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Study of Binding Site for Losartan and Irbesartan As Angiotensin II Receptor Antagonists as Antihypertensive Agents - Part I

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ABSTRACT

This study was designed to examine the importance of interaction in the binding of selective angiotensin II receptor antagonists to angiotensin II type 1 receptor using molecular modeling. AT_1 receptor structural model used in the study is 1Zv0. All possible binding sites for these drugs were suggested to lie between transmembrane domains (TM) 3, 5, and 6 of AT_1 receptor are studiedthrough molecular modeling.

Keywords: losartan, irbesartan, angiotensin II, antihyopertensive

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INTRODUCTION

The angiotensin II (Ang II) type 1 (AT1) receptor belongs to family A of 7 transmembrane (7TM) receptors, also known as G protein-coupled receptors [1,2] that are categorized under the superfamily of membrane proteins. The physiological and pathological importance of the AT1 receptor is underscored by the therapeutic use of AT1 receptor blockers and angiotensin converting enzyme-inhibitors in the treatment of cardiovascular diseases such as hypertension, diabetic nephropathy and cardiac arrhythmia and failure [3]. The human angiotensin II receptors mediate actions of the endogenous agonists such as catecholamine, the neurotransmitter nor-epinephrine, and the hormone epinephrine. Two subtypes of angiotensin receptors have been identifiedand pharmacologically characterized designated as AT1 and AT2 receptors [4-6]. Two more angiotensin II receptors have been described: AT3 and AT4. The first of these two was named ‘‘AT3’’ but it was not included in the update of angiotensin receptor nomenclature proposed by the IUPHAR subcommittee on Angiotensin Receptors [7]. The AT4 receptor, which was originally defined as the specific, high-affinity binding site for the hexapeptide angiotensin IV, has recently been identified as the transmembrane enzyme insulin-regulated membrane amino-peptidase (IRAP) [8].The pioneering efforts of the DuPont Group have generated a promising first non-peptide AT1 antagonist, which represent the prototype of the sartans, was losartan. In the last decades several selective antagonists have been developed and are used to treat both hypertension and damage associated with the diseases such as arthrosclerosis and diabetes [9-19]. All the sartans bind to the AT1 receptors and share common structural features. These sartans are designed to mimic the C-terminal part of Ang II.

All sartans bind with a high affinity to the AT1 receptor and act by same mechanism of action, though the modes of interaction with the receptor are different [20,21]. Sartans such as losartan, eprosartan, and tasosartan bind to the receptor with different degrees of surmountability. Valsartan, irbesartan, candesartan, and the active metabolite of losartan (EXP3174) behave like insurmountable antagonists. A possible explanation for this different response is that the surmountable antagonists interfere with receptor activation by occupying an intra-membrane site that overlaps with the space occupied by the agonist, while insurmountable antagonists induce conformational changes that prevent agonist binding. Another theory hypothesizes that surmountable antagonists dissociate rapidly from the receptor, whereas insurmountable antagonists bind tightly and dissociate so slowly as to cause a prolonged functional loss of the occluded receptors [1].

Knowledge of the 3D structure of AT receptors assists in understanding molecular interactions, and in the rational design of specific ligands, however, as GPCRs are membrane-bound proteins, high-resolution structural characterization is still an extremely difficult task. For this reason, great significance has been placed on molecular modeling studies with emphasis on homology modeling (HM) techniques [22].

The objective behind this study was to investigate, through molecular modeling, the difference in the ligand binding to AT_1 receptor that result from the significant interactions of the functional groups of the ligands with specific amino acid residues of the AT_1 receptor. The functional groups that are pivotal for biological activity of Angiotensin II receptor

blockers is the heterocyclic ring (imidazole in losartan) that binds to amino acids in helix 7 (Asn295). The second group is the biphenyl-methyl group that binds to amino acids in both helices 3, 6 and 7 (Val 108, Phe301, Phe300, Trp253 and His256). The third one is the tetrazole group that interacts with amino acids in helices 4 and 5 (Arg167 and Lys199). The hydroxymethyl group of losartan interacts with amino acids in helix 3 (Ser109). Identification of residues involved in non-peptide ligand binding facilitates studies aimed at elucidating the chemical basis for ligand recognition in the AT receptor [23-27]. Several site-directed mutagenesis studies have been performed to examine the residues involved either in ligand binding or in signal transduction. The key amino acids for binding on non-peptide AT_1 antagonists are Val 108, Ser 109, Ala 163, Arg 167, Lys 199, Trp 253, His 256, Asn 295, Phe300, Phe301 [28].

METHODOLOGY

Molecular docking helps in studying drug/ ligand or receptor/ protein interactions by identifying the suitable active sites in protein, obtaining the best geometry of ligand - receptor complex and calculating the energy of interaction for different ligands to design more effective ligands. The target or receptor is either experimentally known or theoretically generated through knowledge based protein modeling or homology modeling. The molecular docking tool has been developed to obtain a preferred geometry of interaction of ligand - receptor complexes having minimum interaction energy based on different scoring functions viz. only electrostatics, sum of steric and electrostatic (parameters from MMFF force field) Dock Score. This utility allows one to screen a set of compounds for leadoptimization. VLifeMDS [29] uses genetic algorithm (GA), Piecewise Linear Pairwise Potential (PLP) and Grid algorithms to minimize the interaction energy between ligand - receptor.

One key aspect of molecular modeling is calculating the energy of conformations and interactions using methods ranging from quantum mechanics to purely empirical energy functions. Molecular dockingenergy evaluations are usually carried out with the help of a scoring function. Developing these scoring functions is a major challenge in structure based drug design. Efficiency and accuracy of geometric modeling of the binding process to obtain correct docking solutions depends on scoring function. Usually scoring functions are based on force fields that were initially designed to simulate the function of proteins (based on enthalpy).Some scoring functions used in molecular docking have been adapted to include terms such as solvation and entropy.The challenge of the lead-generation phase of the receptor-ligand docking approach is to quickly screen millions of possible compounds that fit a particular receptor and to specifically select those that show a high affinity. The set of ligands thus selected can then be screened further by either more involved computational technique, such as free-energy perturbation theory, or directly in assays. In addition, a flexible ligand docking includes molecules internal degree of freedom along with values of translation and rotation in search of its suitable bound conformation that makes it computationally more expensive than rigid ligand docking. Distinction of good or bad docked conformation is based on scoring or fitness function. The Dock score or XCscore as it is called compute binding affinity of a given protein ligand complex with known 3-D structure. Dock/X-Cscore scoring function include terms for van der Walls interaction, hydrogen bonding, deformation penalty, hydrophobic effects.Genetic algorithm

(implemented in VLifeMDS) offers a successful strategy for globally searching the docked conformers' space. Such an algorithm mirrors Darwinian evolution, representing the solution as a 'chromosome'. Genetic algorithms allow a population of solutions to exist and in each 'generation' these can evolve by processes such 'breeding' and 'mutation'. Poor solutions are killed off, while good ones leave their offspring in future generations. Such algorithms may typically reach an excellent solution is a few tens of generations [30]. VLifeMDS uses following fitness function for searching docking space.Th energy equations for various rigid and flexible docking methodologies are illustrated below:

