Articles

Optimization of the Central Heterocycle of α-Ketoheterocycle Inhibitors of Fatty Acid Amide Hydrolase

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The synthesis and evaluation of a refined series of α-ketoheterocycles based on the oxazole **2** (OL-135) incorporating systematic changes in the central heterocycle bearing a key set of added substituents are described. The nature of the central heterocycle, even within the systematic and minor perturbations explored herein, significantly influenced the inhibitor activity: 1,3,4-oxadiazoles and 1,2,4-oxadiazoles **9** > tetrazoles, the isomeric 1,2,4-oxadiazoles **10**, 1,3,4-thiadiazoles > oxazoles including **2** > 1,2-diazines > thiazoles > 1,3,4-triazoles. Most evident in these trends is the observation that introduction of an additional heteroatom at position 4 (oxazole numbering, N > O > CH) substantially increases activity that may be attributed to a reduced destabilizing steric interaction at the FAAH active site. Added heterocycle substituents displaying well-defined trends may be utilized to enhance the inhibitor potency and, more significantly, to enhance the inhibitor selectivity. These trends, exemplified herein, emerge from both enhancements in the FAAH activity and simultaneous disruption of binding affinity for competitive off-target enzymes.

Introduction

Fatty acid amide hydrolase (FAAH[a])[1,2] is the enzyme that serves to hydrolyze endogenous lipid amides[3–6] including anandamide (**1a**)[7–10] and oleamide (**1b**),[11–13] Figure 1. Its distribution is consistent with its role in degrading and regulating 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,[3,18–20] especially for the treatment of pain,[21–23] inflammatory,[24] or sleep disorders,[13,25] there has been an increasing interest in the development of selective and potent inhibitors of the enzyme.[26] Early studies shortly following the initial characterization of the enzyme led to the discovery that the endogenous sleep-inducing molecule 2-octyl α-bromoacetoacetate is an effective FAAH inhibitor,[27] the disclosure of a series of nonselective reversible inhibitors bearing an electrophilic ketone (e.g., trifluoromethyl ketone-based inhibitors),[28–31] and the reports of 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 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 with some exceptions, the selectivity of such inhibitors has often been low,[31,42,48–50] further complicating the development

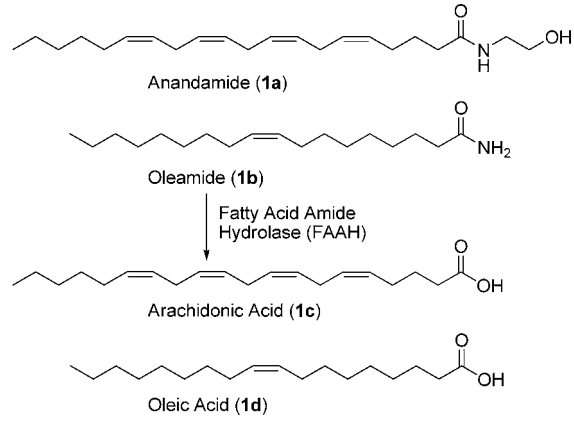

Figure 1. Substrates of fatty acid amide hydrolase (FAAH).

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. Many of these competitive inhibitors are not only potent and extraordinarily selective for FAAH versus other mammalian serine hydrolases, but members of this class have been shown to be efficacious analgesics in vivo.[58,59]

In these 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 (≥100−300 fold)[31] FAAH inhibitor that induces analgesia and increases endogenous anandamide levels.[53,59] It has been shown to exhibit analgesic activity in the tail flick assay, hot plate assay, formalin test of noxious chemical pain (1st and second phase), the mild thermal injury (MTI) model of peripheral pain, and the spinal nerve ligation (SNL) model of neuropathic pain with efficacies that match or exceed those of morphine (@1−3 mg/kg in MTI/SNL),

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[a] Abbreviations: FAAH, fatty acid amide hydrolase, TGH, triacylglycerol hydrolase.

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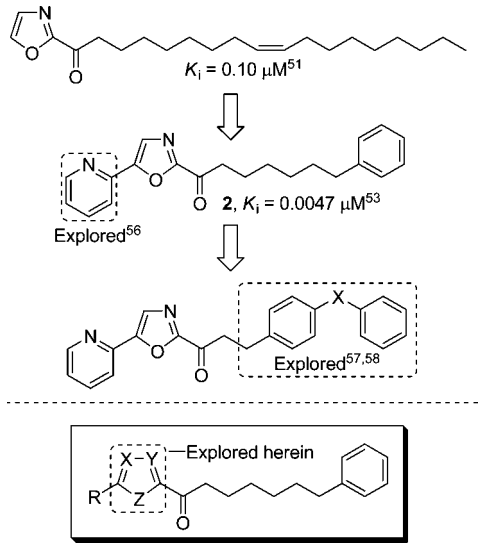


Figure 2. Progression of the inhibitor series.

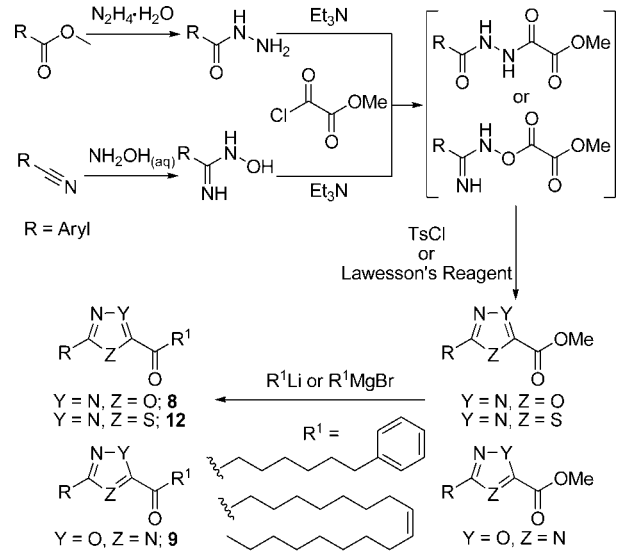
ibuprofen (@100 mg/kg in MTI), or gabapentin (@500 mg/kg in SNL) and at administered doses (10−20 mg/kg, ip) that approach or exceed those of such common pain medications.[59] It has been shown to lack significant offsite target activity (Cerep assay profiling), does not bind cannabinoid (CB1 or CB2) or vanilloid (TRP) receptors and does not significantly inhibit 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.[59]

Consequently, we conducted a series of 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,[53,57,58] both of which have provided extraordinarily potent and selective FAAH inhibitors (Figure 2).[60] Herein, we report results of systematic studies examining candidate inhibitors exploring and further optimizing the central heterocycle of the α-ketoheterocycle within the structure of **2** along with results of the proteome-wide selectivity screening[31] of the resulting candidate inhibitors.

