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AN *IN SILICO* STUDY ON HIV-1 PROTEASE WILD-TYPE AND MUTANT WITH INHIBITORS FROM *ANNONA SQUAMOSA*

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HIV, Glide, Higenamine, Romerolidine, Docking, Molecular dynamics simulation (MDS).

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ABSTRACT: Human immunodeficiency virus (HIV) is a lent virus that cause acquired immunodeficiency syndrome (AIDS), a state in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. HIV-1 protease is a retroviral aspartyl protease (retropepsin) that is essential for the life-cycle of HIV, the retrovirus that causes AIDS. HIV Wild type (PDB: 3EKV) and Mutated (PDB: 3NU9) proteins structures were retrieved from Protein Data Bank in order to check the consistency of ligand's interactions even if mutated viral attack is present. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Docking study of the target protein was done with natural compounds derived from *Annona Squamosa* to find the preferred orientation and binding affinity of drug with target protein using scoring functions. *Annona squamosa* is a small, well-branched tree or shrub from the family Annonaceae that bears edible fruits called sugar-apples. Chemical constituents found in extracts of the seeds, bark, and leaves of this tree. Phytochemicals screened for Glide HTVS followed by SP (Standard Precision) docking showed good interaction with the target. The anti HIV compounds that yielded a fitness score of more than -5 were further subjected to Molecular dynamics simulation (MDS). Higenamine and Romerolidine shows good binding affinity with the target proteins 3EKV and 3NU9 respectively and that can be a potential target for Aids.

INTRODUCTION: We have selected Crystal structure of the wild type HIV-1 protease with the inhibitor, Amprenavir and Crystal Structure of HIV-1 Protease Mutant I84V with Antiviral Drug Amprenavir. Crystal structure of **3EKV** is a 2 chain structure was determined using X-ray diffraction at a resolution of 1.75 Å and Chain A having length of 99.

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The development of HIV-1 protease inhibitors has been the historic paradigm of rational structure-based drug design, where structural and thermodynamic analyses have assisted in the discovery of novel inhibitors [1]. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1.

Inhibitor inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Crystal structure of **3EKV** was determined using X-ray diffraction at a resolution of 1.85 Å Chain A having length of 99 and deposited in 2010[2].

The structural and kinetic effects of amprenavir (APV), a clinical HIV protease (PR) inhibitor, were analyzed with wild-type enzyme and mutants with single substitutions of V32I, I50V, I54V, I54M, I84V and L90M that are common in drug resistance. Crystal structures of the APV complexes at resolutions of 1.02-1.85 Å reveal the structural changes due to the mutations [3].

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets. Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes [4]. Molecular modeling is the general term used to describe the use of computers to construct molecules and perform a variety of calculations on these molecules in order to predict their chemical characteristics and behaviour. The benefit of molecular modeling is that it reduces the complexity of the system, allowing many more particles (atoms) to be considered during simulations [5].

The number of accessible states grows exponentially with the degrees of freedom of the docking molecules. Energy calculations in condensed phases must subtract large numbers to arrive at small differences, almost guaranteeing inaccuracy[6]. The first docking program DOCK worked very similar to the 'lock and key' model: finding complexes with a high degree of shape complementarity between the ligand and the receptor. In this approach, both the ligand and the receptor have very stiff structures, thereby the name rigid body docking. Although simplistic, the method was successfully used for the analysis of the serine proteases specificity [7].

Secondary metabolites are fascinating and important players in mediating plant responses to biotic and abiotic environmental factors. In addition, secondary metabolites also function as defensive compounds or toxins which guard against pathogens and herbivores, and protect plants from abiotic stresses such as ultraviolet (UV) light [8, 9]. Secondary compounds are complex chemicals made by plants that are not essential to the life of the plant.

It is possible that the parasite paralysis and/or death observed may have been attributed to secondary metabolites [10] like tannins, alkaloids salts and saponins among others.

MATERIALS AND METHODS: All the computational analysis were carried out using Schrodinger suite version 9 [11]. Image capturing was carried out using PyMol viewer [12].

