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2,4-Diaminopyrimidines as dual ligands at the histamine H_1 and H_4 receptor—H_1/H_4-receptor selectivity

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ABSTRACT

Distinct diaminopyrimidines, for example, 4-(4-methylpiperazin-1-yl)-5,6-dihydrobenzo[*h*]quinazolin-2-amine are histamine H_4 receptor (H_4R) antagonists and show high affinity to the H_4R, but only a moderate affinity to the histamine H_1 receptor (H_1R). Within previous studies it was shown that an aromatic side chain with a distinct distance to the basic amine and aromatic core is necessary for affinity to the human H_1R (hH_1R). Thus, a rigid aminopyrimidine with a tricyclic core was used as a lead structure. There, (1) the flexible aromatic side chain was introduced, (2) the substitution pattern of the pyrimidine core was exchanged and (3) rigidity was decreased by opening the tricyclic core. Within the present study, two compounds with similar affinity in the one digit μM range to the human H_1R and H_4R were identified. While the affinity at the hH_1R increased about 4- to 8-fold compared to the parent diaminopyrimidine, the affinity to the hH_4R decreased about 5- to 8-fold. In addition to the parent diaminopyrimidine, two selected compounds were docked into the H_1R and H_4R and molecular dynamic studies were performed to predict the binding mode and explain the experimental results on a molecular level. The two new compounds may be good lead structures for the development of dual H_1/H_4 receptor ligands with affinities in the same range.

The G protein-coupled receptors (GPCRs) are a large protein family, being involved in several physiological and pathophysiological processes and represent important drug targets in therapy of several diseases.[1,2] The four histamine receptor subtypes (H_1R, H_2R, H_3R and H_4R) belong to the family A of GPCRs and their endogenous ligand is the biogenic amine histamine.[3–8] For the human histamine H_1 receptor (hH_1R),[9] which couples to $G_{\alpha q}$,[3] a large number of antagonists and (partial) agonists with high differences in structure are known.[5,10–14] H_1R antagonists, for example, desloratadine, levocetirizine or rupatadine, are clinically important drugs for treatment of allergic diseases.[3,5] Furthermore, new experimental studies indicate that the histamine H_4 receptor (H_4R)[15,16] which couples to $G_{\alpha i}$[5] is involved, besides other tasks, like mediation of chemotaxis of different cell types, in allergic reactions like the H_1R.[5,17,18] Thioperamide and the indole derivative JNJ7777120, both antagonists or inverse agonists at the hH_4R, represent reference ligands for the hH_4R.[19–22] However, a large number of (partial) agonists and inverse agonists at the hH_4R with high structural variability were developed within the last years.[23–28] For example, diaminopyrimidine derivatives represent one important class of H_4R ligands,[29,30] for example, **1**[31] (Fig. 1).

In literature, a synergistic effect of H_1R and H_4R antagonists in allergic inflammation is discussed.[18,32–34] Thus, antagonistic compounds with high affinity to hH_1R and hH_4R may be a new option for therapy of the related diseases.[18,32–37] However, selective hH_1R or hH_4R ligands and additionally, dual H_1/H_4-receptor ligands with one joined H_1/H_4-pharmacophor are important tools to study and understand both receptors on a molecular level. As the amino acids forming the orthosteric binding pocket of hH_1R and hH_4R have smallest identity when comparing the human histamine receptors, it may be very challenging to develop ligands with similar affinity to hH_1R and hH_4R.[14]

Within previous studies, H_1R (partial) agonists and antagonists were routinely studied at hH_4R, in order to obtain an extensive pharmacological profile with regard to the selectivity at histamine receptors.[12,32,38] Most of the analyzed (partial) agonists at hH_1R, for

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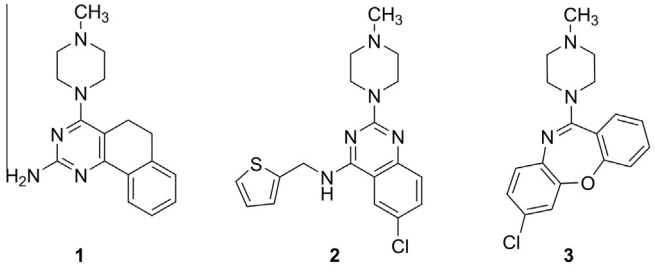


Figure 1. Structures and affinities of some selected H_1R and/or H_4R ligands: diaminopyrimidine derivative, **1**;[31] quinazoline derivative, **2**;[39] loxapine derivative, **3**.[40]

example, phenylhistamine, histaprodifens and phenoprodifens, showed selectivity towards the hH_1R, except three *N*-methylated phenylhistamine derivatives, which showed about 10-fold selectivity towards hH_4R.[38] But due to their partial agonism at both receptor subtypes, those compounds are not relevant in the therapy of H_1R/H_4R-related diseases.[10,11,38] The analysed H_1R antagonists showed only weak affinity to hH_4R.[12,32] Some compounds with high affinity for hH_1R and hH_4R, but with selectivity either to hH_1R or to hH_4R, are described in literature, for example the quinazoline **2** and loxapine derivative **3** (Fig. 1).[39–42]

The diaminopyrimidine **1** is described as a highly affine hH_4R antagonist.[31] Previous studies, based on astemizole derived compounds, suggest that the flexible aromatic side chain at the aromatic core is necessary for affinity to the hH_1R.[14] Furthermore, the experimental data suggest that the increase of the spacer length between the aromatic core and the aromatic moiety of the side chain from one CH_2 moiety to two CH_2 moieties leads to a decrease in affinity to hH_1R, but to an increase in affinity to hH_4R, which corresponds to a decrease in selectivity hH_1R/hH_4R from ~23,000 to ~65 (compds **9** and **13** in Ref. 14).[14] Therefore, the aim of the present study was to introduce different aromatic side chains at the amine moiety of the diaminopyrimidine **1**. Furthermore, the position of the *N*-methylpiperazine and the aromatic side chain were exchanged and the rigidity of the tricyclic core was removed (Fig. 2).

Twenty different diaminopyrimidine derived compounds were synthesized and characterized pharmacologically at hH_1R and hH_4R by radioligand competition binding assays. Within the present study, two new compounds in the one-digit μM affinity range at both receptors with no significant subtype selectivity

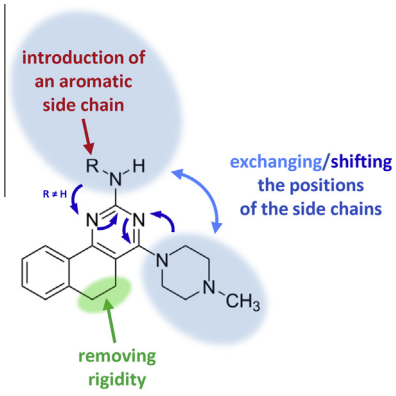


Figure 2. Strategy for the design of dual histamine H_1/H_4-receptor ligands, using the diaminopyrimidine **1** as lead structure.

were identified. Additionally, the binding mode of the parent diaminopyrimidine **1** and two selected derivatives were studied in more detail by means of docking and molecular dynamic (MD) simulations at hH_1R and hH_4R.

