Docking Studies of HIV-1 Protease with Phytochemicals from *Mappia Foetida*

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ABSTRACT
Human immunodeficiency virus type 1 protease (HIV-1 PR) is an essential enzyme for the replication cycle of HIV. HIV-1 PR inhibitors have been extensively investigated as anti-AIDS drugs. In the presence of HIV-1 protease inhibitors, the virion is unable to mature. Natural compounds are important sources of drugs. The present investigation concentrates on discovering anti-HIV compounds that are present in *Mappia foetida*, a medicinal plant belonging to Icacinaceae family. Here, we selected Phytochemicals of *Mappia foetida*- Root identified by the method of Gas chromatography–mass spectrometry (GC-MS Method) to dock against the enzyme HIV-1 protease (PDB ID: 3HAU). The resulted enzyme-substrate interaction energies shows that those compounds are active against HIV-1 protease and further research on plant *Mappia foetida* will be useful in drug designing against HIV.

Keywords
HIV-1 protease, Phytochemicals, *Mappia foetida*, Drug designing, Applications of Computer Science.

1. INTRODUCTION
Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes *Acquired Immunodeficiency Syndrome* (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections [1,2]. When HIV enters the human body, its target is a subset of immune cells that contain CD4 [3]. Without effective HIV PR, HIV remains uninfectious because its work in HIV is to create mature protein components by synthesizing polyproteins [4].

The necessity of this enzyme HIV protease in the virus life cycle makes it a promising target for therapy of the HIV infection. Unless the HIV life cycle is interrupted by specific treatment, the virus infection spreads rapidly throughout the body, which results in the weakness and destruction of the body's immune system [5]. This work aims to produce inhibitor for HIV-1 Protease, so that the growth of HIV can be stopped [4].

2. MAPPIA FOETIDA
Mappia foetida (**Figure 1**) is a medicinal plant belonging to the Icacinaceae family. Globally this species is largely distributed in the Indo-Malaysian region and China. It has been found that *Mappia foetida* contains compounds having anticancer and antiviral properties. Those compounds are present in all parts of the plant [6].

Figure 1: *Mappia foetida*

3. OBJECTIVES
Objectives of this work is to study role of HIV-1 PR in AIDS disease, docking studies to be performed with plant based compounds and to evaluate the binding efficiency of the selected lead molecule with reference Ligand.

The target selected for investigation is HIV-1 PR (PDB ID: 3HAU- *Crystal structure of chemically synthesized HIV-1 protease with reduced isostere MVT-101 inhibitor*) (**Figure 2**) [7] and the ligands are those compounds got from *Mappia foetida*- Root (**Table 1**). Natural compounds are important sources of drugs [3].The compounds from *Mappia foetida*- Root were extracted and their respective ligands got downloaded from PUBCHEM database. HIV-1 protease (3HAU) was got downloaded from Protein data bank database and energy minimized using Universal force field. Using ArgusLab 4.0.1 docking software [8] the compounds of *Mappia foetida* were docked against HIV-1 protease.

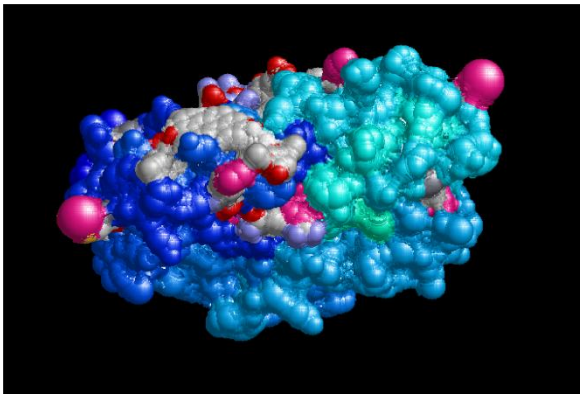

Figure 2:

3HAU (Crystal structure of chemically synthesized HIV-1 protease with reduced isostere MVT-101 inhibitor)

