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ORIGINAL PAPER

An *Insilico* approach to High Altitude Pulmonary Edema - Molecular modeling of human β_2 adrenergic receptor and its interaction with Salmeterol & Nifedipine

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Abstract Knowledge of the three-dimensional structures of protein targets from genomic data has the potential to accelerate researches pertaining to drug discovery. Human β_2 adrenergic receptor is a G-protein-coupled receptor with seven transmembrane helices, and is important in pharmaceutical targeting on pulmonary and cardiovascular diseases. The human β_2 adrenergic receptor has been found to play a very important role in the pathogenesis of high altitude pulmonary edema (HAPE). In the present study, a high quality of protein 3D structure has been predicted for the human β_2 adrenergic receptor sequence with primary accession number P07550. Homologous template protein sequence with known 3D structure was identified and the template-query protein sequence validation was done by multiple sequence alignment method. The homology model was performed through Modeller and depended on the quality of the sequence alignment by BLAST, template structure and the consolidated result performed by Gene silico meta-server. The statistical verification of the generated model was evaluated by PROCHECK which revealed that the structure modeled through Modeller to be of good quality with 84.1% of residues in the most favored region. Docking studies were carried out after modeling with two well known ligands namely Salmeterol and Nifedipine, and the fitness score revealed that Salmeterol has a higher fitness score than Nifedipine. Estimation of binding affinity by X-Score revealed that Salmeterol had −10.40 binding affinity while Nifedipine showed −9.62 binding affinity. From the present study, it can be concluded that the generated model of human β_2 adrenergic receptor can be used for further studies related to this receptor and Salmeterol was found to have a high binding affinity with human β_2 adrenergic receptor.

Keywords Altitude illness · Beta 2 adrenergic receptors · GPCR · High altitude pulmonary edema · Homology modeling · Nifedipine · Salmeterol

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Introduction

HAPE is a severe form of altitude illness that may develop in individuals on rapid ascent to altitudes above 2500 m [1]. The disease is characterized by hypoxia induced pulmonary vasoconstriction caused by endothelial dysfunction and intravascular fluid retention [2–4]. HAPE results from fluid buildup in the lung which prevents effective oxygen exchange. Initially, patients have dyspnea, decreased exertion tolerance, and dry cough followed by pink or bloody sputum and respiratory distress (http://www.merck.com/mmpe/sec21/ch320/ch320a.html). As the condition becomes more severe, the level of oxygen in the bloodstream decreases, and this can lead to cyanosis, impaired cerebral function, and death [5].

The adrenergic receptors are a class of G protein-coupled receptors (GPCR). The members of this receptor class mediate a wide variety of physiological responses, including vasodilatation and vasoconstriction, heart rate modulation, regulation of lipolysis, and blood clotting. These diverse and important functions make the adrenergic receptors a tempting pharmaceutical target [6]. β_2 receptors are present on the smooth muscle of all airways from the trachea to the terminal bronchioles [7, 8]. The β_2 adrenergic receptor has been found to play a very important role in the pathogenesis of HAPE. The prophylactic inhalation of the

β_2-adrenergic agonist such as Salmeterol would stimulate alveolar sodium transport and decrease the incidence of HAPE. Bartsch et al. [9] reported that HAPE can be effectively prevented by prophylactic use of Nifedipine.

Prophylactic inhalation of the β_2-adrenergic agonist Salmeterol to stimulate alveolar sodium transport decreased the incidence of HAPE. Dexamethasone is an alternative therapy to prevent HAPE because it stimulates alveolar sodium and water reabsorption. Lowering pulmonary arterial pressure with a vasodilator like Nifedipine improves symptoms of HAPE and suggests that pulmonary vasoconstriction plays a major role. The logical treatment of HAPE is to increase alveolar pulmonary O_2 (PAO_2) either by administration of oxygen or by descent to lower altitude [10].

For mild symptoms, staying at the same altitude is the best decision, to see if symptoms resolve and ascend when the symptoms have resolved completely. Diamox is used to treat mild-moderate symptoms. Dexamethasone is a very potent steroid used in high altitude cerebral edema temporarily to facilitate descent. This drug improves the symptoms without improving acclimatization. Nifedipine is used for HAPE by lowering pressure in the pulmonary blood vessels and thereby decreasing fluid in the lungs. Inhaled nitric oxide decreased pulmonary arterial pressure and improved ventilation-perfusion mismatch in HAPE-prone subjects exposed to high altitude [11].

