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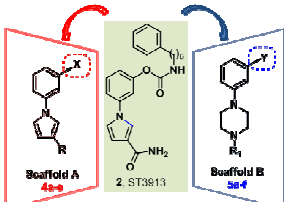

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Identification of a novel arylpiperazine scaffold for fatty acid amide hydrolase inhibition with improved biopharmaceutical properties

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[a] *European Research Centre for Drug Discovery and Development (NatSynDrugs), Università degli Studi di Siena, Via Aldo Moro, 53100 Siena, Italy*
[b]*Sigma-Tau Industrie Farmaceutiche Riunite spa, Via Pontina Km 30,400, 00040 Pomezia, Italy*
[c]*Dipartimento di Scienze Biomediche, Università degli Studi di Teramo, Piazza A. Moro 45, 64100 Teramo, Italy*
[d]*DeFENS,Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente,, Università di Milano, Via Celoria 2, 20133 Milano, Italy*

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ABSTRACT

We herein describe the systematic approach applied (or used to develop) to the development of 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 biopharmaceutical 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, *N*-arachidonoylethanolamine) and other endocannabinoids (ECs). AEA is transported across the plasma membrane and then by intracellular transporters to gain facilitated access to its final targets.[B] 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 resolutely confirmed by X-ray analysis.[1] ECs catabolizing enzymes allow the fascinating possibility of acting on the endocannabinoid system through the modulation of endogenous agonists, rather than by direct global activation of the receptors [B]. Accordingly, inactivation of FAAH elevates the endogenous concentrations of its substrates thus potentiating their beneficial effects on the modulation of pain, inflammation, and anxiety [C] These 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[L.M.1]. 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 a poor biopharmaceutical profile (high lipophilicity).

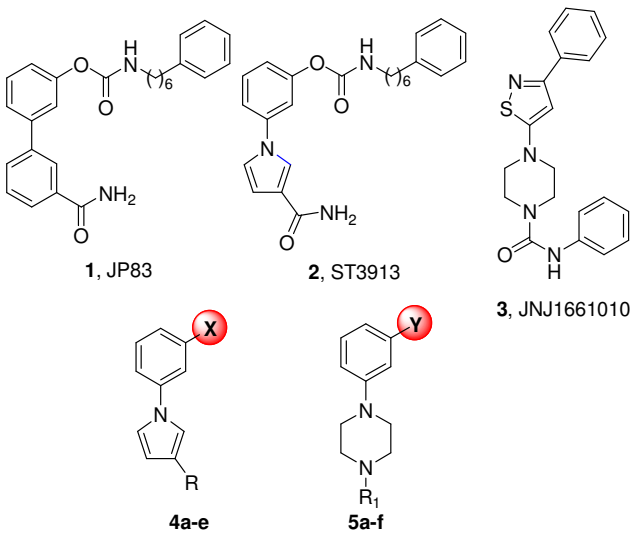


Figure 1. Reference and Title Compounds

[*] Corresponding author. G.C. Tel: 0039-0577-234172. Fax: 0039-0577-234333. E-mail: campiani@unisi.it

The FAAH inhibitors known so far can be classified as irreversible and reversible inhibitors. The early designed irreversible inhibitors were substrate-inspired compounds (e.g. methoxyarachidonoyl fluorophosphonate, IC_{50} = 1-3 nM). Successively, following the wave of a potent electrophile acting as putative active site trap, other structurally diverse FAAH inhibitors were developed (α-ketoheterocycles, ureas and carbamates). Carbamates and ureas act as irreversible FAAH inhibitors (e.g. URB597,[2,D] JP83 **1**,[3] Figure 1), while the α-ketooxazole OL135,[4] and the recently identified ST3913 (**2**, Figure 1),[5,6] were classified as reversible inhibitors. Furthermore, the piperazine urea JNJ-1661010 (**3**, Figure 1)[7] was found a covalent but slowly reversible inhibitor.

