

Topical Perspectives

Can human allergy drug fexofenadine, an antagonist of histamine (H₁) receptor, be used to treat dog and cat? Homology modeling, docking and molecular dynamic Simulation of three H₁ receptors in complex with fexofenadine

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ABSTRACT

Fexofenadine, a potent antagonist to human histamine 1 (H₁) receptor, is a non-sedative third generation antihistamine that is widely used to treat various human allergic conditions such as allergic rhinitis, conjunctivitis and atopic dermatitis. Encouragingly, it's been successfully used to treat canine atopic dermatitis, this supports the notion that it might have a great potential for treating other canine allergic conditions and other mammal pets such as dog. Regrettably, while there is a myriad of studies conducted on the interactions of antihistamines with human H₁ receptor, the similar studies on non-human pet H₁ are considerably scarce. The published studies using the first and second generation antihistamines drugs have shown that the antihistamine response is varied and unpredictable. Thus, to probe its efficacy on pet, the homology models of dog and cat H₁ receptors were built based on the crystal structure of human H₁ receptor bound to antagonist doxepin (PDB 3RZE) and fexofenadine was subsequently docked to human, dog and cat H₁ receptors. The docked complexes are then subjected to 1000 ns molecular dynamics (MD) simulations with explicit membrane. Our calculated MM/GBSA binding energies indicated that fexofenadine binds comparably to the three receptors; and our MD data also showed the binding poses, structural and dynamic features among three receptors are very similar. Therefore, our data supported the application of fexofenadine to the H₁ related allergic conditions of dog and cat. Nonetheless, subtle systemic differences among human, dog and cat H₁ receptors were also identified. Clearly, there is still a space to develop a more selective, potent and safe antihistamine alternatives such as Fexofenadine for dog or cat based on these differences. Our computation approach might provide a fast and economic way to predict if human antihistamine drugs can also be safely and efficaciously administered to animals.

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1. Introduction

Human histamine receptor is a G-protein coupled receptor (GPCR) comprising four subtypes: H₁, H₂, H₃, and H₄. All of them are naturally activated by their specific endogenous ligand histamine. However, H₁, recognized as the only allergy triggering receptor, has become a primary pharmacological target for antihistaminic drugs to treat histamine-mediated allergic conditions: allergic rhinitis, conjunctivitis, contact/atopic dermatitis etc. [1]. Antihistaminic drugs that alleviate the allergic conditions possess antagonistic activity on H₁ receptor, thus inhibiting signaling

transduction pathway that is normally activated by histamine [2]. Fexofenadine (Fig. 1) [3], being a widely used antihistamine drug to treat various human allergic cases, is a metabolite derivative of non-sedative second generation antihistamine Terfenadine. Furthermore, Fexofenadine is informally labelled as a third-generation antihistamine drug because it is associated with a decreased risk of cardiac arrhythmia compared to Terfenadine [4]. Since Fexofenadine was approved to treat human allergy by the Food and Drug Administration (FDA) in 1996, it achieved the blockbuster drug status and was further approved by FDA as an over-the-counter drug in 2011.

Likewise, house-held mammal pets such as dog and cat also suffer from histamine-mediated allergic conditions. Their allergic diseases are attributed to activation of H₁ receptor in a similar way as its counterpart in to human version and consequently creating vasoconstriction, tachycardia, abnormal cardiac output, and

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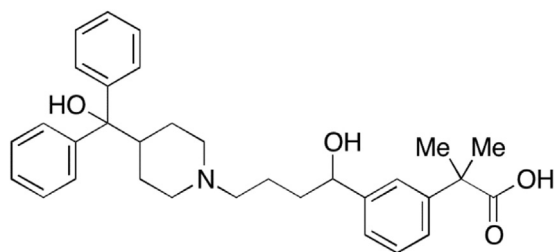


Fig. 1. Chemical structure of fexofenadine.

etc [5]. While there is an abundance of studies conducted on the pharmacological effects of antihistamines on human H_1 receptor [6–8]. The amount of *in vivo* and *in vitro* studies of these antihistaminic agents on dog/cat H_1 subtypes are in literature. The computational studies are essentially absent. Presently, it is understood with the correct doses and frequencies of administration of human H_1 antagonists, antihistamines targeting human can be useful to manage skin disease such as pruritus in cats and dogs in a preventative and slow-active fashion. Unfortunately, the published studies using the first and second generation drugs have shown that the antihistamine response is varied and unpredictable [9,10]. The sequence variation of H_1 receptor for different species is one of the most important factors that determine the efficacy of a particular antihistamine on specific species. In fact, dog and cat H_1 receptors [11] are ~15% genetically different to the human version [12] (Fig. 2). The subtle sequence differences can substantially modulate the receptor-drug interaction and thus the drug efficacy.

Typically, careful clinical trial courses of different antihistamines are required to identify an effective one. As to third generation antihistamine, fexofenadine's antihistaminic efficacy and medical safety in treatment of canine atopic dermatitis (a hypersensitive skin disorder of dogs) has been documented experimentally in 2006 where the severity of pruritus (severe itching of skin) and skin lesions symptomatic of canine atopic dermatitis were reduced after administering fexofenadine to a representative sample of dogs [13]. However, it is not yet reported whether fexofenadine can be used to treat other canine allergic conditions or those of the other mammal pets such as cat. For the purpose of this study, we would like to provide fundamental molecular energetic, structural and dynamic information on the H_1 receptor-ligand (Fexofenadine) interactions toward answering this question. In additions, the species-specific differences can shed light on developing more potent antihistamine drugs specific to specific household pet in the future.

Currently, the computational chemistry techniques of pharmacophore modeling, homology modeling, molecular docking, and virtual screening have been used to screen appropriate human H_1 antagonists as well as to investigate the detailed binding poses between the ligand and human H_1 receptor [14,15–17]. Molecular docking and molecular dynamics (MD) studies at H_1 homology and crystal structure models were used to decipher molecular determinants of antagonists binding at the human H_1 receptor [18]. The quantum-chemical calculations was used to examine the deuteration effects of the H_2 antagonist binding [19]. The three-dimensional quantitative structure-activity relationship (3D-QSAR) techniques, were used to analyze 129 human H_1 antagonist [20]. The activity of 12 human H_1 and guinea pig histamine

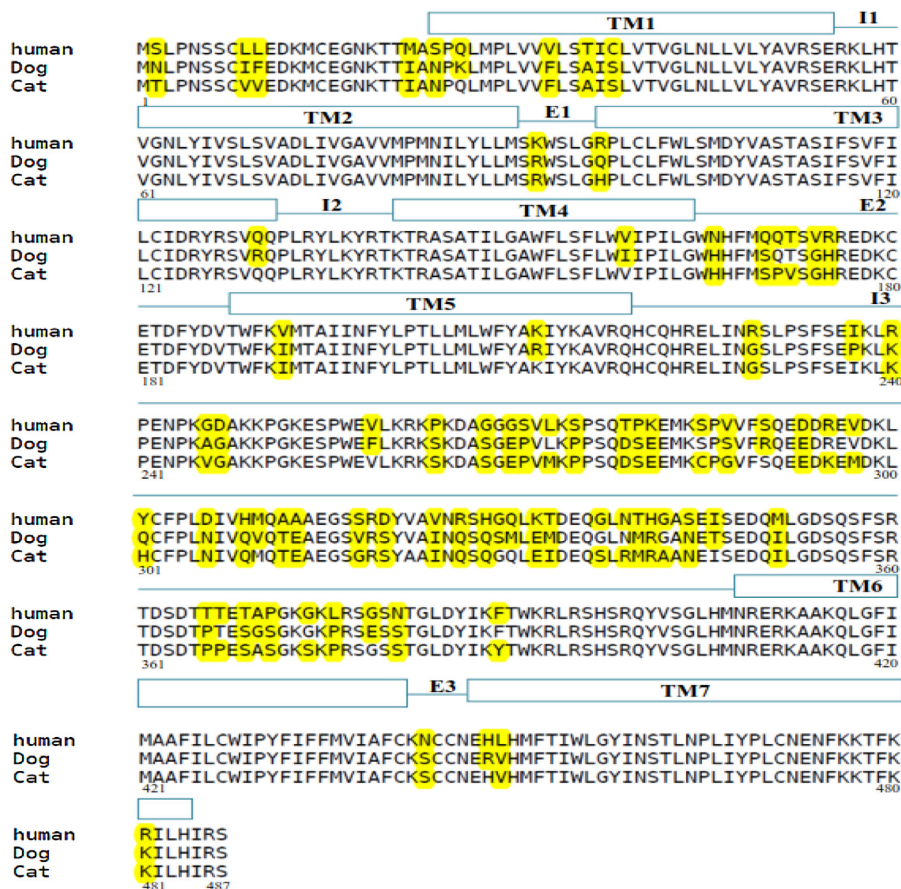


Fig. 2. Sequences of full-length human, dog and cat H_1 receptors. Rectangular boxes refer to the transmembrane helices. The yellow highlight represents non-conserved amino acids with respect to the human H_1 . The intracellular (I1–I3) and extracellular (E1–E3) loops connecting the transmembrane helices are illustrated as threads labelled according to loop location.

