Bioorganic & Medicinal Chemistry Letters 27 (2017) 4525–4530

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel steroidal histamine H_3 receptor antagonists/inverse agonists

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ARTICLE INFO

Article history:
Received 3 July 2017
Revised 28 August 2017
Accepted 29 August 2017
Available online 1 September 2017

Keywords:
Histamine H_3 receptor
Steroid
Estrane derivatives
Antagonist/inverse agonist
Dipsogenia model

ABSTRACT

Emerging from an HTS campaign, novel steroid-based histamine H_3 receptor antagonists were identified and characterized. Structural moieties of the hit compounds were combined to improve binding affinities which resulted in compound **4** as lead molecule. During the lead optimization due to the versatile modifications of diamino steroid derivatives, several *in vitro* potent compounds with subnanomolar binding affinities to histamine H_3 receptors were found. The unfavorable binding to rat muscarinic receptors was successfully reduced by tuning the basicity. Compound **20** showed significant *in vivo* activity in the rat dipsogenia model and could serve as a pharmacological tool in the future.

The biogenic amine histamine mediates its biological actions through the modulation of four G-protein-coupled receptors (H_1R-H_4R).[1] H_1 and H_2 receptors proved to be effective in the therapy of allergy and ulcer, while H_4 receptors play a role in the regulation of immune responses.[2] The histamine H_3 receptor acts as both an autoreceptor controlling the synthesis and release of histamine[3] and a heteroreceptor regulating the release of several key neurotransmitters involved in cognitive processes, such as acetylcholine, norepinephrine, serotonin and dopamine.[4] H_3 receptors are, furthermore, implicated in the regulation of feeding behavior and weight control.[5] Antagonists of the H_3 receptor result in synthesis and release of cerebral histamine and other monoamines, thereby promoting waking, improved cognitive function and normalization of vestibular reflexes. Procognitive activity of H_3 antagonists/inverse agonists seems to involve effects at not only histaminergic but other neurotransmitter systems playing also important roles in cognition.[6] Based on these considerations, significant research has been accomplished in this field, resulting in the identification of a great variety of H_3R antagonists/inverse agonists in the recent years.[7] In spite that several of them were tested in clinical trials, up to now only pitolisant provided positive results in a phase III study in narcolepsy[8] and was already registered.

In the nature, there are several nitrogen-containing steroids with versatile biological activity. The naturally occurring steroid-based alkaloid, conessine was demonstrated to bind to both rat and human H_3 receptors, but not to other histamine receptors.[9]

However, conessine and its analogues also display unfavourable affinity to human alpha$_{2c}$ adrenergic receptors and muscarinic (M_1) receptors.[10] Elimination of these properties may be a challenging synthetic task, due to the complicated synthesis of conessine.[11]

Herein we describe our efforts to identify the new generation of steroid-based H_3R antagonists with improved physico-chemical and pharmacological properties suitable for exploring H_3R function in clinical investigations.

Taking into consideration that the HTS campaign utilized the compound collection of Gedeon Richter Plc. (a company with synthetic experiences in steroid chemistry for more than 60 years), it was not a surprise that we identified 22 steroid derivatives (including conessine, as well as other tertiary and quaternary amine compounds). Having analyzed the hit molecules, several of them showed higher (to rat and human H_3 receptors) or lower (to rat muscarinic receptors, rmAChRs) binding affinities than conessine itself (Fig. 1 and Table 1).

Hit compound **2** possessed robust binding characteristics to both human and rat H_3 receptors with two digit nanomolar K_i values, while its affinity to rmAChRs proved to be weaker than that of conessine (however, it was still prominent). Hit compound **3** showed significant binding affinity to the human but not to the

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http://dx.doi.org/10.1016/j.bmcl.2017.08.060
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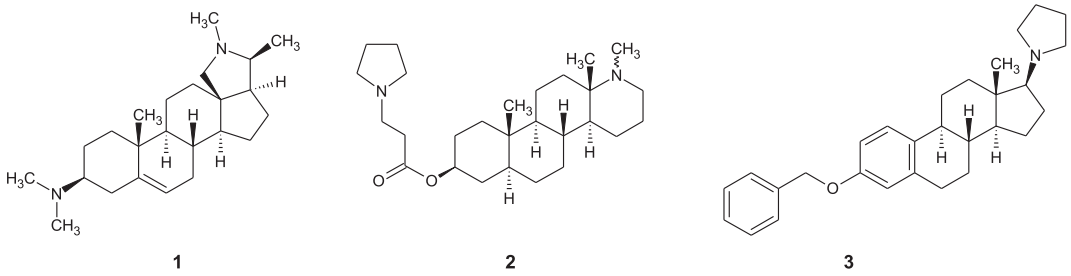

Fig. 1. HTS hits.

Table 1
Binding affinities of **1**, **2** and **3** to human and rat H_3, as well as rat muscarinic ACh receptors (for specific details, see the Supplementary Material) in comparison with reference compounds.