In Rigid docking

E = InterEq (selected option electrostatic)
E = InterEvdW + InterEq (selected option steric + electrostatic)
E = EEPIC (selected option electrostatic)

In Ligand flexible docking

E = InterEq + InterEvdW + IntraEq + IntravdW + IntraEtor

Where,

InterEvdW intermolecular vdW energy of complex
InterEq intermolecular electrostatic energy of complex
EEPIC electrostatic potential for intermolecular complex
IntraEvdWintramolecularvdW energy of ligand
IntraEqintramolecular electrostatic energy of ligand
IntraEtorintramolecular torsion energy of ligand

All the energy components are calculated using MMFF force field [31]. The Grid based docking is a rigid and exhaustive docking method. In this method, after unique conformers of the ligand are generated, the receptor cavity of interest is chosen by the user and a grid is generated around the cavity (default grid interval size 1 Å). Cavity points are found and the centre of mass of the ligand is moved to each cavity point. All rotations of ligand are scanned at each cavity point where ligand is placed (step size of rotation could be typically 100 -150 as an example). For each rotation a pose of the ligand is generated and the corresponding bumps are checked for each pose of ligand. The X-Cscoreis calculated for each valid pose (determined by the cut off criteria fed by user in terms of max no of allowed bumps) and the pose of the ligand with the best score is given as output to user. Though this method is for one ligand for a given receptor, it can also be applied to a set of ligands/their conformers in a batch grid docking mode. MDS also incorporates the Piecewise Linear Pairwise Potential (PLP) function in PLP docking (rigid docking) method that includes ligand-receptor interactions of hydrogen bonding (donor-acceptor), repulsions (donor-donor, acceptor-acceptor) and dispersion (involving non-polar group interactions) types.

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RESULT AND DISCUSSION

The mechanism of binding of antagonists to human AT1 receptor is still not well understood. However, some reports have been published showing the important sites of interaction with different antagonists in AT_1 receptor. In our study, we found that AT1 losartan and irbesartanshowed consistent binding profiles with AT1 receptor and the present molecular modeling study suggests that the different binding affinities are due to their different binding interactions with AT_1R.

Thesite-directed mutagenesis suggested an important role for many residues, in particular, the affinity of imidazole based ARB losartan andirbesartan seemed to be mainly influenced by the presence of Lys 199, His 256, Val 108, Arg163 and Asn 295. Bearing these things in mind, the non-peptide antagonist losartan was docked into the AT_1 receptor model, using the Biopredicta module of Vlife MDS suite. A molecular model 1Zv0 [32] was used for docking studies considering the three interactions between the AT1 receptor and losartan that were suggested from the mutation experiments, Tyr113 and the hydroxyl group, between Lys199 and the carboxyl group, and between Gln257 and the tetrazole group.

Three methodologies were opted for docking of known ligands Losartan and Irbesatan GRIP, PLP and GA methodology.The details of the docking score of losartan are shown in Table 1 for grip docking where Rotation Angle step size of 30° by which the ligand will be rotated for different poses. The 30 best poses as 30 different placement and a with their corresponding scores in terms of ligand - receptor interactions through scoring parameters like H-bonding, repulsion, dispersion etc. It also reports the best of these 30 ligand poses with their corresponding minimum score (interaction energy) and the scoring interaction energy of the original co crystallized ligand for comparison. It is seen that in this GRIPdocking exerciseFigure 1, the minimum score, for molecule name that is losartan_P11 scores 68.003, whereas original ligand score was found to be -51.672.The biphenyl ring of the antagonist was positioned between TM3, TM6, and TM7 in a lipophilic cavity .The anionic tetrazole ring was directed toward the extracellular side of the receptor and interacted with Gly 257, forming a H bond with His 256as shown in Table 2 and Figure 2.The residues Ala 85, Leu 112 and Val 179 were consistent in hydrophobic interactions as in Figure 3. The interactions with Gly 257, His 256 Tyr 292, Trp 253 was maintained in case of vanderwaals interaction and Trp253 was once again observed for pistacking interaction as shown n Figure 4 and Figure 5 respectively.

In PLP methodology in Figure 6 as regards the imidazole ring, it exhibited a new H bond with Tyr 113(2.219 Å) while the $-CH_2OH$ group interacted with Glu 173 also formed a second H bond with tetrazole (1.929 Å).Furthermore, the interactions with Trp 253 (3.2 -4.5 Å) were encountered as hydrophobic interaction and Gly 257 as hydrophobic interactions as shownFigure 7, 8 and 9. The vanderwaals interaction within a radius of 5 Å were shown with Val 108, Leu 112, Tyr 113, Phe 204, Trp253, Gly 257 and Tyr 292 as depicted in Figure 10.

The third docking methodology opted was GA Based Docking (Figure 11) where the GA Parameter Key in 400 as the number of Generations, retain the other GA default parameters with key in translation of ligand inside receptor cavity value of 2 (in Angstroms)

and legend's Rotation step size of 100 by which the ligand would be rotated inside receptor cavity to generate different ligand poses inside the receptor cavity. The ligand translation and rotation is done by the GA algorithm so as to get the final minimum score (dock score interaction/ docking energy of receptor - ligand) for the best ligand pose inside the receptor cavity which was found to be -5.099 with losartan and and -4.990 with irbesartan.

The details of all the interactions achieved with GA based docking is shown in table 3 where His256 (1.259 Å) and Gln 257 (2.326 Å) were the residues in AT1 receptor which exhibited strong hydrogen bonding with hydroxyl and tetrazole moiety of losartan respectively as depicted in Figure 12. Binding to TM III may be important for inhibition of TM III movement by inverse agonists. However, binding to TM VI via His256 and/or Gln257 is essential in most AT1 receptor inverse agonists studied until now, including losartan.. This residue is known to be important for binding the carboxyl group of Ang II in AT1 receptor activation and for binding the acidic group on most biphenyl ARBs. Apart from these residues,Phe 204, Trp 253, His 256, Tyr 292 have shoen hydrophobic and vanderwaals interactions as shown in Table 4 and Figure 13, and 14.A pistacking interaction was also obtained with losartan as shown in Figure 15. Therefore, we propose that different ARBs bind to AT1 receptor primarily docking at His 256 and through different rotations are able to interact with distinct sets of residues in the pocket and induce inverse agonism. The interactions obtained from docking of Irbesartan in Table 5 and 6. The docking pose of Irbesartan is depicted in Figure 16 and 18 for PLP and GA docking respectively.The amino acid interactions obtained for Irbesartan did not show prominent H bond interactions. The hydrophobic and vanderwaals interactions are shown in Figure 17 and 19 for PLP and GA docking respectively. The Vanderwaals interactions of Irbesartan are shown in Figure 20.

CONCLUSION

The detailed study of binding site is performed in this piece of research by opting GRIP, PLPand GA based docking methodologies considering respective parameters for the related approaches. In the study it was concluded that most of the amino acid residues were showing hydrogen bond, hydrophobic, vanderwaals and pi stacking interactions in compliance with the site directed mutagenesis studies reported in literature However some novel amino acid interactions were also encountered whose role cannot be clearly justified at this stage of research. However the known ligand losartan has resulted in interactions which are already justified in mutational studies and the model and methodology used above can be efficiently exploited in design of novel angiotensin II receptor antagonists as antihypertensive agents. The carboxylic acid group and tetrazole ring of molecules possibly interact with His 256 of TM6 and Val 108 of TM3 of AT_1 receptor, Gln257 of TM6 respectively. These findings are in compliance with the data of the radio ligand-binding studies of the antagonists with the AT_1 receptor and the models generated can further be used in design of imidazole ad triazolinone consisting ARBs with angiotensin II receptor antagonists with antihypertensive activity.

