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Contribution of Binding Enthalpy and Entropy to Affinity of Antagonist and Agonist Binding at Human and Guinea- pig Histamine H₁-Receptor

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Abbreviations used: cpd, compound; eff, effective; exp, experimental; gp, guinea pig; GPCR, G-protein coupled receptor; h, human; H₁R, histamine H₁ receptor; QSAR, quantitative structure activity relationships; $\Delta C_p^{\circ}(p,T)$, heat capacity of binding; $\Delta G^{\circ}(p,T)$, Gibbs free energy of binding; $\Delta H^{\circ}(p,T)$, enthalpy of binding; $\Delta S^{\circ}(p,T)$, entropy of binding; or, orientation; or1, orientation 1; or2, orientation 2; TM, transmembrane domain

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Abstract

For several GPCRs discrimination between agonism and antagonism is possible on basis of thermodynamics parameters, like binding enthalpy and entropy. In this study we analyze if agonists and antagonists can also be discriminated thermodynamically at the histamine H₁-receptor (H₁R). Since previous studies revealed species-differences in pharmacology between human H₁R (hH₁R) and guinea pig H₁R (gpH₁R), we analyzed a broad spectrum of H₁R antagonists and agonists at hH₁R and gpH₁R. [³H]Mepyramine competition binding assay were performed at five different temperatures in a range from 283.15 K to 303.15 K. Additionally, we performed a temperature-dependent 3 dimensional quantitative structure activity relationship (3D-QSAR) study in order to predict binding enthalpy and entropy for histaprodiven derivatives, which can bind to H₁R in two different orientations. Our studies revealed significant species-differences in binding enthalpy and entropy between hH₁R and gpH₁R for some antagonists and agonists. Furthermore, in some cases we found changes in heat capacity of the binding process, different from zero. Differences in flexibility of the ligands may be responsible for this observation. For most ligands, the binding process to hH₁R and gpH₁R is clearly entropy-driven. In contrast, for the endogenous ligand histamine, the binding process is significantly enthalpy-driven at both species isoforms. Thus, a definite discrimination between antagonism and agonism based on thermodynamic parameters is neither possible for hH₁R, nor for gpH₁R, but thermodynamic analysis of ligand-binding may be a novel approach to dissect agonist- and antagonist-specific receptor conformations.

Introduction

The histamine H₁ receptor (H₁R) is a biogenic amine receptor that belongs to the G-protein-coupled receptors (GPCRs) and couples to G_q-proteins (Hill et al., 1997). H₁R antagonists (Fig. 1) have relevance in treatment of allergic diseases. In contrast, H₁R agonists (Fig. 1, **9 - 22**) are used as tools to study the pharmacology and functionality of the H₁R on molecular level. Several classes of synthetic H₁R agonists are known, like phenylhistamines (Zingel et al., 1995; Leschke et al., 1995), histaprodifens (Elz et al., 2000; Menghin et al., 2003) and phenoprodifens (Straßer et al., 2008b).

Weiland et al. (1979) were the first to find that antagonists and agonists can be thermodynamically discriminated at the β -adrenergic receptor. Subsequently, several radioligand binding studies at the cholecystokinin CCK₂ receptor (Harper et al., 2007b), β -adrenergic receptor (Weiland et al., 1979; Contreras et al., 1986a,b), serotonin 5-HT_{1A}-receptor (Dalpiaz et al., 1996), histamine H₃-receptor (Harper et al., 2007a), dopamine D₂-receptor (Kilpatrick et al., 1986; Duarte et al., 1988) and adenosine A₁- and A_{2A}-receptor (Borea et al., 1996) were performed at different temperatures to determine thermodynamic parameters like binding enthalpy ΔH° and binding entropy ΔS° of the ligand to the receptor. These studies showed that a thermodynamic discrimination between agonism and antagonism is only possible at some of the receptors, e. g. β -adrenergic receptor, 5-HT₃-, A₁- and A_{2A}-receptors. However, for some receptors, like the D₂- (Kilpatrick et al., 1986; Duarte et al., 1988) and 5-HT_{1A}- (Dalpiaz et al., 1996) receptor, no thermodynamic discrimination between agonists and antagonists was found.

Previous studies from our laboratory (Seifert et al., 2003; Straßer et al., 2008) revealed substantial species-differences in affinity between hH₁R and gpH₁R for some H₁R agonists, like suprahistaprodifen **19**, phenoprodifen **20** and dimeric histaprodifen **22** and for the first-generation H₁R antagonist mepyramine **1**. Several mutational studies identified amino acids

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interacting with antagonists in the H₁R binding pocket (Wieland et al., 1999) and amino acids being responsible for species-differences between hH₁R and gpH₁R (Bruysters et al., 2005; Straßer et al., 2008b) concerning agonists. Since there has been no thermodynamic study of ligand binding to the H₁R, the aim of this study was to analyze the following questions. i) Is there a thermodynamic discrimination between agonists and antagonists at hH₁R and gpH₁R? and ii) Are there species-differences in binding enthalpy ΔH° and binding entropy ΔS° between hH₁R and gpH₁R?

In order to answer those two questions we co-expressed hH₁R or gpH₁R with the regulator of G-protein signalling, RGS4, in Sf9 insect cells and characterized a broad spectrum of H₁R agonists, like phenylhistamines, histaprodifens and phenoprodifens, as well as H₁R antagonists, like flexible and tricyclic antagonists in [³H]mepyramine competition binding assays at five different temperatures in a temperature range from 283.15 K to 303.15 K. Additionally, we did not use the van't Hoff equation to determine the binding enthalpy and entropy, as it is usually the case. Instead we used a more general thermodynamic equation, which also takes into account that binding enthalpy and entropy could be temperature-dependent.

Since we included ligands, like suprahistaprodifen **19**, phenopdifen **20**, and chiral phenoprodifens **21R**, **21S**, which are predicted to bind in two different orientations to the H₁R (Straßer et al., 2008a, 2009), we additionally performed a temperature-dependent 3D-QSAR analysis in order to predict thermodynamic properties for orientation 1 and orientation 2.

Materials and Methods

Materials. [³H]mepyramine (30.0 Ci/mmol) was from PerkinElmer (Waltham, MA). Rotiszint ecoplus scintillation cocktail was from Roth (Karlsruhe, Germany). Compounds **1 - 8** were from Sigma. Compound **14** was synthesized as described (Menghin, 2004). Sources of all other materials were described earlier (Seifert et al., 2003; Straßer et al., 2008a, b, 2009).

Preparation of compound stock solutions. All chemical structures of the analyzed compounds are given in Fig. 1. Compounds **1, 2, 9, 10, 11R and 11S** (10 mM each) were dissolved in double-distilled water. Compounds **3 - 8** (1 mM each) and **12** (5 mM) were dissolved in a solvent containing 50 % (v/v) DMSO and 50 % (v/v) double-distilled water. Compounds **13, 15 – 21** (5mM each) were dissolved in a solvent containing 30% (v/v) DMSO, 30% (v/v) Tris/HCl pH 7.4 (10 mM) and 40% (v/v) double-distilled water. Compounds **14** and **22** (1 mM) were dissolved in 50% (v/v) DMSO and 50% (v/v) Tris/HCl pH 7.4 (10 mM). The final DMSO concentration in all assays was adjusted to 3% (v/v) or 5% (v/v) as appropriate for a given compound.

Competition binding Assays. The [³H]mepyramine competition binding assays were performed as described (Straßer et al., 2008a). The assays were carried out two to four times at each temperatures of 283.15 K, 288.15 K, 293.15 K, 298.15 K, 303.15 K and for some compounds at a temperature of 308.15 K. The temperature was held constant to ± 0.1 °C, using a water thermostat. To guarantee for equilibrium we carried out preliminary experiments at different incubation times for all ligands at all temperatures. Based on these data we determined incubation times of 4 h at 283.15 K, 3.5 h at 288.15 K, 1.5 h at 293.15 K and 298.15 K and 1 h at 303.15 K to ensure equilibrium conditions.

Binding mode of dimeric histaprodiven 22. Dimeric histaprodiven **22** in its bioactive conformation at hH₁R and gpH₁R, resulting of molecular dynamics simulations (Straßer et al., 2008b), was used as template structure for the molecular alignment of all other ligands in the 3D-QSAR study: The quaternary amine moiety, located in the center of dimeric histaprodiven establishes an electrostatic interaction to Asp^{3.32} and Tyr^{7.43}. One imidazole moiety forms stable hydrogen bonds to Ser^{3.36} and Tyr^{6.51}, and the second imidazole moiety forms stable hydrogen bonds to Glu190 (E2-loop) and Trp^{7.40}. There were no differences in hydrogen bond networking of dimeric histaprodiven **22** with hH₁R and gpH₁R. Both diphenyl propyl moieties of **22** are embedded in hydrophobic pockets.

3D-QSAR. The 3D-QSAR calculations concerning histamine **9**, phenylhistamines, **10** - **12**, histaprodivens **13** – **19**, **22** and phenoprodivens **20**, **21R** and **21S** were performed with the software package SYBYL7.0 (Tripos, St. Louis, MO). Into the training set we included all ligands with only one possible orientation in the binding-pocket, i. e. compounds **9** – **18** and **22**. Into the test sets we included all ligands with two possible orientations, i. e. compounds **19**, **20**, **21R** and **21S**. The classification of the ligands in a group with one possible orientation and a group with two possible orientations is based on the following considerations:

Suprahistaprodiven **19** is a substructure of dimeric histaprodiven **22**, and should, therefore, be able to bind in two different orientations. Dimeric phenylhistamine **12** and dimeric histaprodiven **22** bind to the H₁R and thus, phenoprodivens **20**, **21R** and **21S**, containing a phenylhistamine and histaprodiven partial structure should be able to bind in two different orientations into the binding-pocket of the H₁R. Besides, both orientations of **20** led to stable ligand-receptor complexes in molecular dynamics simulations (Straßer et al., 2009). This considerations concerning symmetry of the ligands are not valid for ligands **15** – **18**.

Accordingly, these ligands are expected to bind in only one orientation. We used dimeric histaprodiven **22** in its bioactive conformation at hH₁R and gpH₁R, resulting of molecular

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dynamics simulations, as template structure for the molecular alignment of all other ligands (Straßer et al., 2008b). All other ligands were constructed with SYBYL7.0, except for histamine **9**. Since the binding mode of histamine **9** differs from the binding mode of the histamine partial structures in phenylhistamines and histaprodifens, histamine was docked manually in its bioactive conformation as described (Jongejan et al., 2005) to hH₁R and gpH₁R using SYBYL7.0. All ligands have one positive charge on the amine moiety. For energetic calculations and minimizations we used the Tripos-Force-Field and Gasteiger-Hückel partial charges. The alignment was performed with SYBYL7.0 using the MultiFit tool. All compounds which can bind in two different orientations in the binding-pocket were aligned in both orientations. This alignment was used for the subsequent comparative molecular field analysis (CoMFA) study for each temperature at hH₁R and gpH₁R. The relationship between the ligand structure and the affinities was quantified by the partial least-square (PLS) algorithm (Wold et al., 1993). The cross-validation analysis was performed using the leave-one-out method (LOO). In most cases of the single 3D-QSAR studies at each temperature for hH₁R and gpH₁R, the cross-validated r^2 resulted in an optimum number of 6 components for lowest standard error of prediction. Thus, to obtain comparable results for each 3D-QSAR study, we used 6 components for the subsequent PLS analysis. The predicted pK_i values were used to calculate the thermodynamic parameters $\Delta H^0(p, T_o)$, $\Delta S^0(p, T_o)$ and ΔC_p^0 as described in the next paragraph.

Calculation of thermodynamic parameters. For the ligand-receptor binding equilibrium $L + R \rightleftharpoons LR$ (L = ligand, R = receptor, LR = ligand-receptor-complex) the affinity constants are calculated as

$$K_A = \frac{[LR]}{([L][R])} \quad (1)$$

Ligand-receptor binding at constant temperature T and pressure p is accompanied by a decrease of the Gibbs energy of the system, comprised of the solvent, the free ligand and membrane-bound receptor and ligand-receptor-complex, until equilibrium is reached. The resulting equilibrium constant for the association process K_A is related to the change of Gibbs energy of reference state (infinite dilution for each reactant):

$$\Delta G^\circ(p, T) = -RT \ln(K_A) \quad (2)$$

The binding enthalpy ΔH° and binding entropy ΔS° are related to ΔG° in the following way:

$$\Delta G^\circ(p, T) = \Delta H^\circ(p, T) - T\Delta S^\circ(p, T) \quad (3)$$

The binding enthalpy $\Delta H^\circ(p, T)$ and binding entropy $\Delta S^\circ(p, T)$ are related to enthalpies and entropies of ligand H_L, S_L , of receptor H_R, S_R and of ligand-receptor-complex H_{LR}, S_{LR} , respectively in the following way:

$$\Delta H^\circ(p, T_o) = H_{LR}^\circ - H_L^\circ - H_R^\circ \quad (4)$$

and

$$\Delta S^\circ(p, T_o) = S_{LR}^\circ - S_L^\circ - S_R^\circ \quad (5)$$

In the case of temperature-independent binding enthalpy and binding entropy change, these quantities can be calculated by using the van't Hoff equation:

$$\ln(K_A) = \frac{-\Delta H^\circ(p, T)}{RT} + \frac{\Delta S^\circ(p, T)}{R} \quad (6)$$

In the case of temperature-dependent ΔH° and ΔS° we used the following fundamental thermodynamic relations for calculation of these quantities:

$$\left(\frac{\partial \Delta H^\circ(p, T)}{\partial T} \right)_p = \Delta C_p^\circ(p, T) \quad (7)$$

and

$$\left(\frac{\partial \Delta S^\circ(p, T)}{\partial T} \right)_p = \frac{\Delta C_p^\circ(p, T)}{T}, \quad (8)$$

where ΔC_p° represents the change in the reference molar heat capacity of the given reaction. Considering this quantity as temperature-independent as a first approximation we yield the following relations for $\Delta H^\circ(p,T)$ and $\Delta S^\circ(p,T)$ by integration:

$$\Delta H^\circ(p,T) = \Delta H^\circ(p,T_o) + \Delta C_p^\circ(p,T_o) \cdot (T - T_o) \quad (9)$$

and

$$\Delta S^\circ(p,T) = \Delta S^\circ(p,T_o) + \Delta C_p^\circ(p,T_o) \cdot \ln\left(\frac{T}{T_o}\right) \quad (10)$$

with the reference temperature $T_o = 293.15$ K.

