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Discovery of novel steroidal histamine H_3 receptor antagonists/inverse agonists. Part 2. Versatile steroidal carboxamide derivatives

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ABSTRACT

To further proceed with our previous work, novel steroid-based histamine H_3 receptor antagonists were identified and characterized. Using an 'amine-to-amide' modification strategy at position 17, *in vitro* and *in vivo* potent monoamino steroid derivatives were found during the lead optimization. Usage of the non-basic amide moiety resulted in beneficial effects both in activity and selectivity. The 15α-carboxamido derivative **10** was not only highly active at human and rat H_3 receptors, but also showed negligible activity at rat muscarinic receptors. Furthermore, it proved to be considerably stable in human and rat microsomes and showed significant *in vivo* potency in the pharmacodynamic rat dipsogenia test and in the water-labyrinth cognitive model. Based on all of these considerations, compound **10** was appointed to be a preclinical candidate.

Histamine exerts its biological actions through the modulation of four G-protein-coupled receptors (H_1-H_4).[1] Among them, H_3 receptors are deeply involved in the regulation of cognitive processes,[2] wakefulness,[3] feeding behavior,[4] and body weight.[5] Application of compounds acting on H_3 receptors has been proposed for the treatment of Parkinson's disease,[6] schizophrenia,[7] addiction,[8] sleeping disorders,[9] or attention deficit hyperactivity disorder (ADHD).[10] Therefore, significant research activities were concentrated upon the discovery of novel H_3 receptor-acting ligands, resulting in a wide variety of preclinical projects and clinical candidates.[11] Until now, a single H_3 antagonist, pitolisant proved to be effective in narcolepsy and was registered.[12] and launched under the trademark of Wakix®.

Novel active and selective steroid-based H_3 antagonists with potency values in the subnanomolar range have already been identified by Gedeon Richter Plc. (a detailed summary of this activity was already published in a previous paper).[13] During the optimization campaign, significant affinity of the dibasic compounds towards rat muscarinic receptors appeared as a major drawback. This disadvantageous feature was decreased by reducing the basicity of the molecules.

The present paper aims at giving a summary on the continuation of this approach by complete elimination of one basic center from the structure. To achieve this goal, amides – instead of amines – were synthesized. Usage of the non-basic (but slightly acidic) amide moiety was expected to provide beneficial effects both on activity and selectivity. Furthermore, application of monobasic compounds can significantly reduce the known risk of phospholipidosis – a feature that may be attributed to molecules with amphiphilic nature.

Starting from the former lead molecule **1** (although this compound was already presented in our preceeding paper, its pharmacodynamic result is disclosed here first, and it is used as a reference molecule for the new derivatives), an 'amine-to-amide' modification strategy was attempted either in position 17 or at the end of the linker connected to carbon 3. Results of the biological characterization of the representative examples is summarized in Table 1.

In cases when the left-hand side of the molecule contained a cyclic amide (**2**), the *in vitro* binding affinity to both rat (rH_3) and human H_3 (hH_3) receptors remained high. However, the affinity to muscarinic receptors also remained significant; it proved to be even higher than that of compound **1**. The usage of lactam in position 17 (**3**) resulted in much weaker rH_3 binding, but the affinity to muscarinic receptors also decreased. Based on this promising finding, synthesis and characterization of another amide type – namely the 17β-carboxamido derivatives – was attempted. In spite of the fact that compound **4** (a potent binder to both rat and human H_3 receptors) showed only a 25-fold binding selectivity to rH_3 over rat muscarinic receptors, it seemed clear that the carboxamido group could be a viable alternative of the amino (pyrrolidine) moiety in terms of potency.

Another amide derivative – namely the 15β-acetamido compound **5** – showed weaker affinity to muscarinic receptors with strong affinity to H_3 receptors. Although the suboptimal metabolic stability in rat

