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Novel imidazobenzazepine derivatives as dual H_1/5-HT_{2A} antagonists for the treatment of sleep disorders

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ABSTRACT

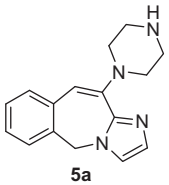
A novel imidazobenzazepine template (**5a**) with potent dual H_1/5-HT_{2A} antagonist activity was identified. Application of a zwitterionic approach to this poorly selective and poorly developable starting point successfully delivered a class of high quality leads, 3-[4-(3-R^1-2-R-5H-imidazo[1,2-*b*][2]benzazepin-11-yl)-1-piperazinyl]-2,2-dimethylpropanoic acids (e.g., **9**, **19**, **20**, and **21**), characterized by potent and balanced H_1/5-HT_{2A} receptor antagonist activities and good developability profiles.

Histamine H_1 and serotonin 5-HT_{2A} receptors mediate two different mechanisms involved in sleep regulation. Brain histamine is involved in the regulation of the sleep–wake cycle, arousal, cognition, and memory mainly through H_1 receptors[1] and H_1 antagonists act as sleep inducers. On the other hand selective blockade of 5-HT_{2A} receptor has been proven in both preclinical[2] and clinical[3] studies to be efficacious in reducing wake after sleep onset (WASO), increasing slow wave sleep (SWS), and total sleep time (TST) therefore providing consolidation of sleep. It can be hypothesized that the combination of these two mechanisms may offer an improved hypnotic profile with respect to marketed gold standard benzodiazepine-like drugs.[4]

As part of a discovery program to identify dual H_1/5-HT_{2A} antagonists the novel imidazobenzazepine compound **5a** (Fig. 1) was designed and prepared in house. Upon characterization it was found to be endowed with good H_1/5-HT_{2A} potency but poor selectivity, in particular towards adrenergic targets. In designing a specific optimization strategy for this compound we reasoned that the introduction of an aminoacid moiety in the top region of the molecule may modulate the developability profile of the series appropriately.

It was well known that zwitterion moieties were tolerated by H_1 receptor antagonists,[5] as they have been originally introduced to reduce the sedating properties of centrally acting first generation anti-histaminic, by reducing their brain penetrating capacities. Furthermore, in the second generation H_1 antagonists (e.g., Cetirizine and Fexofenadine), the introduction of the zwitterion group was responsible for the reduction of the drug–drug interactions and enhanced receptor selectivity profiles with respect of first generation drugs.[6]

Despite the fact that zwitterions were initially introduced to avoid brain penetration of the first generation anti-histamines, scientists at Hypnion, now Lilly, recently demonstrated that zwitterionic structures can penetrate the brain and induce hypnotic activity. In fact they shaped their sleep programme strategy by



5a

In-vitro pharmacology

h-*f*pKi H_1[a] / 5-HT_{2A}[b] = 9.2/8.1
h-*f*pKi[a] 5-$HT_{2B/C}$ = 6.5/7.7
h-*f*pKi[b] $\alpha_{1A/B}$ = 8.4/8.5

Figure 1. In vitro data of compound **5a**. [a]*f*-pK_i, functional p*K*i obtained from the FLIPR (fluorescent imaging plate reader) assay.[11] [b]*f*-pK_i, functional p*K*i obtained from the aequorin assay (intracellular Ca luminescence).[12]

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introducing zwitterion moieties to known H_1/5-HT_{2A} antagonist scaffolds.[7] This approach led Hypnion to quickly progress several compounds into the clinical phase, one of which, HY-10275 (LY-2624803), successfully achieved its Proof of Concept as a sleep drug.[8]

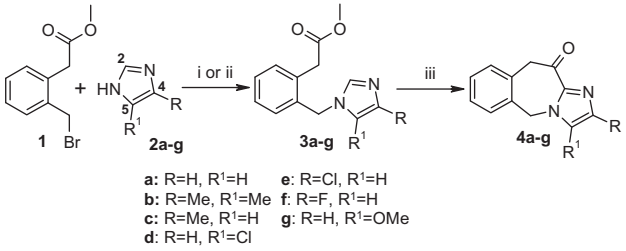
Herein we report our results applying the zwitterionic approach that led to the identification of several advanced leads with excellent developability profiles.