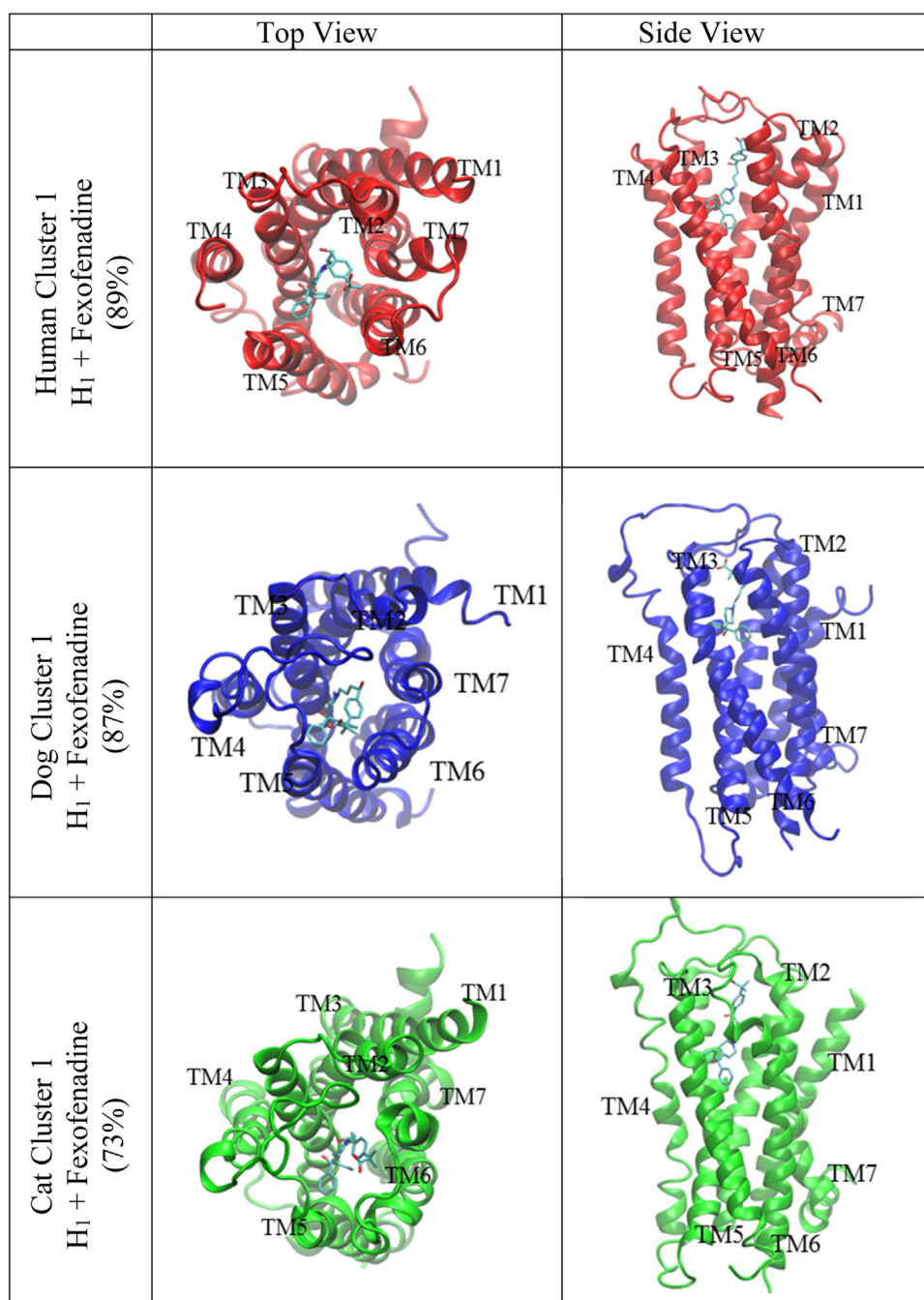


Fig. 3. Representative structures of H₁-Fexofenadine complexes of top structural families from the clustering analysis. The residues (175–187) were removed to improve ligand visualization. Abundance is annotated.

H₁ agonists was analyzed using molecular surface electrostatic potential and other molecular descriptors [21]. Although these computational studies provide important molecular insights on human/pig H₁ receptor and their ligands, the more exhaustive details regarding dog and cat's H₁ receptors and their interactions with ligands remains to be elusive.

Thus, the interactions between human, dog and cat H₁ receptor with fexofenadine were examined in this study. We applied homology modeling, molecular docking and MD simulations to understand how human and non-human (dog and cat) H₁ receptors responded to Fexofenadine binding. Our H₁ receptor homology models for dog and cat were based on the crystal structure of human H₁ bound to antagonist doxepin (3.1 Å, PDB ID: 3RZE) [1]. Fexofenadine were docked to the crystal structure of human H₁ and the

homology models of dog and cat. These complexes were used as initial structures for 1000 ns MD simulations. The detailed energetic, structural and dynamic properties of the three complexes were thoroughly analyzed and compared. The pharmacological implications of our results will be discussed.

2. Computational materials and methods

2.1. Homology modeling of Dog/Cat H₁ receptors

The homology model of dog and cat H₁ receptors were obtained using Prime utility [22,23], which used the FASTA files from Uniprot [24] as the source of protein sequences (Fig. 2) and human H₁ crystal structure (PDB ID: 3RZE, See Fig. S1 in the supporting material)

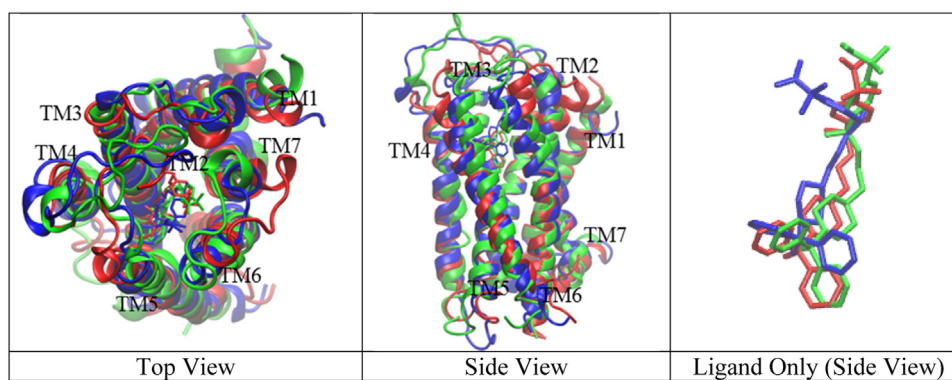


Fig. 4. Superposition of the most abundant complexes from the MD simulations. Red, blue and green colors refer to human, dog and cat H₁ receptors respectively. The residues (175–187) were removed to improve ligand visualization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

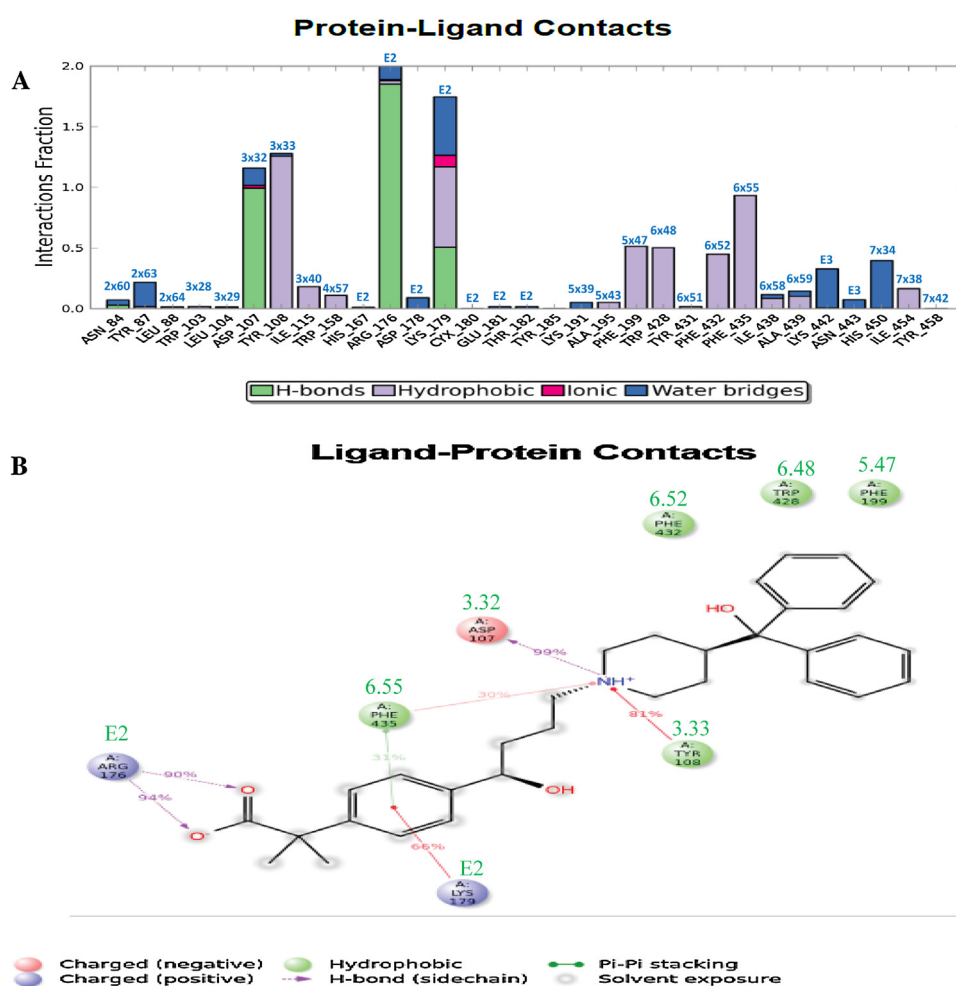


Fig. 5. Protein-ligand contacts between Fexofenadine and human H₁ receptor. A: Histogram of interactions fraction. B: Detailed ligand-protein interactions that persist more than 30% of simulation time. Genetic numbering is annotated for each interacting residue.

as the structural template. Whereas the missing short extracellular loop (residues from 167 to 175) for the three receptors was built by the Prime program, the missing intracellular loop (residues 221–405) was too large to be built. The structures of full-length H₁ receptors of human, dog and cat are shown in (Fig. S2).