Using equations (3), (9) and (10), the following expression for $\Delta G^\circ(p,T)$ can be derived:

$$\Delta G^\circ(p,T) = \Delta H^\circ(p,T_o) + \Delta C_p^\circ(p,T_o) \cdot (T - T_o) - T \cdot \left(\Delta S^\circ(p,T_o) + \Delta C_p^\circ(p,T_o) \cdot \ln\left(\frac{T}{T_o}\right) \right) \quad (11)$$

Using the experimentally determined pK_i values at a pressure p of 101325 Pa and different temperatures the quantities $\Delta H^\circ(p,T_o)$, $\Delta S^\circ(p,T_o)$ and $\Delta C_p^\circ(p,T_o)$ can be calculated based on the last equation by a linear least-square algorithm.

Miscellaneous. The same generation of baculoviruses was used as described earlier (Seifert et al., 2003; Straßer et al., 2008). Cell culture, membrane preparation and determination of protein concentration were performed as described previously (Kelley et al., 2001; Seifert et al., 2003; Straßer et al., 2008a). For data analysis, the software Prism 4.02 (GraphPad, San Diego, CA) was used. pK_i values were calculated according to Cheng and Prusoff (1973). All pK_i values are the means \pm S.E.M. of two to four independent experiments. For comparison of two pairs of data, the significance of the deviation of zero p was calculated using the t test.

Results

Ligand- and receptor-specific differences in temperature dependence of pK_i values. The pK_i values determined for hH₁R and gpH₁R at five temperatures in the range between 283.15 K and 303.15 K (308.15 K for **1**, **4**, **5**, and **7**) are summarized in Table 1 and plotted in Fig. 2. pK_i values exhibited ligand- and receptor-specific differences in temperature-dependence. The H₁R antagonists **1** – **8** showed high affinity to hH₁R and gpH₁R within the analyzed temperature range from 283.15 K to 303.15 (308.15 for **1**, **4**, **5** and **7**). Mepyramine **1**, diphenhydramine **2** and chlorpromazine **8** showed no significant temperature-dependence in affinity. However, some ligands showed a substantial temperature-dependence in affinity, like fexofenadine **3**, terfenadine **4** and clozapine **7**. For clozapine **7** (Fig. 3), the increase in affinity from 283.15 K to 308.15 K was about one unit in pK_i. Most of the ligands showed no species-dependent differences in temperature-dependence of affinities. Nonetheless, for the rigid tricyclic ligands amitriptyline **5**, mianserine **6** and clozapine **7**, the temperature-dependence of pK_i values was larger at gpH₁R than at hH₁R. For these ligands a temperature-dependent selectivity switch between hH₁R and gpH₁R was observed. At 283.15 K the ligands **5** - **7** exhibited higher affinity to hH₁R than to gpH₁R, but at 303.15 K (308.15 K for **5**), the ligands exhibited higher affinity to gpH₁R than to hH₁R. This was most pronounced for amitriptyline **5** (Fig. 4). At temperatures below 295 K, amitriptyline **5** showed higher affinity at hH₁R than at gpH₁R, at a temperature of about 295 K there were no differences in affinity between hH₁R and gpH₁R, but at temperatures above 295 K amitriptyline **5** showed higher affinity at gpH₁R than at hH₁R.

The affinities of histamine, phenylhistamines, histaprodifens and chiraprodifens were in a pK_i range from ~ 4 - 7. In general, these ligands **10** – **22** showed no temperature-dependence in affinity or increasing affinity with increasing temperature, except for histamine **9**. The affinity of histamine **9** decreased slightly with increasing temperature (Fig. 2). For the ligands **9** – **11**, **13** – **18** and **21R** no species-differences between hH₁R and gpH₁R at the

different temperatures were found. Nonetheless, there were species-differences at several temperatures for compounds **12**, **19**, **20**, **21S** and **22**. These species-differences were not significantly temperature dependent.

Ligand- and receptor-specific differences in thermodynamic parameters of ligand binding. All thermodynamic parameters were calculated with Prism using equation 11 and are given in Table 2. Enthalpy ranged from ~ -35 kJ/mol - 80 kJ/mol at hH₁R and from ~ -25 kJ/mol - 100 kJ/mol at gpH₁R. Entropy ranged from ~ -20 J/K/mol - 410 J/K/mol at hH₁R and from ~ 20 J/K/mol - 500 J/K/mol at gpH₁R. The data show that the enthalpy for formation of the ligand-receptor complex at gpH₁R is nearly equal or up to 40 kJ/mol higher than at hH₁R. Additionally, the entropy for formation of the ligand-receptor complex at gpH₁R was nearly equal or up to 135 J/K/mol higher than at hH₁R. This implies that $-T\Delta S^\circ$ at gpH₁R is nearly equal or up to -40kJ/mol smaller than at hH₁R at a temperature of 293.15 K. A correlation between ΔH° and $-T\Delta S^\circ$ (Fig. 5) shows that, in general, the formation of the ligand-receptor complex is entropy-driven at hH₁R and gpH₁R. Interestingly, at hH₁R, the formation of some ligand-receptor complexes was not only entropy-driven, but also slightly enthalpy-driven. A completely different behaviour with regard to the mentioned trend was observed for histamine **9**. At hH₁R and gpH₁R, the formation of the histamine-receptor complex is enthalpy-driven, but not or only slightly entropy-driven at a temperature of 293.15 K. Thus, only for the endogenous ligand histamine **9** the formation of the ligand-receptor complex is significantly enthalpy-driven at 293.15 K. As Fig. 5 shows, for hH₁R and gpH₁R it is not possible to discriminate between agonism and antagonism based on thermodynamical data. The antagonists fexofenadine **3**, terfenadine **4**, mianserine **6** and clozapine **7** showed very high $\Delta H^\circ(p,T_0)$ and $\Delta S^\circ(p,T_0)$ at hH₁R and gpH₁R, outside the region, in which most of the ligands located. This may be explained with specific solvent effects for **3** and **4** or the ligand rigidity for **6** and **7**.

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As shown in Table 2, ΔC_p° -values ranged between -5 kJ/mol/K to 5 kJ/mol/K and are different from zero for some ligand-receptor-systems. Consequently, the enthalpy and entropy of formation of the ligand-receptor complex is temperature-dependent for these systems, e. g. for the formation of the dimeric histaprodifen **22** – gpH₁R receptor complex. If ΔC_p° is zero, ΔH° and ΔS° are independent of temperature, resulting in a linear temperature dependence for ΔG° . However, as can be seen in Fig. 6, ΔG° was not linearly dependent on temperature for dimeric histaprodifen **22** at gpH₁R, indicating a ΔC_p° different from zero. However, it should be noted, that for many compounds the errors in ΔC_p° values are in the same range as the ΔC_p° values themselves. This is a consequence of a non attainable precision in measurement of pK_i values. Nonetheless, we used equation 11 for data analysis, since the van't Hoff equation is only a special case of equation 11. Being aware of this fact, the data, summarized in Table 2 show the trend that ΔC_p° could be higher at gpH₁R, than at hH₁R. Hence, it may be concluded, that either enthalpy or entropy of the free gpH₁R or gpH₁R-ligand-complex are more temperature-dependent than the corresponding parameters at hH₁R. For the D₂-receptor, temperature-dependence of binding enthalpy and entropy (Duarte et al., 1988; Kilpatrick et al., 1986) was described for some cases. At the D₂-receptor ΔC_p° values are ranged between -3 kJ/mol/K up to 3 kJ/mol/K. A similar phenomenon was also described for the A₁-receptor (Casado et al., 1983; Borea et al., 1992) with values ΔC_p° of about -10 kJ/mol and 10 kJ/mol at 293.15 K. Thus, it can be concluded that the heat capacity ΔC_p° should be included into the thermodynamic analysis of ligand-receptor binding in general.

We also calculated ΔH° and $-T\Delta S^\circ$ at 283.15 K, 288.15 K, 298.15 K and 303.15 K. In the temperature range from 283.15 K up to 303.15 K, for some ligands, the formation of the ligand-receptor complex switched from enthalpy-driven to entropy-driven and *vice versa*. For histamine **9**, this switch was species-dependent (Fig. 7).

3D-QSAR. The 3D-QSAR calculations were performed in order to predict the pK_i values of different orientations on the one hand. On the other hand, the thermodynamic properties of the ligands should be predicted, especially the contribution of orientation 1 and 2 onto the thermodynamic properties. The alignment of the ligands, docked in gpH₁R is shown in Fig. 8. For each 3D-QSAR analysis the standard error of estimate was smaller than 0.05 in pK_i , r^2 was larger than 0.997 in all 3D-QSAR calculations. The predicted pK_i values for compounds **19** – **21** for orientation 1 and 2 are given in Table 3 at each temperature. Considering the pK_i values for orientation 1 and 2, the effective pK_i values are calculated, without any implications about the contribution of orientation 1 or 2. The comparison of predicted, effective pK_i values with the experimental data shows deviations smaller than about 0.6 in pK_i .

The predicted thermodynamic parameters for compounds **19**, **20**, **21R** and **21S** for each orientation are given in Table 4. The prediction of the effective values, including orientations 1 and 2, based on the predicted pK_i values, corresponds well to the experimental data for $\Delta H^{\circ}(p, T_o)$, $\Delta S^{\circ}(p, T_o)$ and $\Delta G^{\circ}(p, T_o)$. The prediction of ΔC_p° is not as good, as for the other thermodynamic data, but is in range of the experimental errors.

Species-differences in enthalpy and entropy of the ligand-receptor binding process. At the reference temperature of 293.15 K, we observed, in general, the trend, that $\Delta H^{\circ}(p, T_o)$ at gpH₁R is equal or higher than at hH₁R. In contrast, $-T\Delta S^{\circ}(p, T_o)$ at gpH₁R is equal or smaller than at hH₁R. Consequently we conclude, that the loss in enthalpy at gpH₁R compared to hH₁R is compensated by an increase in entropy. These species-differences are ligand-dependent and are illustrated in Fig. 9. The data shown in Fig. 9 correspond to the equations

$$\Delta\Delta H^{\circ}(p, T_o)_{gp-h} = \Delta H^{\circ}(p, T_o)_{gp} - \Delta H^{\circ}(p, T_o)_h = H_{(gp-L)}^{\circ} - H_{(h-L)}^{\circ} - H_{gp}^{\circ} + H_h^{\circ} \quad (12)$$

and

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$$\Delta\Delta S^o(p, T_o)_{gp-h} = \Delta S^o(p, T_o)_{gp} - \Delta S^o(p, T_o)_h = S^o_{(gp-L)} - S^o_{(h-L)} - S^o_{gp} + S^o_h. \quad (13)$$

Thus, these data include the differences in enthalpies or entropies of ligand-receptor-complexes and the free receptors for hH₁R and gpH₁R, but not enthalpies or entropies for the free ligand. Therefore, the quantities $\Delta\Delta H^o(p, T_o)_{gp-h}$ and $-T_o\Delta\Delta S^o(p, T_o)_{gp-h}$ are ligand-independent, but relative to each other dependent on the ligand-receptor-complex.