Attempts to create effective and specific drugs acting on β_2 receptors have been slowed down due to the lack of a 3D structure for any G protein coupled receptor other than the bovine photoreceptor rhodopsin [6]. The focus of this work is the *insilico* modeling of the three dimensional structure of human β_2 adrenergic receptor which is targeted for the treatment of high altitude pulmonary edema and studying its interaction with Salmeterol and Nifedipine. Three-dimensional models of the adrenergic receptors would be extremely useful in the design of subtype-specific pharmaceutical compounds.

Methodology

Primary sequence analysis

The primary sequence of human β_2 adrenergic receptor was retrieved from Swiss Prot. Its primary accession number is P07550 (entry name ADRB2_HUMAN) and has 413 amino acids. Homologous template protein sequence with known 3D structure was identified through PSI-BLAST.

Sequence alignment

The protein sequence was subjected to PSI-BLAST against PDB database in order to get related sequences from different species whose structure is deposited in PDB. Ten related sequences were taken from the PSI BLAST result and these 10 sequences along with the query were subjected to multiple sequence alignment using Clustal W.

Gene silico metaserver analysis

The Clustal W alignment file was submitted to the Genesilico meta-server [12]. The meta-server sends sequence to several servers which generate sequence alignments. The Genesilico meta-server receives alignments, names of templates and scores from servers. Genesilico meta-server is a gateway to various methods for protein structure prediction and the following predictions are obtained when a query sequence is submitted. The primary structure is predicted by Hmmpfam and HHSearch_cdd. Secondary structure is predicted by TM helices, Protein Order, Protein Solvation and Secondary Structure. Tertiary structure is predicted by PDBBlast, blastp, ffas, hhsearch, mgenthreader, sp4, 3Dpssm server, phyre, inub, and pcons5 [13]. The Pcons consensus server will evaluate to which extent the FR alignments agree with each other. From FR alignments crude three-dimensional models (without variable loops) can be automatically generated.

Tertiary structure prediction and validation

The three dimensional structure of human β_2 adrenergic receptor was modeled using MODELLER. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints. The input to this program comprises of three files namely, (i) the Protein Data Bank atom files with coordinates for the template structures (1U19), (ii) the alignment file produced by Gene Silico meta-server with the alignment of the template structures with the target sequence, and (iii) MODELLER commands in a script file. The 3D model generated was viewed with the help of the visualization tool Pymol (http://www.pymol.org). Energy minimization for the modeled structure was performed using Insight II. 100,000 iterations were performed twice to obtain a good model. Structure analysis and validation of the predicted 3D model of human β_2 adrenergic receptor was done using PROCHECK [14], which is an online meta server accessed from NIH MBI Laboratory for Structural Genomics and Proteomics. Ramachandran plot was generated which shows the allowed and disallowed regions for the modeled molecule.

Retrieval of ligands

Salmeterol and Nifedipine were used as ligands in this study. The two dimensional structures of selected datasets were downloaded from the DrugBank [URL:http://redpoll.pharmacy.ualberta.ca/drugbank] as Smiles strings. These

smiles strings were then converted into three dimensional chemical structures using CORINA [URL: http://www.molecular-networks.com/online_demos/corina_demo.html].

Salmeterol is a novel long acting β_2 agonist (LABA). The drugbank accession number is DB00938. Its IUPAC name is 2-(hydroxymethyl)-4-[1-hydroxy-2-[6-(4-phenylbutoxy) hexylamino]ethyl]-phenol. Its chemical formula is $C_{25}H_{37}NO_4$. It is a bronchodilator. It works by relaxing and opening air passages in the lungs, making it easier to breathe. Nifedipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. The drugbank accession number is DB01115. Its IUPAC name is 3, 5-pyridinedicarboxylic acid, 1, 4-dihydro=2, 6=dimethyl-4=(2=nitrophenyl I)-dimethyl ester. It has the chemical formula $C_{17}H_{18}N_2O_6$. This drug is a calcium channel blocker. Calcium is involved in blood vessel contraction. By blocking calcium, nifedipine relaxes and widens the blood vessels.

