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# Mepyramine–JNJ7777120-hybrid compounds show high affinity to $hH_1R$, but low affinity to $hH_4R$

Eva Wagner [a], Hans-Joachim Wittmann [b], Sigurd Elz [a], Andrea Strasser [c,*]

[a] Department of Pharmaceutical/Medicinal Chemistry I, Institute of Pharmacy, University of Regensburg, D-93040 Regensburg, Germany
[b] Faculty of Chemistry and Pharmacy, University of Regensburg, D-93040 Regensburg, Germany
[c] Department of Pharmaceutical/Medicinal Chemistry II, Institute of Pharmacy, University of Regensburg, Universitätsstraße 31, D-93040 Regensburg, Germany

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ABSTRACT

In literature, a synergism between histamine $H_1$ and $H_4$ receptor is discussed. Furthermore, it was shown, that the combined application of mepyramine, a $H_1$ antagonist and JNJ7777120, a $H_4$ receptor ligand leads to a synergistic effect in the acute murine asthma model. Thus, the aim of this study was to develop new hybrid ligands, containing one $H_1$ and one $H_4$ pharmacophor, connected by an appropriate spacer, in order to address both, $H_1R$ and $H_4R$. Within this study, we synthesized nine hybrid compounds, which were pharmacologically characterized at $hH_1R$ and $hH_4R$. The new compounds revealed (high) affinity to $hH_1R$, but showed only low affinity to $hH_4R$. Additionally, we performed molecular dynamic studies for some selected compounds at $hH_1R$, in order to obtain information about the binding mode of these compounds on molecular level.



Histamine $H_1$ receptor antagonists are used in general for the treatment of allergic reactions, whereas the histamine $H_4$ receptor is suggested to be involved in allergic diseases, like conjunctivitis, rhinitis or bronichal asthma as well as in atopic dermatitis and pruritus.[1–4] Mepyramine **1** (Scheme 1) is a prominent $H_1R$ antagonist, whereas JNJ7777120 **2** (Scheme 1) shows high affinity to the $H_4R$.[4] In 2003 JNJ7777120 **2** was described as a potent and selective $H_4$ antagonist, which has meanwhile established to a $H_4R$ standard antagonist.[5] Further studies revealed that JNJ7777120 acts as inverse agonist at $hH_4R$ but as partial agonist at $mH_4R$.[6] Recently, it was shown experimentally, that the combined application of mepyramine **1** and JNJ7777120 **2** in the acute murine asthma model leads to a synergistic effect.[7] Thus, the development of combined $H_1/H_4$-receptor ligands may be a worthwhile goal for treatment of allergic reactions,[1] since differences in bioavailability are expected if two drugs are administered. This is not the case, if $H_1R$ and $H_4R$ can be addressed with only one drug. Furthermore, ligands addressing both $H_1R$ and $H_4R$ are important pharmacological tools to get deeper insights with regard to ligand binding and selectivity on molecular level. One strategy for development of dual $H_1/H_4$-receptor ligands is the connection of one $H_1$- and one $H_4$-pharmacophor by a spacer. This concept was already applied by Schunack with regard to $H_1R$ and $H_2R$.[8,9] Since the combined application of mepyramine **1** and JNJ7777120 **2** lead to the synergistic effect in the acute murine asthma model,[7] the aim of this study was to synthesize and pharmacologically characterize a number of compounds, combining mepyramine as $H_1$- and JNJ7777120 as $H_4$-pharmacophor.

The hybrid ligands **16–21** were obtained as described (Scheme 2). The structures of compounds **26**, **38** and **45** are presented in Scheme 3, whereas the strategy with regard to synthesis can be found in the supplementary material. Further details with regard to synthesis, as well as analytics of all hybrid compounds are given in the supplementary material.

The synthesized compounds were routinely investigated in competition binding assays. In case of $hH_1R$, Sf9 cell membranes, coexpressing $hH_1R$ and RGS4 were used for competition binding assays in presence of 5 nM [$^3$H]mepyramine.[10] In case of $hH_4R$, Sf9 cell membranes, coexpressing $hH_4R$-RGS19, $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were used for competition binding assays in presence of 10 nM [$^3$H]histamine.[11] Furthermore, some selected compounds were analyzed at $hH_4R$ with the GTP$\gamma$S-assay in order to determine the efficacy.[12] Additionally, most of the new compounds were tested routinely on isolated guinea-pig ileum.[13] Since only $H_1R$, but not $H_4R$ is expressed on ileum, in organ pharmacology, assays at guinea-pig ileum are well established in order to study the affinity and functionality at $gpH_1R$. The histamine-induced contraction of the guinea-pig ileum is measured in presence and absence of an antagonist.[13]

Since the new hybrid compounds showed affinity to $H_1R$, but did not act as (partial) agonists at $H_1R$, a model of $hH_1R$ in the inactive conformation was generated by homology modelling, based on

* Corresponding author. Tel.: +49 941 943 4821; fax: +49 941 943 4820.
*E-mail address:* andrea.strasser@chemie.uni-regensburg.de (A. Strasser).

