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Original Article

Homology modeling, binding site identification and docking study of human angiotensin II type I (Ang II-AT_1) receptor

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ABSTRACT

Ang II-AT_1 receptors play an important role in mediating virtually all of the physiological actions of Ang II. Several drugs (SARTANs) are available, which can block the AT_1 receptor effectively and lower the blood pressure in the patients with hypertension. Currently, there is no experimental Ang II-AT_1 structure available; therefore, in this study we modeled Ang II-AT_1 receptor structure using homology modeling followed by identification and characterization of binding sites and thereby assessing druggability of the receptor. Homology models were constructed using MODELLER and I-TASSER server, refined and validated using PROCHECK in which 96.9% of 318 residues were present in the favoured regions of the Ramachandran plots. Various Ang II-AT_1 receptor antagonist drugs are available in the market as antihypertensive drug, so we have performed docking study with the binding site prediction algorithms to predict different binding pockets on the modeled proteins. The identification of 3D structures and binding sites for various known drugs will guide us for the structure-based drug design of novel compounds as Ang II-AT_1 receptor antagonists for the treatment of hypertension.

1. Introduction

The renin–angiotensin system (RAS) has a central role in blood pressure regulation, fluid and electrolyte homeostasis for the management of hypertension [1]. The linear octapeptide angiotensin II (Ang II) is a potent vasoconstrictor in the RAS cascade, which is produced from angiotensin I (Ang I) by the angiotensin-converting enzyme (ACE) [2]. In humans, Ang II interacts with specific cellular 7-transmembrane, G protein-coupled receptors, namely AT_1 and AT_2. The AT_1 receptors are expressed in the key organ systems including kidney, adrenal gland, immune system, vasculature, heart and both central and peripheral nervous systems and elicit virtually all the known intracellular effects of Ang II [3,4]. Ang II increases blood pressure by different mechanisms in these systems. In vascular smooth muscle it induces vasoconstriction, and in the adrenal cortex it acts on the zona glomerulosa and stimulates aldosterone biosynthesis and secretion; it also modulates fluid balance by affecting tubular sodium reabsorption, apart from constriction of both efferent arterioles and glomerular magnesium [5,6]. AT_1 receptor antagonism is an effective way to control pathogenesis of hypertension and heart failure. Ang II receptor blockers (ARBs) are developed for the treatment of hypertension. ARBs exert their action by blocking the binding of Ang II with the AT_1 receptor rather than by inhibiting its synthesis. ARBs antagonize AT_1-mediated effects of Ang II. Losartan [7] was the first example of this class of drug. Other ARBs are valsartan, irbesartan, telmisartan, candesartan, olmesartan and one recently approved drug azilsartan [8] (Fig. 1). Common structural features of these drugs for binding with the AT_1 receptor are the presence of a biphenyl ring for aromatic hydrophobic interactions, acidic moiety like carboxylate/tetrazole attached to biphenyl moiety for ionic interactions with AT_1 receptor, and an alkyl side chain linked to heterocycles considered to be essential group for lipophilic interaction with the AT_1 receptor. Different heterocyclic ring systems are present in these compounds; among them, benzimidazole ring was particularly found to be very effective heterocyclic system considering its interaction with the AT_1 receptor [9]. To date, there are no experimental structural data available for the Ang-AT_1 receptor. In such cases, homology modeling studies to build and refine 3D models of the Ang-AT_1 receptor, characterization of active sites and docking study with known drugs into the model structure are the most accessible approaches to studying the 3D conformation of protein and binding mode of the known ligand. Homology modeling provides structural insight of modeled receptor, although the quality depends on sequence similarity with the template structure. We

