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Homology Modeling and Molecular Docking Studies of Phytochemical Compounds from Selected Medicinal Plants against HIV-1 Protease

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Abstract:

Background: Despite the availability of anti-retroviral agents, Human Immunodeficiency Virus(HIV)infection continues to be a major global public health issue with a higher rate of morbidity and mortality. The HIV-1 protease is an aspartyl protease that is required for proteolytic processing of the gag and gag-pol polyprotein precursors and is indispensable for proper virion assembly and maturation.The rapid emergence and dissemination of drug-resistant HIV-1 variants and the adverse side effects of currently used HIV-1 protease inhibitors(PIs) remain critical factors that necessitate the discovery of newer phytocompounds with potential anti-viral activity against HIV-1. The study was proposedto evaluate the binding efficiency of the phytochemical compounds from themethanolic extractsof *Ricinus communis*, *Andrographis paniculata* and *Withania somnifera*against the HIV-1 protease, mutant and wild type and to comparewith the standard PI,nelfinavir. **Methods:**The 3D structures of HIV-1 protease with mutations *viz*.,V32I,I47V and V82Iwere developed by homology modeling. A total of 120 phytochemical compounds from the *Ricinus communis*, *Andrographis paniculata* and *Withania somnifera*were virtually screened against the binding site of HIV-1 protease, wild-type and mutant type.

Result: The docking interactions of APC-4 with the HIV-1 protease mutant type exhibited a binding affinity of -27.1425 kJ/mol while, WSC-31 with HIV-1 protease wildtypeexhibited -25.4546 kJ/mol, better than the binding affinity of the conventional protease inhibitor, Nelfinavir.

Conclusion: These phytochemical compounds, APC-4 and WSC-31 could be potential alternatives to the conventional PI, Nelfinavir against HIV-1.

Keywords: HIV-1 Protease, Phytochemicals, Nelfinavir, docking, virtual screening.

Introduction

Acquired immunodeficiency syndrome (AIDS) caused by the retrovirus Human Immunodeficiency Virus (HIV)is one of the most important infectious diseases witha high mortality in many developed and developing countries. According to current data from WHO (2017), it is estimated that there are 36.9 million people areliving with HIV/AIDS worldwide.Majority of the global burden of HIV infections is reported from Africa with approximately 25.7 million people living with HIV,

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followed by 3.5 million in South-east Asia[1].The global coverageof antiretroviral therapy (ART) among people living with HIVis estimated to be 59% (2017) and hence, the HIV related deaths have declined from 1.5 million in 2000 to 0.9 million in 2017. Also, there is a reduction in the number of people newly infected with HIV from 2.8 million in 2000 to 1.8 million in 2017 [2].

Human Immunodeficiency Virus (HIV-1) infection is universally considered as a chronic disease that slowly progresses to Acquired Immunodeficiency Syndrome (AIDS). HIV has a high genetic diversity due to the fast replication cycle of the virus coupled with the high error prone rate of its RT enzyme [3].The HIV polyprotein precursor is encoded by relatively simple genomes consisting of gag, pol and env open reading frames. The *gag* gene encodes the structural capsid, nucleocapsid, and matrix protein; *env* undergoes multiple alternative splicing events to regulatory protein; while, *pol* encodes essential viral enzymes necessary for viral replication [4]. The HIV-1 protease (HIV-1 PR) is an aspartyl protease that is required for proteolytic processing of the gag and gag-pol polyprotein precursors to yield the viral enzyme and structural proteins and is indispensable for proper virion assembly and maturation.HIV-1 PR contains a homodimeric C-2 symmetric structure and each monomer contributes one catalytic aspartic residue along with threonine and glycine residues which are flexible and a flap that favors the binding of substrate and inhibitors [5].

In the absence of an effective vaccine, drugs remain the only therapeutic tool for the treatment ofHIV-1 infections. Unfortunately, ART once initiated, need to be continued lifelong. This places a special burden on the design of anti-HIV drugs. The protease inhibitors (PIs) and reverse-transcriptase inhibitors have resulted in the unprecedented success of HIV/AIDS chemotherapy. However, owing to the rapid emergence of drug-resistant HIV-1 variants and transmission of these resistant viral strains along with the adverse side effects of the currently used HIV-1 PIs, ART remains a clinical challenge [6]. TheFDA approvedHIV-1 protease inhibitors including atazanavir, indinavir, nelfinavir and saquinavir for HIV treatment are very peptide-like and have poor bio-availability [7].

