

Histamine-3 Receptor Inverse Agonists for the Treatment of Obesity: Validation of the Target and Identification of Novel Series

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Abstract: Obesity is a major risk factor for the development of conditions such as hypertension, hyperglycemia, dyslipidemia, coronary artery disease and cancer. Several pieces of evidence, including data in primates, have demonstrated the beneficial effects of histamine-3 receptor (H₃R) inverse agonists in the regulation of food intake and body weight. A pharmacophore model based on selected published H₃R ligands and validated by previous investigations, was used to identify the 5-oxy-2-carboxamide-indole core as a novel series of H₃R inverse agonists. Extensive structure–activity relationship (SAR) investigations were rewarded by the identification of several compounds reversing (*R*)- α -methyl-histamine-induced water intake increase and reducing food intake/body weight in rodent models of obesity. Among those compounds, (4,4-difluoro-piperidin-1-yl)-[1-isopropyl-5-(1-isopropyl-piperidin-4-yloxy)-1*H*-indol-2-yl]-methanone, selected as a lead compound, was exhibiting a promising profile, including excellent pharmacokinetic properties, good *in vitro* safety profile and high efficacy in a chronic rodent model of obesity.

Keywords: hERG · Histamine-3 receptor · Inverse agonist · Obesity · Pharmacophore model

1. Introduction

The histamine receptor family, comprising the four subtypes 1–4,^[1] has already received considerable attention, and led to several blockbusters in the inflammation and gastric secretion fields.^[2] Within this family, the histamine-3 receptor (H₃R) acts both as an autoreceptor, controlling the synthesis and release of histamine, and also as a heteroreceptor, controlling other crucial neurotransmitters (*e.g.* acetylcholine, norephedrine, serotonin, dopamine).^[3]

As a consequence of this rich pharmacology, three distinct therapeutic areas can potentially be envisaged: central nervous system (CNS) disorders (*e.g.* Alzheimer, schizophrenia, anxiety, cognitive deficit),

sleep disorders and metabolic disorders.^[4] Among the latter, we decided to concentrate on obesity, a dramatically increasing burden with tremendous needs for novel and efficient therapies.

H₃R is highly constitutively active; therefore, most of the antagonists should be properly classified as inverse agonists. In term of mechanism, H₃R inverse agonists/antagonists suppress food intake *via* increased histamine release and consequently, increased activation of postsynaptic histamine-1 receptor (H₁R). This H₁R activation is restricted to the CNS, where the H₃R is mainly expressed.

Indeed, in recent years, some potent and selective H₃R antagonists/inverse agonists have demonstrated efficacy in several models of obesity, including in primates. However, some of these compounds also showed clear limitations mainly in terms of safety (*e.g.* hERG affinity/cardiovascular liabilities, genotoxicity, CNS side effects).^[5]

2. Pharmacophore Model

The availability of many published structures of potent H₃R ligands has facilitated the construction of an accurate pharmacophore model.^[6,7] This model is

characterized by a basic sp₃ nitrogen (yellow circle in Fig. 1), acting as the main anchor, most probably through a salt bridge formation with Asp3.32. At a distance of 4–5 Å, an electron-rich entity, typically an ether or a carbonyl oxygen (red circle in Fig. 1) is often observed across different chemical series. Optionally, a second basic nitrogen atom can be present at around 10–11 Å from the first one, most probably interacting with Glu5.46. However, some concerns about risks for brain accumulation and phospholipidosis prompted us to avoid this second basic nitrogen. In contrast, we postulated the importance of a second electron rich entity, probably interacting with Thr6.52. Interestingly, this threonine is only present in the H₃R.

Compared to other scaffolds initially investigated,^[6–8] the indole series appeared to have the widest potential, including the option of easy access to a third exit vector on the indole nitrogen – so far poorly explored – and an excellent chemical tractability.