2.2. Ligand preparation

The 2D structure of fexofenadine, being drawn using the 2D sketcher, was converted into 3D structure. The bond order was fixed for the crystal ligand doxepin, followed by adding hydrogen atoms. Then the protonation or ionization/tautomeric state of the two lig-

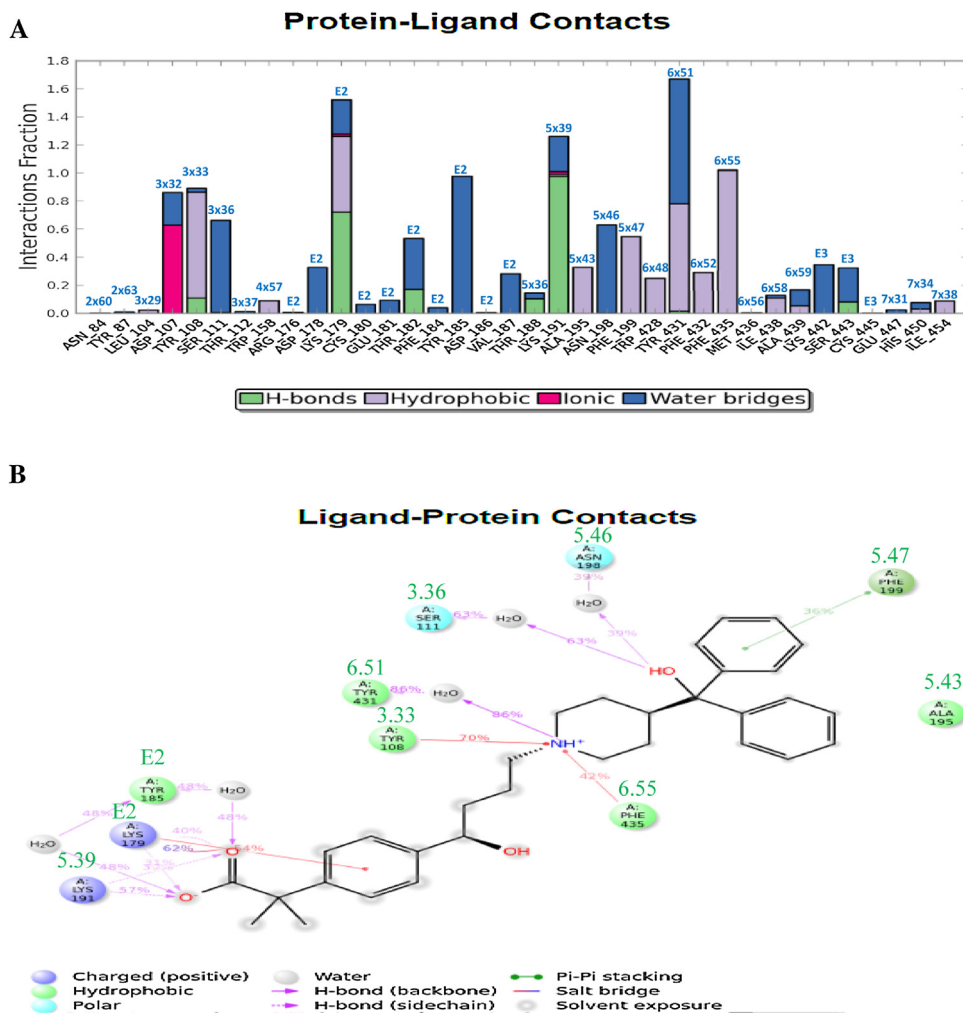


Fig. 6. Protein-ligand contacts between fexofenadine and dog H_1 receptor. A: Histogram of interactions fraction. B: Detailed ligand-protein interactions that persist more than 30% of simulation time. Genetic numbering is annotated for each interacting residue.

ands was generated at pH=7 using the Epik tool [25]. Restrained minimization was used to relax the ligand molecular structure.

2.3. Protein structure preparation

Protein structures of human, dog and cat H_1 receptors were prepared using Schrodinger Protein Preparation Wizard [25]. The charge state of preprocessed proteins were optimized at pH=7. Finally, the restrained minimization of the protein receptors was performed to relax the protein structures using OPLS3 force field [26].

2.4. Ligand docking

The active site of the prepared receptors was specified by the crystal ligand and the prepared ligands (both doxepin and fexofenadine) were docked into the active site using Glide Dock XP scoring function with default procedures, metrics, and parameters [27,28]. The reproduction of the crystal binding pose of doxepin (Fig. S1) provides a validation for our docking protocol. The 3D docking results of Fexofenadine in complex with human, dog and cat H_1 receptor structures are included in Fig. S2 of the supporting material.

2.5. Simulation system setup

Three molecular dynamic simulation systems were constructed using the prepared receptor-ligand complexes from the Glide XP docking. Each system was placed in a membrane of POPC lipids [29], and solvated in an orthorhombic water box with a buffer distance of 10 Å using SPC water model [30]. System was neutralized using Na^+ ions, and was added with a salt concentration of 0.15 M NaCl [17]. OPLS3 force field [26] was used to represent the receptor-ligand complex.

2.6. System relaxation and production runs

Using the Desmond module, simulation systems were first relaxed using the default relaxation protocol for membrane proteins and lipids [31]. This relaxation protocol consists of eight stages: 1). Minimization with restraints on solute heavy atoms; 2) Minimization without any restraints; 3). Simulation with heating from 0 K to 300 K, H_2O barrier and gradual restraining; 4). Simulation under the NPT ensemble (constant number of particles, constant pressure of 1 bar and constant temperature of 300 K), Martyna-Tuckerman-Klein Nosé-Hoover chain coupling scheme [32] with H_2O barrier and with heavy atoms restrained; 5) Simulation under the NPT ensemble with equilibration of solvent and lipids; 6). Simulation under the NPT ensemble with protein heavy atoms annealing from 10.0 kcal/mol to 2.0 kcal/mol; 7). Simulation

under the NPT ensemble with C α atoms restrained at 2 kcal/mol; and 8). Simulation for 1.5 ns under the standard NPT ensemble for partial rigid-body molecular dynamic simulation method with no restraints. After the relaxation, a 1000.0 ns production run was conducted under the NPT ensemble for each of the three systems using the default simulation protocol [33]. M-SHAKE [34] was applied to constrain all bonds connecting hydrogen atoms, enabling a 2.0 fs time step in the simulations. The k-space Gaussian split Ewald method [35] was used to treat long-range electrostatic interactions under periodic boundary conditions (charge grid spacing of ~ 1.0 Å, and direct sum tolerance of 10^{-9}). The cutoff distance for short-range non-bonded interactions was 9 Å, with the long-range van der Waals interactions based on a uniform density approximation. To reduce the computation, non-bonded forces were calculated using an r-RESPA integrator [36] where the short range forces were updated every step and the long range forces were updated every three steps. Temperature value was controlled using the Martyna-Tuckerman-Klein Nosé-Hoover chain coupling scheme with a coupling constant of 1.0 ps. The Desmond trajectories of three system were saved at 40.0 ps intervals for analysis.

2.7. Convergence of simulation

Convergence of simulation were inspected using both protein and ligand Root Mean Standard Deviation (RMSD) relative to the initial frame. RMSD plots of Fexofenadine-H₁ are shown in Fig. S3. A flattened plot at the end of trajectories (i.e. last 200 ns) refer to an equilibrated simulation and the corresponding frames can be used to extract equilibrium properties such as the binding energy.

2.8. Trajectory clustering analysis

Protein backbone RMSD matrix was used in hierarchical cluster average linkage method [37] to group the structures in the simulation trajectory. The merging distance cutoff was set to be 2.5 Å. The centroid structure (i.e. the structure having the largest number of neighbors in the structural family) was used to represent the structural family of the Fexofenadine-H₁ complex. The centroid structures of top structural families (>2% of total structure population) are shown in Fig. S4 of the supporting information.

2.9. Receptor-ligand interaction analysis

Detailed receptor-ligand interaction was analyzed using Simulation Interaction Diagram (SIM) module of Schrödinger suite. The 2D receptor-ligand interaction diagrams were used to explore four types of specific and non-specific protein-ligand interactions: hydrogen bonds, hydrophobic, ionic, and Water Bridge. The results are included in Fig. 5–7.

