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Acidic elements in histamine H_3 receptor antagonists

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ABSTRACT

Antagonists of the human histamine H_3 receptor (hH$_3$R) often contain a second basic moiety, which is well known to boost affinity on this histamine receptor subtype. Here, we prepared compounds with acidic moieties of different pK_a values to figure out that the hH$_3$R tolerates these functionalities when added to a common pharmacophore blueprint. Depending on the acidic, electronic and steric features the designed ligands showed hH$_3$R affinities in the nanomolar concentration range. Additionally, selected ligands were tested but failed as dual acting hH$_3$R/hPPAR (human peroxisome proliferator-activated receptor) ligands.

Human histamine H_3 receptors (hH$_3$R) in the central nervous system (CNS) regulate the synthesis and liberation of the neurotransmitter histamine (autoreceptor function). Additionally, as heteroreceptors, they control the concentration of various other biogene amines and neuropeptides. Hence, these G protein-coupled receptors (GPCR) play a major role in keeping a certain neurotransmitter balance in regions of co-localized neurons and affect central functions like vigilance, learning and attention.[1] Antagonists/inverse agonists of the hH$_3$R have well advanced in their way from bench to bedside and some have passed clinical phase II.[2] Besides their main potential indications in the broad field of neurodegenerative diseases some have shown efficacy in models of metabolic diseases, for example, obesity and diabetes mellitus. In rodents, caloric intake was reduced by administration of hH$_3$R antagonists, like A-331440[3] or NNC-381202.[4] Histaminergic neurons in the CNS interfere with orexinergic pathways. Elevated levels of brain histamine reduce food intake and weight gain in several species.[5] Most probably, this occurs via H$_1$R-mediated downstream signalling because H$_1$R antagonists or antipsychotics evoke the contrary effect.[6] However, contradictory results have been reported[2] and further studies are needed to clear up this complex mechanism.

The hH$_3$R targeting domain and the corresponding antagonist pharmacophore are well investigated. Actually, most hH$_3$R antagonists consist of a basic moiety, which is coupled via an alkyl linker to an aromatic core. The latter might be substituted with a variety of basic, lipophilic or polar moieties of different sizes.[7] A second basic centre usually boosts hH$_3$R affinity due to additional beneficial interactions with the receptor's binding pocket (Glu206)[8] but also covers the risk of central accumulation and induction of phospholipidosis.[9] One of the most prominent examples of these diamine-based structures is JNJ-5207852 (**1**, Fig. 1),[10] which has become a parent structure for this class of dibasic compounds.[6]

Recently, we reported a series of diamine-containing multiple-acting hH$_3$R ligands with antipsychotic properties, amongst it the amitryptiline hybrid **2**. The main focus was on the modification of the in vitro receptor profile of neuroleptic drugs. Additionally we were able to show the robustness of the H$_3$R binding pocket with regard to bulky ligands.[6,11] To expand this series with another piperazine-containing derivative we perchance prepared a ciprofloxacin hybrid (**3**) in an analogue manner and determined its hH$_3$R affinity.

Although ciprofloxacin features not only a second basic moiety but also a weak carboxylic acid it showed a surprising high affinity (pK_i = 8.7). We took this result as a rationale to investigate if the aminergic hH$_3$R tolerates acidic moieties added to the common 1-(3-phenoxypropyl)piperidine pharmacophore. Starting from the dibasic 4,5-dihydro-1*H*-imidazole derivative **7** a series of ligands with different acidic moieties was prepared by modification of the heterocycle in the eastern molecule part.

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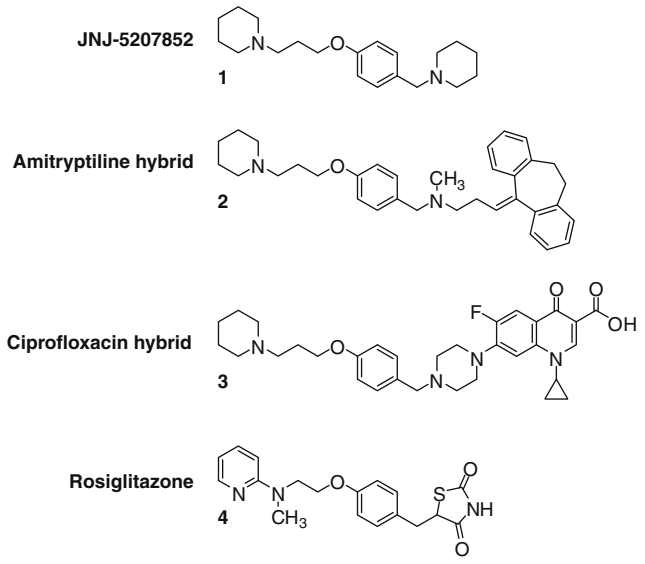


Figure 1. Reference structures for the development of acid-containing hH$_3$R antagonists.