To overcome these issues, there is a need for the development of new PIs with improved activity against drug resistant variants and excellent pharmacokinetic and safety profiles. The pharmaco-informatics approaches including virtual screening and molecular docking have become pivotal techniques in the pharmaceutical industry for lead discovery. Hence in the present study binding efficiencies of the phytochemical compounds from selected medicinal plants wereevaluated against the HIV protease receptor from mutant and wild type in terms of molecular docking and compared with the standard protease inhibitornelfinavir.

Methodology

HIV protease wild type and mutant structures

The 3D structure of HIV-1 protease receptor (HIV-1 PR) an aspartyl protease that is required for proteolytic processing of the gag and gag-pol polyprotein precursors to yield the viral enzyme and structural proteins and is indispensable for proper virion assembly and maturation is retrieved from PDB [8]. The structure of this protein is determined by X-ray crystallography (2R5Q). The 3D structures of protease with mutations such as V32I,I47V and V82Iwere developed by using BLASTP (basic local alignment search tool) [9]similarity search tool against PDB database. The homology modeling of the mutant protease was developed by using the atomic coordinate file of 2R5Q. The sequence alignment and alignment errors were refined using ClustalW[10] program as the sequence alignment reflects the quality of the homology models.

Homology Modeling and model evaluation

A total of five 3D models of the target sequences were built from the starting structure of the templates by satisfying the spatial restraints through random generation[11]and the model with least RMSD value in comparison with template structure was considered for as best model and its energy was minimized by using GROMOS [12]was used for further analysis.The stereo-chemical parameters of the energy-minimized models were considered to evaluate the quality of the generated models. The phi and psi angles representing the stereo-chemical parameters of the model through PROCHECK [13], at SAVES structural analysis server [14].

Binding Pocket Prediction

The anti-viral activities of methanolic extracts of *Ricinuscommunis*(leaf), *Andrographis paniculata*(leaf) and *Withaniasomnifera*(root)were explored against the HIV-1 protease by predicting the binding pocket of modeled HIV-1 protease mutant and wildtype protein structures using DoGSiteScorer[15].

Lead compounds

The 2D structure of the phytochemical compounds from GC–MS analysis of methanolic extracts of *Ricinus communis, Andrographis paniculata* and *Withania somnifera* were drawn in ACD-Chemsketch [16] and their SMILESnotation was obtained. They were converted into SDF files by using 'Online SMILES convertor and Structure file generator' [17] for further docking studies.

Molecular Docking

The obtained 3D structure of phytochemical compounds in SDF format from *Ricinus communis, Andrographis paniculata* and *Withania somnifera* were virtually screened to reveal their binding efficiencies through docking in the predicted binding pockets of modeled protease mutant and wildtype by using FlexX[18] with the default docking parameters. Also the docking interaction was explored with conventional protease inhibitor, nelfinavir against both mutant and wildtypes.

Docking Interactions

The docking interactions that envisage the binding affinities of the phytochemical compounds within the predicted binding pockets amino acids of both mutant and wildtype protease were analyzed by using pose-view module of LeadIT [19] which clearly picturized the Hbond and non-bond interactions.

Results And Discussion

HIVdrug resistance is one of the major hurdles for achieving and maintaining of successful viral suppression. Most data on the genetic mechanisms of HIV-1drug resistance are from studies of sub type B viruses, the predominant sub type in the North American and Europe. Several of studies suggest that the currently available PR(Protease) and RT(Reverse Transcriptase)inhibitors are as active against non-Bviruses as they are against sub typeB viruses [20-24].

Thus, to understand how the screened drug-like virtual hits bind to the receptor, in this study the potential HIV-1 protease ligandnelfinavir interactions with both mutant and wildtype were analyzed using the ligand-receptor interactions through molecular docking. Molecular docking is one of the best filtering methods and a crucial technique in drug design process. The Molecular Docking protocol of the FlexX was used to dock the retrieved compounds by virtual screening. The protocol first analyses the provided cavity and then selects the region of the protein as the active site, and secondly dock the ligands to the selected site. 3D regular grids of points are employed for site detection and also for estimating the interaction energy of the ligand with the protein during docking. The protein receptor of

the HIV protease was selected from Protein Data Bank (RCSB-PDB) for the molecular docking study. Among several HIV protease inhibitors PDB ID: 2R5Q was selected based on the receptor size, resolution and deposited date. The 3D structure of the HIV-1 Protease is shown in Figure. 1.