3. Early SAR and Chemistry

Capitalizing on our previous investigations,^[7,8] the identification of H₃R inverse agonists proved to be straightforward by

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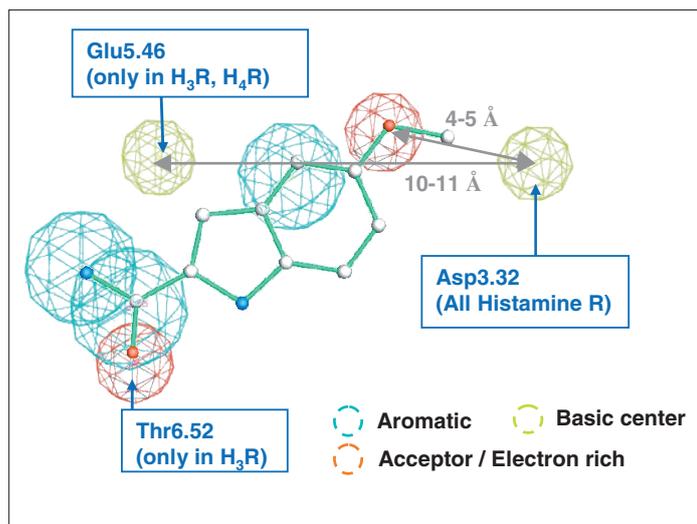


Fig. 1. 5-Oxy-2-carboxamide indole docked into the H_3R pharmacophore model.

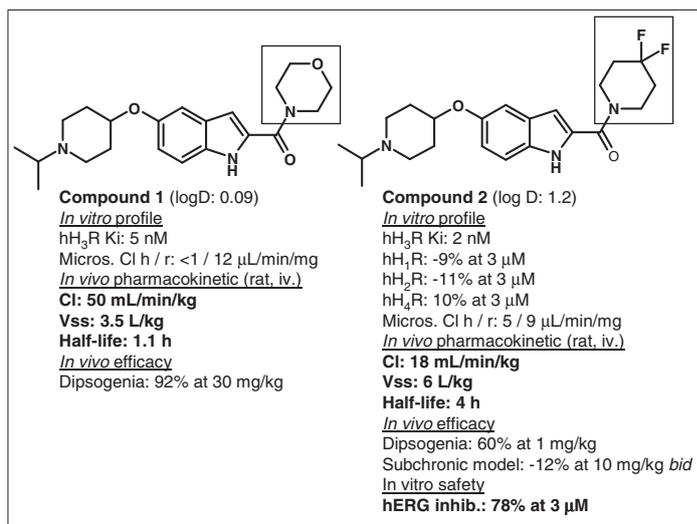


Fig. 2. Selected examples for the influence of the amide substituent on the metabolic stability.

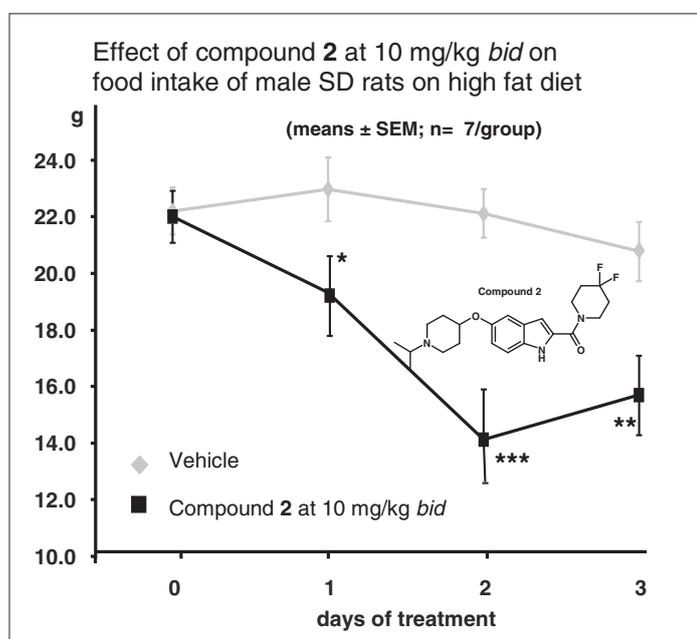


Fig. 3. Efficacy of compound **2** in a subchronic model of obesity at 10 mg/kg *bid*.

combining a 1-isopropyl-piperidin-4-oxy substituent on the 5-position of the indole with a cyclic amide on the 2-position. Being potent (hH_3R Ki = 5 nM) and metabolically stable in microsomal preparation (micros. Cl human/rat = <1/12 μ L/min/mg), compound **1** was an excellent candidate for the first line *in vivo* screening experiment: the dipsogenia assay.^[9] This experiment is based on an antagonist/inverse agonist reversion of the water-intake induced by the H_3R selective agonist (*R*)- α -methyl-histamine (RAMH) in rat.

