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# Novel ketooxazole based inhibitors of fatty acid amide hydrolase (FAAH)

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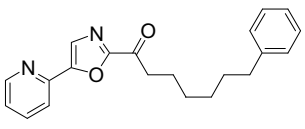
**Abstract**—Efforts to improve the properties of the well studied ketooxazole FAAH inhibitor OL-135 resulted in the discovery of a novel propylpiperidine series of FAAH inhibitors that has a modular design and superior properties to OL-135. The efficacy of one of these compounds was demonstrated in a rat spinal nerve ligation model of neuropathic pain in rats.


The medicinal use of *Cannabis* dates back to early recorded history. As early as 2600 BC, ancient Chinese texts record the use of cannabis for the relief of numerous conditions including rheumatic and menstrual pain.[1] Modern scientific investigations into the mechanisms surrounding the pharmacological effect of cannabis have resulted in the discovery of its principle active component $\Delta^9$-tetrahydrocannabinol (THC),[2,3] as well as the receptors at which THC acts (CB1 and CB2).[4,5] Recently, cannabinoid agonists derived from cannabis extracts have been approved by regulatory agencies for emesis and appetite stimulation in cancer and AIDS patients (Nabilone), and for neuropathic pain (Sativex). However, a limitation of global stimulation of the cannabinoid system appears to be aversive CNS side effects, including motor impairment and dysphoria.[6]

With the discovery of the cannabinoid receptors came a search for the endogenous agonists of these receptors. The first identified was the lipid anandamide.[7] This discovery was followed by the identification of numerous additional lipid signaling molecules that agonize the cannabinoid receptor system, some of which have analgesic and anti-inflammatory properties. Further investigations lead to the discovery of enzymes that hydrolyze these signaling molecules. The most studied member of this family of enzymes is fatty acid amide hydrolase (FAAH).[8] The discovery of catabolizing enzymes for these signaling molecules revealed the intriguing possibility of controlling cannabinergic signaling through the modulation of endogenous agonists, rather than by direct global activation of the receptors. Indeed, pharmacological investigations in animal models of pain and anxiety with FAAH inhibitors have shown efficacy without the motor impairment typical of direct CB1 stimulation, supporting the potential therapeutic utility of this approach.[9]

To date several molecules have been identified that inhibit the hydrolytic activity of FAAH.[10] One of the most potent and selective of these is the ketooxazole OL-135.[11] OL-135 is a competitive and fully reversible inhibitor of FAAH that has been reported to produce analgesia in models of acute and neuropathic pain.[12] However, the compound has poor aqueous solubility and sub-optimal PK properties (0.016 mg/mL in water) (Fig. 1).

The current study reports efforts to improve the pharmaceutical properties of OL-135, while also reducing




**OL-135**

human FAAH $IC_{50}$ = 15 nM

**Figure 1.** Potent FAAH inhibitor OL-135.

*Keywords*: FAAH; Fatty acid amide hydrolase; Pain; Endocannabinoid.
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the complexity of the synthesis to allow for rapid analoging. Because of the challenges associated with the synthesis of the pyridyl oxazole ketones, unsubstituted oxazole ketone **1** was chosen as a starting point for analoging efforts despite its slightly lower affinity for the enzyme.[11]

Replacement of the phenylhexyl group in **1** with a propylpiperidine was envisioned to provide a versatile scaffold from which a variety of analogs could be produced (Fig. 2). In addition, the scaffold would potentially provide functionality capable of reducing the lipophilicity and perhaps increase solubility of the inhibitors.

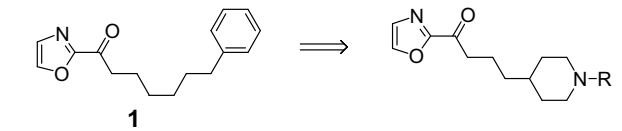
Using the published crystal structure of rat FAAH bound to methyl arachidonyl fluorophosphonate (MAFP) compound **3** was modeled into the active site of FAAH (Fig. 3).[13,14] This modeling shows a good fit of the propylpiperidine group into the active site and suggests that groups appended to the nitrogen of the piperidine might be projected along a vector consistent with binding in the lipophilic pocket occupied by the alkyl chain of MAFP. This provided a reasonable rationale for the pursuit of these analogs.

