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First Metal-Containing Histamine H_3 Receptor Ligands†

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ABSTRACT

Iron-containing ligands targeting the human histamine H_3 receptor (hH_3R) were prepared. The compounds contain ferrocene sandwich complexes coupled via different linkers to a basic hH_3R antagonist/inverse agonist pharmacophore. In a click chemistry approach, a triazole was successfully inserted as a new linking element. Two ferrocenylmethylamines and a ferrocenyltriazole were the most affine hH_3R ligands within this series, showing receptor binding in the nano- and subnanomolar concentration range.

Human histamine H_3 receptors (hH_3R) in the central nervous system (CNS) are auto- and heteroreceptors modulating the synthesis and the release of histamine as well as the liberation of various other neurotransmitters. Hence, histaminergic neurons, which spread from the tuberomamillary nucleus into most parts of the brain, influence the neurotransmitter balance in the respective compartments in dependence of colocalized neurons.[1] Central functions like vigilance, attention, and learning are affected. Modulation of this neuronal interplay by hH_3R antagonists/inverse agonists might be an effective approach in the therapy of neuronal diseases, e.g., cognitive impairment, sleep/wake disorders, epilepsy, and obesity. The diversity of potential indications gives a hint of the complexity of hH_3R modulation.[2]

The well-established construction pattern of hH_3R antagonists/inverse agonists contains an aminergic moiety in the western molecule part that is coupled via an alkyl spacer to a central core. In the eastern part, the receptor binding pocket offers broad possibilities for substitution of polar, acidic, basic, or lipophilic residues.[3]

The goal of this work was the preparation of ferrocene-containing hH_3R antagonists to evaluate the binding behavior of metal-containing residues toward the receptor. The iron-containing sandwich chelates can be used as chemically stable bioisosters of both phenyl and (radioactively labeled) cyclopentadienyl tricarbonyl metal complexes.[4] Hence, a

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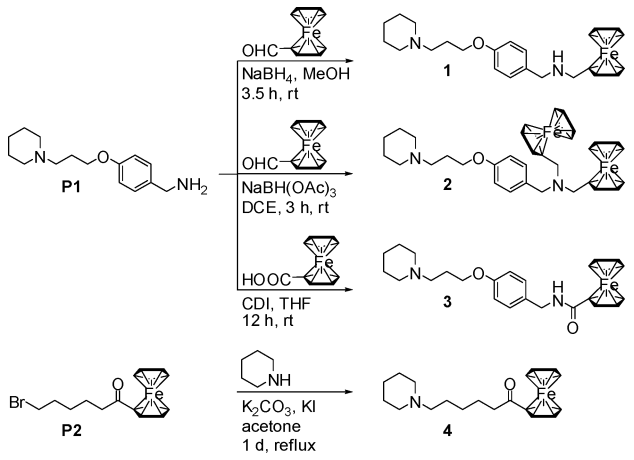
potential application of this type of compounds as imaging ligands was simultaneously investigated.

The project was realized by coupling ferrocene derivatives to the hH$_3$R-targeting domain in the western molecule part using different synthetic approaches. Therefore, the applicability of click chemistry in the design of hH$_3$R ligands was tested. All final compounds were characterized with regard to hH$_3$R binding potencies and selectivity over the closely related hH$_1$R and hH$_4$R subtypes.