Homology modeling and validations

Further, the mutant HIV-1 protease sequences with mutations such as V32I,I47V and V82I was used to build the 3D structure to explore the effect of mutation on the drug binding. Thus, by using the 2R5Q x-ray structure corordinate files, the 3D structure of the mutant HIV-1 protease were developed and validated with Ramachandran plot. The Ramachandran plot of the energy-minimized model showed most of the residues in the most favorable region and 0.0 % in the disallowed region. Thus the model was considered best as it exhibited more number of residues in the most favorable regions and also the low number of residues in disallowed region.

Docking Studies

The docking interactions of phytochemical compounds from *Ricinus communis, Andrographis paniculata* and *Withania somnifera* were used to determine their inhibition activity against the wild type and mutant protease from HIV-1 through docking studies.

Docking interactions of Nelfinavir against HIV-1 protease (Wildtype)

The docking interactions of Nelfinavir against the wildtype HIV-1 exhibited the binding affinity of -19.4109 kJ/mol. This interaction is favored by the formation of Hbond and non-bonded interactions. The hbonds are supported by the aminoacids such as Asp29, Asp30 and Asp25 with the active site of wild type protease. The docking complex and binding interactions of Nelfinavir with wildtype HIV-1 protease is given in Figure.2.

Docking interactions of Nelfinavir against HIV-1 protease (Mutant)

The docking interactions of Nelfinavir against the mutant HIV-1 exhibited the binding affinity of -11.0208 kJ/mol. This interaction is favored by the formation of Hbond and non- bonded interactions. The hbonds are supported by the aminoacids, Asp30 and Asp25 with the active site of mutant protease. The mutation at V32I,I47V and V82I might have the significant role in imposing the low binding affinity. Interestingly, it is observed that the Hbond formation with Asp29 is absent in the mutant when compared to wildtype. The docking complex and binding interactions of Nelfinavir with mutant HIV-1 protease is given in Figure.3. The comparison in the binding affinities of nelfinavir against the wild-type and mutant protease are given in Table.1.

Docking interactions of phytochemical compounds against HIV-1 protease (Wildtype)

A total of 120 phytochemical compounds from the *Ricinus communis, Andrographis paniculata* and *Withania somnifera*were virtually screened against the binding site of HIV-1 protease (wild-type). Theoretically, it is observed that most of the chemical compounds exhibited better docking interactions with better binding affinities in terms of kJ/mol. Among the best docked compounds, the compound (WSC-31) from *Withaniasomnifera*exhibited the highest binding affinity when compared to the conventional protease inhibitor, nelfinavir. Also, that the best five compounds that exhibited better docking score greater than that of nelfinavir were from *Andrographis paniculata* and *Withaniasomnifera*(Table 2). The binding affinities of all the compounds against protease (wild-type) are given in supplementary table. The docking interactions of WSC-31 against the wildtype HIV-1 exhibited the binding affinity of -25.4546kJ/mol. This interaction is favored by the formation of Hbond and non-bonded interactions. The hbonds are supported by the aminoacids such as Asp25 and

Gly48with the active site of wild type protease. The docking complex and binding interactions of WSC-31 with wildtype HIV-1 protease is given in Figure.4.

Docking interactions of phytochemical compounds against HIV-1 protease (mutant)

All the 120 phytochemical compounds from the *Ricinus communis*, *Andrographis paniculata* and *Withania somnifera*were virtually screened against the binding site of HIV-1 protease (mutant). Theoretically, it is observed that most of the chemical compounds exhibited better docking interactions with better binding affinities in terms of kJ/mol. Among the best docked compounds, the compound (APC-4) from *Andrographis paniculata* has exhibited the highest binding affinity when compared to the conventional protease inhibitor,nelfinavir. Also, the best five compounds with docking scores greater than nelfinavir are from *Andrographis paniculata* and *Withaniasomnifera*(Table 2). The binding affinities of all the compounds against protease (mutant) are given in supplementary table. The docking interactions of APC-4 against the mutant HIV-1 exhibited the binding affinity of -27.1425 kJ/mol. This interaction is favored by the formation of Hbond and non-bonded interactions. The h bonds are supported by the aminoacids such asArg8, Asp25 and Gly148with the active site of mutant protease. The docking complex and binding interactions of APC-4 with mutant HIV-1 protease is given in Figure.5.

