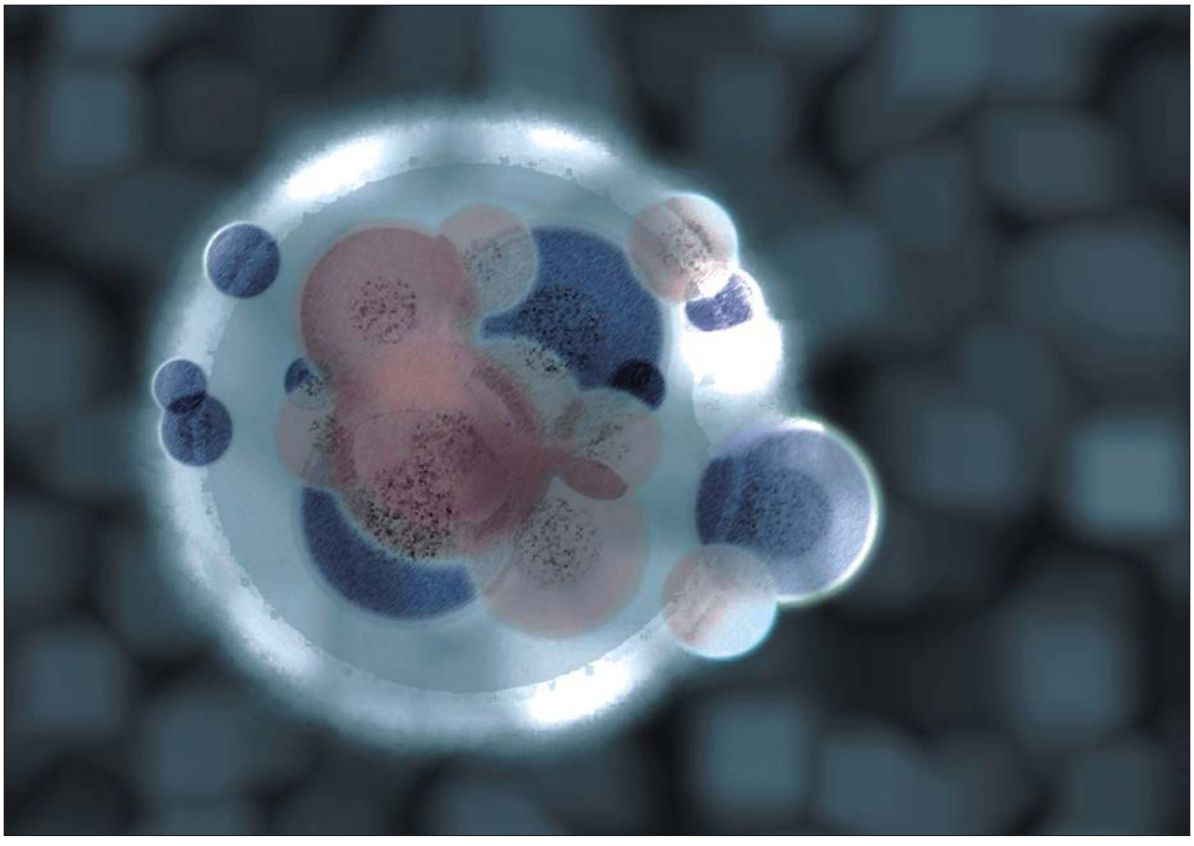

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Research Article
In silico Characterization and Homology Modeling of Histamine Receptors

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Abstract
Background and Objective: Histamine plays a vital role in molecular mechanism of allergic reactions. Therefore, characterization and homology modeling of histamine receptors is of great importance. This study focused on the physicochemical properties, motif analysis, transmembrane region prediction and 3D structure analysis of histamine receptors. **Materials and Methods:** The sequences retrieved from UniProtKB were further analyzed with ExPASy ProtParam tool. Conservancy analysis was done using ClustalW and further resynchronized with Jalview 2. Family and domain prediction, motif analysis and transmembrane region prediction was performed in InterPro, MEME suite and TMHMM Server v. 2.0, respectively. Finally, 3D structure prediction was performed by I-tasser software and result validation was done through RAMPAGE, ERRAT and PROCHECK. **Results:** Physicochemical properties of these histamine receptors were analyzed and found molecular weight around 55.7 KDa, theoretical pI 9.33-9.62, instability index 34.93-47.00, aliphatic index (AI) was above 90 and most of the receptors were hydrophobic except histamine H1 receptor. Histamine receptors lacking satisfactory conserved region but region 75-94 was found promising after multiple sequence alignment. Histamine receptors are members of family G protein-coupled rhodopsin-like receptor. A profound motif from 84-149 for four histamine receptors with significantly lower E-value was observed. These receptors were seven pass transmembrane protein and found the gap between transmembrane helix number 5th and 6th of each histamine receptor except histamine H2 receptor which can be potential drug target candidate. Finally, 3D models of these receptors were developed through homology modeling and best models were selected by applying different model validation tools. **Conclusion:** The potential drug targets of this study can be useful in designing more sustainable antihistamines and relevant drugs in treatment of allergic diseases.

Key words: Histamine receptors, physiochemical properties, motif analysis, homology modeling, drug targets for histamine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Histamine is a biological amine neurotransmitter which is produced due to the response of an allergic reaction of immune system after invasion of antigen. This reaction leads to pruritus, inflammation, sneezing, edema, bronchospasm and increased mucus secretion. There are four histamine receptors within humans body namely, histamine H1 receptor (HRH1), histamine H2 receptor (HRH2), histamine H3 receptor (HRH3) and histamine H4 receptor (HRH4). These receptors provoke allergenic reactions from different dimensions. Histamine H1 receptor is G protein coupled receptor, which up regulates NF-κB activity there by, elicits inflammation. Interestingly, histamine H1 receptor also binds with endogenous histamines and performs as biological clock that influences sleep wake state[1]. Crystal structure of histamine H1 receptor has already been reported[2]. Histamine H2 receptor is also G protein coupled receptor and responsible for cAMP production as it is coupled with adenylate cyclase[3]. Histamine H2 receptor is potent stimulant of gastric acid secretion, vasodilation and smooth muscle relaxation. It also plays pivotal role in immune system as it aids in T-cell proliferation and cytokine production. In contrast to this, histamine H2 receptor inhibits antibody production, neutrophil activation and chemotaxis. Histamine H3 receptor is associated with Central Nervous system (CNS) and controls the synthesis and release of endogenous histamines through feedback mechanism[4]. Like all other histamine receptors, H3 is also a G protein coupled receptor but it inhibits cAMP synthesis. Histamine H3 receptor is vastly distributed in human tissue system, i.e., heart tissue, lungs, endothelial liner cells, gastrointestinal tract (GI), peripheral nervous system and central nervous system. Histamine H4 receptor is another G protein coupled receptor widely expressed in haemopoietic cells. This receptor is frequently found in different parts of human body such as bone marrow, lungs, small intestine, spleen, colon, liver, oral epithelium, testes, thymus, trachea and tonsils[5]. As histamine H4 receptor is highly expressed in bone marrow, it controls the release and activity of neutrophil[6] and it also mediates change in shape of eosinophil as well as its migration and mast cell degranulation. Immunomodulatory functions such as T-cell differentiation and dendritic cell activation are characteristics role of histamine H4 receptor. Altogether this immune responses lead to chronic inflammation, allergy, asthma, chronic pruritus and other autoimmune diseases.