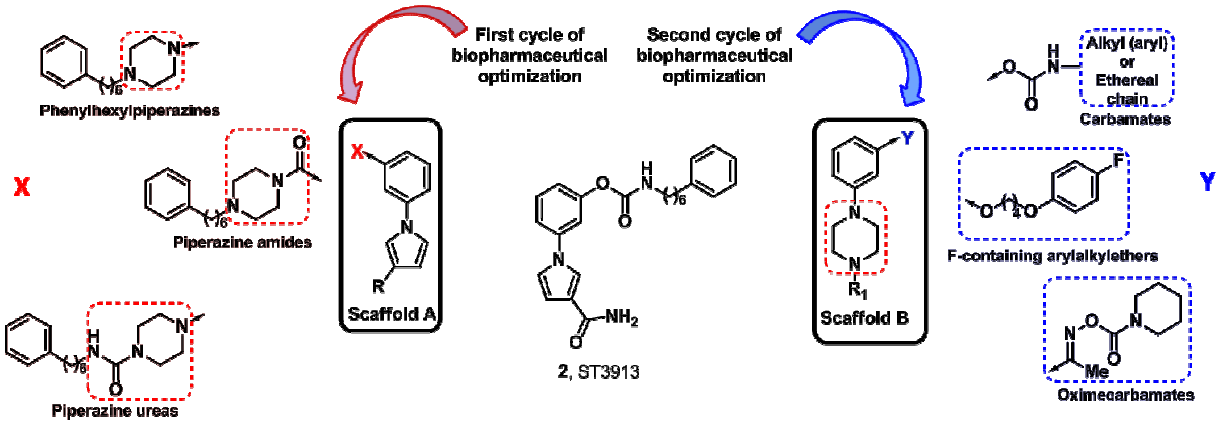
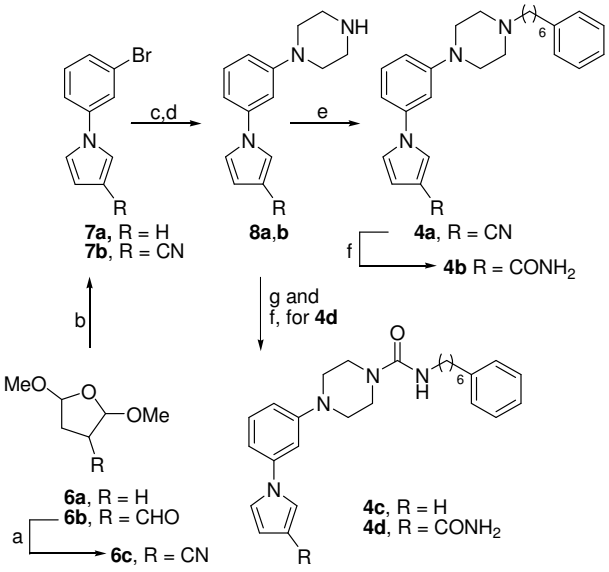


Figure 2. Strategies for biopharmaceutical profile optimization of **2**.

The current study reports the synthesis and the in vitro biological investigation of a series of novel FAAH inhibitors (**4a-e** and **5a-f**, Figure 1) 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 consideration (or account) two key issues for the development of a good (pre)clinical candidate, namely: i) the biopharmaceutical 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 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 the first cycle of biopharmaceutical optimization, leading to scaffold A analogues (Figure 2). The second cycle of biopharmaceutical optimization involved the replacement of the pyrrole system by a piperazine ring (scaffold B analogues, Figure 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**.