Ungwitayatornet al [25]also performed studies on a series of non-peptide HIV-1 protease (HIV-1 PR) inhibitors, chromone derivatives, were docked with the HIV-1 protease binding site for study the binding interaction. The orientation of chromone molecules showed the critical interaction which are important for the inhibition of the enzyme. The chromone molecules form hydrogen bonding interaction with Asp25, Asp25', Ile50 and Ile50' and hydrophobic interaction with Val32,Ile50,Pro81, Val82, and Ile84. These docking studies also implies that the conserved amino acid Aspartic acid and Glycine (Gly26) in the catalytic site of HIV-1Protease receptor are crucial in binding of anti-HIV-1 Protease inhibitors. These docking interactions implies that the NH group and =O present in the compounds favors the hbond interactions. Hence these findings throws light for the design of novel anti-HIV-1 protease inhibitors and also envisages that the amino acids Aspartic acid (Asp 25 and 29) and Glycine (Gly148) should be considered during its design for implying its action as a best anti-HIV-1 Protease compound against the potential target of HIV-1 Protease.

Thus,considering the binding affinities of the phytochemical compounds from *Andrographis paniculata* and *Withaniasomnifera*, it is envisaged that these compounds might possess anti-viral activities against the HIV-1 protease. Also, these docking studies has evidently suggests that these phytochemical compounds could be employed in the treatment of HIV-1 as an alternatives to the conventionally used Nelfinavir. As the binding affinity of the nelfinavir is lesser than the phytochemical compounds. Interestingly, it is observed that the binding affinity of Nelfinavir has decreased in mutant protease, while the binding affinities of the phytochemical compounds have shown higher affinities both in mutant and wild type proteases. Thus this study, significantly suggest that this compound might lead to the design of novel PI inhibitors against the drug resistant HIV-1.

Conclusion

The 3D structure of HIV-1 protease with mutations V32I,I47V and V82Iwas developed through homology modeling with reference to wild type protease as template (PDBID:2R5Q) and validated. Further, the effect of mutations on the drug resistance was determine through docking studies. The conventionally used protease inhibitor, Nelfinavir was docked against both wild type and mutant protease. Also, the anti-reteroviral activity of the phytochemical compounds from *Ricinus communis*, *Andrographis paniculata* and *Withania somnifera* were evaluated. It is observed that nelfinavir exhibited -19.4109 kJ/mol and -11.0208 kJ/mol against HIV-1 protease wild type and mutant

respectively. Interestingly, the binding affinities of the phytochemical compounds exhibited better score when compared to that of standard protease inhibitor. The docking interactions of compound APC-4 exhibited -27.1425 kJ/mol and WSC-31 exhibited-25.4546 kJ/mol against HIV-1 protease mutant and wildtype respectively. Thus, this docking study evidently suggests that these phytochemical compounds could be employed in the treatment of HIV-1 as alternatives to the conventionally used Nelfinavir.

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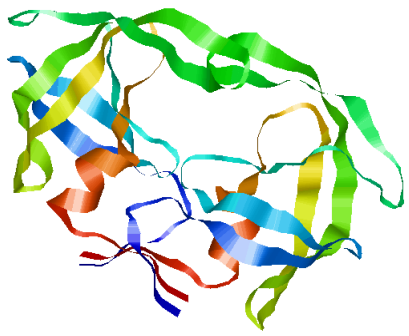
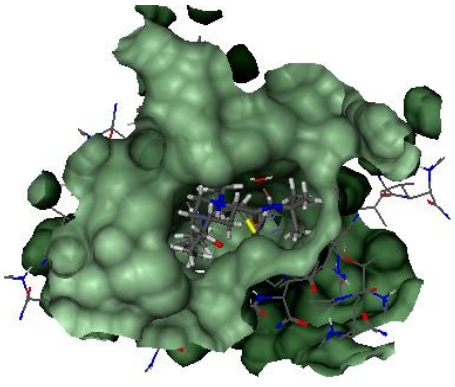