A novel synthetic pathway was put in place for the synthesis of the compounds reported in this paper; in particular the first two steps that allow access to the key intermediate **4** (Scheme 1) proved critical.

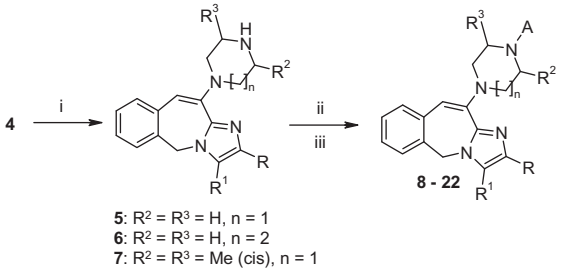
The first step is the crucial one, in particular for substituted imidazoles **2c**–**g**, in order to obtain the target cycloketone **4** with the desired regiochemistry of substituents R and R^1. Imidazole **2a** and symmetric dimethyl imidazole **2b**, were reacted with **1** in DMF, without the use of any additional base, to give the correspondent alkylated imidazoles **3a** and **3b**.

Subsequent cyclization with LDA gave **4a** and **4b**, respectively, exploiting the acidity of the hydrogen in the 2-position of the imidazole. Regarding the mono-substituted imidazoles, two approaches were developed for the selective syntheses of the imidazole derivatives **3**. To obtain the 5-substituted benzyl imidazoles **3c**, **3d**, and **3g**, at least as the major regioisomers, we employed the same base free conditions as reported above, while for the 4-substituted benzyl imidazoles **3e** and **3f**, we were able to obtain high levels of selectivity by introducing a base to the reaction mixture. An optimization process was required for this reaction as the bases examined initially, such as K_2CO_3 and NaH, allowed formation of the desired 4-substituted product with satisfactory selectivity but in low yield as these bases promoted oligomerisation of the bromoester **1**. This problem was overcome by the use of a Verkade proazaphosphatrane[9] base to effect the alkylation. This nonionic reagent is a strong base readily able to deprotonate the imidazole and hence favors the 4-substituted products but it is a very weak Lewis acid and therefore avoids the oligomerisation side reaction. The ring closure step of compounds **3c**–**g**, to the corresponding tricyclic ketones **4c**–**g**, was carried out using LDA as described for **4a**,**b**. The ketones **4** obtained were then treated with trimethylsilyl triflate (TMSOTf) in the presence of the appropriate piperazine derivative to give the intermediates **5**, **6** and **7**[10] (Scheme 2). The amino acid side chain was introduced either by means of a reductive amination reaction or, in the case of compounds **8** and **11**, an aza-Michael reaction of the intermediates **5a** and **7a**, respectively, with an acrylate. Compound **12** was synthesized using methyl 2-(bromomethyl)-2-propenoate. Finally, hydrolysis of the esters furnished the desired final zwitterionic compounds **8**–**22** (see Table 1).

The SAR exploration was principally focused on three elements: first, substitution/homologation of the piperazine in order to study the influence of the most basic center of the compounds; second, modulation of the acidic moiety; third, introduction of substituents



Scheme 1. Reagents and conditions: (i) (used for compounds **3a**, **3b**, **3c**, **3d**, and **3g**) DMF, room temperature, 8 h; (ii) (used for compounds **3e** and **3f**) THF, Verkade's Superbase, room temperature, 2 h; (iii) THF, LDA, from −78 °C to room temperature, 12 h.



Scheme 2. Reagents and conditions: (i) appropriate piperazine derivative (see Table 1), TMSOTf, 130°C, 2 h; (ii) appropriate ester (A, see Tables 1), DCE, $NaBH(OAc)_3$, rt, 12 h; (iii) KOH or LiOH, $MeOH/H_2O$; reflux, 3 h.