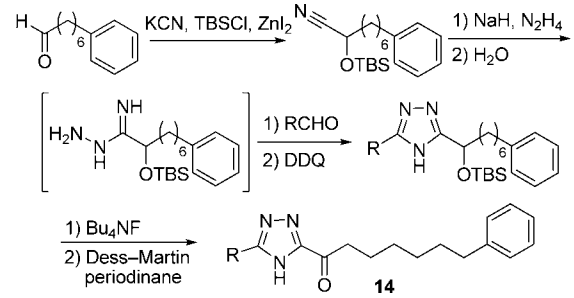
Chemistry

The preparation of the 1,3,4-thiadiazole (**12**), 1,3,4-oxadiazole (**8**), and 1,2,4-oxadiazole (**9**) inhibitors is illustrated in Scheme 1. A one-pot procedure was utilized to prepare the C-5 aryl substituted 1,3,4-thiadiazole, 1,3,4-oxadiazole, and 1,2,4-oxadiazole methyl esters. In the case of the 1,3,4-thiadiazole and 1,3,4-oxadiazole, the corresponding aryl(R) methyl ester was converted to the hydrazide with hydrazine monohydrate, whereas the aryl(R) nitrile was transformed to the *N*-hydroxycarbamide with hydroxylamine for the 1,2,4-oxadiazole, all of which were subsequently treated with methyl oxalyl chloride in the presence of Et_3N to give a diacyl hydrazide intermediate. These were cyclized upon treatment with Lawesson's reagent to give the desired 1,3,4-thiadiazole or with *p*-toluenesulfonyl chloride (TsCl) for the 1,3,4-oxadiazole and 1,2,4-oxadiazole. Subsequent addition of the requisite side chain (R^1) to the methyl ester was

Scheme 1



Scheme 2

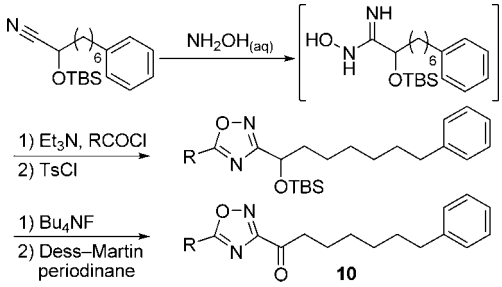


accomplished via a metal−halogen exchange of the corresponding alkylbromide to give the α-ketooxadiazole or α-ketothiadiazole.

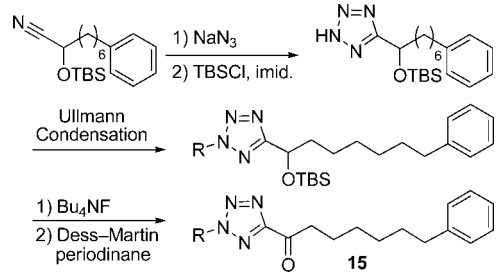
Synthesis of the 1,2,4-triazole series (**14**) in an analogous fashion was unsuccessful in which the side chain nucleophilic addition to the corresponding methyl ester failed to afford product. Therefore, an alternative approach was utilized to obtain the desired 1,2,4-triazoles (Scheme 2). 7-Phenylheptanal was converted to the silyl-protected cyanohydrin upon treatment with potassium cyanide, TBSCl, and catalytic ZnI_2.[61] The corresponding amidrazone[62] was formed by in situ generation of sodium hydrazine followed by dropwise addition of the cyanohydrin, which was treated with an aryl(R) acid chloride. Surprisingly, the cyclization afforded mainly the 1,3,4-oxadiazole product along with a small amount of the desired 1,2,4-triazole product. Although this offered an additional route to the 1,3,4-oxadiazoles, it required modification for 1,2,4-triazole formation. The alternative was a two-step condensation and oxidation pathway via reaction of the amidrazone with aryl(R) aldehydes to give a stable imine, which undergoes oxidative cyclization to the triazole upon treatment with DDQ.[63] The desired 1,2,4-triazoles **14** were obtained after TBS deprotection (Bu_4NF) and oxidation of the liberated alcohol with Dess−Martin periodinane[64] (Scheme 2).

The TBS-protected cyanohydrin was also a key intermediate for the preparation of two additional inhibitor series, the isomeric 1,2,4-oxadiazoles (**10**) and the tetrazoles (**15**). For the 1,2,4-oxadiazoles, the cyanohydrin was converted to the *N*-hydroxycarbamide upon treatment with hydroxylamine. Its treatment with acid chlorides in the presence of Et_3N to give the diacyl

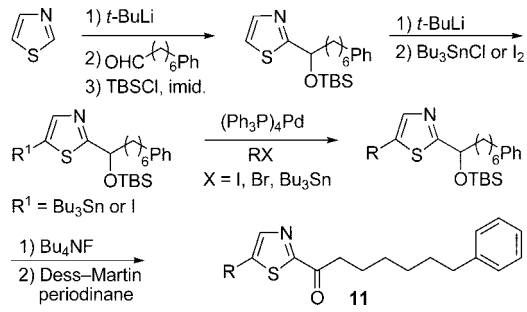
Scheme 3



Scheme 4



Scheme 5



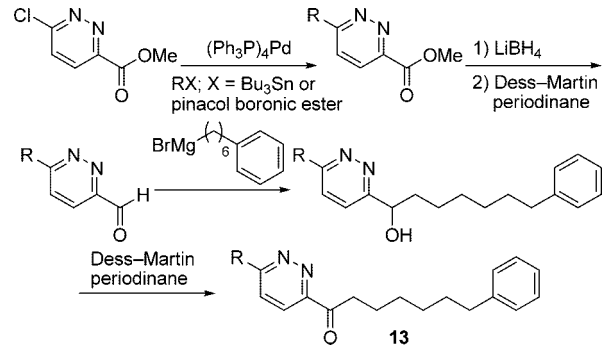
hydrazide intermediates followed by dehydration with *p*-toluenesulfonyl chloride (TsCl) at elevated temperatures gave the desired 1,2,4-oxadiazoles **10** after TBS deprotection (Bu_4NF) and oxidation of the liberated alcohol with Dess−Martin periodinane[64] (Scheme 3).

The tetrazoles (**15**) were obtained by treatment of the cyanohydrin with sodium azide.[65] Reprotection of the alcohol with TBSCl followed by regioselective *N*-arylation using a copper-catalyzed Ullmann condensation[66] with iodopyridine or with mixed-aryl hypervalent iodonium salts[67] afforded N2-substituted tetrazoles, which were converted to the corresponding products upon TBS deprotection (Bu_4NF) and oxidation of the liberated alcohol with Dess−Martin periodinane[64] (Scheme 4).

The synthesis of the 1,3-thiazole inhibitors (**11**) entailed a selective C2-lithiation of thiazole followed by condensation with 7-phenylheptanal. TBS protection of the resulting alcohol followed by selective C5-lithiation (*t*-BuLi)[68] and stannylation or iodination and subsequent Stille coupling[69] produced the substituted thiazoles, which were converted to the corresponding ketones by TBS deprotection (Bu_4NF) and oxidation of the liberated alcohol with Dess−Martin periodinane[64] (Scheme 5).

Synthesis of the 1,2-diazine inhibitors (**13**) began with Stille coupling of a known chloro-pyridazine[70] and a series of 2-(tributylstannyl)arenes or 2-aryl boronic esters to afford the series of 6-aryl substituted pyridazine-3-carboxylates. The corresponding methyl ester was converted to the aldehyde by a reduction−oxidation process in order to increase the electrophilicity of the intermediate for subsequent side chain addition, which was accomplished via metal−halogen exchange of the corresponding alkylbromide. The alcohol precursor was oxidized with Dess−Martin periodinane[64] to give the 1,2,-diazines **13** (Scheme 6).