In this *in vivo* functional assay, compound **1** reversed the RAMH-induced water intake by 92% at 30 mg/kg. Therefore, we decided to further profile this promising compound in a disease-relevant model. Compound **1** was applied at a dose of 30 mg/kg *bid* (twice daily) over three days to Sprague Dawley (SD) rats fed with a high

fat diet (HFD, 42% of energy intake as fat), and their body weight was recorded. To our disappointment, compound **1** did not show any significant effect in this sub-chronic model. Although the compound was able to permeate the CNS (brain to plasma ratio = 0.3), pharmacokinetics disappointingly revealed a fast metabolism (Cl tot. = 50 ml/min/kg) and a short half-life ($t_{1/2}$ = 1 h). We hypothesized that a longer half-life might be required to cover the whole active period of the animal (*i.e.* 12 h during the dark phase) and started searching for compounds with such a property.

The replacement of the morpholine by the more lipophilic piperidine, shielded by two fluorine atoms, led simultaneously to a reduction of the *in vivo* clearance and an enlarged volume of distribution, both leading towards an improved half-life. Compound **2** exhibited a promising half-life

($t_{1/2}$ = 4 h) while maintaining an excellent affinity (hH_3R Ki = 2 nM). It was moreover extremely selective over the three other histamine isoforms (Fig. 2).

To our delight, this profile translated into a robust efficacy in the sub-chronic model of obesity, in line with our hypothesis on the critical importance of the half-life (Fig. 3).

However, compound **2** demonstrated some inhibitory potential for the human ether-a-go-go related gene (hERG). hERG inhibition is considered to be an *in vitro* predictor of potential cardiovascular liabilities. Taking into account the late failure of some competitors' compounds because of cardiovascular-related issues,^[5] the 78% inhibition value of the hERG channel at 10 μ M was therefore judged suboptimal (Fig. 2).

As far as hERG inhibition is concerned, a basic nitrogen is often suspected as the *usual culprit*, although definitely not the only one.^[10]

Therefore the relationship between pKa, hH_3R affinity and hERG inhibition was studied by varying the *needle* at the 5-position. Compound **3**, an analog of the original compound **1** decorated with an additional N-2,2,2-trifluoroethyl group side-chain, exhibited a pKa of 9.7. In the same series, replacement of the N-isopropyl by a N-cyclopropyl reduced the pKa by about 2 log units (compound **4**: pKa = 7.5) without a major change on the hH_3R affinity. On the other hand, using the linear propoxy linker, it was demonstrated that the phenolic ether in compound **5** could be advantageously replaced by a carbonyl group (compound **6**). We decided to combine both modifications – into a unique *needle*. As a result, the carbonylpiperazine **7** featured a very low pKa value (pKa = 6.8) while keeping an excellent binding affinity (hH_3R

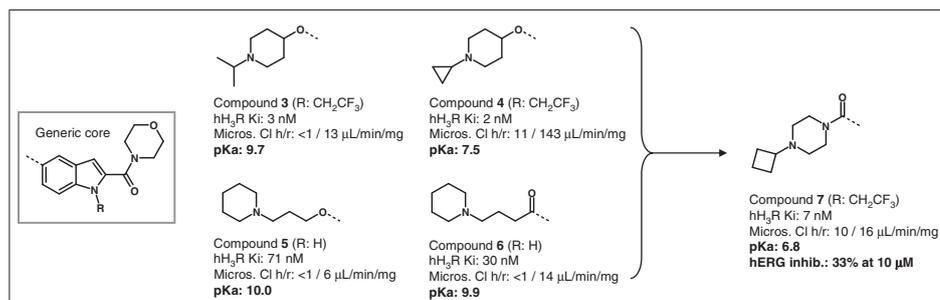


Fig. 4. Selected examples exploring the side chain basicity.