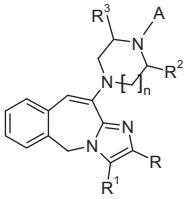
on the tricyclic portion of the compound, in particular on the imidazole ring, with the aim to mask and reduce its basicity.

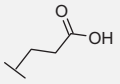
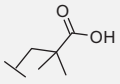
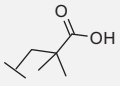
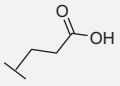
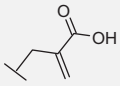
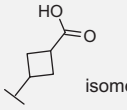
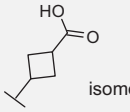
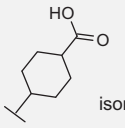
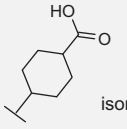
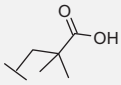
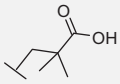
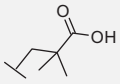
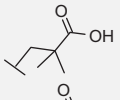
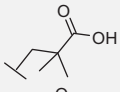
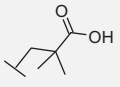
All the newly prepared chemical entities were assayed for their agonistic and antagonistic properties using two kinds of functional assays: FLIPR[11] (fluorescent imaging plate reader) assays, using either adherent transfected Chinese hamster ovary (CHO) cells stably expressing the recombinant human H_1 receptor or adherent SHSY5Y cells stably expressing recombinant human 5-HT_{2B} or 5-HT_{2C} receptors; luminescence[12] (aequorin) assays using either frozen Chinese hamster ovary (CHO) cells stably expressing the human H_1 receptor and aequorin apo-protein or frozen human embryonic kidney (HEK) cells stably expressing the human 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptors and aequorin apo-protein.

Before going into a detailed discussion of the results obtained, a few general observations need to be made. No agonistic activity was observed for any of the amino acids considered; high selectivity versus 5-HT_{2B} and 5-HT_{2C} receptors proved elusive, however this was not considered a criterion to preclude compound progression. While there are few evidences of slight increases in wakefulness and motor activity after blockade of the 5-HT_{2B} receptor,[13] no significant alteration in the percentage distribution of any sleep stage, arousal and light SWS (SWS1), was observed with the inhibition of the 5-HT_{2C}.[14] Therefore the presence of 5-HT_{2B} and 5-HT_{2C} activity in our compounds may not represent a potential issue for their hypnotic profile. With regards to selectivity versus adrenergic α_{1A} and α_{1B} receptors, which was a concern for our initial hit **5a** (Fig. 1), for the zwitterionic compounds synthesized and tested in this assay, we never observed any significant activity on these two receptors.[15]

From the outset of our exploration we noticed that in terms of activity versus the primary targets the zwitterionic modification always led to a reduction of both H_1 and 5-HT_{2A} activities, but while a suitable level of potency is generally maintained at H_1, potency at 5-HT_{2A} is much more sensitive, thus since the beginning it was clear that the challenge for the exploration was to find compounds with balanced H_1/5-HT_{2A} activity. The first two zwitterionic compounds in the table are testimony to this behavior. Compound **8** shows good H_1 activity while its 5-HT_{2A} activity is unsatisfactory for progression. In contrast, compound **9**, characterized by additional geminal dimethyl groups α to the acidic moiety that have the dual function of increasing lipophilicity and 'masking' the acid, demonstrates good H_1/5-HT_{2A} activity and selectivity versus 5-HT_{2B} but not versus 5-HT_{2C}. Due to its good overall in vitro profile, compound **9** was progressed into a rat pharmacokinetics (PK) study,[16] see Table 2. It showed moderate blood clearance, moderate volume of distribution, a half-life of 0.7 h and good oral bioavailability. Compound **9** was also characterized by a high brain fraction unbound (>50%) and low brain penetration (0.02). As a result of this low brain penetration there is a substantial imbalance between the free drug concentrations in the brain and in the blood (free blood concentrations ~50-fold higher than