Docking studies and determination of binding affinity

Docking studies were carried out between the predicted model and the two ligands Salmeterol and Nifedipine. Protein - ligand docking was carried out with the help of GOLD v3.0 (http://gold.ccdc.cam.ac.uk/index.php). GOLD stands for Genetic Optimisation Ligand Docking. GOLD is a genetic algorithm for docking flexible ligands into protein binding sites. GOLD provides all the functionality required for docking ligands into protein binding sites from *prepared* input files. Docking procedure was performed using Gold-score scoring functions. Human β_2 adrenergic receptor and ligand datasets were docked with 10 runs for each docking procedure. The binding affinity of the protein-ligand complex was determined using X-Score (http://sw16.im.med.umich.edu/software/xtool/). X-Score is a scoring function which has its major applications to structure-based drug design studies. It computes a binding score for a given protein-ligand complex structure, and this binding score correlates to experimental binding constants well. Three individual empirical scoring functions have been implemented in this program, which are named as HPScore, HMScore, and HSScore respectively. X-Score is an empirical scoring function developed to estimate the binding affinity of a given protein-ligand complex with known three-dimensional structure.

Results and discussion

The sequence of human β2 adrenergic receptor (P07550) containing 413 amino acids with molecular weight of 46557 Da was primarily analyzed using PSI-BLAST which revealed that the crystal structure of bovine rhodopsin at 2.2 Angstroms resolution has more sequence identity with human β2 adrenergic receptor (ADRB2_HUMAN). This was used as a template for modeling ADRB2_HUMAN. The PDB ID for this molecule is 1U19. The PDB keyword is crystal structure of bovine rhodopsin at 2.2 Angstroms resolution. It is the best known template for GPCRs [15].

Clustal W was used to generate the multiple sequence alignment of the 10 related sequences along with the query. The Clustal W alignment file showed alignment for only residues ranging from 1 to 380. All the servers of Gene silico meta-server predicted various alignments and templates. Based on the results of tertiary structure prediction servers, the template with highest score and identity were taken (Table 1).

The templates identified were 1U19 A, 1F88 A, 2R4R A, 2RH1 A, 1BOK, 1LN6 A, 1FDF A, 1BOJ and 1L9H A with identity ranging from 46 to 65. It was identified that the chain A of the crystal structure of bovine rhodopsin at 2.2 Angstroms resolution (1U19A) was found to have maximum identity of 65% and a good score of 1e–129 as revealed by blastp analysis. The results produced by PdbBlast, hhsearch, phyre, fugue, and pcons5 servers also revealed 1U19 to have the maximum identity of 65%. Based on the assessment of reliability by each of these servers, 1U19 A was found to have a good reliability level also. Since 1U19 had the maximum identity, score and good reliability level, it was used as a template to model the three dimensional structure of human β_2 adrenergic receptor.

The crystal structure of Bovine rhodopsin with PDB Id 1F88 had 65% identity and structure of bovine rhodopsin (Metarhodopsin II) with PDB Id 1LN6 had 62% identity. 1F88 and 1LN6 were identified as template only by a few servers. The bovine rhodopsin (7-helix bundle) with 11-cis retinal with PDB Id 1BOK had 64% identity and a score of 0.0027. Since it was a theoretical model, 1BOK was not considered as a template for further analyses. 1BOJ is also a theoretical model of the bovine rhodopsin (7-helix bundle). The crystal structure of the human β2 adrenoceptor with PDB Id 2R4R and the high resolution crystal structure of human β2 adrenergic G protein-coupled receptor with PDB Id 2RH1 had low identity of 46% and 47%, respectively. The helix 7 bovine rhodopsin with PDB Id 1FDF had 60% identity but it comprised the sequence of only the seventh transmembrane segment of rhodopsin. 1L9H is the PDB Id of the crystal structure of bovine rhodopsin at 2.6 Angstroms resolution. It was found to have 64% identity according to the result produced by pcons5 server.