Compounds identified from *Annona Squamosa*: *Annona squamosa* is a small, well-branched tree or shrub[13] from the family Annonaceae that bears edible fruits called sugar-apples. Many Phytochemicals obtained from *Annona squamosa*. The diterpenoid alkaloid atisine is the main component of the root. Other constituents of *Annona squamosa* include oxophoebine[14], reticuline, atidine, histisine, hetisine, hetidine, heterophyllisine, heterophylline, heterlophylline, isoatisine, dihydroatisine, hetisinone benzoyl heteratisine and citronella oil. In US patent 4689232, Bayer AG patented the extraction process and molecular identity of squamocin. This molecule is known as an annonaceous acetogenin. Bayer also patented its use as a biopesticide. Many others have found other acetogenins in extracts of the seeds, bark, and leaves.

For docking study, we have used 81 chemical compounds obtained from extracts of the seeds, bark, and leaves of this tree. They are (+)-O-methylarmepavine, (2,4-cistrans)-squamoxinone, 1H-Cycloprop[e]azulene, 1-Tritriacontanol, 4, 4-Dimethylcholesterol, 4, 4-Tert- Butylcalix(4)arene, 4-deoxyannoreticuin, 6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium, Annomosin A, Annonacin A, Annonacin, Annosquamosin B, Annosquamosin D, Annosquamosin, Anolobine, Anomuricine, Anonaine, Alpha-pinene, Aporphine, Armepavine, Benzoquinazoline, Benzyl-tetrahydroisoquinoline, Beta sitosterol, Beta-Caryophyllene, Bisabolene epoxide, Bisabolene, Borneol, Beta-pinene, Bullatacin, Bullatacinone, Camphene, Camphor, Car-3-ene, Carvone, Caryophyllene oxide, Cis-4-deoxyannoreticuin, Corydine, Cyclosquamosin B, Cyclosquamosin BC, Diterpene, Duguevalline, Eugenol, Farnesol, Geraniol, Germacrene D,

Glaucine, Hexacontanol, Higenamine, Hydroxyl ketones, Isoamylacetyate, Isocorydine, Kaur-16-ene, Lanuginosine, Limonin, Linalool acetate, Liriodenine, Liriodenine, Menthone, Methyl anthranilate, Methylheptenone, Molvizarin-, Mosin B, Mosin C, Mosinone A, Nmethylcorydaldine, N-Octacosanol, Norcorydine, Norisocorydine, Norlaureline, N-triacontanol, Reticulatacin, Reticuline, Roemerine, Roemerolidine, Rutin, Samoquasine A, Sodium benzoate, Squamotacin, Stigmasterol acetate, Stigmasterol, Thymol.

Protein preparation: The PDB is a key resource in areas of structural biology, is a key repository for 3D structure data of large molecules. The molecule which taken is HIV-1 protease wild-type and mutant for our consideration. The PDB ID for HIV Wild type is 3EKV and for Mutated is 3NU9. Glide is a ligand docking program for predicting protein-ligand binding modes and ranking ligands via high-throughput virtual screening. Protein preparation wizard was used in Schrodinger 2009. All the water molecules and RNA were removed from the original crystal structure before protein preparation process, to analyze the structure and the bond order assigned, hydrogen atoms were added and the geometry of all the hetero groups were corrected. Hydrogen bonds assignment tool was used to optimize the hydrogen bond network. Finally, impref optimized the position of hydrogen bonds and keeping all the atoms in place. Energy minimization was carried out using default constraint of the 0.3Å of RMSD and the OPLS_2005 force field. Glide utilizes two different scoring functions, SP and XP GlideScore, to rank-order compounds. Three modes of sampling ligand conformational and positional degrees of freedom are available to determine the optimal ligand orientation relative to rigid protein receptor geometry. Here it describes the protocols for flexible ligand docking with Glide, optionally including ligand constraints or ligand molecular similarities [15].

Flexible ligand docking with Glide: The target proteins were prepared for Glide Docking calculations using the Protein Preparation Wizard. The ligand, water molecules and residues were removed from the protein and H atoms were added to the structure; and the chemistry of the protein was corrected for missing hydrogen. Following the above steps of preparation, the protein was subjected to energy minimization using the options from Schrodinger 9 [16].