Table 1
Functional activity (*f*-pK_i[a]) at the human H_1,[b] 5-HT_{2A},[c] 5-HT_{2B},[b] and 5-HT_{2C}[b] receptors, for compounds **5a** and **8**–**22**



Compound	R	R^1	R^2	R^3	*n*	A	*f*-pK_i[a]			
							H_1[b]	5-HT_{2A}[c]	5-HT_{2B}[b]	5-HT_{2C}[b]
5a	H	H	H	H	1	H	9.2	8.1	6.5	7.7
8	H	H	H	H	1		7.3[c]	6.5	<6.1[c]	<6.1[c]
9	H	H	H	H	1		7.6	7.1	5.8	7.3
10	H	H	H	H	2		7.3	<5.3	<5.7	<5.7
11	H	H	Me	Me	1		7.7	7.4	6.2	6.8
12	H	H	H	H	1		7.5[c]	6.7	<6.1	6.3
13	H	H	H	H	1	 isomer 1	7.9[c]	<6.1	<6.1	<6.1
14	H	H	H	H	1	 isomer 2	7.6[c]	<6.1	<6.1	<6.1
15	H	H	H	H	1	 isomer 1	6.9	6.2	7.4	<5.7
16	H	H	H	H	1	 isomer 2	7.2	6.3	7.6	<5.7
17	Me	Me	H	H	1		7.4	6.4	7.8	7.2
18	Me	H	H	H	1		7.3	6.9	7.2	7.5
19	Cl	H	H	H	1		7.1[c]	7.3	7.0[c]	6.3[c]
20	H	Cl	H	H	1		7.4[c]	7.2	7.6[c]	6.9[c]
21	F	H	H	H	1		7.4[c]	7.0	6.5[c]	6.5[c]
22	H	MeO	H	H	1		6.9[c]	6.6	6.8[c]	6.3[c]

[a] *f*-pK_i, functional p*Ki* obtained from the FLIPR and aequorin assays. SEM for H_1, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} data sets is <0.2 with a minimum of two replicates.
[b] FLIPR, fluorescent imaging plate reader.[11]
[c] Aequorin assay (intracellular Ca luminescence).[12]

Table 2
Rat pharmacokinetic profile for compounds **9**, **19**, **20**, and **21**

Compound	Br/Bl fu[a] (%)	In vivo PK[b]				
		CLb (ml/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	F_{po} (%)	Br:Bl
9	>50/>50	38	1.9	0.7	61	0.02
19	30/34	11	1.9	2.4	58	0.07
20	18/22	3	1.1	5.0	37	0.07
21	45/40	10	1.1	1.8	45	0.04

[a] fu, fraction unbound in brain/blood rat.
[b] In vivo data determined following 0.5 mg/kg iv and 1 mg/kg po administration in rat, for compounds **19**, **20**, and **21** and by 1 mg/kg iv and 3 mg/kg po administration in rat for compound **9**. Br:Bl measured as brain to blood AUC_{0-8h} ratio following po dosing.

free brain concentrations) that will greatly reduce the possibility of achieving an acceptable therapeutic index. Therefore, an additional goal for the exploration was to modify the physicochemical properties of the newly synthesized derivative **9** in order to obtain a compound with a more balanced profile in terms of the free concentration in blood and brain. This topic will be discussed in detail below.

With regard to modification of the piperazine, two compounds were prepared: the homopiperazine derivative **10**, which maintained comparable H_1 activity but completely lost 5-HT_{2A} potency, and the dimethylpiperazine derivative **11**, that was characterized by balanced potency at the target receptors and reasonable selectivity versus 5-HT_{2B}.

Some efforts to move away from the 2,2-dimethylpropanoic acid moiety were also made. A propenoic acid derivative **12**, the two diastereoisomers of cyclobutanecarboxylic acid **13**, **14** and the two diastereoisomers of cyclohexanecarboxylic acid **15**, **16** were synthesized. In general they presented good H_1 potency but had poor activity at 5-HT_{2A}; the only compound from this series that showed interesting target activities was compound **12**.