Modeller used 1U19 as template to generate the tertiary structure of human β_2 adrenergic receptor. However, only residues ranging from 1 to 380 were modeled and visualized (Fig. 2). Figure 1 represents the ribbon model of bovine rhodopsin at 2.2 Angstroms resolution (1U19) while Fig. 2 represents the predicted model of human β_2

Table 1 Tertiary structure prediction results of genesilico metaserver

S.No	Tool	Template	Score	Identity	SCOP	Fssp
1	PdbBlast	1U19 A	0.0	65		
2	Blastp	1U19 A	1e–129	65		
3	Ffas	1F88 A	−120	65	f.13.1.2	1.5.13.1
		2R4R A	−101	47		
		2RH1 A	− 80.7	46		
4	hhsearch	2RH1 A	483.74	50		
		1U19 A	470.3	65		
5	Fugue	1U19 A	74.96	65		
6	3dpssm	1F88 A	1.7 e –08	65	f.13.1.2	1.5.13.1
		1BOK	0.0027	64		
7	Phyre	1U19 A	0	65		
		1LN6 A	0	62	f.13.1.2	
		1FDF A	0.00011	60	j.35.1.1	
8	Inub	1F88 A	973.58	65	f.13.1.2	1.5.13.1
		1BOJ	385.79	60		
9	Pcons5	1F88 A	4.0475	65	f.13.1.2	1.5.13.1
		1U19 A	3.7587	65		
		1L9H A	3.4741	64	f.13.1.2	

Summary of the templates predicted by various tools of Genesilico metaserver. 1U19 A had the best score, maximum identity and was identified as template by many tertiary structure prediction servers.
1U19 crystal structure of bovine rhodopsin at 2.2 Angstroms resolution
1F88 Crystal structure of Bovine rhodopsin
2R4R Crystal structure of the Human Beta2 adrenoceptor
2RH1 High resolution Crystal structure of Human B2-adrenergic g protein-coupled receptor
1BOK Bovine rhodopsin (7-helix bundle) with 11-cis retinal, theoretical model
1LN6 Structure of Bovine rhodopsin (metarhodopsin II)
1FDF Helix 7 Bovine rhodopsin
1BOJ Bovine rhodopsin (7-helix bundle) with all-trans retinal, metarhodopsin II model, theoretical model
1L9H Crystal structure of Bovine rhodopsin at 2.6 angstroms resolution

adrenergic receptor. Okada et al. [15] reported the high resolution crystal structure of bovine rhodopsin. This is the visual pigment in red photoreceptor cells. The crystal structure of bovine rhodopsin at 2.2 Angstroms resolution was used as template in this study because it shares maximum identity with human β_2 adrenergic receptor. The hydrophobic domain 2 of the human β_2 adrenergic receptor shares 30% identity with the comparable region of human rhodopsin. Hydrophobic domain 5 of the human β_2-adrenergic receptor shares 25% identity with the comparable regions of human and bovine rhodopsin [16]. Figure 3 represents the superimposed structure of 1U19 A with the predicted model of human β_2 adrenergic receptor. The red colored ribbons represent 1U19 A and green colored ribbons represent the predicted model. It shows that they have about the same steric similarity. The overall structure of the human β_2-adrenergic receptor is similar to rhodopsin with seven transmembrane helices. However, in the crystal structure deposited recently, only 365 amino acids were included for crystallographic study but in the present study 380 residues were used to build homology model.

The predicted model was compared with bovine rhodopsin which revealed that the predicted binding site is in good agreement with the experimental results [6]. Studies by Freddolino et al. [6] reported the prediction of 3D structure of human β_2-adrenergic receptor. They used Membstruk first principles method to predict the structure and Hierdock first principles to validate the generated structure and to predict the ligand binding sites and reported that ASP -113 is a good binding site for epinephrine. The predicted human β_2-adrenergic receptor shows the same general topology as Bovine rhodopsin and the ASP -113 is chosen to be the binding site in the further analyses. The results of PROCHECK are summarized in Table 2 and Fig. 4. The residues from 1 MET to 380 ASP were involved in the generation of Ramachandran plot. The Ramachandran plot generated by this analysis showed that 290 residues occurred in the most favored regions which make up 84.1% of the total residues, 46 residues occurred in additionally allowed regions which makes up 13.3%, and six residues occurred in the generously allowed regions which makes up 1.7% and three residues in the disallowed region. The PROCHECK analysis generated the main chain bond lengths and bond angles of protein structures as a function of resolution [14]. From this we can conclude that the generated model has almost an overall good quality.