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Table 1: Docking score of losartan at different poses with GRIP docking

Placement	Score
losartan_P1	-54.427688
losartan_P2	-63.691186
losartan_P3	-54.817640
losartan_P4	-61.019699
losartan_P5	-56.183984
losartan_P6	-63.726652
losartan_P7	-54.318776
losartan_P8	-63.321508
losartan_P9	-54.181445
losartan_P10	-55.515197
losartan_P11	-68.003371
losartan_P12	-53.866205
losartan_P13	-53.791598
losartan_P14	-55.986524
losartan_P15	-53.572289
losartan_P16	-59.289793
losartan_P17	-54.721509
losartan_P18	-59.407550
losartan_P19	-55.089529
losartan_P20	-56.670621
losartan_P21	-53.636210
losartan_P22	-53.503420
losartan_P23	-53.881455
losartan_P24	-56.418923
losartan_P25	-53.857697
losartan_P26	-63.735435
losartan_P27	-56.554896
losartan_P28	-56.014253
losartan_P29	-54.479023
losartan_P30	-53.982914

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Table 2: Details of interactionof losartan with amino acid residues in AT_1 receptor model GRIP docking

Residue Atom LigandAtom	Interaction Type
HIS256B	HYDROGENBOND_INTERACTION
GLN257B	HYDROGENBOND_INTERACTION
LEU81B	HYDROPHOBIC_INTERACTION
ALA85B	HYDROPHOBIC_INTERACTION
LEU112B	HYDROPHOBIC_INTERACTION
VAL179B	HYDROPHOBIC_INTERACTION
TYR35B	VDW_INTERACTION
ALA85B	VDW_INTERACTION
LEU112B	VDW_INTERACTION
THR178B	VDW_INTERACTION
PHE204B	VDW_INTERACTION
PHE249B	VDW_INTERACTION
TRP253B	VDW_INTERACTION
HIS256B	VDW_INTERACTION
GLN257B	VDW_INTERACTION
TYR292B	VDW_INTERACTION
TRP253B	PI_STACKING_INTERACTION

Table 3: Details of interaction of losartan with amino acid residues in AT1 receptor model PLP docking

Resident	Interaction Type
TYR113B	HYDROGENBOND_INTERACTION
GLU173B	HYDROGENBOND_INTERACTION
LEU112B	HYDROPHOBIC_INTERACTION
TRP253B	HYDROPHOBIC_INTERACTION
GLN257B	HYDROPHOBIC_INTERACTION
LEU81B	VDW_INTERACTION
VAL108B	VDW_INTERACTION
LEU112B	VDW_INTERACTION
TYR113B	VDW_INTERACTION
GLU173B	VDW_INTERACTION
THR178B	VDW_INTERACTION
VAL179B	VDW_INTERACTION
PHE204B	VDW_INTERACTION
PHE208B	VDW_INTERACTION
PHE249B	VDW_INTERACTION
TRP253B	VDW_INTERACTION
GLN257B	VDW_INTERACTION
TYR292B	VDW_INTERACTION

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Table 4: Details of interactionof losartan with amino acid residues in AT1 receptor model GA docking

Residue Atom	Interaction Type
HIS256B	HYDROGENBOND_INTERACTION
GLN257B	HYDROGENBOND_INTERACTION
LEU81B	HYDROPHOBIC_INTERACTION
ALA85B	HYDROPHOBIC_INTERACTION
VAL179B	HYDROPHOBIC_INTERACTION
TYR35B	VDW_INTERACTION
LEU81B	VDW_INTERACTION
ALA85B	VDW_INTERACTION
LEU112B	VDW_INTERACTION
THR178B	VDW_INTERACTION
PHE204B	VDW_INTERACTION
PHE208B	VDW_INTERACTION
PHE249B	VDW_INTERACTION
TRP253B	VDW_INTERACTION
HIS256B	VDW_INTERACTION
GLN257B	VDW_INTERACTION
TYR292B	VDW_INTERACTION
TRP253B	PI_STACKING_INTERACTION

Table 5: Details of interactions of irbesartan with amino acid residues in AT1 receptor model PLP docking

Residue Atom	**Interaction Type**
LEU81B	HYDROPHOBIC_INTERACTION
VAL108B	HYDROPHOBIC_INTERACTION
LEU112B	HYDROPHOBIC_INTERACTION
ILE288B	HYDROPHOBIC_INTERACTION
ASN294B	HYDROPHOBIC_INTERACTION
PHE77B	VDW_INTERACTION
LEU81B	VDW_INTERACTION
LEU112B	VDW_INTERACTION
THR178B	VDW_INTERACTION
TYR184B	VDW_INTERACTION
ASN200B	VDW_INTERACTION
TRP253B	VDW_INTERACTION
HIS256B	VDW_INTERACTION
GLN257B	VDW_INTERACTION
THR260B	VDW_INTERACTION
ILE288B	VDW_INTERACTION
TYR292B	VDW_INTERACTION

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Table 6: Details of interactions of irbesartan with amino acid residues in AT1 receptor model GA docking

Residue Atom	Interaction Type
ILE38B	HYDROPHOBIC_INTERACTION
LEU81B	HYDROPHOBIC_INTERACTION
THR178B	HYDROPHOBIC_INTERACTION
ILE288B	HYDROPHOBIC_INTERACTION
ILE38B	VDW_INTERACTION
LEU81B	VDW_INTERACTION
TYR113B	VDW_INTERACTION
ASN174B	VDW_INTERACTION
THR178B	VDW_INTERACTION
THR178B	VDW_INTERACTION
HIS183B	VDW_INTERACTION
TYR184B	VDW_INTERACTION
TYR184B	VDW_INTERACTION
TRP253B	VDW_INTERACTION
HIS256B	VDW_INTERACTION
GLN257B	VDW_INTERACTION
THR260B	VDW_INTERACTION
ILE288B	VDW_INTERACTION

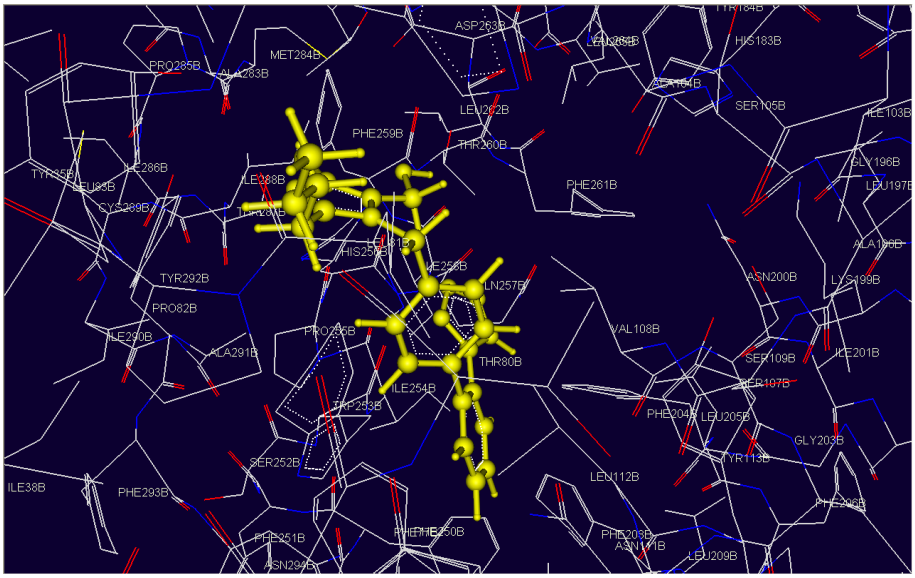

Figure 1: Docking pose of losartan with Angiotensin II AT1 receptor 1Zv0GRIP docking