Table 1: List of the Phytochemicals present in *Mappia foetida*- Root obtained from PUBCHEM Database

S. No.	Compound name	PUBCHEM ID
1	3-BENZYL-6,7-DIPHENYL-2-METHYL-4H-PYRAZOLO(5,1-C)(1,4)OXAZINE	30204
2	METHYL 13-OXO-14-(3-PENTYNYL)PODOCARP-8-EN-18-OATE	3267
3	1,4,4-TRIMETHYL-2,6-DIPHENYL-1,4-DIHYDROPYRIDINE-3,5-DICARBONITRILE	628258
4	1,2,3,6-TETRAHYDROPHTHALIC ACID $$ 4-CYCLOHEXANE-1,2-DICARBOXYLIC ACID(CAS)	4728
5	DICYCLOHEXYLPHOSPHINE	13239
6	(14Z,17Z)-21-ETHYL-2,6-EPOXY-1-OXACYLOHENICOSA-2,5,14,17,20-PENTAEN-H-YN-4-ONE	5053
7	ANDROSTAN-H-AMINE,N,N-DIMETHYL-,(5-ALPHA,H-ALPHA)-(CAS)	250282
8	OXIRANE ,2-(CHLOROMETHYL)-2-CYCLOBUTYL	576261

4. MATERIAL AND METHODS

4.1 Preparations

The three dimensional structure of target HIV Protease (PDBID: 3HAU *Crystal structure of chemically synthesized HIV-1 protease with reduced isostere MVT-101 inhibitor*) was retrieved from protein data bank at 1.3 Å RMSD resolution and active sites were analysed using Q-SiteFinder [9] (**Table 2**) (**Figure 3**). The compounds from *Mappia foetida* were made identified and separated by method of Gas chromatography–mass spectrometry (GC-MS Method) [10].The 2D structures of Phytochemicals from *Mappia foetida* with anti-HIV activity were retrieved from PUBCHEM compound database and then with the help of Open Babel software [11] these 2D structures are converted to 3D structures and energy minimized using Universal force field (UFF).

Table 2: Residues of active site (3HAU)-

Q-SITEFINDER

Residues
265 N GLU A 35
266 CA GLU A 35
267 C GLU A 35
268 O GLU A 35
269 CB GLU A 35
270 CG GLU A 35
271 CD GLU A 35
272 OE1 GLU A 35
273 OE2 GLU A 35

274	N	NLE	A	36
275	CA	NLE	A	36
276	C	NLE	A	36
277	O	NLE	A	36
282	N	ASN	A	37
283	CA	ASN	A	37
287	CG	ASN	A	37
288	OD1	ASN	A	37
326	CE3	TRP	A	42
327	CZ2	TRP	A	42
328	CZ3	TRP	A	42
329	CH2	TRP	A	42
339	N	PRO	A	44
340	CA	PRO	A	44
341	C	PRO	A	44
342	O	PRO	A	44
343	CB	PRO	A	44
344	CG	PRO	A	44
346	N	LYS	A	45
347	CA	LYS	A	45
348	C	LYS	A	45
349	O	LYS	A	45
415	CA	LYS	A	55
416	C	LYS	A	55
417	O	LYS	A	55
418	CB	LYS	A	55
419	CG	LYS	A	55
420	CD	LYS	A	55
421	CE	LYS	A	55
422	NZ	LYS	A	55
423	N	VAL	A	56
424	CA	VAL	A	56
425	C	VAL	A	56
426	O	VAL	A	56
427	CB	VAL	A	56
430	N	ARG	A	57
431	CA	ARG	A	57
434	CB	ARG	A	57
435	CG	ARG	A	57
436	CD	ARG	A	57
437	NE	ARG	A	57
438	CZ	ARG	A	57
439	NH1	ARG	A	57
440	NH2	ARG	A	57