The histamine receptors are widely distributed in different parts of human organelles and different animal species especially in mammals[7]. Drug design of allergen is solely depending on these receptors agonists and antagonists, thus knowledge on the physicochemical properties of these receptors are of utmost importance. As it is discussed earlier, crystal structure of histamine H1 receptor is available in PDB database but for other histamine receptor, no such data is available. In order to better understand the functions and molecular biology of these receptors, there is need to analyze their 3D structures.

This *in silico* study planned to characterize and model of histamine receptors. This study focused on the physiochemical properties, motif, domain, transmembrane region and three-dimensional structure of *Homo sapiens* histamine receptors to better understand histamine's biological events. Physicochemical properties of histamine receptors can provide significant knowledge on biological events of these G protein coupled receptors. *In silico* computational study can be useful to attain desired information. Furthermore, sequence alignment provides insight on homology, dissimilarities and conserved region of a group of protein[8]. In this regard, multiple sequence alignment data is more effective in order to acquire clarified information of sequence homology. Protein motif is crucial part of its feature and can be analyzed through computational tool. Finally, authors planned to develop 3D model which can be helpful to study complex structure of protein and target specific region of a targeted protein to design effective drugs or vaccines.

MATERIALS AND METHODS

The *in silico* study has multi-folds to fulfill the objectives. Different resources were applied to analyze biological features of histamine receptors. Figure 1 illustrates schematic outline of this study and the steps are explained in the following sub-sections.

Data collection and sequence retrieval: Four histamine receptors of human were retrieved from UniProtKB database[9]. The accessions of different histamine receptors were as follows: P35367 (HRH1_HUMAN histamine H1 receptor), P25021 (HRH2_HUMAN histamine H2 receptor), Q9Y5N1 (HRH3_HUMAN histamine H3 receptor) and Q9H3N8 (HRH4_HUMAN histamine H4 receptor). Full FASTA sequences of these receptors were collected from the UniProtKB database.

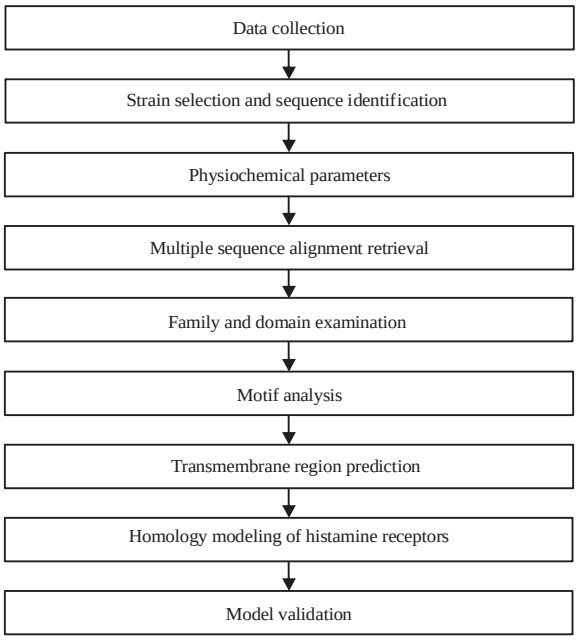

Fig. 1: Schematic outline of methodology

Analysis of physiochemical parameters: Physiochemical parameters such as: Molecular weight (M.wt), theoretical pI (isoelectric point), most redundant amino acids, instability index (II), aliphatic index (AI) and grand average of hydropathicity (GRAVY) were calculated by using ExPASy ProtParam tool[10]. Isoelectric point was computed in order to determine the acidic or basic nature of protein[11]. From the amino acid distribution chart, most redundant amino acid was obtained. From the chart, top two highly occurring amino acids were counted to analyze the redundant amino acids pattern. The instability index denotes the stability of the enzyme in *in vitro* condition. Instability index below 40 is generally regarded as the enzyme is stable whereas greater than 40 are considered as unstable[12]. Aliphatic index defines thermal stability based on position occupied and redundancy of amino acids alanine, valine and leucine of globular proteins[13].

Multiple sequence alignment: In order to analyze sequence similarities of histamine receptors, multiple sequence alignment was performed. ClustalW[14] was used for sequence alignment of histamine receptors. Data attained from ClustalW further analyzed with Jalview 215 in a quest of finding conserved or core region of histamine receptors.

Prediction of histamine family and domain: Evolutionary pattern can be exposed for a group of protein by identifying their family and domain. The family and domain of a protein possess signature of its function. Protein family denotes on clan of this protein and domain illustrates its distinctive functions. InterPro was used for predicting family and domain of histamine receptors[16].

Motif analysis: Protein motifs were vital signature of the belonging domain. In order to predict motifs of histamine receptors, MEME suit[17] was used.

Prediction of transmembrane region: Prediction of transmembrane helices is of utmost importance in functional analysis of protein. TMHMM Server v. 2.0[18] was applied for predicting transmembrane helices in histamine receptors.

Homology modeling of histamine receptors: Homology modeling of histamine receptors was conducted by using I-tasser software[19]. As previously mentioned, crystal structure of histamine H1 receptor is already available but homology modeling of all four receptors were performed in order to cross check the validation of predicted models with crystal structure of histamine H1 receptor. This approach can justify researchers prediction of other histamine receptors (HRH2, HRH3 and HRH4) 3D model.

Model validation: Predicted models usually contain some errors in their primitive structure. Trouble shooting step is prerequisite to overcome these issues. Therefore, validation of predicted models were performed by using different software those were frequently used for model verification, such as RAMPAGE, ERRAT and PROCHECK[20-22].

RESULTS

Physicochemical properties of human histamine receptors: Analysis through ExPASy ProtParam tools, has been found that molecular weight of histamine receptors ranged from 40-55 KDa. Among the histamine receptors, HRH1 weighed highest (55.784 KDa) and HRH2 weighted lowest (40.098 KDa). Molecular weight varied with their relative amino acid number. Higher number of amino acid residues in the protein sequence resulted higher molecular weight as shown in Table 1. The isoelectric point of these receptors has been found in the range of 9.33-9.62. *In vitro* stability of histamine receptors were also studied and found that, most of these receptors scored more than 40, except histamine H2 receptor (HRH2). This indicates relative instability of these receptors in *in vitro* conditions. Aliphatic index of these receptors were