The reference compound **1** was prepared from 1-tetralone in four steps according to procedures described by Cowart.[31] The other ligands which were synthesized and evaluated within this study are derived from three major scaffolds (Schemes 1–3).

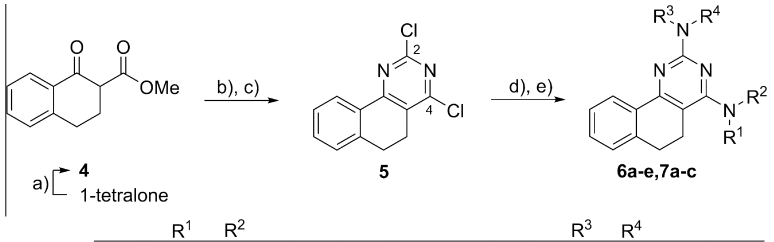
The starting material **4** required for the synthesis of the first group of ligands **6a–e** and **7a–c** (Scheme 1) was readily obtained from 1-tetralone and dimethylcarbonate via deprotonation with sodium hydride.[43] Methyl 1-tetralone-2-carboxylate (**4**) was then condensed with urea at a high temperature and the resulting pyrimidine-2,4-dione was converted to its corresponding dichloropyrimidine derivative **5** using phosphorus oxychloride.[43] At 40 °C in dimethylformamide, nucleophilic substitution of **5** with the first amine (HNR^1R^2) selectively occurred at the 4-position of the pyrimidine ring as evinced by NOESY experiments (see Supporting information). The second amino group was introduced in reactions using the amine HNR^3R^4 as solvent at 90 °C. According to this strategy, eight ligands **6a–e** and **7a–c** were prepared.

The ligands of the second group (Scheme 2) were synthesized from 2,4-dichloro-6-phenylpyrimidine **9**, which was itself obtained through a Suzuki coupling between phenylboronic acid and trichloropyrimidine **8**.[44] As in the nucleophilic substitution of **5**, the attack of the first amine HNR^1R^2 exclusively occurred at the 4-position of the dichloropyrimidine **9**, which was again verified by NOESY experiments (see Supporting information). A reaction with the amine HNR^3R^4 at 90 °C provided the final compounds **10a–c** and **11a–c**. The ligands **12a** and **12b** bearing a dimethylamino group in 2-position were accessible from **9** through a single reaction step at 90 °C. In this particular double substitution the attack of the amine HNR^1R^2 occurred at the 4-position and the dimethylamino group, which was most likely transferred from the solvent dimethylformamide, was subsequently introduced into the 2-position of the pyrimidine core.

The regioisomeric dichloropyrimidine **14** was used as central precursor for the ligands of the third group (Scheme 3). Under conditions identical to those shown in Scheme 1, methyl 2-tetralone-1-carboxylate **13** (this compound was obtained from 2-tetralone and dimethylcarbonate following the procedure reported by Harris)[43] was first condensed with urea and the resulting pyrimidine-dione was then reacted with phosphorous oxychloride to give dichloropyrimidine **14**.[43] In contrast to the nucleophilic substitutions described above (Schemes 1 and 2), the attack of the first amine HNR^1R^2 now occurred at the 2-position of the pyrimidine moiety (see Supporting information for NOESY experiments). In the final step, *N*-methylpiperazine was attached in 4-position through a reaction at 90 °C to yield **15a–c**. Unexpectedly, but possibly related to the stronger nucleophilicity of *N*-methylpiperazine compared to the primary aliphatic amines,[45] the double substitution of **14** with *N*-methylpiperazine to yield **15d** was already observed at 40 °C in dimethylformamide.

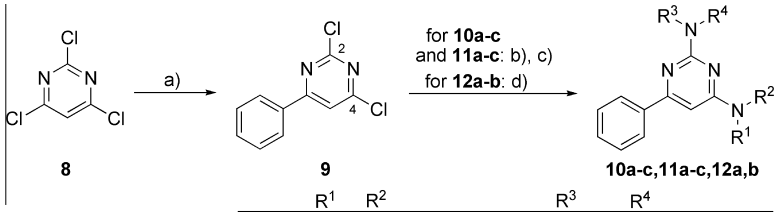
In the present study, several compounds derived from the diaminopyrimidine **1** were pharmacologically characterized at hH_1R and hH_4R by radioligand competition binding assays with Sf9 insect cell membranes, expressing either hH_1R and RGS4 or hH_4R, $G\alpha_{i2}$ and $G\beta_1\gamma_2$ as described previously (see Supporting information).[10,38] The resulting pharmacological data are given in Table 1.

The diaminopyrimidine derivative **1** has a weak affinity ($pK_i = 5.04$) to the hH_1R, whereas the affinity to hH_4R is about 49 fold higher (Table 1). Within the present study, we determined a pK_i value of 6.73 (Table 1) for compound **1** at hH_4R, in contrast to a pK_i of 7.81 which is described in literature.[31] Similar discrepancies in affinity between literature data and the data determined



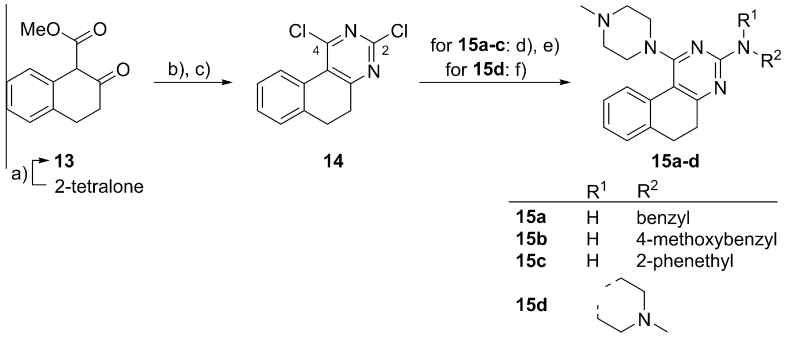
	R^1	R^2	R^3	R^4
6a			H	benzyl
6b			H	4-methylbenzyl
6c			H	4-fluorobenzyl
6d			H	4-methoxybenzyl
6e			H	2-phenethyl
7a	H	benzyl		
7b	H	2-phenethyl		
7c	H	3-(4'-chlorophenyl)-4-methoxybenzyl		

Scheme 1. Reagents and conditions: (a) NaH, dimethyl carbonate, MeOH, 80 °C, 3 h; (b) urea, 190 °C, 45 min; c) $POCl_3$, DMF (cat.), 110 °C, 46 h; (d) HNR^1R^2, DMF, 40 °C, 18 h; (e) HNR^3R^4, 90 °C, 18 h.