Scheme 6



Enzyme Assay

Enzyme assays were performed at 20−23 °C with purified recombinant rat FAAH expressed in *Escherichia coli*[71] (unless 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

In the course of early studies,[51] a range of oleyl α-ketoheterocycles were explored in which the benzoxazolyl[51] and oxazolyl[53] derivatives were found to be among the most potent and were chosen for further examination. Their examination, which included the elaboration to the exceptionally potent oxazolopyridines[51] or 5-(2-pyridyl)oxazoles[53] including **2**, defined important features of the heterocycle substituents and the C2-aryl side chain that enhance inhibitor potency or inhibitor selectivity.[51–58] With this knowledge of the generalized SAR surrounding these two classes of α-ketoheterocycles and with the benefit of insightful modeling and computational studies[72a] indicating that the active site hydrogen bonding and steric interactions favor a 5-membered versus 6-membered heterocycle, we reexamined the central heterocycle, extending the series to include systems not yet explored.

Oleyl-Based Inhibitors. Even before a systematic examination of the central heterocycle of **2** was undertaken, a small series of additional substituted heterocycles bearing an oleyl acyl side chain were examined beyond those originally disclosed.[53] This included the candidate 1,3,4-oxadiazole and 1,3,4-thiadiazole inhibitors **4a**−**4e** and **5a**−**5c**, respectively, bearing C5 substituents that probe a well-defined trend reflecting the hydrogen bond acceptor capabilities of the substituent (Figure 3). This provided inhibitors that followed the identical substituent trends (K_i for 2-pyr < 2-furyl < 2-thienyl < H) and that exhibited potencies that exceeded (**4a**−**4e**, 1,3,4-oxadiazoles, 2−10 fold) or roughly matched (**5a**−**5c**, 1,3,4-thiadiazoles) those

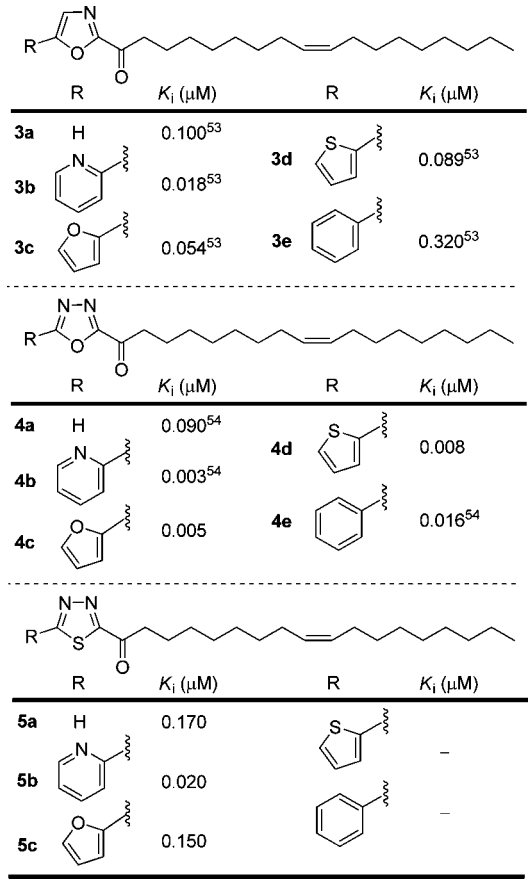


Figure 3. Oleyl-based inhibitors.

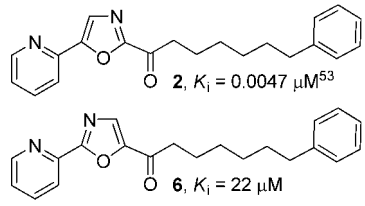


Figure 4. Activity of the isomeric oxazole.

of the corresponding oxazole (**3a**−**3e**).[53] Thus, the introduction of an additional heteroatom into the central heterocycle had a beneficial impact that in the case of the 1,3,4-oxadiazole could be attributed to a reduced destabilizing steric interaction in the active site (position 4 N vs CH) and a lower torsional energy penalty for near coplanar binding of the central heterocycle and its C5 substituent.[72a] These, and related studies,[54] indicated that systematic modifications to the central oxazole of **2** would be productive.

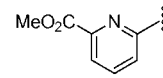
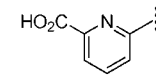
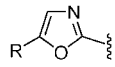
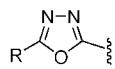
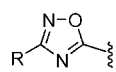
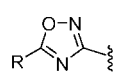
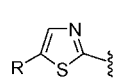
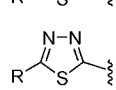
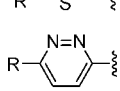
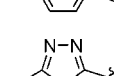
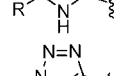
Phenhexyl-Based Inhibitors. The first of the inhibitors examined was the oxazole isomer **6**. Simply switching the location of the oxazole nitrogen atom within **6** resulted in a >4000-fold loss of FAAH inhibition (Figure 4). This result was anticipated based on the reduced electrophilicity of such ketones, and additional members of this class were not examined.

Much more interesting was the behavior observed in the systematic examination of alternative 5-membered heterocycles bearing two or more heteroatoms. In these comparisons, a constant series of representative C5 substituents was examined that permitted an assessment of not only the central heterocycle trends but also served to generalize the substituent trends defined with the oxazole series.[53,56] In principle and because the substituent trends remain analogous across each of the heterocycles, this matrix examination confidently permits the relative extrapolation of the extensive SAR surrounding **2** and the oxazole-based inhibitors onto each of the new α-ketoheterocycles (Figure 5).

Thus, the substituent impact within each heterocycle series was found to follow the well-defined potency order of 2-pyr >2-furanyl >2-thienyl > phenyl, reflecting their relative hydrogen bonding ability[53] and for which the 2-pyr derivatives were 10−20 fold more active than the corresponding phenyl derivative. Moreover, the unsubstituted derivative (R = H) was always less potent than the corresponding 2-pyr derivative but more potent than the corresponding phenyl derivative displaying a K_i that approached or exceeded those of the 2-furanyl or 2-thienyl derivative. Typically, the 2-pyr-6-CO_2Me derivative was less potent than the corresponding 2-pyr derivative and the corresponding 2-pyr-6-CO_2H derivative was 2−10 fold less potent than its ester. However, the results with these latter carboxylic acid derivatives represent K_i's measured at pH 9 under conditions where they are fully ionized destabilizing their active site binding. These K_i's improve when measured at pH 8 and 7.4 (physiological pH); for example, oxazole **7g** was examined and displayed an increase in inhibitory potency with decreasing pH (**7g**: pH = 9, K_i = 20 nM; pH = 8, K_i = 14.5 nM; pH = 7.4, K_i = 10.3 nM). These overall substituent trends are analogous to those defined with the oxazole-based inhibitors (**7** series)[53−58] that includes **2**.

