhibitor was ineffective at promoting analgesia in FAAH knockout mice,[58,59] verifying that FAAH is the only relevant target responsible for the in vivo analgesic effects of **2**. Moreover, the analgesic effects were observed without the respiratory depression or chronic dosing desensitization characteristic of opioid administration[59] or the increased feeding and decreased mobility and motor control characteristic of a cannabinoid (CB1) agonist administration.[58,59]

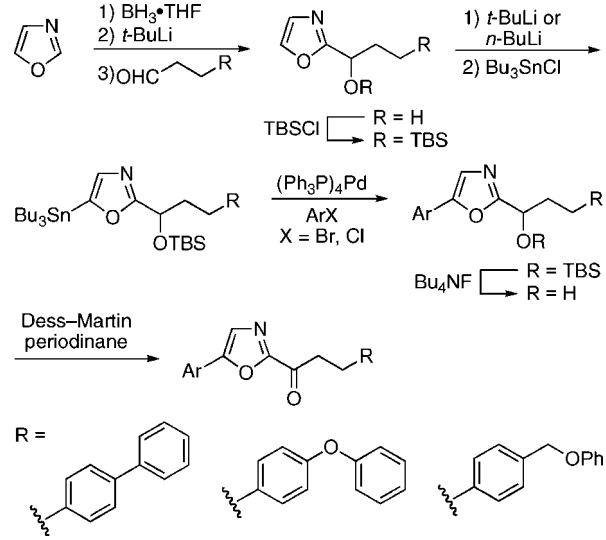
Consequently, we have conducted systematic structure–activity relationship (SAR) studies on **2** independently targeting the 5-position of the central oxazole (aryl and nonaromatic substituents)[53,55,56] and the C2 acyl side chain,[53,57] both of which have provided extraordinarily potent and selective FAAH inhibitors (Figure 2).[60] Herein, we report results of studies examining candidate inhibitors formed by combining the optimized or most representative C5 oxazole substituents with optimized C2 acyl side chains along with results of the proteome-wide selectivity screening[31] of the resulting candidate inhibitors.

Chemistry

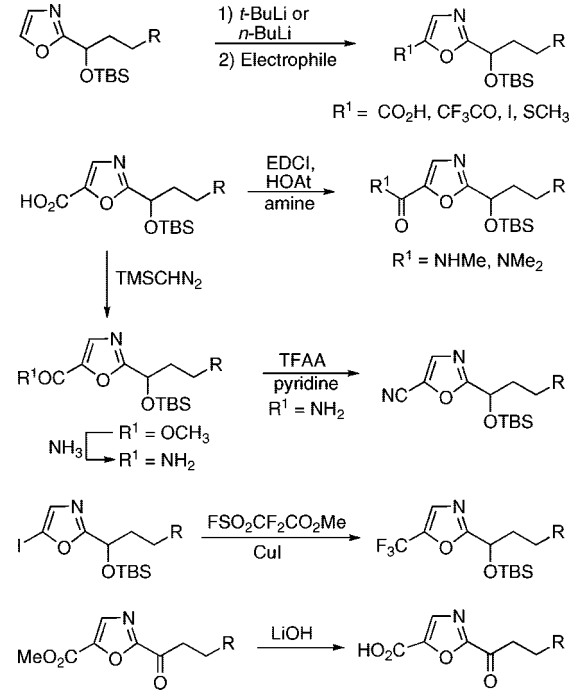
The general method for the synthesis of the C5 aryl inhibitors containing a conformationally restricted C2 acyl side chain is shown in Scheme 1. Vedejs oxazole metalation[61] and condensation with the appropriate side chain aldehyde were followed by TBS protection of the resulting alcohol. Selective C5 oxazole lithiation[62] of these intermediates followed by treatment with Bu_3SnCl afforded the corresponding stannanes. Stille coupling[63] of the stannanes with the arylpyridine halides produced the substituted oxazoles, which could be readily converted to the corresponding ketones via TBS deprotection (Bu_4NF) and oxidation of the liberated alcohol with Dess−Martin periodinane.[64]

The synthesis of the candidate inhibitors that bear a nonaromatic C5 oxazole substituent is summarized in Scheme 2. Following the analogous C5 oxazole lithiation, treatment with various electrophiles (CO_2(g), $CF_3CON(CH_3)_2$, I_2, dimethyl disulfide) provided the corresponding C5 substituted oxazoles

Scheme 1



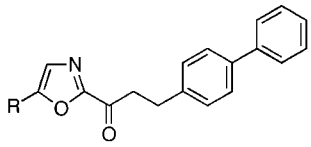
Scheme 2



in good conversions. In each case, deprotection of the TBS ether followed by Dess−Martin periodinane oxidation of the liberated alcohol yielded the corresponding α-ketooxazoles. Many such substitution products served as intermediates en route to additional key inhibitors. The C5 carboxylic acids were directly converted to corresponding amides (RNH_2 or R_2NH, EDCI, and HOAt) as well as to their corresponding methyl esters by treatment with $TMSCHN_2$. In turn, the esters were converted to the carboxamides by treatment with methanolic ammonia and the carboxamides were dehydrated with TFAA and pyridine to provide the C5 nitriles. Similarly, the C5 iodides were transformed ($FSO_2CF_2CO_2CH_3$, CuI) to candidate inhibitors bearing a C5 trifluoromethyl substituent.[65,66]

Enzyme Assay

Enzyme assays were performed at 20–23 °C with purified recombinant rat FAAH expressed in *Escherichia coli*[67] (unless



compd	R	K_i, μM	compd	R	K_i, μM
3	–H	0.016	**9**	$-CONH_2$	0.0035
4	–I	0.004	**10**	$-CONMe_2$	0.003
5	$-CF_3$	0.0007	**11**	$-COCF_3$	0.008*
6	–CN	0.0002	**12**	$-SO_2CH_3$	0.0012
7	$-CO_2H$	0.010	**13**	$-SCH_3$	0.003
8	$-CO_2Me$	0.0015			

*binds as gem diol

Figure 3. Effect of nonaromatic substituents.

indicated otherwise) or with solubilized COS-7 membrane extracts from cells transiently transfected with human FAAH cDNA[2] (where specifically indicated) in a buffer of 125 mM Tris/1 mM EDTA/0.2% glycerol/0.02% Triton X-100/0.4 mM Hepes, pH 9.0. The initial rates of hydrolysis (<10–20% reaction) were monitored using enzyme concentrations (typically 1 nM) at least 3 times below the measured K_i by following the breakdown of ^{14}C-oleamide, and K_i values (standard deviations are provided in the Supporting Information tables) were established as described (Dixon plot). Lineweaver–Burk analysis previously established reversible, competitive inhibition for **2** and related inhibitors.[53]

Results and Discussion

Many of the most potent inhibitors that emerged from previous SAR studies of **2** contained conformational constraints in the flexible C2 acyl side chain removing many of its rotatable bonds and introducing additional π-unsaturation. This portion of the inhibitors binds in a hydrophobic channel of the FAAH active site reserved for the unsaturated lipid chain of the fatty acid amide. Consequently, both their hydrophobic character and π-unsaturation mimic the nature and location of the fatty acid chain and its unsaturation (arachidonyl $\Delta^{5,6}$, $\Delta^{8,9}$, and $\Delta^{11,12}$ double bonds or oleyl $\Delta^{9,10}$ double bond) and the added conformational restriction may contribute to their enhanced binding affinity while simultaneously improving their drug-like characteristics. Three such improved C2 side chains identified in these studies were now incorporated into candidate inhibitors that contain representative or optimized C5 oxazole aryl and nonaryl substituents that impact FAAH potency, selectivity, and physical properties (e.g., solubility).