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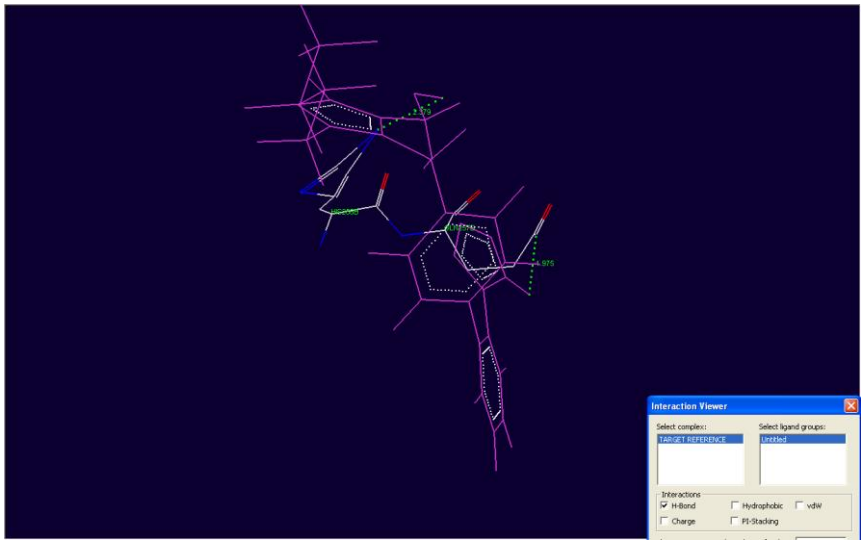

Figure 2: Hydrogen bond interactions of losartan with amino acid residues in AT_1 receptor modelGRIP docking

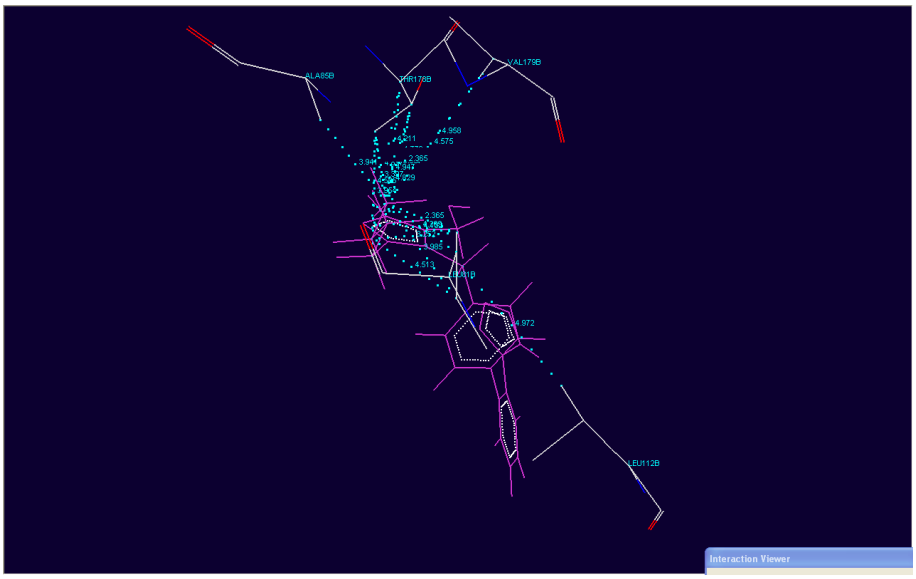

Figure 3: Hydrophobic interactions of losartan with amino acid residues in AT_1 receptor modelGRIP docking

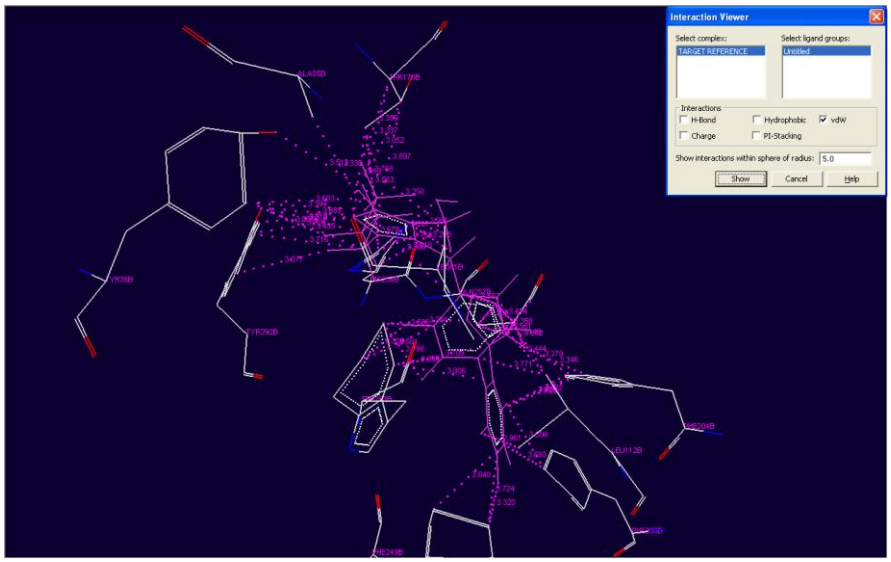

Figure 4: Vanderwaal's interactions of losartan with amino acid residues in AT_1 receptor modelGRIP docking

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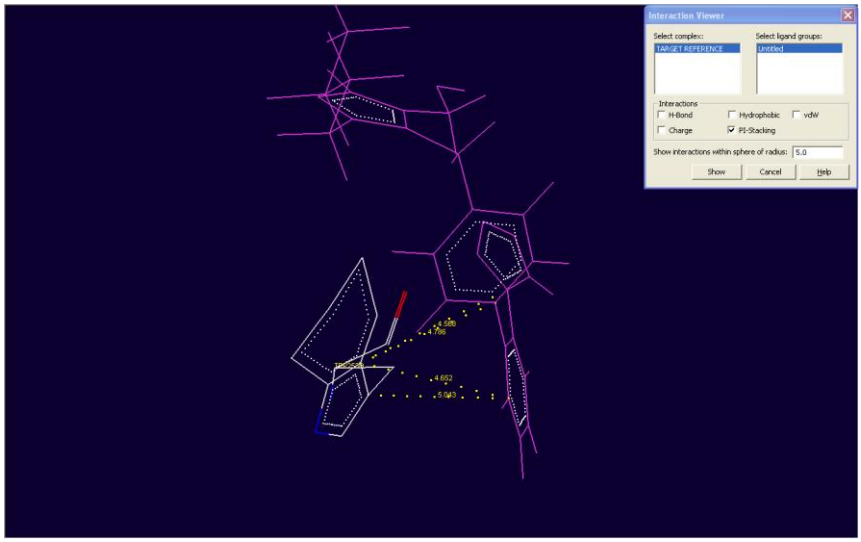