2.10. Binding energy calculations and decomposition methods

To calculate respective binding energies of Fexofenadine to human, cat, and dog H₁ receptor, Molecular Mechanics-Generalized Born Surface Area (MM/GBSA) binding energies were calculated based on snapshots from the last 200 ns of each trajectory. A surface-area-based version of the Generalized Born model [38,39] with implicit membrane model was used (VSGB 2.0) [40]. The implicit membrane is a slab-shaped region with a low dielectric constant between 1 and 4, and the regions to exclude from membrane is assigned with the solvent (water) dielectric constant of 80. The OPLS3 force field and the default Prime protocol was used [41]. The OPLS3 force field employs a CM1A-BCC based charge model based on a combination of Cramer-Truhlar CM1A charges [42] with an extensive parameterization of bond charge correction terms (BCC). The step wise energy calculations as follows: 1) Receptor

alone (minimization). 2) Ligand alone (minimization). 3) Receptor-ligand complex (minimization) 4) Ligand extracted from optimized receptor-ligand complex (energy calculation) 5) Receptor extracted from optimized receptor-ligand complex (energy calculation) [43]. As for analyzing the MM-GBSA binding energy decompositions in this study, we merge the original terms (Coulombic + H-bond + GB solvation + van der Waals + π - π packing + self-contact + lipophilic) into three components: $E_{\text{electrostatics}}$, E_{vdw} , and $E_{\text{lipophilic}}$, where $E_{\text{electrostatics}} = (H_{\text{bond}} + E_{\text{coulomb}} + E_{\text{GB.solvation}})$, $E_{\text{vdw}} = (E_{\text{vdW}} + E_{\pi-\pi} + E_{\text{self-contact}})$ and $E_{\text{lipophilic}}$. Systematic benchmarking studies up to 1864 crystal complexes have shown that MMGBSA is a powerful tool to rank ligands [44–48].

3. Results

3.1. Homology modeling yielded similar human, dog, and cat H₁ structures

Homology models of the H₁ receptors for dog and cat were obtained based on experimentally resolved crystal structure of human H₁ complex with doxepin (PDB ID: 3RZE) [1] as described in the methods section. Cartoon representations are shown in Fig. S2 from top and side viewpoints. The sequence differences between human H₁ receptor and household pet H₁ receptors provide a base for understanding the different responses to the same drug and for designing more selective pharmacological ligands toward specific species. These homology models can be good starting structures for molecular docking and simulations to inspect species specific ligand-protein interactions.

3.2. Fexofenadine glide XP docking data indicates similar binding poses in human and non-human H₁ receptors

First of all, we validated our docking protocol by docking the crystal ligand back to the crystal structure of human H₁ receptors (Fig. S1). The excellent overlap between the crystal pose and the docked pose of the ligand suggest a satisfactory docking method. Using the same protocol, Fexofenadine was docked into three receptors, resulting in similar binding poses (Fig. S2).

3.3. RMSD data indicates the convergence of MD simulations

The complexes from the docking were utilized as input structures to perform 1 μ s molecular dynamic simulation of each system. In the C α -RMSD plots (Fig. S3) for all simulation systems (human, dog, and cat), both protein RMSD and ligand RMSD are approaching stable values with the last 200 ns. This indicates that the three H₁ complexes had been adequately and sufficiently equilibrated, allowing for rigorous analysis. Subtle differences were also notified: 1). Human H₁ and cat H₁ receptor with the RMSD of ~ 2.9 Å had slightly larger conformation change than dog H₁ receptor of the RMSD ~ 2.0 Å. 2). Fexofenadine had a larger binding pose change in the human system at ~ 500 ns. 3). Fexofenadine in the human and dog system had slightly larger conformation change (ligand RMSD of ~ 2.0 Å) than that in the cat system (ligand RMSD of 1.6 Å).

3.4. MM/GBSA calculations show fexofenadine binds comparably to human and non-human H1 receptors

MM/GBSA calculation was utilized to estimate binding energy (ΔG_{bind}) and relative binding energy ($\Delta \Delta G_{\text{bind}}$) of Fexofenadine to the three H₁ receptors (Table 1). Compared with human H₁, $\Delta \Delta G_{\text{bind}}$ of dog and cat systems were 1.4 kcal/mol and -0.2 kcal/mol, respectively. These small binding energy differences revealed that fexofenadine possesses practically similar binding

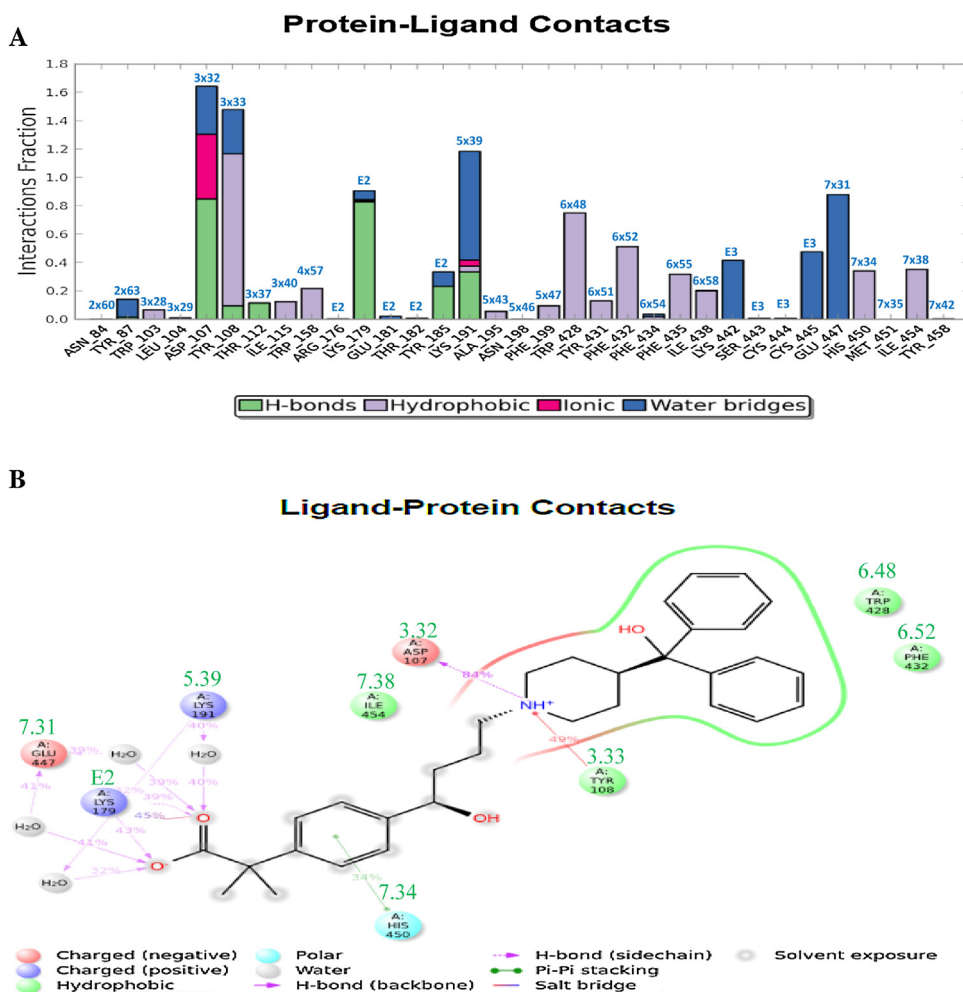


Fig. 7. Protein-ligand contacts between fexofenadine and cat H_1 receptor. A: Histogram of interactions fraction. B: Detailed ligand-protein interactions that persist more than 30% of simulation time. Genetic numbering is annotated for each interacting residue.

Table 1
MM/GBSA binding energies of Fexofenadine to human, dog and cat H_1 receptors.

	VDW ^a (kcal/mol)	GBELE ^b (kcal/mol)	LIPO ^c (kcal/mol)	ΔG^d (kcal/mol)	$\Delta \Delta G^e$ (kcal/mol)
Human	-52.1 ± 3.9	-70.4 ± 7.2	-45.6 ± 2.4	-168.1 ± 8.7	0.0
Dog	-67.8 ± 3.9	-39.1 ± 6.7	-62.7 ± 2.5	-169.5 ± 7.0	1.4
Cat	-62.1 ± 3.0	-48.7 ± 8.0	-57.1 ± 3.0	-167.9 ± 9.0	-0.2

^a Change of van Der Waals energy in gas phase upon complex formation.

^b Change of electrostatic energy upon complex formation.

^c Change of hydrophobic energy upon complex formation.

^d Change of total potential energy upon complex formation.

^e Change of the binding energy relative to human H_1 receptor.

affinity for human and house pet's H_1 receptors. It is worth mentioning that MM/GBSA scoring function efficacy and efficiency in estimating ΔG_{bind} and $\Delta \Delta G_{\text{bind}}$ of ligand-protein complexes were investigated recently by numerous literature [44–47,49]. Most remarkable contribution was made by Hou et al. [50] where they evaluated MM/GBSA scoring function based on MD simulation and proved its superiority over MM/PBSA (Molecular Mechanics/Poisson Boltzmann Surface Area) in ranking multiple ligands based on relative binding energies. However, the latter was better than former in calculating the absolute ΔG_{bind} .

Our MM/GBSA decomposition results indicated there is appreciable discrepancies (10–15 kcal/mol) in contribution of van der Waals energy (ΔG_{VDW}) to the ΔG_{bind} between human and non-human H_1 receptor. Likewise, contribution of ΔG_{liPO} terms for each activating Fexofenadine complex was observed to be different by

12–18 kcal/mol for human compared to cat/dog. However, the differences between these two terms is offset by the contribution of electrostatic energy, where human is about $-31/-21$ kcal/mol more favorable than dog and cat. The different composition of the binding energy between human and non-human reflects the change of the interaction nature due to their sequence differences. Nonetheless, the overall binding does not change significantly from human to dog and cat.