Biphenyl Series. The series most extensively examined contained the C2 biphenylethyl side chain. A set of candidate inhibitors bearing representative small nonaryl C5 oxazole substituents were examined and found to follow a well-defined correlation[55] between the electronic character of the substituent and the inhibitor potency (Figure 3). Thus, the inhibitor potency was found to smoothly increase as the electron-withdrawing character of the substituent increased. As observed in an earlier study,[55] the magnitude of the effect is remarkably large and a plot of the substituent Hammett σ_p constant versus $-\log K_i$ provided a ρ value of 2.70 (slope = ρ, $R^2 = 0.97$), indicating that a unit change in σ_p leads to an increase in the K_i of nearly 1000-fold (Figure 4). As such and although such substituents may engage in additional more subtle interactions at the enzyme active site (H-bonding or hydrophobic interactions), the magnitude of electronic effect indicates that the substituent electronic character dominates the behavior of this series of relatively small substituents. Presumably this reflects the electronic effect of the

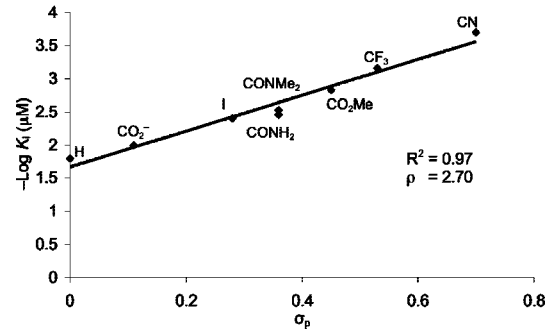
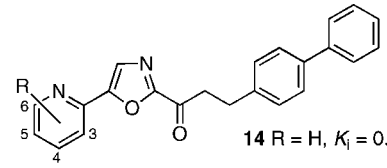


Figure 4. Plot of $-\log K_i$ (μM) versus σ_p for biphenyl series.



14 R = H, K_i = 0.00075 μM

compd	R	K_i, μM	compd	R	K_i, μM
15	6-CO_2Me	0.0012	**20**	6-$CONH_2$	0.0012
16	6-CO_2Et	0.003	**21**	5-$CONH_2$	0.0013
17	6-CO_2H	0.022	**22**	6-CN	0.0029
18	5-CO_2Me	0.0022	**23**	6-tetrazole	0.025
19	5-CO_2H	0.014			

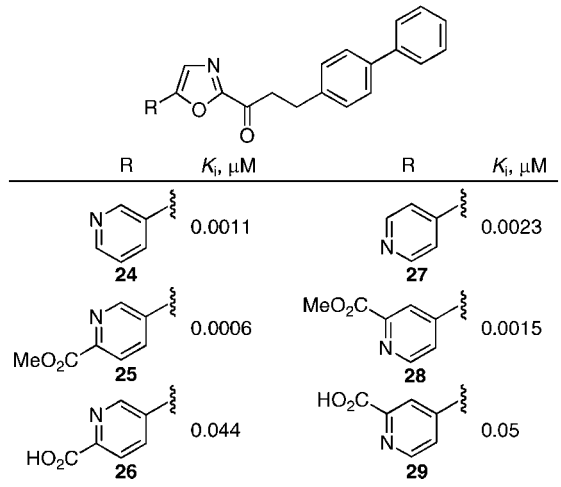
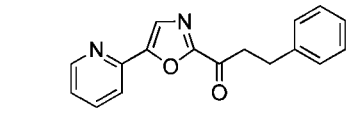


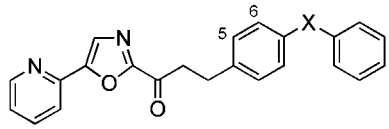
Figure 5. Effect of pyridyl derivatives.

substituent on activating the electrophilic carbonyl toward nucleophilic attack by the FAAH active site catalytic Ser.

A series of aryl C5 oxazole substituents were also examined including a key set of substituted 2-pyridyl derivatives (Figure 5). Preceding studies have shown that such substituents exhibit well defined potency trends (2-Pyr > 3-Pyr > 4-Pyr > Ph) that correlate with the location and H-bond acceptor capabilities of the weakly basic heterocycle substituent. Although this has been discussed in detail elsewhere,[53] the potency of such 2-Pyr substituents exceeds that predicted by their intrinsic electron-withdrawing properties further benefiting from a key H-bonding interaction at the enzyme active site.[56,68] Consistent with prior SAR observations with **2**,[53] the inhibitor potencies followed the order **14** > **24** > **27** (2-Pyr > 3-Pyr > 4-Pyr), suggesting that the extensive heterocycle series explored with **2**[51,53,56] would provide analogous, albeit more potent, inhibitors in this biphenylethyl series with **14** being among the most potent. Similarly, the addition of electron-withdrawing and water-solubilizing substituents weakly modulates the potency of such inhibitors (**15**–**23** vs **14**) and many (e.g., $-CO_2H$ and 2-tetrazole) further



K_i = 0.20 μM[53]



X	K_i, μM	X	K_i, μM
CH_2O	0.0010	CH_2	0.0032
O	0.0034	S	0.0022
NH	0.002	OCH_2	0.0013
biphenyl derivative →		–	0.00075

Figure 6. Conformational restriction in the C2 side chain.

enhance the intrinsic FAAH selectivity. Several such inhibitors exhibit subnanomolar K_i values, and those of the carboxylic acids (**17**, **19**, **26**, and **29**) are artificially underestimated with an in vitro enzymatic assay conducted at pH 9 where they are fully deprotonated.

4-Phenoxyphenethyl and 4-(Phenoxymethyl)phenethyl Series. In the preceding SAR study of **2** and beyond the biphenylethyl derivative **14**,[56] the 4-phenoxyphenethyl and 4-(phenoxymethyl)phenethyl C2 acyl side chains represented the least effective and next most effective members of the conformationally restricted series examined (Figure 6).

