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ORIGINAL PAPER

In silico binding characteristics between human histamine H_1 receptor and antagonists

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Abstract It is widely acknowledged that the H_1 receptor antagonists have important therapeutic significance in the treatment of various allergic disorders, but little was known about the binding mode between the receptor and antagonists since the crystal structure of G-protein coupling receptors (GPCRs) were hard to obtain. In this paper, a theoretical three-dimensional model of human histamine H_1 receptor (HHR1) was developed on the basis of recently reported high resolution structures of human A_{2A} adenosine receptor, human β_2-adrenoceptor and turkey β_1-adrenoceptor. Furthermore, three representative H_1 receptor antagonists were chosen for docking studies. Subsequently, a qualitative pharmacophore model was developed by Hiphop algorithm based on the docking conformations of these three antagonists. In this paper, active environment, certain key residues, and the corresponding pharmacophore features of H_1 receptor were identified by such combinations of receptor-based and ligand-based approaches, which would give sufficient guidance for the rational design of novel antihistamine agents.

Keywords Docking · H_1 receptor · Homology modeling · Molecular dynamics · Pharmacophore modeling

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Introduction

Histamine has been widely recognized as an important intercellular messenger which plays an important role in several physiological processes [1, 2]. So far four histamine receptor subtypes (H_1, H_2, H_3 and H_4) have been identified, characterized, and cloned by pharmacological analysis and genetic technology [3–6] as critical members of the G-protein coupled receptors (GPCRs) superfamily. H_1 receptor that is located in most smooth muscle contains seven transmembrane (TM 1-7) helices in which several conserved residues are very important for the signal transduction [1]. H_1 receptor protein that has a molecular weight of 53 to 58 kDa in different animal tissues is regarded as the target of allergy since it controls the contraction of smooth muscle and is responsible for allergy and immunity courses. Many drugs have been developed and applied in clinical trials during the 1950s–1990s. Further SAR and mutation researches shows that H_1 receptor antagonists have a protonated amine function, which interacts with carboxylic acid of Asp107 in TM3 [7, 8]. This electrostatic interaction is crucial for the binding affinity of the antagonists. The lipophilic antagonist binding pocket consisted of Phe432, Phe435 and Trp158 in human H_1 receptor (HHR1) also seems to be essential for ligand binding [9]. Moreover,

some zwitterionic H_1 antagonists, such as Levocetirizine, may also hold an ionic interaction between their carboxylic moiety and Lys191 of HHR1 [10].

The development of new antihistamine compounds was often based on the modifications of some marketed H_1 antagonists (*e.g.* Mepyramine). As first generation H_1 antagonists, Mepyramine and Diphenhydramine were therapeutically effective in allergic rhinitis conjunctivitis and dermatitis. However, they were not very active in bronchial asthma. The explanation is that histamine is not the only mediator in allergic diseases. Although the first generation H_1 antagonists were proven to possess significant antihistamine effects, many of them also display important antimuscarinic effects. Moreover, at therapeutic dosages, they usually give rise to sedative and other effects that related to the action of H_1 receptors in the brain [2, 11]. The second-generation H_1 antagonists like Loratadine and Cetirizine that penetrate poorly into the CNS appear to be devoid of central depressant effects. Nevertheless, the second generation has much lower affinity than the first-generation, and some of them such as Tefenadine and Astemizole have been withdrawn from the market due to their cardiotoxicity triggered from intensive blockade of *h*ERG potassium channel [12]. Therefore, the aim to discover new generation of H_1 antagonists, based on deep investigation of H_1 receptor-antagonist interaction, is to reduce the side effect caused by traditional H_1 receptor antagonists, like sedation and the potential cardiovascular toxicity.

The basic SAR of H_1 receptor antagonists could be easily summarized since most antagonists share almost the same chemical structures. The general scaffold consists of two neighboring aromatic rings and a side chain with basic nitrogen. The side chain can be saturated, unsaturated, branched as long as if they can keep a certain distance (about 3–4 bonds) between the nitrogen atom and the aromatic rings. The atom thatis between the side chain and the aromatic part could be either carbon, oxygen or nitrogen atom, and the *para*-substitution with a small lipophilic