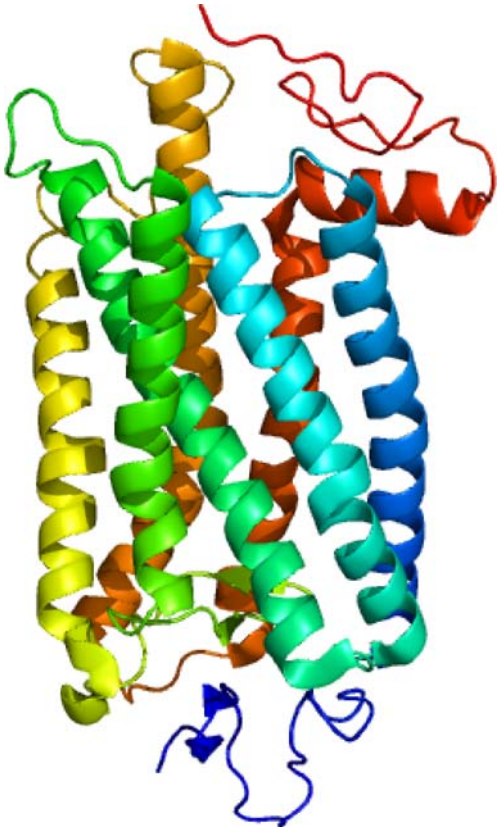

Fig. 1 Crystal structure of bovine rhodopsin at 2.2 Angstroms resolution (1U19A). This molecule was used as template in this study since it had maximum identity of 65% as revealed by Gene silico meta server analysis

Gabry et al. [17] and Raymond et al. [18] supported the view of using Salmeterol or Nifedipine for HAPE. Prophylactic inhalation of β_2-adrenergic agonists such as Salmeterol stimulates the alveolar sodium transport and

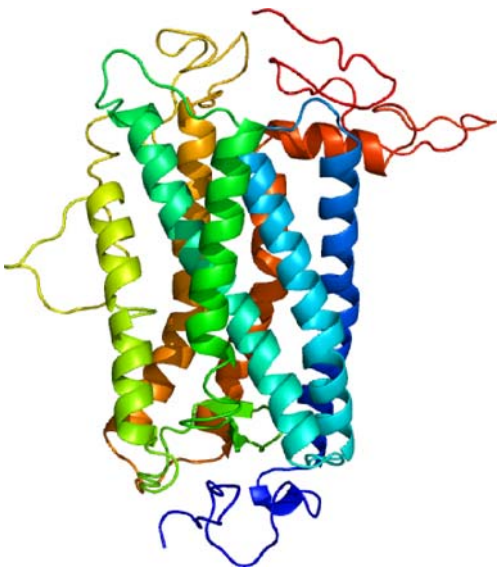

Fig. 2 Ribbon representation of the modeled human β_2 adrenergic receptor. 1U19 was used as template and was modeled using Modeller

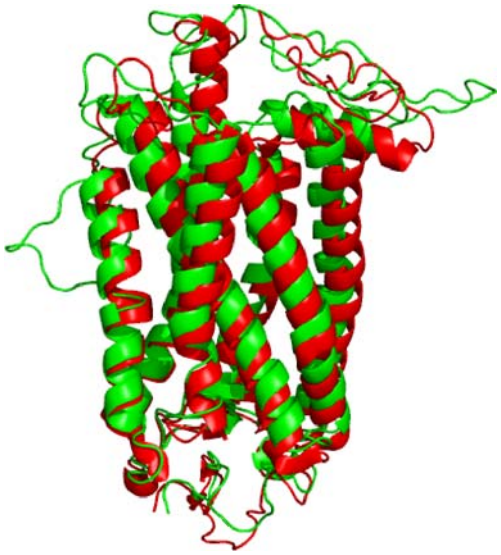

Fig. 3 Represents the superimposed structure of 1U19 A with the predicted model of human β_2 Adrenergic Receptor. Red colored ribbons represent 1U19 A and green colored ribbons represent the predicted model. It shows that they have almost the same structural similarity

decreased the incidence of HAPE [10] and Nifedipine in the form of single randomized placebo - controlled trial in persons susceptible to HAPE [19]. Based on these findings, Salmeterol and Nifedipine were used as ligands.

The Drugbank accession number for Salmeterol is APRD00277 (Fig. 5). The Drugbank accession number for Nifedipine is APRD00590 (Fig. 6). CORINA was used to generate a theoretical 3D structure for Salmeterol (Fig. 7) and Nifedipine (Fig. 8).