Figure 5: Pi stacking interactions of losartan with amino acid residues in AT_1 receptor modelGRIP docking

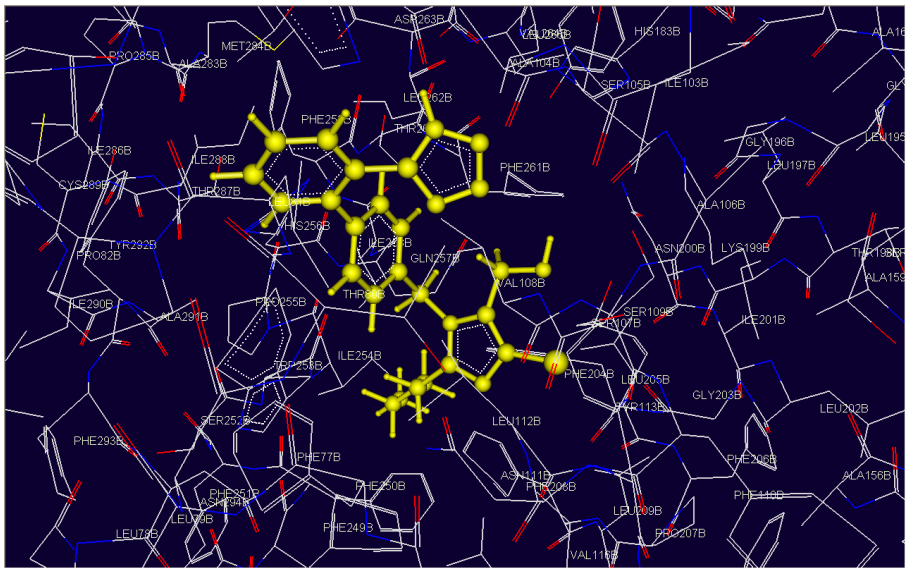

Figure 6: Docked Pose of Losartan in receptor 1Zv0PLP docking

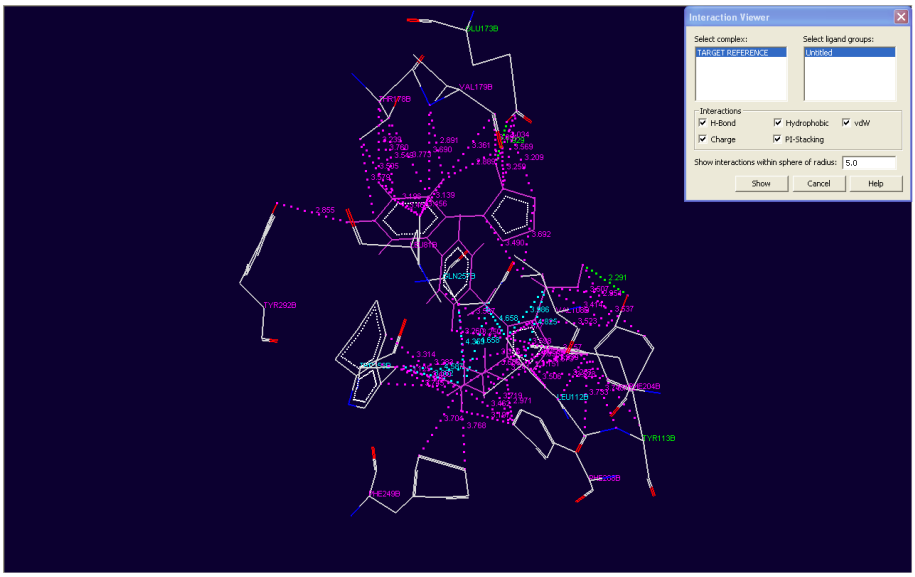

Figure 7: Interaction with amino acid residues in AT_1 receptor modelPLP docking

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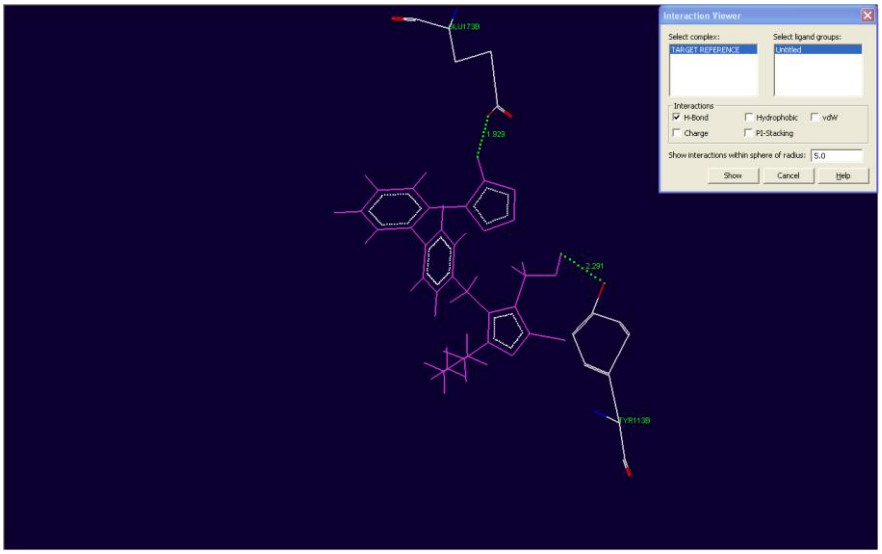

Figure 8: Hydrogen bond interactions of losartan with amino acid residues in AT_1 receptor modelPLP docking

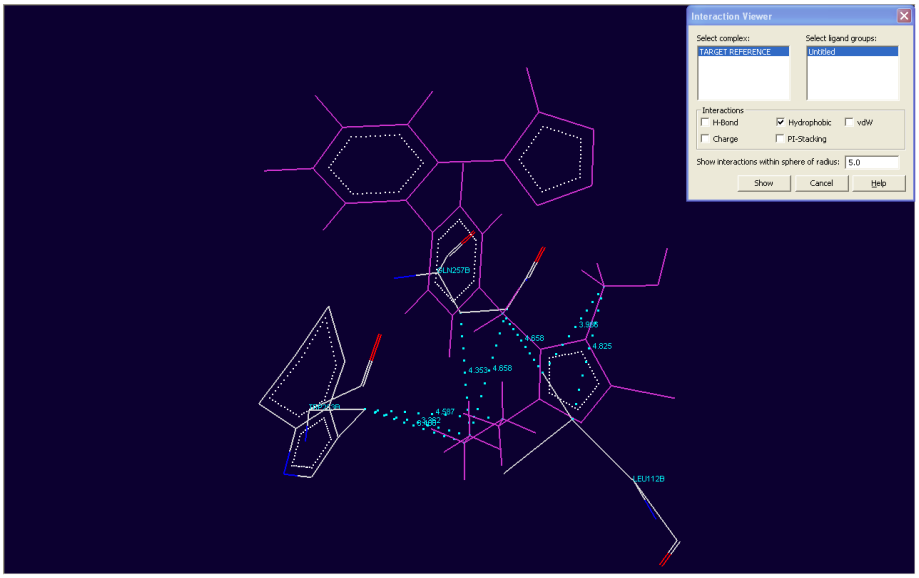

Figure 9: Hydrophobic interactions of losartan with amino acid residues in AT_1 receptor modelPLP docking