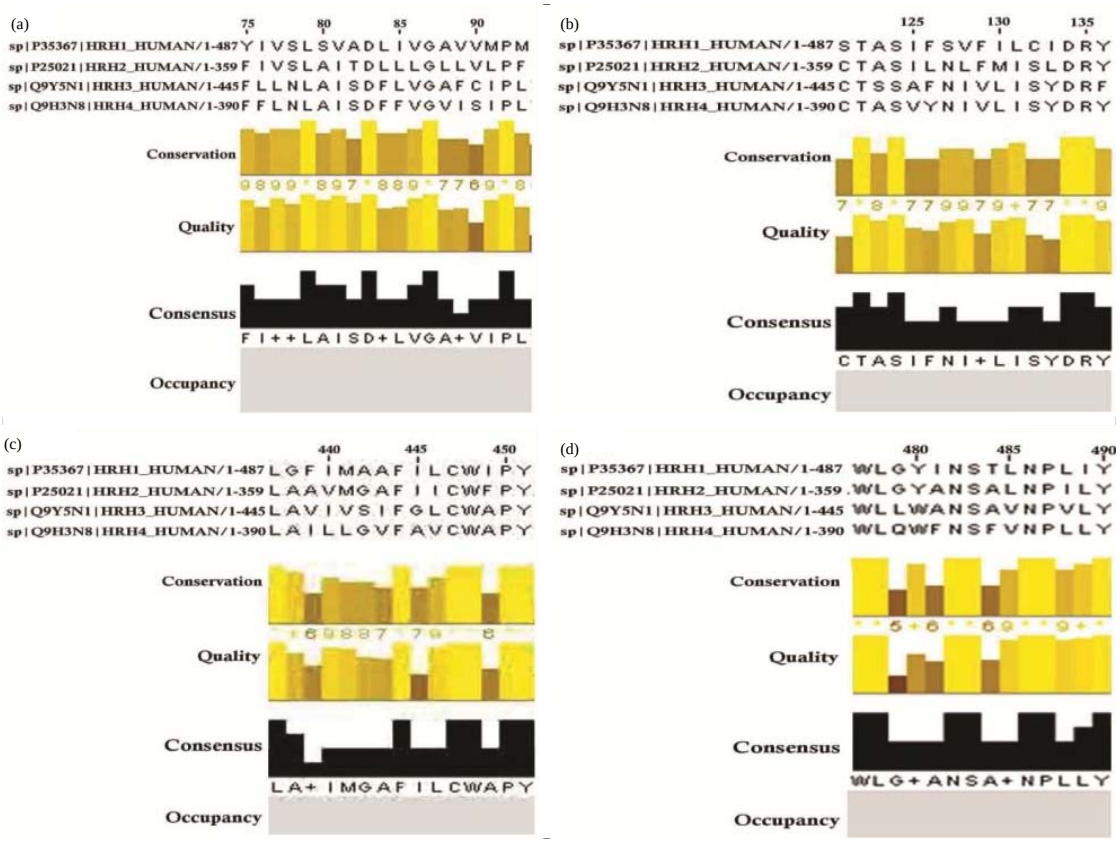

Fig. 2(a-d): Multiple sequence alignment and conservancy analysis

In four different sites, partially conserved regions have been found. From the figure, A and D sites carry most conservancy among the four notable sites found from multiple sequence alignment data

Table 1: Physicochemical properties of human histamine receptors

Receptors	Accession	Number of A.A	Molecular weight	Theoretical pI	Instability index	Aliphatic index	GRAVY	Two most redundant AA (%)
HRH1	P35367	487	55784.12	9.33	47.00	90.06	-0.086	Leu (L) 11.3%, Ser (S) 8.8%
HRH2	P25021	359	40098.12	9.36	34.93	113.51	0.396	Leu (L) 13.1%, Val (V) 8.9%
HRH3	Q9Y5N1	445	48671.37	9.43	42.23	89.30	0.166	Leu (L) 12.1%, Ala (A) 11.2%
HRH4	Q9H3N8	390	44495.89	9.62	45.36	100.21	0.314	Leu (L) 11.8%, Ser (S) 13.6%

AA: Amino acids, pI: Isoelectric point, GRAVY: Grand range of hydropathicity

well above 90. GRAVY value was calculated as 0.086 for histamine H1 receptor (HRH1). In contrast, other histamine receptors showed positive GRAVY value. Finally, it was observed that these four histamine receptors were rich in leucine as shown in Table 1.

Sequence similarities/conserved region of histamine receptor: No significant similarities have been observed among four histamine receptors. Partially conserved regions have been found from amino acid residue No. 75-94 (Fig. 2a), which is about 18 amino acids long and this is the most conserved region found in multiple sequence alignment. Another partially conserved region was found 14 amino acids long, from residue number 477-490 (Fig. 2d). Two other sites (Fig. 2b, c) lacking conservancy quality and consensus value comparing to sites are shown in Fig. 2a and d.

Family and domain of human histamine receptors: All of these four histamine receptors were belong to major protein family, G protein-coupled receptor. Functional domain of four receptors was same as well, which is GPCR, rhodopsin-like, 7TM (Seven pass transmembrane).

Motif prediction: Motifs of histamine receptors were predicted by using MEME suite. By default set up, it can predict up to three motifs and distribution of motifs can be selected in three different parameters. For this experiment, number of motifs as five and site distribution as any number of

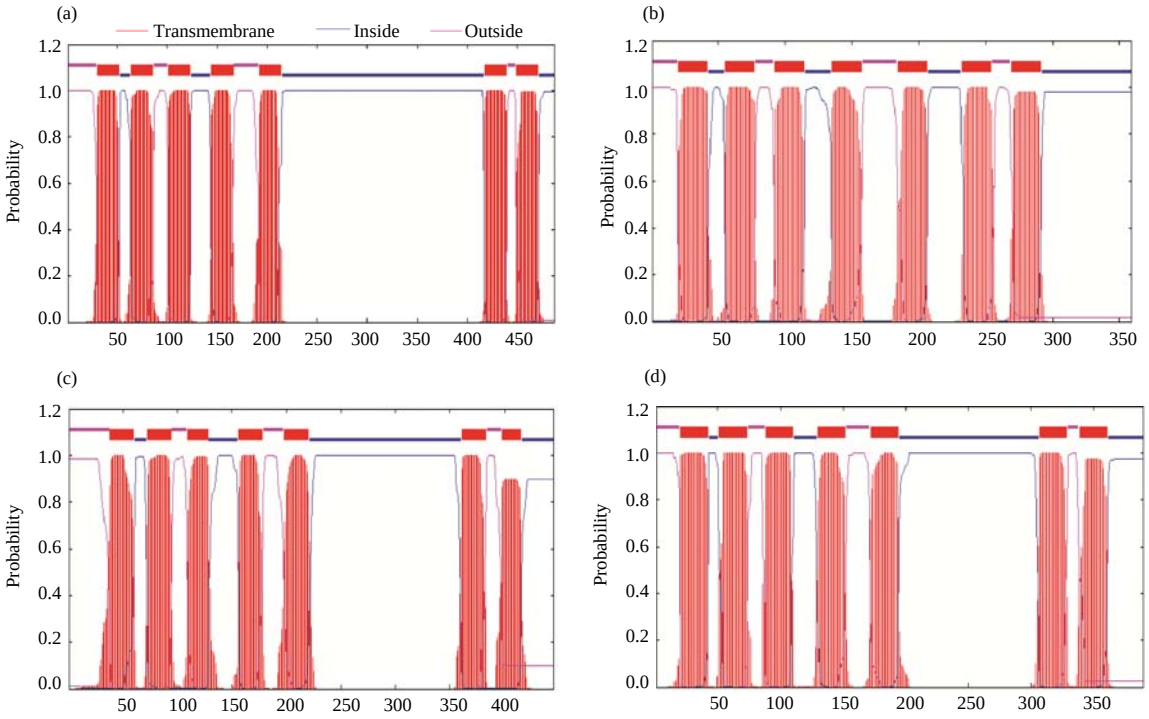

Fig. 3(a-d): Transmembrane regions of histamine (a) H1, (b) H2, (c) H3 and (d) H4 receptor
Transmembrane regions are showed in thick red lines

Table 2: Motif analysis of four histamine receptors

Motif No.	Width	E-value	Sites	Position in HRH1	Position in HRH2	Position in HRH3	Position in HRH4
1	50	3.00E-23	4	93-142	84-133	100-149	80-129
2	41	2.20E-20	4	446-486	266-306	352-374	297-319
3	23	2.10E-10	4	409-431	228-250	332-374	297-319
4	49	1.80E-09	4	41-89	32-80	48-96	29-77

repetitions were selected. All other parameters were left as provided in default value. MEME suite automatically predicts the width and occurrence number of motif, in order to minimize the E-value of predicted motif.