Human β_2 adrenergic receptor and ligand datasets were docked using GOLD with 10 runs for each docking procedure. The well docked complexes in lowest docked energy with average GOLD fitness score were enumerated. Following the completion of all docking runs on a ligand, the results from the different runs are compared in the ligand log file.

Table 2 Ramachandran plot statistics

Residues in most favored regions (A,B,L)	290	84.1%
Residues in additional to allowed regions (a,b,l,p)	46	13.3%
Residues in generously allowed regions (−a,−b,−l,−p)	6	1.7%
Residues in disallowed regions	3	0.9%
	—	—
Number of non - glycine and non - proline residues	345	100.0%
Number of end - residues (excl. Gly and Pro)	2	
Number of glycine residues \(shown as triangles)	23	
Number of proline residues	10	
	–	
Total number of residues	380	

Summary of the generated Ramachandran plot showing the percent of allowed and disallowed regions for human β_2 adrenergic receptor.

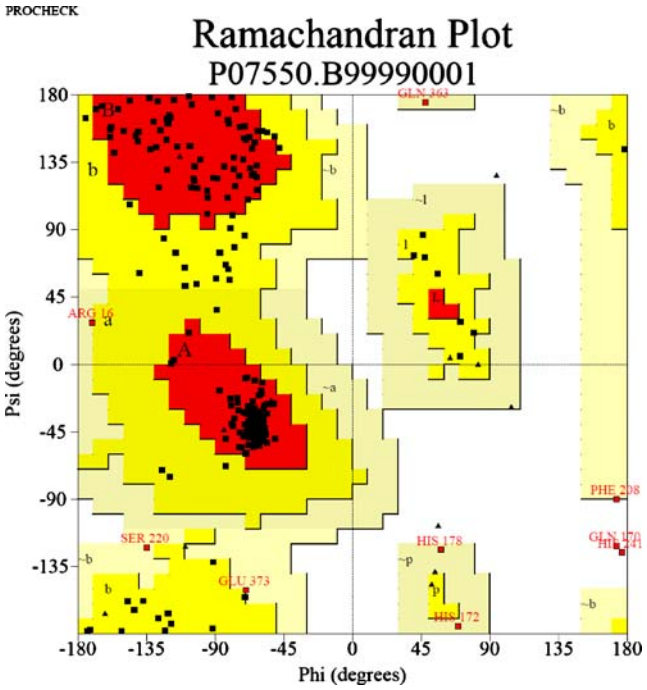

Fig. 4 Ramachandran plot generated by PROCHECK. White areas correspond to sterically disallowed region, red areas corresponds to allowed regions, yellow areas correspond to the atoms in the generously allowed regions

In the case of Salmeterol, solution number 2 had the largest fitness score while solution number 5 had the worst fitness. Figure 9 represents this best fit solution for salmeterol. The total Chemscore fitness value of Salmeterol is 44.89. Since it has the high score, it is the best fitness score and the best docking result. The contribution to Chemscore value by free energy change (that occurs on ligand binding) is −53.59. Protein-ligand H-bond contribution to Chemscore Value is 1.84. The metal-binding contribution to Chemscore value is 0. The protein-ligand lipophilic contribution to the Chemscore value is 460.36. The protein-ligand clash penalty to the Chemscore value is 2.14. The internal ligand torsional strain penalty to the Chemscore value is 6.57.

In the case of Nifedipine, solution number 5 had the largest fitness score while solution number 7 had the worst fitness. Figure 10 represents this best fit solution for Nifedipine. The total Chemscore fitness value of Nifedipine is −17.12. The contribution to Chemscore value by free

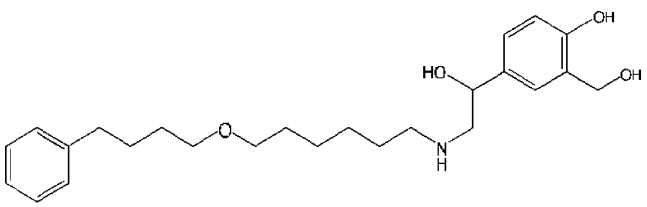

Fig. 5 Chemical structure of Salmeterol or 2-(hydroxymethyl)-4-[1-hydroxy-2-[6-(4-phenyl butoxy) hexylamino] ethyl]-phenol which is one of the ligand used

