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Delineation of a Fundamental α-Ketoheterocycle Substituent Effect for Use in the Design of Enzyme Inhibitors

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Anandamide[1] (**1a**) and oleamide[2−4] (**1b**) have emerged as the prototypical members of a class of endogenous fatty acid amides that serve as chemical messengers (Figure 1). Anandamide binds and activates the central (CB1) and peripheral (CB2) cannabinoid receptors where it has been implicated in the modulation of nociception, feeding, and anxiety.[5] Oleamide was found to accumulate in the cerebrospinal fluid of animals under conditions of sleep deprivation and induces physiological sleep in a dose-dependent manner.[3,4] The pharmacological action of both anandamide and oleamide is terminated by the enzyme fatty acid amide hydrolase (FAAH, Figure 1).[6−9] It degrades neuromodulating fatty acid amides at their site of action and is currently the only characterized mammalian enzyme that is in the amidase signature family bearing an unusual catalytic Ser-Ser-Lys triad.[6−9] As such, FAAH has emerged as an exciting therapeutic target for a range of clinical disorders.[5,10]

One class of FAAH inhibitors that exhibit potent and selective enzyme inhibition and in vivo efficacy is the α-ketoheterocycles.[11−16] Since their introduction by Edwards,[17−19] the use of such α-ketoheterocycles has emerged as a powerful design concept for the development of inhibitors of serine and cysteine proteases and hydrolases.[20] Possessing electrophilic carbonyls, they reversibly form enzyme-stabilized covalent hemiketals or hemithioketals with the enzyme catalytic nucleophile analogous to more conventional aldehyde,[21] trifluoromethyl ketone,[22] or α-keto ester[23] and amide inhibitors.[24] By virtue of the ability of the heterocycle to hydrogen bond to the adjacent hemiketal of the enzyme adduct[17−19] and because of interactions of the heterocycle itself with the enzyme active site independent of its role in activating the carbonyl,[13] they offer advantages over the simpler predecessors. Although the potency of such α-ketoheterocycles has been anticipated to be related to the intrinsic electron-withdrawing properties of the heterocycles,[17−19] attempts to draw such correlations are weak, accompanied by deviations from expectations, and the more potent heterocycles in a series are empirically derived.[25]

Herein, we report the synthesis and evaluation of a series of 5-substituted 7-phenyl-1-(oxazol-2-yl)heptan-1-ones that define an alternative and fundamental α-ketoheterocycle substituent effect that led to the discovery of FAAH inhibitors with K_i's as low as 400 pM. Its intrinsic basis, which relates K_i with the Hammett σ_p constant of the substituent, as well as the magnitude of the effect ($\rho = 3.01$), and its predictive value ($R^2 = 0.91$) suggest a widespread applicability in studies beyond FAAH inhibition.

Key to the divergent synthesis of the inhibitors was the preparation of intermediate **3**[11] from which all the compounds could be derived (Scheme 1). Intermediate **3** was obtained by Vedejs oxazole metalation,[26] condensation with 7-phenylheptanal, and TBS protection of the resulting alcohol. Selective C5 lithiation[27] of **3** followed by treatment with various electrophiles (CO_2(g), CF_3CONMe_2, CH_3CONMe_2, DMF, I_2, Br_2, NCS, *N*-fluorobenzenesulfonimide, CH_3I, $(MeS)_2$) afforded **4b**, **4f**−**h**, **4j**, and **4l**−**p**, many of which served as precursors to additional candidate inhibitors bearing further modified C5 substituents. Carboxylic acid **5b** was directly converted to its corresponding methyl ester **5c** by treatment with $TMSCHN_2$. The ester **5c** was converted to the carboxamide **5d** by treatment with methanolic ammonia, which, in turn, was dehydrated with TFAA and pyridine to provide nitrile **5i**. Using a method developed by Chen et al., iodide **4l** was transformed to **4k** ($FSO_2CF_2CO_2CH_3$, CuI) bearing a C5 trifluoromethyl substituent.[28,29] In each case, deprotection of the TBS ether followed by Dess−Martin periodinane oxidation[30] of the liberated alcohol yielded the corresponding α-ketoheterocycles.

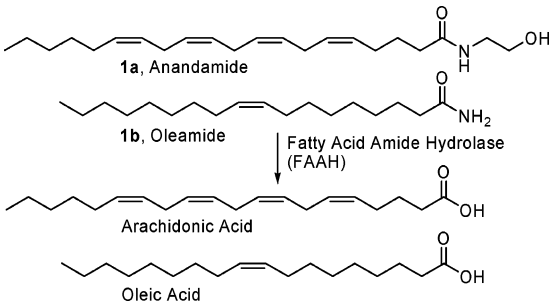
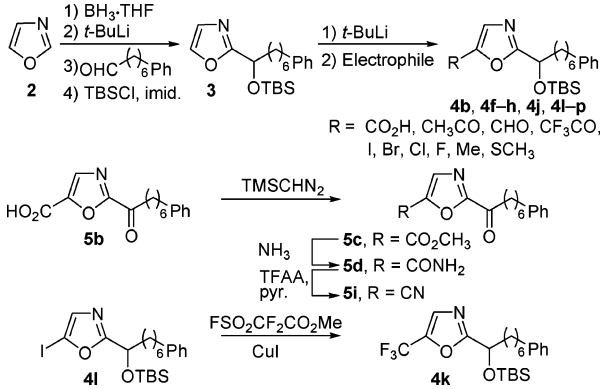


Figure 1. Substrates of fatty acid amide hydrolase (FAAH).