The synthesis of **2** and its analogs was complicated by the lack of high yielding general methods for the synthesis of 2-acyl oxazoles. This challenge arises from the propensity of 2-lithiated oxazole to exist in an opened-chain enolate-isonitrile form, rather than in the desired closed-chain oxazole form.[16]

To overcome this we developed new technology for reliable access to this class of compounds. Specifically, we demonstrated that reactions between 2-magnesiated oxazoles and Weinreb amides proceed to form the desired 2-acyl oxazole products in excellent yield.[17] Thus, our synthesis of these compounds began with the commercially available 4-piperidine butyric acid hydrochloride (Scheme 1). A simplified one-pot, two-step process was developed to first esterify and then Boc protect this starting material. The ester was then transformed to the corresponding Weinreb amide according to a known literature method (Scheme 2).[18]

Deprotonation of oxazole with *i*-PrMgCl followed by reaction with the Weinreb amide afforded the desired 2-acyl oxazole **2** in 77% yield.

As our modeling suggested, when the Boc protected intermediate **2** was tested for inhibition of the human FAAH enzyme it was found to be a very potent inhibitor.[19] However, deprotection resulted in the poor




**1**

human FAAH $IC_{50}$ = 43 nM

**Figure 2.** Concept to improve properties of ketooxazole FAAH inhibitors.

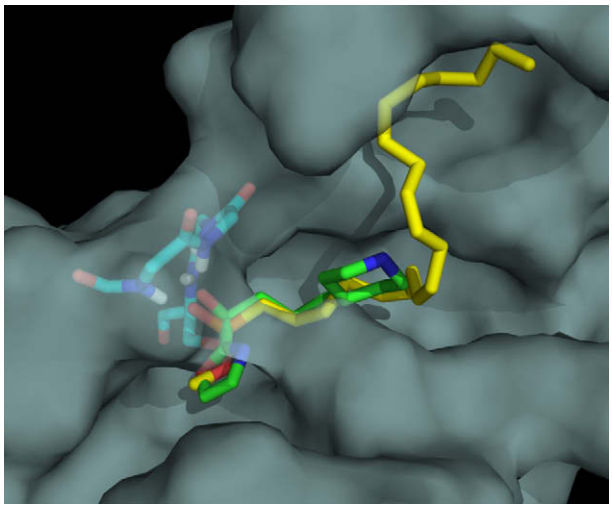
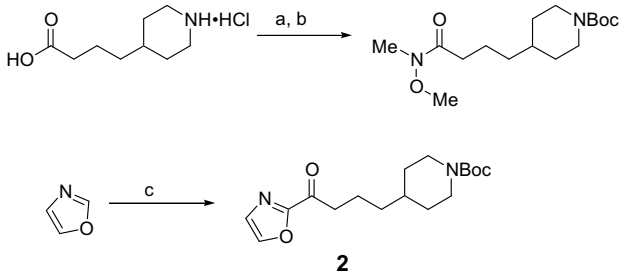


**Figure 3.** Modeling of compound **3** (green) into the active site of FAAH, overlaid with MAFP (yellow). Important active site residues of FAAH are shown in blue (Ser241 and the backbone atoms of residues 238–240 which form the oxyanion hole).[15]