The trends for the heterocycles themselves proved even more interesting. The thiazole-based inhibitors **11** proved significantly less active than the oxazole-based inhibitors **7**, and replacing the position 4 CH of the oxazole or thiazole with a heteroatom uniformly and substantially improved the potency. The most potent of these were the 1,3,4-oxadiazoles **8** and 1,2,4-oxadiazoles **9**, which were typically 10−70 fold more active than the corresponding oxazoles, followed closely by the isomeric 1,2,4-oxadiazoles **10**. Perhaps the most dramatic change in potency occurred between the thiazole **11** and 1,3,4-thiadiazole **12** series, where incorporation of the position 4 nitrogen improved potencies 30−600 fold. As such, the 1,3,4-thiadiazoles **12** typically exceed the potency of the corresponding oxazoles **7**, approaching the activity of the 1,2,4-oxadiazoles **10**, albeit still being less potent than the isomeric 1,2,4-oxadiazoles **9** or 1,3,4-oxadiazoles **8**. Notably, this position 4 heteroatom effect (N > O > CH) is consistent with its role in reducing a destabilizing steric interaction at the active site as well as lowering the torsional energy penalty for coplanar binding of the heterocycle and its aryl C5 substituent.[72] Given that this latter effect is not contributing to the differences observed with the heterocycles when R = H, the result suggests that it is the former steric effect or an as yet unrecognized effect that dominates the differences in binding affinity. Such unrecognized effects could include the introduction of a stabilizing hydrogen bond acceptor site (N > O ≫ CH), heteroatom effects leading to increases in the heterocycle pK_b and intrinsic hydrogen bonding capabilities or even simply productive increases in the intrinsic electron-withdrawing character of the central heterocycle.

Interestingly, the isosteric replacement of sulfur in the 1,3,4-thiadiazoles **12** with CH═CH, providing the corresponding 1,2-diazines **13**, afforded effective but significantly less potent inhibitors (typically 5−50 fold). Nonetheless, such inhibitors were still more effective than the corresponding triazoles **14**. The 1,2,4-triazoles **14** proved inactive, but this behavior represents the destabilizing binding of the deprotonated acidic

heterocycle \ R =	H						
	48 (**7a**)[56]	4.7 (**2**)[53]	12 (**7c**)[53]	55 (**7d**)[53]	80 (**7e**)[56]	8.0 (**7f**)[56]	20 (**7g**)[a,56]
	1.0 (**8a**)	0.29 (**8b**)[54]	0.56 (**8c**)[54]	0.80 (**8d**)	2.2 (**8e**)[54]	3.0 (**8f**)	14 (**8g**)[a]
	1.0 (**9a**)	0.34 (**9b**)	1.0 (**9c**)	1.6 (**9d**)	–	3.0 (**9f**)	13 (**9g**)[a]
	7.0 (**10a**)	1.1 (**10b**)	8.5 (**10c**)	44 (**10d**)		7.0 (**10f**)	53 (**10g**)[a]
	800 (**11a**)	24 (**11b**)	1500 (**11c**)	2000 (**11d**)	–	500 (**11f**)	10,000 (**11g**)[a]
	1.4 (**12a**)	0.80 (**12b**)	7.4 (**12c**)	27 (**12d**)	–	7.7 (**12f**)	50 (**12g**)[a]
	140 (**13a**)	25 (**13b**)	40 (**13c**)	46 (**13d**)	–	110 (**13f**)	360 (**13g**)[a]
	>1000 (**14a**)[a]	>100000 (**14b**)[a]	>1000 (**14c**)[a]	>1000 (**14d**)[a]	–	–	–
	>1000 (**15a**)[a]	1.1 (**15b**)	5.0 (**15c**)	8.5 (**15d**)	16 (**15e**)	–	–

[a]Deprotonated under the pH = 9 assay conditions, see text.

Figure 5. Inhibitors containing alternative central heterocycles, K_i (nM).

heterocycle under the pH 9 assay conditions resulting from the C2 acyl substitution (Figure 6).

One of the most interesting series to emerge from these studies was the tetrazoles **15**. In addition to being the most electron-deficient of the heterocycles examined, position 4 (oxazole numbering) incorporates a N (N > O > CH), and all three accessible sites on the heterocycle constitute potential hydrogen bond acceptor sites (N). Consistent with these features, the tetrazoles **15** proved substantially more active than the oxazoles **7**, comparable in potency with the 1,2,4-oxadiazoles **10**, albeit not quite as potent as the isomeric 1,2,4-oxadiazoles **9** or 1,3,4-oxadiazoles **8**. Clearly, there are subtle and unrecognized effects that account for the small differences in binding affinity between the most potent classes of heterocycles (**8**, **9** > **10**, **12**, **15**) that in turn are easily distinguished from the less active series (**13** > **11** > **14**). These latter studies with the tetrazoles seems to suggest that the most potent activity within the 5-membered heterocycles is observed with those that incorporate a hydrogen bonding acceptor N and O (vs N and N) bracketing the electrophilic carbonyl attachment site. This may account for the subtly more potent activity of the 1,3,4-oxadiazoles **8** and 1,2,4-oxadiazoles **9** (N and O) relative to the isomeric 1,2,4-oxadiazoles **10** and the comparable tetrazoles **15** (N and N). Each incorporate a heteroatom at the oxazole position 4 that enhance their activity relative to the oxazole series, and the results with the tetrazole series **15** suggest the subtle differences in the **7**−**15** series has more to do with the location of the oxygen atom than a preference for an added nitrogen versus oxygen at the oxazole position 4. The exception to the behavior in the tetrazole series **15** is the unsubstituted derivative **15a**. This unsubstituted and acidic central heterocycle, like the 1,2,4-triazole series **14**, is deprotonated under the pH 9

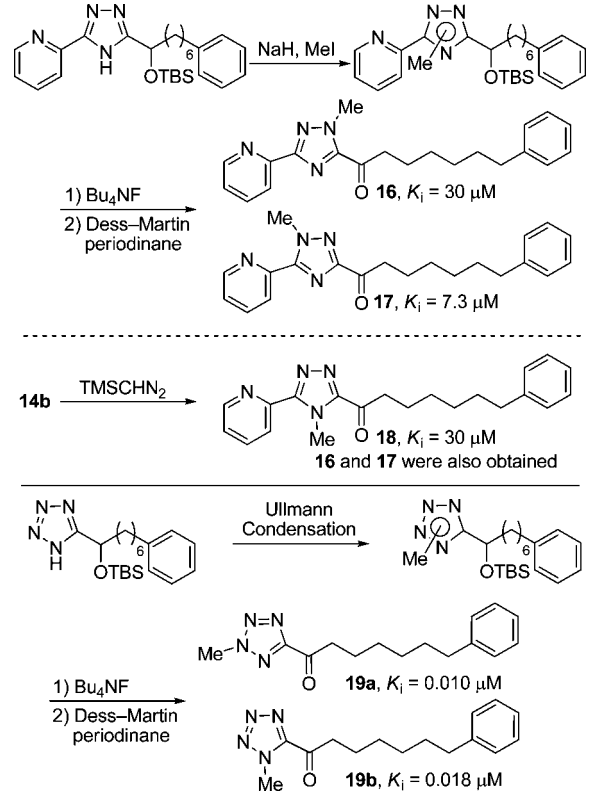


Figure 6. Effect of *N*-methylation on inhibitor potency of 1,2,4-triazoles and tetrazoles.

assay conditions, destabilizing active site binding or its activation of the reversible hemiketal formation with the otherwise

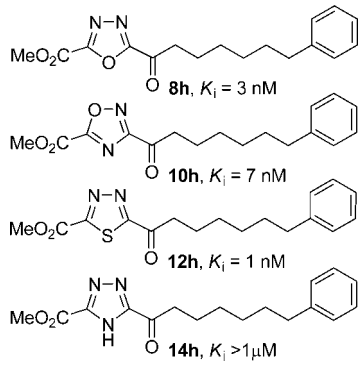


Figure 7. Methyl ester substituted α-ketoheterocycle inhibitors of FAAH.