4.2 Docking

The ligands and crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for the missing hydrogen. Following the above steps of preparations, the protein was subjected to energy minimization using the Universal force field (UFF). Q-SiteFinder interface server was used to detect the active sites and docking was performed by ArgusLab molecular docking software. Docking was performed with all the potential active sites detected on HIV-1 protease enzyme. During docking at first the molecules were prepared and bonds, bond orders, explicit hydrogen's, charges, flexible torsions were assigned to both the protein and ligands. From the docking wizard ligands were selected and the scoring function used is ArgusLab score. If hydrogen bonding is possible, the hydrogen bond energy contribution to the docking score is assigned a penalty based on the deviations from the ideal bonding angle. Using this option can significantly reduce the number of unlikely hydrogen bonds reported also internal electrostatic interaction; internal hydrogen bond sp2-sp2 torsions are calculated from the pose by enabling the ligand evaluation terms. The search algorithm is taken as ArgusLab and numbers of runs are taken 10 and max interactions were 2000 with population size 50 and with an energy threshold of 100 also at each step least 'min' torsions/translations/rotations are tested and the one giving lowest energy is chosen. If the energy is positive (i.e. because of a clash or an unfavorable electrostatic interaction) then additional 'max' positions will be tested. If the pose being docked is closer to one of the ligands in the list than specified by the RMSD threshold, an extra penalty term (the Energy penalty) is added to the scoring function. This ensures a greater diversity of the returned solutions since the docking engine will focus its search on poses different from earlier poses found.

The energy penalty was set to 100, RMSD threshold was 2.00 and RMSD calculation by atom ID (fast) were set. Docking was conducted between Protein and Inhibitor which results binding affinities in kcal/mol and docking run time. The Phytochemical which gives lowest binding energy is chosen

as best inhibitor. ArgusLab showed better overall performance in docking simulations when compared with other software.

4.3 Adverse effect prediction

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties were predicted using PreADMET server to know whether the Phytochemicals has the potential of adverse effect in human [12].

5. RESULTS

Molecular docking predicts the binding affinity of the Ligand to the protein based on complex geometry. If an effective inhibitor, or drug, can be found to stop the protease from carrying out its functions in the virus, the replication of HIV could be stopped or the virus could be rendered non-infective. Here compounds of *Mappia foetida* were docked against HIV -1 Protease using Argus Lab 4.0.1 and the best result was shown (**Figure 4**). Physiochemical properties (**Table 3**) of compounds from *Mappia foetida* were checked and results of docking were tabulated (**Table 4**). 3-BENZYL-6,7-DIPHENYL-2-METHYL-4H-PYRAZOLO(5,1 C)(1,4)OXAZINE, the compound of *Mappia foeida* got best ligand pose energy of -10.8761 kcal/mol. Further research on the plant *Mappia foetida* will be useful in designing drug for inhibiting HIV-1 PR.

Table 3: Physiochemical properties of compounds from *Mappia foetida*

S. No.	Compound name	Molecular formula	Molecular weight [g/mol]	XLogP3	H-Bond Donor	H-Bond Acceptor
1	3-BENZYL-6,7-DIPHENYL-2-METHYL-4H-PYRAZOLO(5,1-C)(1,4)OXAZINE	$C_{26}H_{22}N_2O$	378	5.9	0	2
2	METHYL 13-OXO-14-(3-PENTYNYL)PODOCARP-8-EN-18-OATE	$C_{23}H_{32}O_3$	356	6	1	3
3	1,4,4-TRIMETHYL-2,6-DIPHENYL-1,4-DIHYDROPYRIDINE-3,5-DICARBONITRILE	$C_{22}H_{19}N_3$	325	4.4	0	3
4	1,2,3,6-TETRAHYDROPHTHALIC ACID $$ 4-CYCLOHEXANE-1,2-DICARBOXYLIC ACID(CAS)	$C_8H_{10}O_4$	170	0.6	1	4
5	DICYCLOHEXYLPHOSPHINE	$C_{12}H_{23}P$	198	3.5	0	0
6	(14Z,17Z)-21-ETHYL-2,6-EPOXY-1-OXACYLOHENICOSA-2,5,14,17,20-PENTAEN-H-YN-4-ONE	$C_{22}H_{26}O_3$	338	6.1	0	3
7	ANDROSTAN-H-AMINE,N,N-DIMETHYL-,(5-ALPHA,H-ALPHA)-(CAS)	$C_{21}H_{37}N$	303	7.8	1	1
8	OXIRANE ,2-(CHLOROMETHYL)-2-CYCLOBUTYL	$C_7H_{11}ClO$	146	1.6	0	1