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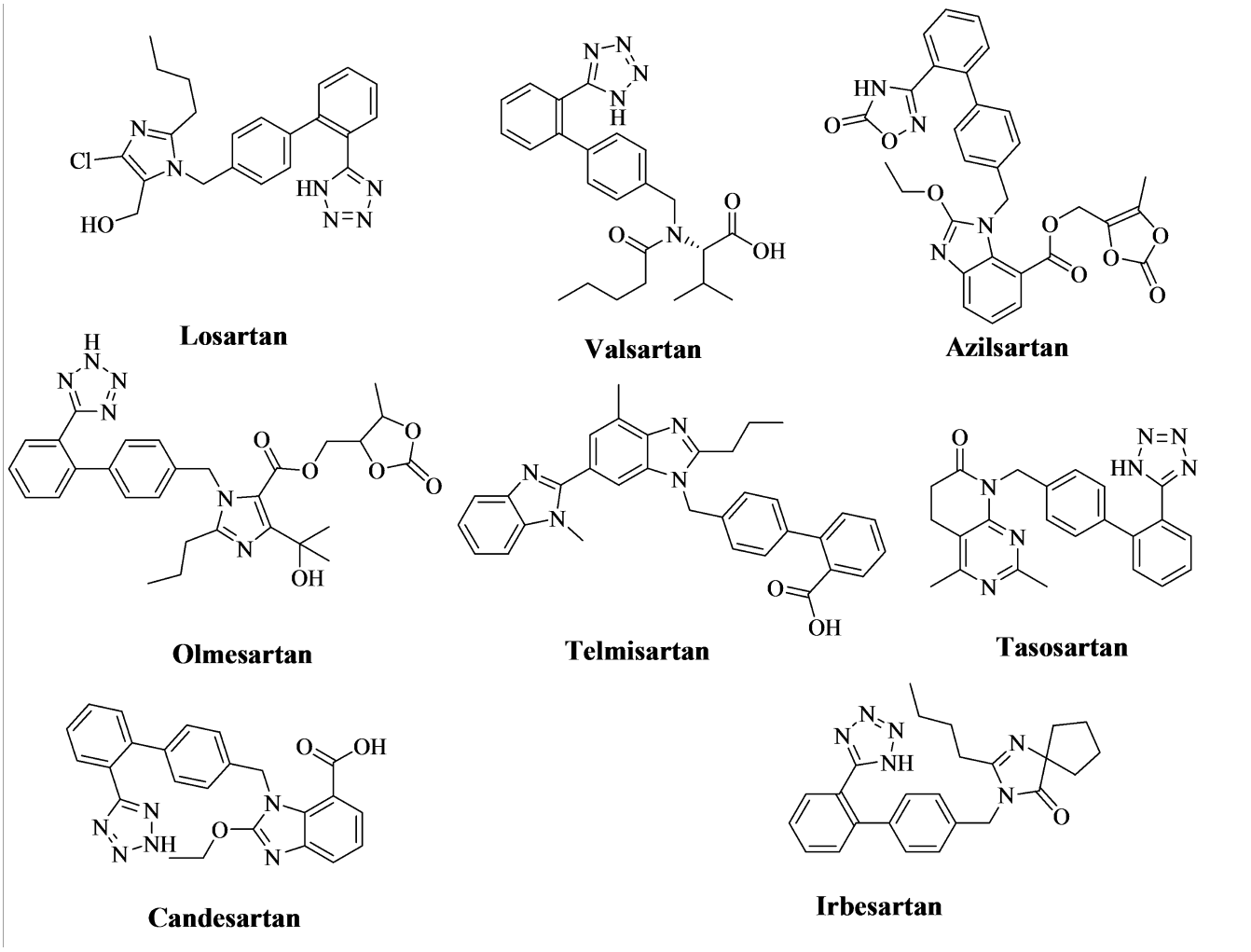

Fig. 1. Chemical structure of known angiotensin II-AT_1 receptor blocker (ARBs) drugs.