Four motifs for four histamine receptors with lower E-value were noticed. Lowest E value of 3E-23 for motif 1 with four sites and frame width of 50. Highest width was observed in motif 1 and lowest was found in motif 3. All motifs consisted of four sites and E values were ranged from 3.00E-23-1.80E-09. From study observation, motifs 1 and 4 showed compactness as potential conserved regions of histamine receptors. Details of motif analysis are presented in Table 2.

Transmembrane region: It was found that all four histamine receptors were seven pass transmembrane protein. Transmembrane locations were different in each protein structure. From researchers observations, histamine H2 receptor contained evenly distributed transmembrane helix than other three histamine receptors. Histamine H1 receptor, histamine H3 receptor and histamine H4 receptor posses 100-200 amino acid residue gap between helix number 5th and 6th , whereas this gap was only 28 amino acid long in case of histamine H2 receptor as shown in Fig. 3. With this signature, histamine H2 receptor functionally different from other receptor and this might aids in binding endogenous histamine molecule with histamine H2 receptor.

Homology modeling and validation: The homology modeling had been performed by using I-tasser19. For histamine H1 receptor and histamine H2 receptor, five models had been generated separately (Fig. 4 and 5) and for histamine H3 receptor and histamine H4 receptor, four models had been produced separately (Fig. 6 and 7).

These models were then validated by using ERRAT, RAMPAGE and Ramachandran plot. Validation scores attained from ERRAT, RAMPAGE and Ramachandran plot were showed

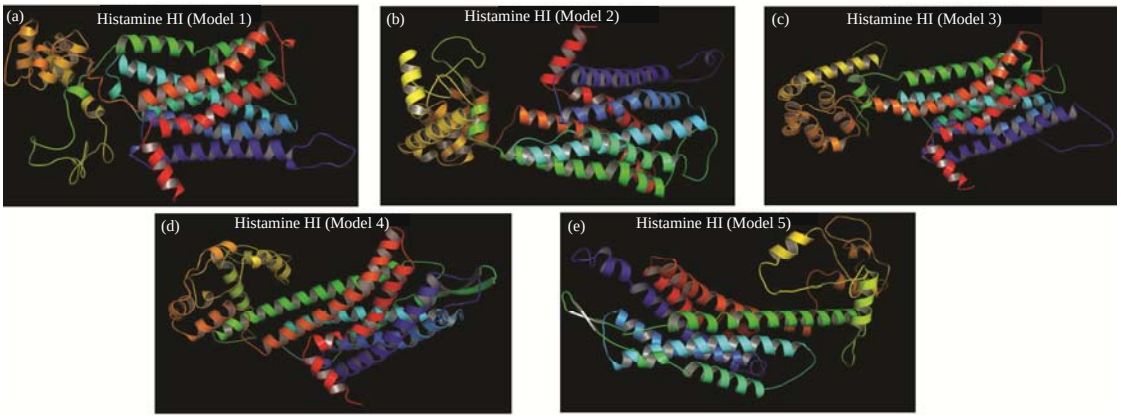

Fig. 4(a-e): Homology modeling of histamine H1 receptor by I-tasser

Five models were generated by I-tasser. All models were heavily loaded with alpha helix and coil-coil regions. No sign of beta sheets in primitive structures. From these models of histamine H1 receptor, one model will be selected as the best model after analyzing with model validation tools, i.e., ERRAT, RAMPAGE and PROCHECK

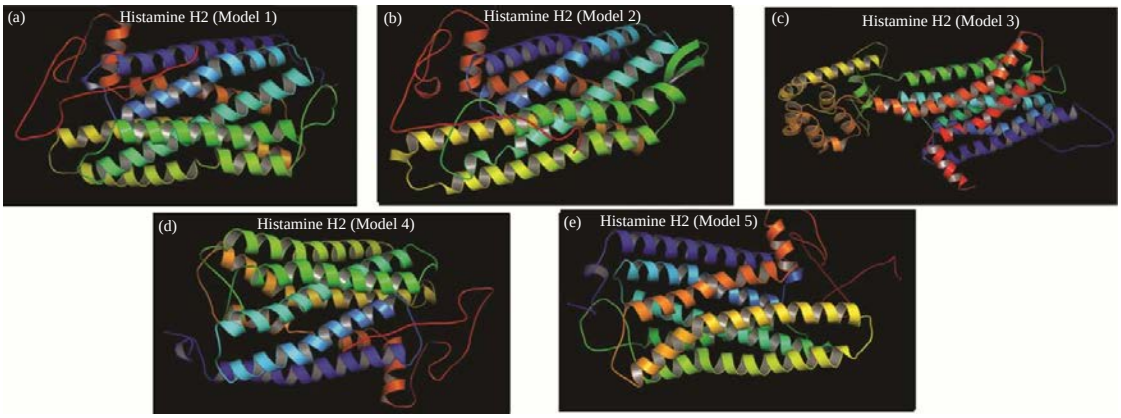

Fig. 5(a-e): Homology modeling of histamine H2 receptor by I-tasser

Five models were generated by I-tasser. All models were heavily loaded with alpha helix and coil-coil regions.. From these models of histamine H2 receptor, one model will be selected as the best model after analyzing with model validation tools, i.e., ERRAT, RAMPAGE and PROCHECK

in Table 3. Data from Table 3 suggest that, model 2 of histamine H1 receptor, model 4 of histamine H2 receptor, model 1 of histamine H3 receptor and model 2 of histamine H4 receptor were the best models according to model validation scores.

ERRAT validated models by statistical relation of non-bonded interactions among different atom types based on characteristic atomic interaction[20]. It assesses overall quality of a model at 0.01 and 0.05 level of significance and presents result as overall quality factor. Standard high resolution structures generally produces values around 95% or higher. Low resolution structures produced values around 91%. Table 3 illustrate ERRAT score of predicted models, which ranged from 82.710-94.017%. This range suggests significance of current predicted models according to the algorithm of ERRAT software. Best model from each histamine receptor group also scored above 90% (Fig. 8).

RAMPAGE is another 3D model validation tool, which presents result based on amino acids geometry and deviation. It provides result in three main categories, such as number of residues in favored region (expected value ~98.0%), number of residues in allowed region (~2.0% expected) and number of residues in outlier region. Table 3 illustrates RAMPAGE score in percentage, which ranges from 75.1-86.5%. The best models form each histamine group that was selected on the basis of RAMPAGE scored more than 82% (Fig. 9). This range suggests quality models were predicted by using I-Tasser.