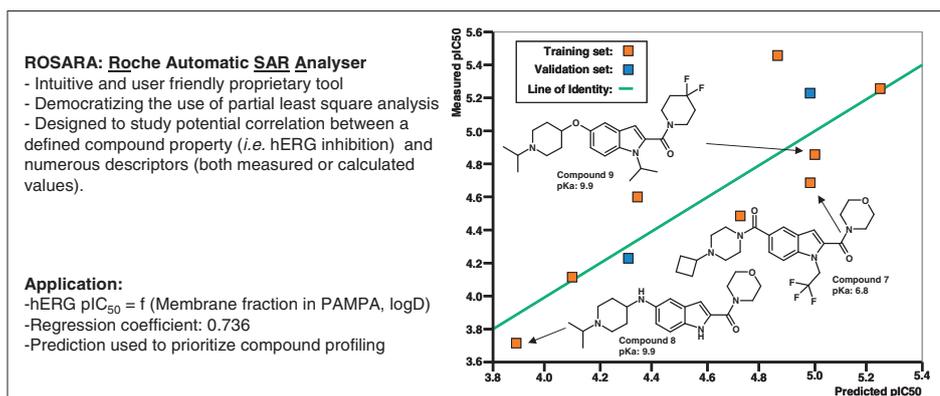
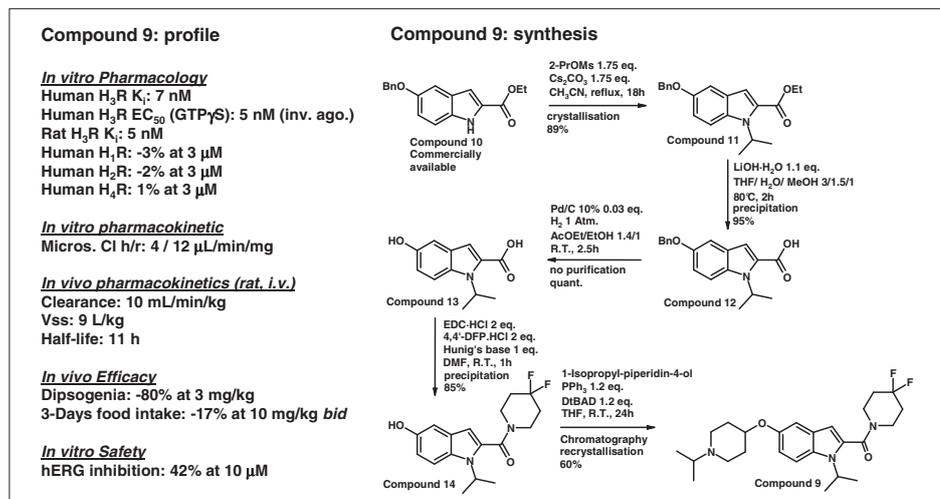
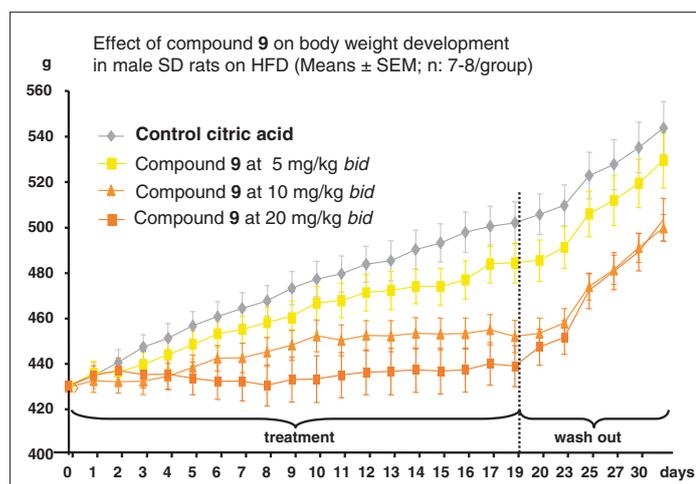
Fig. 5. Correlation of experimental vs. calculated hERG pIC_{50} , using Roche Automatic SAR Analyser (ROSARA).