have been engaged since last half a decade in the *in silico* design, synthesis and pharmacological evaluation of novel angiotensin II receptor blocker agents. In the year 2009 [10], we have performed a QSAR study on 2-alkyl-4-(biphenylmethoxy)quinolone derivatives as angiotensin II receptor antagonists; then, in the year 2011 [9], we have compiled the literature of substituted benzimidazole derivatives as Ang II-AT_1 receptor antagonist in a review paper. In the year 2013 [11], we have performed CoMFA and CoMSIA study on substituted benzimidazole derivatives as ARBs, and based upon *in silico* design, [12] we have synthesized substituted benzimidazole derivatives and screened them as Ang II-AT_1 receptor antagonists in the year 2014. In continuation of our work, now we are focused on structure-based *in silico* design of ARBs, and in an attempt to that in the present study, we have constructed 3D structure of Ang-AT_1 receptor and performed binding site analysis using various algorithms. The 3D models of Ang-AT_1 receptor were used to carry out the binding analysis with known ARBs (antihypertensive drugs) in docking study to understand the interactions with the functional residues in the modeled receptor structure.

2. Materials and methods

2.1. Modeling platform and software

Model building and all *in silico* simulations were carried out on windows platform with core i5 processor. The multiple sequence alignment was performed using CLUSTALW [13] program. MODELLER [14] and I-TASSER [15,16] server were used for the protein homology modeling and PROCHECK [17] was used for structure validation. DoGSiteScorer [18] was used for binding site prediction study. Surflex-Dock (SYBYL X 1.2) [19] was used for docking study. Chemical structures of known drugs (Fig. 1) were drawn and energy minimized in SYBYL.

2.2. Sequence alignment

The amino acid sequence of Ang II-AT_1 receptor was obtained from UniProt database (accession no. P30556) [20] at http://www.uniprot.org. The first requirement in the construction of homology models is the sequence alignment. The basic local alignment search tool (BLAST) [21] program against protein data bank (PDB) was used to select a template structure for protein modeling of Ang II-AT_1 receptor. A search with BLAST against the database for optimal local alignments with the query gives a list of known protein structures that matches the sequence. The crystal structures of the *human* chemokine (C-X-C motif) receptor 1 (CXCR1) in phospholipid bilayers resolved with solid NMR accessed with PDB ID: 2LNL [22], *human* kappa opioid receptor in complex with JDTic resolved at 2.90 Å with PDB ID: 4DJH [23], CXCR4 and viral chemokine antagonist vMIP-II complex (PSI community target) resolved at 3.10 Å with PDB ID: 4RWS and CCR5 [24], respectively and chemokine receptor resolved at 2.71 Å with PDB ID: 4MBS [25] were used as templates for protein modeling and retrieved from protein data bank (PDB) at http://www.rcsb.org/pdb. The

target-templates alignment was carried out using the program CLUSTALW [13] with default parameters, which seemed to be optimum for our target protein. The multiple sequence alignment is based on identifying structurally conserved regions (SCR) common to the template and target.

2.3. Homology modeling

The 3D protein models of Ang II-AT_1 receptor were constructed using the MODELLER program implemented on a windows platform [14] and I-TASSER server [15,16]. Using the sequence alignment and the crystal structure of the template protein as an input, MODELLER extracts large number of spatial restraints from the template structure and builds a homology model of the target protein. In total, five models of Ang II-AT_1 receptor were generated with differing geometric conformations showing the least root mean square deviation (RMSD) with respect to trace (Cα atoms) of the crystal structure of the template. Further, refinement was performed in order to obtain the best conformation of structure resulting from MODELLER. The 3D models of Ang II-AT_1 were also constructed using the I-TASSER server. I-TASSER server was used to predict an automated full-length 3D structure of Ang II-AT_1, where the benchmarked scoring system helps to obtain quantitative assessments of the I-TASSER models. The output of the I-TASSER server for each query includes up to five full-length models, the confidence score (*C*-score), the estimated TM-score and RMSD, and the standard deviation of the estimations. To select the final models, I-TASSER uses the SPICKER program to cluster all the decoys based on the pair-wise structure similarity, and reports up to five models which corresponds to the five largest structure clusters.