All were more effective than the parent **2** itself ($K_i = 0.0047$ μM) approaching the potency of **14** indicating that all benefit from the conformational restriction and incorporation of two phenyl rings in the C2 acyl side chain. Consequently, we examined the candidate inhibitors incorporating the 4-phenoxyphenethyl and 4-(phenoxymethyl)phenethyl side chains with a somewhat smaller series of the same representative or optimized C5 oxazole aryl and nonaryl substituents. The results of their examination are presented in Figure 7 alongside the results of the corresponding phenhexyl series,[56] of which **2** is a member, and the biphenylethyl series disclosed herein. With a few notable exceptions, the phenhexyl inhibitor was less potent than each of the corresponding inhibitors in the biphenylethyl and (phenoxymethyl)phenethyl series and comparable in potency with the phenoxyphenethyl series. Thus, the potency for any given C5 substituent typically followed the order of 4-biphenylethyl > 4-(phenoxymethyl)phenethyl > phenhexyl > phenoxyphenethyl. However, the activity of each such inhibitor is so good that the potency distinctions between members of the new classes are subtle especially with data collected over a 1–2 year period. Nonetheless, for the most interesting inhibitors that bear a pyridyl (**14** > **68** > **54** > **2**) or substituted pyridyl C5 oxazole substituent that modulates the solubility properties of the inhibitors (**74** > **23** > **44**), the trends are readily discernible.

Like observations made with **2** ($\rho = 3.0$, $R^2 = 0.91$)[55] and the biphenylethyl series (Figure 4), a plot of the Hammett σ_p constant versus $-\log K_i$ for the small C5 oxazole substituents provided well defined correlations ($R^2 = 0.95$ and 0.93) with ρ values of 2.99 and 2.67 (Figures 8 and 9). Thus, a consistent magnitude ($\rho = 3.0$–2.7) of the substituent effect was observed for all four series, suggesting that the correlation represents one of a fundamental nature with predictive value. Importantly, this allows one to quantitatively predict a K_i from an existing relationship in a given C2 acyl series, to qualitatively estimate or extrapolate to members of a new C2 series if a single member has been examined, and to confidently draw inferences from a measured K_i about active site binding that may not be known a priori (e.g., $-CO_2H$ vs $-CO_2^-$ binding).[55,56]

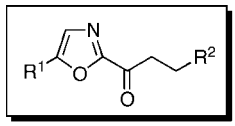
Additional Derivatives. A small series of additional derivatives in the biphenylethyl series were also examined which incorporate a basic amine at the terminus of the C2 acyl side chain. These derivatives, which contain substituents that impact the physical properties of compounds (solubility) and might be expected to alter the PK properties, were prepared by late stage reductive amination incorporation of the tertiary amine as shown in Scheme 3. Interestingly and although not investigated in detail, conducting the reductive amination on the intermediate aldehyde containing the α-ketooxazole resulted only in reductive amination of the aldehyde and not the α-ketooxazole. Moreover and perhaps more surprisingly, the reactive α-ketooxazole was not even reduced to the corresponding alcohol when the reaction was conducted with $NaBH(OAc)_3$, whereas it was competitively reduced if $NaCNBH_3$ was employed.

The results of the examination of these derivatives are summarized in Figure 10. Despite the incorporation of a polar, weakly basic amine in the acyl side chain that occupies the active site hydrophobic channel, the candidate inhibitors proved to be effective FAAH inhibitors that typically displayed activity that approached that of the parent biphenylethyl derivative. Moreover, albeit in a small series, the activity smoothly parallels the relative hydrophobic character of the derivatives (**77** > **78** > **79** > **80**, **76**) with **75**, **77**, **78**, **83**, and **84** most effectively approaching the activity of the corresponding parent derivatives **3**, **15**, and **17**. In contrast, the much more basic and polar *N*-methylpiperizine derivative **81** was much less active. Thus, while polar functionality is poorly tolerated in the portion of the enzyme hydrophobic channel that binds C1−C7 of the fatty acid amide substrates,[17] it is much more readily accommodated at the far end of this channel.

Similarly, a small series of derivatives were examined in which the second aryl ring in the C2 acyl side chain was replaced with one containing a secondary, basic amine (Scheme 4). Although those that contained the free amine (**87** and **89**) were relatively poor inhibitors of FAAH, the precursors in which the free amine was protected as a carbamate (Boc derivative) proved to be effective FAAH inhibitors (Figure 11).

Electrophilic Carbonyl. A select set of the candidate inhibitors were also examined that bear a secondary alcohol or a methylene in place of the ketone. The former were prepared en route to the α-ketoheterocycles and simply entailed examination of this alcohol intermediate. The candidate inhibitors bearing a methylene were prepared by enlisting an alkylative substitution of the Vedejs metalated oxazole as a key step (Scheme 5). Consistent with a mechanism of reversible Ser addition to the electrophilic carbonyl forming a hemiketal at the enzyme active site, the corresponding alcohol and methylene inhibitors were found to be >1000-fold less active than the corresponding ketone (Figure 12). Although not investigated in detail for the inhibitors disclosed herein, even this level of reduced inhibition (0.1% activity) is most likely attributable to contaminant ketone in the samples of the alcohol and methylene compounds that can arise from air oxidation upon storage or even while undergoing assay.[56] Important in these comparisons is the fact that the ketone is essential to the potent activity of the inhibitors and that their reduction to an alcohol or removal altogether leads to $>10^3$ reductions in activity.

Inhibition of Recombinant Human FAAH. Rat and human FAAH are very homologous (84% sequence identity),[2] exhibit near identical substrate selectivities and inhibitor sensitivities in studies disclosed to date, and embody an identical amidase



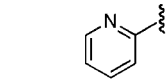
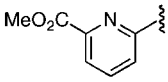
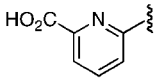
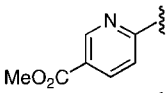
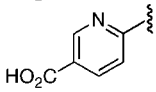
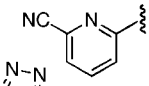
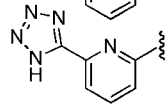
R1	R2 =		R2 =		R2 =		R2 =	
	K_i, μM[56]		K_i, μM		K_i, μM		K_i, μM	
–H	0.048	(**30**)	0.016	(**3**)	0.077	(**45**)	0.025	(**59**)
–I	0.003	(**31**)	0.004	(**4**)	0.01	(**46**)	0.004	(**60**)
$–CF_3$	0.0008	(**32**)	0.0007	(**5**)	0.002	(**47**)	0.0008	(**61**)
–CN	0.0004	(**33**)	0.0002	(**6**)	0.0008	(**48**)	0.0005	(**62**)
$–CO_2H$	0.03	(**34**)	0.010	(**7**)	0.041	(**49**)	0.022	(**63**)
$–CO_2Me$	0.0009	(**35**)	0.0015	(**8**)	0.0017	(**50**)	0.0016	(**64**)
$–CONH_2$	0.005	(**36**)	0.0035	(**9**)	0.0066	(**51**)	0.0045	(**65**)
–CONHMe	0.007	(**37**)	–		0.011	(**52**)	0.0036	(**66**)
$–CONMe_2$	0.002	(**38**)	0.003	(**10**)	0.0054	(**53**)	0.0067	(**67**)
	0.0047	(**2**)	0.00075	(**14**)	0.0034	(**54**)	0.0010	(**68**)
	0.008	(**39**)	0.0012	(**15**)	0.013	(**55**)	0.002	(**69**)
	0.02	(**40**)	0.022	(**17**)	0.065	(**56**)	0.0061	(**70**)
	0.0035	(**41**)	0.0022	(**18**)	0.054	(**57**)	0.0035	(**71**)
	0.007	(**42**)	0.014	(**19**)	0.033	(**58**)	0.0046	(**72**)
	0.0029	(**43**)	0.0029	(**22**)	–		0.003	(**73**)
	0.040	(**44**)	0.025	(**23**)	–		0.021	(**74**)

Figure 7. FAAH inhibitors with conformationally restricted C2 side chains.

