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Heterocyclic sulfoxide and sulfone inhibitors of fatty acid amide hydrolase

Wu Du, Christophe Hardouin, Heng Cheng, Inkyu Hwang and Dale L. Boger*

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

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Abstract—A novel series of heterocyclic sulfoxides and sulfones was prepared and examined as potential inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for inactivation of neuromodulating fatty acid amides including anandamide and oleamide.

1. Introduction

Oleamide (**1**)[1,2] and anandamide (**2**)[3,4] are prototypical members of a class of endogenous fatty acid amides that serve as chemical messengers.[5] Anandamide is an endogenous fatty acid ethanolamide that binds and activates the central (CB1) and peripheral (CB2) cannabinoid receptors, and the vanilloid (VR1) receptor through which it is thought to exhibit its analgesic and cannabinoid effects.[4] Oleamide was found of accumulate in the cerebrospinal fluid under conditions of sleep deprivation and to induce physiological sleep in animals where it reduces motility, shortens the sleep induction period, and lengthens the time spent in slow wave sleep 2 at the expense of wakening.[1,6]

Fatty acid amide hydrolase (FAAH)[7] is an integral membrane protein that degrades fatty acid primary amides and ethanolamides including oleamide and anandamide (Fig. 1).[8,9] The distribution of FAAH in the central nervous system suggests that it degrades neuromodulating fatty acid amides at their sites of action and is responsible for or intimately involved in their regulation.[10] FAAH hydrolyzes a wide range of fatty acid substrates and it appears to work most effectively on arachidonyl and oleyl substrates.[11] This enzyme belongs to the amidase family of hydrolytic enzymes for which FAAH is the only member found in mammalians.[12] This unique mammalian distribution,[12–14] its attractive and targetable catalytic mechanism (Ser-Ser-Lys triad), and the consequences of inhibition (increase endogenous level of anandamide and/or oleamide) have made FAAH an attractive therapeutic target[8,9] for the development of a new class of analgesics, nonhypnotic sleep-aids, and a range of additional clinical disorders.

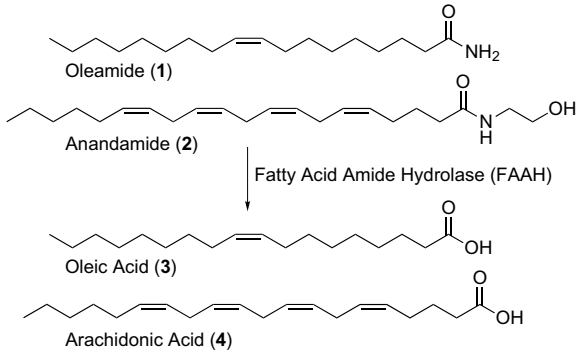

Figure 1.

In spite of this interest, only a selected series of FAAH inhibitors have been disclosed. These include the discovery that the endogenous sleep-inducing compound 2-octyl-γ-bromoacetoacetate is an effective inhibitor of FAAH,[15] an early series of irreversible FAAH inhibitors (fatty acid sulfonyl fluorides,[16] fluorophosphonates,[17] and α-diazoketones[18]), and an early series of fatty acid-based reversible FAAH inhibitors bearing nonselective electrophilic carbonyls.[19–21] More recently, two additional promising classes of FAAH inhibitors

* Corresponding author. Tel.: +1 8587847522; fax: +1 8587847550; e-mail: boger@scripps.edu

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have emerged. The first of these is a series of reversible α-ketoheterocycle inhibitors that exhibit an extraordinary potency (K_i = 100–200 pM) and/or selectivity,[22] and the second constitutes a class of aryl carbamates that acylate (carbamoylate) a FAAH active site catalytic serine.[23] Herein, we report the extension of the studies on the former class to the examination of a unique series of heterocyclic sulfoxide and sulfone FAAH inhibitors.

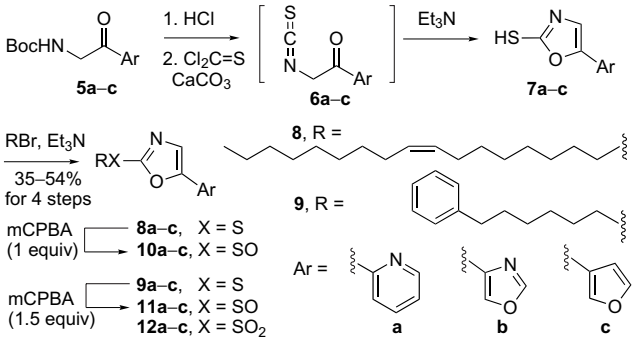
2. Chemistry

The heterocyclic sulfoxides and sulfones examined contain three classes of heterocycles established to be effective with the α-ketoheterocyclic FAAH inhibitors:[22] substituted oxazoles, a fused oxazole, and substituted oxadiazoles. They were prepared by oxidation of the corresponding oleyl and phenylalkyl sulfides, which were prepared by alkylation of corresponding thiols.