Ligand preparation: The three dimensional structure of compounds, that is 81 compounds from Annona squamosa taken for binding analysis were downloaded in sdf format from PubChem database. Generate low energy conformations. Lipinski properties such as Molecular weight, XLog P, number of hydrogen bond donors and acceptors for the compounds were obtained from PubChem (**Table 1).** These structures were used for docking and pharmacokinetic studies. Output file created after LigPrep was *ligandout.maegz*

TABLE 1. PHYSICOCHEMICAL PROPERTIES OF BEST COMPOUNDS IDENTIFIED IN *ANNONA SQUAMOSA.*

S.No	Compound name	Compound ID	Mol. wt	Xlogp	H-Bond donor	H-Bond acceptor
1	Higenamine	CID 114840	271.31 [g/mol]	2.2	4	4
2	Romerolidine	CID 183517	311.33 [g/mol]	1.8	2	5

Here ligand Higenamine and Roemerolidine is taken for binding affinity studies. The validation process consisted of two parts;

- Hydrogen bond details of the top-ranked docked pose.
- Prediction of binding energy between the docked ligand and the enzyme using various scores calculated using Schrodinger.

Active site Prediction: In biology, the active site is the small portion of an enzyme where substrate molecules bind and undergo a chemical reaction. The active sites for the target proteins 3EKV and 3NU9 were predicted using Sitemap of Schrödinger shown in **Figure 1.**

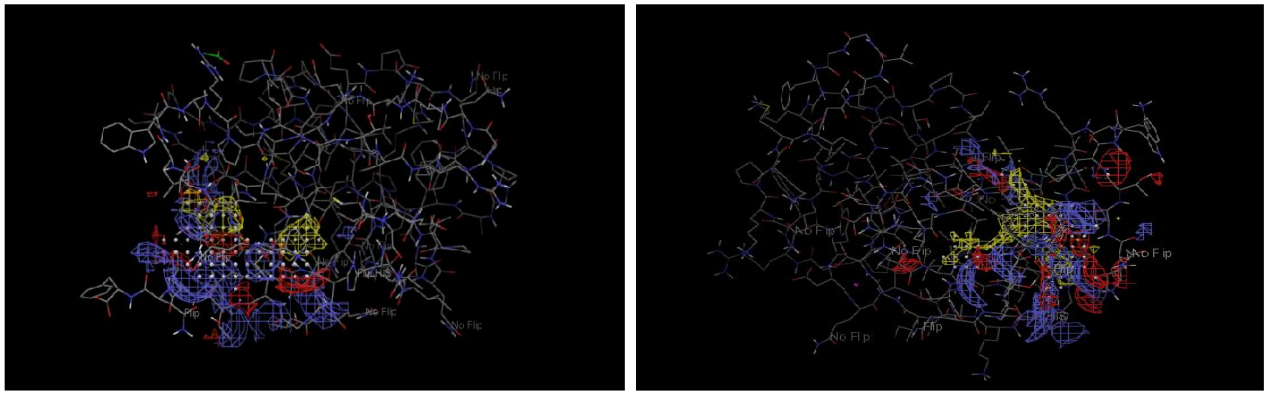


FIGURE 1: PREDICTED ACTIVE SITES OF 3EKV AND 3NU9 USING SITE MAP

Receptor Grid Generation: The outputs from sitemap prediction were given as the input for Receptor Grid generation. Grid box generated for about 20 Angstrom within the DNA binding site of the proteins (3EKV and 3NU9). Grids were generated by Receptor Grid Generation panel which defines receptor structure by excluding any other co-crystallized ligand that may be present, settle on position and size of the active site was represented by receptor grids. The out file generated was 1.*zip* which was given as an input for Protein- ligand docking.

Docking studies:

Ligand Docking: Output files generated from receptor grid generation (1.zip) and LigPrep (ligandout.mae) were loaded in the ligand docking panel. Output file generated after ligand docking was lig1.pv.maegz.

High throughput virtual screening: Virtual screening is the easiest method to identify and rank potential drug candidates from a set of compounds. Based on the active site of 3EKV and 3NU9, high throughput virtual screening was performed using the 81 compounds from *Annona squamosa.* The compounds were subjected to Glide based docking strategy in which all the compounds were docked by two stages of the docking protocol, High Throughput Virtual Screening (HTVS), Standard Precision (SP). First stage of HTVS docking screens the ligands that are retrieved and all the screened compounds are passed on to the second stage of SP docking. Based on the glide score and glide energy, the protocol gives the leads in SP. Glide includes ligand-protein interaction energies, hydrophobic interactions, hydrogen bonds, internal energy, p-p stacking interactions and root mean square deviation (RMSD) and desolvation.