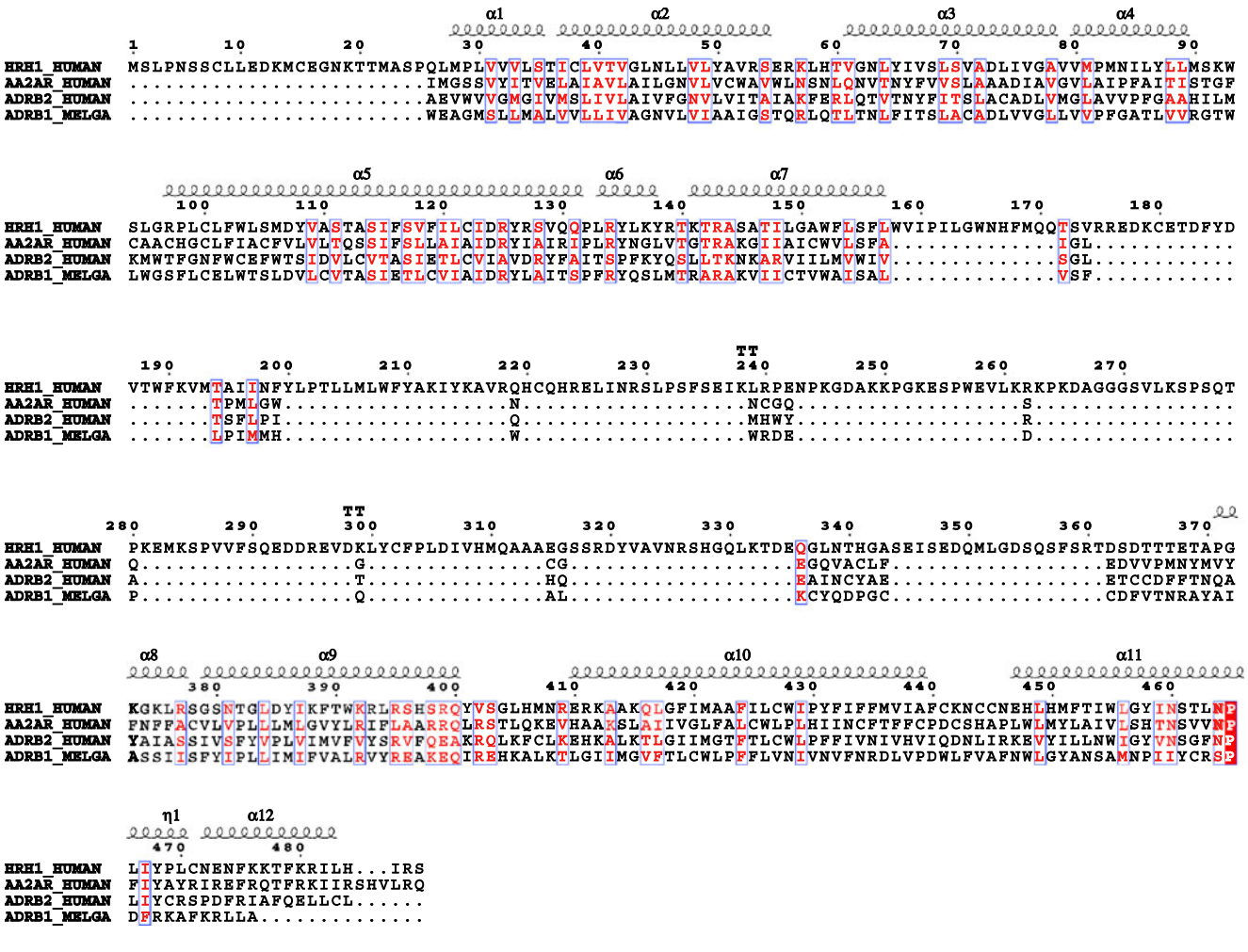

Fig. 1 Secondary structure-based sequence alignment between human H_1 receptor and human A_{2A} adenosine receptor, human β_2-adrenoceptor and turkey β_1-adrenoceptor

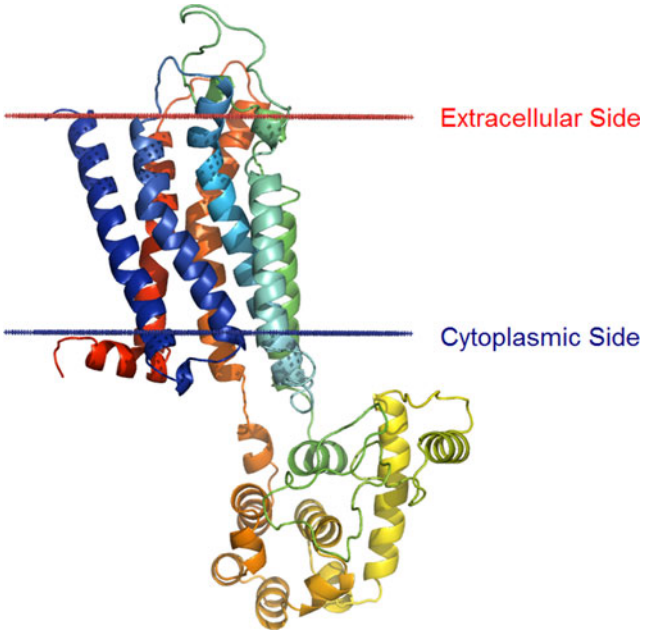

Fig. 2 Initial conformation of full-length H_1 receptor in the GBSW implicit membrane model

group (*e.g.*, -CH_3, -Cl) is favorable for only one of the two aromatic rings of H_1 antagonists [13].