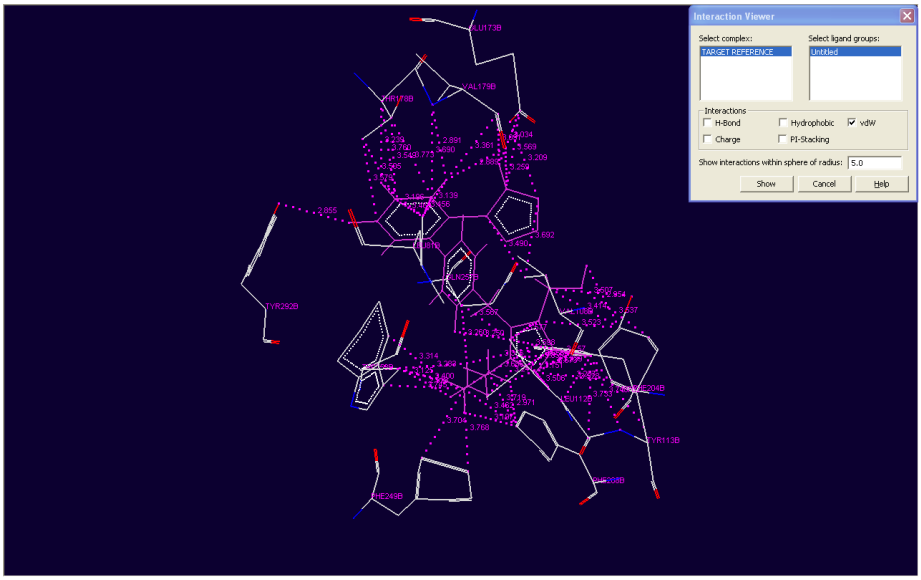

Figure 10: Vanderwaals interactions of losartan with amino acid residues in AT_1 receptor modelPLP docking

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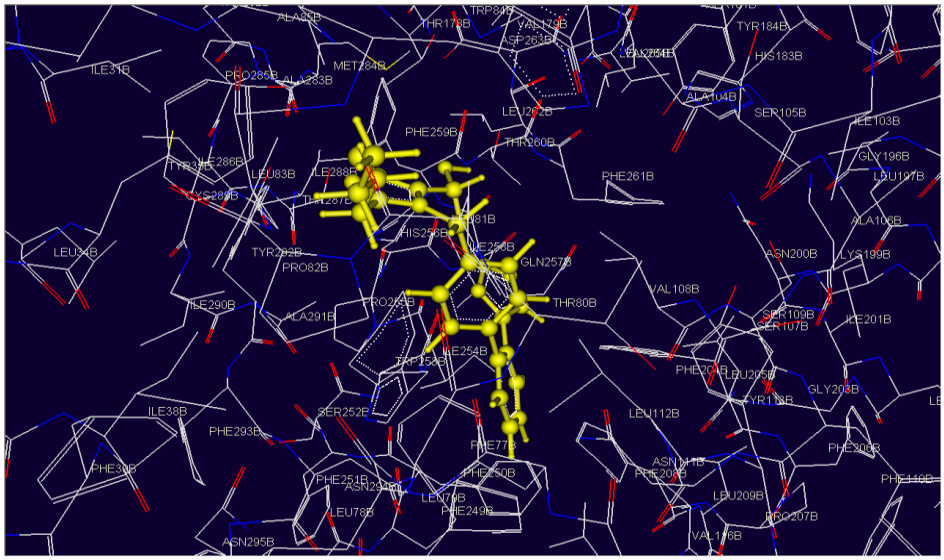

Figure 11:Docked Pose of Losartan in receptor 1Zv0 GA docking

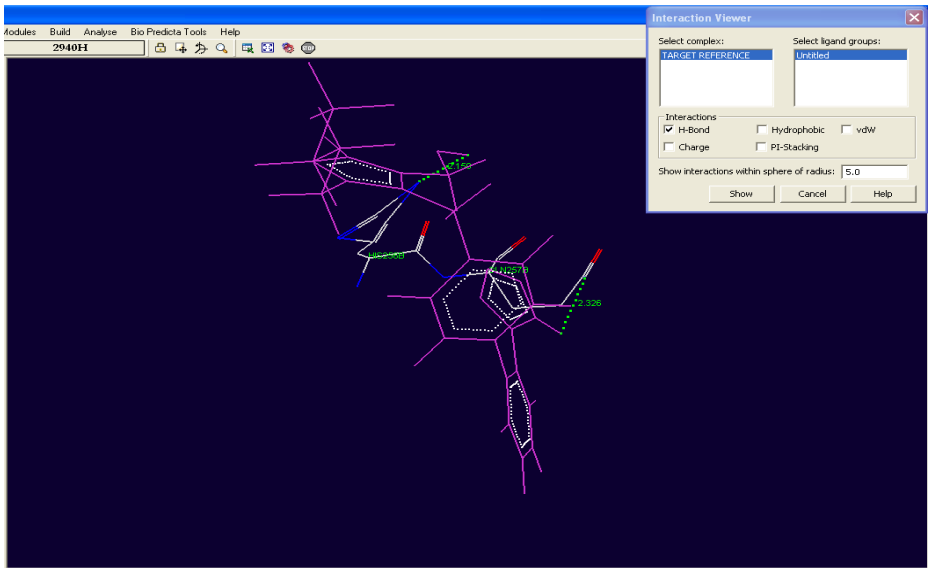

Figure 12:Hydrogen bond interactions of losartan with amino acid residues in AT_1 receptor model GA docking

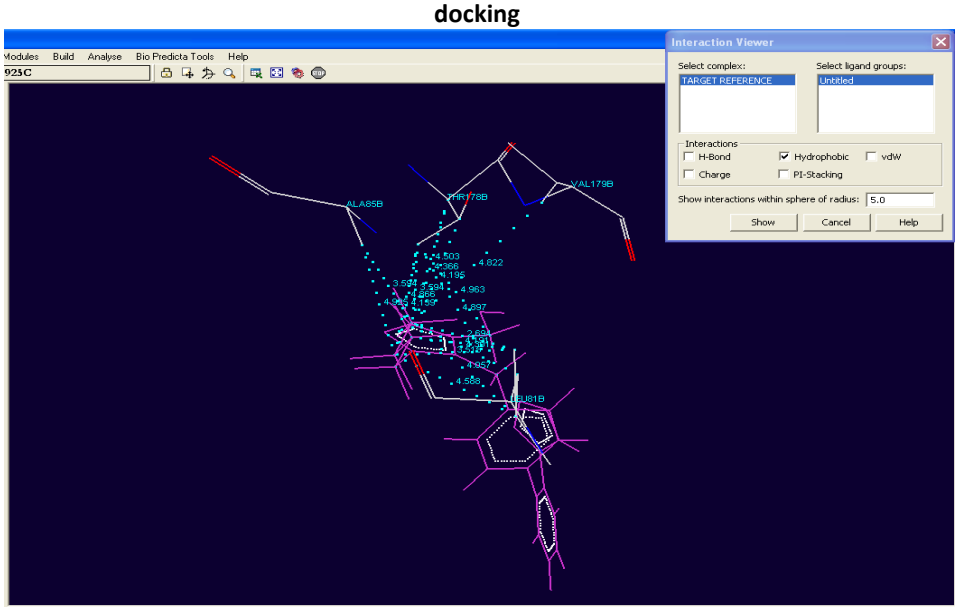

Figure 13:Hydrophobic interactions of losartan with amino acid residues in AT_1 receptor model GA docking