2.4. Protein structure refinement and energy minimization

Structure refinement of the modeled Ang II-AT_1 receptor was performed using the KoBaMIN [26] web server (a knowledge-based minimization web server for protein structure refinement) in order to obtain the best conformation of modeled structures resulting from MODELLER and I-TASSER sever. The KoBaMIN web server provides an online interface to a simple, consistent and computationally efficient protein structure refinement protocol based on minimization of a knowledge-based potential of mean force.

2.5. Evaluation of models

The stereochemical quality of the protein structure was examined by Ramachandran plot using the PROCHECK program [17]. The number of residues that were in the allowed and disallowed regions of Ramachandran plot determines the quality of the model. The Ang II-AT_1 receptor model was found with an ERRAT quality factor [27] of 80.060 and the Ramachandran plot provided by PROCHECK analysis.

2.6. Binding pocket analysis

Surface pockets and sub-pockets for catalytic domain of modeled Ang II-AT_1 receptor structure were identified using program DoGSiteScorer [18]. DoGSiteScorer is automated pocket detection and analysis tool, which can be used for protein druggability assessments. Predictions with DoGSiteScorer are based on calculated size, shape and chemical features of automatically predicted pockets, incorporated into a support vector machine (SVM) for druggability estimation. A druggability score between zero and one is returned, and the higher the drug score, the more druggable the pocket is estimated to be for docking with the ligands.

2.7. Molecule building

Losartan, valsartan, telmisartan, candesartan and irbesartan were built using 'sketch' option in SYBYL and minimized using conjugate gradient algorithm with a gradient convergence value of 0.01 kcal/mol Å. Partial atomic charges were calculated using the Gasteiger–Huckel method.

2.8. Molecular docking studies

Docking experiments were performed using Surflex-Dock [19] module of SYBYL X 1.2. Five known ARBs were docked into the binding pocket of Ang II-AT_1 protein model generated using MODELLER. Surflex-Dock is a fully automatic flexible molecular docking algorithm that combines the scoring function from the Hammer-head docking system with a search engine that relies on a surface-based molecular similarity method as a mean to rapidly generate suitable putative poses for molecular fragments. Blank protomol generation was applied for the determination of the active site of the homologous proteins. The amino acid residues of the side chains were minimized energetically and Gasteiger–Huckel (GH) charges were applied.

3. Results and discussion

3.1. Sequence analysis

Database search was performed using the BLAST against PDB from all the crystal structures that were available at the time of this study. Human AGTR1 (*human* angiotensin II type 1 receptor) FASTA sequence shares 34% identity and 78% query coverage with 2LNL, 28% identity and 83% query coverage with 4MBS, 37% identity and 63% query coverage with 4DJH and 36% identity and 82% query coverage with 4RWS (Table 1). The multiple sequence alignment was accomplished using the program CLUSTALW, which included the sequences of AGTR1, and the selected PDB templates (Fig. 2). The resulting sequence alignment showed that AGTR1 contained most of the highly conserved residues that were found in PDB ID: 2LNL.

3.2. Ang II-AT_1 receptor structure construction and validation

3D structures of the Ang II-AT_1 receptor were modeled using the I-TASSER server (5 models) and MODELLER (5 models) program. These 3D structures were superimposed on the experimental structures (2LNL, 4DJH, 4RWS and 4MBS), and the RMSD of the

Table 1
Template identification using BLAST against protein data bank (PDB).

Type	Method	Template		Identity (%)	Query cover	Score	*E*-value
		PDB ID	Chain				
Angiotensin II type I	PSI-BLAST	2LNL	A	34	78	141	2e−38
		4MBS	A	28	83	148	5e−40
		4DJH	A	37	63	164	5e−34
		4RWS	A	36	82	132	2e−33

Fig. 2. Multiple sequence alignment between AGTR1 (P30556) query sequence and template structures (2LNL, 4DJH, 4RWS and CCR5).

Table 2
Ramachandran plots qualities refined by SYBYL X 1.2 program.