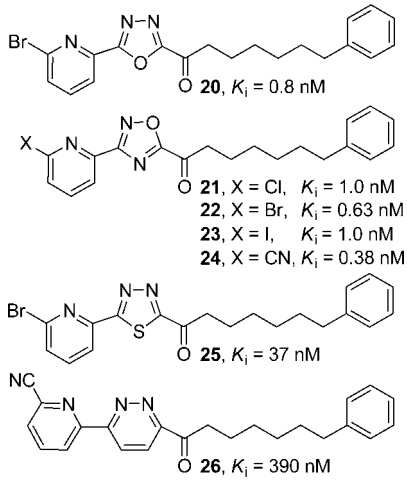


Figure 8. Additional C6 substituted 2-pyridyl substituents.

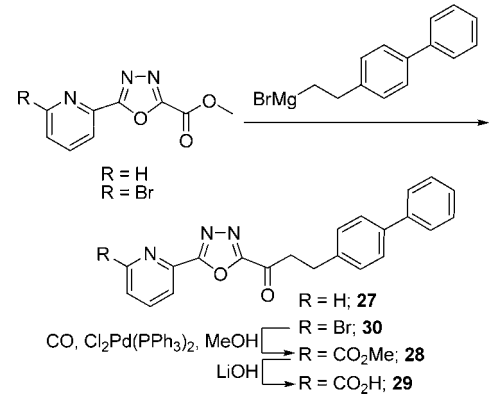
electrophilic carbonyl. However, simple methylation of **15a** rendered the isomeric 2- and 1-methyltetrazole derivatives **19a** and **19b**, respectively, incapable of this deprotonation and which now displayed FAAH inhibition at levels consistent with this series (Figure 6). Notably, the isomer assignments for **16**−**19** were made by HMBC (long-range proton−carbon correlation) NMR analysis.

An additional smaller series was examined in which a methyl ester substituent was placed directly on the heterocycle (Figure 7). Such candidate FAAH inhibitors in the oxazole-based series typically display activity reflecting the strength of the conjugated electron-withdrawing substituent[55,58] but lack the intrinsic enzyme selectivity observed with **2** and related inhibitors.[58] With the exception of the acidic triazole **14h**, which was inactive presumably because of its deprotonation under the assay conditions, this substitution with **8h**, **10h**, and **12h** provided potent FAAH inhibitors (K_i < 10 nM). However and unlike the behavior of the corresponding oxazole,[56] in each case the activity approached but did not exceed that of the unsubstituted heterocycles (**8a**, **10a**, and **12a**).

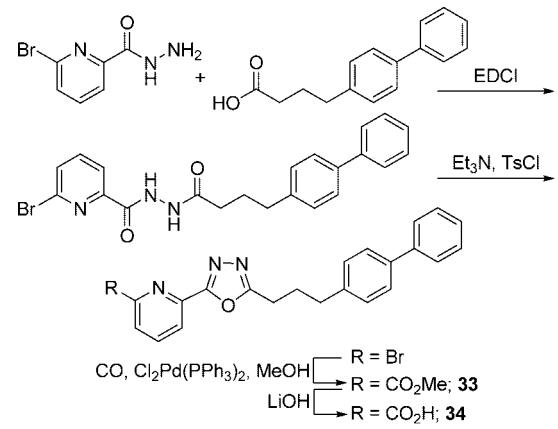
A final series of inhibitors incorporating a substituted pyridine substituent on the central heterocycle was examined and constituted potential synthetic intermediates en route to **8f**−**13f** (Figure 8). Each displayed activity that approached the unsubstituted pyridine derivative (**b** series in Figure 5) or, in the case of **24** and **26**, the corresponding methyl esters **9f** and **13f**.

Biphenylethyl-Based Inhibitors. After having established that several central heterocycle replacements further improve on the potency of the oxazole-based inhibitors and that its trends in the substituent effects carry forward onto these additional

Scheme 7



Scheme 8



α-ketoheterocycles, we sought to establish that analogous modifications or substitutions in the C2 acyl side chain would be possible. Thus, a small preliminary series of key 1,3,4-oxadiazoles bearing the conformationally restricted biphenylethyl side chain was prepared (Scheme 7) and examined (Figure 9). Analogous to trends observed in the oxazole-based series,[58] the replacement of the phenhexyl side chain with the biphenylethyl side chain maintained or further enhanced the extraordinary potency of the candidate inhibitors (e.g., K_i of **27** = 300 pM). Such studies continue and will be disclosed in due course.

The 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 often prepared en route to the α-ketoheterocycles and simply entailed examination of this alcohol intermediate. The candidate 1,3,4-oxadiazole inhibitors bearing a methylene were prepared by simple dehydrative ring closure of the corresponding diacylhydrazide as the key step (Scheme 8). 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

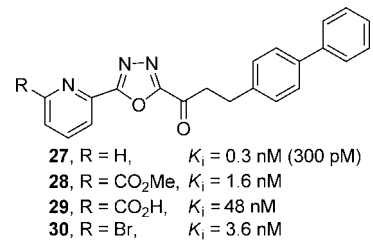
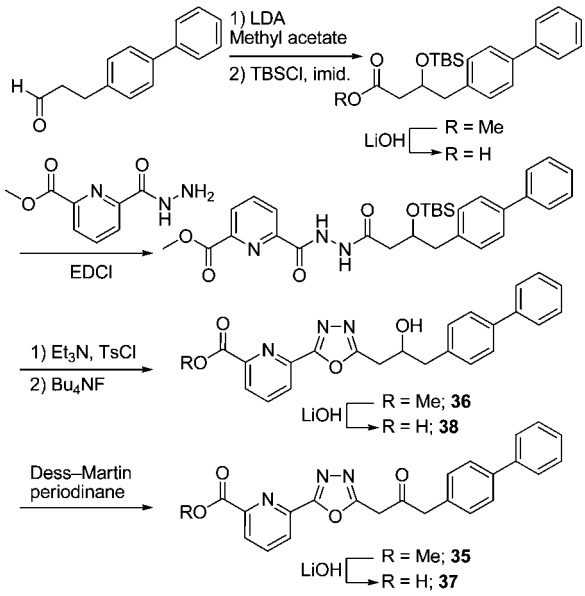


Figure 9. Biphenylethyl-based inhibitors.

Scheme 9



be ≥10000-fold less active than the corresponding ketone, Figure 10. Although not investigated in detail for the inhibitors disclosed herein, even this level of reduced inhibition (0.01% 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 the assay.[56] Important in these comparisons is the fact that the ketone is essential to the potent activity of the inhibitors and that reduction to an alcohol or removal altogether leads to $\geq 10^4$ reductions in activity.

The results of an additional and unique series that were prepared (Scheme 9) and examined are summarized in Figure 11. These constitute ketone inhibitors with a methylene inserted between the reactive carbonyl and heterocycle in efforts to establish whether the inductive electron-withdrawing properties of the electron-deficient heterocycle would be sufficient to activate the carbonyl for active site hemiketal formation. Unlike the potent activity of the α-ketoheterocycles, these *β*-ketoheterocycles and their corresponding alcohols were found to be inactive against FAAH.