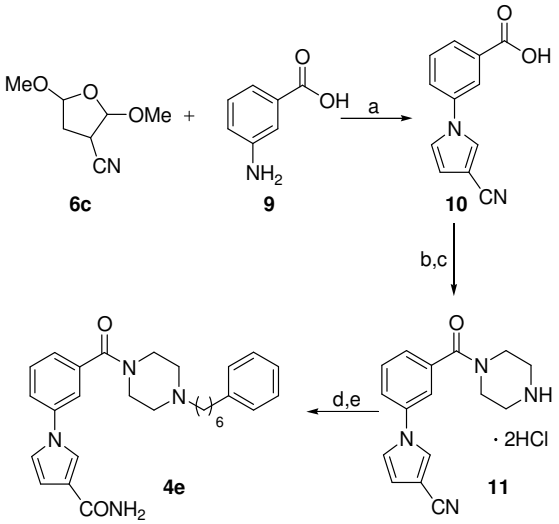
Schemes 1 and 2 describe the synthesis of compounds **4a-e** belonging to the first template. Compounds **4a-d** (Scheme 1) were synthesized starting from the appropriate dimethoxytetrahydrofuran derivatives (**6a,c**) which were subjected to a Clauson-Kaas reaction for providing the corresponding 1-phenylpyrrole derivatives (**7a,b**). A palladium-catalyzed insertion of the piperazine moiety[8] followed by in situ hydrochloric acid generation for Boc-deprotection[9] led to 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.[10] Boc-deprotection followed by piperazine N^4-alkylation and partial nitrile hydrolysis led to **4e**.



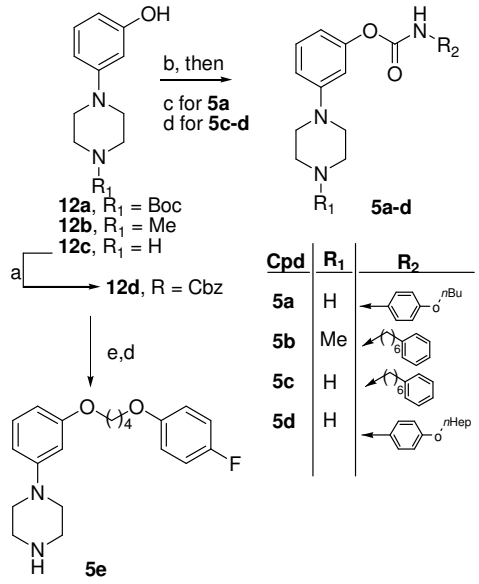
Scheme 1. Reagents and conditions: (a) I_2, NH_4OH, THF, rt, 3 h, 46%; (b) 3-bromoaniline, HCl 5N, dioxane, reflux 30 min, 92%; (c) Boc-piperazine, $Pd_2(dba)_3$, (±)-BINAP, NaO-*t*-Bu, dry toluene, 70 °C 10 h, 70%; (d) AcCl, MeOH, rt, 30 min, 99%; (e) phenylhexyl bromide, MeCN, TEA, reflux, 15 h, 82%;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

Schemes 3 and 4 describe the synthesis of compounds **5a-f**. The phenylpiperazines **12a-d** were reacted with functionalized arylalkylisocyanates, 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.

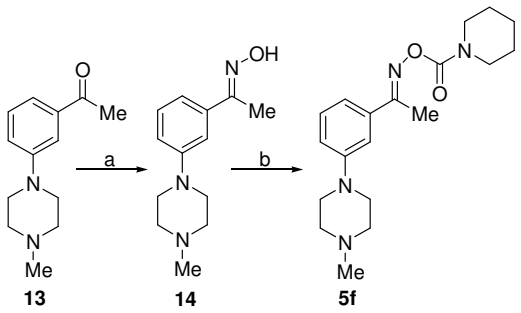


Scheme 2. Reagents and conditions: (a) 5N HCl, dioxane, reflux, 30 min, 45%; (b) Boc-piperazine, *N*-bromosuccinimide, Ph_3P, pyridine, dry DCM, rt, 5 h, 87%; (c) TFA, CH_2Cl_2 rt, 2 h, 99%; (d) phenylhexylbromide, MeCN, TEA, reflux 15 h, 77%; (e) 6N NaOH, 35% wt H_2O_2, EtOH, rt, 12 h, 36%.