PROCHECK tests stereochemical quality of protein structure by evaluating residue-by-residue geometry and

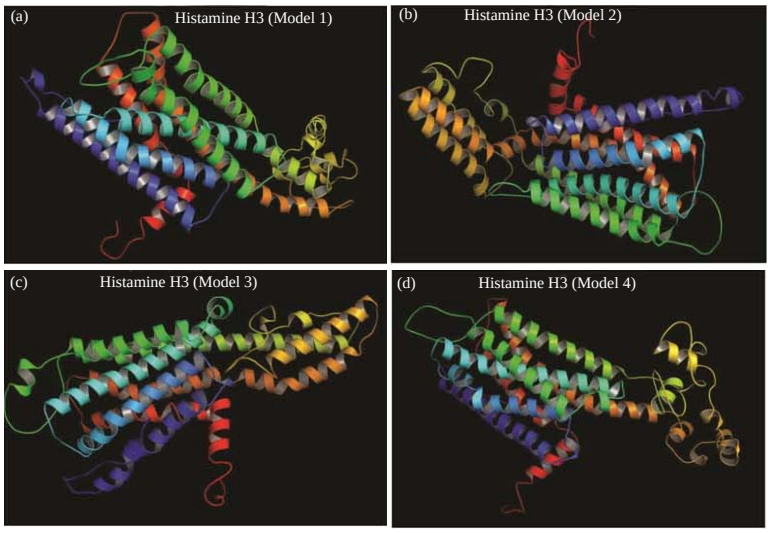

Fig. 6(a-d): Homology modeling of histamine H3 receptor by I-tasser

Four models were preliminary generated by I-tasser. Redundancy of alpha helix also observed in 3D structures of these models. From these models of histamine H3 receptor one model will be selected as the best model after analyzing with model validation tools, i.e., ERRAT, RAMPAGE and PROCHECK

Table 3: Model validation scores by different tools e.g., ERRAT, RAMPAGE and PROCHEK

Receptors	Model number	ERRAT* (%)	RAMPAGE** (%)	Ramachandran plot*** (%)
Histamine H1 receptor (HRH1)	Model 1	86.221	75.7	69.9
	Model 2	93.946	83.5	77.2
	Model 3	88.703	80.4	74.7
	Model 4	87.891	81.6	75.2
	Model 5	90.337	75.1	69.9
Histamine H2 receptor (HRH2)	Model 1	86.895	81.5	76.7
	Model 2	92.308	79.8	76.1
	Model 3	92.000	83.8	77.6
	Model 4	92.877	83.2	77.9
	Model 5	94.017	81.2	76.4
Histamine H3 receptor (HRH3)	Model 1	90.618	82.6	82.5
	Model 2	85.981	83.5	80.1
	Model 3	82.710	76.7	75.3
	Model 4	90.337	86.5	78.0
Histamine H4 receptor (HRH4)	Model 1	86.649	80.7	79.5
	Model 2	92.147	87.4	83.7
	Model 3	90.814	81.7	77.3
	Model 4	93.651	85.8	81.7

*Good high resolution structures generally produces values around 95% or higher. Low resolution structures produces values around 91%, **Amino acid residues in most favoured region. Expected or standard of good quality model around 98% or higher, ***Residues in most favoured regions. a good quality model would be expected to have over 90% in the most favoured regions

overall structural geometry. It provides amino acid residues distribution on Ramachandran plot divided into four colour coated regions. The regions are residues in most favored regions, residues in additional allowed regions, residues in generously allowed regions and residues in disallowed regions. According to PROCHECK standard, a good quality model should posses over 90% amino acid resided in the most favored regions. Table 3 suggests that, study predicted models were ranged from 69.9-83.7%. The best models form each histamine group suggest that more than 77% amino acid residues were in most favored region for these models (Fig. 10).

Analyzing the results of different validation tools, top model for each histamine receptor was selected. From the Table 3, it was analyzed that model 2 of histamine H1 receptor, model 4 of histamine H2 receptor, model 1 of histamine H3 receptor and model 2 of histamine H4 receptor are outperformed other models according to the validation scores attained from different tools. Figure 11 represents best model for different histamine receptor.

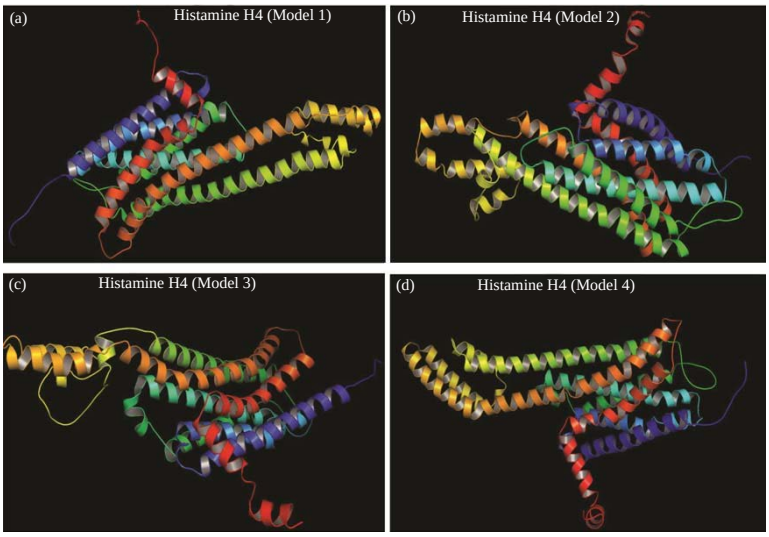

Fig. 7(a-d): Homology modeling of histamine H4 receptor by I-tasser

Four models were generated by I-tasser. Redundancy of alpha helix also observed in 3D structures of these models. Some coiled coil regions also observed in histamine H4 receptor primitive structure. From these models of histamine H4 receptor, one model will be selected as the best model after analyzing with model validation tools, i.e., ERRAT, RAMPAGE and PROCHECK