Molecular dynamics simulations: Molecular dynamics (MD) is a computer simulation of physical movements of atoms and molecules in the context of N-body simulation. The atoms and molecules are allowed to interact for a period of time, giving a view of the motion of the atoms. Molecular dynamics can be used to explain protein structure function problems, such as folding, conformational flexibility and structural stability. In the simulations, we monitored the backbone atoms and the C-α-helix of the modeled protein. The RMSD values of the modeled structure's backbone atoms were plotted as a time-dependent function of the MD simulation. The results support our modeled structure, as they show constant RMSD deviation throughout the whole simulation process. Graphs of potential energy, temperature, pressure and volume and time dependence of the RMSD (Å) of the backbone atoms of the modeled protein during a 2 ns simulation is shown in **Figure 2 & 3.**

MOLECULAR DYNAMICS RESULTS

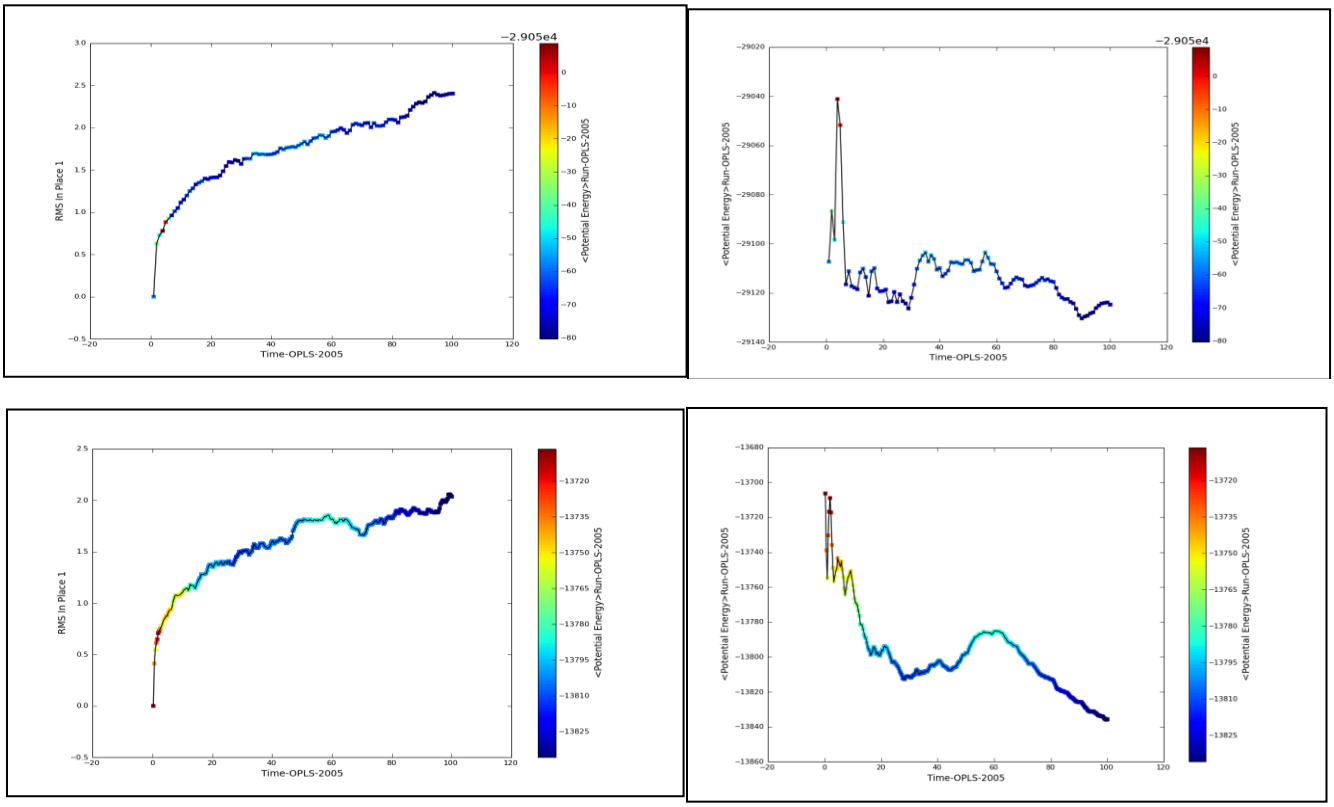


FIGURE 2: RMSD OF THE BACKBONE ATOMS OF THE MODELED AND DOCKED 3EKV PROTEIN OVER A TIME PERIOD OF 1 NANO SECONDS

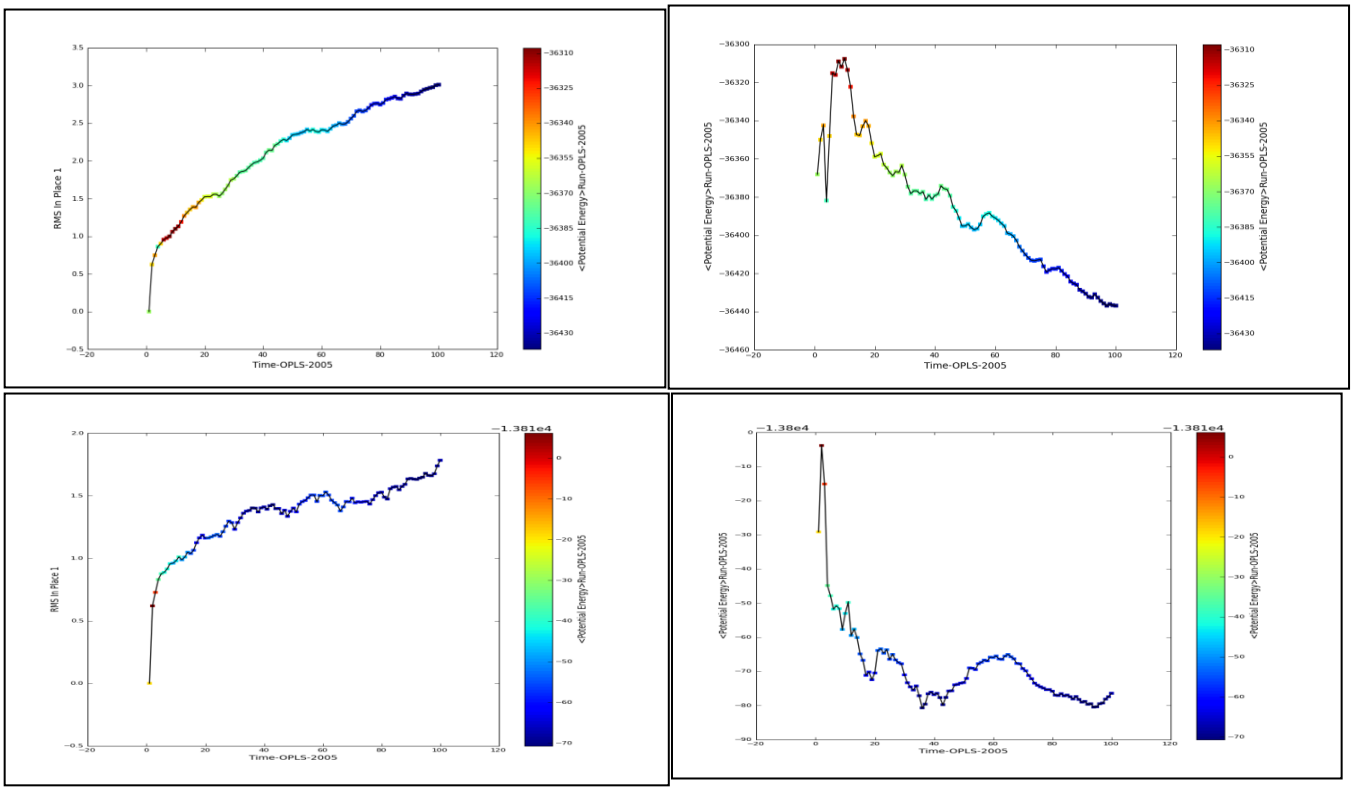


FIGURE 3: RMSD OF THE BACKBONE ATOMS OF THE MODELED AND DOCKED 3NU9 PROTEIN OVER A TIME PERIOD OF 1 PICOSECONDS

The RMSD values of the backbone atoms in the system tend to converge after 2000 ps, showing fluctuations of around 1 Å. The low RMSD and the simulation time indicate that, as expected, the 3D structural model of 3EKV and 3NU9 represents a stable folding conformation.