However, the lack of crystal structure of H_1 receptor hinders the development of new types of antagonists and further investigations on the H_1 receptor. So far several homology models for HHR1 have been reported [14–16], but the template for all the models is bovine rhodopsin [17–19] that was extensively used in many GPCR modeling studies. For the length of the amino acid sequences of the HHR1 is much shorter than the sequence of bovine rhodopsin, the two receptors do not share the commonly accepted sequence identity. Therefore, the models based on rhodopsin are not accurate enough to elucidate the structure of H_1 receptor and the binding site of histamine and other antagonists. We have therefore undertaken molecular modeling studies to explore the antagonist-H_1 receptor interactions by using recently reported high resolution structures of human A_{2A} adenosine receptor, β_1-adrenoceptor and β_2-adrenoceptor which are more suitable as templates for GPCRs.

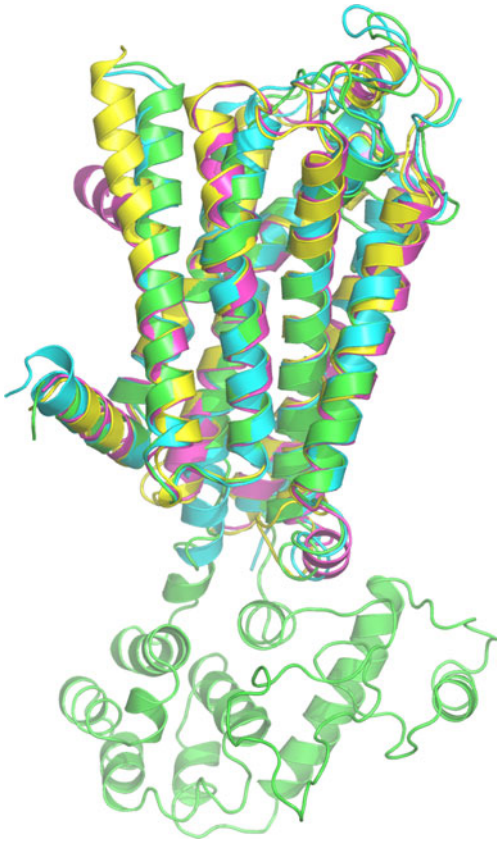

Fig. 4 The superposition of templates A_{2A} adenosine receptor (blue ribbons), β_1-adrenoceptor (red ribbons) and β_2-adrenoceptor (yellow ribbons) crystal structures and H_1 receptor homology model (green ribbons)

Methods

Construction and refinement of the homology model

The construction of protein models by homology modeling normally proceeds along a series of well-defined and commonly accepted steps we have successfully practiced in the earlier publications [20–24]: (1) sequence alignment between the target and the template; (2) building an initial model; (3) refining the model; and (4) evaluating the quality of the model. The recent availability of new high

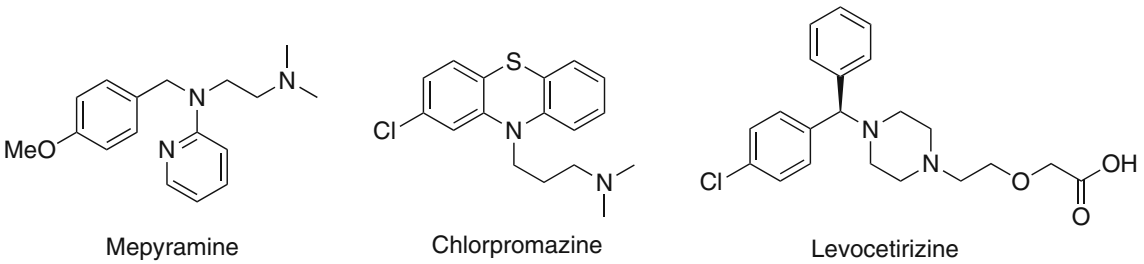

Fig. 3 Chemical structures of Mepyramine, Chlorpromazine, and Levocetirizine

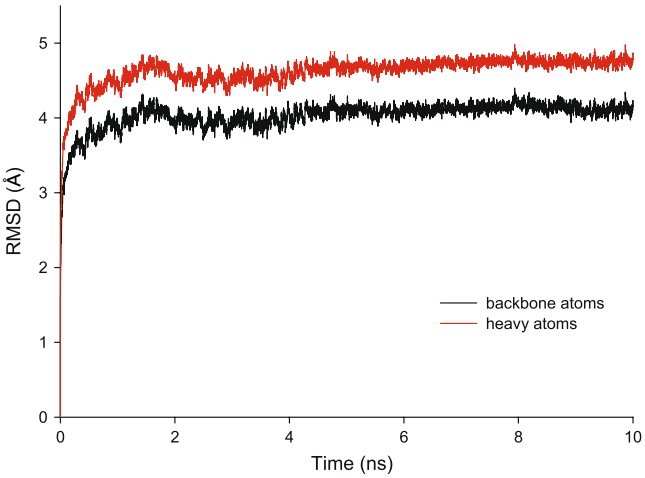

Fig. 5 Time-dependent RMSD (Å) from H_1 receptor homology model for the backbone and heavy atoms in a 10 ns MD simulation