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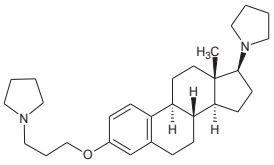
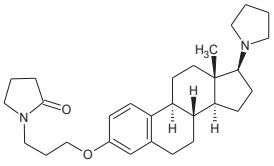
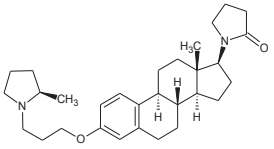
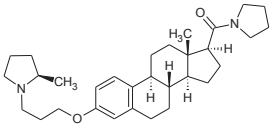
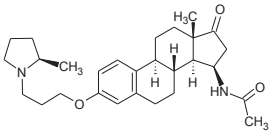
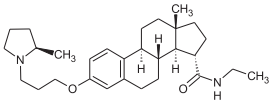
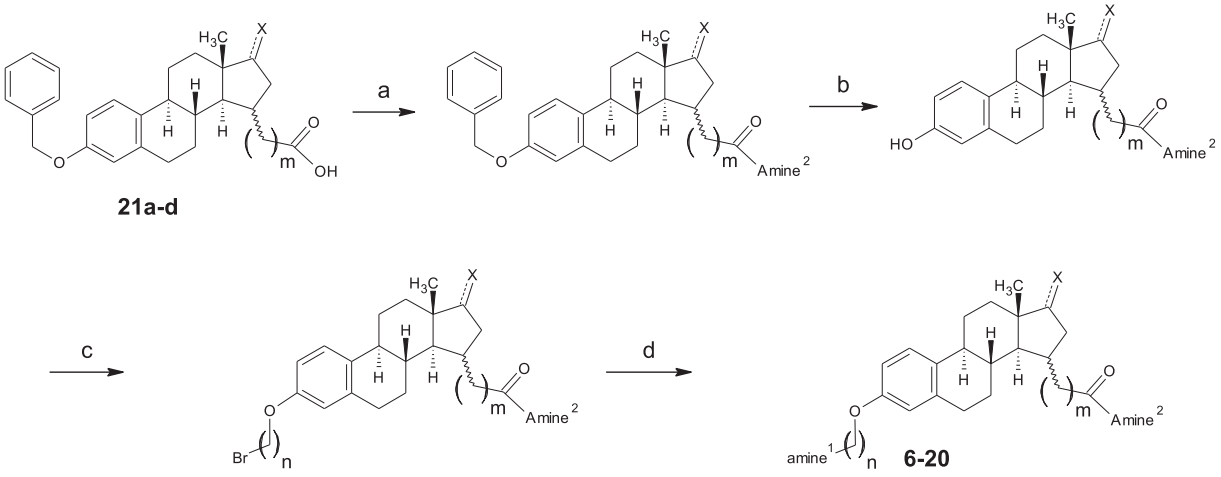
Table 1
Derivatives with 'amine to amide' modification strategy.

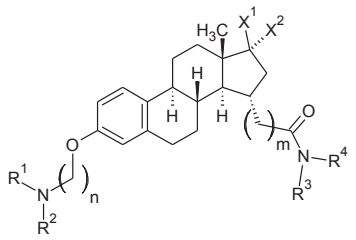
Compound	Steroid structure	hH_3 K_i (nM)	rH_3 K_i (nM)	rmAChR Ki (nM) or %@3 μM	μS F_M% (h/r)	hERG inh. IC_{50} (μM)	Dipsogenia inh. (p.o) ED_{50} (mg/kg)
1		0.6	2.4	110	–	7.5	[a]19% 12 (i.p.)
2		9	22	700	77/85	–	–
3		–	135	51%	–	–	–
4		2	18	454	–	–	–
5		10	39	20%	85/46	–	–
6		9	24	24%	62/66	4,1	2.26

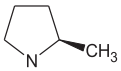
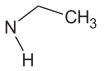
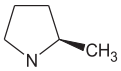
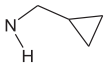
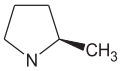
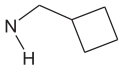
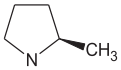
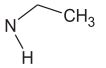
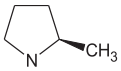
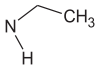
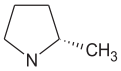
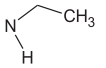
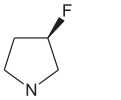
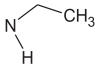
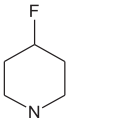
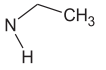
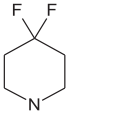
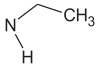
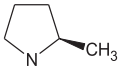
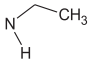
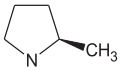
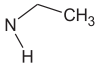
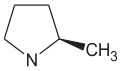
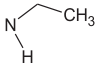
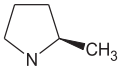
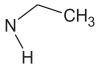
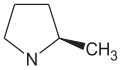
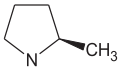
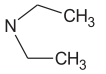
[a] Measured at 30 mg/kg (p.o.).



Scheme 1. General synthetic route to 15-carboxamides (**6–20**): (a) 1. $(COCl)_2$, DMF, CH_2Cl_2, 2. amine[2], CH_2Cl_2, Et_3N; (b) H_2, Pd/C, EtOH; (c) $Br(CH_2)_nBr$, KOH, H_2O, THF, reflux; (d) amine[1], EtOH, reflux.

Table 2
15-Carboxamido derivatives.