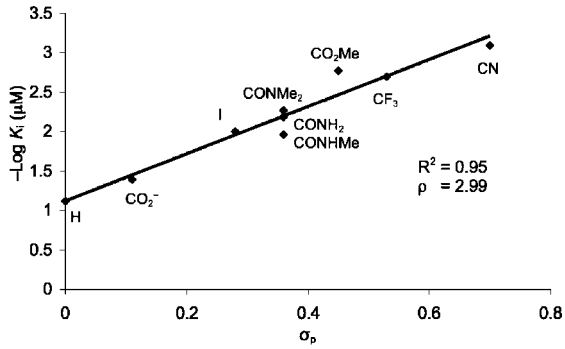


Figure 8. Plot of $-\log K_i$ (μM) versus σ_p for 4-phenoxyphenethyl series.

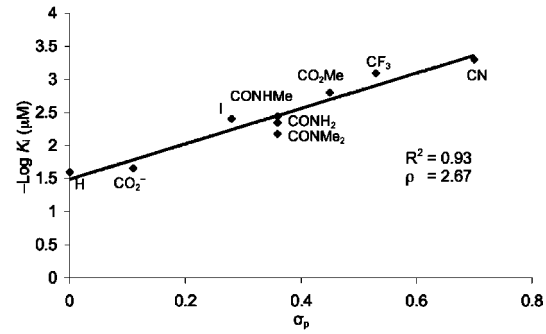


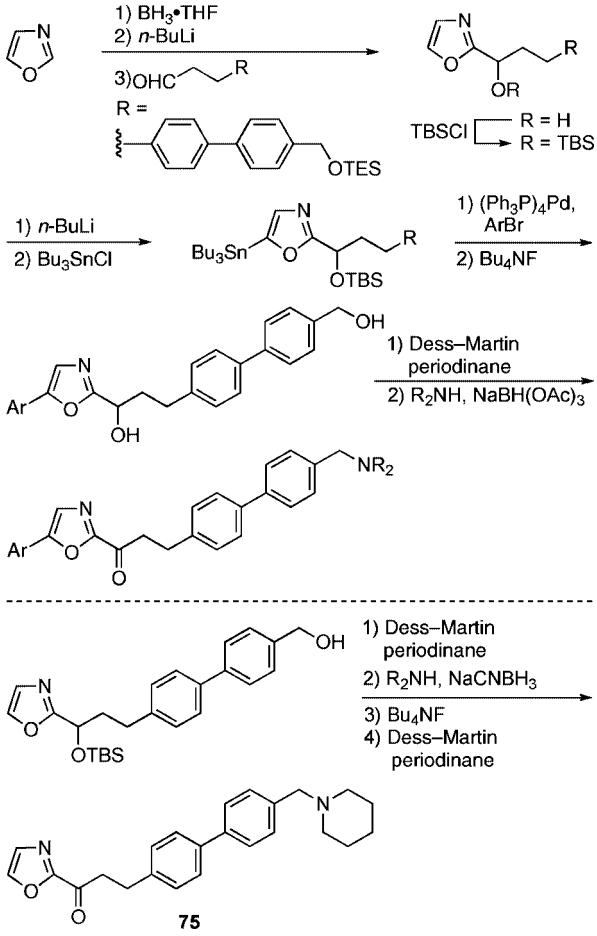
Figure 9. Plot of $-\log K_i$ (μM) versus σ_p for 4-(phenoxymethyl)phenethyl series.

signature sequence, suggesting the observations made with rat FAAH would be analogous to those made with the human enzyme. Consequently, key inhibitors in the series were examined against the human enzyme and, consistent with previous observations, were found to exhibit the same relative and absolute potencies (Figure 13).

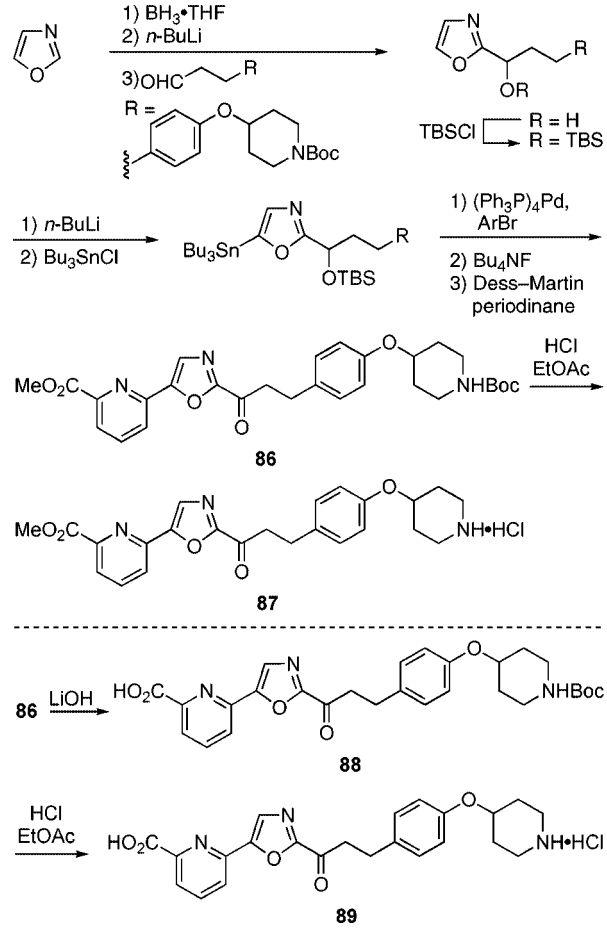
Selectivity. Early assessments of α-ketoheterocycle inhibitors of FAAH against possible competitive enzymes (e.g., phospholipase A2, ceramidase) revealed no inhibition. Consequently, a method for proteome-wide screening capable of globally profiling all mammalian serine hydrolases was developed,[31,69] and studies have shown that the α-ketoheterocycle class of inhibitors generally are exquisitely selective for FAAH. However, two enzymes did emerge as potential competitive targets: triacylglycerol hydrolase (TGH) and an uncharacterized membrane-associated hydrolase that lacked known substrates or function (KIAA1363).[70] In this screen, IC_{50} values are typically higher than the measured K_i values, but the relative potency, the magnitude of binding affinity differences, and the rank order binding determined in the assay parallel those established by standard substrate assays.