For mianserine **6**, the largest species-difference is observed (Fig. 9, group I). In the next group (Fig. 9, group II) are the tricyclic compounds amitryptilline **5**, clozapine **7** and chlorpromazine **8** as well as the histaprodifens, which can bind in two different orientations into the binding pocket, like suprahistaprodifen **19**, phenoprodifen **20** and the chiral phenoprodifens **21R** and **21S**. In group III (Fig. 9), ligands with enthalpic species-differences in a range from -9 kJ/mol to -16 kJ/mol and entropic species-differences in a range from -9 kJ/mol to -19 kJ/mol are found. These ligands include histamine **9**, phenylhistamine **10**, dimeric phenylhistamine **11**, histaprodifen **13**, and the histaprodifen derivatives **15** - **17** and **22**. The last group (Fig. 9, group IV), with smaller enthalpic and entropic species-differences in a range from -5 to 5 kJ/mol the flexible antagonists **1**, **2**, **3**, **4**, the chiral phenylhistamines **11R**, **11S**, the histaprodifen derivatives **14** and **18** are found. The location of the effective enthalpic and entropic species-differences between hH₁R and gpH₁R for **19**, **20**, **21R** and **21S** far away from the other phenylhistamines and histaprodifens is *a priori* unexpected. However the 3D-QSAR prediction with regard to enthalpy and entropy of these ligands for orientation 1 and orientation 2 shows a shift away from the non-flexible antagonists (group II) in direction to phenylhistamines and histaprodifens (Fig. 9). For **19**, **20**, **21R** and **21S**, orientation 1 is found in the same group (group III), as histaprodifen and most histaprodifen derivatives. In contrast, for **19** and **20**, orientation 2 is found in the same range (group III) as histamine **9** and phenylhistamine **10**, but for **21R** and **21S**, respectively, orientation 2 is found in the same range (group IV) as the chiral phenylhistamines **11R** and **11S**.

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The separation of the effective $\Delta\Delta H^\circ(p, T_o)_{gp-h}$ and $-T\Delta\Delta S^\circ(p, T_o)_{gp-h}$ into the predicted data for orientation 1 and 2 (Fig. 9) shows, that for **19**, **20**, **21R** and **21S** the quantities for orientation 1 are found in group III. In orientation 1, the histaprodifen moiety of **19**, **20**, **21R** and **21S** are located in the same part of the binding-pocket as histaprodifen **13** itself. In group III, **13** and dimeric histaprodifen **22** are found, too. In contrast, for **19**, **20**, **21R** and **21S** the quantities for orientation 2 are found in group III only for **19** and **20**, but in group IV for **21R** and **21S**. In orientation 2, the histaprodifen moiety of **19**, **20**, **21R** and **21S** is located not in the histaprodifen binding-pocket itself, but in a pocket possessed by the second histaprodifen moiety of **22**, located near to TM2. The histamine moiety of **19** and phenylhistamine moieties of **20**, **21R** and **21S** in orientation 2 are located in the binding-pocket of histamine **9** and phenylhistamines **10**, **11R** and **11S** itself. **9** and **10** are located in group III, as well as orientation 2 of compounds **19** and **20**. Additionally, the chiral phenylhistamines **11R** and **11S** belong to group IV, as well as orientation 2 of compounds **21R** and **21S**. Furthermore, the data suggest that there is no change in species difference of $\Delta\Delta H^\circ(p, T_o)_{gp-h}$ and $-T\Delta\Delta S^\circ(p, T_o)_{gp-h}$ in case of introduction of a second phenylhistamine moiety into **10**, resulting in **12**, or in case of introduction of a second histaprodifen moiety into **13**, resulting in **22**. Based on these data we suggest that the part of the entire binding-pocket of phenylhistamine **10** and histaprodifen **13**, located near to TM5 is responsible for the membership of **19**, **20**, **21R** and **21S** (Straßer et al., 2009) dependent from orientation to group III or group IV.

Discussion

Thermodynamic discrimination between antagonism and agonism. Previous studies showed that it is possible to discriminate between agonism and antagonism based on binding enthalpy and binding entropy for some GPCRs. Discrimination was found for the β -adrenergic receptor and the A_1 - and A_{2A} -receptor. The formation of ligand-receptor-complexes is enthalpy-driven for agonists and enthalpy- and entropy-driven for antagonists at β -adrenergic receptor (Weiland et al., 1979; Contreras et al., 1986). In contrast, the formation of ligand-receptor-complexes is entropy-driven for agonists and enthalpy- and entropy-driven for antagonists at A_1 - (Dalpiaz et al., 1999) and A_{2A} -receptors (Borea et al., 1995). For the D_2 - (Duarte et al., 1988) and 5-HT_{1A} -receptor (Dalpiaz et al., 1996) discrimination between agonism and antagonism based on ΔH° and ΔS° is not possible. A study on the H_3 -receptor (Harper and Black, 2007a) showed that discrimination between agonism and antagonism depends of the experimentally used buffer. ΔH° is found in a range from about -225 kJ/mol - 100 kJ/mol, ΔS° is in a range from \sim -600 J/mol/K - \sim 450 J/mol/K for GPCRs (Borea et al., 2000).

To our best knowledge we are the first to study the binding thermodynamics of the H_1 -receptor, including H_1R antagonists and agonists with large structural differences. At 293.15 K, $\Delta H^\circ(p, T_o)$ ranges from \sim -40 kJ/mol - \sim 90 kJ/mol, and $\Delta S^\circ(p, T_o)$ ranges from \sim -20 J/mol/K - \sim 500 J/mol/K. These findings for the H_1R are in very good accordance to the findings for other GPCRs. The correlation between $\Delta H^\circ(p, T_o)$ and $-T\Delta S^\circ(p, T_o)$ for hH_1R and gpH_1R (Fig. 5) shows that discrimination between agonism and antagonism based on binding enthalpy and entropy is not possible for hH_1R and gpH_1R . Thus, the H_1R is more related to the D_2 - and 5-HT_{1A} -receptor than to β -adrenergic or adenosine receptors, concerning thermodynamic discrimination between agonism and antagonism. To obtain a more detailed insight in thermodynamic agonist – antagonist discrimination, even more compounds with high structural diversity have to be studied at several GPCRs.

For the process of ligand-binding to a receptor including receptor activation in case of agonists several subprocesses have to be considered: i) Disruption of ligand-solvent-complex, ii) formation of ligand-receptor-complex, iii) conformational change of the ligand, iv) disruption of hydrogen-bonds in the receptor and formation of new hydrogen-bonds and, v) change in receptor conformation. Thus, the energetic contribution of these subprocesses leads to the effective change in binding free energy, binding enthalpy and binding entropy, which can be determined experimentally. Because of the complexity of this process we propose, that in dependence of ligand structure, but independent of agonistic or antagonistic function of the ligand, some of the subprocesses i) to v) energetically cancel each other out or intensify each other. Thus, for a thermodynamic study of ligand-receptor interactions, a broad structural variety within the agonists and antagonists has to be guaranteed. Since changes in heat capacity for formation of ligand-receptor processes different from zero were observed, it should also be taken into account that an entropy-driven binding process can change to an enthalpy-driven binding process and *vice versa* with increasing temperature. Thus, the discrimination between agonism and antagonism may be a function of temperature.

Thermodynamic measurements – a new approach for the dissection of ligand-specific receptor conformations? A number of experimental data, based on fluorescence spectroscopy (Ghanouni et al., 2001) suggest the existence of agonist-dependent receptor conformations. Within the series of analyzed compounds in this study our data suggest that the endogenous ligand histamine stabilizes the ligand-receptor-complex enthalpical, in contrast to the other analyzed agonists, where no enthalpic stabilization is observed (Fig. 5). Further studies, including more natural or synthetic compounds have to be carried out in order to improve our knowledge about compounds being enthalpically favored in binding to the H₁R. One reason for the different behaviour of histamine in contrast to phenylhistamines and histaprodifens, concerning the enthalpy may be the different binding-mode of histamine in

comparison to phenylhistamines and histaprodifens (Fig. 8). It was shown experimentally, that Lys^{5.39} forms a hydrogen-bond to the imidazole moiety of histamine (Jongejan and Leurs, 2005). In contrast, further experimental data show that an interaction between Lys^{5.39} and histaprodifens is not established (Jongejan and Leurs, 2005). Additionally, Gln^{5.46} interacts with histamine but not with phenylhistamine (Leurs et al., 1994). If there is a compensation of the loss of enthalpy of solvation of histamine by a benefit of enthalpy of histamine-receptor-interaction, the data suggest a receptor conformation specifically stabilized by histamine. Additionally it has to be considered, that histamine, acting as full agonist, should promote a change of receptor conformation from inactive to active state. Thus, it may be speculated, that $\Delta H^{\circ}(p, T_o)$ mainly includes the enthalpy related to changes in receptor conformation. These results are in good accordance with the description of agonist-dependent receptor conformations (Xie et al., 2006; Kobilka et al., 2007).

The data (Fig. 9) suggest that the species-differences in $\Delta\Delta H^{\circ}(p, T_o)_{gp-h}$ and $\Delta\Delta S^{\circ}(p, T_o)_{gp-h}$ are closely related to flexibility of the ligand and of the receptor. Antagonist **6** is very rigid and belongs to group I with largest species-differences. Antagonists **5**, **7** and **8**, belonging to group II with smaller species-differences, have also a rigid, tricyclic moiety, but additionally, there are flexible side chains. For the highly flexible antagonists **1**, **2**, **3** and **4**, belonging to group IV, no significant-species differences are found. The flexible antagonists are able to undergo conformational changes to fit optimally into the binding-pocket and to establish ligand-receptor interactions. Thus, these antagonists should be able to compensate for differences in receptor-flexibility between hH₁R and gpH₁R, which is not possible for the rigid antagonists. Since $\Delta H^{\circ}(p, T_o)$ is significantly higher at gpH₁R than at hH₁R for the rigid antagonists, it can be supposed that hH₁R is more flexible than gpH₁R, resulting in a better ligand-receptor interaction at hH₁R. Additionally, it should be considered, that it is proposed that antagonists do not induce a change of the receptor conformation from the inactive state to the active state. Thus, only the loss of enthalpy of solvation of the ligand and the benefit of

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ligand-receptor interactions should contribute to $\Delta H^{\circ}(p, T_0)$. Since there are no differences in amino acids, interacting with antagonists in the binding-pocket, between hH₁R and gpH₁R, there should be no differences in binding enthalpies between hH₁R and gpH₁R. This was observed for the flexible antagonists. Due to their flexibility they can undergo conformational changes to fit optimally into the binding-pocket of hH₁R and gpH₁R. Thus, there is no necessity for the receptor to change its conformation. In contrast, we observed species differences in binding enthalpy between hH₁R and gpH₁R in the range of ~25 – 40 kJ/mol for the rigid antagonists. Since there are no obvious differences in the binding-pocket between hH₁R and gpH₁R this result is surprising. Thus, these results may be explained by differences in flexibility of the receptor or compactness of the receptor structure, caused by changes of H-bonding network of the receptor, due to the differences in amino acid sequences between hH₁R and gpH₁R (Straßer et al., 2008b), especially concerning N-terminus and E2-loop. Another possible explanation for the observed species differences of the rigid antagonists may be antagonist-dependent receptor conformations in the inactive state. Our data, based on thermodynamic measurements suggest that different receptor conformations not only exist for the active state, but also for the inactive state of the receptor. The species-differences observed for the flexible agonists may be explained in contrast to the antagonists not with flexibility of the ligands, but with species-dependent conformational changes of the receptor, due to its activation by the agonists. The division of the agonists in two groups (Fig. 9) may indicate that there exist agonist-dependent receptor conformations.

Conclusions. In our study we have analyzed the binding of a large variety of antagonists and agonists to the hH₁R and gpH₁R thermodynamically. Our data suggest species-differences between hH₁R and gpH₁R are observed not only in affinity, but also in $\Delta H^{\circ}(p, T)$ and $\Delta S^{\circ}(p, T)$. Additionally, our study shows that, in contrast to some other GPCRs, antagonists and agonists cannot be distinguished thermodynamically for the H₁R. But our data

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may suggest that there are not only different conformations of the active state of the receptor, but also of the inactive state for the H₁R.

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Footnotes

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Legends for Figures

Fig. 1. Chemical structures of H₁R antagonists 1 - 8 and H₁R agonists. Mepyramine **1**, diphenhydramine **2**, fexofendadine **3**, terfenadine **4**, amitriptyline **5**, mianserine **6**, clozapine **7**, chlorpromazine **8**, histamine **9**, phenylhistamines **10 – 12**, histaprodifens **13 – 18**, suprahistaprodifen **19**, phenoprodifens **20 – 21** and dimeric histaprodifen **22**.

Fig. 2. pK_i values as function of temperature for ligands 1 - 22 at hH₁R and gpH₁R.

[³H]Mepyramine competition binding assays in Sf9 membranes expressing hH₁R or gpH₁R in combination with RGS4 were performed at different temperatures in presence of 5 nM [³H]mepyramine as described under *Materials and Methods*. Data were analyzed by non-linear regression and were best fit to one-site (monophasic) competition curves. pK_i values were calculated according to Cheng and Prusoff (1973). Data shown are the means ± SEM of at least two to four experiments with independent membrane preparations in duplicates each.

Fig. 3. Competition binding isotherms for clozapine 7 at various temperatures at hH₁R.

The experiments were performed using Sf9 cell membranes expressing hH₁R and RGS4 in presence of 5 nM [³H]mepyramine ([³H]MEP) as described under *Materials and Methods*. Data were analyzed by nonlinear regression and were best fit to one-site (monophasic) competition curves.

Fig. 4. Temperature-dependence of pK_i and ΔG⁰(p,T) for amitriptyline 5 at hH₁R and gpH₁R.

a, temperature-dependence of pK_i values for amitriptyline **5** at hH₁R and gpH₁R, based on the data given in Table 2; **b**, temperature-dependence of ΔG⁰(p,T) values for amitriptyline **5** at hH₁R and gpH₁R; the data were calculated using equation 2 as described under *Materials and Methods*.

