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Identification of a novel arylpiperazine scaffold for fatty acid amide hydrolase inhibition with improved drug disposition properties

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ABSTRACT

We herein describe the systematic approach used to develop new analogues of compound **2**, recently identified as a potent and selective fatty acid amide hydrolase (FAAH) inhibitor. Aiming at identifying new scaffolds endowed with improved drug disposition properties with respect to the phenylpyrrole-based lead, we subjected it to two different structural modification strategies. This process allowed the identification of derivatives **4b** and **5c** as potent, reversible and non-competitive FAAH inhibitors.

Fatty acid amide hydrolase (FAAH) is a membrane bound enzyme which regulates the intracellular level of anandamide (AEA) and other endocannabinoids (ECs).[1] AEA is transported across the plasma membrane and then by intracellular transporters to gain facilitated access to its final targets.[2–6] Subsequent hydrolysis by FAAH drives AEA uptake by creating and maintaining a concentration gradient across the plasma membrane. FAAH utilizes a Ser-Ser-Lys catalytic triad as confirmed by X-ray analysis.[7] Inactivation of FAAH elevates the endogenous concentrations of its substrates thus potentiating their beneficial effects on the modulation of pain, inflammation, and anxiety.[8] This modulation of ECs catabolizing enzymes for controlling the endocannabinoid system is an intriguing possibility with respect to direct global activation of the receptors.[2] Indeed, the therapeutic effects could be elicited by avoiding cannabinoid agonists side effects (hypomotility, hypothermia, and catalepsy). The design of selective and druggable small-molecule inhibitors of FAAH remains an essential step in the exploitation of this enzyme as a therapeutic target.[9–12] However, for the identification of potential clinical candidates the use of the substrate as template may lead to inhibitors characterized by a number of drawbacks such as poor drug disposition properties (high lipophilicity).

The FAAH inhibitors can be classified as irreversible and reversible inhibitors. The early designed irreversible inhibitors were substrate-inspired compounds (e.g., the one digit nanomolar inhibitor methoxyarachidonoyl fluorophosphonate). Successively, other structurally diverse FAAH inhibitors were developed. Among them, carbamates and ureas act as irreversible FAAH inhibitors such as URB597[13] and JP83[14] (**1**, Fig. 1), while the α-ketooxazole OL135,[15] and the recently identified enol carbamates,[16] and ST3913[17,18] (**2**, Fig. 1), were classified as reversible inhibitors. Furthermore, the piperazine urea JNJ-1661010[19] (**3**, Fig. 1) was found as a covalent but slowly reversible inhibitor.

The current study reports the synthesis and the in vitro biological investigation of a series of novel FAAH inhibitors (**4a–e** and **5a–f**, Fig. 2) the development of which was based on the model of our previously identified potent and selective FAAH reversible inhibitor **2**. The new scaffolds were conceived following the lines of approach described in Figure 2, by taking into account two key issues for the development of a good (pre)clinical candidate, namely: (i) the drug disposition profile and (ii) the possibility of a rapid analoging. Since a successful drug candidate should possess a balance of potency and drug-like properties (e.g., water solubility), our lead

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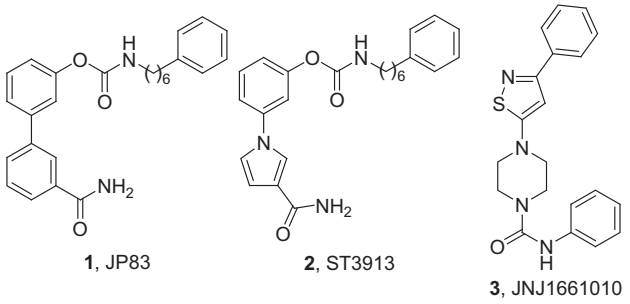
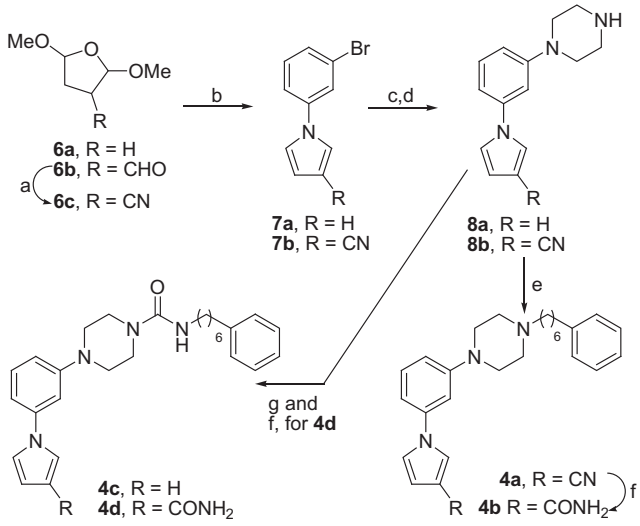


Figure 1. Reference FAAH inhibitors.

optimization strategies include solubility enhancement, performed through introduction of ionizable groups and of H-bond donors/acceptors. The insertion of a piperazine moiety represents a common approach for providing reduced lipophilicity while increasing solubility. We therefore rationally modified the core structure of our lead **2** by appending a piperazine ring on the 1-phenylpyrrole system. This represented a drug disposition properties optimization, leading to scaffold A analogues (Fig. 2). An additional cycle of optimization involved the replacement of the pyrrole system by a piperazine ring (scaffold B analogues, Fig. 2). Accordingly, from the structural point of view, all the newly developed piperazine-containing compounds can be clustered in two main subgroups (i) the bis-aryl derivatives **4a**–**e**, and (ii) the arylpiperazines **5a–f**.