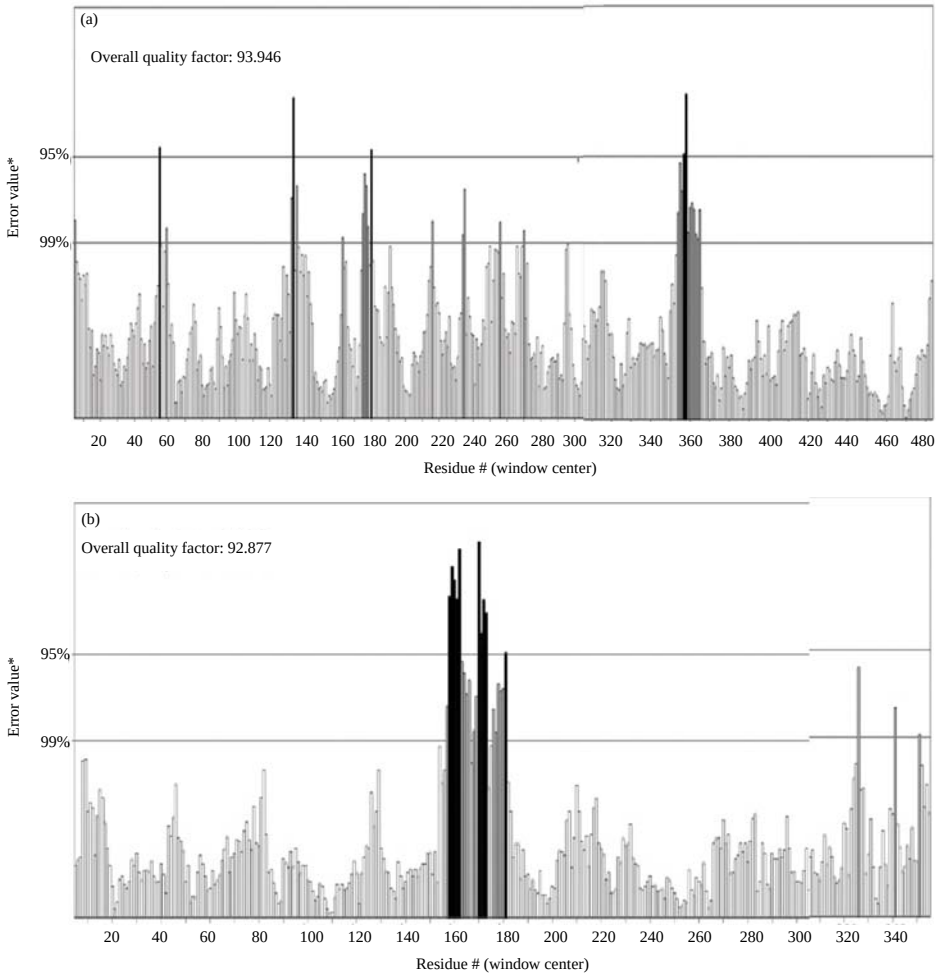

Fig. 8(a-d): Continue

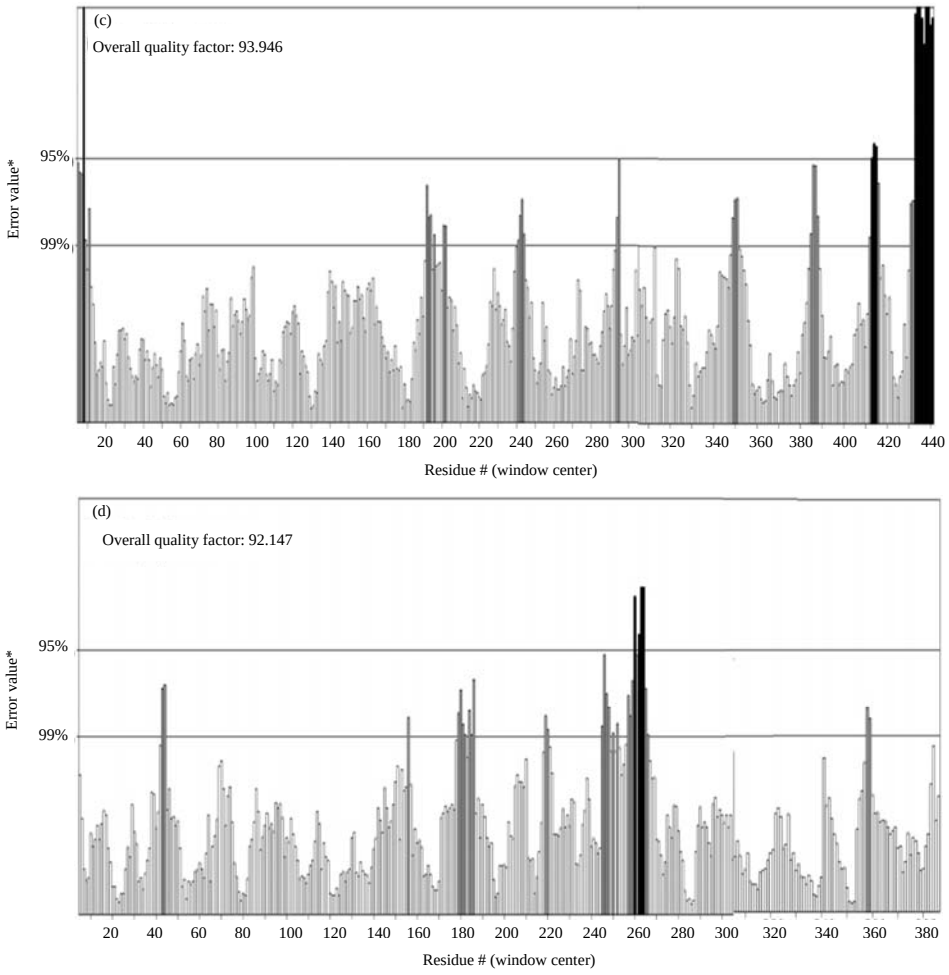

Fig. 8(a-d): ERRAT score of best models for histamine (a) H1, (b) H2, (c) H3 and (d) H4 receptor

Model 2 of histamine H1 receptor posses ERRAT score of 93.946%, RAMPAGE score (residues in favoured region) 83.5% and RAMACHANDRAN PLOT score (percentage of residues in most favoured regions) 77.2%. Similarly, model 4 of histamine H2 receptor carries ERRAT score of 92.8776%, RAMPAGE score (residues in favoured region) 83.2% and RAMACHANDRAN PLOT score (percentage of Residues in most favoured regions) 77.9%. Model 1 of histamine H3 receptor carries ERRAT score of 90.618%, RAMPAGE score (residues in favoured region) 82.6% and RAMACHANDRAN PLOT score (percentage of residues in most favoured regions) 82.5%. Lastly, model 2 of histamine H4 receptor possess ERRAT score of 92.147%, RAMPAGE score (residues in favoured region) 87.4% and RAMACHANDRAN PLOT score (percentage of residues in most favoured regions) 83.7%.

DISCUSSION

Histamine receptors are crucial G protein coupled receptor for endogenous and exogenous histamine receive and transfer[23]. From current study, it was found that molecular weight of histamine H1 receptor (HRH1) is around 55.7 KDa which is relatively higher than other receptors and histamine H2 receptor's (HRH2) molecular weight was lowest (40 KDa) among four histamine receptors of human. This indicates that there might be heavy amino acid side chains in its tertiary structure. Also, it can be seen in Fig. 3 that, 3D structure of histamine receptor 1 (HRH1) is heavily loaded with alpha helix. All four receptors are basic in nature as the isoelectric point hits the value near 9.5. From the theory, instability index value greater than 40 regarded as the protein is unstable[24]. From the instability index, it is found that histamine H2 receptor (HRH2)

is stable and other three receptors value are marginally over 40, those have a higher tendency of becoming stable from slightly unstable conditions. Aliphatic index states relative volume poised by aliphatic side chains such as alanine, valine, isoleucine and leucine. It also denotes thermal stability of a protein[25]. Aliphatic index of histamine receptors are ranging from 89.3-113.5, which indicates that the tendency of having a wide range of temperature sensitivity as aliphatic index (AI). This aliphatic index is well supported by data of most two redundant amino acids in the histamine receptors sequences. GRAVY value of protein denotes hydropathicity of a protein and indicates whether the protein side chains are hydrophilic or hydrophobic in nature[26]. Leaving the histamine H1 receptor (HRH1), all other receptors are hydrophobic in nature and