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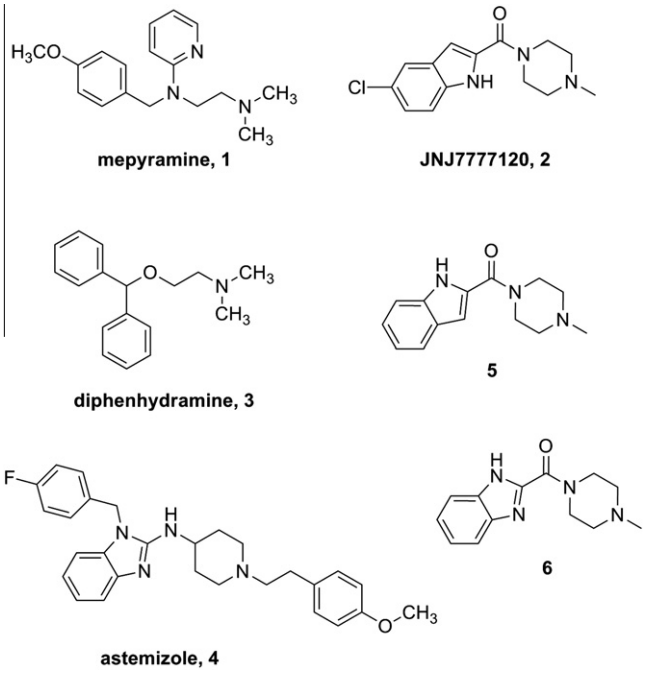


**Scheme 1.** Structures of $H_1$ (mepyramine, **1**; diphenhydramine, **3**; astemizole, **4**) and $H_4$ (JNJ7777120, **2**; JNJ-derivative, **5**; JNJ-derivative, **6**) receptor ligands.

the crystal structure 2RH1,[14] analogue, as already described.[15] A comparison of our $H_1R$ homology model, refined by molecular dynamic simulations, with the recently published $hH_1R$ crystal[16] showed no significant differences. The compounds **16**, **19** and **38** were docked manually into the binding pocket of $hH_1R$ using the software package SYBYL 7.0 (Tripos Inc.). Molecular dynamic simulations, using the software GROMACS 4.0.2 (http://www.gromacs.org), were performed, as already described.[10] Ligand parameterization was obtained from the PRODRG server (http://davapc1.bioch.dundee.ac.uk/prodrg/). For both compounds a 6 ns productive phase in molecular dynamic simulations was performed subsequent to a 1 ns equilibration phase.

The pharmacological and modeling data of reference compounds and the new hybrid compounds are given in Tables 1–4. For compounds **16** and **19**, the experimental pharmacological data are shown in Figure 1.