Compound	hH_3 binding K_i or % @300 nM	rH_3 binding K_i or % @300 nM	rmAChR % @1 μM
Thioperamide	191	23	ND
Pitolisant	3	ND	19
1 (conessine)	45%	34%	84%
2	10 nM	39 nM	44%
3	216 nM	3%	5%

rat H_3 receptor, while it possessed very low muscarinic binding activity. These two efficient structures were chosen as starting points for the Hit-to-Lead campaign. Several structural elements of the original hit compounds were incorporated into novel molecules to improve their pharmacological characteristics.

First, the 5-membered D ring with a pyrrolidine from compound **3** was merged with compound **2** using a rather simple synthetic route below (see Fig. 2).

In the first step, the hydroxyl group at position 3 of the steroid skeleton was alkylated with 1,3-dibromopropane (in case of estrone derivatives) or with 3-chloropropan-1-ol through its tosylate (in case of dehydroepiandrosterone derivatives). The obtained halogen compounds were then transformed to the 3-pyrrolidinylpropyl 17-oxo key intermediates which were reacted with a wide variety of secondary amines to afford the corresponding 17-amino derivatives via reductive amination.

Already the first synthesized compound **4** showed significant improvement in its H_3 receptor binding characteristics: its affinity to the hH_3 receptor proved to be in the subnanomolar range (for details, see Table 2.). At the same time, it possessed high selectivity over the other human histamine receptor subtypes (at 1 μM ligand concentration, the displacement values for hH_1, hH_2 and hH_4 receptors were 64, 44 and 36%, respectively), while its affinity to the rat H_3 receptor was also in the low nanomolar range. Binding of Compound 4 to estrogen-α and β receptors was negligible (19 and 26% displacement at 1 μM, respectively), which was not surprising, due to the lack of structural elements (aromatic 3-OH, 17-OH or 17-oxo) responsible for estrogen-like hormonal activity. Affinity to H_1, H_2, H_4, ERα and ERβ was further tested using a diverse set of compounds without any significant results (data not shown), therefore regular testing of these targets were later omitted. The compound showed inverse agonist/antagonist characteristics in the respective *in vitro* functional assays (detailed description of the applied *in vitro* and *in vivo* methodologies are summarized in the Supporting information.[12]) Taking the putative adverse effects of the activation of certain mAChRs into consideration (being M_2 the most evident, with its high expression in the heart, where it controls myocyte contraction and consequently, heart rate), one of the aims of the optimization process was to get rid of this phenomenon. Based on the available dataset, compound **4** was selected as lead compound and the optimization was initiated with special emphasis of elimination of the mAChR binding.

Since a great variety of steroid skeletons (androstane, androstene, estrane) appeared in the hit structures, first it seemed reasonable to evaluate the importance of the steroid skeleton in the affinity towards H_3 and mAChRs. To address this question, several compounds with modified steroid skeletons were synthesized. Among them, addition of a methyl group to the estrane skeleton (**5**, 18βMe; **6**, 7αMe) or the usage of an androstene-like steroid skeleton (**7**) resulted in a retained human and concurrently a ten-fold decreased rat H_3 affinity. Similar conclusion was drawn from studying the influence of the linker length that changes the distance between the two basic nitrogens. It was found that neither shortening (n = 2, **8**) nor lengthening (n = 4, **9**) of the linker chain resulted in significant changes in the affinity compared to

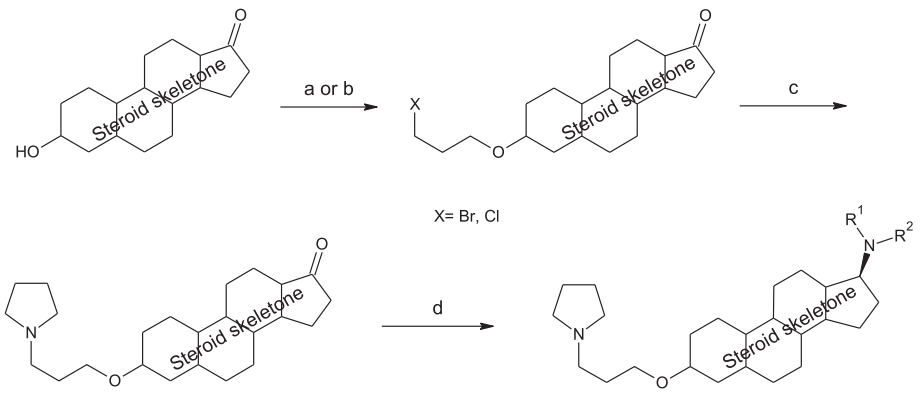

Fig. 2. Synthesis of compound **4** and its analogues **5**–**27**: (a) $Br(CH_2)_3Br$, KOH, H_2O, MeOH, reflux (in case of aromatic A ring); (b) 1: TsCl, THF, Et_3N; 2: $Cl(CH_2)_3OH$, toluene, 80 °C (in case of aliphatic A ring); (c) pyrrolidine, EtOH, reflux; (d) R^1R^2NH, HCOOH, 160 °C. (Detailed synthetic and analytical description of Compound 4 can be seen in the Supplementary Material).