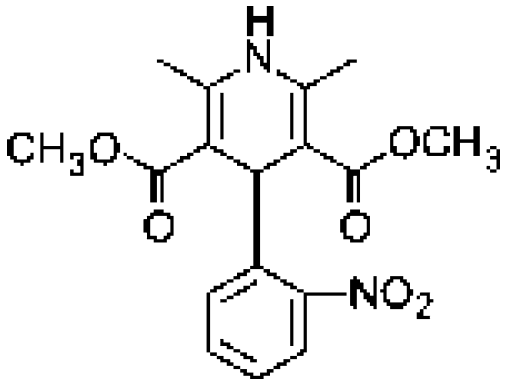

Fig. 6 Chemical structure of Nifedipine or 3, 5-pyridinedicarboxylic acid, 1, 4-dihydro=2, 6=dimethyl-4=(2=nitrophenyl I)-dimethyl ester which is another ligand used

energy change (that occurs on ligand binding) is −36.61. Protein-ligand H-bond contribution to Chemscore value is 0. The Metal-binding contribution to Chemscore value is 0. The protein-ligand lipophilic contribution to the Chemscore value is 298.30. The protein-ligand clash penalty to the Chemscore value is 8.11. The internal ligand torsional strain penalty to the Chemscore value is 45.61.

Gold docking study was performed in order to find the interactions between the human β_2 adrenergic receptor and the selected ligands Salmeterol and Nifedipine. After 10 docking runs for each of the ligand, the fitness scores were generated. The fitness score is taken as the negative of the sum of the component energy terms, so that larger fitness scores are better. The fitness function has been optimized for the prediction of ligand binding positions. The fitness score reveals that the ligand Salmeterol has the best fitness score. It has the higher score of 44.89 than Nifedipine which has −17.12. Thus it was found that Salmeterol binds best to the target human β_2 adrenergic receptor.

The binding affinity for the generated protein - ligand complex of human β_2 adrenergic receptor and salmeterol; and human β_2 adrenergic receptor and Nifedipine was estimated using X - Score. Table 3 shows that Salmeterol showed HPScore of 8.36, HMScore of 8 and HSScore of 6.51. The predicted average is 7.62 with a predicted binding energy of −10.40. Nifedipine showed HPScore of 7.25, HMScore of 6.82 and HSScore of 6.33. The predicted average is 6.80 with a predicted binding energy of −9.27. Salmeterol has a high binding affinity of −10.40.

X-Score is typically applied in combination with a molecular docking program, such as DOCK, AutoDock,

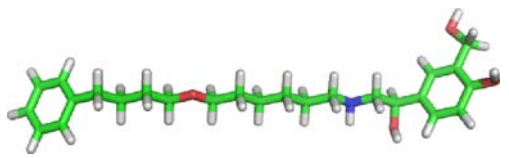

Fig. 7 Generated 3D structure of Salmeterol using CORINA. This 3D structure is used for docking

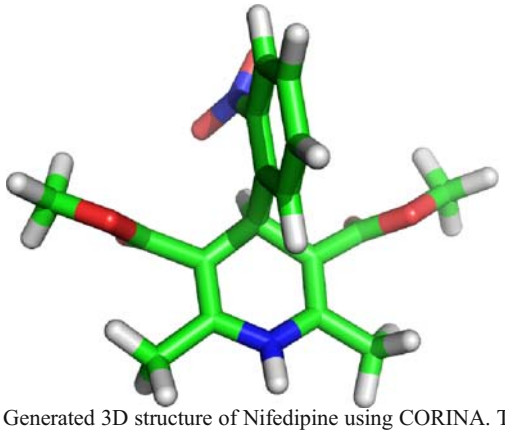

Fig. 8 Generated 3D structure of Nifedipine using CORINA. This 3D structure is used for docking

FlexX or GOLD, in structure-base drug design studies. The molecular docking program will provide the binding model of the molecules of interests to the given target protein. Then, X-Score can be applied to give more accurate estimation of the binding free energies of these molecules and re-rank them accordingly [20]. Evaluation of binding free energy in receptor-ligand complexes is one of the most important challenges in theoretical drug design. Free energy is directly correlated to the thermodynamic affinity constant, and, as a first step in drug likeness, a lead compound must have this constant in the range of micro- to nanomolar activity [21].