Scheme 1



This series, which constitutes a set of relatively small substituents that can occupy accessible space in the FAAH active site, exhibited FAAH inhibition that tracked with the electron-withdrawing properties of the substituents (Figure 2). A plot of the inhibition ($-\log K_i$) versus the Hammett σ_p constant for the substituents (Figure 3) was found to follow a well-defined correlation ($\rho = 3.01$, $R^2 = 0.91$). In addition, this substituent effect was established to be large ($\rho = 3.01$), resulting in a 1000-fold increase in K_i per unit change in σ_p and indicating that the electronic character of the substituent is the dominant factor contributing to the differences in binding affinity. Presumably, this arises from the increased electrophilic

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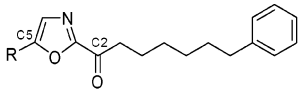

compd	R	K_i, nM	compd	R	K_i, nM
5a	–H	48	**5i**	–CN	0.4
5b	$-CO_2H$	30	**5j**	$-CH_3$	80
5c	$-CO_2CH_3$	0.9	**5k**	$-CF_3$	0.8
5d	$-CONH_2$	5	**5l**	–I	3
5e	$-CONMe_2$	2	**5m**	–Br	3
5f	$-COCH_3$	2	**5n**	–Cl	5
5g	–CHO	6	**5o**	–F	30
5h	$-CF_3CO$	3.5	**5p**	$-SCH_3$	25

Figure 2. FAAH inhibition. K_i measurement errors are provided in Supporting Information.

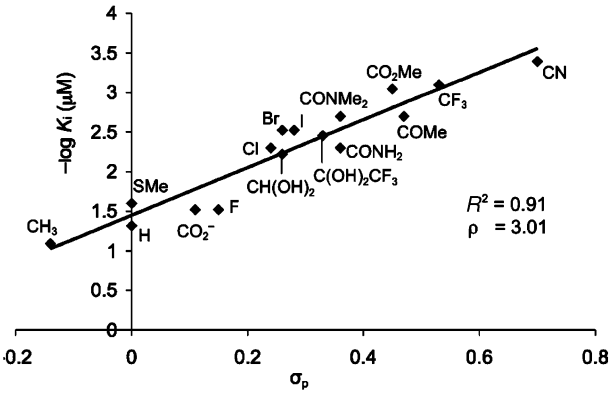

Figure 3. Value of $-\log K_i$ (μM) versus σ_p.

character of the C2 carbonyl imparted by the electron-withdrawing C5 substituent that leads to an increased strength of the covalent bond formed with the catalytic Ser241 OH, thereby enhancing the stability of the tetrahedral adduct and lowering the K_i value.[31] The definition of this fundamental relationship between the K_i and substituent property (σ_p) permits the prediction of an expected K_i. For example, we can assert that the carboxylic acid **5b** binds FAAH as the carboxylate anion ($-CO_2^-$ vs $-CO_2H$, $\sigma_p = 0.11$ vs 0.44) under the conditions of the assay (from the K_i value, pH 9). Even more interestingly, we are able to establish that both the aldehyde **5g** and trifluoromethyl ketone **5h** exist in solution as gem diols (at C5, but not C2; ^1H and ^{13}C NMR) and inhibit the enzyme with potencies ($K_i = 6$ and 3.5 nM) at a level more consistent with this C5 substituent gem diol versus carbonyl active site binding and providing the first σ_p estimates for such substituents (0.26 for $CH(OH)_2$ and 0.33 for $C(OH)_2CF_3$).[32] That is, the correlation between σ_p and K_i is sufficiently dependable that deviations from expectations can be utilized to establish features of active site binding that are not a priori known. Similarly, with this correlation in hand, two of the more potent inhibitors in Figure 2 (**5f** and **5g**) were retrospectively prepared and examined based on this relationship. Notably, **5c**, **5i**, and **5k** bearing the strongest electron-withdrawing substituents display subnanomolar FAAH inhibitory potency. While additional substituent features can and will further modulate the binding affinity of the candidate inhibitors (e.g., H-bonding, hydrophobic or steric interactions),[33] the magnitude of the electronic effect of the substituent ($\rho = 3.01$) on the activity of a conjugated α-ketoheterocycle (K_i) suggests the latter will dominate, especially with small and simple substituents.

The delineation of a fundamental correlation that relates the Hammett σ_p constant of a substituent with its enzyme inhibition ($-\log K_i$) and the magnitude of the effect ($\rho = 3.01$) provides a useful new predictive tool for the rational design of serine and cysteine protease and hydrolase inhibitors.

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

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(31) A reviewer has suggested that this may also arise from an increased H-bond capability of the oxazole nitrogen known to stabilize such enzyme adducts.
(32) An anomalous σ_p of 0.22 is occasionally reported for −CHO (vs 0.42) that may more accurately reflect the analogous but unrecognized gem diol.
(33) An example is provided in Supporting Information.

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