Fig 1A:Wild type protease from HIV-1

Fig 1B:Modelled protease (mutant) from HIV-1 with mutations such as V32I,I47V and V82I

Figure.1: The 3D structure of wild type(1A) and modelled protease (mutant) (1B) from HIV-1



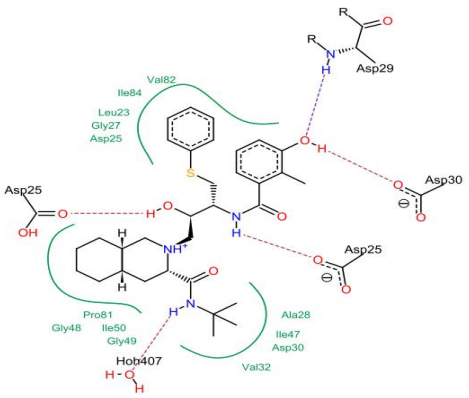


Figure.2: Docking complex and interactions of Nelfinavir with wild-type protease (Binding affinity: -19.4109 kJ/mol)

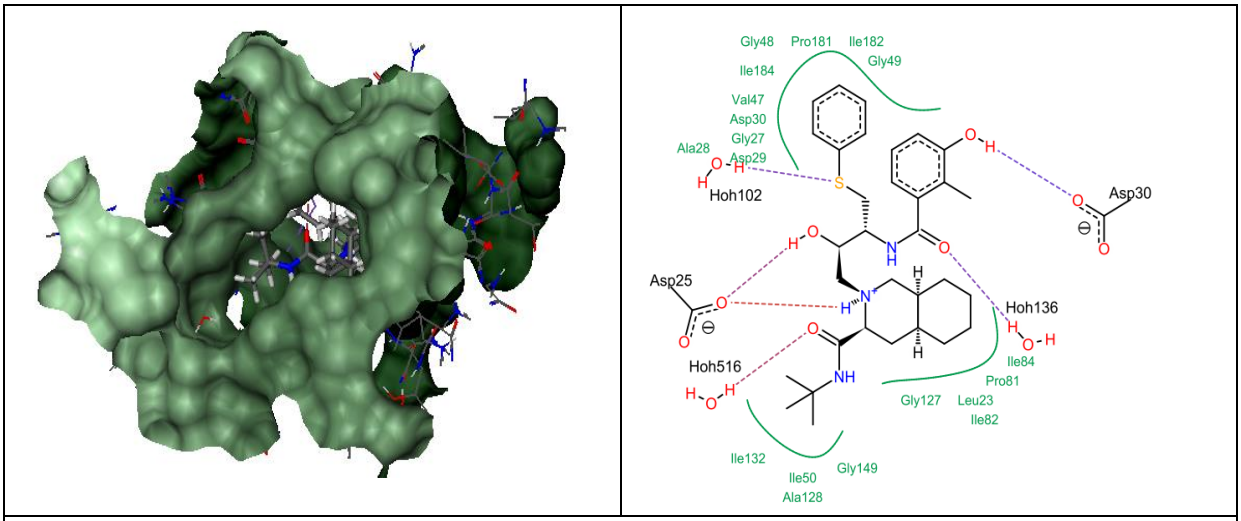


Figure.3: Docking interactionsof Nelfinavir with protease mutant (V32I,I47V and V82I) (Binding affinity: -11.0208 kJ/mol)