	R^1	R^2	R^3	R^4
10a			H	benzyl
10b			H	4-methoxybenzyl
10c			H	2-phenethyl
11a	H	benzyl		
11b	H	4-methoxybenzyl		
11c	H	2-phenethyl		
12a	H	4-methoxybenzyl	methyl	methyl
12b	H	2-phenethyl	methyl	methyl

Scheme 2. Reagents and conditions: (a) $PhB(OH)_2$, Na_2CO_3, $Pd(OAc)_2$ (cat.), PPh_3 (cat.), 60 °C, 3 h; (b) HNR^1R^2, DMF, 40 °C, 18 h; (c) HNR^3R^4, 90 °C, 18 h; (d) HNR^1R^2, DMF, 90 °C, 18 h.



	R^1	R^2
15a	H	benzyl
15b	H	4-methoxybenzyl
15c	H	2-phenethyl
15d		

Scheme 3. Reagents and conditions: (a) NaH, dimethyl carbonate, MeOH, 80 °C, 3 h; (b) urea, 190 °C, 45 min; (c) $POCl_3$, DMF (cat.), 110 °C, 46 h; (d) HNR^1R^2, DMF, 40 °C, 18 h; (e) *N*-methylpiperazine, 90 °C, 18 h; (f) *N*-methylpiperazine, DMF, 40 °C, 18 h. Positions 2 and 4, mentioned in **14**, are according to the numbering of the pyrimidine ring only.

within our lab were found for the quinazoline-derivative **2** at hH_1R and hH_4R and for the loxapine derivative **3** at hH_4R (Table 1).[14,27,39] A reason for those differences may be different assay conditions or the use of different cell membranes. In contrast, for JNJ7777120 ((5-chloro-1*H*-indol-2-yl)(4-methylpiperazin-1-yl)methanone), one of the pK_i values determined by Leurs and co-workers (pK_i 7.8, hH_4R expressed in SK-N-MC cells)[40] and (pK_i 8.31, hH_4R expressed in HEK 293T cells)[46] is in acceptable accordance with our data (pK_i 7.45).[14] This may indicate that the cells used for hH_1R- or hH_4R expression or the assay conditions, like buffer or other ingredients may have an influence on the affinity of some compounds to hH_1R and hH_4R.

The affinities of the compounds **6a**–**6e**, **7a**–**7c**, **10a**–**10c**, **11a**–**11c**, **12a**–**12b** and **15a**–**15d** at hH_1R and hH_4R are summarized in Table 1. At hH_1R, the pK_i values of **6a**–**6e**, **7a**–**7c**, **10a**–**10c**, **11a**–**11c** and **15a**–**15d** are in a range from 5.0 to 6.0 at hH_1R and from 4.1 to 6.0 at hH_4R (Table 1). As expected, for the compounds **12a** and **12b** affinity with neither hH_1R nor hH_4R was observed (Table 1). A reason for that is the missing basic amine moiety in an appropriate distance to the aromatic core.

Table 1
Affinities of compounds **1–3**, **6a–6e**, **7a–7c**, **10a–10c**, **11a–11c**, **12a–12b** and **15a–d** determined by radioligand competition binding assays at hH_1R and hH_4R

Compd	pK_i (hH_1R)	pK_i (hH_4R)
1	5.04 ± 0.15	6.73 ± 0.09
2	6.26 ± 0.11[a]	7.37 ± 0.06[a]
3	8.08 ± 0.07[b]	6.99 ± 0.01[b]
6a	5.68 ± 0.03	5.80 ± 0.06
6b	5.47 ± 0.06	4.70 ± 0.01
6c	5.71 ± 0.17	4.96 ± 0.01
6d	5.24 ± 0.01	5.37 ± 0.04
6e	5.03 ± 0.02	5.04 ± 0.05
7a	6.05 ± 0.05	4.84 ± 0.02
7b	5.09 ± 0.01	4.82 ± 0.01
7c	5.20 ± 0.11	4.71 ± 0.05
10a	5.95 ± 0.04	6.02 ± 0.06
10b	5.28 ± 0.04	5.76 ± 0.05
10c	5.48 ± 0.01	5.01 ± 0.03
11a	5.60 ± 0.01	4.50 ± 0.05
11b	5.38 ± 0.09	4.14 ± 0.68
11c	5.71 ± 0.15	4.44 ± 0.14
12a[c]	No binding	No binding
12b[c]	No binding	No binding
15a	5.72 ± 0.03	4.58 ± 0.04
15b	5.97 ± 0.05	4.86 ± 0.05
15c	5.73 ± 0.02	5.17 ± 0.01
15d	5.93 ± 0.01	5.11 ± 0.04

At least three independent assays were performed.
[a] pK_i values, determined by Smits et al.[39]: hH_1R: 7.70 ± 0.10; hH_4R: 8.12 ± 0.02.
[b] pK_i values, determined by Smits et al.[40]: hH_1R: 8.11 ± 0.10; hH_4R: 7.55 ± 0.09.
[c] Compounds **12a** and **12b** were expected to show no affinity at hH_1R and hH_4R; they were only synthesized for reason of verification.

The most important structure–activity relationships, based on the pharmacological data (Table 1) are presented in Figure 3.

Compared to the lead structure **1**, the introduction of a benzyl side chain (**6a**) led to an increase in affinity at hH_1R in contrast to a phenethyl moiety (**6e**), whereas the affinity decreased significantly at hH_4R. However, the elongation of the side chain ($n = 1$ (**6a**) → $n = 2$ (**6e**)) led to a decrease in affinity at both receptors. The 'opening' of the tricyclic core (**6a** → **10a**, **6e** → **10c**) had only small influence on affinity at hH_1R and hH_4R. Similar to **6a** and **6e**, the elongation of the spacer ($n = 1$ (**10a**) → $n = 2$ (**10c**)) led to a decrease in affinity at both receptors. An exchange of the positions of the aromatic side chain and the piperazine moiety (**6a** → **7a**, **6e** → **7b**, **10a** → **11a**, **10c** → **11c**) led to a decrease in affinity up to ~1.5 orders of magnitude at hH_4R, indicating that the hH_4R is highly sensitive to the substitution pattern at the tricyclic core. The opening of the tricyclic moiety ($n = 2$, **7b** → **11c**) led to an increase in affinity at hH_1R, which indicates that due to the increased flexibility the aromatic moieties are able to adopt a more favored binding conformation. The shift of the piperazine moiety and the aromatic side chain led to an increase in affinity ($n = 2$, **7b** → **15c**) at hH_1R. Furthermore, the elongation of the side chain (**15a** → **15c**) led to an increase in affinity at hH_4R.