3.5. Fexofenadine has a very similar binding pose in human and non-human H_1 receptors

Fexofenadine binding poses in human and non-human H_1 receptors were analyzed based on trajectories of MD simulation. Each trajectory was clustered as described in the method section

and the representative structures of the most abundant structural family for each system are shown in Fig. 3. Clearly, fexofenadine binding to both human and household pet H₁ receptor adopts three slightly different but virtually similar poses. To facilitate the comparison, we also superimposed the three complexes (human, dog and cat H₁ receptor) for the same ligand fexofenadine in (Fig. 4). Interestingly, the fexofenadine poses from cat (red) and human (green) system have a better overlap than that (blue) from dog system. This result is consistent with our MM/GBSA binding energy data that the ligand binding energy of cat H₁ receptor system is very similar to that of human (−0.2 kcal/mol); and the binding energy of dog system is weaker by a difference of 1.4 kcal/mol.

3.6. 2D interaction data from the trajectories show key residues contributing to the fexofenadine binding among the three complexes are highly conserved

To identify critical residues contributing to Fexofenadine bindings in each receptor, we performed simulation interaction diagram analysis implemented in Maestro program. The raw data are included in Fig. 5–7 and Fexofenadine interacts with a total of 23 residues in three receptors. More importantly, the trans-membrane helices 3, 5, 6 and 7, involving in the H₁ receptor activation, are all participating in ligand interacting. The key interacting residues for each receptor system were tabulated in Table 2. Cat H₁ shares more identical interacting residues with human H₁ receptor, while Dog H₁ shows slightly more difference to human H₁ receptor such as Ser111^{3,36}, Asp186^{E2}, Val187^{E2}, Thr188^{5,36} and Met436^{6,56}. Snake plots of three receptors is shown in Fig. S5 to visualize the key interaction residues.

3.7. The H₁ receptor secondary structure elements (SSE) for human, dog and cat are notably homogeneous throughout the simulation except for subtle differences in the SSE distribution

To investigate the secondary structure of the receptors, the abundance of SSE composition of H₁ receptor over the course of simulation is shown in Fig. 8. In the 3 complexes, the extreme similar abundance of helices (Human: 63.48% vs. Dog: 61.87% vs. Cat: 60.59%) is observed. Seven helices are easily identified, more subtle differences are located at TMs 2, 4, 5 and 7 as well as at the loop regions.

3.8. P-RMSF data indicates similar dynamic responses for human, dog and cat H₁ in complex with fexofenadine

The three P-RMSF plots are shown in Fig. 9. Characteristically, we observed the general trend that the most rigid parts of the receptor (i.e. secondary structure elements such as the seven trans-membrane helices) exhibit lower RMSF values (fluctuate less) while loose secondary loop structures such as the N- and C-terminals and intra- and extra- cellular loops show higher RMSF values (fluctuate more) based on the visual inspection of the comparative height of the peaks. Given the hypothesized biological responses of the different histamine receptors in response to the same stimulating ligand, these over-all dynamic similarities could contribute to the similar biological responses (i.e. inhibit the activation of H₁ receptors) by modulating the interaction between the H₁ receptors and down-stream signal transduction proteins such as G-protein or β -arrestin. Subtle differences are identified to be located at flexible loop regions. Biological consequence of these differences remains to be elusive with the biological activity and other studies.

Table 2

Interacting residues of Fexofenadine with the three H₁ receptors.

GEN-NO.	Human H ₁	Dog H ₁	Cat H ₁
2.60	ASN84	ASN84	ASN84
2.63	TYR87	TYR87	TYR87
2.64	LEU88		
3.28	TRP103		TRP103
3.29	LEU104	LEU104	LEU104
3.32	ASP107	ASP107	ASP107
3.33	TYR108	TYR108	TYR108
3.36		SER111	
3.37		THR112	THR112
3.40	ILE115		ILE115
4.57	TRP158	TRP158	TRP158
E2	HIS167		
E2	ARG176	ARG176	ARG176
E2	ASP178	ASP178	
E2	LYS179	LYS179	LYS179
E2	CYS180	CYS180	
E2	GLU181	GLU181	GLU181
E2	THR182	THR182	THR182
E2		PHE184	
E2	TYR185	TYR185	TYR185
E2		ASP186	
E2		VAL187	
5.36		THR188	
5.39	LYS191	LYS191	LYS191
5.43	ALA195	ALA195	ALA195
5.46		ASN198	ASN198
5.47	PHE199	PHE199	PHE199
6.48	TRP428	TRP428	TRP428
6.51	TYR431	TYR431	TYR431
6.52	PHE432	PHE432	PHE432
6.54			PHE434
6.55	PHE435	PHE435	PHE435
6.56		MET436	
6.58	ILE438	ILE438	ILE438
6.59	ALA439	ALA439	
E3	LYS442	LYS442	LYS442
E3	ASN443	SER443	SER443
E3			CYS444
E3		CYS445	CYS445
7.31		GLU447	GLU447
7.34	HIS450	HIS450	HIS450
7.35			MET451
7.38	ILE454	ILE454	ILE454
7.42	TYR458		TYR458

3.9. L-RMSF plots shows similar pattern when binding with human and non-human H₁ receptors

L-RMSF analysis is useful in detecting ligand fluctuations at atomic level during trajectory. L-RMSF of Fexofenadine binding to human, cat and dog H₁ receptors shows a consistent conformation dynamics patterns as can be observed in Fig. 10. Another interesting observation is the higher L-RMSF value in human H₁ compared to non-human H₁ (Dog and Cat). The torsion angle distribution of every rotatable bond in Fexofenadine is included in Fig. S6. The very similar distribution for the three systems indicates that fexofenadine undergoes similar conformational strain to maintain protein-bound conformation. Again, subtle differences are also identified, reflecting the sequence differences of the three receptors.

4. Discussion & conclusion

The compelling clinical success of third-generation antihistamines such as fexofenadine in treating human allergic diseases necessitates more research to probe its potential therapeutic antihistaminic benefit in common household pets such as dog and cat. Yet, the dog and cat H₁ characteristics and their antihistamines binding properties were not explored previously. In this study,

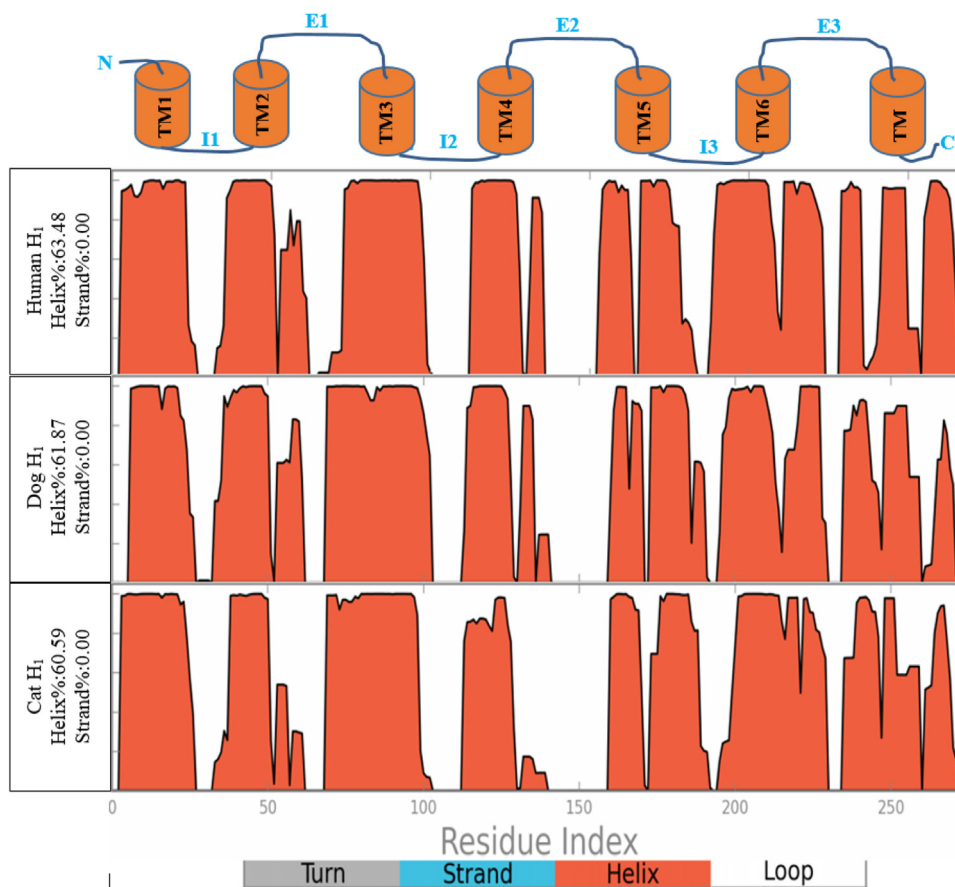


Fig. 8. Protein secondary structures elements profiles of fexofenadine binding to the human, dog and cat H₁ receptors. Left panel shows the percentages of abundances of each secondary structure element throughout simulation time. X-axis represents residue index of H₁ receptor.