The first attempts to introduce substituents onto the imidazole ring gave compounds **17** and **18**. These methylated derivatives were prepared with the objective of increasing lipophilicity thus potentially improving brain penetration with respect to **9**. In both cases the target receptor potencies were suboptimal and they were not progressed into PK studies. Attention then turned to substituents that could modulate more effectively the electronic nature, and therefore the basicity, of the imidazole ring.

Introduction of the electron donating methoxy substituent in compound **22** was not well accepted, leading to a slight drop in activity at both target receptors. In contrast, electron withdrawing substituents appear to be better tolerated with halogenated derivatives of **9** maintaining similar levels of activity to the parent. Compounds **19** and **20**, carrying a chlorine in the 5- and the 4-position of the imidazole, respectively, and compound **21**, carrying a fluorine in the 4-position, all demonstrated good H_1/5-HT_{2A} activity but little or no selectivity versus 5-HT_{2B} and 5-HT_{2C}.

As remarked earlier, the parent compound **9** is characterized by low brain penetration and a goal was set to modify the physicochemical properties with the objective of improving brain penetration. In particular, compounds **19**, **20**, and **21** were the result of a tailored medicinal chemistry strategy focused on reducing the basicity of the imidizole moiety and increasing the lipophilicity of compound **9** through substitution with electron withdrawing groups; it was hypothesized that these modifications should lead to a reduction of the volume of distribution, and consequently a reduction in half-life, as well as reducing the imbalance between free concentrations in brain and blood.

Results obtained in in vivo PK studies with these halogenated derivatives of **9** are detailed in Table 2. All three compounds showed low clearance, a moderate volume of distribution, a half-life comprised between 1.8 and 5 h and good oral bioavailability. Additionally, all of them were characterized by high fraction unbound in brain and blood and a brain penetration comprised between 0.04 and 0.07.

To understand if the low brain penetration observed is under the control of an efflux system, the interaction with P-gp (P-glycoprotein) was investigated in vivo in rat with a pre-treatment with a known inhibitor of efflux transporters (GF120918).[17] Compounds **19**, **20**, and **21** were tested in this paradigm and while the fold change in the Br:Bl ratio in the presence and in the absence of the inhibitor was significant for **20** and **21**, suggesting a possible role of efflux processes, for compound **19** it was not, suggesting no role of efflux processes.

Compounds **19**, **20**, and **21** were specifically prepared to test the hypothesis that reducing the basicity of the imidazole ring and increasing lipophilicity would have a positive impact both on brain penetration and on the half-life, via reduction of the volume of distribution.

Recalling that compounds **19**, **20**, and **21** were prepared to test a specific hypothesis, an analysis of the rat PK data demonstrates that these modifications have indeed led to improvements in brain penetration (Br:Bl = 0.04–0.07 vs 0.02 for **9**), attenuating somewhat the imbalance between free concentrations in brain and blood.

Furthermore, the volumes of distribution for two of the three derivatives were lower than for the parent (V_{ss} = 1.1 L/kg vs 1.9 for **9**) as speculated, however this did not translate into a shorter half-life due to greater metabolic stability (half-life = 1.8–5.0 h vs 0.7 h for **9**).

In conclusion, a novel imidazobenzazepine template (**5a**) with potent dual H_1/5-HT_{2A} antagonist activity was identified. Application of a zwitterionic approach to this poorly selective and poorly developable starting point successfully delivered a class of high quality leads, 3-[4-(3-R^1-2-R-5H-imidazo[1,2-*b*][2]benzazepin-11-yl)-1-piperazinyl]-2,2-dimethylpropanoic acids (e.g., **9**, **19**, **20**, and **21**), characterized by potent and balanced H_1/5-HT_{2A} receptor antagonist activities and good developability profiles. Compound **9** was also progressed into a pharmacodynamic model for sleep disorders,[16,18] where it exhibited a robust effect (statistical significant effect in the TST was reached at 1.0 mg/kg), thus confirming a possible application as an alternative therapy to currently available hypnotic drugs with the potential for a reduced side-effect burden.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.029.

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