Table 4: Docking results

S. No.	Compound name	Pose energy (kcal/mol)
1	3-BENZYL-6,7-DIPHENYL-2-METHYL-4H-PYRAZOLO(5,1-C)(1,4)OXAZINE	-10.8761
2	METHYL 13-OXO-14-(3-PENTYNYL)PODOCARP-8-EN-18-OATE	-8.73998
3	1,4,4-TRIMETHYL-2,6-DIPHENYL-1,4-DIHYDROPYRIDINE-3,5-DICARBONITRILE	-8.10513
4	1,2,3,6-TETRAHYDROPHTHALIC ACID $$ 4-CYCLOHEXANE-1,2-DICARBOXYLIC ACID(CAS)	-6.62486
5	DICYCLOHEXYLPHOSPHINE	-8.55526
6	(14Z,17Z)-21-ETHYL-2,6-EPOXY-1-OXACYLOHENICOSA-2,5,14,17,20-PENTAEN-H-YN-4-ONE	-6.54708
7	ANDROSTAN-H-AMINE,N,N-DIMETHYL-,(5-ALPHA,H-ALPHA)-(CAS)	-10.4041
8	OXIRANE ,2-(CHLOROMETHYL)-2-CYCLOBUTYL	-10.1031

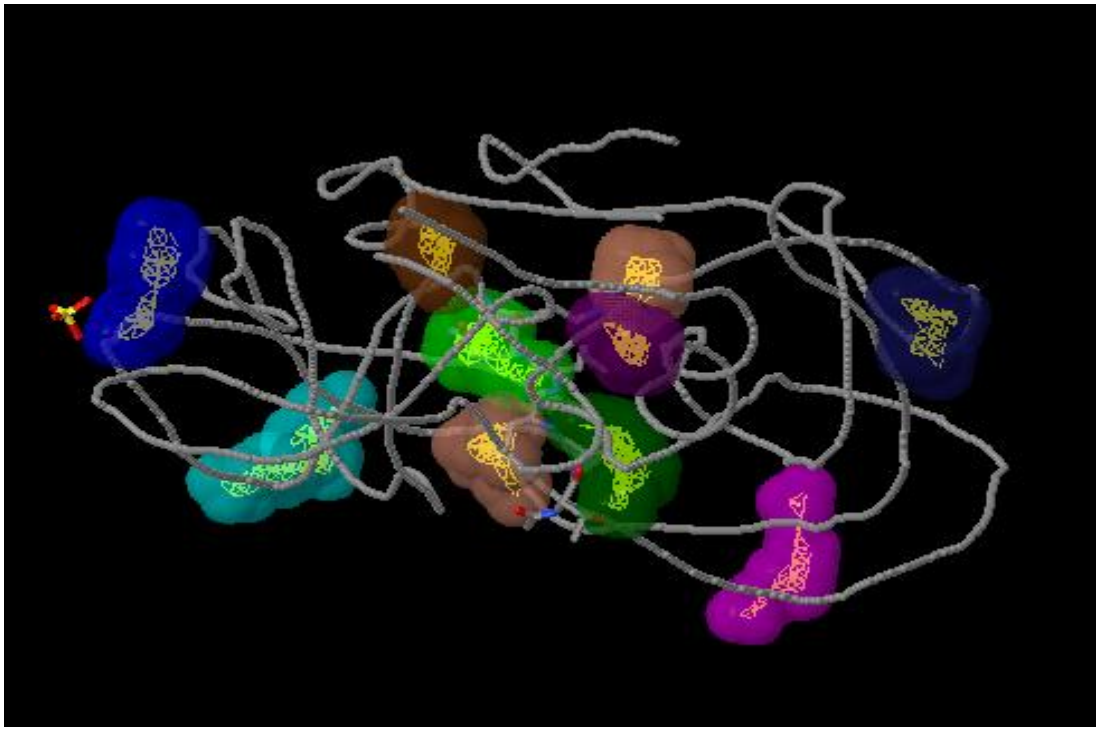

Figure 3: Active site analysis using Q-SiteFinder

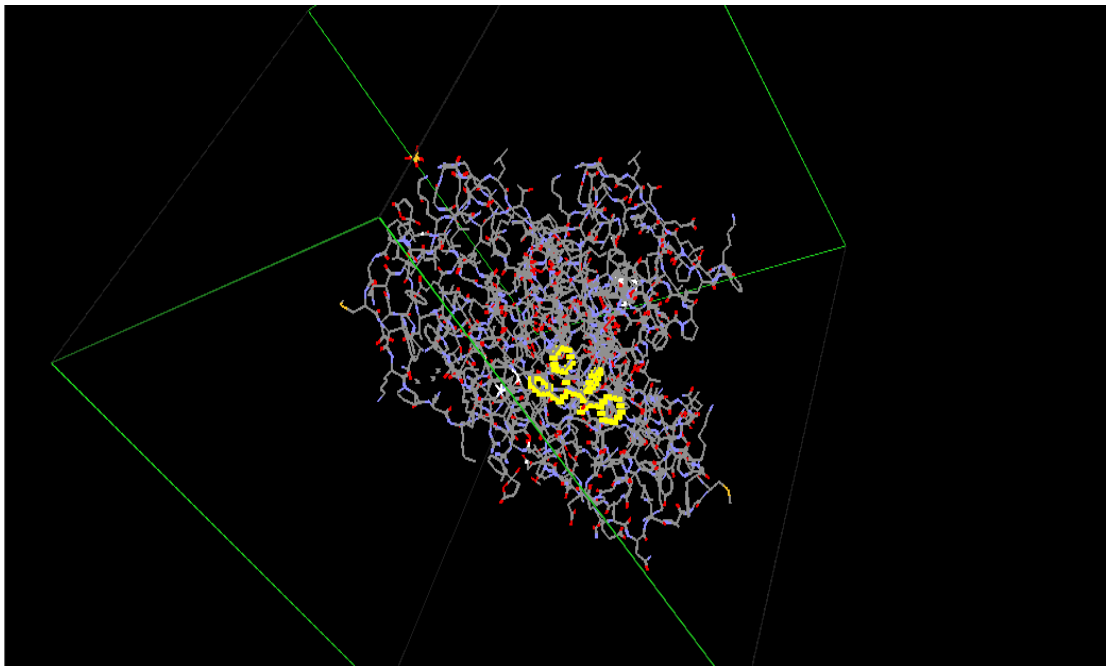

Figure 4:

Docking of 3-BENZYL-6,7-DIPHENYL-2-METHYL-4H-PYRAZOLO(5,1-RC)(1,4)OXAZINE against HIV-1 protease

6. CONCLUSIONS

1. The best binding Phytochemicals of *Mappia foetida* were identified by best binding energies obtained in docking studies of Phytochemicals with HIV-1 protease (3HAU).
2. The docking studies are also helpful for understanding the binding mode and interaction of Phytochemicals of *Mappia foetida* with HIV-1 protease (3HAU).
3. The present study also provides an insight into the mechanism of action of Phytochemicals against diseases.
4. This In-silico approach can be further investigated to generate more effective and potential HIV-1 protease inhibitors through ligand based drug designing approaches.
5. Hence it can be concluded that Phytochemicals of *Mappia foetida* can act as HIV-1 protease inhibitors. From this analysis it was found that 3-BENZYL-6,7-DIPHENYL-2-METHYL-4H-PYRAZOLO(5,1-C)(1,4)OXAZINE can effectively inhibit HIV-1 protease (3HAU).

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