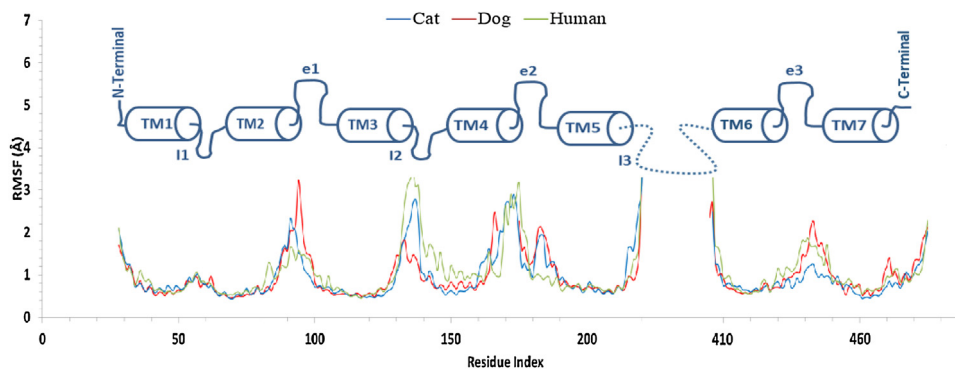


Fig. 9. Protein Root Mean Square Fluctuation (P-RMSF) of human, dog and cat H₁ receptors in complex with fexofenadine.

we explored these non-human H₁ characteristics by probing the energetic, dynamic and structural properties of dog and cat H₁ receptors in complex with the antagonist fexofenadine. We hope these detailed information might be helpful in theoretically and computationally predicting the efficacy of fexofenadine in treating H₁ related allergic conditions of dog and cat. The success would substantially improve success rate in identifying effective antihistamines for common household pet.

Because the interaction between antihistamine antagonist fexofenadine and human H₁ has been previously studied experimentally [51,2], and computationally [18,52], we want to validate our methods by comparing our human H₁-fexofenadine results to them. Being a small and compacted molecule, fexofenadine could adopt different binding poses within binding pocket; however,

the key residues involved in our human H₁-fexofenadine binding pose have been consistent with most of previous experimental and computational studies. For example, most of the important interaction residues contacting fexofenadine observed in early molecular determinant studies such as Trp^{6.48}, Phe^{6.52}, Tyr^{3.33}, Asn^{5.46}, Thr^{5.42} etc. [18] are also consistent across the board with our computational results. This validated and substantiated our computational methodologies.

Interestingly, our MD data show that human, dog and cat H₁ receptors adopt very similar conformations and have comparable binding affinities to the antihistamine fexofenadine. Fexofenadine adopts a similar binding pose and mainly interacts with TMs 3, 5, 6 and 7 in the three H₁ receptors. The three receptors have similar structural fluctuation at the E2 and I3 loops and the ligand has sim-

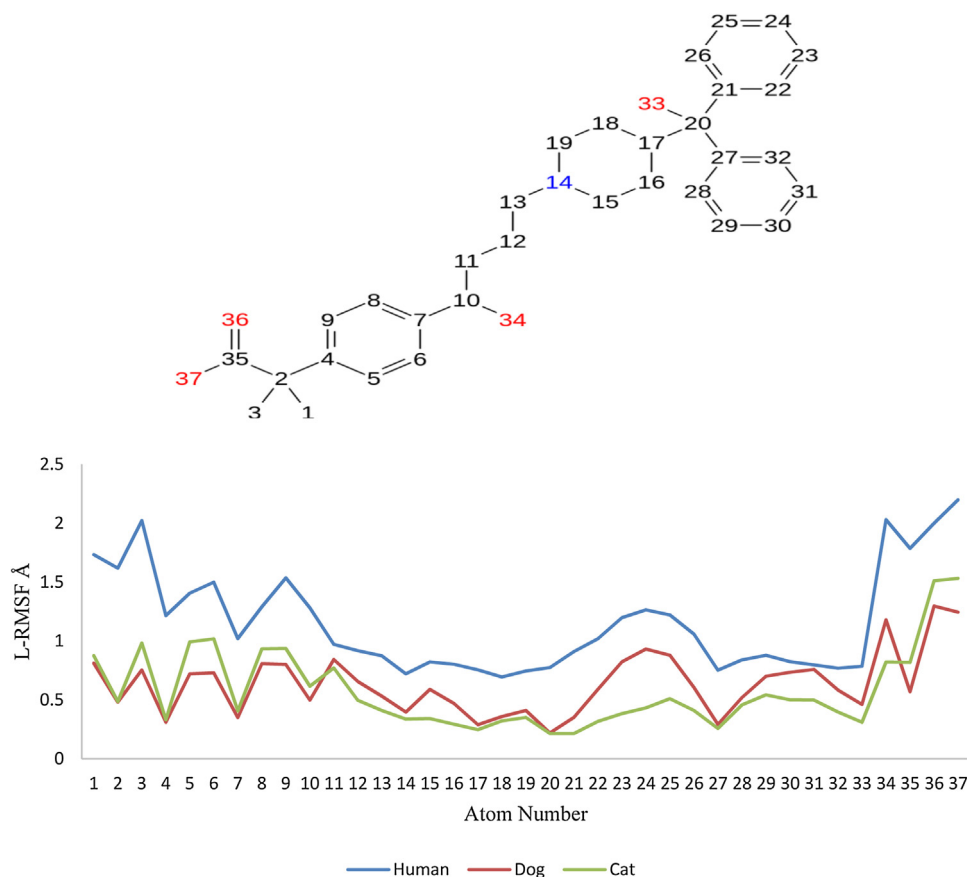


Fig. 10. Ligand Root Mean Square Deviation (L-RMSF) diagrams of fexofenadine in complex with human, dog and cat H₁ receptors.

ilar conformation fluctuation. Therefore, our energetic, structural and dynamic data cautiously supports similar efficacy of fexofenadine to dog and cat. This conclusion is partially supported by the report that canine atopic dermatitis were reduced after administering Fexofenadine to a representative sample of dogs [13]. Along the same line, our results cautiously supports potential applications of fexofenadine to cat and to other allergenic conditions that have not been tried. Given the complex of biology, further *in vitro/in vivo* studies are required to assess our predictions. On the other hand, our data indeed show subtle systemic differences among human, dog and cat H₁ receptors. First, although the overall MM/GBSA binding energy of Fexofenadine to the three receptors is very similar, the energetic composition are significantly different between human and non-human receptors. This reflects the change of the interaction nature due to their sequence differences. Second, Cat H₁ receptor shares more identical interacting residues with human H₁ receptor, while Dog H₁ shows slightly more difference to human H₁ receptor such as Ser111^{3.36}, Asp186^{E2}, Val187^{E2}, Thr188^{5.36} and Met436^{6.56}. This is also consistent with our MM/GBSA binding energy data that the ligand binding energy of cat system is very similar to that of human (−0.2 kcal/mol); and the binding energy of dog system is weaker by a difference of 1.4 kcal/mol. Third, the different receptors have more subtle structural differences located at TMs 2, 4, 5 and 7 as well as at the loop regions. Fourth, Fexofenadine has a higher position fluctuation in human H₁ receptor compared to non-human H₁ (Dog and Cat) receptors. Therefore, there is still a space to develop more selective, potent and safe antihistamines for dog or cat. Our detailed findings will provide important insights into the development of more safe, potent and selective agents assisting veterinarian to treat household pet allergic illnesses with specific therapeutic agent. This will also encourage future attempts

to design library of antihistamine compounds selective to other classes of Histamine receptors to achieve high efficacy and lowest side effects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jmngm.2017.05.010>.

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Supporting Information

The 3D-structures of Fexofenadine-H₁ complexes obtained from Glide XP Docking, the RMSD plots of each trajectory, the representative complex structures of top structural families obtained the clustering analysis, the ligand torsion profiles and the PDB files of the most abundant conformation for each species are included in the supporting documents.

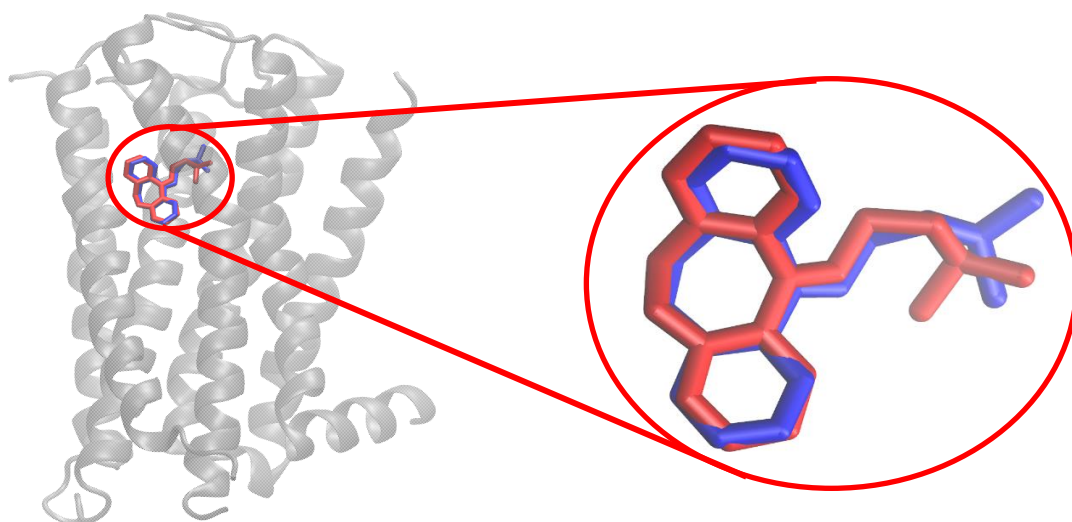


Figure S1 Superimposition between the crystal and docked pose of the crystal ligand in 3RZE.