Summarized in Figure 14 are the results of the selectivity screening of selected candidate inhibitors. In general, the inhibitors were very selective for FAAH over TGH and

Scheme 3



Scheme 4



KIAA1363. The trends within the series examined are very clear and mirror the observations disclosed in our preceding studies.[53,54,56–58] Thus, the inhibitors bearing an unsubstituted oxazole (**3**, **30**, **45**, and **59**) were selective for FAAH over KIAA1363 but more selective for TGH over FAAH (1- to 125-fold). The potent inhibitors incorporating the small C5 nitrile (**6**, **33**, **48**, and **62**) were found to be sufficiently selective for FAAH versus KIAA1363 (>10^3- to 10^4-fold) but only modestly selective for FAAH versus TGH (3- to 50-fold). Here, the subtle impact of the nature of the C2 acyl side is also apparent with the series following the selectivity order of (phenoxymethyl)phenethyl > biphenylethyl > phenoxyphenethyl = phenhexyl. In line with prior observations, the addition of a 2-pyridyl C5 substituent (**2**, **14**, **54**, and **68**) increases not only the FAAH potency but also FAAH selectivity such that they no longer inhibit KIAA1363 (10^4-fold selective) and are typically >100-fold selective for FAAH versus TGH. The corresponding tetrazolyl substituted 2-pyridyl derivatives **23**, **44**, and **74**

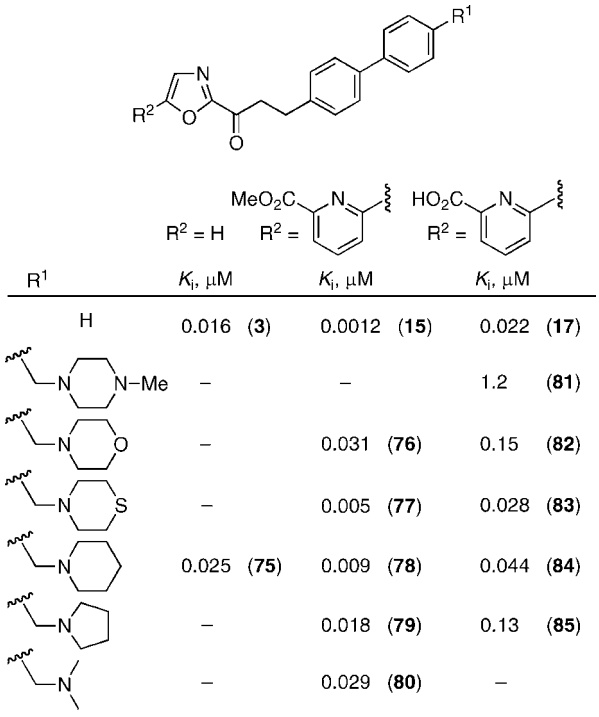


Figure 10. Effect of tertiary amine derivatives.

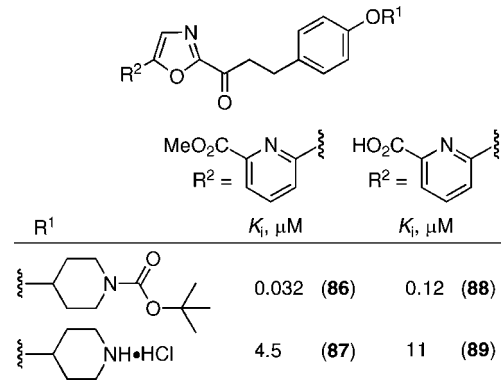
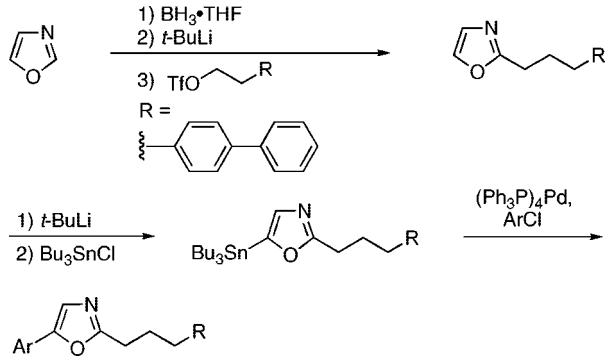


Figure 11. Effect of secondary amine derivatives.

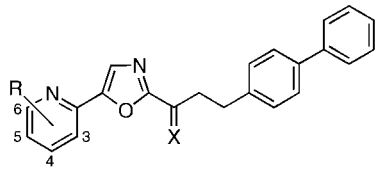
Scheme 5



exhibited essentially identical selectivity trends exhibited by **2**, **33**, and **62**, whereas the addition of a carboxylic acid to the 2-pyridyl C5 substituent (−CO_2H: **17**, **40**, **56**, and **70**) further enhances this intrinsic selectivity such that the resulting inhibitors are no longer viable competitive inhibitors of either KIAA1363 or TGH. In fact, the selectivity impact of the addition of a weakly acidic C5 oxazole substituent is so large that the candidate inhibitors bearing only a C5 carboxylic acid (**7**, **34**, **44**, and **63**) were found to be surprisingly selective for FAAH (typically >100-fold). Superimposed on this impact of the oxazole C5 substituent is the impact of the C2 acyl side chain. In general, the increased size and conformational restraints of the biphenylethyl, phenoxyphenethyl, and (phenoxymethyl)phenethyl side chains matched or enhanced the intrinsic selectivity of the phenhexyl series that includes **2** typically following the order of (phenoxymethyl)phenethyl > biphenylethyl > phenoxyphenethyl > phenhexyl (Figure 15).

Conclusions

Herein, we report the synthesis and evaluation of a key series of α-ketooxazoles based on **2** incorporating conformationally constrained C2 acyl side chains and optimized or representative C5 oxazole substituents that provided extraordinarily potent (K_i = 200 pM) inhibitors of fatty acid amide hydrolase. Those

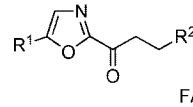


compd	R	X	K_i, μM
15	6-CO_2Me	O	0.0012
90		H, OH	9
91		H, H	7
17	6-CO_2H	O	0.022
92		H, OH	1.1
93		H, H	10
18	5-CO_2Me	O	0.0022
94		H, OH	1.1
95		H, H	50
19	5-CO_2H	O	0.014
96		H, OH	10
97		H, H	20
20	6-$CONH_2$	O	0.0012
98		H, OH	20
99		H, H	4
21	5-$CONH_2$	O	0.0013
100		H, OH	>1
101		H, H	30

Figure 12. Effect of the electrophilic carbonyl.

compd	K_i, μM (human)	K_i, μM (rat)
6	0.00026	0.0002
14	0.0029	0.00075
15	0.0012	0.0012
47	0.0010	0.0020
48	0.00085	0.0008
61	0.00067	0.0008
62	0.00051	0.0005
68	0.005	0.001

Figure 13. Inhibition of recombinant human fatty acid amide hydrolase.