Refined model	Ramachandran plot quality (%)			Average *G*-factor	RMSD (Å) from template backbone
	Allowed	Generally allowed	Disallowed		
2LNL	96.3	2.6	1.1	−0.13	–
Ang II-AT$_1$	96.9	1.5	1.5	−0.14	1.603

models with the experimentally determined structures of templates (all backbone atoms) was calculated using SuperPose v1.0. The modeled Ang II-AT$_1$ receptor structure showed lowest RMSD with PDB ID: 2LNL (Table 2). Further refinement of modeled structures was carried out using KoBaMIN web server, and out of all the 10 models (5 from I-TASSER and 5 from MODELLER), one best model (Fig. 3) was selected based upon its lowest RMSD (1.603 Å) and energy value (−8889.135 kcal/mol). The best possible model was selected for further *in silico* studies. The resulting model was evaluated using the program PROCHECK. The Ramachandran plot obtained from PROCHECK is shown in Fig. 4. It is not possible for the Ang II-AT$_1$ model to be completely accurate, and validation by Ramachandran plot (RAMPAGE and PROCHECK) supports the reliability of modeled Ang II-AT$_1$ structure. Ramachandran plot qualities showed the amount (%) of residues belonging to the favoured, allowed, generally allowed and disallowed region of the plot. Ramachandran plot showed probably 88.4% of 290 amino acid residues in the most favoured regions, 8.5% of 28 amino acid residues in allowed regions, and 1.5% of 5 amino acid residues in generally allowed regions and 1.5% of 5 amino acid residues in disallowed region of Ang II-AT$_1$ modeled structure (Table 3). The constructed and validated model was used for active site prediction.

3.3. Analysis of binding pocket and druggability assessment

Amino acids detected in the binding pocket are mainly hydrophobic in character, and some charged amino acids were also found in the binding pocket. DoGSiteScorer has detected 0–9 potential pockets (P0–P9) and 0 to 30 sub-pockets on the surface of modeled Ang II-AT$_1$ structure. The first pocket (P0) is estimated to be druggable with highest drug score of 0.67. A large pocket volume (3821.30 Å^3), high depth (39.77 Å) and a high apolar amino acid ratio (0.58) are the properties of the pocket that generally characterized the druggable pocket. Since the detected pocket is rather large, the analysis based on sub-pocket level may provide a

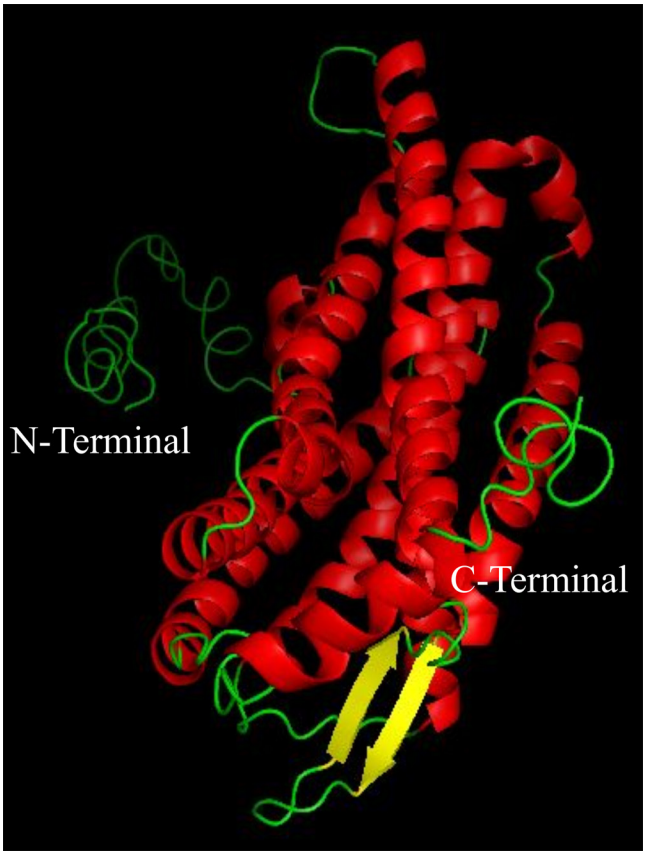

Fig. 3. Homology models (3D structures) Ang II-AT$_1$ receptor. Colors are according to the secondary structure. Red color in the figure indicates α-helix, yellow color indicates β-sheet, and turns are in green color structures with C- and N-terminal ends. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