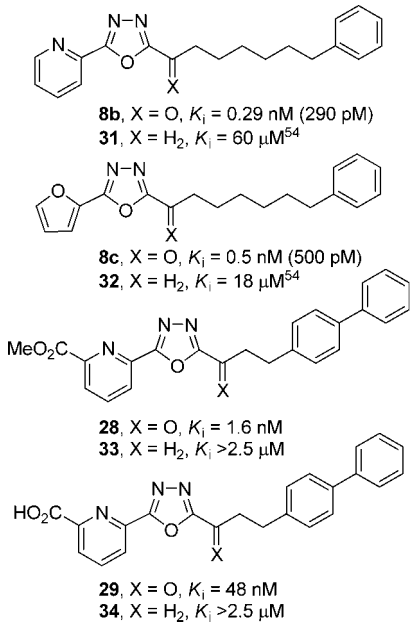


Figure 10. Effect of the electrophilic carbonyl.

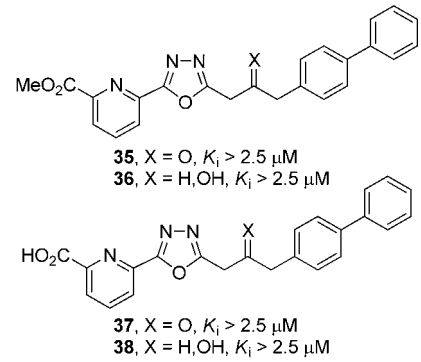


Figure 11. *β*-Keto-1,3,4-oxadiazoles.

compd	K_i, μM (human)	K_i, μM (rat)
7b	0.0090	0.0047
7g	0.026	0.020
8b	0.0012	0.00029
8g	0.037	0.014
9b	0.00092	0.00034
10b	0.016	0.0011
10g	0.110	0.053
12b	0.003	0.0008
15b	0.0055	0.0011

Figure 12. Inhibition of recombinant human fatty acid amide hydrolase.

Inhibition of Recombinant Human FAAH. Rat and human FAAH are very homologous (82% sequence identity),[2] exhibit near identical substrate selectivity and inhibitor sensitivity in our studies disclosed to date and embody an identical amidase 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 were found to exhibit the same relative and absolute potencies consistent with previous observations (Figure 12).

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,[31,73] and studies have shown that the α-ketoheterocycle class of inhibitors can be exquisitely selective for FAAH. However, two enzymes did emerge as potential competitive targets: triacylglycerol hydrolase (TGH) and a previously uncharacterized membrane-associated hydrolase that lacked known substrates or function (KIAA1363).[74] 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 13 are the results of the selectivity screening of selected candidate inhibitors. In general, the inhibitors were selective for FAAH over TGH and 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 the unsubstituted heterocycles (**7**−**13a**) were selective for FAAH over KIAA1363 but were typically more selective for TGH over FAAH (1−125-fold). The potent inhibitors incorporating even the small methyl ester substituent (**7**−**12h** series) on the heterocycle were found to be selective for FAAH versus KIAA1363 (>100-fold) and now

	FAAH K_i, μM	FAAH IC_{50}, μM	KIAA1363 IC_{50}, μM	TGH IC_{50}, μM
Oxazole series				
7a R = H	0.048	2.5	20 (8)	0.02 (0.008)[56]
2 R = 2-pyr	0.0047	0.002	>100 (>50000)	0.6 (300)[53]
7c R = 2-furanyl	0.012	0.08	>100 (>1250)	0.3 (4)[53]
7d R = 2-thiophenyl	0.055	0.8	>100 (>125)	0.2 (0.25)[53]
7e R = Ph	0.08	0.9	>100 (>110)	0.7 (0.8)
7f R = 2-pyr-6-CO_2Me	0.008	0.17	40 (240)	1.0 (6)
7g R = 2-pyr-6-CO_2H	0.02	0.1	>100 (>1000)	>100 (>1000)[56]
7h R = CO_2Me	0.0009	0.03	>100 (>3300)	0.3 (10)
1,3,4-Oxadiazole series				
8a R = H	0.001	0.016	>100 (>6250)	0.025 (1.5)
8b R = 2-pyr	0.00029	0.001	90 (90000)	0.14 (140)[54]
8c R = 2-furanyl	0.00056	0.001	>100 (>10^5)	0.08 (80)[54]
8e R = Ph	0.0022	0.025	>100 (>4000)	0.25 (10)[54]
8f R = 2-pyr-6-CO_2Me	0.003	0.008	>100 (>12500)	0.04 (5)
8g R = 2-pyr-6-CO_2H	0.014	0.1	>100 (>1000)	40 (400)
8h R = CO_2Me	0.001	1.0	>100 (>100)	2.0 (2)
1,2,4-Oxadiazole series 9				
9a R = H	0.001	0.004	25 (6250)	0.0007 (0.18)
9b R = 2-pyr	0.00034	0.003	30 (10000)	0.03 (10)
9f R = 2-pyr-6-CO_2Me	0.003	0.003	40 (13000)	0.15 (50)
9g R = 2-pyr-6-CO_2H	0.013	0.04	>100 (>2500)	40 (1000)
1,2,4-Oxadiazole series 10				
10a R = H	0.007	0.5	>100 (>200)	0.01 (0.02)
10b R = 2-pyr	0.0011	0.007	>100 (>14000)	0.4 (60)
10f R = 2-pyr-6-CO_2Me	0.007	0.3	>100 (>330)	3.0 (10)
10g R = 2-pyr-6-CO_2H	0.053	0.07	>100 (>1400)	>100 (>1400)
10h R = CO_2Me	0.007	0.01	>100 (>10000)	0.2 (20)
Thiazole series				
11a R = H	0.8	>100	>100 (1)	2 (<0.02)
11b R = 2-pyr	0.024	2	>100 (>50)	8 (4)
11f R = 2-pyr-6-CO_2Me	0.5	>100	>100 (1)	40 (<0.4)
11g R = 2-pyr-6-CO_2H	10	8	>100 (>12)	30 (4)
1,3,4-Thiadiazole series				
12a R = H	0.0014	0.3	>100 (>330)	0.1 (1)
12b R = 2-pyr	0.0008	0.03	>100 (>3300)	4.5 (150)
12f R = 2-pyr-6-CO_2Me	0.0077	0.8	>100 (>125)	0.8 (1)
12g R = 2-pyr-6-CO_2H	0.050	3	>100 (>33)	>100 (>33)
12h R = CO_2Me	0.001	0.06	>100 (>1700)	11 (180)
1,2-Diazine series				
13a R = H	0.14	3	>100 (>30)	5 (1)
13b R = 2-pyr	0.025	6	>100 (>20)	>100 (>20)
13f R = 2-pyr-6-CO_2Me	0.110	10	>100 (>10)	>100 (>10)
13g R = 2-pyr-6-CO_2H	0.360	20	>100 (>5)	>100 (>5)
13h R = 2-pyr-6-CN	0.390	13	>100 (>8)	12 (1)
Tetrazole series				
15a R = Me	0.010	0.6	60 (100)	0.08 (0.13)
15b R = 2-pyr	0.0011	0.003	>100 (>30000)	0.12 (40)