This analysis for the estimation of binding affinity revealed that the predicted X-Score for Salmeterol is 7.62 with a predicted binding energy of −10.40 whereas the predicted X-Score for Nifedipine is 6.80 with a predicted binding energy of −9.27. Thus, Salmeterol was found to have the best binding affinity out of these two ligands.

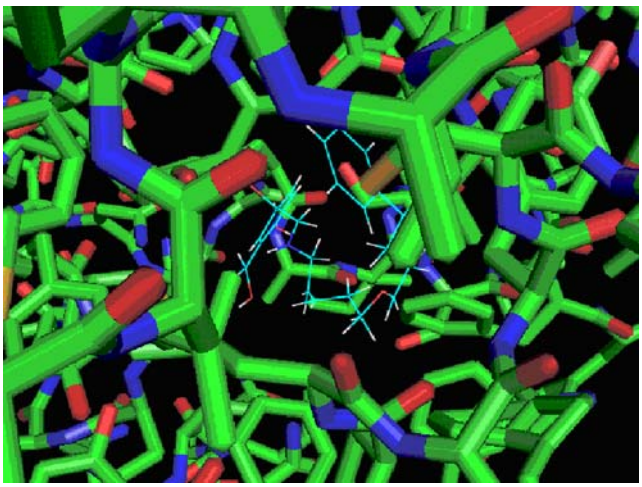

Fig. 9 Salmeterol's interaction with human β_2 adrenergic receptor. This figure represents the structure of the best docked solution of Salmeterol

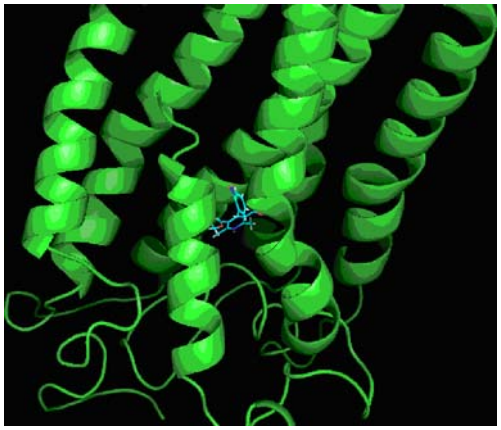

Fig. 10 Nifedipine's interaction with human β_2 adrenergic receptor. This figure represents the structure of the best docked solution of Nifedipine

The most stable docking models were selected according to the best-scored conformation predicted by the GoldScore and X-Score scoring functions [22]. The best predictability was achieved using the average of the three scores calculated by X-score [23].

Conclusions

This work aimed at the modeling of the 3D structure and studies on its interaction with agonists such as Salmeterol and Nifedipine. β_2 adrenergic agonists have been identified to up-regulate the clearance of alveolar fluid by stimulating transepithelial sodium transport and attenuate alveolar flooding. Salmeterol acts in preventing edema formation at high altitude. Nifedipine offers a potential emergency treatment for HAPE when descent or evacuation is impossible and oxygen is not available.

Docking studies were carried out with two well known ligands namely Salmeterol and Nifedipine to study their interaction with the modeled human β_2 adrenergic receptor. From the docking studies it can be concluded that Salmeterol binds best to human β_2 adrenergic receptor. X-

Table 3 X-score results

Ligands (-log(Kd)	HPScore (-log(Kd)	HMScore (-log(Kd)	HSScore (-log (Kd)	Predicted average (-log(Kd) (*)	Predicted Binding Energy (kcal/mol)
Salmeterol	8.36	8.00	6.51	7.62	−10.40
Nifedipine	7.25	6.82	6.33	6.80	−9.27

HPScore - hydrophobic pair score, HMScore - hydrophobic match score, HSScore - hydrophobic surface score, * is the average of X Score averages.

Score analysis revealed the binding affinity of the given ligands with the generated three-dimensional structure.

Finally, we wish to conclude that this work can be considered as the preliminary approach in determining the functional characteristics of β_2 adrenergic receptor. It will expand our knowledge of the role of β_2 adrenergic receptor in pathological conditions and suggest new therapeutic approaches. The generated model is very reliable and can be used for further studies. A computer generated molecular model such as that reported here cannot substitute for a crystal structure. The modeled structure proves to be a useful for exploiting the increasing amount of information on β_2 adrenergic receptors. Since only two ligands were used in this study, similar work could be done using other new molecules which could interact with this receptor.

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