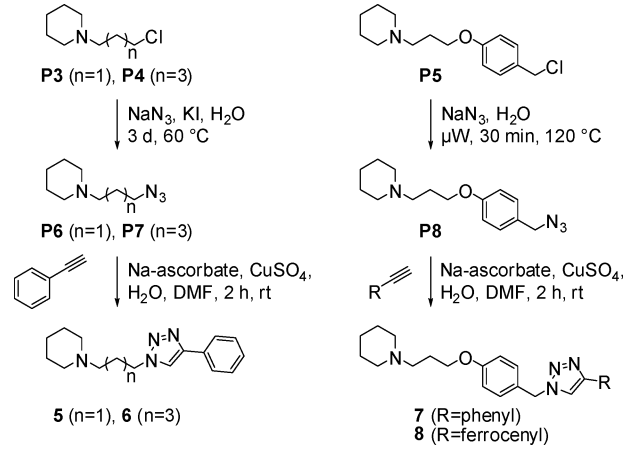
Synthesis of precursors **P1** and **P3**−**P5** as well as compound **4** was performed as described before. Briefly, ω-piperidinylalkanoles were chlorinated and subsequently coupled to *para*-substituted phenols. The high-yielding Williamson-like reactions completed by a Finkelstein exchange were used to generate a small selection of precursors.[5] Alkyl- and benzyl-substituted chlorides **P3**−**P5** were converted into the corresponding azides **P6**−**P8** using water as solvent. Microwave irradiation was used as an additional means to obtain **P8**.[6] Precursor compound **P2** was comercially available.

Scheme 1. Synthesis of Compounds **1**−**4**



Following the typical hH$_3$R affine structural blueprint, the receptor-targeting domain, 1-(3-phenoxypropyl)piperidine, was obtained. Different substituents in the *para*-position of the phenyl ether element allowed the connection to a variety of residues that have a major influence on physicochemical properties of the compounds. Ligands **1**−**3** were prepared via reductive amination and *N*,*N*′-carbonyldiimidazole-provided amidation, respectively (Scheme 1).[7] Compounds **5**−**8** were prepared via the efficient click-chemistry approach. In a Huisgen 1,3-dipolar cycloaddition of azides and acetylenes, 1,4-disubstituted 1,2,3-triazoles were formed under copper(I) catalysis (Scheme 2).[8]

Scheme 2. Synthesis of Compounds **5**−**8**



The hH$_3$R affinities (i.e., receptor binding strenghts) of all final compounds **1**−**8** were determined by measuring the displacement of [^{125}I]iodoproxyfan from hH$_3$R stably expressed in HEK-293 cells.[9] Compounds with pK_i values >7.0 (**1**−**3**, **7**, **8**) were investigated with regard to their hH$_1$R and hH$_4$R affinities as described before (Table 1). The hH$_3$R inverse agonist efficacy was confirmed in a [^{35}S]GTPγS binding assay with compounds **1** and **8**.[10] The potencies in the CNS evoked by compounds with hH$_3$R affinities in the picomolar concentration range (**1**, **2**) was determined in vivo by measuring histamine turnover in a radioimmunoassay after peroral (p.o.) application.[11]

Compounds **1** and **2** belong to the class of diamine-based ligands. A second basic moiety usually boosts hH$_3$R potency due to a second ionic interaction between receptor protein and ligand.[12] As expected, both amines strongly interact with the binding pocket showing affinities in the subnanomolar concentration range. However, this class

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of compounds offers the risk of accumulation in the CNS and induction of phospholipidosis.[13] In an effort to avoid potentially occurring side effects in an early step of ligand development, other linker moieties were inserted.

Amide **3**, an analogue of compound **1**, exhibited good hH$_3$R affinity in the nanomolar concentration range; however, compared to that of amine **1** it is decreased to about 2 orders of magnitude. Ketone **4** as well as triazole-containing compounds **5** and **6** were prepared in order to investigate if parts of the central core in the hH$_3$R pharmacophor, in this case the phenoxy ether, could be substituted by a carbonyl or triazole moiety. All possess hydrogen bond acceptor properties; phenyl ether and triazole additionally share aromaticity. All together, this could avoid unfavorable physicochemical properties inducing metabolic instability and reduce the ligands' size to accomplish sufficient CNS penetration. Compound **7** with an enlarged central core was designed to test the triazole moiety as a linker.