Schemes 1 and 2 describe the synthesis of compounds **4a**–**e** belonging to the first template. Compounds **4a**–**d** (Scheme 1) were synthesized starting from the appropriate dimethoxytetrahydrofuran derivatives (**6a**,**c**) which were subjected to a Clauson–Kaas reaction to provide the corresponding 1-phenylpyrrole derivatives (**7a**,**b**). A palladium catalyzed amination of Boc-piperazine[20,21] followed by Boc-deprotection[22] afforded the key intermediates **8a**,**b**. Reaction of the piperazine-derivatives with phenylhexyl bromide provided the alkylated piperazine **4a**, and after partial hydrolysis of the nitrile functionality the amido-derivative **4b**. Reaction of **8a**,**b** with phenylhexylisocyanate provided urea derivatives **4c** and **4d**. For the synthesis of compound **4e** (Scheme 2), the key intermediate **10** was reacted with Boc-piperazine by a highly yielding coupling procedure performed in the presence of *N*-bromosuccinimide and triphenylphosphine.[23] Boc-deprotection followed by piperazine N^4-alkylation and partial nitrile hydrolysis led to **4e**.



Scheme 1. Reagents and conditions: (a) I_2, NH_4OH, THF, rt, 3 h, 46%; (b) 3-bromoaniline, HCl 5 N, dioxane, reflux, 30 min, 92%; (c) Boc-piperazine, $Pd_2(dba)_3$, (±)-BINAP, NaO-*t*-Bu, dry toluene, 70 °C, 10 h, 70%; (d) AcCl, MeOH, rt, 30 min, 99%; (e) phenylhexyl bromide, MeCN, TEA, reflux, 15 h, 82%; (f) 6 N NaOH, 35% wt H_2O_2, EtOH, reflux, 12 h, 55–63%; (g) phenylhexyl isocyanate, TEA, dry THF, reflux, 8 h, 52%.

Schemes 3 and 4 describe the synthesis of compounds **5a**–**f**. The phenylpiperazines **12a**,**b**,**d** were reacted with functionalized aryl or aralkyl isocyanates, and after appropriate deprotection of the piperazine functionality, compounds **5a**–**d** were obtained. The preparation of compound **5e** was accomplished by an alkylation reaction of the Cbz-protected 3-hydroxyphenylpiperazine **12d** with 4-fluorophenoxybutyl bromide, followed by Cbz removal. Oxime carbamate derivative **5f** (Scheme 4) was prepared by reaction of the ketone **13** with hydroxylamine in ethanol, followed by treatment of the oxime **14** with 1-piperidine carbonylchloride.

For the development of novel FAAH inhibitors endowed with an optimized solubility profile, we modified our potent and selective lead candidate **2** (K_i = 0.16 nM, on *m*FAAH)[17] while preserving nanomolar inhibitory activity. Accordingly, starting from the core scaffold of **2**, we adopted a strategy involving the two main structural tunings outlined in Figure 2 (see calculated Log*S* in Table 1). The first subgroup of compounds (scaffold A analogues, **4a**–**e**)

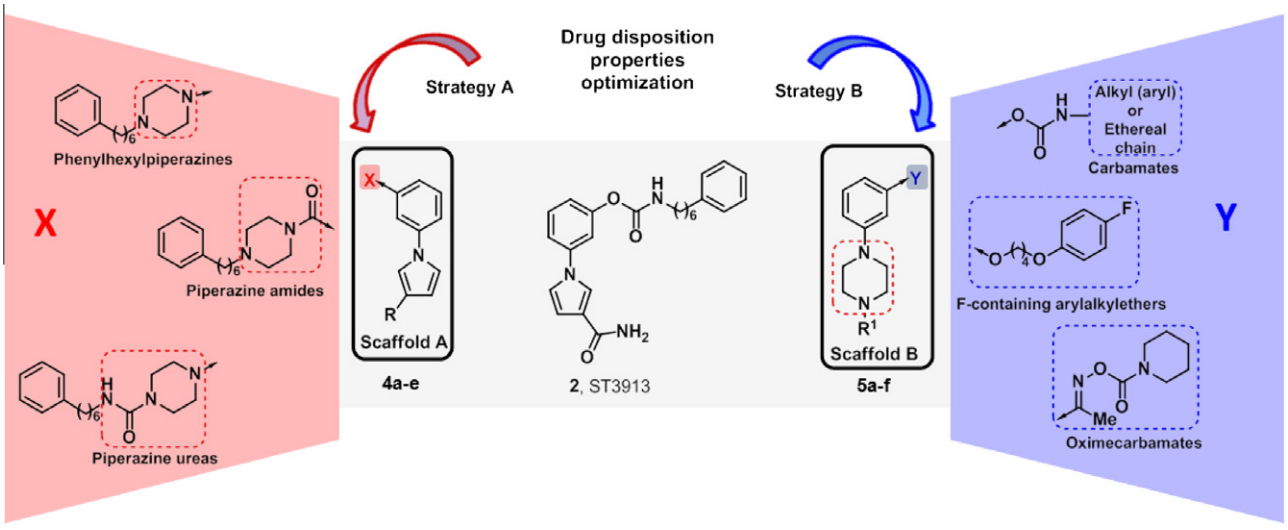


Figure 2. Strategies for optimizing the drug disposition properties of **2**.