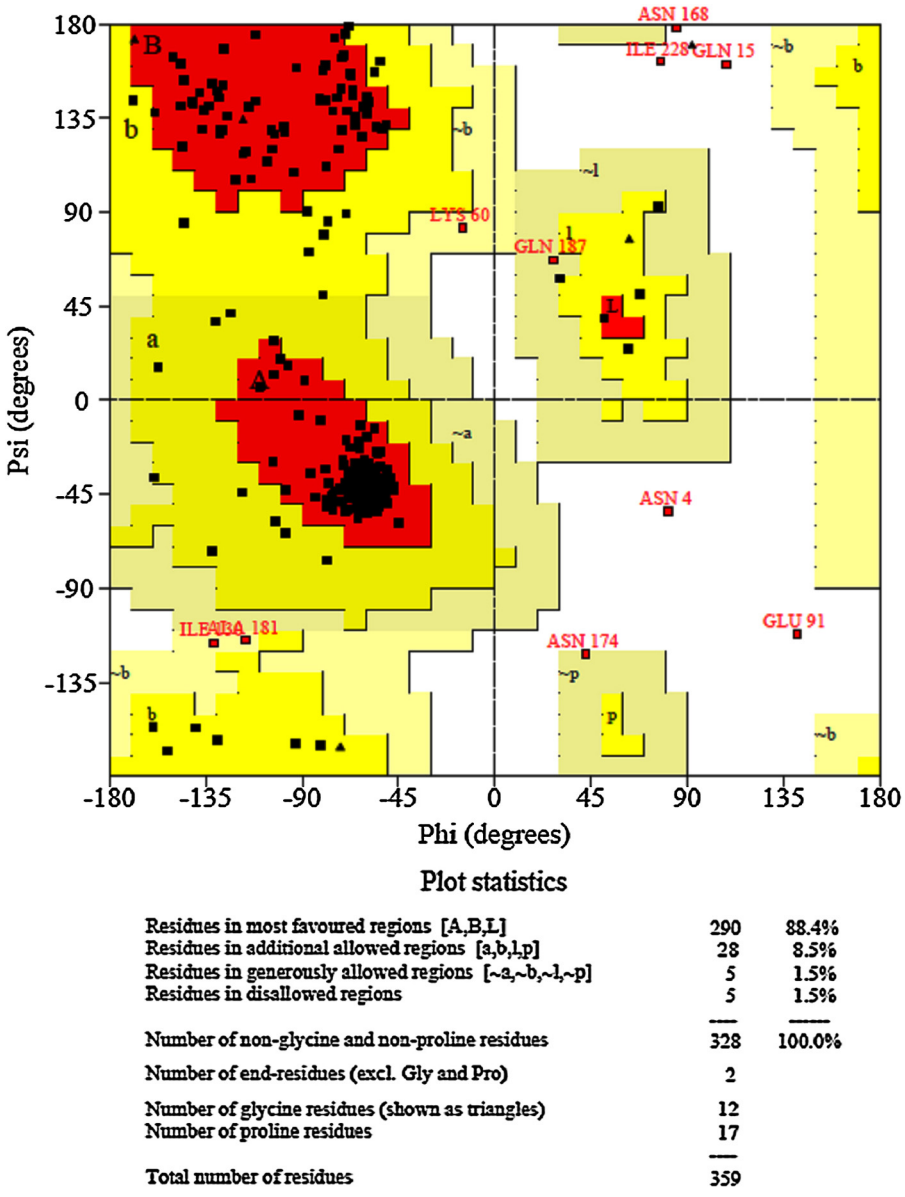

Fig. 4. Stereochemical analysis of Ang II-AT$_1$ receptor obtained from Ramachandran plots. Red color in the plots indicates the most favorable region, yellow represents additionally allowed, light yellow indicates generously allowed and white field indicates disallowed region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 3
The calculated RMSD values between Ang II-AT$_1$ modeled structure and, the superimposed template structures.