Table 1. hH$_3$R, hH$_1$R, and hH$_4$R Affinities of Compounds **1**−**8**

	affinity (pK_i)		
compd	hH$_3$R[a]	hH$_1$R[(b)]	hH$_4$R[(c)]
1 (ST-892)	9.5	5.3	<5
2 (ST-853)	9.8	5.5	5.2
3	7.7	5.3	<5
4	<5		
5	<5		
6	6.6		
7	8.2	5.9	<5
8 (ST-1036)	8.7	<5	<5

[a] [^{125}I]Iodoproxyfan binding (HEK-293 cells stably expressing hH$_3$R). [(b)] [^{3}H]Mepyramine binding (CHO-K1 cells stably expressing hH$_1$R). [(c)] [^{3}H]Histamine binding (Sf9 cells expressing hH$_4$R, coexpressed with Gα_{i2} and $\beta_1\gamma_2$ subunits).

Affinity data showed that the receptor's binding pocket hardly interacts with pentyltriazole **6** and not at all with compounds **4** and **5**, indicating the lack of important structural elements within these small compounds. By inserting the known phenoxy core (**7**, **8**) affinity was retrieved, independent of the coupled moieties (phenyl or ferrocenyl rest) in the eastern part of the molecules. The introduction of a triazole as a linker (**7**) offers new possibilities in the design of hH$_3$R ligands. From an extrapolation of these results, the corresponding ferrocene **8** was synthesized and even exhibited a slightly improved affinity.

Compounds with pK_i values >7.0 were investigated with regard to their affinities to hH$_1$R and hH$_4$R (cf. Table 1). hH$_3$R and hH$_4$R are structurally closely related.[14] Although it is not yet clear which role the hH$_4$R plays in the CNS, cross-affinities have to be excluded for hH$_3$R ligands. The hH$_1$R is according to the phylogenetic tree the next related histamine receptor subtype and, besides hH$_3$R, most involved in the regulation of central histamine-mediated functions.[15] Compounds **1**−**3**, **7**, and **8** clearly exhibited a selective binding behavior with a high preference for the targeted hH$_3$R subtype. Affinities differed to more than 2 orders of magnitude.

Functional potency of the 1-(3-phenoxypropyl)piperidine scaffold was confirmed in a [^{35}S]GTPγS binding assay. Compounds **1** and **8** exhibited efficacies in the same range as the reference full inverse agonist thioperamide. The extraordinarily affine and selectively acting amines **1** and **2** were further investigated in vivo. In a radioimmunoassay determining histamine turnover in the CNS no effect was observed after p.o. administration to Swiss mice. However, the rough screening model does not elucidate the reasons for such an outcome. Trials with these promising ligands are next required, including the determination of their pharmacokinetic properties and/or their intracerebroventricular application, because others have shown that cyclopentadienylmetal-containing ligands are able to access the brain.[16] The iron-containing ligands are suitable single-photon emission computed tomography (SPECT) ligand precursors since the exchange of cyclopentadienyl iron into tricarbonylrhenium or -technetium has been successfully performed.[17] Because of the electron-withdrawing properties of the linker, triazole **8** is the most promising compound to be radiolabeled by recomplexation.

The presented hH$_3$R ligands are, to our knowledge, the first metal-containing histamine receptor ligands communicated. Using different chemical approaches, ferrocene moieties were coupled to the hH$_3$R pharmacophore using amines, carbonyl-containing groups, or triazoles as linkers. For the first time, click chemistry was successfully applied in the design of hH$_3$R ligands. The triazole moiety is a qualified extension of the hH$_3$R pharmacophore offering improved pharmacodynamic properties. Compounds **1** (ST-892), **2** (ST-853), and **8** (ST-1036) resulted in excellent hH$_3$R ligands selectively binding to this receptor subtype in the nano- to subnanomolar concentration range.

The new molecules exhibit exceptional structural features rarely used in medicinal chemistry. They are promising SPECT precursor compounds and constitute excellent model substances for the ongoing characterization of the hH$_3$R binding pocket.

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CHO-K1 cells stably expressing hH$_1$R were generously provided by Prof. H. Timmermann and Prof. R. Leurs (Vrije Universiteit Amsterdam, The Netherlands).

Supporting Information Available: Synthetic procedures, analytical data, and NMR spectra of precursors and final compounds as well as descriptions of pharmacological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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