resolution crystal structure for mammalian GPCRs, including human A_{2A} adenosine receptor (PDB entry: 3EML) [25], human β_2-adrenoceptor (PDB entry: 2R4S) [26] and turkey β_2-adrenoceptor (PDB entry: 2VT4) [27], would provide high insights into the molecular mechanisms of GPCR activation as well as GPCR modeling [28]. However, it's hard to specify which template benefits for HHR1 homology modeling [29]. Therefore, in the current study all three crystal structures were used as templates for homology modeling. The secondary structure-based sequence alignment between HHR1 and these three templates conducted by the T-Coffee multiple alignment program [30] and the ESPript program [31] were shown in Fig. 1. The homology model was then built. By using the MODELLER 9v7 program [32]. It should be noted that after building the model, the loop region was refined at high level by using the DOPE-based loop modeling protocol. Molecular dynamic simulations were carried out on an implicit membrane model, GBSW (Generalized Born model with a simple Switching function) [33] by using the CHARMM c33b1 program [34]. The protein atoms were parameterized by using the CHARMM22 force field [35] on CHARMM-GUI online platform [36]. The surface tension coefficient (representing the non-polar solvation energy) was set to 0.03 kcal $mol^{-1}\cdot Å^{-2}$, which was consistent with literature precedents in the calculation of non-polar contributions in soluble proteins [33]. The membrane thickness centered at Z=0 was set to 30.0 Å with a membrane smoothing length of 5.0 Å (w_m=2.5 Å) (Fig. 2). Such implicit membrane settings were in accordance with the commonly believed membrane environment [33]. All bond lengths involving hydrogen atoms were fixed using the SHAKE algorithm [37]. No cutoff for the non-bonded and GB energy calculations was used. Simulation temperature was at 300 K. Minimizations were carried out using 1500 steps of steepest descent, followed by adopted basis Newton-Raphson (ABNR) minimization until the root mean square gradient was less than 0.001 kcal mol^{-1} $Å^{-1}$. The whole system was then equilibrated for 50 ps, followed by another 10 ns of canonical ensemble (NVT)-MD simulation run.

Assessment of the homology model

In order to get an accurate homology model, the quality assessment is an awfully critical step [38]. In this case, PROCHECK Ramachandran plot statistics [39], ERRAT [40], VERIFY-3D [41], WHAT-IF [42], PROSA2003 [43],

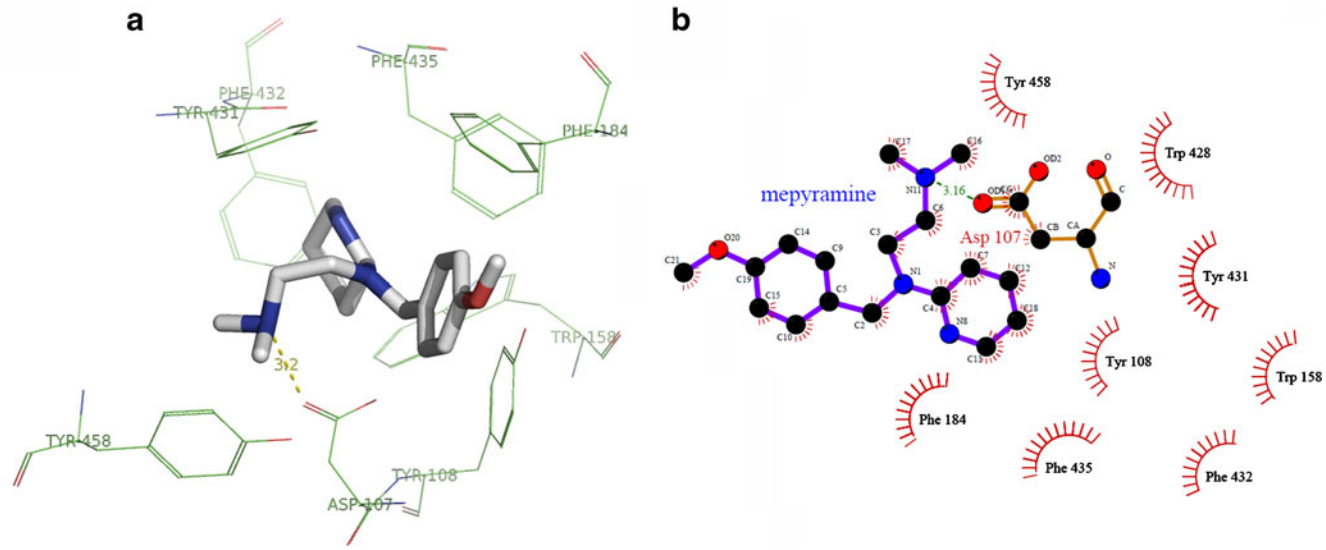

Fig. 6 (**a**) Predicted binding models for Mepyramine; (**b**) The structure of Mepyramine-H1 receptor homology model complex was analyzed using the Ligplot 4.22 program to identify some specific contacts between atoms of ligand and receptor