Compared to the diaminopyrimidine **1** the compounds **6a–6e**, **7a–7c**, **10a–10c**, **11a–11c** and **15a–15d** showed affinities in the same range or an increase up to one order of magnitude at hH_1R. As the introduction of an aromatic side chain is a common element of the compounds **6a–6e**, **7a–7c**, **10a–10c**, **11a–11c** and **15a–15d** compared to the parent compound **1**, it can be suggested that the aromatic moiety in a distinct distance to the core of the molecule, as well as the substitution pattern, is relevant for the affinity to the hH_1R. In contrast, the compounds **6a–6e**, **7a–7c**, **10a–10c**, **11a–11c** and **15a–15d** except **12a** and **12b** showed affinities to the hH_4R of about 0.7 up to 2.7 orders of magnitude smaller than the diaminopyrimidine **1**. This indicates that the introduction of an aromatic moiety is not tolerated by the hH_4R within this series of compounds. Furthermore, the hH_4R is more sensitive than the

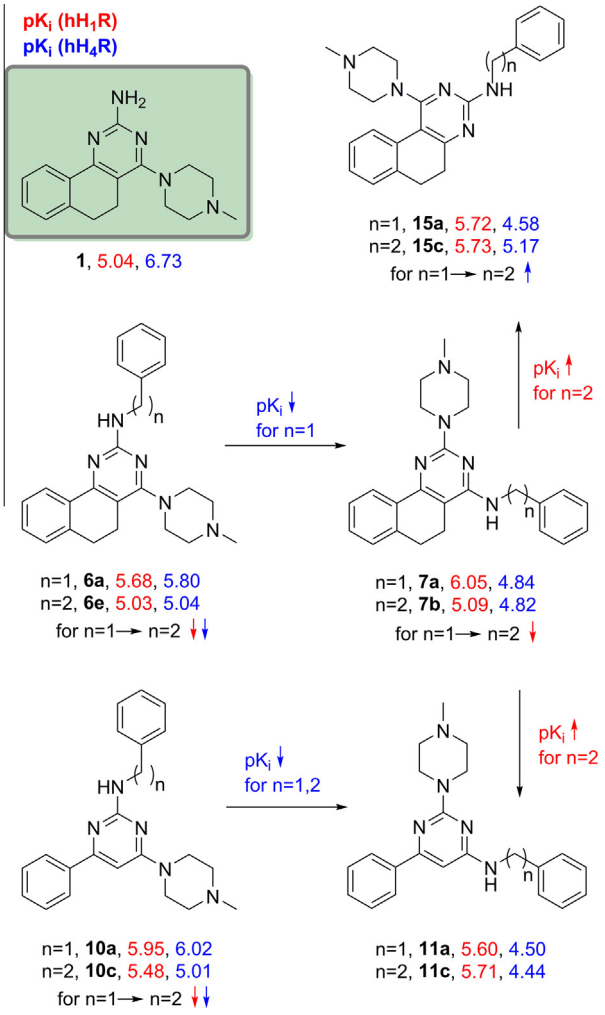


Figure 3. Structure–activity-relationships of selected diaminopyrimidine derived compounds at hH_1R and hH_4R. Absolute changes ⩾0.5 in pK_i are indicated by an arrow (upwards arrow: increase in pK_i, downwards arrow: decrease in pK_i).

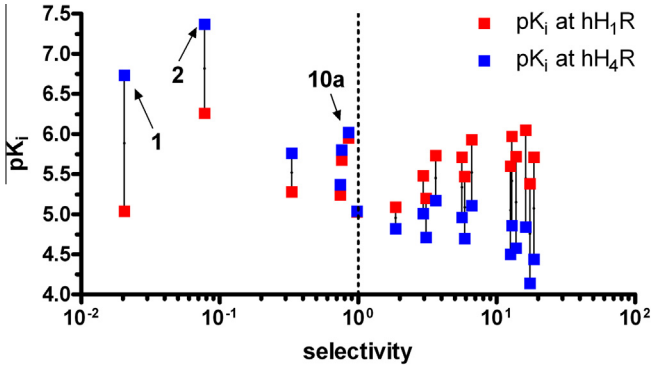


Figure 4. 'Affinity–selectivity' profile of the compounds **1**, **2**, **6a–6e**, **7a–7c**, **10a–10c**, **11a–11c** and **15a–15d**. The profile is based on the data provided in Table 1 and was obtained by radioligand competition binding assays at hH_1R and hH_4R under comparable conditions.

hH_1R regarding the structural variations (Table 1). A correlation of the affinities of the parent diaminopyrimidine **1** and the derived compounds **6a–11c**, **15a–15d** between hH_1R and hH_4R, determined in radioligand competition binding assays under comparable conditions is given in Figure 4. The aim of the study was to

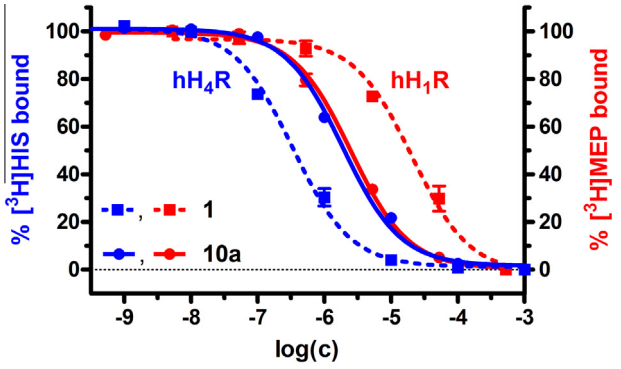


Figure 5. Competition binding curves (specific binding shown) for compounds **1** and **10a** at hH_1R (coexpressed with RGS4) and hH_4R (coexpressed with $G\alpha_{i2}$ and $G\beta_1\gamma_2$) in Sf9 cell membranes.

identify ligands with high affinity to hH_1R and hH_4R, while the selectivity between both receptors should be as small as possible. The correlation shows that compound **10a**, with an affinity in the one-digit µM range at both receptors (Fig. 5), fits best to this requirement, because the pK_i of **10a** to hH_1R and hH_4R (Table 1) are not significantly different and there is rather no selectivity (0.85) between both receptor subtypes (Fig. 4).