Fig. 5. Scatter plot of $\Delta H^{\circ}(p, T_o)$ versus $-T\Delta S^{\circ}(p, T_o)$ for H_1R antagonists and agonists.

The data points are given for the temperature 293.15 K and are based on Table 2. Region H: enthalpy-driven, Region S: entropy-driven, Region H and S: enthalpy- and entropy-driven.

Fig. 6. Temperature-dependence of $\Delta G^{\circ}(p, T)$ for dimeric histaprodiven 22 at hH_1R and gpH_1R .

ΔG° values at the different temperatures were calculated using equation 2 as described under *Materials and Methods*, based on the data given in Table 1.

Fig. 7. $\Delta H^{\circ}(p, T)$ and $-T\Delta S^{\circ}(p, T)$ as function of temperature for histamine 9 at hH_1R and gpH_1R .

The quantities $\Delta H^{\circ}(p, T)$ and $-T\Delta S^{\circ}(p, T)$ were calculated based on the data in Table 2, using the equation $\Delta H^{\circ}(p, T) = \Delta H^{\circ}(p, T_o) + \Delta C_p^{\circ}(p, T_o) \cdot (T - T_o)$ for temperature-dependence of $\Delta H^{\circ}(p, T)$ and equation $\Delta S^{\circ}(p, T) = \Delta S^{\circ}(p, T_o) + \Delta C_p^{\circ}(p, T_o) \cdot \ln(T/T_o)$ for temperature-dependence of $-T\Delta S^{\circ}(p, T)$.

Fig. 8. Alignment of histamine, phenylhistamines, histaprodivens and phenoprodivens in the active gpH_1R .

The alignment was performed as described under *Materials and Methods*.

Fig. 9. Species differences in $\Delta H^{\circ}(p, T_o)$ and $-T\Delta S^{\circ}(p, T_o)$ between hH_1R and gpH_1R .

The data points are calculated using the equations $\Delta\Delta H^{\circ}(p, T_o)_{gp-h} = \Delta H^{\circ}(p, T_o)_{gp} - \Delta H^{\circ}(p, T_o)_h$ and $-\Delta\Delta S^{\circ}(p, T_o)_{gp-h} = -T\Delta S^{\circ}(p, T_o)_{gp} + T\Delta S^{\circ}(p, T_o)_h$ based on the data given in Table 2. Data points, exclusively based on experimental data are indicated by exp.. The compounds are divided into the four groups in the following manner: I: black box, unfilled: **6**; II: black box, unfilled: **5, 7, 8**; red circle, filled: **19, 20, 21R, 21S**; III: black triangle, filled: **9, 10, 12, 13, 15, 16, 17, 22**; green circle, filled: **19, 20, 21R, 21S**; blue circle, filled: **19, 20**; IV: black triangle, unfilled: **1, 2, 3, 4**; black box, filled: **11R, 11S, 14, 18**; blue circle, filled: **21R, 21S**.

Table 1. pK_i values for H₁-receptor antagonists and agonists at hH₁R and gpH₁R co-expressed with RGS4 in Sf9 cell membranes in the competition binding assays at different temperatures. [³H]Mepyramine competition binding assays in Sf9 membranes expressing hH₁R or gpH₁R in combination with RGS4 were performed at different temperatures in presence of 5 nM [³H]mepyramine as described under *Materials and Methods*. Data were analyzed by non-linear regression and were best fit to one-site (monophasic) competition curves. pK_i values were calculated according to Cheng and Prusoff (1973). Data shown are the means ± SEM of at least two to four experiments with independent membrane preparations in duplicates each.

cpd.	species	283 K	288 K	293 K	298 K	303 K
1 ¹	hH ₁ R	8.33 ± 0.03	8.35 ± 0.03	8.35 ± 0.03	8.33 ± 0.03	8.31 ± 0.03
	gpH ₁ R	8.57 ± 0.02	8.59 ± 0.02	8.58 ± 0.03	8.58 ± 0.03	8.60 ± 0.02
2	hH ₁ R	7.67 ± 0.05	7.67 ± 0.06	7.83 ± 0.03	7.78 ± 0.03	7.95 ± 0.03
	gpH ₁ R	7.52 ± 0.03	7.57 ± 0.07	7.53 ± 0.04	7.66 ± 0.01	7.78 ± 0.01
3	hH ₁ R	6.77 ± 0.07	6.93 ± 0.08	7.23 ± 0.11	7.45 ± 0.07	7.73 ± 0.08
	gpH ₁ R	6.39 ± 0.10	6.51 ± 0.06	6.83 ± 0.04	7.08 ± 0.07	7.42 ± 0.04
4 ¹	hH ₁ R	7.66 ± 0.09	7.76 ± 0.08	8.05 ± 0.01	8.22 ± 0.01	8.41 ± 0.06
	gpH ₁ R	7.39 ± 0.09	7.45 ± 0.09	7.52 ± 0.07	7.96 ± 0.03	8.21 ± 0.11
5 ¹	hH ₁ R	8.78 ± 0.04	8.94 ± 0.01	8.89 ± 0.04	9.04 ± 0.06	8.99 ± 0.04
	gpH ₁ R	8.60 ± 0.02	8.82 ± 0.01	8.86 ± 0.06	9.01 ± 0.04	9.22 ± 0.01
6	hH ₁ R	8.62 ± 0.02	8.67 ± 0.02	8.81 ± 0.05	8.92 ± 0.02	8.99 ± 0.04
	gpH ₁ R	8.52 ± 0.01	8.57 ± 0.05	8.81 ± 0.06	9.08 ± 0.07	9.32 ± 0.06
7 ¹	hH ₁ R	8.01 ± 0.03	8.29 ± 0.09	8.50 ± 0.06	8.87 ± 0.04	8.80 ± 0.08
	gpH ₁ R	7.71 ± 0.02	8.07 ± 0.01	8.25 ± 0.06	8.82 ± 0.08	8.96 ± 0.04
8	hH ₁ R	7.91 ± 0.03	7.89 ± 0.05	7.83 ± 0.03	7.81 ± 0.02	7.91 ± 0.04
	gpH ₁ R	7.93 ± 0.07	7.97 ± 0.01	8.09 ± 0.06	8.16 ± 0.06	8.22 ± 0.01
9	hH ₁ R	5.75 ± 0.10	5.77 ± 0.06	5.66 ± 0.04	5.48 ± 0.02	5.35 ± 0.05
	gpH ₁ R	5.75 ± 0.05	5.58 ± 0.04	5.50 ± 0.03	5.49 ± 0.01	5.42 ± 0.04
10	hH ₁ R	5.36 ± 0.03	5.32 ± 0.01	5.38 ± 0.08	5.48 ± 0.03	5.28 ± 0.05
	gpH ₁ R	5.58 ± 0.10	5.58 ± 0.01	5.63 ± 0.02	5.72 ± 0.02	5.69 ± 0.02
11R	hH ₁ R	4.29 ± 0.01	4.38 ± 0.03	4.50 ± 0.02	4.46 ± 0.06	4.48 ± 0.03
	gpH ₁ R	4.65 ± 0.01	4.70 ± 0.02	4.71 ± 0.03	4.77 ± 0.03	4.87 ± 0.01
11S	hH ₁ R	5.24 ± 0.03	5.20 ± 0.03	5.18 ± 0.03	5.17 ± 0.04	5.16 ± 0.03
	gpH ₁ R	5.50 ± 0.02	5.47 ± 0.02	5.45 ± 0.02	5.42 ± 0.02	5.44 ± 0.01

12	hH ₁ R	5.39 ± 0.04	5.42 ± 0.03	5.45 ± 0.03	5.43 ± 0.02	5.46 ± 0.08
	gpH ₁ R	5.82 ± 0.06	5.87 ± 0.04	5.93 ± 0.03	6.04 ± 0.09	6.06 ± 0.12
13	hH ₁ R	6.50 ± 0.04	6.43 ± 0.04	6.45 ± 0.04	6.43 ± 0.01	6.43 ± 0.03
	gpH ₁ R	6.27 ± 0.04	6.24 ± 0.05	6.37 ± 0.02	6.25 ± 0.04	6.42 ± 0.03
14	hH ₁ R	5.08 ± 0.01	5.15 ± 0.03	5.24 ± 0.02	5.33 ± 0.03	5.39 ± 0.04
	gpH ₁ R	5.00 ± 0.07	5.09 ± 0.03	5.12 ± 0.04	5.23 ± 0.03	5.29 ± 0.01
15	hH ₁ R	5.79 ± 0.03	5.79 ± 0.04	5.76 ± 0.02	5.76 ± 0.02	5.77 ± 0.04
	gpH ₁ R	5.78 ± 0.04	5.81 ± 0.03	5.86 ± 0.04	5.89 ± 0.07	5.91 ± 0.11
16	hH ₁ R	5.54 ± 0.02	5.54 ± 0.01	5.56 ± 0.03	5.57 ± 0.02	5.58 ± 0.03
	gpH ₁ R	5.73 ± 0.01	5.76 ± 0.02	5.79 ± 0.04	5.84 ± 0.06	5.89 ± 0.09
17	hH ₁ R	5.55 ± 0.06	5.44 ± 0.04	5.59 ± 0.04	5.54 ± 0.04	5.53 ± 0.05
	gpH ₁ R	5.45 ± 0.04	5.52 ± 0.04	5.50 ± 0.05	5.58 ± 0.05	5.59 ± 0.06
18	hH ₁ R	5.39 ± 0.05	5.38 ± 0.02	5.41 ± 0.03	5.44 ± 0.04	5.46 ± 0.01
	gpH ₁ R	5.69 ± 0.10	5.65 ± 0.05	5.72 ± 0.04	5.73 ± 0.03	5.81 ± 0.06
19	hH ₁ R	6.38 ± 0.05	6.39 ± 0.08	6.35 ± 0.02	6.32 ± 0.11	6.33 ± 0.02
	gpH ₁ R	7.18 ± 0.04	7.17 ± 0.01	7.14 ± 0.02	7.27 ± 0.05	7.35 ± 0.01
20	hH ₁ R	6.45 ± 0.07	6.59 ± 0.11	6.58 ± 0.02	6.55 ± 0.01	6.55 ± 0.07
	gpH ₁ R	7.00 ± 0.12	7.23 ± 0.02	7.32 ± 0.01	7.42 ± 0.02	7.35 ± 0.02
21R	hH ₁ R	5.90 ± 0.06	6.01 ± 0.06	6.05 ± 0.02	6.02 ± 0.01	6.07 ± 0.02
	gpH ₁ R	6.67 ± 0.01	6.68 ± 0.01	6.76 ± 0.02	6.88 ± 0.01	7.10 ± 0.02
21S	hH ₁ R	6.05 ± 0.04	6.15 ± 0.05	6.29 ± 0.03	6.24 ± 0.01	6.28 ± 0.05
	gpH ₁ R	6.27 ± 0.04	6.18 ± 0.05	6.35 ± 0.04	6.52 ± 0.06	6.72 ± 0.11
22	hH ₁ R	6.42 ± 0.01	6.46 ± 0.02	6.65 ± 0.01	6.64 ± 0.01	6.67 ± 0.01
	gpH ₁ R	6.94 ± 0.08	7.22 ± 0.01	7.33 ± 0.03	7.42 ± 0.01	7.38 ± 0.06

¹ We additionally determined the pK_i values at 308 K for **1**, hH₁R: 8.32 ± 0.02, gpH₁R: 8.58 ± 0.03; for **4**, hH₁R: 8.70 ± 0.02, gpH₁R: 8.37 ± 0.08; for **5**, hH₁R: 8.96 ± 0.07, gpH₁R: 9.22 ± 0.06 and for **7**, hH₁R: 9.05 ± 0.06, gpH₁R: 9.09 ± 0.13.