Compared to mepyramine, the affinity of compounds **16**–**18** is significantly reduced of about 1.5–2 log units at $hH_1R$. The introduction of one chlorine atom in the indole moiety **17** leads to a slight decrease in affinity to $hH_1R$, compared to **16**. The exchange of the indole moiety **16** into a benzimidazole **18** leads to a decrease in affinity at $hH_1R$. For compound **19**, an affinity comparable to that of mepyramine **1** at $hH_1R$ could be observed. The introduction of one chlorine atom on the corresponding position in the JNJ7777120 partial structure **20** leads to a significant decrease in affinity at $hH_1R$, compared to **19**. In compounds **16–18**, the basic nitrogen atom is embedded in a piperazine moiety, which shows a higher rigidity than an ethylene spacer. This more voluminous piperazine moiety is suggested to disturb the electrostatic interaction between the positively charged amine and $Asp^{3.32}$, leading to a significantly decreased affinity. Based on the molecular dynamic studies, a mean coulomb energy (short range) between **16** and $hH_1R$ of about $-157 \pm 1$ kJ/mol was detected (Fig. 2). In contrast, a coulomb energy (short range) of $-197 \pm 1$ kJ/mol was detected between **19** and $hH_1R$ (Fig. 2, Table 2). Both interaction energies are, according to a t-test, significant different to each other ($p < 0.0001$). In contrast, there is no significant difference with regard to the Lennard-Jones energy (short-range) interaction energies between **16** ($-252 \pm 1$ kJ/mol) or **19** ($-253 \pm 1$ kJ/mol) and $hH_1R$ (Table 2). Thus, the dynamic studies support the hypothesis that the piperazine moiety disturbs the electrostatic interaction between **16** and $hH_1R$. This difference in the short range coulomb interaction is reflected by the experimentally determined $pK_i$ values of **16** and **19** at $hH_1R$ (Fig. 1A). However, during the molecular dynamic simulations, a stable hydrogen bond interaction could be detected between the carbonyl moiety of **16** and $Asn^{2.61}$ (Fig. 2). Additionally, an aromatic interaction between the indole moiety of **16** and $Tyr^{2.64}$ was observed during the simulation (Fig. 2). In compound **19**, the amino moiety, suggested to interact with $Asp^{3.32}$ is flexible, analogous to mepyramine itself and in contrast to compounds **16–18**. Thus, the interaction between the amine moiety and $Asp^{3.32}$ can be established well. This is also confirmed by the stronger electrostatic interaction between $hH_1R$ and **19**, compared to **16** (Fig. 2). However, the elongation of mepyramine by the JNJ7777120 partial structure did not lead to an increased affinity at $hH_1R$, compared to mepyramine **1**. Since there is a significant difference in affinity of **19** and **20** at $hH_1R$, it may be suggested, that the additional JNJ7777120 partial structure interacts specifically with the $hH_1R$. A stable hydrogen bond was detected during the molecular dynamic simulation between the carbonyl moiety of **19** and Thr182 (E2-loop) (Fig. 2). The exchange of the piperazine moiety by a more flexible aminopyrrolidine moiety **26** leads only to a slight decrease in affinity at $hH_1R$, compared to **16**. The diphenhydramine—JNJ-hybrid compound **21**, analogue to the mepyramine—JNJ-hybrid compound **16**, leads to a decrease in affinity of about 1 log unit at $hH_1R$, compared to diphenhydramine **3**. For the analogoue astemizole–JNJ-hybrid compound **45**, only a slight decrease in affinity was observed at $hH_1R$, compared to astemizole **4**. Thus, the introduction of a JNJ partial structure into mepyramine and diphenhydramine leads to a stronger decrease in affinity, compared to the corresponding $H_1$ antagonists. In contrast, the JNJ–astemizole hybrid shows an affinity in the same range as found for astemizole (Fig. 3). Compound **38** shows a significant decrease in affinity at $hH_1R$, compared to **19**. In **38**, the JNJ partial structure is connected to mepyramine via the indole moiety, whereas in **19**, the JNJ partial structure is connected via the piperazine moiety to mepyramine. Thus, this switch is suggested to be responsible for the observed differences in affinity. Compound **38** was obtained experimentally as racemate, but in molecular modelling, both enantiomers were analyzed (Fig. 2). Molecular dynamic simulations revealed a stable binding mode for both enantiomers. The mepyramine partial structure (for both enantiomers) is located in the same part of the binding pocket, as already described for **16** or **19** and the positively charged amino moiety of **38** (both enantiomers) interacts electrostatically with $Asp^{3.32}$. Molecular dynamic simulations revealed a stable hydrogen bond interaction between the carbonyl moiety of **38** (R- and S-configuration) and $Trp^{7.40}$. For **38** (S-configuration), the carbonyl moiety establishes an additional hydrogen bond to $Asn^{2.61}$. Aromatic interactions between the indole moiety of **38** and the receptor were not detected. However, both enantiomers showed slight differences in conformation in its receptor bound state. Theses differences are reflected in the interaction energy between **38** and $hH_1R$. Between the R enantiomer of **38** and $hH_1R$, a coulomb energy (short range) of $-166 \pm 3$ kJ/mol and a Lennard–Jones energy (short range) of $-285 \pm 2$ kJ/mol was observed. In contrast, between the S enantiomer of **38** and $hH_1R$, a coulomb energy (short range) of $-241 \pm 2$ kJ/mol and a Lennard–Jones (short range) of $-284 \pm 1$ kJ/mol was observed (Table 2).

As shown in Table 2, a comparison of the calculated ligand-receptor-interaction energies (C+LJ LR, Table 2) does not reflect the observed $pK_i$ values of **16**, **19** and **38**. However, this observation can be explained: During molecular dynamic simulations, the