In order to study the selectivity at the four human histamine receptor subtypes, the affinities of the parent compound **1** and the selected compounds **6a** and **10a** at human histamine H_2 receptor (hH_2R) and humane histamine H_3 receptor (hH_3R) were determined additionally to complete the pharmacological profile (Table 2) (see Supporting information).[12]

The data show that the aminopyrimidine **1** binds selective to the hH_4R. The affinity of the compounds **6a** and **10a** to the hH_2R is decreased compared to hH_1R and hH_4R. The compounds **6a** and **10a** show a slightly decreased affinity at hH_3R compared to hH_1R or hH_4R.

Additionally, the reference compound **1** and the two selected compounds **6a** and **10a** were analysed functionally at the hH_4R with the help of the [^{35}S]GTPγS assay (Fig. 6, Table 3) (see Supporting information).[47]

The potencies of **1**, **6a** and **10a** are comparable to the corresponding affinities (Table 1). The potency of the aminopyrimidine **1** is increased compared to **6a** and **10a**. Compound **1**, which is described in literature as an H_4R antagonist,[31] is identified as a partial inverse agonist at hH_4R in our assay system. The E_{max} values of **6a** and **10a** are decreased compared to **1** and are comparable to the E_{max} of thioperamide. Thus, the compounds **6a** and **10a** were identified as inverse agonists at hH_4R.

To study the binding mode of **1**, **6a** and **10a** at hH_1R and hH_4R, MD simulations were performed, as described previously (see Supporting information), and the resulting stable binding modes are shown (Fig. 7).[14,48]

The MD simulation of the diaminopyrimidine **1** within the binding pocket of hH_1R revealed a stable binding mode (Fig. 7A). The tricyclic core of **1** is located in a hydrophobic pocket between TM III, TM IV, TM V, TM VI and the E2-loop, next to the amino acid side chains of Tyr$^{3.33}$, Ser$^{3.36}$, Trp$^{4.56}$, Ala$^{5.43}$, Phe$^{5.47}$, Trp$^{6.48}$, Phe$^{6.52}$, Phe$^{6.55}$, Phe^{184} (E2-loop) and Tyr^{185} (E2-loop). As described for other aminergic GPCRs, a stable electrostatic interaction between the positively charged piperazine moiety of **1** and the highly conserved Asp$^{3.32}$ was formed (Fig. 7A). Analogous MD simulations for **6a** (Fig. 7B) and **10a** (Fig. 7C) in the binding pocket of hH_1R revealed different binding modes compared to **1**. The aromatic core of **6a** and **10a** is embedded in a similar hydrophobic pocket, as observed for **1**, formed by the amino acids Tyr$^{3.33}$, Ser$^{3.36}$, Trp$^{4.56}$, Phe$^{5.38}$, Ala$^{5.43}$, Phe$^{5.47}$, Trp$^{6.48}$, Phe$^{6.52}$, Phe$^{6.55}$ and Tyr^{185} (E2-loop). The additional aromatic side chain of **6a** and **10a** is embedded in a stable manner in a small but deep pocket between TM III, TM V and TM VI and is in direct neighbourhood to the hydrophobic parts of the amino acids Thr$^{3.37}$, Ile$^{3.40}$, Phe$^{3.41}$ and Trp$^{6.48}$. The MD simulations suggest that the aromatic side chain of **6a** and **10a** stabilizes the highly conserved Trp$^{6.48}$, which is involved in an early step of the receptor activation in a more vertical conformation, typical of the inactive state.[49] Since this aromatic side chain is missing in **1**, the smallest distance between **1** and Trp$^{6.48}$ is larger than 5 Å and therefore a direct contact between **1** and Trp$^{6.48}$ was not observed. This may explain the lower affinity of **1** compared to **6a** or **10a** to the hH_1R. This observation is supported by previous studies on astemizole derived compounds: Here, the introduction of a flexible aromatic side chain results in an up to ~1000 fold increase in affinity, in dependence on the substitution pattern of the aromatic side chain.[14]

The diaminopyrimidine **1** was docked into the binding pocket of the hH_4R in two different poses (mode 1 and 2) (Fig. 8A). To study, if one of both modes is the preferred binding mode, MD simulations were performed for both models. Within both models, the positively charged piperazine moiety was observed to form a

Table 2
Affinities of the selected compounds **1**, **6a** and **10a** determined by radioligand competition binding assays at the four human histamine receptor subtypes hH_1R, hH_2R, hH_3R and hH_4R

Compd	pK_i (hH_1R)	pK_i (hH_2R)	pK_i (hH_3R)	pK_i (hH_4R)
1	5.04 ± 0.15	4.50 ± 0.28	5.01 ± 0.16	6.73 ± 0.09
6a	5.68 ± 0.03	5.16 ± 0.10	5.22 ± 0.16	5.80 ± 0.06
10a	5.95 ± 0.04	4.99 ± 0.15	5.60 ± 0.20	6.02 ± 0.06

At least four independent assays were performed.

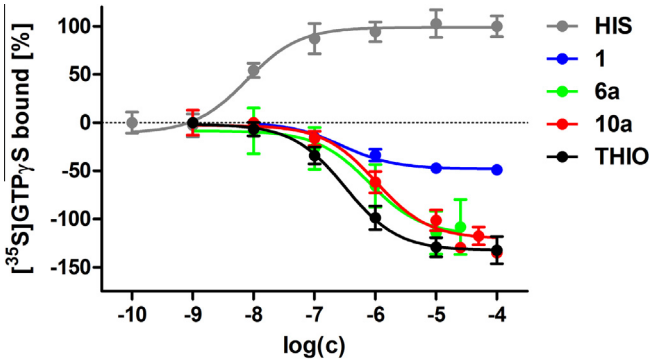


Figure 6. [^{35}S]GTPγS binding curves (specific binding shown) for selected compounds **1**, **6a**, **10a** and histamine (**HIS**) and thioperamide (**THIO**) as reference compounds at hH_4R (coexpressed with $G\alpha_{i2}$ and $G\beta_1\gamma_2$) in Sf9 cell membranes. All E_{max} values are determined relative to the E_{max} of histamine.