Table 2. Calculated thermodynamic properties for H₁ receptor antagonists and agonists at hH₁R and gpH₁R. All thermodynamic quantities were calculated, as described under *Materials and Methods* at a reference temperature of $T_o = 293.15$ K.

cpd.	species	$\Delta H^{\circ}(p, T_o)$ [kJ/mol]	$\Delta S^{\circ}(p, T_o)$ [J/K/mol]	$-T\Delta S^{\circ}(p, T_o)$ [kJ/mol]	$\Delta C_p^{\circ}(p, T_o)$ [J/mol/K]	$\Delta G^{\circ, \text{calc}}(p, T_o)$ [kJ/mol]	$\Delta G^{\circ, \text{exp}}(p, T_o)$ [kJ/mol]
1	hH ₁ R	-1.0 ± 2.1	156.2 ± 7.1	-45.8 ± 2.1	-300.7 ± 492.4	-46.8 ± 3.0	-46.8 ± 0.1
	gpH ₁ R	1.0 ± 1.9	167.7 ± 6.6	-49.2 ± 1.9	-200.5 ± 454.5	-48.2 ± 2.7	-48.1 ± 0.1
2	hH ₁ R	22.6 ± 5.0	225.7 ± 17.1	-66.2 ± 5.0	1536 ± 1708	-43.6 ± 7.1	-43.9 ± 0.1
	gpH ₁ R	20.9 ± 4.0	216.0 ± 13.5	-63.3 ± 4.0	3023 ± 1348	-40.4 ± 5.6	-42.2 ± 0.2
3	hH ₁ R	80.2 ± 7.4	411.6 ± 25.3	-120.7 ± 7.4	2116 ± 2597	-40.5 ± 10.4	-40.5 ± 0.6
	gpH ₁ R	87.1 ± 8.4	427.4 ± 28.5	-125.3 ± 8.4	4281 ± 2863	-38.2 ± 11.9	-38.3 ± 0.2
4	hH ₁ R	64.7 ± 5.9	373.9 ± 20.3	-109.6 ± 5.9	1917 ± 1817	-44.9 ± 8.4	-45.1 ± 0.1
	gpH ₁ R	65.3 ± 7.9	369.3 ± 27.1	-108.2 ± 7.9	3500 ± 2174	-42.9 ± 11.2	-42.2 ± 0.4
5	hH ₁ R	16.3 ± 4.9	227.1 ± 17.0	-66.6 ± 5.0	-2142 ± 1247	-50.3 ± 7.0	-49.8 ± 0.2
	gpH ₁ R	44.6 ± 3.8	322.7 ± 13.0	-94.6 ± 3.8	-734.1 ± 981.4	-50.0 ± 5.4	-49.7 ± 0.3
6	hH ₁ R	32.6 ± 4.0	279.6 ± 13.4	-82.0 ± 3.9	116.7 ± 1251	-49.4 ± 5.6	-49.4 ± 0.3
	gpH ₁ R	72.2 ± 6.7	415.1 ± 23.0	-121.7 ± 6.7	3943 ± 2221	-49.5 ± 9.5	-49.4 ± 0.3
7	hH ₁ R	72.0 ± 4.7	409.0 ± 16.3	-119.9 ± 4.8	-2290 ± 1300	-47.9 ± 6.7	-47.7 ± 0.3
	gpH ₁ R	98.8 ± 5.9	497.8 ± 20.3	-145.9 ± 6.0	-1756 ± 1571	-47.1 ± 8.4	-46.3 ± 0.3
8	hH ₁ R	-1.5 ± 3.5	144.7 ± 12.0	-42.4 ± 3.5	2772 ± 1186	-43.9 ± 4.9	-43.9 ± 0.1
	gpH ₁ R	25.9 ± 6.4	242.9 ± 21.9	-71.2 ± 6.4	102.2 ± 1936	-45.3 ± 9.1	-45.4 ± 0.3
9	hH ₁ R	-37.6 ± 7.0	-19.9 ± 23.8	5.8 ± 7.0	-3847 ± 2366	-31.8 ± 9.9	-31.7 ± 0.2
	gpH ₁ R	-24.1 ± 4.2	23.3 ± 14.3	-6.8 ± 4.2	2474 ± 1543	-30.9 ± 5.9	-30.9 ± 0.1
10	hH ₁ R	-2.1 ± 6.9	96.5 ± 23.2	-28.3 ± 6.8	-2984 ± 2271	-30.4 ± 9.7	-30.2 ± 0.4
	gpH ₁ R	12.1 ± 4.4	149.3 ± 15.0	-43.8 ± 4.4	-125.9 ± 1483	-31.7 ± 6.2	-31.6 ± 0.1
11R	hH ₁ R	14.5 ± 3.5	134.9 ± 12.1	-39.5 ± 3.5	-2707 ± 1192	-25.0 ± 4.9	-25.2 ± 0.1
	gpH ₁ R	17.3 ± 2.1	149.3 ± 7.2	-43.8 ± 2.1	1627 ± 709.6	-26.5 ± 3.0	-26.4 ± 0.1
11S	hH ₁ R	-6.2 ± 2.5	77.9 ± 8.5	-22.8 ± 2.5	565.2 ± 842.6	-29.0 ± 3.5	-29.0 ± 0.1
	gpH ₁ R	-4.8 ± 1.6	87.7 ± 5.4	-25.7 ± 1.6	767.7 ± 534.1	-30.5 ± 2.3	-30.6 ± 0.1
12	hH ₁ R	5.3 ± 4.0	122.0 ± 13.8	-35.8 ± 4.0	-473.6 ± 1361	-30.5 ± 5.7	-30.6 ± 0.2
	gpH ₁ R	21.4 ± 6.8	186.8 ± 23.1	-54.8 ± 6.8	133.0 ± 2285	-33.4 ± 9.6	-33.3 ± 0.2

13	hH ₁ R	-3.9 ± 3.9	109.9 ± 13.5	-32.2 ± 4.0	1062 ± 1326	-36.1 ± 5.6	-36.2 ± 0.2
	gpH ₁ R	11.1 ± 5.4	158.4 ± 18.3	-46.4 ± 5.4	1568 ± 1834	-35.3 ± 7.6	-35.7 ± 0.1
14	hH ₁ R	25.6 ± 2.2	187.6 ± 7.5	-55.0 ± 2.2	64.9 ± 703.7	-29.4 ± 3.5	-29.4 ± 0.1
	gpH ₁ R	23.6 ± 3.5	178.8 ± 12.1	-52.4 ± 3.5	209.9 ± 1194	-28.8 ± 4.9	-28.7 ± 0.2
15	hH ₁ R	-2.3 ± 2.9	102.5 ± 9.9	-30.0 ± 2.9	408.9 ± 982.2	-32.3 ± 4.1	-32.3 ± 0.2
	gpH ₁ R	11.1 ± 5.5	149.8 ± 18.7	-43.9 ± 5.5	-303.1 ± 1843	-32.8 ± 7.8	-32.8 ± 0.2
16	hH ₁ R	3.6 ± 1.8	118.7 ± 6.1	-34.8 ± 1.8	22.1 ± 601.8	-31.2 ± 2.5	-31.2 ± 0.1
	gpH ₁ R	13.4 ± 4.7	156.6 ± 16.0	-45.9 ± 4.7	746.9 ± 1580	-32.5 ± 6.6	-32.5 ± 0.2
17	hH ₁ R	1.9 ± 5.6	112.5 ± 19.1	-33.0 ± 5.6	-108.7 ± 1887	-31.1 ± 7.9	-31.3 ± 0.2
	gpH ₁ R	11.1 ± 4.3	143.7 ± 14.6	-42.1 ± 4.3	-202.8 ± 1439	-31.0 ± 6.1	-30.9 ± 0.3
18	hH ₁ R	6.4 ± 3.0	125.4 ± 10.1	-36.8 ± 3.0	505.0 ± 993.6	-30.4 ± 4.2	-30.3 ± 0.2
	gpH ₁ R	10.6 ± 5.3	145.2 ± 18.1	-42.6 ± 5.3	1801 ± 1786	-32.2 ± 7.5	-32.1 ± 0.2
19	hH ₁ R	-5.0 ± 5.7	104.5 ± 19.4	-30.6 ± 5.7	-1.4 ± 1943	-35.6 ± 8.1	-35.6 ± 0.1
	gpH ₁ R	16.1 ± 3.8	192.3 ± 13.0	-56.4 ± 3.8	3350 ± 1215	-40.3 ± 5.4	-40.1 ± 0.1
20	hH ₁ R	5.6 ± 6.3	145.4 ± 21.5	-42.6 ± 6.3	-2912 ± 2282	-37.0 ± 8.9	-36.9 ± 0.1
	gpH ₁ R	27.5 ± 4.8	234.5 ± 16.3	-68.7 ± 4.8	-5578 ± 1612	-41.2 ± 6.8	-41.1 ± 0.1
21R	hH ₁ R	10.8 ± 4.5	152.3 ± 15.4	-44.6 ± 4.5	-1743 ± 1537	-33.8 ± 6.4	-33.9 ± 0.1
	gpH ₁ R	36.2 ± 1.3	252.9 ± 4.4	-74.1 ± 1.3	4606 ± 442.5	-37.9 ± 1.8	-37.9 ± 0.1
21S	hH ₁ R	17.3 ± 4.2	178.5 ± 14.3	-52.3 ± 4.2	-2734 ± 1411	-35.0 ± 5.9	-35.3 ± 0.2
	gpH ₁ R	41.8 ± 7.0	263.9 ± 23.9	-77.4 ± 7.0	5581 ± 2489	-35.6 ± 9.9	-35.6 ± 0.2
22	hH ₁ R	22.6 ± 3.9	203.2 ± 13.4	-59.6 ± 3.9	-2196 ± 1295	-37.0 ± 5.5	-37.3 ± 0.1
	gpH ₁ R	33.6 ± 4.6	255.3 ± 15.7	-74.8 ± 4.6	-5989 ± 1545	-41.2 ± 6.5	-41.1 ± 0.2

Table 3. Predicted pK_i values for suprahistaprodifen 19, phenoprodifen 20, and the chiral phenoprodifens 21R, 21S based on 3D-QSAR calculations. The calculations were performed as described under *Materials and Methods*. Δ describes the difference pK_i(predicted) – pK_i(experimental). The effective pK_i value (eff), including orientation 1 (or1) and orientation 2 (or2) was calculated using equation $\log_{10}(K_i(\text{or1}) + K_i(\text{or2}))$ as described (Straßer et al., 2009).

cpd.	species	or	283 K		288 K		293 K		298 K		303 K	
			pred.	Δ								
19	hH ₁ R	or1	5.95	-	5.96	-	6.01	-	6.01	-	6.08	-
		or2	5.45	-	5.53	-	5.71	-	5.71	-	5.60	-
		eff	6.07	-0.31	6.10	-0.29	6.19	-0.16	6.19	-0.13	6.20	-0.13
	gpH ₁ R	or1	6.03	-	6.07	-	6.12	-	6.19	-	6.26	-
		or2	6.40	-	6.48	-	6.56	-	6.63	-	6.70	-
		eff	6.55	-0.63	6.62	-0.55	6.69	-0.45	6.76	-0.51	6.83	-0.52
20	hH ₁ R	or1	6.43	-	6.42	-	6.46	-	6.43	-	6.52	-
		or2	5.42	-	5.50	-	5.67	-	5.68	-	5.64	-
		eff	6.47	0.02	6.47	-0.12	6.52	-0.06	6.50	-0.05	6.57	0.02
	gpH ₁ R	or1	6.56	-	6.61	-	6.67	-	6.76	-	6.84	-
		or2	6.41	-	6.52	-	6.64	-	6.74	-	6.83	-
		eff	6.79	-0.21	6.87	-0.36	6.95	-0.37	7.05	-0.37	7.14	-0.21
21R	hH ₁ R	or1	5.99	-	5.98	-	6.06	-	6.06	-	6.09	-
		or2	4.75	-	4.91	-	5.16	-	5.14	-	5.14	-
		eff	6.01	0.11	6.02	0.01	6.11	0.06	6.11	0.09	6.14	0.07
	gpH ₁ R	or1	6.48	-	6.54	-	6.60	-	6.70	-	6.76	-
		or2	5.87	-	5.99	-	6.11	-	6.23	-	6.25	-
		eff	6.58	-0.09	6.65	-0.03	6.72	-0.04	6.83	-0.05	6.88	-0.22
21S	hH ₁ R	or1	5.88	-	5.89	-	5.98	-	5.99	-	6.05	-
		or2	5.15	-	5.29	-	5.52	-	5.50	-	5.58	-
		eff	5.95	-0.10	5.99	-0.16	6.11	-0.18	6.11	-0.13	6.18	-0.10
	gpH ₁ R	or1	5.98	-	6.06	-	6.11	-	6.22	-	6.27	-
		or2	6.20	-	6.30	-	6.42	-	6.55	-	6.63	-
		eff	6.40	0.13	6.50	0.32	6.59	0.24	6.72	0.20	6.79	0.07

Table 4. Predicted thermodynamic properties for suprahistaprodifen 19, phenoprodifen 20 and the chiral phenoprodifens 21R, 21S at a temperature of 293.15 K. The thermodynamic parameters are calculated based on the predicted pK_i values (Table 3) as described in *Materials and Methods*. The difference predicted quantity – experimental quantity for the effective quantities is given by Δ.