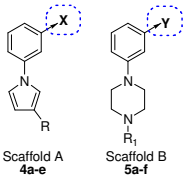
Scheme 3. Reagents and conditions: (a) $KHCO_3$, benzyl chloroformate, H_2O/acetone, 0 °C, 1 h, 89%; (b) aryl(alkyl)isocyanate, TEA, dry THF, rt, 8 h, 46-67%; (c) AcCl, MeOH, rt, 1 h, 99%; (d) H_2, Pd/C, AcOH, rt, 12 h, 59%; (e) K_2CO_3, 4-fluorophenoxybutyl bromide, acetone, 60 °C, 10 h, 70%.

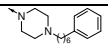
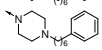
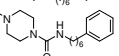
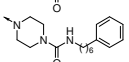
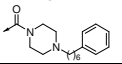
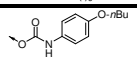
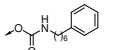
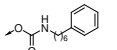
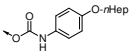
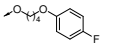
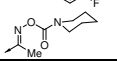
Oxime carbamate derivative **5f** (Scheme 4) was prepared by reaction of the ketone **13** with hydroxylamine in ethanol, followed by treatment of the oxime **14** with 1-piperidine carbonylchloride.



Scheme 4. Reagents and conditions (a) NH_2OH·HCl, NaOH, EtOH, reflux 8 h, 62%; (b) 1-piperidine carbonylchloride, NaH, THF, reflux, 30 min, 50%.

Table 1. Inhibition of Mouse Brain FAAH Activity (K_i, nM) by Compounds **4a-e** and **5a-f**



Cpd	R/R_1	X/Y	K_i (nM)[a]	Log*S*[b]
4a	CN		270	-6.18
4b	$CONH_2$		**10**	**-5.39**
4c	H		263	-6.91
4d	$CONH_2$		>500	-6.49
4e	$CONH_2$		>500	-5.10
5a	H		>500	-1.93
5b	Me		240	-3.39
5c	H		**13**	**-2.38**
5d	H		>500	-2.89
5e	H		>500	-1.69
5f	Me		>500	-2.69
2[c]	-	-	**0.16**	**-5.69**

[a]Enzyme inhibition tests were performed as in ref [5], each value is the mean of at least three experiments (all SD are within 10%); [b]Calculated Log*S* in water at pH = 7, ACD/Labs V12.0, Toronto, Canada; [c]data From ref [5].

For the development of novel FAAH inhibitors endowed with an optimized solubility profile, we performed some rational modifications of our potent and selective lead candidate **2** (K_i = 0.16 nM, on *m*FAAH)[5] while preserving a 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**) springs from the goal of optimizing our lead **2** while maintaining the 1-phenylpyrrole template. Accordingly, we substituted the carbamoyl moiety of **2** by: i) a piperazine (**4a,b**); ii) a piperazine urea (**4c,d**); iii) a piperazine amide (**4e**). Moreover, these modifications were combined with a different functionalization at position 3 of the pyrrole ring of **2** (such as removal of the amide moiety (**4c**) or insertion of a nitrile group (**4a**)).