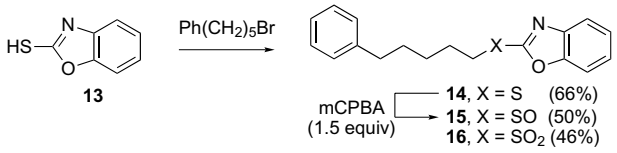
The synthesis of the substituted 2-mercaptooxazoles is shown in Scheme 1. The Boc protected 2-aminoketones **5** (Ar = 2-pyridyl, 2-furanyl, 4-oxazolyl groups) were first prepared from the corresponding heterocycles.[24] Treatment of **5** with HCl followed by condensation of the liberated free amine with thiophosgene in the presence of $CaCO_3$ as a mild base yielded the isothiocyanates **6**.[25] Without purification, these labile intermediates were treated with Et_3N to give the 2-mercaptooxazoles **7** through a base-catalyzed cyclization reaction. Without purification, crude **7** was treated with *cis*-1-bromoheptadec-8-ene[26] or 6-bromohexylbenzene in the presence of Et_3N to give sulfides **8** or **9**, respectively.

Oxidation of sulfides **8** with 1 equiv mCPBA gave the sulfoxides **10** in approximately 80% yields with good chemoselectivity over epoxidation. When **8** were exposed to a larger excess of mCPBA, epoxidation occurred in preference of the formation of sulfone. Oxidation of the sulfides **9** with ca 1.5 equiv mCPBA provided a mixture of the sulfoxides **11** (60–82%) and sulfones **12** (15–32%) in excellent combined yields (92–97%), which were easily separated by chromatography.

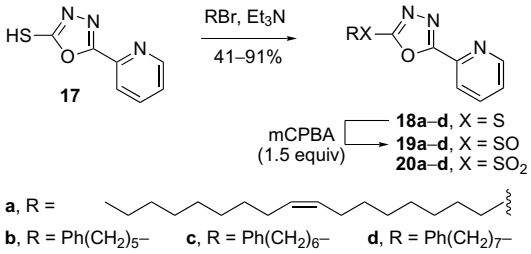
Alkylation of commercially available 2-mercaptobenzoxazole (**13**) with 5-bromopentylbenzene gave sulfide



Scheme 1.



Scheme 2.



Scheme 3.

14, which was treated with mCPBA to afford sulfoxide **15** and sulfone **16** (Scheme 2).

The synthesis of the substituted 1,3,4-oxadiazole sulfoxides and sulfones is shown in Scheme 3. 2-Mercapto-1,3,4-oxadiazole **17** was prepared as previously described through condensation of pyridine-2-carboxhydrazide with CS_2 in the presence of KOH in refluxing ethanol.[27] Alkylation of **17** with four alkyl bromides provided sulfides **18a–d**. When sulfide **18a** was treated with 1 equiv of mCPBA, the reaction gave a mixture of the desired sulfoxide **19a** in 35% yield and other epoxidation products. The poor chemoselectivity in this reaction compared to sulfide **8** may be attributed to the greater electron deficient nature of oxadiazole, which further deactivates sulfide **18a**. Treatment of sulfides **18b–d** with 1.5 equiv of mCPBA yielded the sulfoxides **19b–d** (33–56%) and sulfones **20b–d** (42–62%) accordingly (81–98% combined).

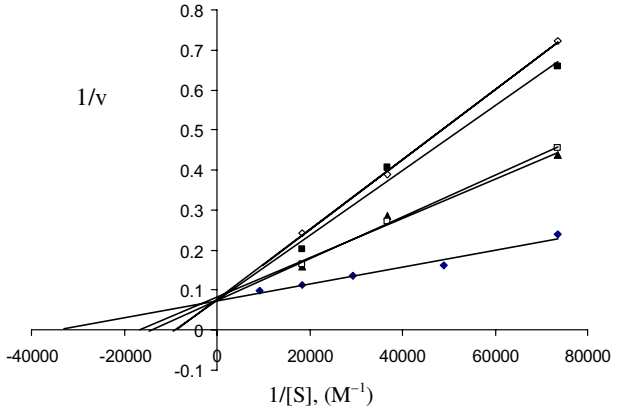
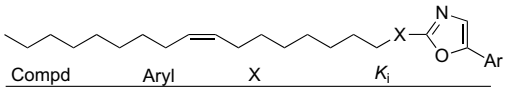


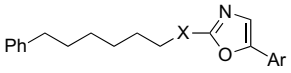
Figure 2. Lineweaver–Burk plot of competitive FAAH inhibition by **11a** and **16**. FAAH activity was monitored in the presence of 0 μM (filled diamond), 10 μM (filled triangle), and 20 μM (filled square) **11a**; and 0 μM (filled diamond), 5 μM (unfilled square), and 10 μM (unfilled diamond) **16** with an oleamide concentration varied from 13.6 to 108.8 μM.