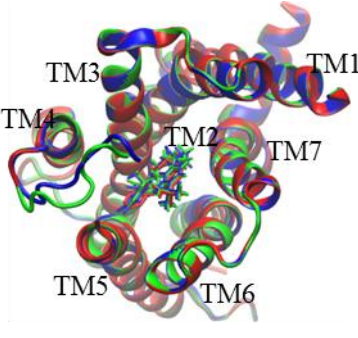
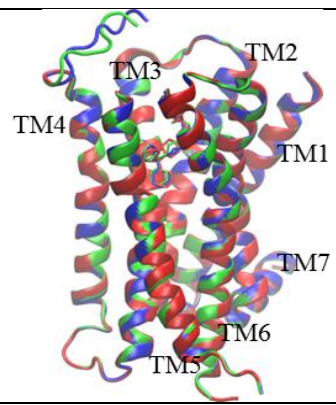
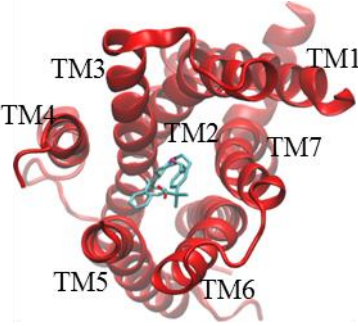
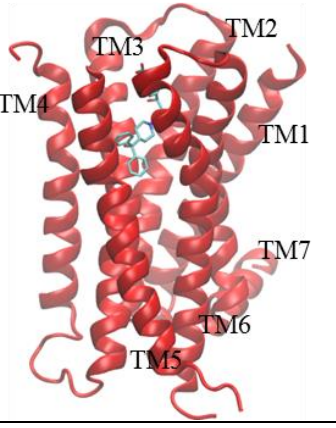
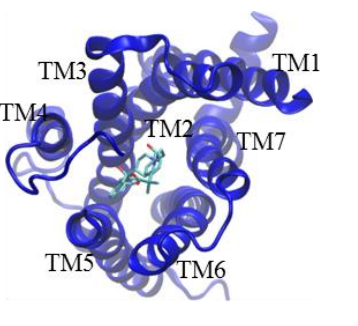
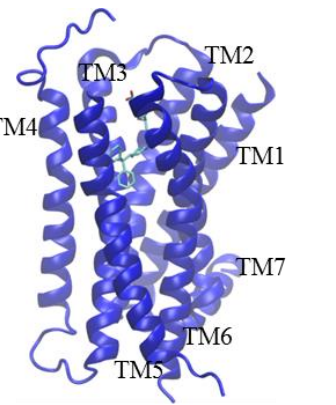
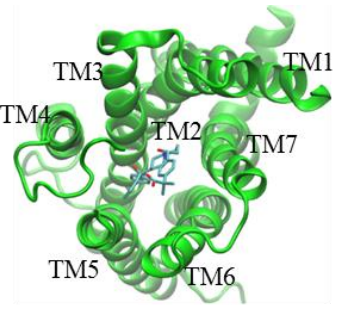
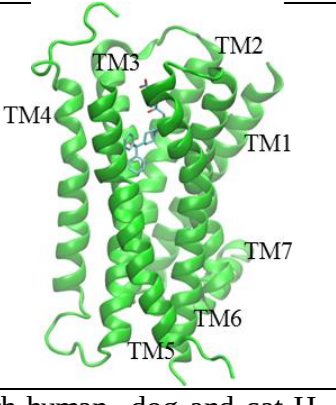
Receptor	Top View	Side View
Superimposed Human + Dog + Cat H1R with Fexofenadine		
Human H1R + Fexofenadine		
Dog H1R + Fexofenadine		
Cat H1R + Fexofenadine		

Figure S2 Structures of fexofenadine in complex with human, dog and cat H₁ receptor obtained from Glide XP docking. The residues (175-187) were removed to improve ligand visualization.

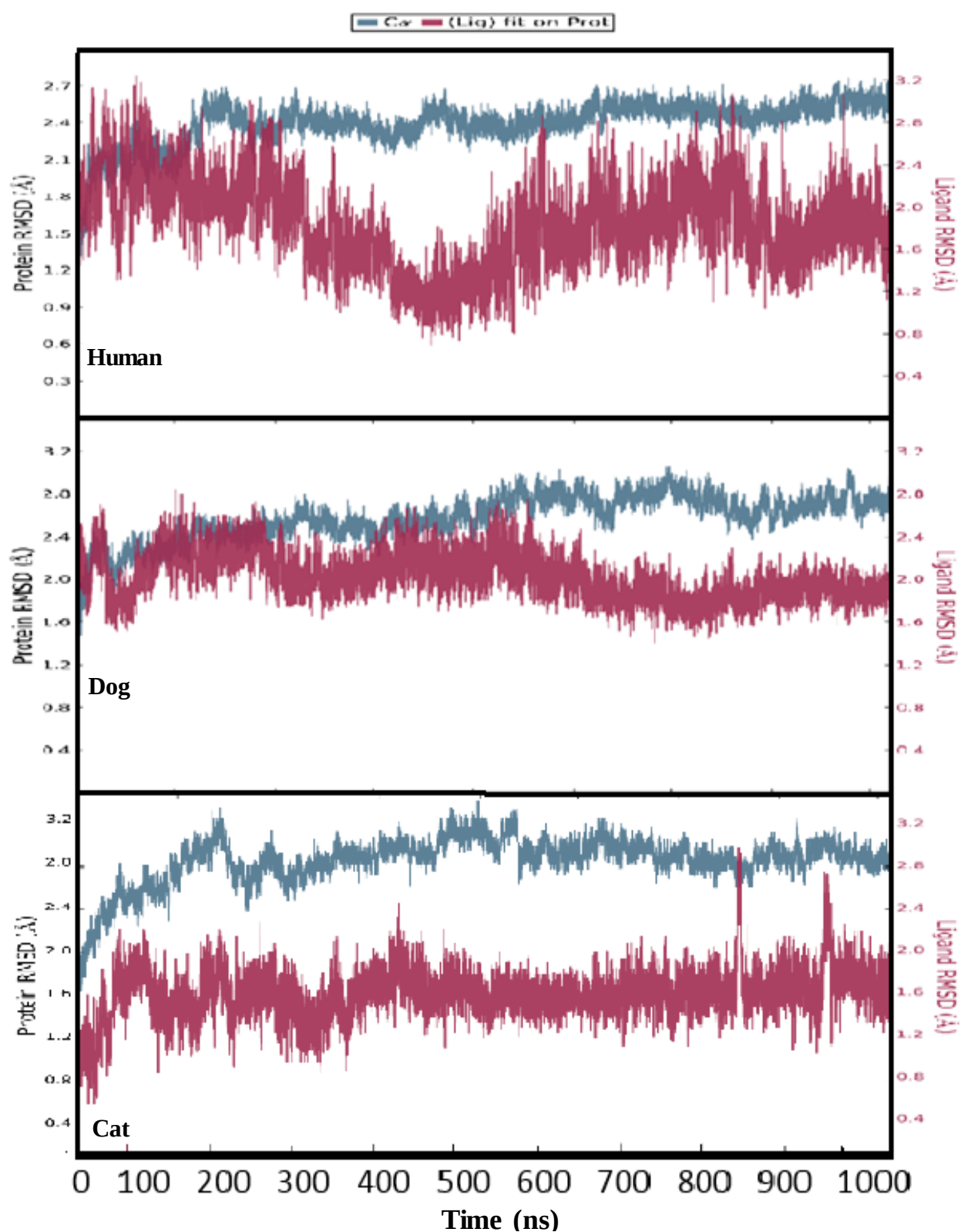
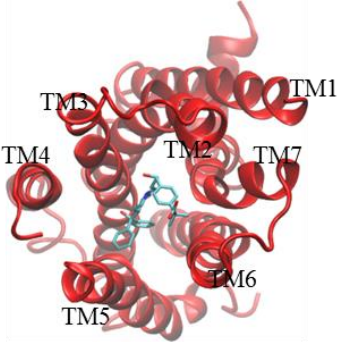
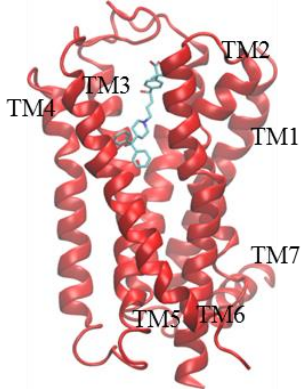
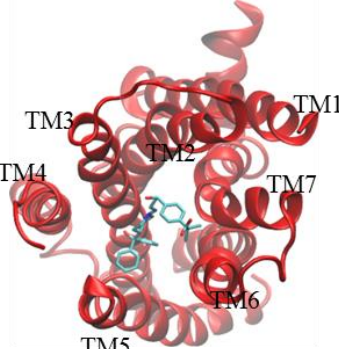
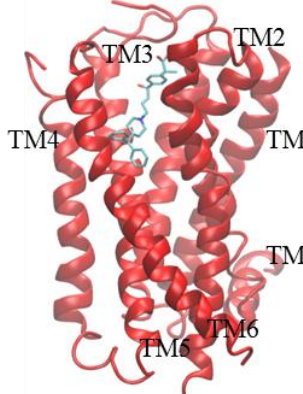
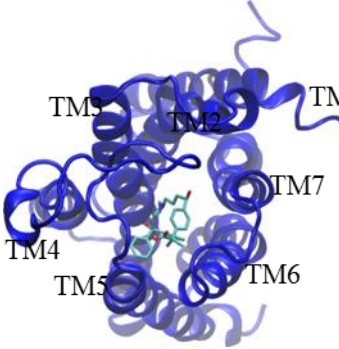
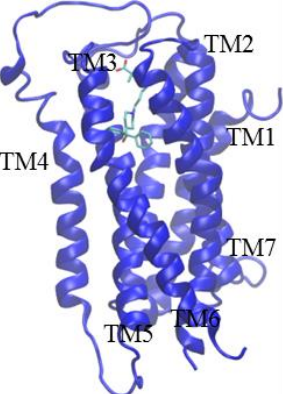


Figure S3 Root Mean Square deviation diagrams (RMSD) of Fexofenadine- H_1 trajectories in three species. Red and blue plots refer to ligand and protein RMSDs, respectively.