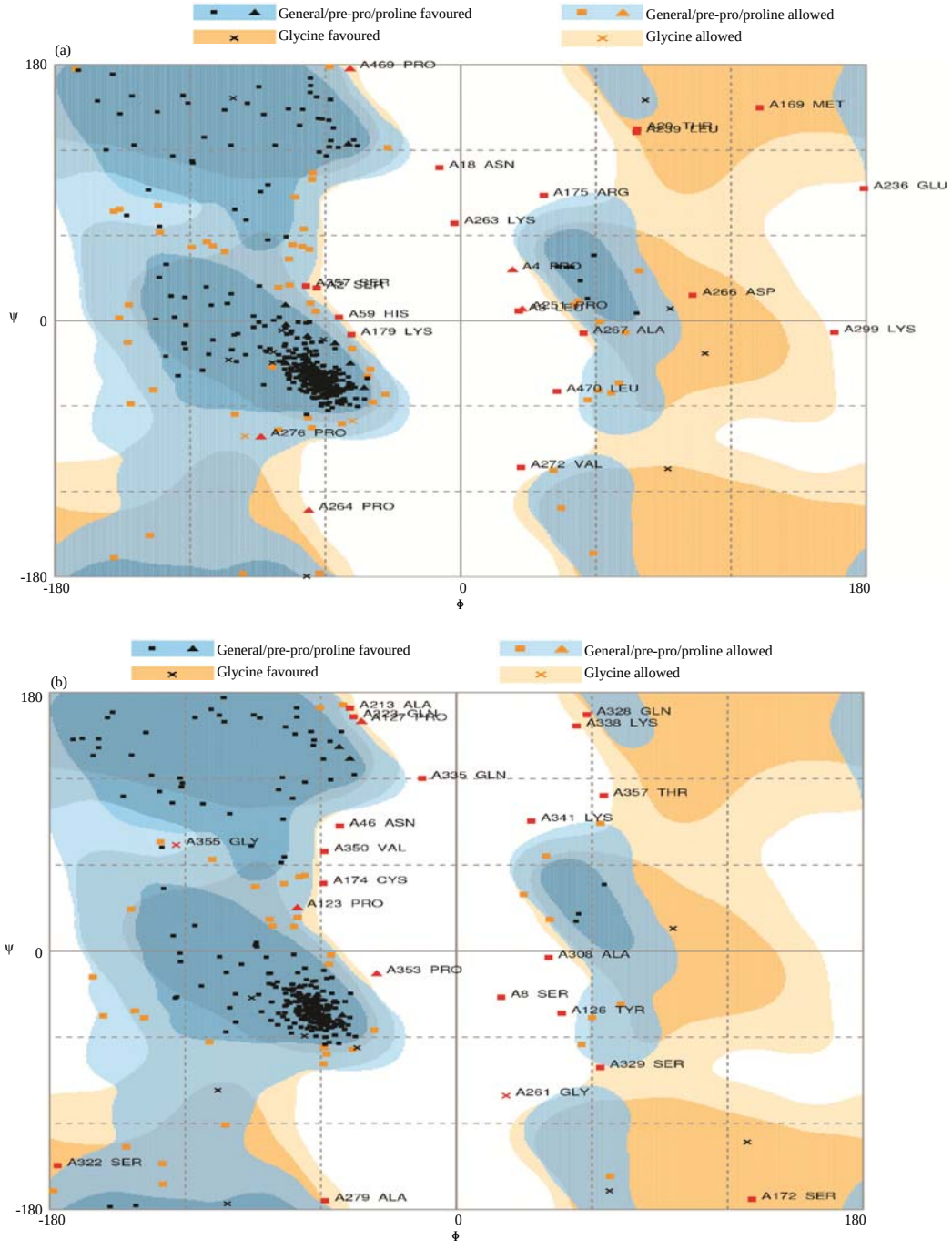

Fig. 9(a-d): Continue

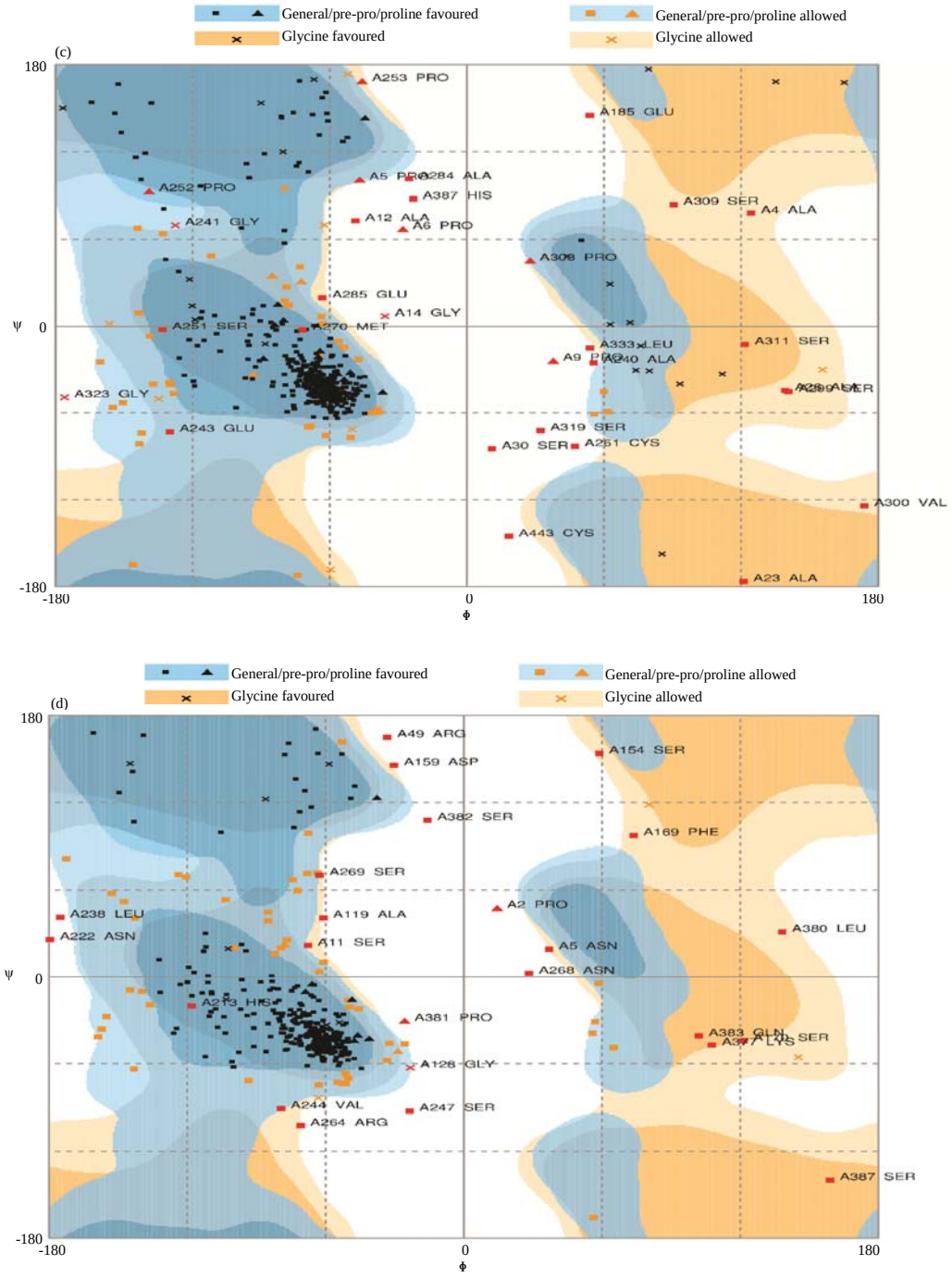

Fig. 9(a-d): RAMPAGE output of best models for histamine (a) H1, (b) H2, (c) H3 and (d) H4 receptor

histamine H1 receptor (HRH1) can be interpreted as very slightly hydrophilic to hydrophobic. The probabilities of common conserved regions of these receptors were checked also and few partially conserved regions were found (Fig. 2a-d) which can be employed as drug targets[27]. From the family analysis of these receptors, it can be said that, these are members of 7TM family and these receptors are integral component of cellular membrane. Histamine H1 receptor regulates vascular permeability, up regulates vasoconstriction and responsible for eosinophil chemotaxis[2]. On the other hand, histamine H2 receptor positively influences vasoconstriction and it stimulates gastric acid secretion[3]. As it has been already discussed, histamine H3 receptor is associated with Central Nervous System (CNS), it regulates