RESULTS AND DISCUSSION: Two different levels of docking and scoring processes were used for this study starting with HTVS, followed by SP. HIV protease wild type and mutant protein target was docked with 81 chemical constituents from *Annona squamosa* using Glide. HTVS selected compounds based on Glide score. SP docking filtered by another Glide score criteria value (Glide score < -4.0 Kcal/mol) which led to 13 ligands for 3EKV and 3 ligands for 3NU9. The final docking with SP ranked the 16 best-scored compounds for both the proteins; two compounds were selected based on the Glide score. The ID's and H-Bond interactions of the top two scored compounds are shown in Figure 4 and Table 2. Docking results showed that CID 114840 and CID 183517 from *Annona squamosa* tree got glide score of -5.98 and -6.67 Kcal/mol.

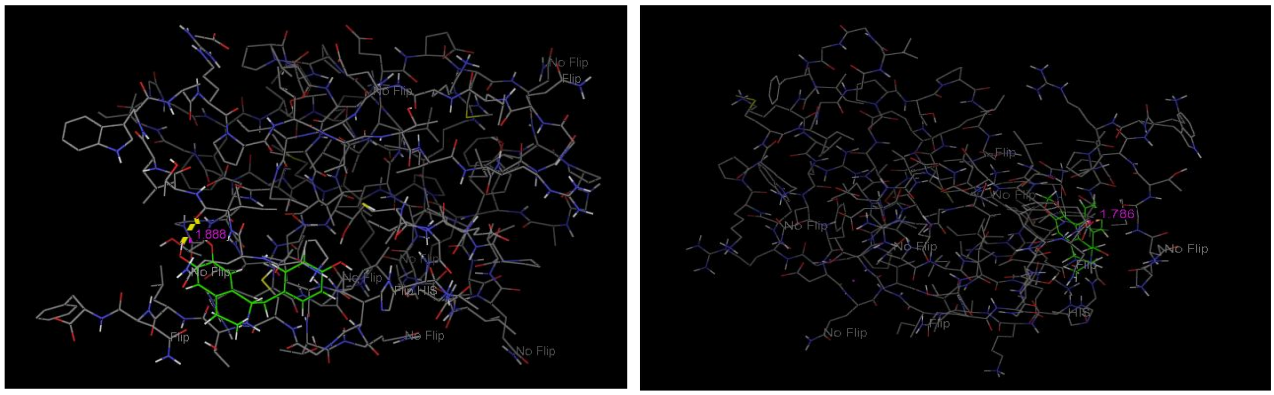


FIGURE 4: DOCKING INTERACTION OF LEAD COMPOUNDS WITH HIV PROTEASE WILD AND MUTATED TYPE 3EKV, 3NU9.

TABLE 2: GLIDE SCORE AND NUMBER OF H BONDS OF BEST COMPOUNDS IDENTIFIED IN *ANNONA SQUAMOSA*

3EKV

Sl. No.	id	Compound Name	G score	No of H bonds	Distance	Protein residues	Lig atm
1	114840	Higenamine	-5.98	1	1.88	THR 26:(H) H34	(H)

3 NU9

Sl No	id	Compound Name	G score	No of H bonds	Distance	Protein Residues	Lig atm
1	183517	Roemerolidine	-6.67	1	1.78	ASN 98:(H) H39	(H)

Binding mode of Higenamine with 3EKV

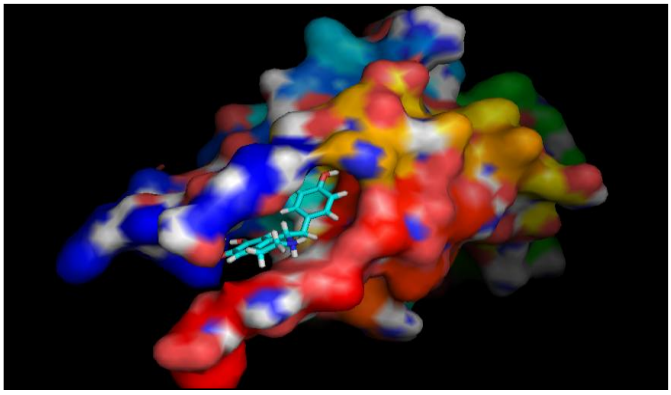
Docking results showed that the ligand Higenamine occupied the binding region of 3EKV with a Glide score of -5.98 and the Glide energy is -39.78 Kcal/mol. One hydrogen bond interaction was identified with the amino acid residue Thr 26 in the binding region of 3EKV **(Figure 4).**