cpd.	species	or	ΔH ⁰ (p,T ₀) [kJ/mol]		ΔS ⁰ (p,T ₀) [J/K/mol]		-TΔS ⁰ (p,T ₀) [kJ/mol]		ΔC _p ⁰ (p,T ₀) [J/mol/K]		ΔG ⁰ (p,T ₀) [kJ/mol]	
			pred.	Δ	pred.	Δ	pred.	Δ	pred.	Δ	pred.	Δ
19	hH ₁ R	or1	10.4 ± 2.0	-	150.3 ± 6.8	-	-44.1 ± 2.0	-	730.5 ± 676.4	-	-33.7 ± 2.8	-
		or2	14.0 ± 5.9	-	156.5 ± 20.2	-	-45.9 ± 5.9	-	-5177 ± 2000	-	-31.9 ± 8.3	-
		eff	11.1 ± 2.5	16.1	156.0 ± 8.5	51.5	-45.7 ± 2.5	-15.1	-1148 ± 840.1	-1147	-34.6 ± 3.5	1.0
	gpH ₁ R	or1	19.3 ± 0.3	-	183.3 ± 1.1	-	-53.7 ± 0.3	-	879.9 ± 112.3	-	-34.4 ± 0.4	-
		or2	24.6 ± 0.2	-	209.5 ± 0.6	-	-61.4 ± 0.2	-	-113.4 ± 59.5	-	-36.8 ± 0.3	-
		eff	23.0 ± 0.1	6.9	206.7 ± 0.1	14.4	-60.6 ± 0.1	-4.2	157.0 ± 0.6	-3193	-37.6 ± 0.1	2.5
20	hH ₁ R	or1	6.3 ± 3.3	-	144.7 ± 11.1	-	-42.4 ± 3.3	-	1457 ± 1100	-	-36.1 ± 4.7	-
		or2	19.3 ± 4.9	-	174.0 ± 16.7	-	-51.0 ± 4.9	-	-3745 ± 1653	-	-31.7 ± 6.9	-
		eff	7.8 ± 2.9	2.2	151.0 ± 9.8	5.6	-44.3 ± 2.9	-1.7	770.2 ± 967.5	3682	-36.5 ± 4.1	0.4
	gpH ₁ R	or1	24.1 ± 0.7	-	209.9 ± 2.3	-	-61.5 ± 0.7	-	1108 ± 229.7	-	-37.4 ± 1.0	-
		or2	34.7 ± 0.7	-	245.5 ± 2.5	-	-72.0 ± 0.7	-	-244.5 ± 90.46	-	-37.3 ± 1.0	-
		eff	29.9 ± 0.5	2.4	235.5 ± 1.7	1.0	-69.0 ± 0.5	-0.3	551.7 ± 126.7	6130	-39.1 ± 0.7	2.0
21R	hH ₁ R	or1	9.2 ± 2.8	-	147.0 ± 9.6	-	-43.1 ± 2.8	-	55.1 ± 945.0	-	-33.9 ± 4.0	-
		or2	31.3 ± 6.0	-	204.7 ± 20.4	-	-60.0 ± 6.0	-	-5322 ± 2019	-	-28.7 ± 8.5	-
		eff	11.4 ± 2.8	0.6	155.3 ± 9.5	3.0	-45.5 ± 2.8	-0.9	-397.4 ± 940.3	1346	-34.1 ± 4.0	-0.2
	gpH ₁ R	or1	23.8 ± 1.4	-	207.9 ± 4.8	-	-60.9 ± 1.4	-	532.0 ± 476.7	-	-37.1 ± 2.0	-
		or2	32.3 ± 2.6	-	227.3 ± 8.9	-	-66.6 ± 2.6	-	-1666 ± 874.9	-	-34.3 ± 3.7	-
		eff	25.7 ± 1.9	-10.5	216.5 ± 6.4	-36.4	-63.5 ± 1.9	10.6	167.3 ± 636.0	-4439	-37.8 ± 2.7	0.1
21S	hH ₁ R	or1	14.5 ± 2.6	-	163.7 ± 9.0	-	-48.0 ± 2.6	-	282.6 ± 886.1	-	-33.7 ± 3.7	-
		or2	34.0 ± 6.1	-	220.7 ± 20.8	-	-64.7 ± 6.1	-	-3233 ± 2050	-	-30.7 ± 8.6	-
		eff	18.9 ± 3.4	1.6	180.8 ± 11.7	2.3	-53.0 ± 3.4	0.7	-434.5 ± 1159	2300	-34.1 ± 4.8	1.2
	gpH ₁ R	or1	24.4 ± 2.0	-	200.4 ± 6.8	-	-58.7 ± 2.0	-	162.2 ± 672.1	-	-34.3 ± 2.8	-
		or2	36.4 ± 1.7	-	247.4 ± 5.8	-	-72.5 ± 1.7	-	-41.6 ± 576.8	-	-36.1 ± 2.4	-
		eff	32.8 ± 1.8	-9.0	238.5 ± 6.1	-25.4	-69.9 ± 1.8	7.5	30.2 ± 602.9	-5551	-37.1 ± 2.6	-1.5

Fig. 1

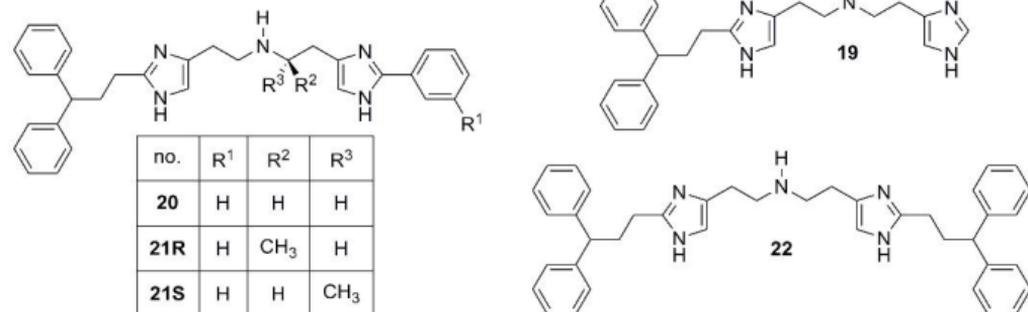
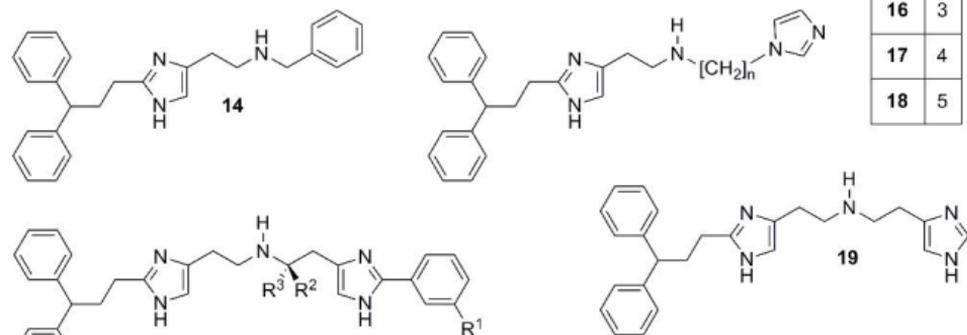
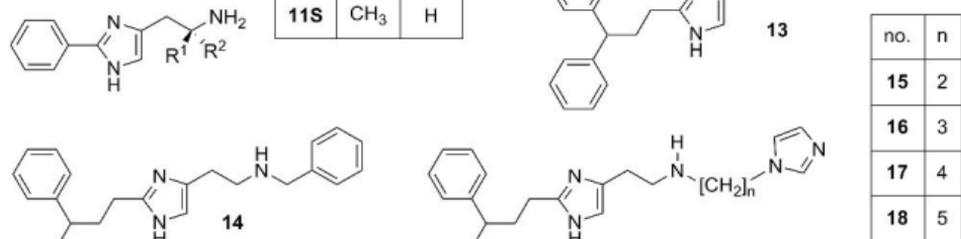
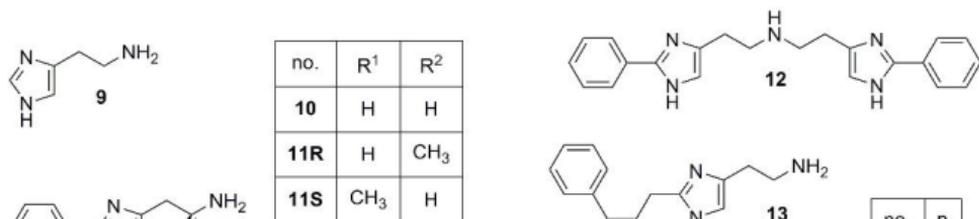
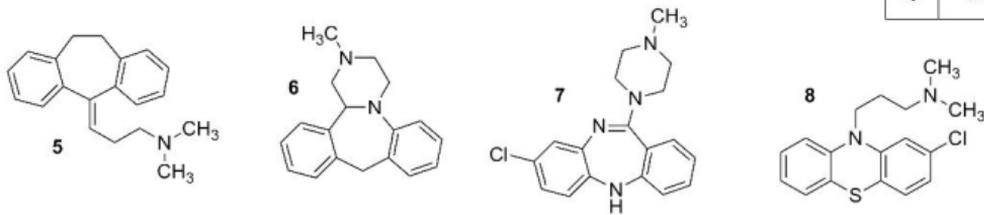
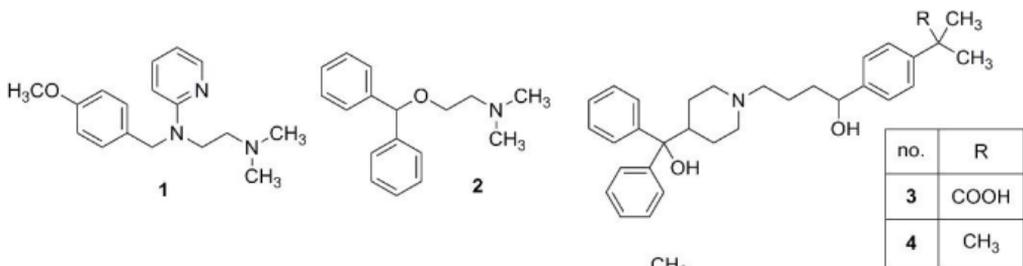