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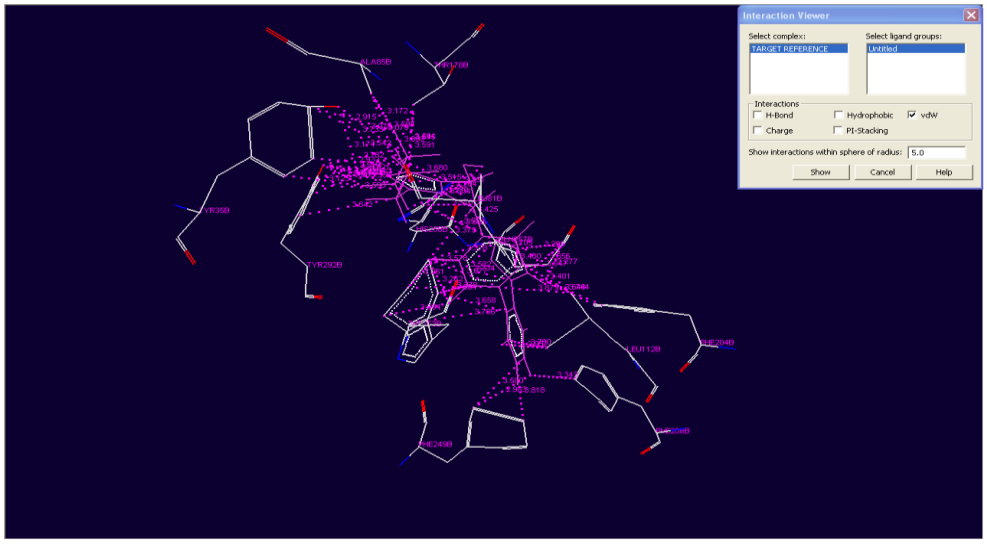

Figure 14:Vanderwaal's interactions of losartan with amino acid residues in AT_1 receptor model GA docking

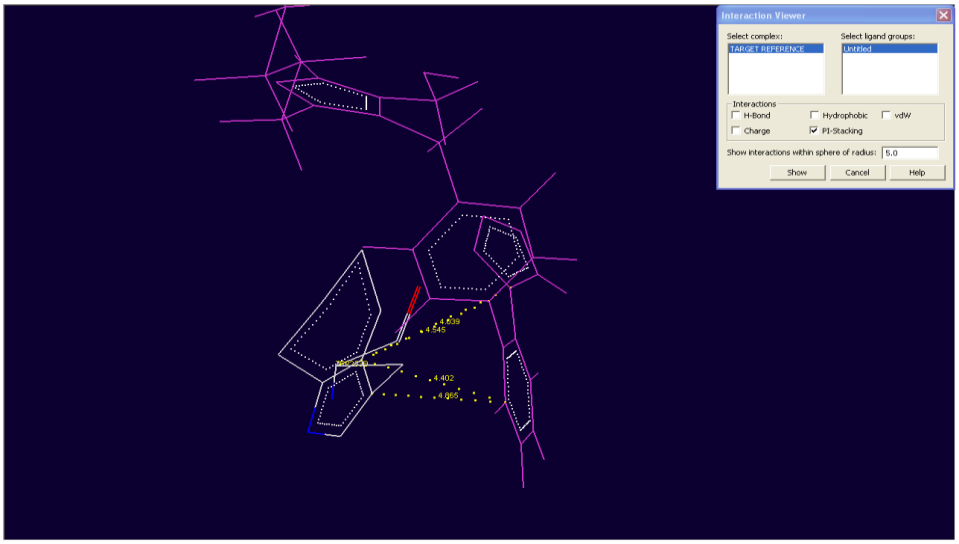

Figure 15:Pistacking interactions of losartan with amino acid residues in AT_1 receptor model GA docking

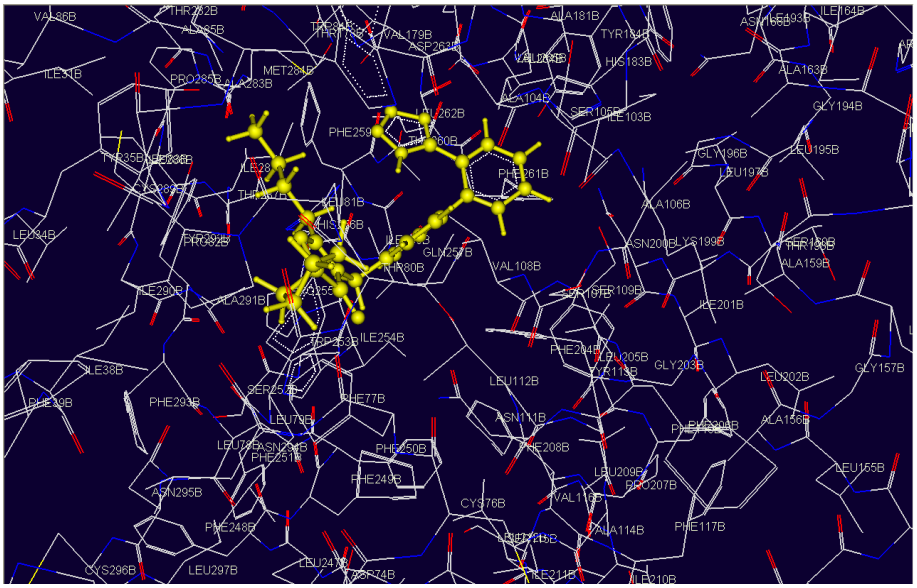

Figure 16: Docking pose of irbesartan with Angiotensin II AT1 receptor 1Zv0 PLP docking