Binding mode of Romerolidine with 3NU9: Docking results showed that the ligand Romerolidine occupied the binding region of 3NU9 with a Glide score of -6.67 and the Glide energy is -36.40 Kcal/mol. One hydrogen bond interaction was identified with the amino acid residue Asn 98 in the binding region of 3NU9 **(Figure 4).**

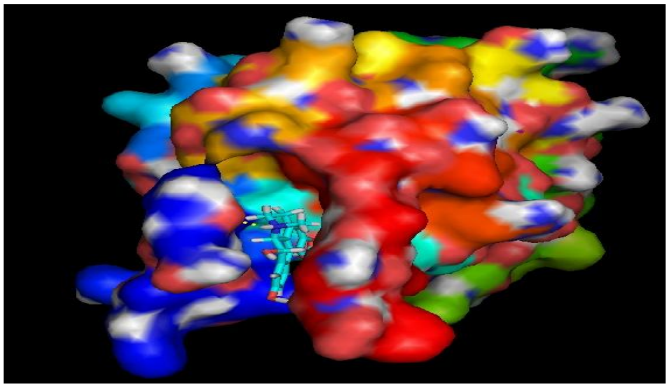
The structure of Wild type and Mutated HIV protease with PDB ID 3EKV and 3NU9 is retrieved from PDB. The resolution factor is retrieved from PDB. Both proteins structures have total 2 chains viz. A, B.

The 81 chemical compounds from *Annona squamosa* taken as a ligand for docking analysis with the Wild type and Mutated HIV protease (3EKV and 3NU9). The active site of the target protein determined using site map prediction. Docking done using Glide and the Receptor and ligand can browse for prepared output files. Docked pose of the compound with proteins is presented in **Figure 5**.

The ligands Higenamine and Romerolidine docked with protein Wild type and Mutated HIV protease with -5.98 and -6.67 dock score value **(Table 3).** Molecular docking is an efficient technique to predict the predominant binding modes of the ligand with the protein of known three-dimensional structure. Studies on binding modes are essential to elucidate key structural characteristics and interactions and they provide helpful data for designing effective inhibitors. The two selected compounds were in the acceptable range of Lipinski's rule of five, indicating their potential for use as drug like molecules [17].



DOCKING POSE OF HIGENAMINE WITH 3EKV



DOCKING POSE OF ROMEROLIDINE WITH 3NU9

FIGURE 5: DOCKING POSES OF BEST COMPOUNDS IDENTIFIED IN *ANNONA SQUAMOSA*

TABLE 3. GLIDE SCORE AND GLIDE ENERGY OF BEST COMPOUNDS IDENTIFIED IN *ANNONA SQUAMOSA*

Target protein	Glide score	Glide energy
3EKV	-5.98	-39.78
3NU9	-6.67	-36.40

CONCLUSION: It is important to understand the role of chemical constituents found in extracts of the seeds, bark, and leaves of *Annona squamosa*. Chemical compounds bind to specific receptors on target protein. *In silico* molecular docking is one of the most powerful techniques to discover novel ligand for proteins of known structure and thus play a key role in structure based drug designing. Investigators often use docking computer programs to find the binding affinity for molecules that fit a binding site on the protein. Hence in this present work we have carried out *in silico* molecular docking to analyze the binding properties of the wild and mutant type HIV protease with chemical constituents from *Annona squamosa*.

The target protein wild and mutant type HIV protease was validated by molecular dynamics simulation. HTVS screened compounds from *Annona squamosa* tree were subjected to SP docking which resulted in 16 compounds. Using SP docking, based on the glide score, glide energy and H-bond interactions with the amino acid residues 2 compounds were shortlisted. 2 compounds were within the acceptable range of Lipinski's Rule of Five. This suggests that these two compounds could be potential inhibitors of the HIV protease wild type and mutated protein. CID 114840, CID 183517 has a good glide score and glide energy (-5.98 & -6.67 kcal/mol).

The ligand Higenamine has a good contact with the specific amino acid residue THR 26 with the hydrogen bond distances of 1.78 Å. The ligand Romerolidine has a good contact with the specific amino acid residue ASN 98 with the hydrogen bond distances of 1.88 Å. The ligand Romerolidine could be a potent inhibitor for HIV. So the present study may act as supportive evidence that substantiates property of tree extract may be a good drug because of the inhibiting ability of Higenamine and Roemerolidine identified from this tree parts with 3EKV and 3NU9.

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