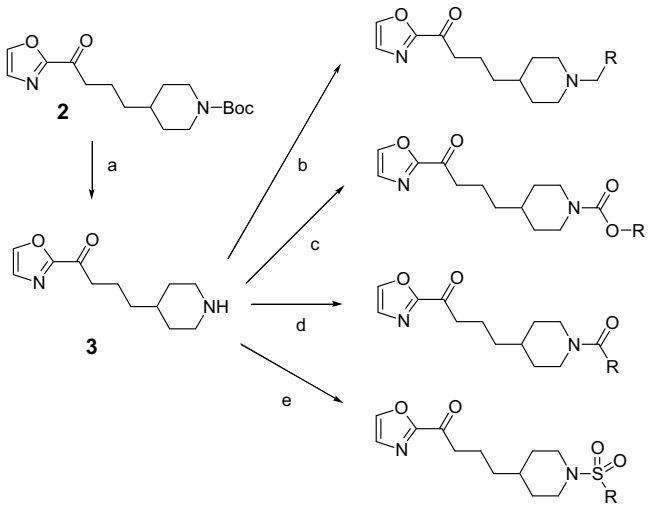

**2**

**Scheme 1.** Reagents and conditions: (a) Ethanol, HCl, reflux, 2 h, then $Na_2CO_3$, Boc anhydride, THF, water, rt, 16 h, 97%; (b) *N*,*O*-dimethylhydroxylamine hydrochloride, THF, *i*-PrMgCl, −5 °C, 2 h, 98%; (c) *i*-PrMgCl, THF, −15 °C, 30 min then Weinreb amide, THF, rt, 15 h, 77%.

FAAH inhibitor **3** (Fig. 4). This supported the modeling that shows a large pocket occupied by the MAFP alkyl chain in the crystal structure where the BOC group would presumably reside (Fig. 3).

As a result of the activity of compound **2**, a number of amide, sulfonamide, and carbamate derivatives were prepared with lipophilic substituents (Table 1).[20] When these compounds were tested for FAAH inhibition it was found that the distance between the piperidine nitrogen and lipophilic group had a dramatic effect on activity. A phenyl ring directly attached to the carbonyl of the amide or with a two carbon spacer was relatively active (**4** and **6**). In contrast the analog containing the one carbon spacer was relatively inactive (**5**). This suggests that a very specific orientation of the piperidine substituent is required for good inhibition of FAAH. This same trend was seen when the phenyl ring was replaced with a cyclohexyl ring (**7**–**9**), or when the carbonyl was replaced with a sulfonyl group (**10**–**12**).

Additionally, replacing the Boc group with other carbamates also resulted in potent FAAH inhibitors (**16** and




**Scheme 2.** Reagents and conditions: (a) HCl (2 N soln. in $Et_2O$), rt, 24 h, 98%. (b) $NaBH(OAc)_3$, RCHO, $Et_3N$, $CH_2Cl_2$, rt, 24 h; (c) ROCOCl, $NaHCO_3$, EtOAc, rt, 2 h; (d) RCOCl, $Et_3N$ or pyridine, $CH_2Cl_2$, rt, 1–24 h; (e) $RSO_2Cl$, $Et_3N$, $CH_2Cl_2$, rt, 24 h.

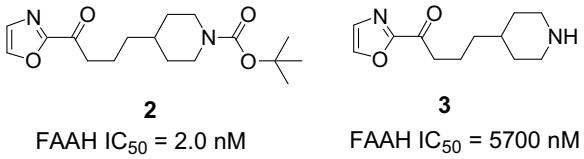
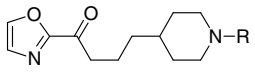



**Figure 4.** FAAH activity of propylpiperidine intermediates.

**Table 1.** Inhibition of human FAAH by amide, carbamate, and sulfonamide analogs of **3**




| # | R | $IC_{50}$ (nM) | # | R | $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **3** | H | 5700 | **10** | $SO_2Ph$ | 2.5 |
| **2** | $CO_2t$-Bu | 2.0 | **11** | $SO_2CH_2Ph$ | 600 |
| **4** | COPh | 77 | **12** | $SO_2(CH_2)_2Ph$ | 6.0 |
| **5** | $COCH_2Ph$ | 500 | **13** | CO*i*-Pr | 250 |
| **6** | $CO(CH_2)_2Ph$ | 22 | **14** | $COCH_2(t$-Bu) | 90 |
| **7** | CO(cyclohex) | 45 | **15** | $SO_2i$-Pr | 140 |
| **8** | $COCH_2$(cyclohex) | 100 | **16** | $CO_2i$-Pr | 4.0 |
| **9** | $CO(CH_2)_2$(cyclohex) | 3.0 | **17** | $CO_2CH_2Ph$ | 4.0 |

**17**). Because there are known inhibitors of FAAH that make use of carbamates as mechanism-based electrophiles,[10] we evaluated the kinetics of inhibition with compound **2** and found the inhibition to be non-time-dependant and fully dialyzable. This suggests that the carbamate is likely not interacting directly with the active site serine of FAAH. Unfortunately, while these compounds were potent FAAH inhibitors they suffered from very poor solubility. In addition, the carbamates proved to be unstable in plasma presumably due to hydrolysis by plasma esterases.