R^2 presented in order of phenhexyl, phenoxyphenethyl, biphenylethyl, (phenoxymethyl)phenethyl

compd	K_i, μM	FAAH IC_{50}, μM	KIAA1363 IC_{50}, μM	TGH IC_{50}, μM
R^1 = H				
30	0.048	2.5	19 (8)	0.02 (0.008)
45	0.077	0.5	>100 (>200)	0.03 (0.06)
3	0.016	0.18	>100 (>550)	0.0045 (0.025)
59	0.025	0.27	>100 (>370)	0.27 (1)
R^1 = CN				
33	0.0004	0.0065	>100 (>15400)	0.02 (3)
48	0.0008	0.005	20 (4000)	0.016 (3)
6	0.0002	0.001	64 (64000)	0.026 (26)
62	0.0005	0.002	19 (9500)	0.100 (50)
R^1 = 2-pyridine				
2	0.0047	0.002	>100 (>50000)	>0.6 (>300)
54	0.0034	0.002	>100 (>50000)	0.2 (100)
14	0.00075	0.0007	>100 (>140000)	1.2 (1700)
68	0.001	0.002	>100 (>50000)	2 (1000)
R^1 = 6-(1*H*-tetrazol-5-yl)pyridin-2-yl				
44	0.040	0.5	>100 (>200)	>100 (>200)
23	0.025	0.1	>100 (>1000)	7 (70)
74	0.021	0.18	>100 (>550)	90 (500)
R^1 = 6-picolinic acid				
40	0.020	0.1	>100 (>1000)	>100 (>1000)
56	0.0065	0.02	>100 (>5000)	>100 (>5000)
17	0.022	0.006	>100 (>17000)	>100 (>17000)
70	0.0061	0.04	>100 (>2500)	26 (650)
R^1 = CO_2H				
34	0.03	0.002	>100 (>50000)	2 (1000)
49	0.041	0.08	>100 (>1250)	2.4 (30)
7	0.010	0.02	>100 (>5000)	17 (850)
63	0.022	0.05	>100 (>2000)	37 (740)

Figure 14. Selectivity screening.

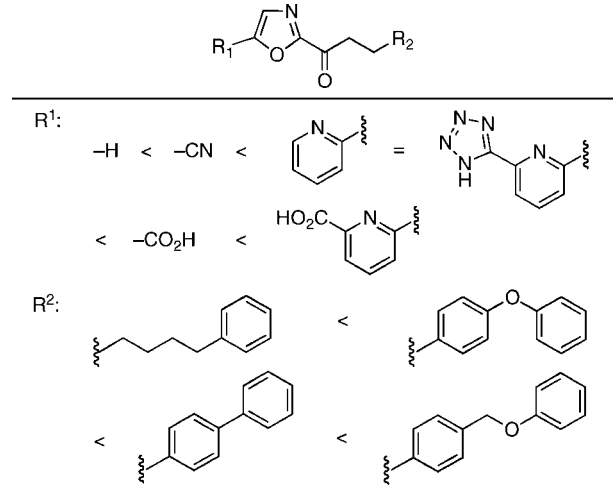


Figure 15. Selectivity trends.

incorporating small C5 oxazole substituents were found to follow a well-defined linear relationship between −log K_i and Hammett σ_p of predictive value and that is of a magnitude (ρ = 2.7–3.3) that indicates that the substituent electronic properties dominate its inhibitor effects. Presumably this reflects the substituent electron-withdrawing effect on enhancing the reactivity of the electrophilic carbonyl. Additional C5 aryl substit-

uents (e.g., 2-Pyr or 2-PyrCO_2H) similarly increase FAAH potency, enhance FAAH selectivity, and significantly modify physical properties (e.g., solubility) in a manner that may impact PK and PD behavior of the inhibitors. Just as significantly, the nature of the C5 oxazole substituent substantially impacts the selectivity of the FAAH inhibitors whereas the effect of the C2 acyl substituent was more subtle but still significant in the small series examined. These trends proved to be general across a range of inhibitors examined to date.[53–58] This is especially evident with the TGH selectivity for the unsubstituted oxazole **30** (125-fold selective for TGH vs FAAH), which can be improved by the independent choice of the C2 side chain (compare **59**) as well as the C5 oxazole substituent (compare **33**, **34**, **2**, **40**, and **44**). Combination of these independent features that simultaneously improve FAAH potency and disrupt TGH or KIAA1363 binding can provide exquisitely selective and potent FAAH inhibitors.

Experimental Section

Methyl 6-(2-(3-(Biphenyl-4-yl)propanoyl)oxazol-5-yl)picolinate (15). A solution of triethyl phosphonoacetate (13.73 mL, 1.25 equiv) in 100 mL of anhydrous THF at −78 °C was treated with *n*-BuLi (2.5 M in hexanes, 28.5 mL, 1.5 equiv). After the mixture was stirred for 45 min, 4-biphenylcarboxaldehyde (10 g, 1 equiv) in 80 mL of anhydrous THF was added dropwise. The reaction mixture was allowed to warm to 25 °C and was stirred overnight. The reaction was quenched with the addition of saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated to yield the crude α,β-unsaturated ester. The α,β-unsaturated ester (13.85 g) was dissolved in EtOH (250 mL), and 10% Pd/C (1.17 g, 0.2 equiv) was added. The solution was purged with H_2 and stirred for 16 h. The mixture was filtered through a Celite pad, and the solution was concentrated. Column chromatography (SiO_2, 5–20% EtOAc/hexanes) afforded ethyl 3-(biphenyl-4-yl)propanoate as a clear oil (9.60 g, 69%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (d, 2H, J = 7.0 Hz), 7.66 (d, 2H, J = 8.0 Hz), 7.55 (t, 2H, J = 7.5 Hz), 7.47–7.46 (m, 3H), 4.28 (q, 2H, J = 5.6 Hz), 3.13 (t, 2H, J = 8.0 Hz), 2.79 (t, 2H, J = 7.0 Hz), 1.38 (t, 3H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 141.4, 140.2, 139.7, 129.2, 129.2, 127.7, 127.6, 127.5, 60.9, 36.4, 31.1, 14.7.