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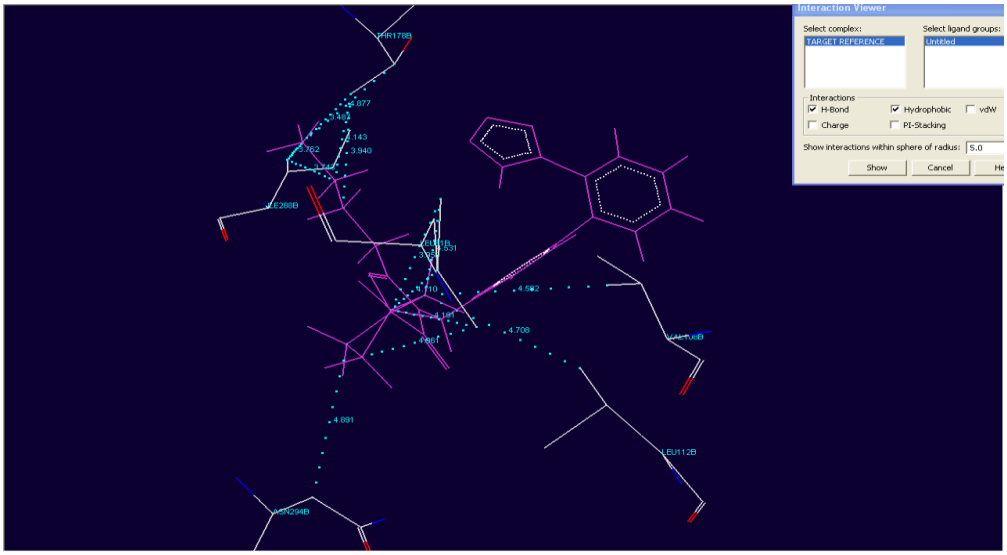

Figure 17: Hydrophobic interactions of irbesartan with amino acid residues in AT_1 receptor model PLP docking

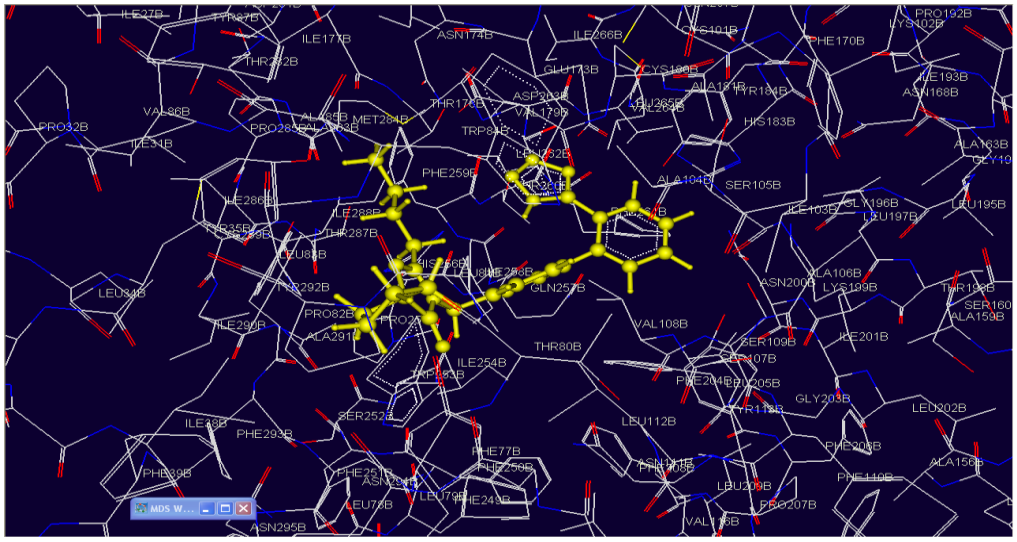

Figure 18:Docking pose of irbesartan with Angiotensin II AT_1 receptor 1Zv0GA Docking

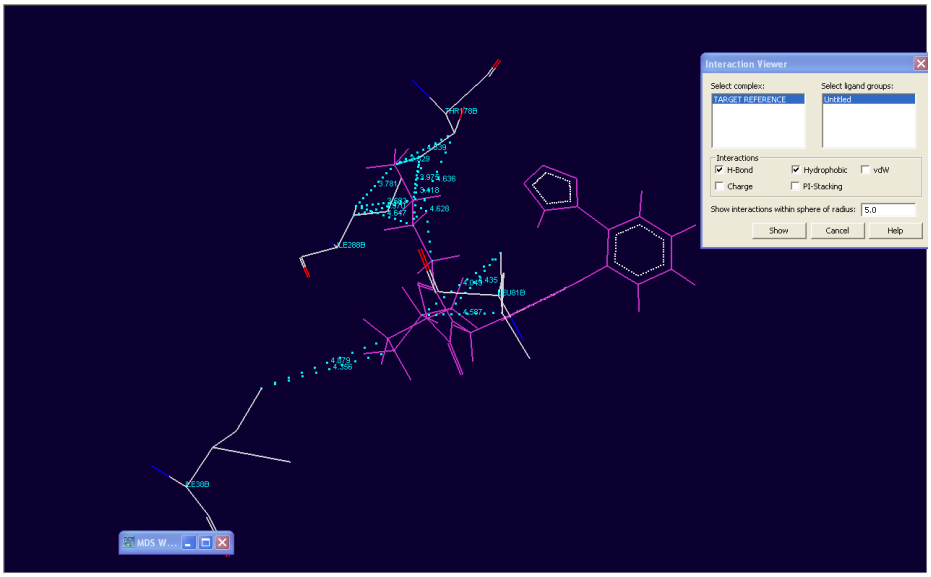

Figure 19: Interactions of irbesartan with Angiotensin II AT_1 receptor 1Zv0

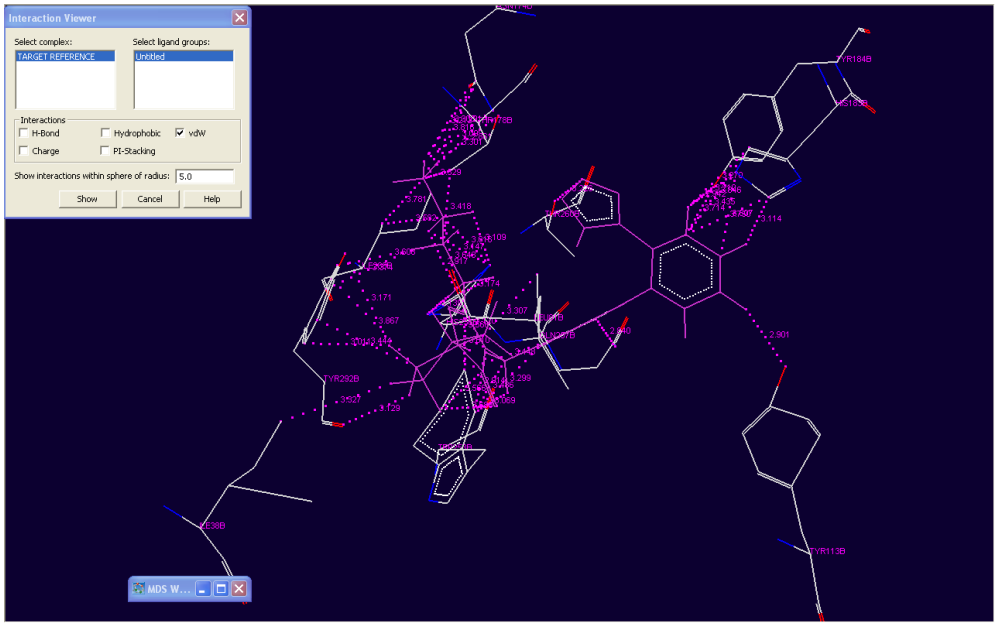

Figure 20: Docking pose of irbesartan with Angiotensin II AT_1 receptor Z1v0

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