Drugs that are commonly used in the treatment of metabolic diseases, that is, fibrates or glitazones (cf. Fig. 1 for rosiglitazone **4**),[12] usually request an acidic head group to interact with their targets, that is, human peroxisome proliferator-activated receptors (hPPARs; cf. Fig. 2).[13] A second goal of this study was to explore if the newly designed compounds with both, basic and acidic moieties, are efficacious as dual acting hH$_3$R/hPPAR ligands. Such compounds could be useful in the treatment of metabolic diseases like obesity and diabetes mellitus. Additionally, because PPARs induce neuroprotection[14] and reduce neuroinflammation in rodent models,[15] such hybrids might be efficacious in the treatment of neurodegenerative diseases.

Starting from piperidine the preparation of the precursors, aldehyde **5** and nitrile **6**, was performed as described before.[11,16] Cyclization of nitrile **6** by refluxing it in ethane-1,2-diamine under sulfur catalysis in the microwave oven resulted in 4,5-dihydro-1H-imidazole derivative **7** (Scheme 1).[17] All other compounds were prepared using aldehyde **5** as reactant. Ciprofloxacin hybrid **3** was synthesized in a reductive amination with the piperazine moiety of the fluoroquinolone.[18] Thiazolidines **8** and **9** were obtained in reductive nucleophilic substitution reactions with cysteine derivatives,[19,20] whereas thiazolidindiones **10** and **11** are the products of a Knoevenagel reaction with thiazolidindione[21,22] and rhodanine,[23] respectively. According to previous publications[24] this condensation exclusively leads to the Z configuration of the exocyclic double bond, which was confirmed by X-ray investigation showing the expected configuration and the dimerization of molecules in the crystal structure (CCDC No. 760848, cf. Supplementary data). Compound **12** was prepared in a catalytic hydrogenation of **10** (Scheme 2).[22]

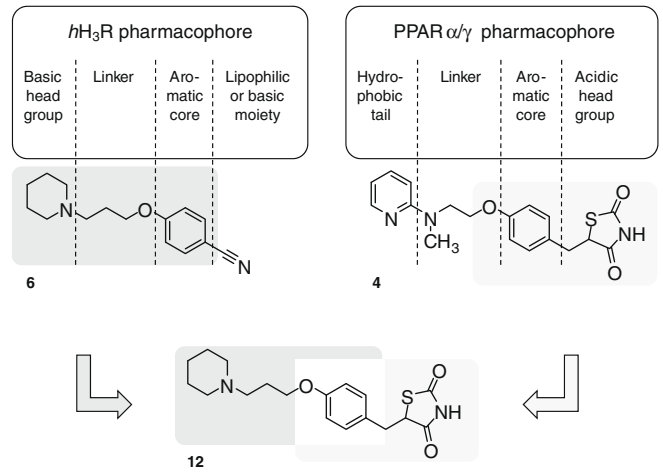
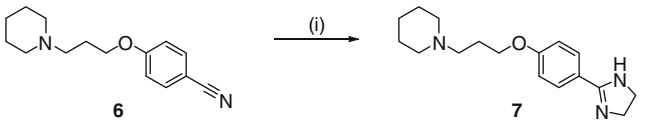


Figure 2. Development of the potential merged hH$_3$R/hPPAR hybrid **12**.