3. Inhibition studies

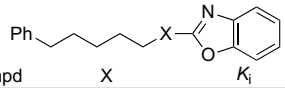
Enzyme assays were performed at 20–23 °C with purified recombinant rat FAAH expressed in *E. coli*[28] in a 125 mM Tris/1 mM EDTA/0.2% glycerol/0.2% Triton X-100/0.4 mM Hepes, pH 9.0 buffer. The initial rates of hydrolysis (⩽10–20% reaction) were monitored using enzyme concentrations at least 3 times below the measured K_i by following the hydrolysis of ^{14}C-oleamide and K_i's established as described (Dixon plot).[22a] Lineweaver–Burk analysis established reversible, competitive inhibition (Fig. 2).



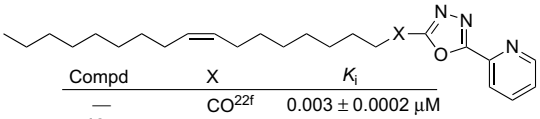
Compd	Aryl	X	K_i
—		CO[22d]	0.018 ± 0.005 μM
8a		S	160 ± 7 μM
10a		SO	8 ± 0.5 μM
—		CO[22d]	0.012 ± 0.001 μM
8b		S	130 ± 10 μM
10b		SO	2.2 ± 0.2 μM
—		CO[22d]	0.054 ± 0.004 μM
8c		S	180 ± 10 μM
10c		SO	8 ± 1 μM



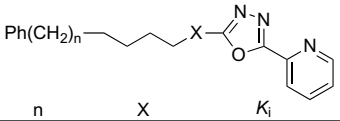
Compd	Aryl	X	K_i
—		CO[22d]	0.0047 ± 0.0013 μM
9a		S	110 ± 10 μM
11a		SO	11 ± 0.4 μM
12a		SO_2	16 ± 4.5 μM
—		CO[22d]	0.0046 ± 0.0004 μM
9b		S	80 ± 5 μM
11b		SO	20 ± 4 μM
12b		SO_2	10 ± 2 μM
—		CO[22d]	0.012 ± 0.001 μM
9c		S	120 ± 10 μM
11c		SO	30 ± 10 μM
12c		SO_2	40 ± 10 μM



Compd	X	K_i
—	CO[22a]	0.22 ± 0.01 μM
14	S	150 ± 10 μM
15	SO	2.6 ± 0.1 μM
16	SO_2	3.3 ± 0.7 μM



Compd	X	K_i
—	CO[22f]	0.003 ± 0.0002 μM
18a	S	140 ± 10 μM
19a	SO	13 ± 1 μM[29]



Compd	n	X	K_i
18b	1	S	720 ± 50 μM
19b	1	SO	8 ± 1 μM
20b	1	SO_2	7.5 ± 1 μM
18c	2	S	110 ± 10 μM
19c	2	SO	4.9 ± 1.0 μM
20c	2	SO_2	6.8 ± 0.7 μM[30]
18d	3	S	860 ± 50 μM
19d	3	SO	7 ± 0.3 μM
20d	3	SO_2	3.3 ± 0.5 μM

Figure 3. FAAH inhibition.

4. Results and discussion

The results of the examination of the sulfoxide and sulfone FAAH inhibitors are summarized in Figure 3.[29,30] In each case, the sulfoxides and sulfones proved to be 10–100-fold more potent than the corresponding sulfides, consistent with an expected enhanced binding affinity derived from a polarized oxygen binding an enzyme oxyanion pocket mimicking the tetrahedral intermediate of the amide hydrolysis reaction. Moreover, little distinction and no discernable trends were observed in comparing the sulfoxides with the corresponding sulfones and both inhibit FAAH to the same extent.

Unlike the behavior of the corresponding α-ketoheterocycles, the potency of the corresponding sulfoxides and sulfones was relatively insensitive to the nature of the attached heterocycle as well as the side chain, although each system examined represent those known to convey effective FAAH active site binding.[22] Finally, the sulfides were roughly 10^4–10^5-fold less potent than the corresponding α-ketoheterocycles, whereas the sulfoxides and sulfones were roughly 10^2–10^3-fold less potent than the α-ketoheterocyles. The notable exceptions are **15** and **16**, which proved to be only 10-fold less potent than the corresponding α-ketobenzoxazole, which constitutes one of the least potent α-ketoheterocycles examined in this series. Thus, the heterocyclic sulfoxides and sulfones exhibit a potency intermediate of the corresponding sulfides and α-ketoheterocycles illustrating that the polarized oxygen binding in an enzyme oxyanion binding pocket increases affinity up to 100-fold and that the reversible covalent binding of the α-ketoheterocycles to an active site serine increases binding up to 1000-fold beyond that of the corresponding sulfoxide and sulfone.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.10.025. Details of the synthesis and characterization of all new compounds is provided.

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29. The corresponding epoxide exhibited a $K_i = 1.0 \pm 0.1\,\mu M$.
30. The corresponding pyridine N-oxide exhibited a $K_i = 590 \pm 50\,\mu M$.