ID	n	m	R^1R^2N	R^3R^4N	X^1	X^2	hH_3 K_i (nM)	rH_3 K_i (nM)	rmAChR K_i (nM) or %@3 μM	μS F_M% (h/r)	hERG inh. IC_{50} (μM)	Dipsogenia inh. ED_{50} (mg/kg)
6	3	0			H	H	9	24	24%	62/66	4.1	2.3
7	3	0			H	H	9	13	27%	90/76	4.1	–
8	3	0			H	H	5	19	1708	48/50	–	–
9	3	0			OMe	H	9	28	7%	96/96	11.4	2.5
10	3	0			O		3	9	4700	99/99	17.5	0.47
11	3	0			O		21	–	–	–	–	–
12	3	0			O		230		–	–	–	–
13	3	0			O		530		–	–	–	–
14	3	0			O		1371		–	–	–	–
15*	3	0			O		43	–	–	–	–	–
16	3	1			OMe	H	4	20	6%	59/80	–	–
17	4	0			O		234		17%	–	–	–
18	2	0			O		260		4%	–	–	–
19	3	0			O		30	9	16%	75/85	80	0.6
20	3	0			O		34	44%	–	–	–	–

**Measured at @300 nM.
* In this single case the configuration of C15 was beta.

microsomes averted further development of this molecule, synthesis of certain 15-carboxamido derivatives seemed to be a rational approach. Indeed, 15α-carboxamido compound **6** exhibited one-digit nanomolar affinity to hH_3 receptors with good selectivity vs. rat muscarinic receptors. Furthermore, it showed a robust efficacy in the pharmacodynamic dipsogenia test *in vivo* (ED_{50} = 2.26 mg/kg, p.o.) which is a significant improvement compared to compound **1** (19% inhibition at 30 mg/kg, p.o. or ED_{50} = 12 mg/kg, i.p.). Unfortunately, two drawbacks – namely the relatively low microsomal metabolic stability and the robust inhibitory potential on the hERG channel activity – were also identified. Still, usage of the carboxamido moiety in position 15α resulted in a very promising compound that was selected for further

Table 3
Physico-chemical properties of compound **10**.

Molecular weight	Melting point (°C)	Solubility in DMSO (mM)	Kinetic solubility (μM)	Measured Log P	Measured Log D (pH 7.4)	$[\alpha]_D^{20}$ (c = 0.1%, CH_2Cl_2)
466.7	122–124	> 50	250	4.2	1.7	+75,1°

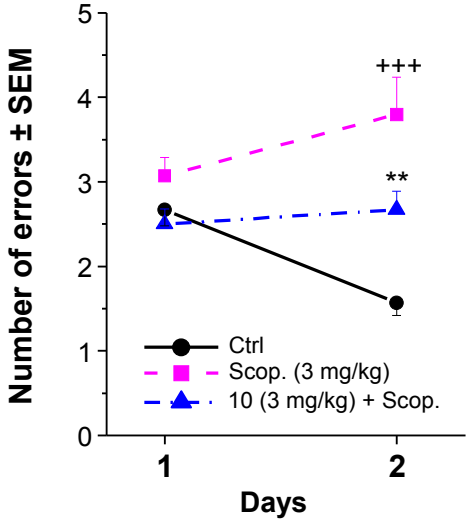


Fig. 1. Effect of compound **10** (3 mg/kg) on the scopolamine-induced amnesia in the rat water labyrinth test (n = 9–10). +++p < 0.001 vs. control, **p < 0.01 vs. scopolamine-treated group (ANOVA, followed by Duncan-test).

exploration.

Based on our previous findings, the central (estrane) core was left unchanged, since this structural element had already proved to be the best choice.[9] In order to further explore the chemical space around the steroidal core, modifications at position 17 (X^1 and X^2), usage of several different amines at the end of the linkers (R^1R^2N and R^3R^4N) and different linkers' lengths (m, n) were tested.

The general synthetic route is summarized in Scheme 1. The benzyl-protected carboxylic acid starting materials were transformed to the desired amides through the corresponding acyl chlorides by reacting with various amines. After debenzylation and subsequent formation of the aminopropoxy moiety, the desired carboxamido derivatives were easily obtained (detailed chemical syntheses of the different starting materials (**21a–d**) and a representative compound (**10**) are described in the Supplementary Material).