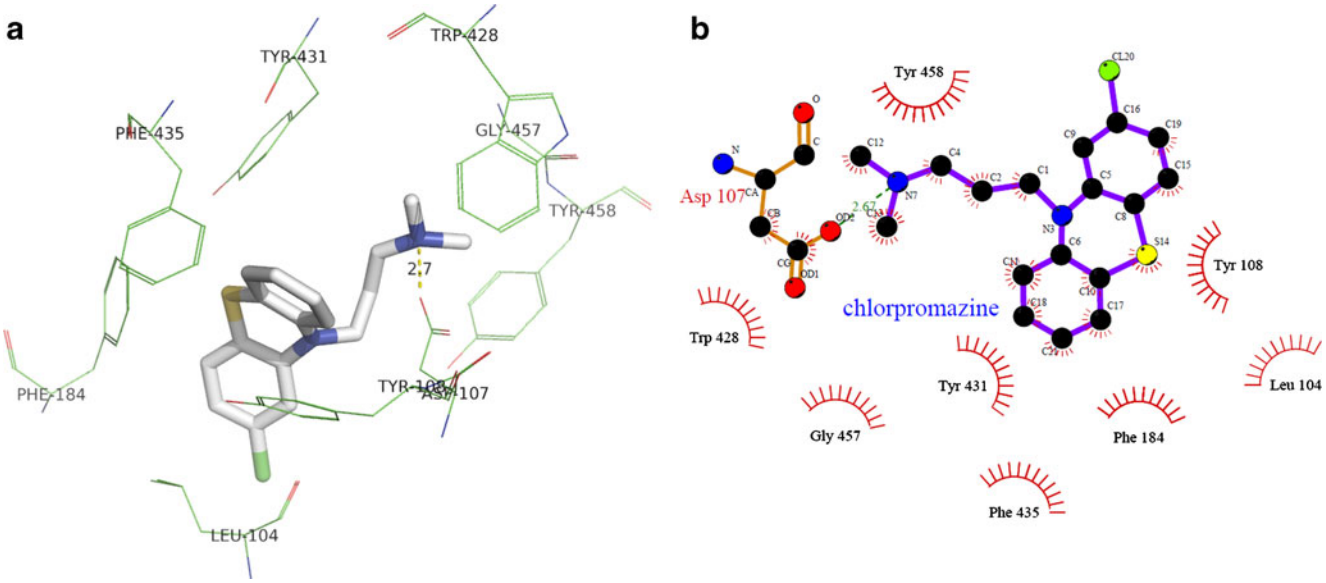

Fig. 7 (**a**) Predicted binding models for Chlorpromazine; (**b**) The structure of Chlorpromazine-H1 receptor homology model complex was analyzed using the Ligplot 4.22 program to identify some specific contacts between atoms of ligand and receptor

and DOPE [44] were applied to evaluate the geometric quality of the backbone conformation and the appropriateness of residue interactions, residue contacts, and energy profiles of the homology model.

Molecular docking

To further predict the accuracy of the resulting homology model, the binding patterns of three structural diverse H_1 receptor antagonists, Mepyramine, Chlorpromazine and Levocetirizine (Fig. 3), were in silico examined and compared with available experimental mutagenesis data. All partial charges on the atoms of homology model were derived from the AMBER 8 force field parameters. The 3D structures for those three antagonists were refined using the PM3 method in the MOPAC 7 program [45] and assigned with AM1-BCC partial charges [46–48] by using the QuACPAC program [49]. Molecular docking of the antagonists into the active site of H_1 receptor homology model was performed by DOCK 5.4 program [50]. After molecular docking, the ligand-receptor complexes were then optimized using the same parameters as depicted in the refinement phase and then analyzed by HBPLUS [51], LIGPLOT [52], and Pymol [53] programs.