Fig. 2

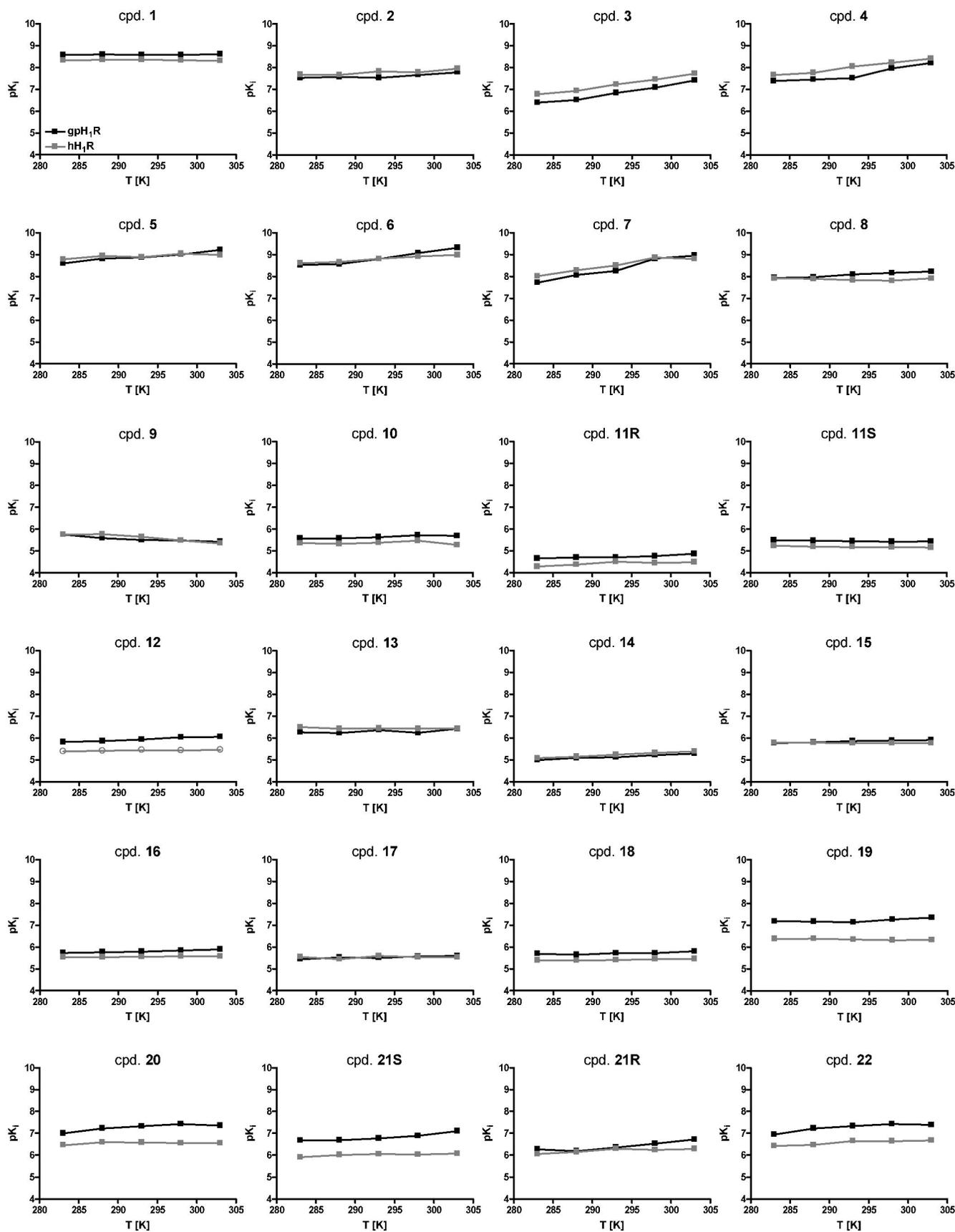


Fig. 3

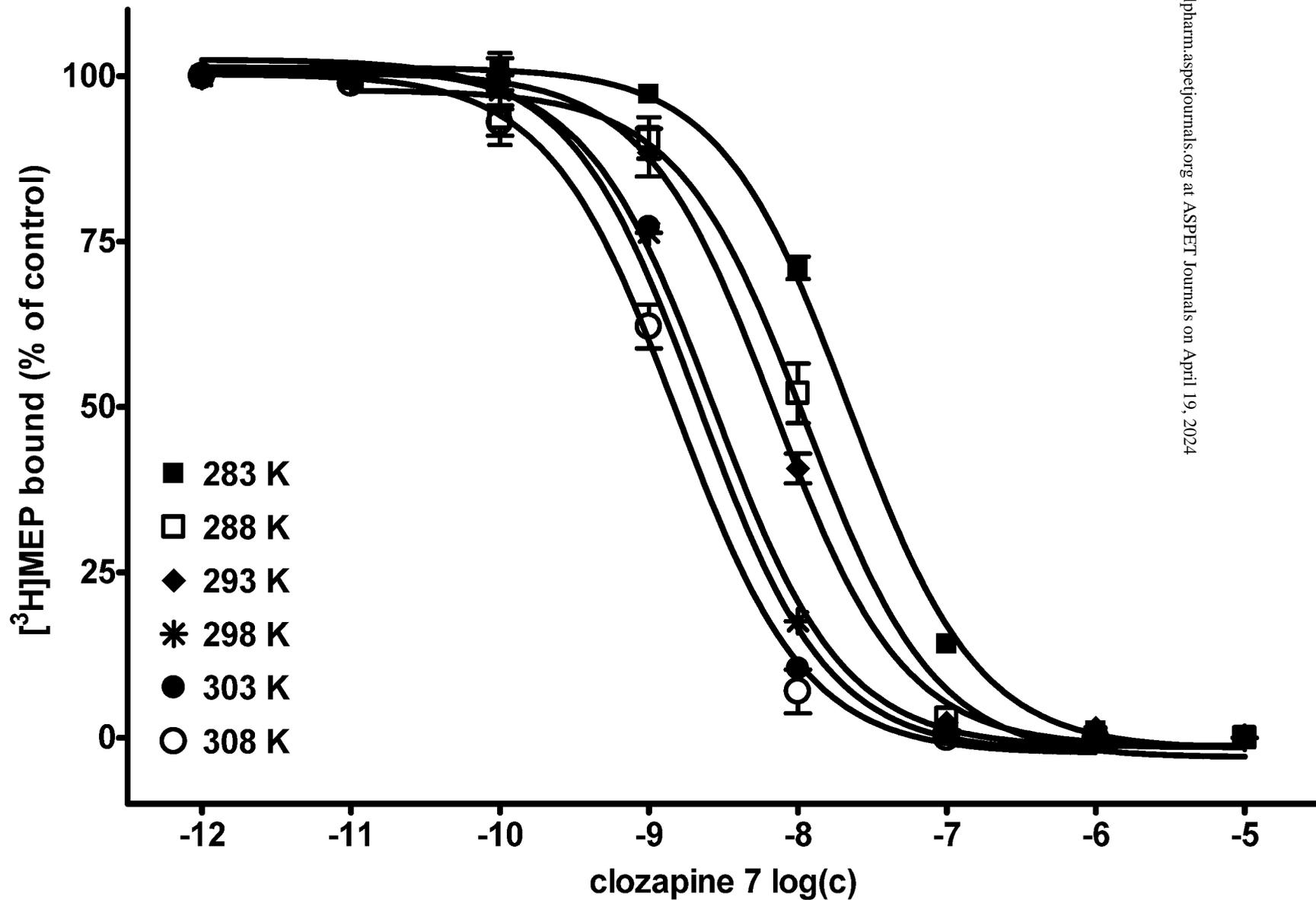


Fig. 4

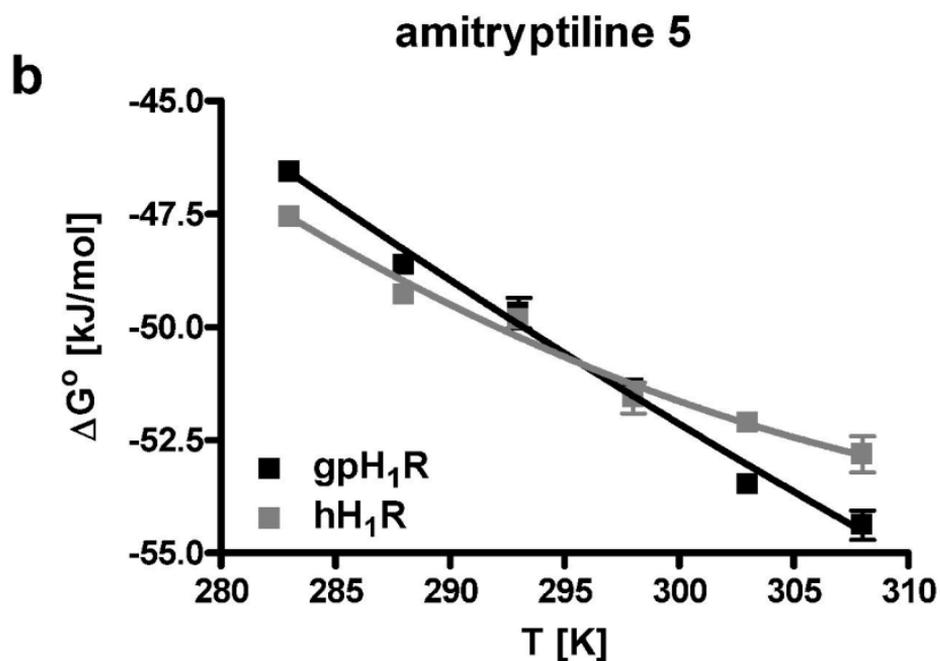
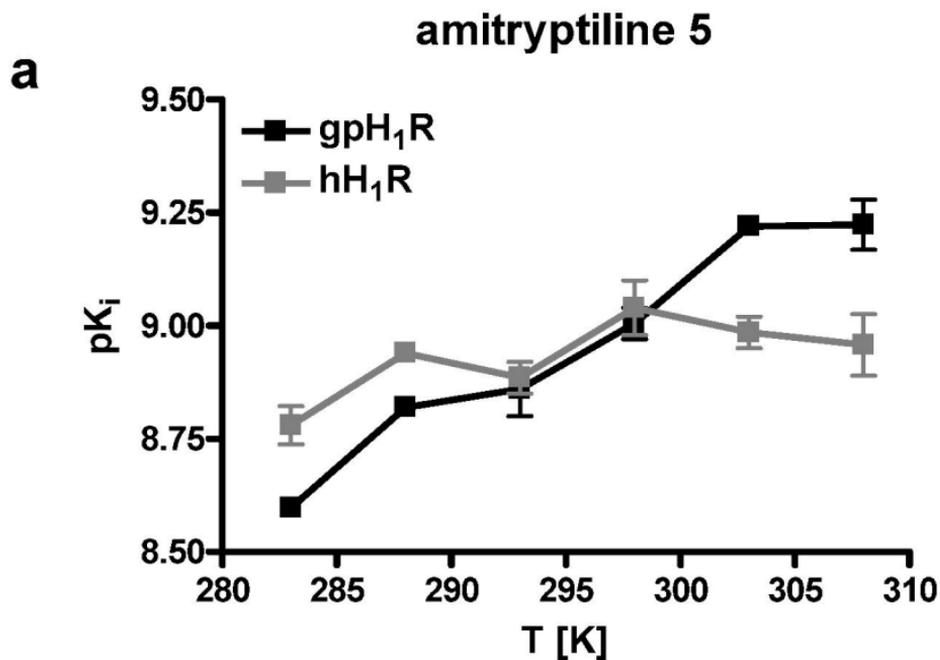


Fig. 5

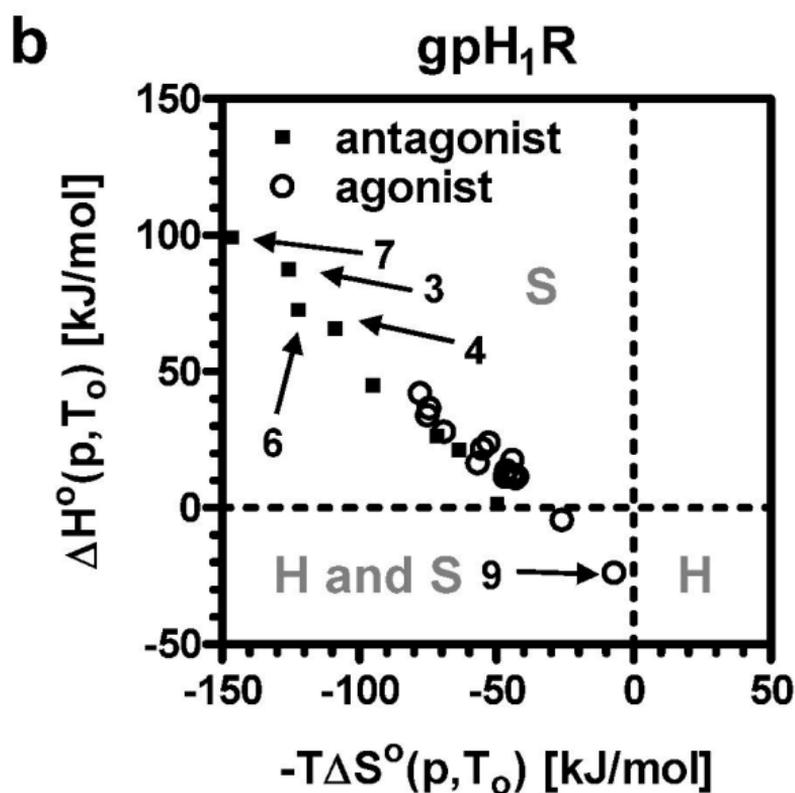
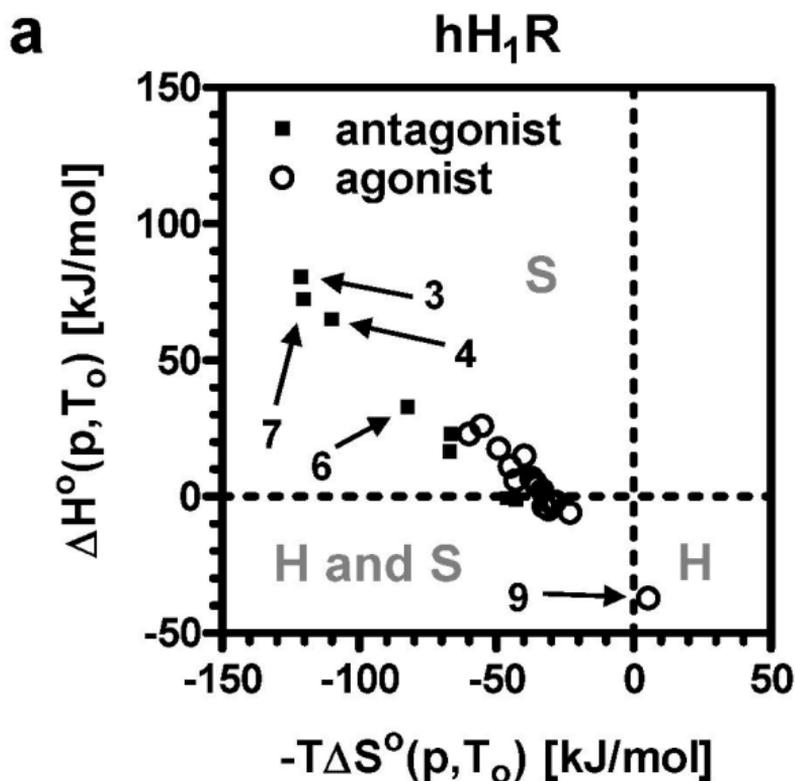