In an effort to improve solubility and/or stability, reductive amination of piperidine **3** was performed to obtain compounds that contained a basic nitrogen (Table 2).

The unsubstituted benzylpiperidine **18** had modest activity for FAAH ($IC_{50}$ = 315 nM). This probably reflects some negative interactions resulting from a protonated nitrogen being located in the lipophilic region of the FAAH enzyme. Additional simple substitutions on benzyl amine **18** generally increased potency. We believe the lipophilic substituents may compensate for the negative effect of the protonated nitrogen ultimately providing highly potent analogs.

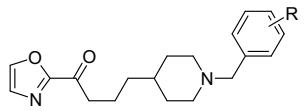
Regardless of the exact nature of the substituent, meta-substitution appeared to provide the greatest FAAH inhibition. Para-substitution was typically less potent than meta by a small degree, and ortho substitution was generally the least active (**19–30**). Following the observed activity trend 3-Me < 3-Cl < 3-OMe < l 3-Br substituents such as O*i*-Pr and *i*-Pr were added, resulting highly potent analogs (**31–34**).

Modeling of compound **34** into the active site of FAAH provides a reasonable rationale for the potency trends seen in this series (Fig. 5). The substituent on the aryl ring can occupy the lipophilic pocket that binds the alkyl chain of MAFP in the published crystal structure of FAAH. It is reasonable to assume that additional van der Waals interactions made with these larger substituents would result in increased inhibition of FAAH.

Compound **34** was selected for further profiling in rat pain models due to its potent FAAH inhibition and improved solubility properties (>0.4 mg/mL in water as the HCl salt). Compound **34** was administered ip to spinal nerve ligated (SNL) rats and fully reversed the tactile allodynia induced by spinal nerve ligation with an $ED_{50}$ of 7.3 mpk (SE ± 4.2 mpk). Furthermore, **34** showed no motor impairment, as assessed by rotorod, at the doses evaluated (Fig. 6).[21]

Additionally, an issue of interest in the use of FAAH inhibitors as potential analgesics is whether sustained

**Table 2.** Inhibition of human FAAH by substituted benzyl piperidines




| # | R | $IC_{50}$ (nM) | # | R | $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **18** | H | 315 | **28** | 2-Br | 315 |
| **19** | 2-Me | 1200 | **29** | 3-Br | 10 |
| **20** | 3-Me | 90 | **30** | 4-Br | 45 |
| **21** | 4-Me | 400 | **31** | 3-O*i*-Pr | 5.0 |
| **22** | 2-OMe | 4000 | **32** | 4-O*i*-Pr | 14 |
| **23** | 3-OMe | 34 | **33** | 3-*i*-Pr | 1.0 |
| **24** | 4-OMe | 130 | **34** | 4-*i*-Pr | 3.6 |
| **25** | 2-Cl | 260 | | | |
| **26** | 3-Cl | 60 | | | |
| **27** | 4-Cl | 66 | | | |