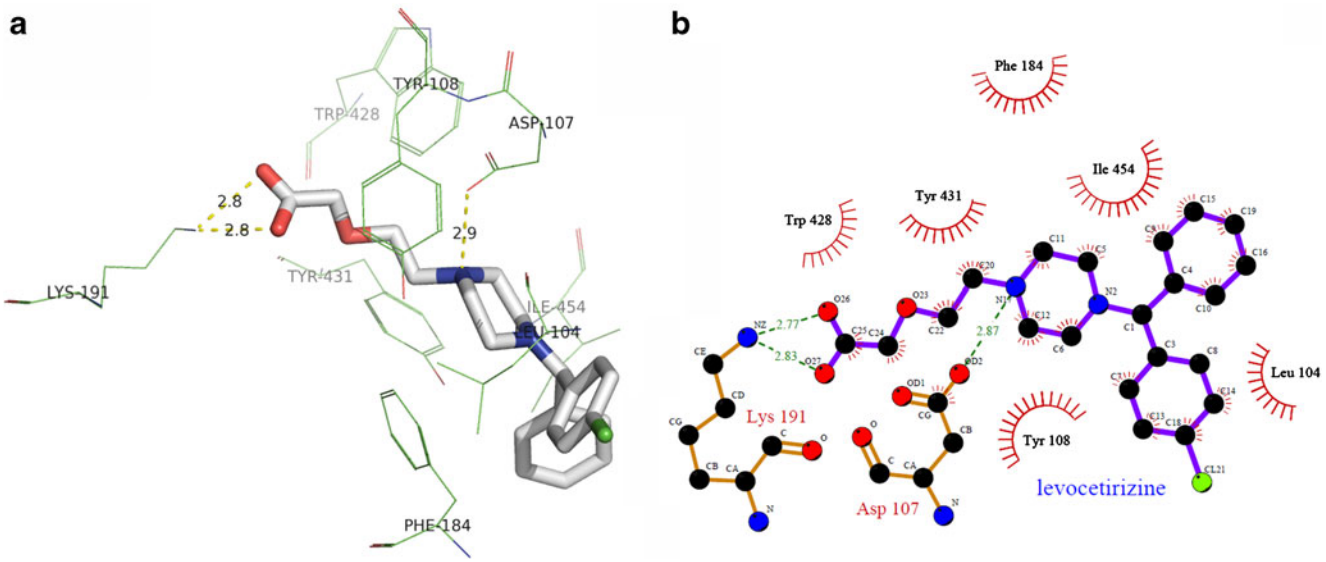

Fig. 8 (**a**) Predicted binding models for Levocetirizine, (**b**) The structure of Levocetirizine-H1 receptor homology model complex was analyzed using the Ligplot 4.22 program to identify some specific contacts between atoms of ligand and receptor

Table 1 Results of the common feature hypothesis run[a]

Hypothesis No.	Composition[b]	Ranking score	Direct hit	Partial hit
1	RPYZ	35.742	111	000
2	RPYZ	35.652	111	000
3	RPYZ	35.609	111	000
4	RPYZ	35.532	111	000
5	RPYZ	35.501	111	000
6	RPYZ	35.499	111	000
7	RPYZ	35.487	111	000
8	RPYZ	35.414	111	000
9	RPYZ	35.356	111	000
10	RPYZ	35.322	111	000

[a] Direct hit, all the features of the hypothesis are mapped.
Direct hit=1 means yes and Direct hit=0 is no;
Partial hit, partial mapping of the hypothesis.
Partial hit=1 means yes and Partial hit=0 means no.
[b] Abbreviation used for features:
R: ring aromatic;
P: positive ionizable;
Y: hydrophobic aromatic;
Z: hydrophobic aliphatic.

Pharmacophore modeling

Based on the above computational results, a docking-based pharmacophore model was established by using HipHop algorithm implemented in the Catalyst 4.11 package, which could reflect common structural features of H_1 receptor antagonists among a set of highly active compounds without the use of activity data. This 'qualitative model' represents the essential 3D arrangement of functional groups common to active molecules for interacting with a specific biological target [54]. In the pharmacophore generation, the docking structures of all compounds were used. A default uncertainty factor of 3 for each compound was defined, and six chemical features, including hydrogen-bond acceptor (A), hydrogen-bond donor (D), aromatic ring (R), positive ionizable (P), hydrophobic aromatic (Y) and hydrophobic aliphatic (Z) group, were selected to generate the HipHop pharmacophore hypothesis. A principal number of 2 and MaxOmitFeat number of 0 was defined for the good mapping of all features of these compounds on the hypothetic model [55].

Hardwares and softwares

Homology modeling, DOPE analysis (MODELLER 9v7), binding analysis (HBPLUS 3.06 and Ligplot 4.22), and visualization of models (PyMOL 0.99) were carried out on a Linux workstation. The pharmacophore modeling (Catalyst 4.11) was executed on a SGI Origin 3800 workstation equipped with 48×400 MHz MIPS R12000 processors. MM and MD simulations (CHARMM c33b1) were performed on URSA, a 160-processor computer based on Power5+ processor and IBM's P series architecture. PROCHECK, WHAT-IF (http://swift.cmbi.kun.nl/WIWWWI/), VERIFY-3D and ERRAT (http://nihserver.mbi.ucla.edu/SAVS/) validations were computed on-line.

Results and discussion

Verification of homology model

The final sequence alignment of HHR1 with human A_{2A} adenosine receptor, human β_2-adrenoceptor and turkey β_1-adrenoceptor shows about 26%, 31% and 31% homology identity, respectively (Fig. 1). In addition, the root-mean-square deviation (RMSD) between the backbone atoms of human A_{2A} adenosine receptor, human β_2-adrenoceptor and turkey β_1-adrenoceptor, and the rough HHR1 homology model is only 2.44, 2.33 and 2.57 Å, respectively. Moreover, because of the evidence that specific parts in the transmembrane domains of the GPCRs' helices are less flexible than others as suggested by Rayan [56], the RMSD values of the whole TMDs in the predicted HH1R model versus those counterparts in templates were also compared. The RMSD value between the transmembrane domains of human A_{2A} adenosine receptor, human β_2-adrenoceptor and turkey β_1-adrenoceptor, and the rough HHR1 homol-