Ethyl 3-(biphenyl-4-yl)propanoate (9.27 g, 36.45 mmol) in anhydrous CH_2Cl_2 (150 mL) was cooled to −78 °C, and DIBAL-H (1 M in hexanes, 47.4 mL, 47.4 mmol) was added dropwise. The reaction mixture was stirred for 2 h at −78 °C before methyl formate (2.25 mL, 36.45 mmol) was added dropwise to quench the reaction. The reaction mixture was warmed to 0 °C, saturated aqueous NH_4Cl (20 mL) and saturated aqueous Na^+K^+ tartrate (25 mL) were added, and the reaction mixture was stirred vigorously overnight. The aqueous phase was separated and extracted with CH_2Cl_2, and the combined organic layers were washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl and dried over Na_2SO_4. Evaporation in vacuo yielded the crude aldehyde, which was purifed by flash chromatography (SiO_2, 2–10% EtOAc/hexanes) to yield 3-(biphenyl-4-yl)propanal as a white solid (6.83 g, 89%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.94 (s, 1H), 7.66 (d, 2H, J = 7.0 Hz), 7.62 (d, 2H, J = 8.0 Hz), 7.52 (t, 3H, J = 7.5 Hz), 7.43 (t, 1H, J = 7.5 Hz), 7.36 (d, 2H, J = 8.0 Hz), 3.10 (t, 2H, J = 7.5 Hz), 2.91 (t, 2H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 201.9, 141.3, 139.9, 139.8, 129.2, 129.2, 127.8, 127.6, 127.4, 45.7, 28.2.

Oxazole (1.0 g, 14.5 mmol) in anhydrous THF (100 mL) was treated with BH_3·THF (1 M, 15.9 mL, 15.9 mmol), and the solution was stirred at room temperature for 1 h before being cooled to −78 °C and treated with 1.5 M *t*-BuLi (12.6 mL, 18.9 mmol) dropwise. The reaction mixture was stirred at −78 °C for 40 min before a solution of 3-(biphenyl-4-yl)propanal (3.04 g, 14.5 mmol) in THF (20 mL) was added. The reaction mixture was stirred at −78 °C for 2 h before being warmed to room temperature. A 5% HOAc/EtOH solution (50 mL) was added, and this mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue was dissolved in EtOAc and washed with H_2O, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl before the organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Flash chromatography (SiO_2, 10−40% EtOAc/hexanes) afforded 3-(biphenyl-4-yl)-1-(oxazol-2-yl)propan-1-ol (3.32 g, 82%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.62 (s, 1H), 7.57 (d, 2H, J = 7.2 Hz), 7.51 (d, 2H, J = 7.8 Hz), 7.43 (t, 2H, J = 6.6 Hz), 7.34–7.28 (m, 4H), 7.10 (s, 1H), 4.90–4.88 (m, 1H), 4.34 (s, 1H), 2.85–2.82 (m, 2H), 2.30–2.25 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 167.1, 141.9, 141.0, 140.0, 139.9, 129.8, 129.6, 128.1, 128.0, 127.9, 127.1, 67.6, 37.7, 31.7.

A solution of 3-(biphenyl-4-yl)-1-(oxazol-2-yl)propan-1-ol (5.70 g, 20.4 mmol), TBSCl (4.62 g, 30.7 mmol), and imidazole (2.09 g, 30.7 mmol) in DMF (50 mL) was stirred at room temperature for 72 h before it was diluted with ether and washed with H_2O and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. Flash chromatography (SiO_2, 2–10% EtOAc/hexanes) yielded 2-(3-(biphenyl-4-yl)-1-(*tert*-butyldimethylsilyloxy)propyl)oxazole (7.54 g, 94%) as a thick colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.63 (s, 1H), 7.60 (d, 2H, J = 7.8 Hz), 7.53 (d, 2H, J = 7.8 Hz), 7.44 (t, 2H, J = 7.8 Hz), 7.34 (t, 1H, J = 7.8 Hz), 7.28 (d, 2H, J = 8.4 Hz), 7.10 (s, 1H), 4.92 (t, 1H, J = 6.0 Hz), 2.86–2.81 (m, 1H), 2.74–2.69 (m, 1H), 2.34–2.19 (m, 2H), 0.93 (s, 9H), 0.11 (s, 3H), −0.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.9, 142.0, 141.4, 139.8, 139.5, 129.7, 129.6, 128.0, 127.9, 127.9, 127.8, 68.8, 38.9, 32.0, 26.7, 19.1, −4.2, −4.3.

A solution of 2-(3-(biphenyl-4-yl)-1-(*tert*-butyldimethylsilyloxy)propyl)oxazole (842 mg, 2.14 mmol) in THF (20 mL) was cooled to −78 °C before it was treated with 1.6 M *t*-BuLi (1.74 mL, 2.78 mmol) dropwise. The reaction mixture was stirred at −40 °C for 2 h, cooled to −78 °C, treated with a solution of Bu_3SnCl (1.15 mL, 3.53 mmol) in THF (5 mL), and stirred for 5 min. The solution was warmed to room temperature, diluted with EtOAc, and washed with saturated aqueous NaCl. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. Flash chromatography (SiO_2, 0–2% EtOAc/hexanes) yielded 2-(3-(biphenyl-4-yl)-1-(*tert*-butyldimethylsilyloxy)propyl)-5-(tributylstannyl)oxazole (1.09 mg, 90%) as a thick colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.58 (d, 2H, J = 7.8 Hz), 7.51 (d, 2H, J = 7.8 Hz), 7.43 (t, 2H, J = 7.4 Hz), 7.44 (t, 2H, J = 7.8 Hz), 7.32 (t, 1H, J = 7.2 Hz), 7.27 (d, 2H, J = 7.8 Hz), 7.10 (s, 1H), 4.92 (t, 1H, J = 6.6 Hz), 2.84–2.79 (m, 1H), 2.72–2.67 (m, 1H), 2.30–2.18 (m, 2H), 1.58–1.55 (m, 6H), 1.36–1.32 (m, 6H), 1.13–1.10 (m, 6H), 0.90 (s, 18H), 0.08 (s, 3H), −0.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.9, 155.8, 142.0, 141.7, 139.7, 138.1, 129.7, 129.6, 128.0, 127.9, 127.9, 68.9, 39.0, 32.0, 29.8, 28.0, 26.6, 19.1, 14.5, 11.1, −4.2, −4.3.