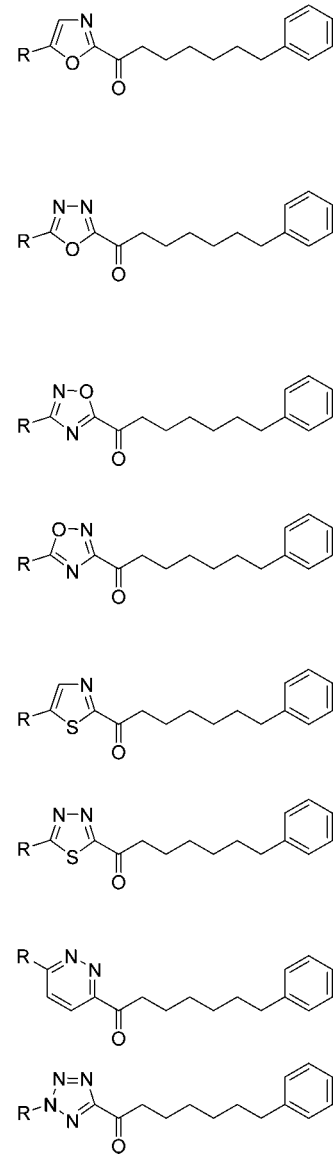


Figure 13. Activity based protein profiling of α-ketoheterocycles.

modestly selective for FAAH versus TGH (2−200-fold). In line with prior observations, the addition of a 2-pyridyl substituent (**7**−**15b** series) increased not only the FAAH potency but also FAAH selectivity such that the most potent inhibitors failed to inhibit KIAA1363 (>10^4-fold selective) and are now typically 4−300-fold selective for FAAH versus TGH. Addition of a C6-carboxylic acid to the 2-pyridyl substituent (**7**−**15g** series) further enhanced this intrinsic selectivity such that the resulting inhibitors are typically no longer viable competitive inhibitors of KIAA1363 or TGH (Figures 13 and 14). Notably, many of the central heterocycle features and added substituents that were found to increase FAAH potency were also found to enhance FAAH selectivity by simultaneously disrupting KIAA1363 and TGH affinity.

Conclusions

Herein, we report the synthesis and evaluation of a key series of α-ketoheterocycles based on **2** incorporating systematic changes in the central heterocycle that provided extraordinarily potent (K_i = 300 pM) inhibitors of fatty acid amide hydrolase. The nature of the central heterocycle, even with the modest and systematic perturbations explored herein, significantly influenced the inhibitor activity that can be defined as 1,3,4-oxadiazoles (50) and 1,2,4-oxadiazoles **9** (50) > tetrazoles (5), the isomeric 1,2,4-oxadiazoles **10** (7), 1,3,4-thiadiazoles (2−30) > oxazoles including **2** (rel activity = 1) > 1,2-diazines (0.3) > thiazoles (0.06) > 1,3,4-triazoles (−). Most evident in the observations is that the introduction of an additional position 4 heteroatom (oxazole numbering) substantially increases the activity (N > O > CH) that may be attributed in part to a reduced destabilizing steric interaction at the FAAH active site. Additionally the position 1 and 3 requisite heteroatoms exhibit a N,O > N,N > N,S preference that is reflected in the observed heterocycle trends. Within each heterocycle series, the impact of an added substituent was systematically explored and was found to follow well defined trends first observed with **2**. Exemplifying these effects, additional aryl substituents (e.g., 2-pyr or 2-pyr-6-CO_2H) placed on the central heterocycle increase FAAH potency 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 substituent substantially impacts the selectivity of the FAAH inhibitors, and these trends proved general across the range of inhibitors examined to date.[53–58] This is especially evident with the TGH selectivity for the unsubstituted heterocycles (**7**−**13a** series, 1−100-fold selective for TGH vs FAAH), which can be improved by the independent choice of the C2 side chain (compare **2**)[58] as well as the heterocycle substituent (compare **7**−**13a** vs **7**−**15b** and **7**−**15g**). A combination of these independent features which simultaneously improve FAAH potency and disrupt TGH or KIAA1363 binding provide exquisitely selective and potent FAAH inhibitors.

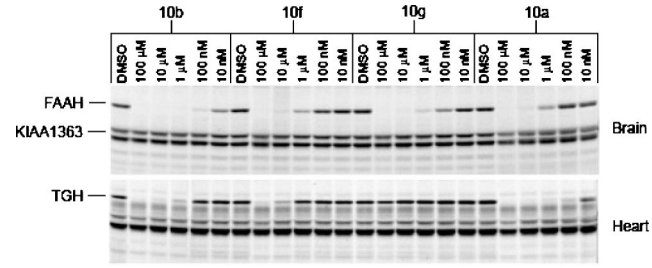


Figure 14. Activity based protein profiling of FAAH inhibitors **10b**, **10f**, **10g**, **10a** with FP-Rh in brain and heart membrane proteome. Enzyme targets such as FAAH, KIAA1363, and TGH are highlighted.

Experimental Section

7-Phenyl-1-(2-(pyridin-2-yl)-2*H*-tetrazol-5-yl)-heptan-1-one (15b). A solution of 7-phenylheptanal (1.93 g, 0.01 mol) in MeCN (64 mL) was treated with KCN (2.65 g, 0.04 mol), ZnI_2 (77.7 mg, 0.0002 mol), and TBSCl (3.06 g, 0.02 mol) under Ar at room temperature. The reaction mixture was stirred vigorously and the progress monitored by TLC (SiO_2, 5% EtOAc−hexanes). After 72 h, the solvent was removed in vacuo and the residue resuspended in Et_2O. The salts were removed by filtration and rinsed thoroughly with Et_2O. The filtrate was washed with water, dried over Na_2SO_4, and concentrated in vacuo to give a yellow oil that was purified by flash chromatography (SiO_2, 4 × 25 cm, 1% EtOAc−hexanes) to afford 2-(*tert*-butyldimethylsilyloxy)-8-phenyloctanitrile (2.6 g, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.30 (m, 2H), 7.20 (m, 3H), 4.43 (t, 1H, J = 6.4 Hz), 2.63 (t, 2H, J = 7.7 Hz), 1.80 (m, 2H), 1.65 (m, 2H), 1.49 (m, 2H), 1.39 (m, 4H), 0.94 (s, 9H), 0.21 (s, 3H), 0.16 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 142.7, 128.5, 128.4, 125.7, 120.2, 62.0, 36.4, 36.0, 31.4, 29.1, 28.9, 25.6, 24.6, 18.2 (3C), −5.0, −5.2. HRMS-ESI-TOF *m*/*z* 354.2221 ([M + Na]$^+$, $C_{20}H_{33}NOSi$ requires 354.2223).

A sample of 2-(*tert*-butyldimethylsilyloxy)-8-phenyloctanitrile (335 mg, 1.01 mmol) was dissolved in a mixture of 2-propanol (1.4 mL):water (2.9 mL). NaN_3 (197 mg, 3.04 mmol) and ZnBr (250 mg, 1.11 mmol) were added to the reaction mixture as solids, which was subsequently warmed at 100 °C for 90 h. Upon disappearance of starting material, the solution was cooled to room temperature and diluted with EtOAc. Then 2 N HCl was added to the reaction mixture, which was stirred until all solids dissolved. The organic layer was isolated, and the aqueous layer was washed several times with EtOAc. The combined organic phases were dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford 7-phenyl-1-(2*H*-tetrazol-5-yl)-heptan-1-ol as a colorless oil that was used without further purification (270 mg, quant). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.25 (m, 2H), 7.15 (m, 3H), 5.26 (m, 1H), 2.57 (t, 2H, J = 7.7 Hz), 2.02 (m, 1H), 1.90 (m, 1H), 1.59 (m, 2H), 1.44 (m, 2H), 1.34 (m, 4H). HRMS-ESI-TOF *m*/*z* 261.1707 ([M + H]$^+$, $C_{14}H_{20}N_4O$ requires 261.1710).