The second subgroup of compounds (scaffold B, analogues **5a-f**) stems from a further simplification streamline of the structure of **2**. Accordingly, we replaced the pyrrole ring with a piperazine ring thus transforming the original 1-phenylpyrrole core in the arylpiperazine core system, characterized by a protonatable function (**5b, c**). The arylpiperazine represents a new scaffold for the development of FAAH inhibitors. Based on this versatile system, we synthesized analogues decorated by specific groups that characterize the structure of URB597, **1**, **2** and the oximecarbamate analogues.[11] As a further modification aiming at improving the solubility profile of phenylpiperazines, we explored the outcome of derivatives bearing an ethereal chain in place of the carbamate moiety (**5e**, Table 1 for Log*S*) or an oximecarbamate function (**5f**). The potency of inhibition of FAAH for the newly developed compounds (Table 1) was assessed through inhibition studies performed on the mouse brain enzyme. The inhibitory activity of **4b** and **5c**, the most interesting inhibitors of these two series of analogues, was further evaluated on the human recombinant enzyme (Table 2). For the same compounds dialysis studies were also performed in order to evaluate the reversibility of FAAH inhibition. A SAR analysis of the data reported in Table 1 evidences the best substitution for the 1-phenylpyrrole series of analogues. Starting from our lead **2**, the replacement of the carbamoyl functionality by a phenylhexylpiperazine, while maintaining the amide function at the pyrrole ring, led to a new inhibitor (**4b**) characterized by a nanomolar potency at *m*FAAH. We further modified the structure of **4b**. Conversely, the introduction of a nitrile group at 3 position of the pyrrole system brought to a loss of activity (**4a**) of one order of magnitude. Similarly to **3**, an isothiazole inhibitor lacking the amide functionality, introduction of the piperazine urea moiety, combined or not with the presence of the amide at the pyrrole ring (**4c,d**) led to submicromolar FAAH inhibitors with **4c** slightly more potent than **4d**. Spacing the piperazine ring from the 1-phenylpyrrole scaffold by a carbonyl unit (piperazine amide **4e**) was not tolerated and the compound was found not effective in inhibiting the enzyme at the tested concentrations. Taking into account the series of compounds **4** the modifications performed on lead **2** did not provide a significant improvement of Log*S*. In fact compound **2** and its analogue **4b** show a comparable calculated Log*S* value at pH 7 (Table 1).

Starting from the core structure of **2** we decided also to explore novel arylpiperazines for FAAH inhibition. Compounds **5a-d** based on scaffold B (Figure 2) were characterized by the presence of the urethane function and the lack of the pyrrole-3-amide group. Among these compounds **5c** was found as the most potent of this sub-series, being identified as a nanomolar inhibitor of *m*FAAH. Furthermore, as shown in Table 1, its calculated Log*S* indicate a much higher solubility with respect to compound **2** and **4b**. Replacement of the urethane function by a polyethereal arylalkyl chain (**5e**) and the introduction of an oximecarbamate function led to poorly active analogues.

Table 2. Further Characterization of Mouse and Human FAAH Inhibition, for Compounds **4b** and **5c**.

Cpd	Type of inhibition[a]	IC_{50} (nM)[b]	K_i (nM)[b]
4b	reversible non competitive	>1000	N.D.[c]
5c	reversible non competitive	**44**	26

[a]*mouse FAAH;* [b]*human FAAH;* [c]N.D. not determined

In line with the pharmacological in vitro characterization of our lead **2**, dialysis studies performed on compounds **4b** and **5c** revealed a reversible non competitive profile for these analogues towards *m*FAAH (Table 2). Furthermore for compound **5c** but not for 4b, the efficacy profile was maintained when tested against human FAAH.

Selectivity profile was assessed by Cerep, testing **5c** towards 54 different receptors, ion channels and transporters. The compound was tested at 1 μM concentration and the % of inhibition is shown in Table 1 of the Supporting Information. At this concentration, **5c** showed low affinity for the majority of receptors tested, except for 5-HT_1, 5-HT_2 serotonin receptors, D_1, δ_2 dopamine transporter. Low affinity for AEA-binding cannabinoid receptors was also demonstrated.

In summary, we performed some biopharmaceutical optimization of compound **2** with the aim of balancing biological activity and chemico-physical properties. While **4b** shows properties similar to our lead compound **2**,exclusively inhibiting *m*FAAH, the arylpiperazine **5c** may be considered prototypic of a new class of potent inhibitors of human and murine FAAH with improved calculated solubility profile. Furthermore, dialysis studies showed that **5c** behaves as a reversible and non competitive inhibitor of the mouse enzyme.

Acknowledgments

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