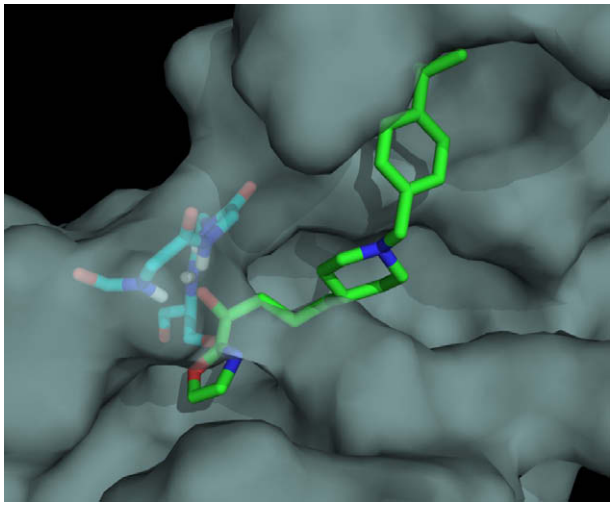


**Figure 5.** Modeling of compound **34** into the active site of rat FAAH.[15]

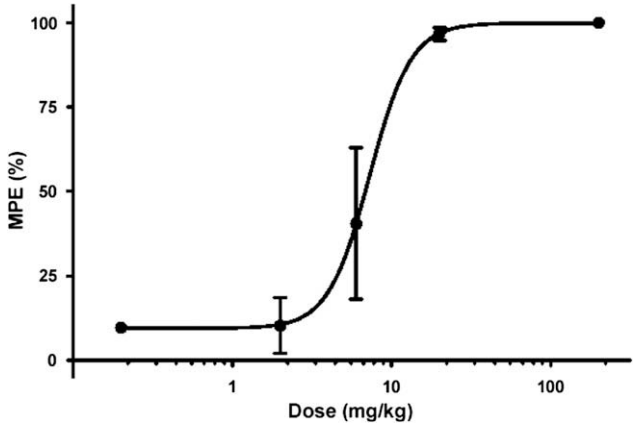



**Figure 6.** Compound **34** reversal of tactile allodynia in SNL rats. Compound dosed ip at 2, 6, and 20 mpk ($n$ = 8/dose group). Tactile thresholds measured 30 min after administration of compound. %MPE is % maximal possible effect based upon pre- and post-ligation withdrawal thresholds.[21]

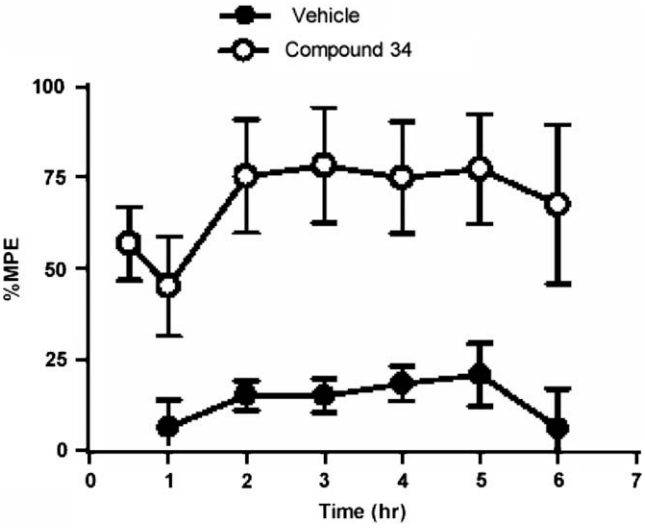



**Figure 7.** Rats with a spinal nerve ligation were given a bolus dose of 4 mg/kg iv **34** followed by infusion from an implanted mini pump at 6 mg/kg/h. Mechanical allodynia was assessed at hourly intervals until the termination of the experiment at 6 h. %MPE is % maximal possible effect based upon pre- and post-ligation withdrawal thresholds.[21]

efficacy can be achieved. Little data on this question have previously appeared in the literature. Due to the high solubility of compound **34**, we were able to approach this question using a mini pump to achieve and maintain an appropriate sustained plasma concentration of **34** (4 mg/kg iv bolus dose followed by infusion at 6 mg/kg/h to maintain a target plasma concentration of 2 μM). We tested the efficacy of the compound in reversing tactile allodynia throughout a 6 h time period. As shown in Figure 7, high levels of efficacy were maintained at a plateau for the 6 h of the experiment, during the course of which the actual Cp was 1.2 μM (SEM 0.5 μM; $n$ = 5). This encourages the concept that enduring pain relief may be achieved by inhibition of FAAH.[21]

In conclusion a highly versatile and novel series of ketooxazole FAAH inhibitors was identified. The series was optimized to produce a highly potent soluble analog **34** that was appropriate for evaluation in rat pain models and proved efficacious in the SNL model of neuropathic pain.

## Acknowledgments

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