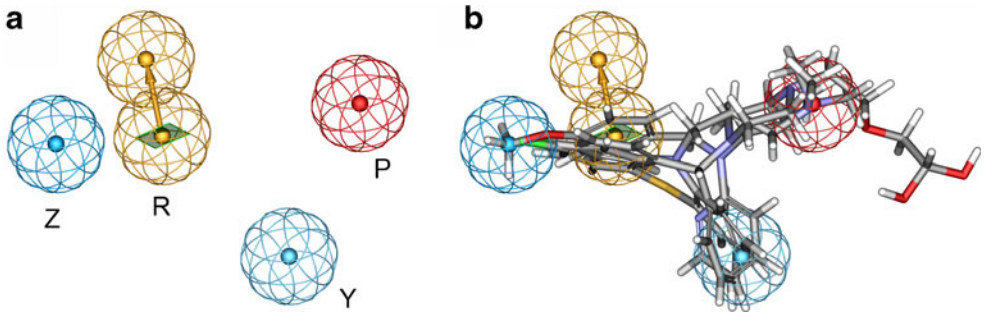

Fig. 9 (**a**) Top scoring pharmacophore model of H1 antagonists. The pharmacophore model contains aromatic ring (**R**), hydrophobic aromatic (**Y**), hydrophobic aliphatic (**Z**) and positive ionizable (**P**) features; (**b**) The top hypothesis is mapped onto the docking conformation of Mepyramine, Chlorpromazine, and Levocetirizine

Table 2 The distances (Å) between pharmacophore features for H1 antagonists[a]

Feature	R	P	Y	Z
R	–	7.16	5.98	3.75
P	7.16	–	6.13	11.05
Y	5.98	6.13	–	8.85
Z	3.75	11.05	8.85	–

[a] Abbreviation used for features: R: ring aromatic; P: positive ionizable; Y: hydrophobic aromatic; Z: hydrophobic aliphatic

ogy model is 1.57, 1.49 and 1.63 Å, respectively. These low RMSD values indicate a very similar conformation with templates and a reliable initial structure for further MM and MD simulations (Fig. 4).

It is well known that classical MD simulations in vacuum and in solution can severely distort loop structures, especially for membrane proteins [57]. Therefore, we conducted a MD simulation using a GBSA/IM implicit membrane model in CHARMM. During the MD optimization, the RMSD values of the HHR1 backbone atoms and all heavy atoms in the whole system are plotted as time-dependent functions in Fig. 5. The RMSD value of backbone atoms and all heavy atoms in the whole system tends to be convergent after 3 ns with fluctuations around 4 Å and 4.5 Å, respectively. Such evidence indicates that the system was equilibrated after 3 ns of MD simulation to provide a stable model for HHR1.

In the validation phase, the quality of H_1 receptor has been checked by five generally used criteria, including PROCHECK [39], ERRAT [40], VERIFY-3D [41], WHAT-IF [42], PROSA2003 [43], and DOPE [44] methods. The overall results show that the geometric quality of the backbone conformation, the residue interaction, the residue contact, and the energy profile of the structure seem well within the limits established for reliable structures, which suggest that a reasonable homology for H_1 receptor has been obtained to allow for examination of protein-substrate interaction.

Molecular docking studies

The ligand-binding cavities between the active site of H_1 receptor and Mepyramine, Chlorpromazine, Levocetirizine have been thoroughly investigated. As the representative of the first generation of H_1 receptor antagonists, Mepyramine is chosen as typical H_1 receptor antagonist for the docking study. According to Fig. 6, the cationic nitrogen on the side chain creates ionic interaction (or so-called salt bridge) with Asp107, which was highlighted as a crucial action for the ligand-receptor binding. Moreover, the two aromatic pyridine and methylbenzene groups are engaged in the π-stacking with Trp428, Tyr431, Phe435, Tyr108, and Phe184 Chlorpromazine which bears a rigid tricyclic scaffold is selected as another sample for docking research. As shown in Fig. 7, the aromatic framework of Chlorpromazine is trapped in an aromatic/hydrophobic pocket formed by