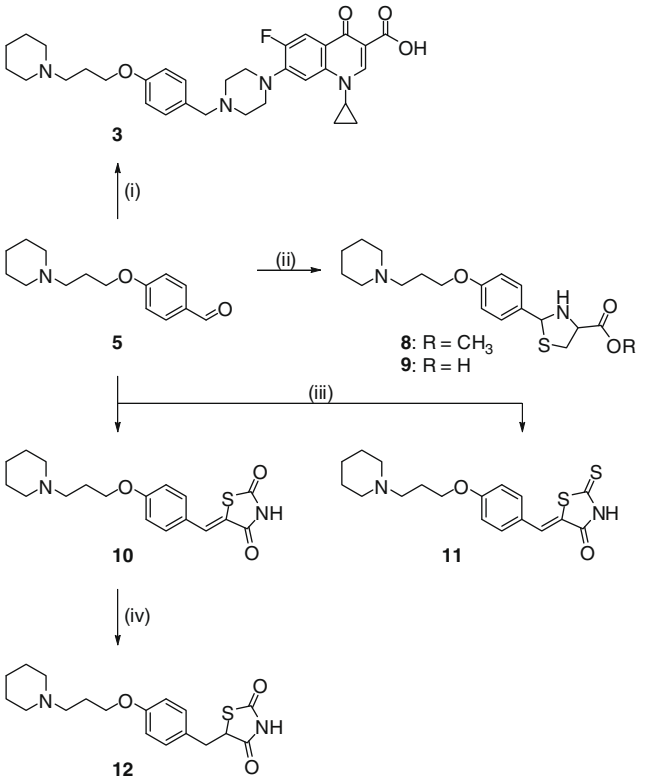


Scheme 1. Synthesis of 4,5-dihydro-1H-imidazole derivative **7**. Reagents and conditions: (i) ethan-1,2-diamine, sulfur, μW, 30 min, 110 °C.

With regard to their hH$_3$R affinity compounds **3** and **7**–**12** were tested in a [^{125}I]iodoproxyfan displacement assay, which was performed on a membrane preparation of HEK-293 cells stably expressing the hH$_3$R (Table 1).[25–27] Compounds **10** and **12** were additionally tested in a hPPARα/γ reporter gene assay. Receptor activation was determined by quantification of the luminescence evoked by the expressed luciferase in Cos-7 cells (Table 2).[13,28]

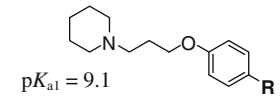
The 4,5-dihydro-1H-imidazole-containing compound **7** showed a hH$_3$R affinity in the low nanomolar concentration range (pK_i = 8.1) and hence served as a lead structure for the development of this series of acidic compounds (Table 1).

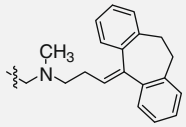
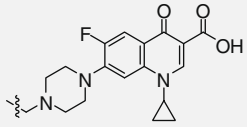
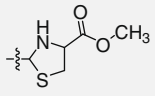
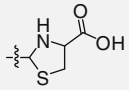
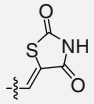
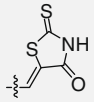
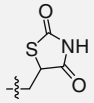
To insert acidic elements cyclic cysteine derivatives **8** and **9** were prepared implying a heteroatom exchange and the complete



Scheme 2. Synthesis of ciprofloxacin hybrid **3**, thiazolidines **8** and **9** as well as thiazolidinediones **10**–**12**. Reagents and conditions: (i) ciprofloxacin, NaBH(OAc)$_3$, 1,2-dichloro ethane, 16 h, rt; (ii) cysteine methylester (**8**)/cysteine (**9**), ethanol/H$_2$O, 3 h, rt; (iii) thiazolidindione (**10**)/rhodanine (**11**), piperidine, glacial acetic acid, toluene, 6 h, reflux. (iv) Pd(OH)$_2$/C, H$_2$, 5 bar, 48 h, 60 °C.

Table 1
Acidity and hH$_3$R affinities of references 1,2 and compounds **3**, **7**–**12**



Compds	R	Acidity of R[a] pK_{a2}		hH$_3$R affinity[b] pK_i[c]
		Base	Acid	(nM)
1[d]		8.6		9.2
2[e]		8.6		9.5
3		8.6	6.1	8.7
7		11.2		8.1
8		6.6		8.4
9		8.7	2.2	8.0
10			6.2	8.1
11			7.8	8.3
12			6.5	7.3

[a] pK_s Values were assessed with ALOGPS 2.1 program.[29]
[b] [^{125}I]Iodoproxyfan binding assay (HEK-293 cells stably expressing hH$_3$R).[25–27]
[c] Values were determined in one experiment, at least in triplicates for each concentration tested.
[d] Ref. 10.
[e] Ref. 6.