Fig. 6

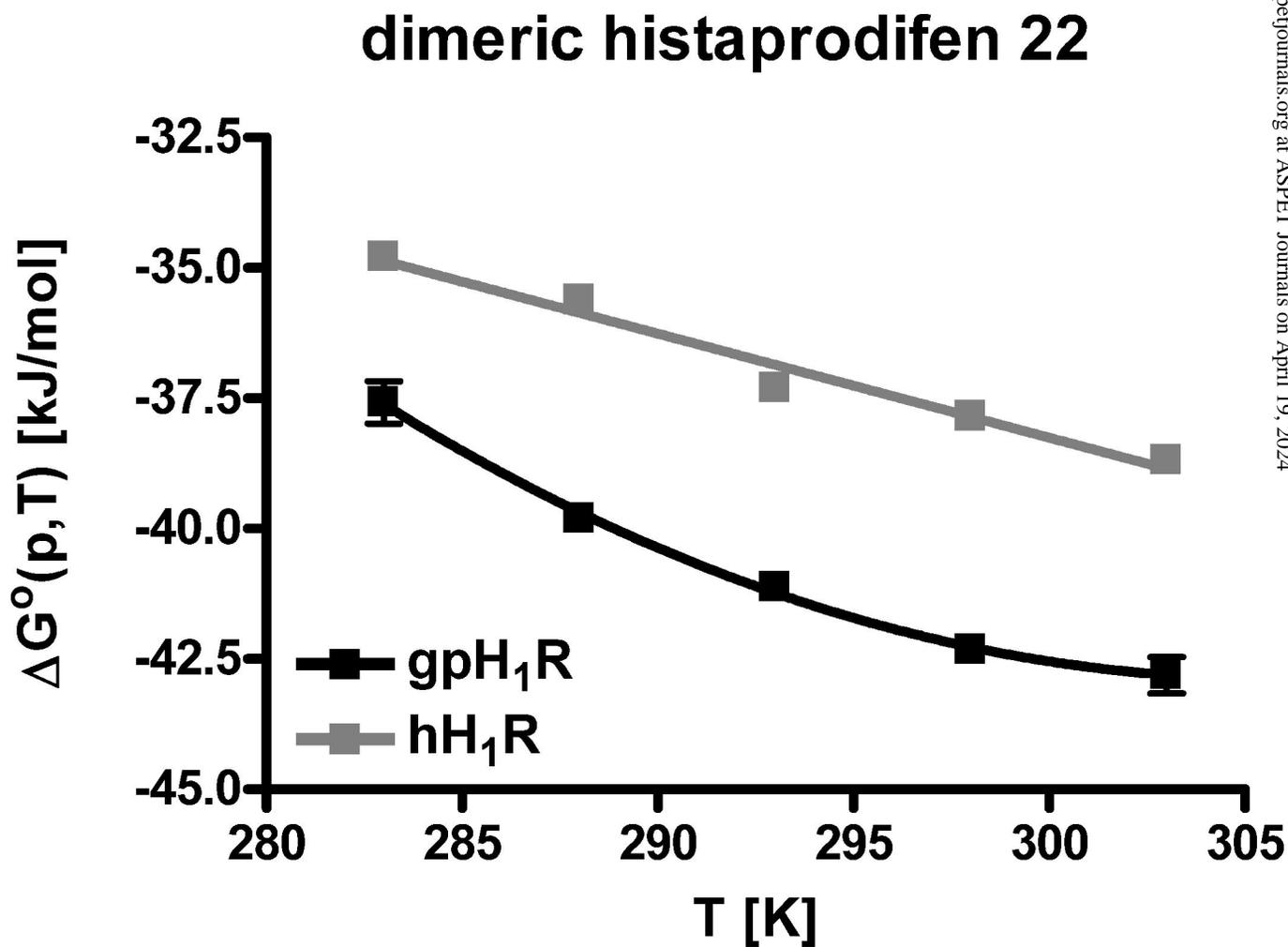


Fig. 7

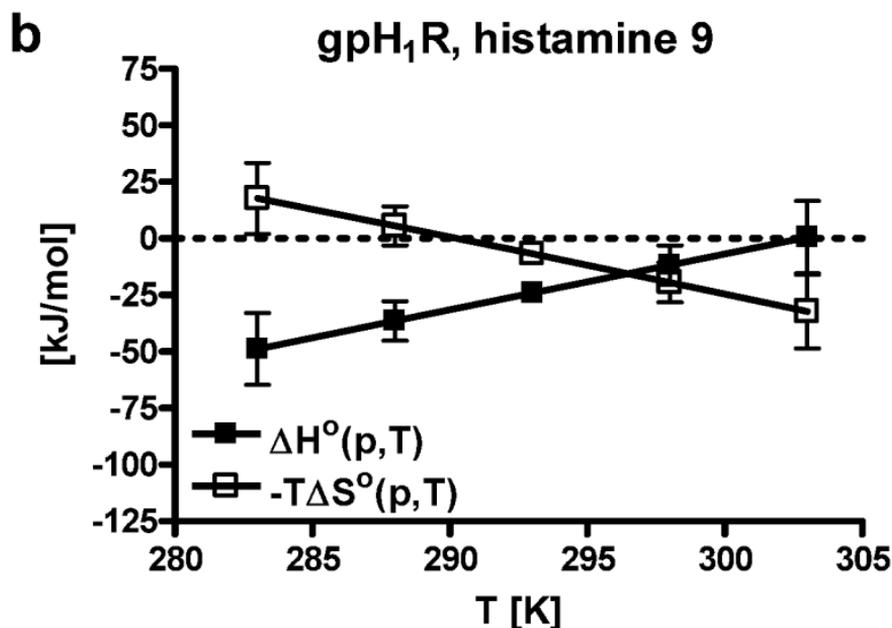
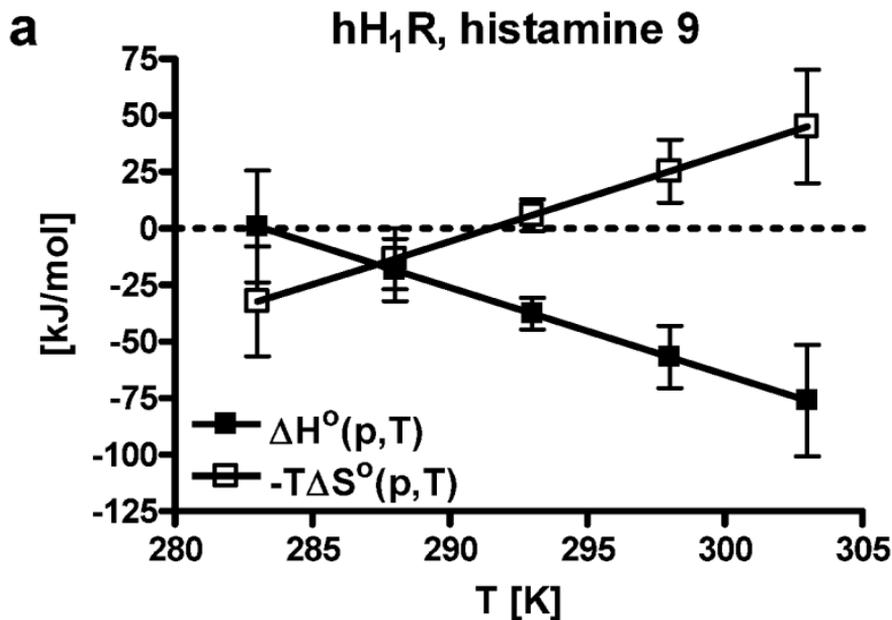


Fig. 8

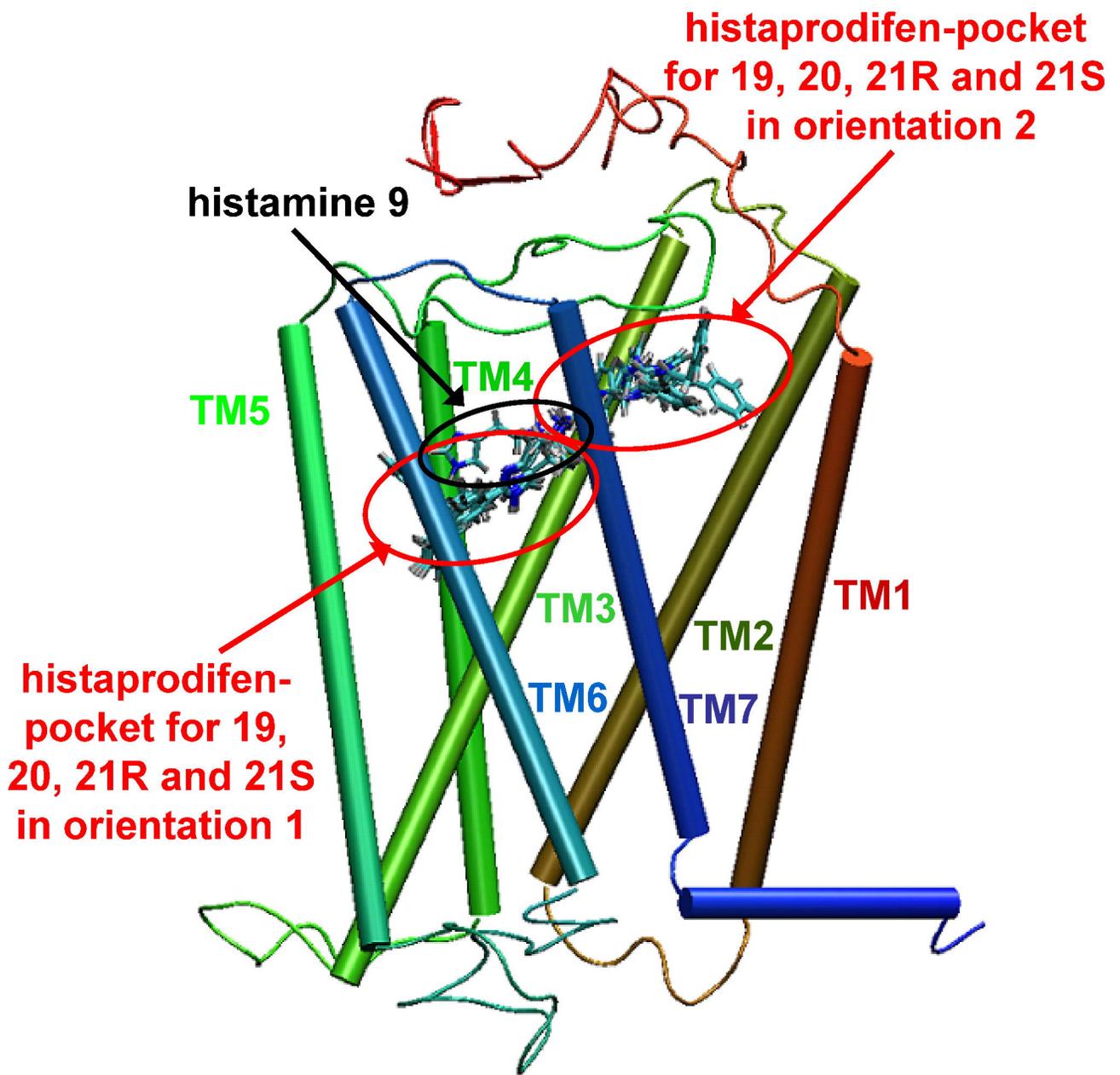
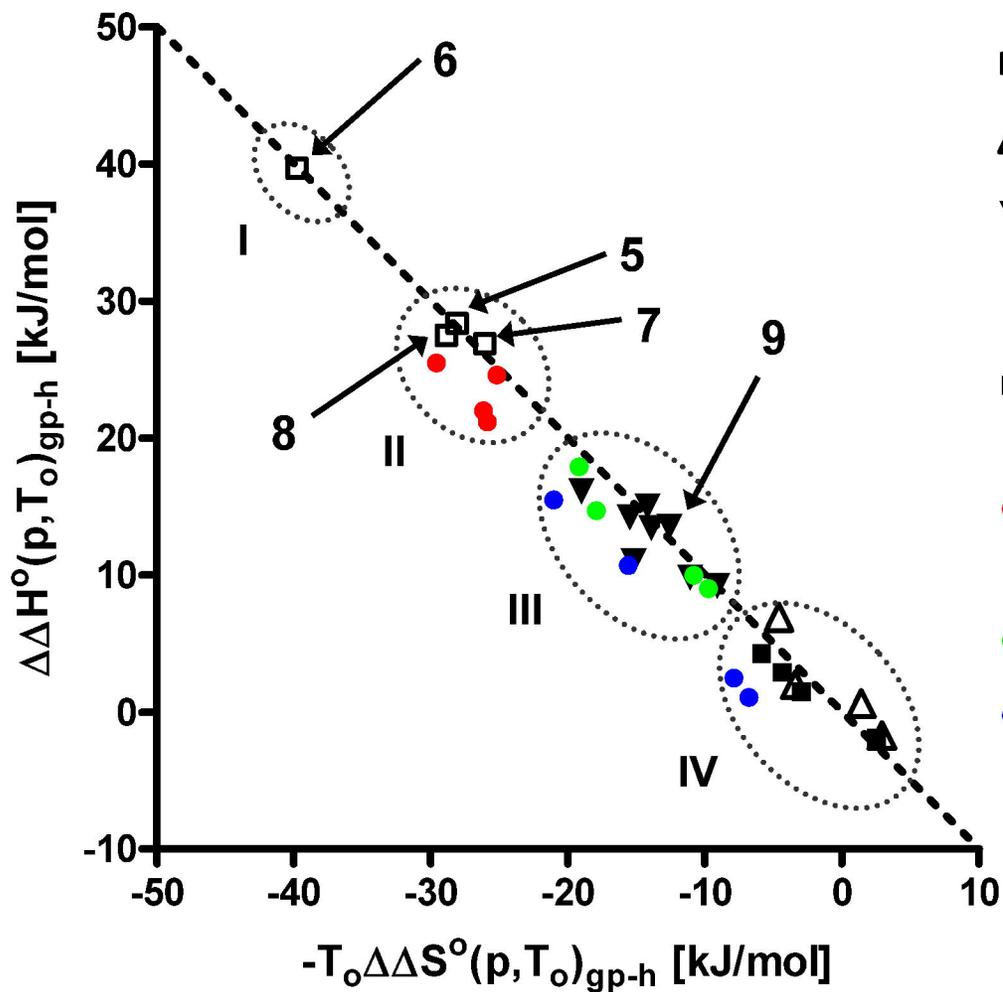


Fig. 9



- non-flexible antagonists (exp.)
- △ flexible antagonists (exp.)
- ▼ histamine, phenylhistamines and histaprodifens with larger species-differences (exp.)
- phenylhistamines and histaprodifens with smaller species-differences (exp.)
- effective values for ligands with two orientations (exp.)
- prediction for orientation 1
- prediction for orientation 2