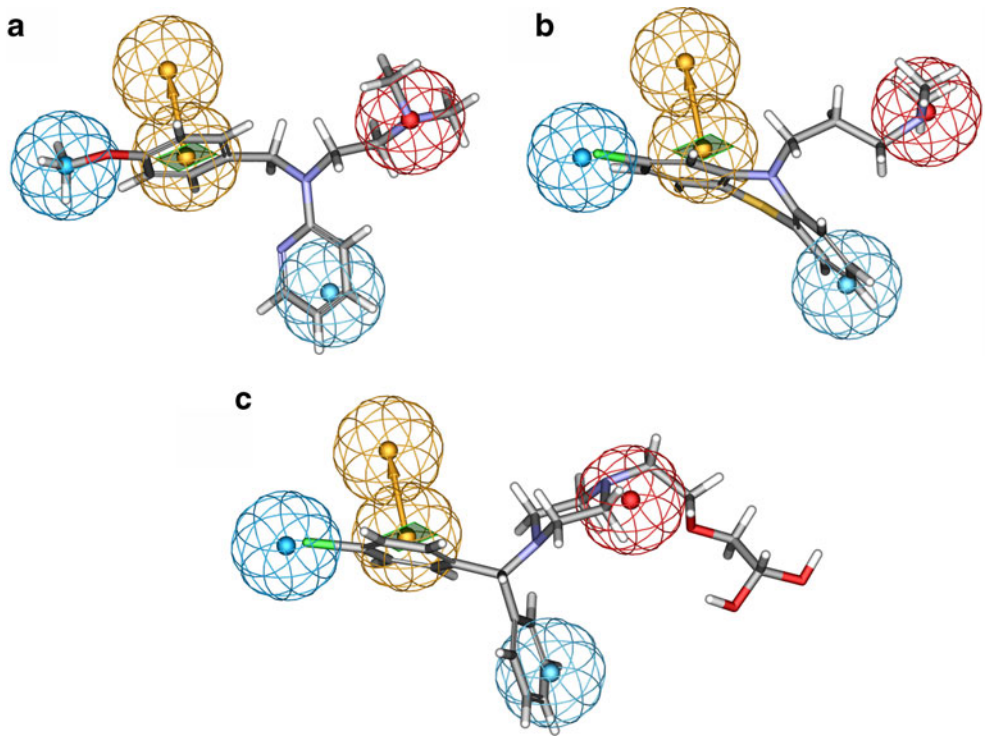

Fig. 10 The top scoring pharmacophore model is mapped onto the docking conformation of (**a**) Mepyramine, (**b**) Chlorpromazine and (**c**) Levocetirizine, respectively

Leu104, Tyr108, Trp428, Tyr431, Phe435, Tyr458, and Phe184, meanwhile the positive ionizable nitrogen on the side chain can also touch Asp107 by ionic interaction. Some structures of second generation H_1 receptor antagonists contain a carboxylate group in order to avoid the possible blood-brain barrier (BBB) side effect. The mutagenesis study illustrated that the interaction between these zwitterionic molecules and the H_1 receptor is novel and Lys191 plays an important role for the type of antagonists binding [58]. However, these results failed to conclude the accurate interaction mode. Therefore, Levocetirizine, a well-known antihistamine drug, is chosen for our docking research to find out the binding pattern between the zwitterionic ligands and the H_1 receptor. More than the previous ironic interaction on the Asp107 and the hydrophobic interaction, the computational results also discloses that the carboxylate group of Levocetirizine binds to Lys191 through an additional but critical ionic interaction (Fig. 8). This strengthened interaction explains the strong potency of Levocetirizine against H_1 receptor.

Docking-based pharmacophore modeling

The pharmacophore model of H_1 receptor antagonists was established on the basis of the docking conformations of Mepyramine, Chlorpromazine, and Levocetirizine. The common-feature generation approach Hiphop was employed to test the coherence of the homology model with the ligand-based model. In the current study, Hypo 1 is the best pharmacophore model among the 10 hypothesis that Hiphop produced with the highest ranking score (Table 1). It is worth noted that the docking conformations of these three compounds could match the features generated in Hypo 1 very well respectively (Fig. 9). The distances between the pharmacophoric features have been presented in Table 2, which are identical with the previous study on SAR which elucidates the essential criterion for antagonists binding. Then the docking conformations of Mepyramine (fitness value: 4), Chlorpromazine (fitness value: 3.454), and Levocetirizine (fitness value: 3.205) have been mapped onto the pharmacophore model (Fig. 10).

For a more restricted validation of this pharmacophore model, an external set, including 17 active and 33 inactive HHR1 antagonists, was selected for a fishing test from the literature [59]. Out of 17 active molecules, 15 (88.2%) were estimated to have >3 fitness scores as depicted in supplemental Table S1. In the meantime, 31 (93.3%) out of inactive compounds were predicted to have <3 fitness score by our pharmacophore model as represented in Table S2. These results again confirmed our confidence on the accuracy of both homology model and pharmacophore hypothesis for HHR1. Therefore, the pharmacophore features around the active site not only solidifies confidence on the homology model, but also augments the description on the detailed information of the SAR results. We do think that such combinations of homology model and docking-based pharmacophore model would be useful in designing new leads.

Conclusions

In this paper, a highly accurate homology model of human H_1 receptor is developed on the basis of A_{2A} adenosine receptor crystal structure. After MD simulations, this homology model is validated by several methods, including PORCHECK, ERRAT, WHAT-IF, PROSA2003, and DOPE. Subsequently, three structural diverse H_1 receptor antagonists, Mepyramine, Chlorpromazine and Levocetirizine, which belong to three generations of H_1 receptor antagonists, were chosen for the forthcoming molecular docking. The docking results discloses that around the active site of H_1 receptor, some residues, such as Asp107, Tyr431, Phe432, Phe184, Tyr108, and Phe435, have been proven to play important roles in ligand binding. Significantly, in the case of ligand-receptor research of zwitterionic drugs Levocetirizine with the H_1 homology model established herein, it has been disclosed that Lys191 could form an additional ionic interaction with its carboxylic acid, which provides important guidance for designing new leads with low risk on BBB. On the other hand, the docking-based qualitative pharmacophore model could also provide useful information for ligand-based drug design. In a word, the combination of homology modeling and pharmacophore would be very useful in understanding the binding modes of H_1 receptor antagonists. We believe that these results may be of great help in understanding of the action mechanism of H_1 receptor and the design of potent agents for the treatment of allergy.

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