Main characteristics of the synthesized derivatives are summarized in Table 2. Compounds **6**, **7** and **8** did not have any functional group on carbon 17 (both X^1 and X^2 are hydrogens). However, this feature seemed to be the reason of suboptimal metabolic stability and hERG liability, as improved characteristics were found in the case of the 17-methoxy (**9**) and 17-oxo (**10**) derivatives. Most probably, increasing the molecules' polarity resulted in beneficial effects not just on metabolic stability and hERG liability (the hERG IC_{50} values reached [**9**] or even exceeded [**10**] the 10 μM limit), but also on *in vitro* potency. In addition, both compounds showed activity in the dipsogenia test *in vivo*. It was particularly true for compound **10,** which showed extraordinary *in vivo* potency with an ED_{50} value of 0.47 mg/kg. To further proceed with the optimization, effects of other R^1R^2N moieties were tested. Usage of the *S*-2-methylpyrrolidne enantiomer resulted in a somewhat decreased (however, still significant) affinity to hH_3 receptors in case of compound **11**. However, the replacement with several fluorinated pyrrolidine and piperidine moieties (compounds **12**, **13** and **14**) resulted in significant loss of binding affinity to rH_3 receptors. In case of **15** (which is a 15β-carboxamide compound), the *in vitro* hH_3 K_i was increased with one order of magnitude compared to the 15α-carboxamide compound **10**. Thus, the best *in vitro* potency values were found among the 15α-carboxamido-*R*-2-methylpyrrolidine derivatives.

Having attempted to provide the structure with more flexibility by lengthening the chain with one carbon at position 15 (**16**) resulted in high H_3 and low muscarinic affinity. However, metabolic stability was lower than what was seen with the corresponding methoxy derivative **9**. Furthermore, lengthening and shortening (n = 2, 4) the original propoxy linker with one carbon in compounds **17** and **18** seemed to be not beneficial for rH_3 binding.

Various R^3R^4N groups may be tolerated regarding the binding activity at hH_3 receptors. The overall characteristics of pyrrolidino (**19**) derivative was almost as good as that of compound **10**. The cyclopropylmethylamino (**7**) and cyclobutylmethylamino (**8**) derivatives were also very potent *in vitro*. Only the *N*,*N*-diethylamino derivative (**20**) showed weak affinity towards rH_3 receptors.

As we demonstrated above, the best derivatives were among the 17-oxo and 17-metoxy analogues. They possessed more advantageous properties than the 17-deoxo derivatives, however the presence of the oxo and the hydroxy functions might raise the issue of possible binding to various hormone receptors. As compound **10** showed ten times higher affinity to hH_3 receptors than that of compound **19**, in-depth characterization of **10** was carried out with special emphasis on its physico-chemical properties and binding affinity to various steroid receptors. Physico-chemical properties of compound **10** showed promising data (molecular weight, solubility, lipophilicity) concerning its drug-likeness (Table 3). Furthermore, detailed receptor profiling of **10** did not reveal any affinity to other steroid receptors (data not shown, as a more detailed summary on the pharmacological properties of **10** is planned to be published in a separate paper). As it is evident from the data summarized in Table 2, compound **10** was not only highly active at hH_3 and rH_3 receptors, but also showed negligible activity at rat muscarinic receptors (K_i = 4700 nM). Furthermore, **10** proved to be considerably stable in human and rat microsomes (F_M% = 99 in both species) and acted in a very potent manner in the rat dipsogenia test following p.o. administration with an ED_{50} value of 0.47 mg/kg.

Oral bioavailability following 3 or 10 mg/kg (iv. and po.) administration of compound **10** was investigated in fasted male Wistar rats. Oral bioavailability of compound **10** was 62.3%, calculated from the AUC_{0-24h} values of the 3 mg/kg iv. and 3 mg/kg po. treatments. Drug exposure (AUC_{0-24h}) increased more than dose proportionally between 3 and 10 mg/kg both after iv. and po. administrations; the increase was 64% (iv.) and 39% (po.) higher than it would have been in case of linearity. Clearance (Cl) and volume of distribution (Vd) decreased, elimination half-life (T_{half}) of the compound slightly increased with dose. Brain penetration of compound **10** was investigated at 10 mg/kg oral dose. Compound **10** showed rapid brain penetration with maximal brain concentrations at 1 hr. Brain to plasma AUC_{0-inf} ratio was 0.24, t_{half} was 3.02–3.58.

To demonstrate beneficial effects on cognitive performance, compound **10** was also tested in the water-labyrinth test.[14] in rats. At the dose of 3 mg/kg, compound **10** significantly improved the scopolamine-induced impairment on Day 2 with significant (52.3%) restoring effect (Fig. 1). Further models (including place and novel object recognition paradigms as well as inhibitory avoidance test) will be discussed in detail in a separate paper.

In summary, as a continuation of our previous work, novel, estrane

based steroidal H_3 receptor antagonists with *in vivo* activity in the dipsogenia and water labyrinth tests were identified. The best compound **10** was appointed to be preclinical candidate and detailed pharmacological characterization was initiated which is also about to be published in a separate paper.

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Appendix A. Supplementary data

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