Model	Local RMSD (Å)				Global RMSD (Å)			
	Alpha carbons	Back bone	Heavy	All	Alpha carbons	Back bone	Heavy	All
2LNL	1.522	1.51	2.125	2.407	1.522	1.51	2.125	2.407
4MBS	2.643	2.617	2.797	2.797	2.643	2.617	2.797	2.797
4DJH	6.517	6.517	6.84	6.84	6.517	6.517	6.84	6.84
4RWS	5.463	5.337	5.617	5.617	5.463	5.337	5.617	5.617

more sensible view on binding site. The sub-pockets P0SP00 achieved a highest druggability score of 0.77 having high apolar amino acid ratio of 0.74.

3.4. Molecular docking analysis

To study the binding mode of the known ARBs with the Ang II-AT$_1$ receptor, we performed molecular docking study (Table 4)

Table 4
Docking score of known ARBs.

S. No.	Known ARBs	Total score	Crash	Polar
1	Telmisartan	8.740	−9.678	0.720
2	Losartan	5.333	−4.821	0.013
3	Candesartan	3.302	−7.256	0.799
4	Valsartan	3.807	−9.166	0.518
5	Irbesartan	2.682	−12.903	0.001

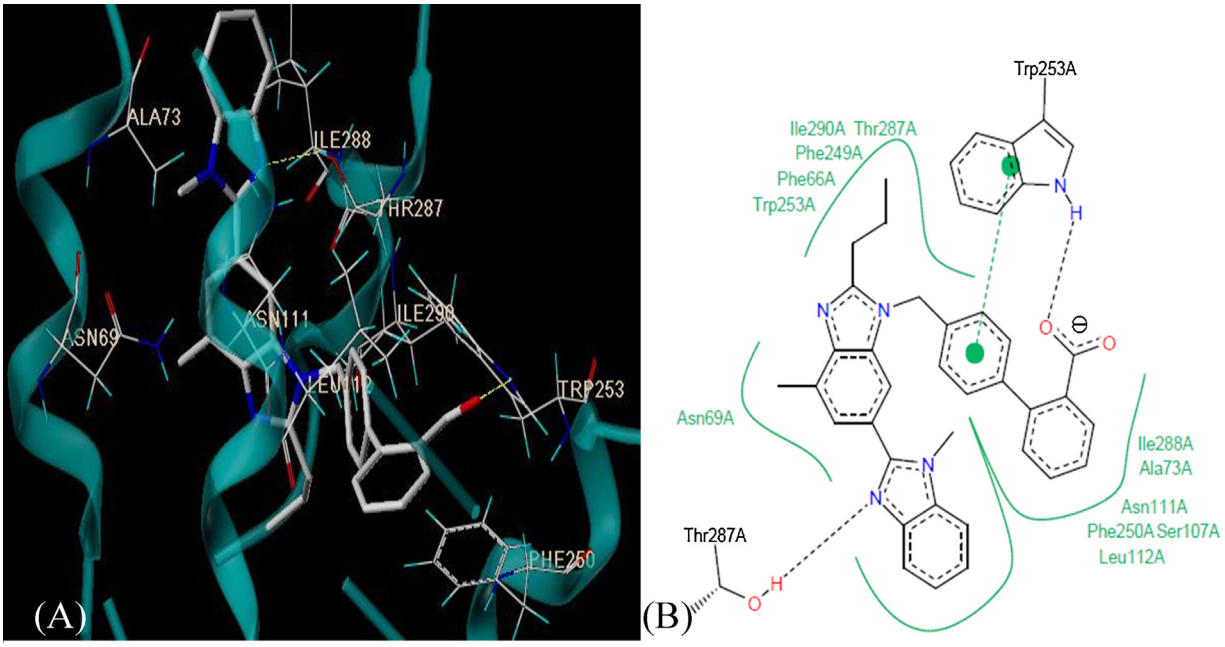

Fig. 5. Binding mode of telmisartan in the modeled Ang II-AT$_1$ receptor proposed by docking studies using Surflex-Dock (A), telmisartan is in stick model with color by atoms, H-bond interactions were highlighted using yellow dotted lines. The labeled protein residues are in capped stick model with color by atom. Two dimensional (2D) interaction model of telmisartan within the active site of Ang-AT$_1$ receptor according to chemical drawing conventions generated using PoseView at http://poseview.zbh.uni-hamburg.de/ (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

into the best predicted binding pocket of the modeled structure using the Surflex-Dock module. After running Surflex-Dock, the scores of the active docked conformers were ranked in a molecular spreadsheet. We selected the best total score conformers and speculated regarding the detailed binding patterns in the cavity. Surflex-dock results mainly contain 3 information, total score, which is the total Surflex-Dock score expressed as $-\log(K_d)$ to represent binding affinities, which include hydrophobic, polar, repulsive, entropic and solvation. Crash value is the degree of inappropriate penetration by the ligand into the protein and of interpenetration between ligand atoms (self-clash) that are separated by rotatable bonds. Crash score close to 0 is favourable. Polar value is the contribution of the hydrogen bonding and salt bridge interactions to the total score.

3.5. Binding pose of telmisartan in Ang II-AT$_1$ receptor