	Top View	Side View
Human Cluster 1 H1R + Fexofenadine (89%)		
Human Cluster 2 H1R + Fexofenadine (11%)		
Dog Cluster 1 H1R + Fexofenadine (87%)		

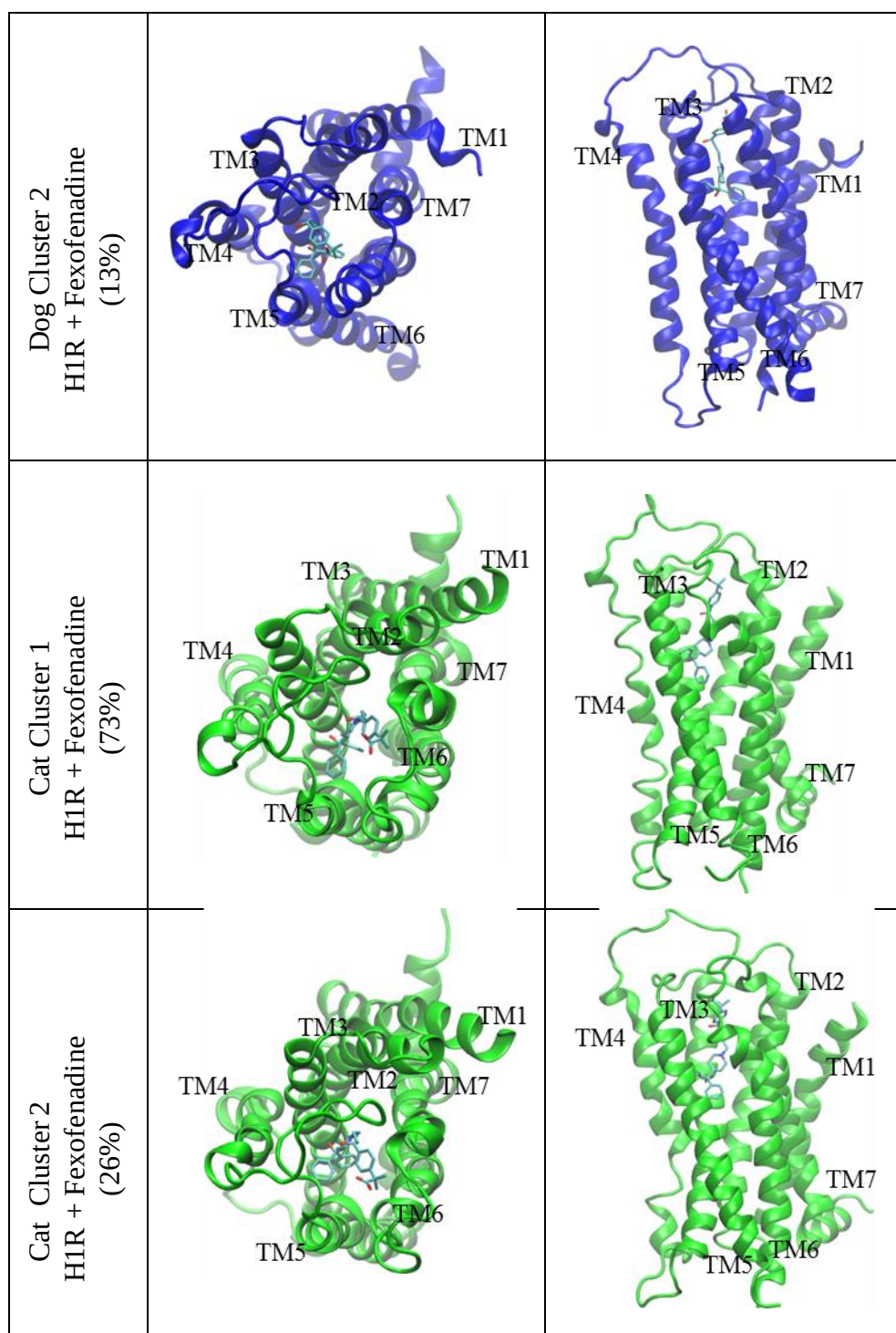


Figure S4 Representative structures of top structural families of fexofenadine-H1 complexes from the clustering analysis. The residues (175-187) were removed to improve ligand visualization. Abundance is annotated.

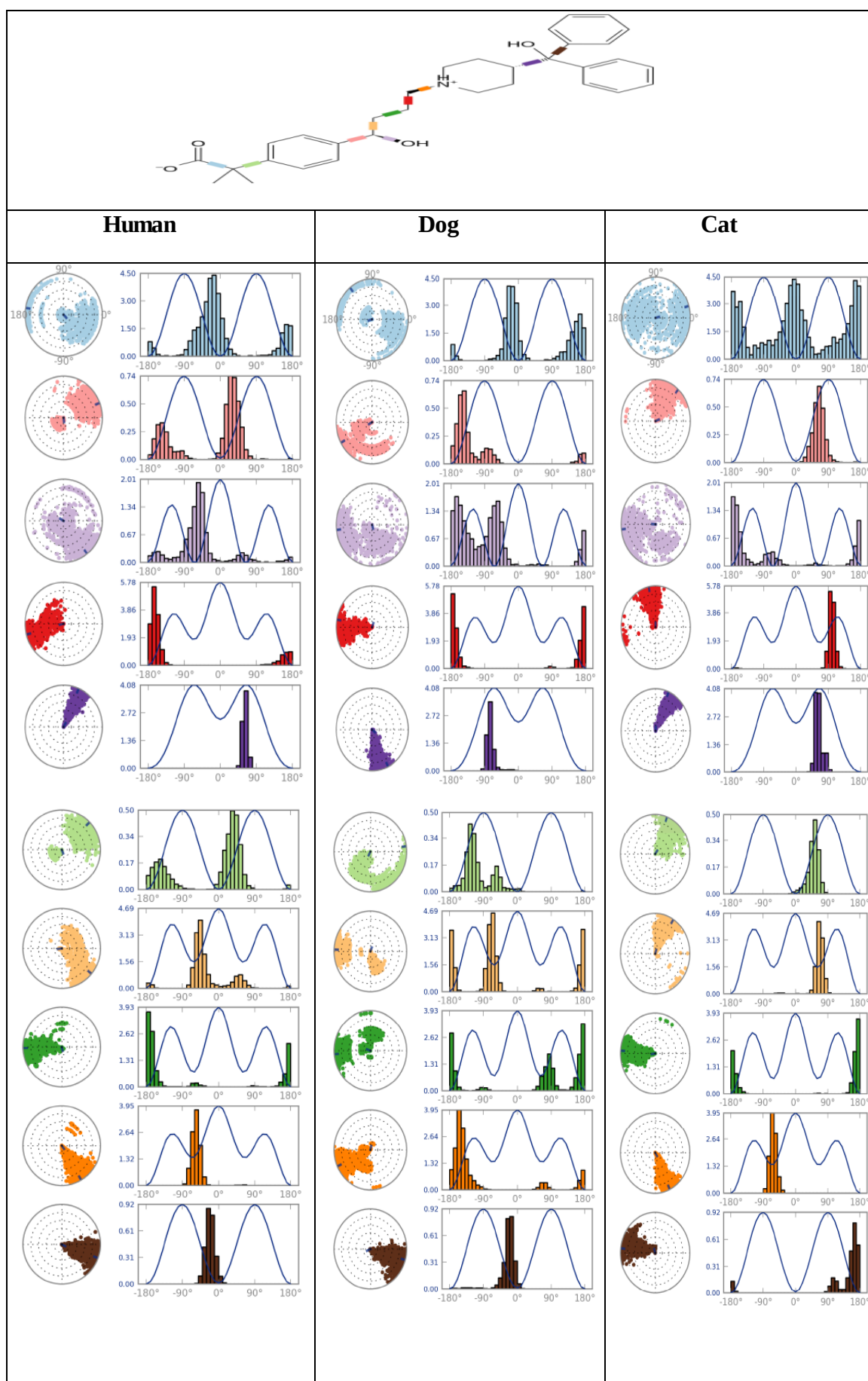


Figure S6 Plots of ligand torsion profiles of fexofenadine binding to the three H₁ receptors.