Fig. 6. Profile and chemical synthesis of compound 9.

Fig. 7. Chronic application of compound 9 at 5 (yellow), 10 (orange) and 20 (red) mg/kg *bid* in a Diet-Induced Obesity rat model.

$K_i = 7$ nM). The hERG inhibition was also reduced (Fig. 4). Although, thanks to this exercise it became clear that H_3R affinity was independent of the basicity within a broad pK_a range (*ca.* 6.8–10.0), the relationship between pK_a and hERG inhibition remained elusive.

In order to gain a better understanding, we have used ROSARA, the Roche Automatic SAR Analyser.^[11] ROSARA is an intuitive and user friendly proprietary tool which democratizes the use of partial least square analysis. It is designed to allow a fast study of the putative correlations between a defined compound property (in this case: hERG inhibition, converted to pIC_{50}) and numerical descriptors. These descriptors may be both measured (*e.g.* LYSA^[12] solubility, logD, hH_3R K_i , pK_a , *etc.*) or calculated (*e.g.* $clogP$, PSA, *etc.*). In our case, ROSARA was able to generate a valuable hERG inhibition model (regression coefficient = 0.736) by selecting only two *in vitro* descriptors: the percentage of membrane-bound compound in the Parallel Artificial Membrane Permeation Assay (PAMPA)^[13] and the logD value (measured lipophilicity at $pH = 7.4$). As some small molecule hERG inhibitors are assumed to interact with this channel through the membrane section, this correlation did indeed make sense. Interestingly, although the pK_a spanned over more than three log units in the training set, no correlation with hERG affinity was observed. The model has been validated with two new compounds from the same series (blue squares, Fig. 5).

The independence of pK_a and hERG inhibition encouraged us to reinvestigate compounds having rather strong basicity ($pK_a = 9$ –10). Among them, compound 9 (Fig. 6) attracted our attention by its striking combination of a high H_3R binding affinity (hH_3R $K_i = 7$ nM), low clearance *in vivo* (rat $Cl_{tot.} = 10$ $ml \cdot min^{-1} \cdot kg^{-1}$), extended half life ($t_{1/2} = 11$ h in rat), rather low hERG inhibition (42% hERG inhibition at 10 μM) and, eventually, excellent performance in the 3-day obesity model (–17% reduction of food intake at 10 mg/kg *bid* compared to control).

The synthesis of compound 9 was straightforward: commercially available 5-benzyloxy-1H-indole-2-carboxylic acid ethyl ester 10 was N-alkylated with isopropyl methane sulfonate. Both the acidic and the phenolic functionalities were liberated by saponification with lithium hydroxide and hydrogenolysis, respectively, followed by an amine coupling reaction. The ether was obtained by applying a Mitsunobu reaction. This end-game reaction required extensive optimization in order to minimize the elimination of the activated alcohol as a side-reaction. The overall yield was satisfactory (43% over five steps).

Applied in a chronic model of obesity (DIO model in male SD rats) over 17 days, compound **9** led to an impressive and dose-dependent slower progression of body weight gain in all groups (−4, −10, −13% respectively), statistically significant for the 10 and 20 mg/kg groups (Fig. 7).

4. Conclusion

The 5-indole-2-carboxamide-indole scaffold was identified as a valid chemistry entry point by the use of a pharmacophore model. A series of derivatives was identified as novel class of H₃R inverse agonists.^[14] After SAR development, extensive multidimensional optimization, and careful evaluation of the underlying drivers for the H₃R binding affinity and hERG inhibition, compound **9** proved to combine suitable overall characteristics for advancement. In the indirect acute pharmacodynamic dip-sogenia model, compound **9** reversed the water intake induced by the H₃R selective agonist RAMH. Furthermore, in a 17-day Diet-Induced Obesity (DIO) rat model, compound **9** demonstrated a robust and dose dependant reduction in body weight gain. On the basis of its efficacy, metabolic stability and safety profile, compound **9** was selected for further evaluation of its potential for the treatment of obesity.

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