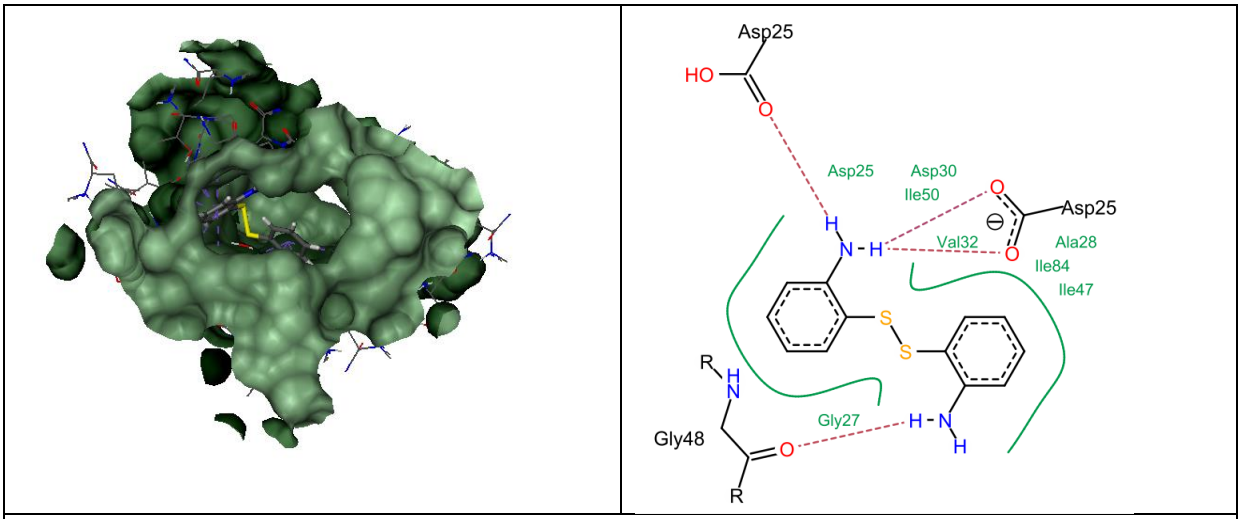


Figure.4: Docking complex and interactionsof protease wild type with WSC-31 (Binding affinity:-25.4546 kJ/mol)

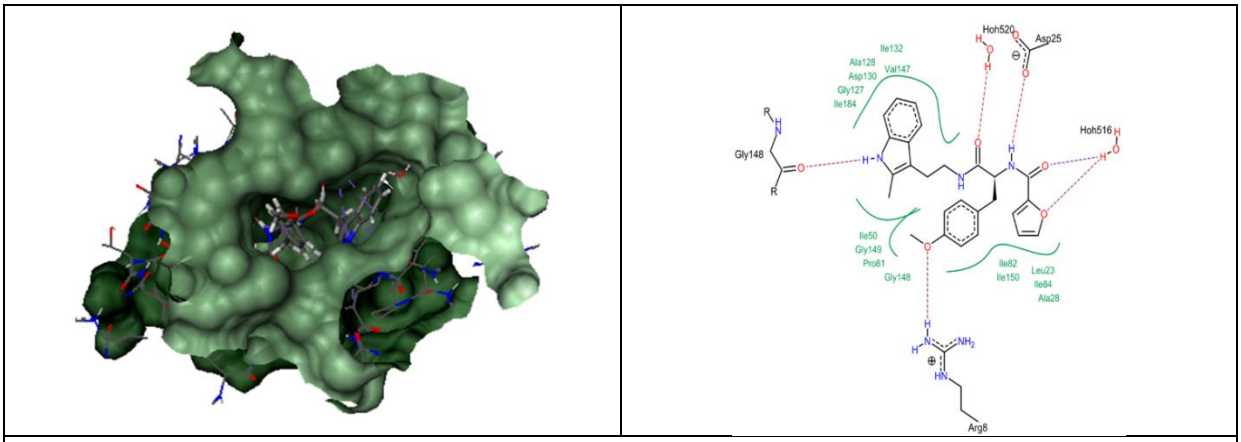


Figure.5: Docking complex and interactions of protease mutant with APC-4 (Binding affinity: -27.1425 kJ/mol)

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Table.1: Comparison of binding affinities of Nelfinavir against wild-type and mutant HIV-1 protease.

Table.2: The binding interactions of best five phytochemicals against mutant and wild type HIV-1protease.

Table.1: Comparison of binding affinities of Nelfinavir against wild-type and mutant HIV-1 protease.

Protein	Wild type/ mutant	Docking score (kJ/mol)
Protease	No mutation (Wild type)	-19.4109
	V32I,I47V and V82I (mutant)	-11.0208

Table.2: The binding interactions of best five phytochemical against mutant and wild type HIV-1protease.

Phytochemicals	Protease mutant	Phytochemicals	Protease wild type
(4) APC-4_001	-27.1425	(98) WSC-31_001	-25.4546
(100) WSC-33_001	-26.2197	(5) APC-5_001	-24.2563
(5) APC-5_001	-23.2949	(106) WSC-39_001	-23.6818
(93) WSC-26_001	-22.133	(4) APC-4_001	-21.3861
(98) WSC-31_001	-21.5536	(100) WSC-33_001	-20.4196

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