saturation of the heterocyclic system of **7**. Although both derivatives showed good hH$_3$R affinities, the more lipophilic ester **8** evoked slightly stronger receptor interactions than acid **9** did (pK_i values of 8.4 and 8.0, respectively). Nevertheless, the amino acid moiety with estimated pK_a values of 2.2 and 8.7 (acid and base, respectively) was well tolerated. The thiazolidine-containing compounds **8** and **9** represent new lead structures for the development of neuroprotective agents. Thiazolidines coupled to aromatic (hetero)cycles have shown some potential to modify the conformational properties of amyloid beta peptides and to prevent their aggregation.[30] A combination with the hH$_3$R pharmacophore might be a new approach to design ligands for the treatment of Alzheimer's disease.[31]

The attenuation of acidity by implementation of NH-acidic groups slightly improved receptor binding as could be seen in methylene-thioxo/azolidinone structures **10** and **11**. Comparing the characteristics of these two compounds the less electronegative and therefore less acidic and more lipophilic thioxothiazolidinone **11** evoked a stronger affinity. This small series of compounds shows a tendency regarding the binding potency of acid-containing ligands: the less acidic the more affine. However, differences in affinity values of all compounds **9**–**11** range within half an order of magnitude (pK_i values between 8.0 and 8.3) indicating that the hH$_3$R tolerates acidic moieties very well. Regarding its affinity but not its acidity compound **12** constitutes an exception within this series. The pK_a value resembled that of **10** due to similar structural features of the thiazolidindione moiety. Compared to the receptor binding of compounds **10** and **11** the hydrogenated derivative **12** showed a decrease of hH$_3$R affinity in the range of almost one order of magnitude (pK_i = 7.3). This might be caused by electronic and steric effects. Because of the saturation of the methylene linker the high electronegativity of the thiazolidindione moiety cannot be attenuated by the phenyl ring, which creates a molecule part of high electron density. In contrast, the double bond in compounds **10** and **11** generates a conjugated planar system with improved electron distribution.

Compound **12** incorporates parts of both pharmacophores, the hH$_3$R phenoxypropylpiperidine and the (alkoxybenzyl)thiazolidindione moiety of glitazones. Hence, it exhibits the structural features of a merged hybrid ligand (Fig. 2).[32] To investigate if the moderate affine hH$_3$R ligand **12** and its precursor **10** interact with hPPAR subtypes, especially hPPARγ, the main target of the glitazone class, these two compounds were tested in a transactivation assay but none of them was able to activate hPPARs (Table 2). The main reason for this might be the aliphatic piperidino moiety, which exhibits a considerably higher pK_a value than the aromatic pyridine structures of the glitazones (cf. Supplementary data).

The presented results confirm that hPPARs do not interact with compounds that contain strong basic moieties.[34] Because the closely related compounds did not show PPAR activity testing of the other compounds was closed. Hence, it has not been possible to design dual acting hH$_3$R/hPPAR ligands with the actual approach because the exchange of tertiary amines in the basic head of the hH$_3$R pharmacophore into weaker basic moieties, that is, aromatic nitrogen-containing heterocycles found in the glitazone class, leads to a loss of hH$_3$R affinity.[35] Nevertheless, the dual approach may be useful in the therapy of metabolic diseases. Considering the other side of the coin we successfully implemented acidic moieties into the hH$_3$R antagonist pharmacophore, which until now was only known to tolerate basic, polar or lipophilic moieties of different sizes. This small series of compounds clearly shows that the hH$_3$R is able to bind acid-containing ligands in the low nanomolar concentration range as long as the ionic bond between the basic head and the receptor binding pocket (Asp114) as well as the π and edge-to-face interactions of the aromatic core (Tyr115 and Trp371) are kept intact.[8,36] With these results the so far broadly accepted doctrine among hH$_3$R researchers is broadened offering new possibilities for the development of hH$_3$R antagonists especially targeted on the treatment of metabolic diseases.

Table 2
hPPARα/γ transactivation activities of compounds **10** and **12**

Compds	hPPARα activation[a] EC_{50}[b] (μM)	hPPARγ activation[a] EC_{50} [b] (μM)
4[c]	> 10	γ1: 0.03 γ2: 0.1
11	> 10	> 10
13	> 100	> 100

[a] Luciferase reporter gene assay (Cos-7 cells transfected with Lipofectamine™ reagent (Invitrogen) according to the manufacturer's protocol with pFR-Luc (Stratagene), pRL-SV40 (Promega) and the Gal4-fusion receptor plasmids (pFA-CMV-hPPAR-LBD) of the respective subtype).[13,28]
[b] Values are means of three independent experiments, each in triplicates.
[c] Ref. 33.

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Supplementary data

Supplementary data (synthetic procedures, analytical data and assay descriptions) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.089.

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