A solution of 7-phenyl-1-(2*H*-tetrazol-5-yl)-heptan-1-ol (36 mg, 0.14 mmol), TBSCl (63 mg, 0.42 mmol), and imidazole (28 mg, 0.42 mmol) in DMF (0.7 mL) was stirred at room temperature for 72 h before it was diluted with EtOAc and washed with H_2O and saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford the crude material that was purified by flash chromatography (SiO_2, 1.5 × 15 cm, 10% acetone−hexanes) to afford 5-(1-(*tert*-butyldimethylsilyloxy)-7-phenylheptyl)-2*H*-tetrazole (39 mg, 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.26 (dd, 2H, J = 6.3, 8.4 Hz), 7.16 (m, 3H), 5.25 (t, 1H, J = 5.8 Hz), 2.57 (t, 2H, J = 7.7 Hz), 1.85 (m, 2H), 1.58 (m, 2H), 1.31 (m, 6H), 0.90 (s, 9H), 0.13 (s, 3H), 0.01 (s, 3H).

In a gas-tight vessel, a solution of 5-(1-(*tert*-butyldimethylsilyloxy)-7-phenylheptyl)-2*H*-tetrazole (16 mg, 0.043 mmol), 2-iodopyridine (7 μL, 0.064 mmol), CuI (1 mg, 0.004 mmol), K_2CO_3 (12 mg, 0.085 mmol), and *N*,*N*-dimethylethylene diamine (1 μL, 0.006 mmol) in DMF (200 μL) was purged with Ar and sealed. The reaction mixture was warmed at 100 °C for 18 h before it was cooled to room temperature, diluted with EtOAc, and washed with H_2O, 9:1 NH_4OH:saturated aqueous NH_4Cl, and saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford the crude material that was purified by flash chromatography (SiO_2, 1.5 × 15 cm, 5−10% acetone−hexanes) to afford 2-(5-(1-(*tert*-butyldimethylsilyloxy)-7-phenylheptyl)-2*H*-tetrazol-2-yl)-pyridine (4.0 mg, 21%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.69 (dd, 1H, J = 0.9, 4.8 Hz), 8.16 (d, 1H, J = 7.5 Hz), 7.98 (dt, 1H, J = 1.8, 7.9 Hz), 7.48 (m, 1H), 7.26 (m, 2H), 7.16 (m, 3H), 5.16 (dd, 1H, J = 5.9, 7.4 Hz), 2.58 (t, 2H, J = 7.7 Hz), 1.99 (m, 2H), 1.60 (m, 2H), 1.34 (m, 6H), 0.88 (s, 9H), 0.10 (s, 3H), −0.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 169.7, 149.6, 149.0, 142.9, 139.5, 128.5, 128.4, 125.7, 125.0, 115.2, 67.4, 37.5, 36.1, 31.5, 29.3, 25.9 (3C), 25.4, 18.4, −4.1, −4.8. HRMS-ESI-TOF *m*/*z* 452.2823 ([M + H]$^+$, $C_{25}H_{37}N_5OSi$ requires 452.2840).

2-(5-(1-(*tert*-Butyldimethylsilyloxy)-7-phenylheptyl)-2*H*-tetrazol-2-yl)-pyridine (4.4 mg, 0.009 mmol) was dissolved in THF (122 μL), treated with Bu_4NF (1 M in THF, 0.013 mL, 0.013 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 that was purified by flash chromatography (SiO_2, 0.5 × 4 cm, 20−50% EtOAc−hexanes) to afford 7-phenyl-1-(2-(pyridin-2-yl)-2*H*-tetrazol-5-yl)-heptan-1-ol (2.7 mg, 79%) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.69 (dd, 1H, J = 1.1, 4.7 Hz), 8.18 (d, 1H, J = 8.1 Hz), 8.00 (m, 1H), 7.50 (ddd, 1H, J = 0.7, 4.8, 7.4 Hz), 7.26 (m, 2H), 7.16 (m, 3H), 5.16 (m, 1H), 2.59 (t, 2H, J = 7.7 Hz), 2.48 (d, 1H (−OH), J = 6.1 Hz), 2.07 (m, 2H), 1.61 (m, 2H), 1.52 (m, 2H), 1.40 (m, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 169.5, 149.6, 148.8, 142.9, 139.6, 128.5, 128.4, 125.7, 125.3, 115.3, 67.0, 36.7, 36.1, 31.5, 29.3 (2C), 25.2. HRMS-ESI-TOF *m*/*z* 338.1966 ([M + H]$^+$, $C_{19}H_{23}N_5O$ requires 338.1975).

7-Phenyl-1-(2-(pyridin-2-yl)-2*H*-tetrazol-5-yl)-heptan-1-ol (2.7 mg, 0.007 mmol) was dissolved in CH_2Cl_2 (0.24 mL), and Dess−Martin periodinane (4.5 mg, 0.011 mmol) was added. The mixture was stirred at room temperature for 2 h before the reaction mixture was reduced to half-volume, and this mixture was directly loaded onto silica gel and purified by flash chromatography (SiO_2, 0.5 × 4 cm, 10−30% EtOAc−hexanes) to afford 7-phenyl-1-(2-(pyridin-2-yl)-2*H*-tetrazol-5-yl)-heptan-1-one (**15b**, 2.7 mg, 99%) as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.74 (m, 1H), 8.23 (m, 1H), 8.05 (dt, 1H, J = 1.6, 7.9 Hz), 7.57 (dd, 1H, J = 4.8, 7.5 Hz), 7.27 (t, 2H, J = 7.6 Hz), 7.17 (m, 3H), 3.26 (t, 2H, J = 7.4 Hz), 2.61 (t, 2H, J = 7.7 Hz), 1.83 (m, 2H), 1.64 (m, 2H), 1.43 (m, 4H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.5, 162.5, 149.9, 148.6, 142.8, 139.8, 128.5, 128.4, 126.0, 125.8, 115.9, 41.0, 36.0, 31.4, 29.1, 29.0, 23.5. HRMS-ESI-TOF *m*/*z* 336.1813 ([M + H]$^+$, $C_{19}H_{21}N_5O$ requires 336.1819). Purity 99%.

FAAH Inhibition. ^{14}C-labeled oleamide was prepared from ^{14}C-labeled oleic acid as described.[13] The truncated rat FAAH (rFAAH) was expressed in *E. coli* and purified as described.[71] 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 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 described.[13] In brief, the enzyme reaction was initiated by mixing 1 nM of rFAAH (800, 500, or 200 pM rFAAH for inhibitors with $K_i \leq 1-2$ nM) with 10 μM of ^{14}C-labeled oleamide in 500 μL of reaction buffer (125 mM TrisCl, 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, reversible inhibition.[53]

Selectivity Screening. The selectivity screening was conducted as 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 disclosed. This material is available free of charge via the Internet at http://pubs.acs.org.

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