2-(3-(Biphenyl-4-yl)-1-(*tert*-butyldimethylsilyloxy)propyl)-5-(tributylstannyl)oxazole (191 mg, 0.337 mmol), $Pd(PPh_3)_4$ (39 mg, 0.034 mmol), and methyl 6-chloropicolinate (116 mg, 0.674 mmol) were dissolved in anhydrous 1,4-dioxane (18 mL), and the mixture was warmed to reflux for 24 h under Ar. The mixture was diluted with EtOAc, washed with saturated aqueous NaCl, and dried over Na_2SO_4. Evaporation in vacuo yielded the crude coupling product. Flash chromatography (SiO_2, 10–20% EtOAc/hexanes) yielded methyl 6-(2-(3-(biphenyl-4-yl)-1-(*tert*-butyldimethylsilyloxy)propyl)oxazol-5-yl)picolinate as a yellow oil (145 mg, 81%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.09 (d, 1H, J = 7.5 Hz), 7.95 (t, 1H, J = 7.5 Hz), 7.88–7.86 (m, 2H), 7.61 (d, 2H, J = 7.8 Hz), 7.57 (d, 2H, J = 7.8 Hz), 7.47 (t, 2H, J = 7.8 Hz), 7.39–7.34 (m, 3H), 5.04 (t, 1H, J = 6.0 Hz), 4.08 (s, 3H), 2.98–2.92 (m, 1H), 2.86–2.80 (m, 1H), 2.47–2.33 (m, 2H), 1.00 (s, 9H), 0.20 (s, 3H), 0.07 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.0, 165.8, 150.5, 148.7, 148.0, 141.4, 140.8, 139.3, 138.4, 129.3, 129.1, 128.5, 127.6, 127.5, 127.4, 126.9, 122.5, 68.5, 53.3, 38.4, 31.5, 26.2, 18.7, −4.5, −4.6.

Methyl 6-(2-(3-(biphenyl-4-yl)-1-(*tert*-butyldimethylsilyloxy)propyl)oxazol-5-yl)picolinate (139 mg, 0.263 mmol) was dissolved in

THF (5 mL), treated with Bu_4NF (1 M in THF, 0.316 mL, 0.316 mmol), and stirred at room temperature for 2 h under Ar. The reaction mixture was diluted with EtOAc, washed with saturated aqueous NaCl, and dried over Na_2SO_4. Evaporation in vacuo yielded the crude alcohol, which was filtered through a short silica gel pad. The silica gel pad was washed with 10% EtOAc/hexanes followed by 60% EtOAc/hexanes to afford the alcohol, which required no further purification. The alcohol was dissolved in CH_2Cl_2 (12 mL), and Dess−Martin periodinane (167 mg, 0.395 mmol) was added. The mixture was stirred at room temperature for 2 h before silica gel was added, and the reaction mixture was evaporated in vacuo to afford the crude ketone absorbed on silica gel. Flash chromatography (SiO_2, 10–40% EtOAc/hexanes) yielded methyl 6-(2-(3-(biphenyl-4-yl)propanoyl)oxazol-5-yl)picolinate (**15**) as a white solid (37 mg, 75%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.10 (d, 1H, J = 7.5 Hz), 8.01–8.00 (m, 2H), 7.95 (t, 1H, J = 7.5 Hz), 7.56 (d, 2H, J = 7.8 Hz), 7.52 (d, 2H, J = 7.8 Hz), 7.41 (t, 2H, J = 7.8 Hz), 7.34–7.30 (m, 3H), 4.02 (s, 3H), 3.50 (t, 2H, J = 7.2 Hz), 3.15 (t, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 188.2, 166.0, 158.2, 153.3, 149.4, 147.4, 141.8, 140.3, 140.1, 139.2, 129.8, 129.6, 128.9, 128.1, 128.0, 127.9, 126.1, 124.2, 54.0, 41.5, 30.1; HR ESI-TOF m/z 413.1491 ($M + H^+$, $C_{25}H_{21}N_2O_4$, requires 413.1496).

6-(2-(3-(Biphenyl-4-yl)propanoyl)oxazol-5-yl)picolinic Acid (17). Methyl 6-(2-(3-(biphenyl-4-yl)propanoyl)oxazol-5-yl)picolinate (**15**) (15 mg, 0.036 mmol) was dissolved in a mixture of 3:2 THF/H_2O (3 mL), and LiOH (3 mg, 0.1 mmol) was added. The reaction mixture was stirred for 2 h at room temperature before the mixture was made acidic with the addition of aqueous 1 N HCl. The solution was diluted with EtOAc, and the organic layer was separated from the aqueous layer. The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with saturated aqueous NaCl and dried over Na_2SO_4. Evaporation in vacuo yielded the crude acid, which was purified by flash chromatography (SiO_2, 0–2% AcOH/EtOAc) to yield 6-(2-(3-(biphenyl-4-yl)propanoyl)oxazol-5-yl)picolinic acid (**17**) as a white solid (10 mg, 71%): ^{1}H NMR (THF-d_8, 600 MHz) δ 8.12–8.06 (m, 4H), 7.60 (d, 2H, J = 7.8 Hz), 7.55 (d, 2H, J = 7.8 Hz), 7.40–7.36 (m, 4H), 7.28 (t, 1H, J = 7.8 Hz), 3.47 (t, 2H, J = 7.2 Hz), 3.10 (t, 2H, J = 7.2 Hz); ^{13}C NMR (THF-d_8, 150 MHz) δ 187.0, 165.4, 158.6, 153.3, 149.8, 146.9, 141.8, 141.0, 139.8, 139.5, 129.6, 129.3, 128.4, 127.6, 127.4, 125.2, 123.4, 41.2, 29.9; HR ESI-TOF m/z 399.1334 ($M + H^+$, $C_{24}H_{19}N_2O_4$, requires 399.1339).

FAAH Inhibition. ^{14}C-Labeled oleamide was prepared from ^{14}C-labeled oleic acid as previously described.[13] The truncated rat FAAH (rFAAH) was expressed in *E. coli* and purified as previously described.[67] The purified recombinant rFAAH was used in the inhibition assays unless otherwise indicated. The full-length human FAAH (hFAAH) was expressed in COS-7 cells as previously described,[2] and the lysate of hFAAH-transfected COS-7 cells was used in the inhibition assays where explicitly indicated.

The inhibition assays were performed as previously described.[13] In brief, the enzyme reaction was initiated by mixing 1 nM rFAAH (800, 500, or 200 pM rFAAH for inhibitors with $K_i \leq$ 1–2 nM) with 10 μM ^{14}C-labeled oleamide in 500 μL of reaction buffer (125 mM Tris-Cl, 1 mM EDTA, 0.2% glycerol, 0.02% Triton X-100, 0.4 mM Hepes, pH 9.0) at room temperature in the presence of three different concentrations of inhibitor. The enzyme reaction was terminated by transferring 20 μL of the reaction mixture to 500 μL of 0.1 N HCl at three different time points. The ^{14}C-labeled oleamide (substrate) and oleic acid (product) were extracted with EtOAc and analyzed by TLC as detailed.[13] The K_i of the inhibitor was calculated using a Dixon plot as described (standard deviations are provided in the Supporting Information tables). Lineweaver–Burk analysis was performed as described, confirming competitive and reversible inhibition.[53]

Selectivity Screening. The selectivity screening was conducted as previously detailed.[31]

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Supporting Information Available: Full experimental details and characterization of the candidate inhibitors, FAAH assay measurement errors, and purities of the FAAH inhibitors. This material is available free of charge via the Internet at http://pubs.acs.org.

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