Table 3
Potencies and efficacies of histamine and the compounds **1**, **6a** and **10a** determined by the [^{35}S]GTPγS assay at hH_4R

Cpmpd	pEC_{50}	E_{max}	*n*
Histamine (HIS)[a]	8.10 ± 0.36	1.00	3
1	6.53 ± 0.20	−0.47 ± 0.04	5
6a	6.03 ± 0.03	−1.01 ± 0.38	3
10a	6.04 ± 0.17	−1.27 ± 0.17	3
Thioperamide (THIO)[b]	6.38 ± 0.16	−1.34 ± 0.17	4

All E_{max} values are determined relative to the E_{max} of histamine.
n: number of independent experiments
[a] pEC_{50}: 8.13 ± 0.06, determined by Wifling et al.[47]
[b] pEC_{50}: 6.58 ± 0.06, E_{max}: −1.39 ± 0.08, determined by Wifling et al.[47]

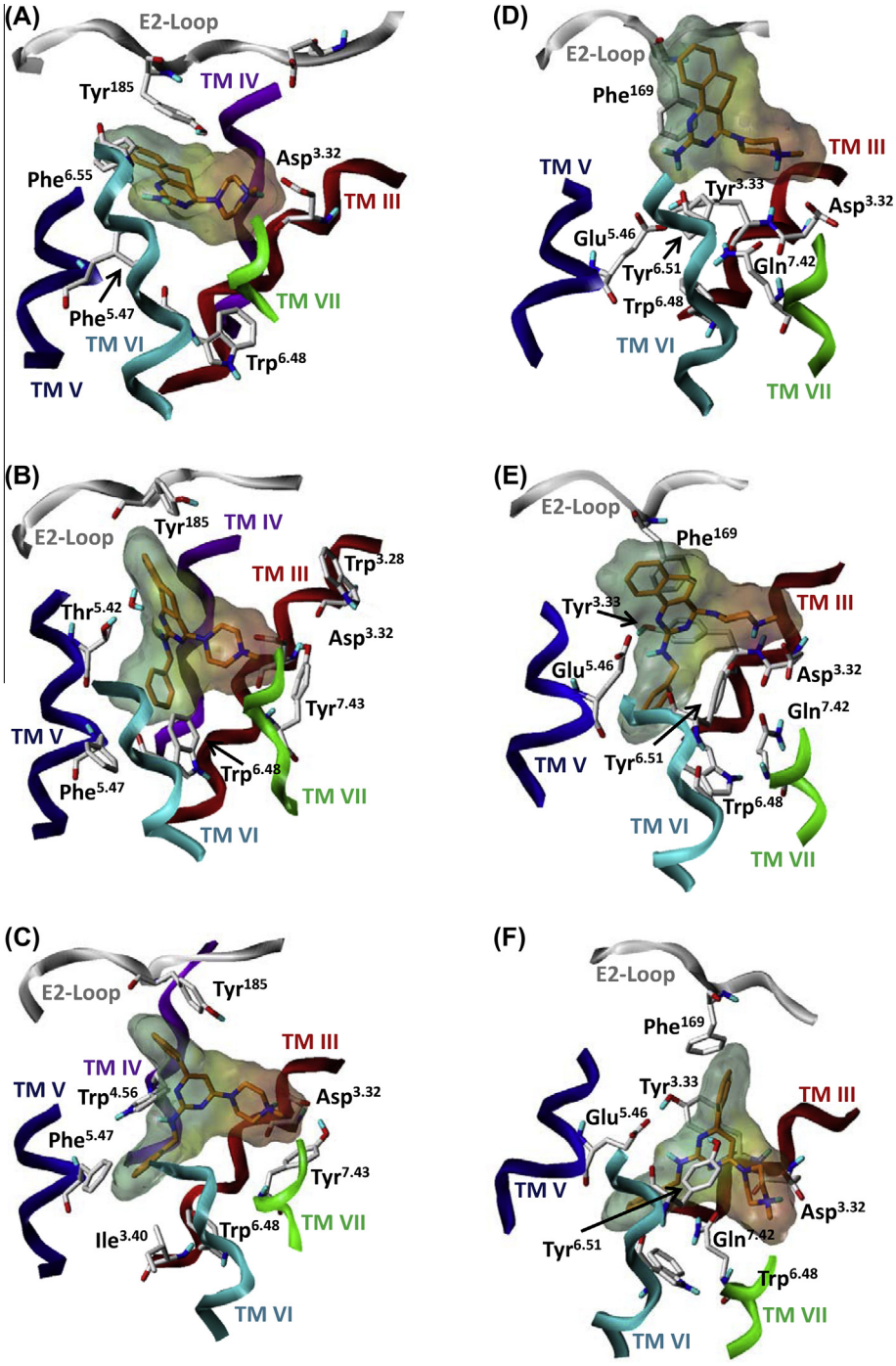


Figure 7. Binding mode of compounds **1**, **6a** and **10a** at the hH_1R and hH_4R, based on molecular dynamic simulations. The surface of the ligand is coloured according to its electrostatic potential (red: positive, green: neutral). (A) **1** at hH_1R; (B) **6a** at hH_1R; (C) **10a** at hH_1R; (D) **1** at hH_4R; (E) **6a** at hH_4R; (F) **10a** at hH_4R. For reason of clarity only the most important amino acids are shown.

stable electrostatic interaction with $Asp^{3.32}$ (Fig. 8A). The analysis of the side chain conformation of amino acids within the orthosteric binding side shows large differences for $Glu^{5.46}$, $Trp^{6.48}$, $Tyr^{6.51}$ and $Gln^{7.42}$ (Fig. 8A). The side chain conformation of these four amino acids obtained for mode 2 (Fig. 8A) is similar to the conformation of these amino acids in the ligand-free inactive hH_4R, obtained by MD simulation (Fig. 8B): In the ligand-free state, the $Tyr^{6.51}$ points towards $Glu^{5.46}$ and forms a stable interaction. In contrast to mode 2, in mode 1, $Tyr^{6.51}$ points into the opposite direction and interacts with $Gln^{7.42}$, forcing the $Gln^{7.42}$ side chain more downwards (Fig. 8).

The analysis of the short range energy terms (Coulomb and Lennard-Jones) between compound **1** and the hH_4R or the water molecules within the orthosteric binding site, respectively and additionally the short range energy terms between the four amino acids $Glu^{5.46}$, $Trp^{6.48}$, $Tyr^{6.51}$ and $Gln^{7.42}$, which differ mainly in their conformation between mode 1 and mode 2, suggests that mode 2 is the preferred one (Table 4). Switching from binding mode 1 to mode 2, the Coulomb and Lennard-Jones terms of the ligand-receptor interaction energy increase by +113.4 kJ/mol. However, the decrease of the analogous interaction terms for the amino acids ($Glu^{5.46}$, $Trp^{6.48}$, $Tyr^{6.51}$ and $Gln^{7.42}$) and the