Docking study suggested that most of the compounds have a common binding mode in the active binding site pocket of Ang II-AT$_1$ receptor. Docking results suggested that telmisartan has the highest docking score of 8.7401 (Table 4). The overall binding of telmisartan in Ang II-AT$_1$ receptor structure is illustrated in Fig. 5. Telmisartan formed two hydrogen bonds (H-bond) in the active site of Ang II-AT$_1$ receptor. The oxygen atom of carboxylate group substituted on biphenyl ring formed one H-bond with –HN of indole ring of Trp253. Second H-bond was formed between –N atom of terminal benzimidazole ring of telmisartan with –H atom of hydroxy group of Thr287. Lipophilic propyl side chain was found in contact with lipophilic amino acid residues like Ile290, Phe249, Phe66 and aromatic hydrobhopic biphenyl ring was found in contact with aromatic amino acid residue Phe250 and hydrophobic amino acid residues like Ile280, Ala73, Leu111, which are important residues for the hydrophobic interactions with the receptor. The parent benzimidazole ring was found in contact with Asn69. Docking study explored the interaction mechanism and reasonable binding mode of these known drug molecules in the active site of Ang II-AT$_1$ receptor. Known ARBs showed interactions with several residues near the active site, including Phe66, Asn69, Ala73, Asn111, Leu112, Ser115, Phe204, Phe249, Phe250, Trp253 Thr287 Ile288, Ile290 and Ala291 which seem to be important residues for aromatic hydrophobic, ionic and lipophilic interactions of small molecule as antagonist of Ang II-AT$_1$ receptor.

4. Conclusions

In this study, 3D structures of the Ang II-AT$_1$ receptor were constructed based on homology modeling principles. The models were validated by well-known procedures. Binding site analysis was performed to identify binding pocket and the functional residues involved in it. Known angiotensin II receptor blockers were docked into the resulted homology models and the ligand–protein complexes were analyzed. The results gave insights into the characterization of key H-bonding, hydrophobic residues, and defining the binding pocket of Ang II-AT$_1$ receptor. The present study is a step towards characterization of Ang II-AT$_1$ proteins and will be very helpful for the structure-based design and discovery of novel angiotensin II receptor blockers as antihypertensive agents.

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