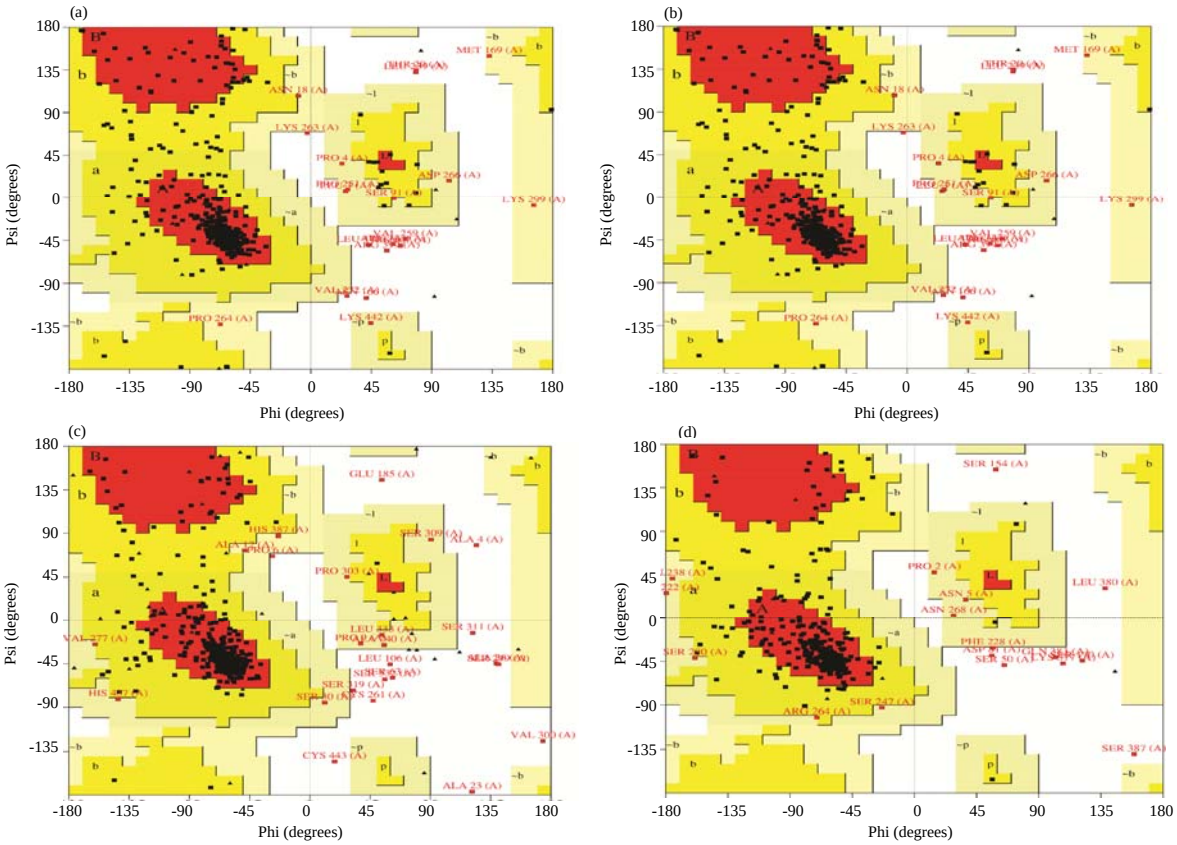

Fig. 10(a-d): PROCHECK analysis result for best models of histamine (a) H1, (b) H2, (c) H3 and (d) H4 receptor
Color codes are red color- most favorable regions, yellow color region- allowed region and pale yellow-generously allowed region and white color-disallowed regions

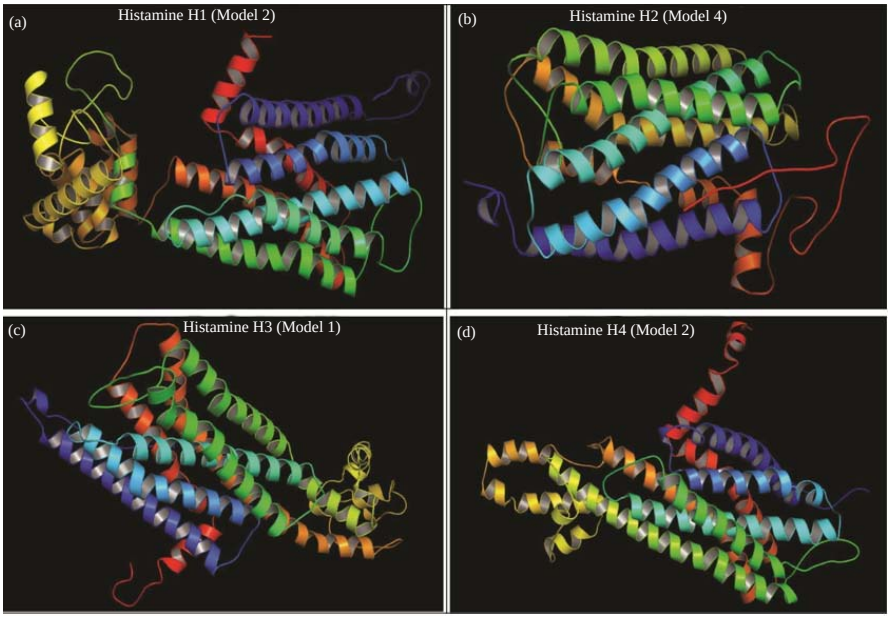

Fig. 11(a-d): Confirmation of best model for histamine (a) H1, (b) H2, (c) H3 and (d) H4 receptor by different model validation tools such as ERRAT, RAMPAGE and PROCHECK
For histamine H1 receptor the best model was model-2, in case of histamine H2 receptor the best model was model model-4 and for histamine H3 and H4 receptor the best model was model-1 and model-2, respectively

release of some neurotransmitter and also controls the level in cellular matrix upon activating feedback mechanisms. Lastly, histamine H4 receptor plays pivotal role in inflammation response, regulates MAPK cascade and positively regulates cytosolic calcium ion concentration. In addition, Motif of a protein carries significant importance in proteomics as it denotes function of a protein domain and conserved regions. For designing primer, it is prerequisite to find out the conserved region of the protein. The MEME suite especially designed for predicting Motifs in different types of sequences[17]. E-value of the predicted motif designates its statistical significance. Motifs with a lower E-value possess the probability of finding out an equally well conserved region in the test sequence[28]. From study analysis, total four motifs with lower E-value was found. Among these, motif No. 1 has lower E value, moderate width and four sites of occurrence. These receptors are also member of G protein coupled receptor and contain seven pass transmembrane regions. These transmembrane regions position vary from each other but the notable thing is approximately 200 amino acid residue gap between 5th and 6th transmembrane helix. This phenomenon is very common in histamine H1, H3 and H4 receptors but not observed in histamine H2 receptor. Homology modeling of these receptors has shown heavy load of alpha helix in each receptor's 3D structure. Validation of 3D structure of these receptors also performed to check the quality of our predicted models as well as to select the best model of each histamine receptor.

CONCLUSION

Histamine receptors are very significant in studying molecular mechanism of allergy. A rigorous characterization of different histamine receptors has been done in this study as well as their 3D models have been developed. Physicochemical properties, instability index and aliphatic index have been examined as well as suitable motifs and potential regions have been suggested for targeted drug binding site. The findings of this study might be helpful in designing more suitable antihistamines and relevant drugs in treatment of allergic diseases. Much study need to be done in order to analyze why histamine provokes immune system to perform cascade of allergic reactions and how it could be sensitized without any side effects.

SIGNIFICANCE STATEMENT

This study shows potential regions of histamine receptors those can be targeted as drug binding site. 3D models for all four histamine receptors are also proposed. Among these receptors, crystal structure of histamine H2, histamine H3 and histamine H4 receptors yet to be discovered. With 3D models, potential therapeutic peptides can be docked to active sites for blocking burden amount of histamine by these receptors. Overall, this study might be useful in designing new generations of antihistamines.

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