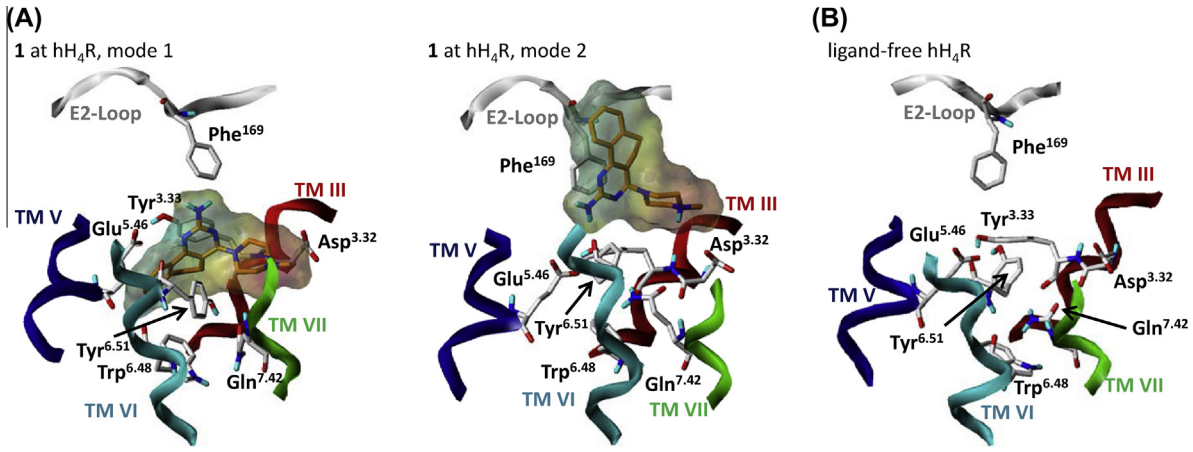


Figure 8. (A) Binding mode 1 and 2 of compound **1** in the orthosteric binding site of hH_4R, based on molecular dynamic simulations. The surface of the ligand is coloured according to its electrostatic potential (red: positive, green: neutral); (B) Ligand-free orthosteric binding site of the hH_4R, based on molecular dynamic simulations.

Table 4
Interaction energies (Coulomb short range (C^{SR}) and Lennard-Jones (LJ^{SR})) between compound **1** and the hH_4R or water (W) in the orthosteric binding pocket (W^{osbp}), respectively

Interaction energy (kJ/mol)	Mode 1	Mode 2
$\mathbf{C^{SR}}$ (**1**–hH_4R)	−149.8 ± 0.7	−71.7 ± 0.8
$\mathbf{LJ^{SR}}$ (**1**–hH_4R)	−168.6 ± 0.4	−133.3 ± 0.6
$\mathbf{C^{SR}}$ (**1**–W^{osbp})	5.4 ± 0.4	−55.5 ± 0.9
$\mathbf{LJ^{SR}}$ (**1**–W^{osbp})	−18.9 ± 0.2	−27.4 ± 0.4
$\mathbf{C^{SR}}$ ($Glu^{5.46}$, $Trp^{6.48}$, $Tyr^{6.51}$ and $Gln^{7.42}$)	−82.1 ± 0.3	−154.3 ± 0.4
$\mathbf{LJ^{SR}}$ ($Glu^{5.46}$, $Trp^{6.48}$, $Tyr^{6.51}$ and $Gln^{7.42}$)	−50.2 ± 0.2	−57.2 ± 0.3
$\sum$	−464.2 ± 2.2	−499.4 ± 3.4

Interaction energies between $Glu^{5.46}$, $Trp^{6.48}$, $Tyr^{6.51}$ and $Gln^{7.42}$ which show large differences in conformation between mode 1 and mode 2.

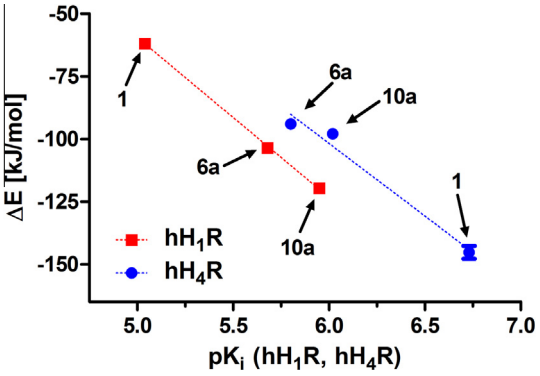


Figure 9. Correlation between the experimentally determined pK_i values and the change in interaction energies (ΔE) between ligand and surrounding for the transfer of the ligand from the aqueous phase into the orthosteric binding pocket.

ligand-water interaction by −58.6 kJ/mol and −79.2 kJ/mol, respectively favours mode 2.

Since mode 2 is suggested to be the preferred one for compound **1**, the compounds **6a** and **10a** were docked into the orthosteric binding site of the hH_4R in a similar manner and the subsequent MD simulations revealed stable binding modes for **6a** and **10a**. The positively charged piperazine moiety of **6a** and **10a** forms a stable electrostatic interaction with $Asp^{3.32}$ (Fig. 7E and F). For both compounds, the (opened) tricyclic moiety is embedded in the same part of the pocket as found for **1** (Fig. 7D–F) and forms an aromatic interaction with Phe^{169} (E2-loop) (Fig. 7E and F). The additional aromatic side chain is embedded in a subpocket between TM III, TM V and TM VI, formed by $Thr^{3.37}$ (CH_3 group), $Val^{3.40}$, $Phe^{5.47}$, $Trp^{6.48}$ and $Tyr^{6.51}$ (Fig. 7E and F). In case **6a** or **10a** is bound to the orthosteric binding site, the MD simulations suggest that the interaction between $Tyr^{6.51}$ and $Glu^{5.46}$, which is present if no ligand or diaminopyrimidine **1** is bound, is interrupted (Figs. 7D–F, 8B). This destabilization of the receptor may be the reason for the smaller affinity of **6a** and **10a** compared to **1**.

In addition to the structural analysis, the change in interaction energies between ligand and surrounding for the transfer of the ligand from the aqueous phase into the orthosteric binding pocket (ΔE) was approximately calculated (see Supporting information) for **1**, **6a** and **10a**. The linear correlation between ΔE and the experimentally determined pK_i values is quite good (Fig. 9) and supports the binding modes, predicted by the MD simulations. Furthermore, this model may help to identify dual ligands with high affinity to the H_1- and H_4-receptor.

The present modelling results suggest that the binding orientation of **6a** and **10a** in the orthosteric binding pocket is quite similar (Fig. 7). However, a detailed analysis of the most important amino acids, forming the orthosteric binding site of hH_1R and hH_4R, showed that ~70% of these amino acids are different (positions: 2.61, 3.36, 3.40, 4.56, 5.38, 5.39, 5.43, 5.46, 6.52 and 7.42) between both receptor subtypes.[14] At hH_1R, it was shown by mutagenesis studies that the amino acids at positions 2.61, 4.56, 5.39 and 6.52 are involved directly or indirectly in ligand binding.[50–52] Furthermore, experimental studies at hH_4R suggest that the amino acid at position 5.46 is involved in binding of selected ligands.[53] Additionally, it was shown experimentally and by modelling studies that the amino acid in position 3.40 is responsible for species differences between hH_3R and rH_3R.[54–56] Thus, it may be speculated that the amino acid at 3.40 (hH_1R: I, hH_4R: V) may be involved in subtype differences between hH_1R and hH_4R, too. Furthermore, it has to be taken into account that the differences in the E2-loop may also be involved in subtype differences between hH_1R and hH_4R, as indicated by experimental and modelling studies at H_1R and H_4R.[46,57–59] Especially Phe^{169} (E2-loop) was shown to be involved in species differences between hH_4R and mouse H_4R.[46] These results indicate that the amino acids at the mentioned positions may be involved in subtype differences between hH_1R and hH_4R, hampering the identification of dual, high affinity H_1/H_4 receptor ligands with only marginal selectivity. However, it has to be proven by mutagenesis studies, which of the mentioned amino acids, being different between hH_1R and hH_4R, are involved in subtype differences. These results may increase

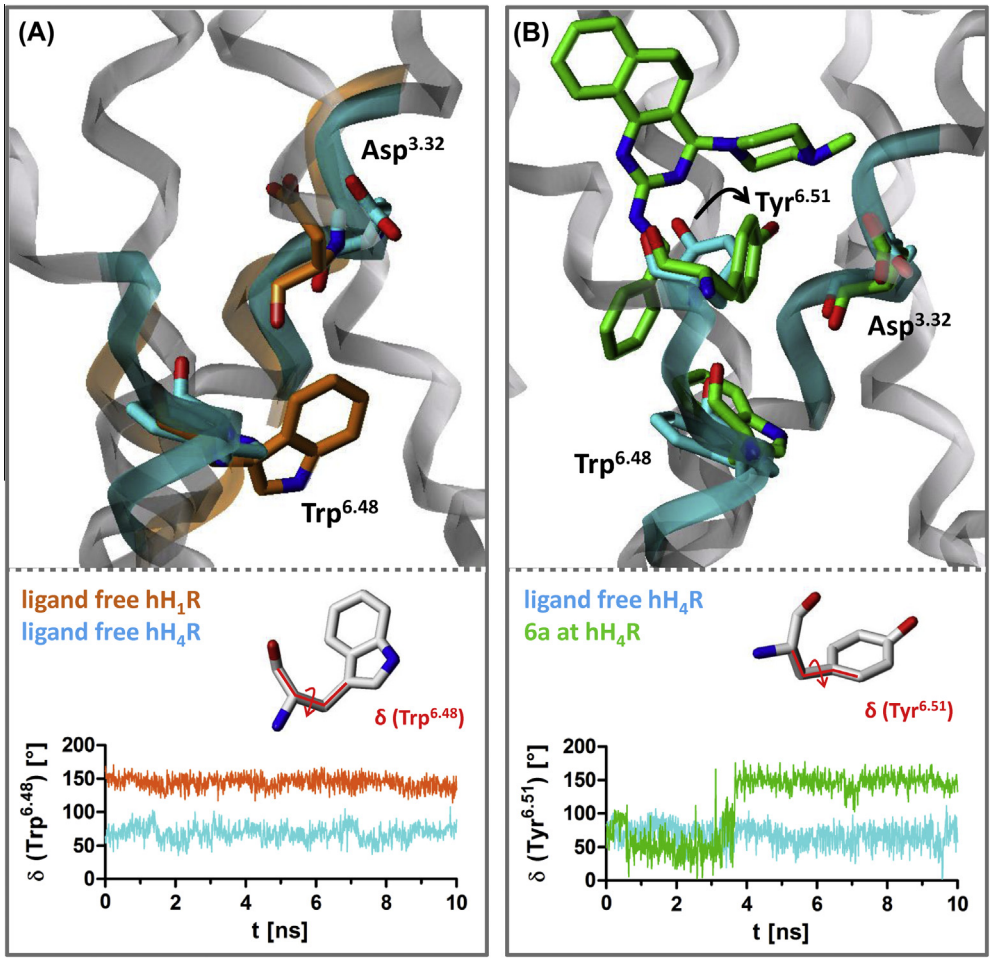


Figure 10. (A) Difference in side chain conformation of $Trp^{6.48}$ between hH_1R and hH_4R indicated by the corresponding dihedral angle. (B) Difference in side chain conformation of $Tyr^{6.51}$ between the ligand-free hH_4R and the **6a**–hH_4R complex.

the understanding of the selectivity profile between hH_1R and hH_4R on a molecular level.

The present simulation results indicate that the different orientation of the $Trp^{6.48}$, which is discussed to be involved in activation of GPCRs[49] between hH_1R and hH_4R, may play an important role. Due to the more horizontal orientation of the $Trp^{6.48}$ at the ligand-free hH_4R (Fig. 10A), a small subpocket between TM III, TM V and TM VI is closed in contrast to the hH_1R and results in case of **6a** and **10a** in an energetically disfavoured reorientation of $Tyr^{6.51}$ (Fig. 10B), which is indicated by an corresponding change of its dihedral angle. This may be a reason why the aromatic side chain of some ligands is not well tolerated by the hH_4R. However, it has to be taken into account that an analogous side chain is tolerated for the quinazoline derivative **2**.[39] However, for the quinazoline derivative **2**, MD simulations predict a different binding mode, compared to **6a** or **10a**.[14] Although the present study provides suggestions for the binding mode of the analysed ligands, more experimental studies, for examples, mutagenesis studies, have to be performed to support these models.

Within previous studies, it was shown that the affinity of compounds to hH_1R and hH_4R is strongly dependent on the Cl-substitution pattern at the aromatic core.[14,30,39] Thus, the affinity of compounds **6a** or **10a** to hH_1R and hH_4R may be improved by introduction of a chlorine at the aromatic core, which will be a subject of future studies.

Within the present study twenty diaminopyrimidine derived compounds were synthesized and pharmacologically characterized at hH_1R and hH_4R. Compared to the lead structure **1**, the affinity to hH_1R was increased by the introduction of an aromatic side chain, as suggested by previous studies with astemizole derived compounds. However, the introduction of the flexible aromatic side chain led to a decrease in affinity of about one or two orders of magnitude at hH_4R, compared to the originally developed hH_4R ligand, resulting in two compounds with an affinity in the single-digit μM range at hH_1R and hH_4R and no selectivity between hH_1R and hH_4R. The present study may indicate that due to the highly different binding pockets of hH_1R and hH_4R, the development of dual H_1R/H_4R-ligands may be a challenge. However, the two compounds developed within this study represent a good starting point for the development of dual hH_1R/hH_4R ligands with high affinity and no selectivity between both receptor subtypes.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.12.035.

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