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Structural analysis and interaction of w*ild-type* and *mutant HIV-1 protease in complex with curcumin derivatives* using molecular Docking

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HIV-1 protease is an important drug target for AIDS therapy. To date, curcumin, a substance in turmeric, has been reported as an inhibitor of HIV-1 protease. Our previous work on molecular dynamics simulations of HIV-*1 protease*–curcumin *complexes showed* that curcumin is flexible and can move toward closing the flap region because the structure of curcumin is smaller than the binding cavity of HIV-*1 protease*. Moreover, only hydrogen bonds are in the flap region. In this study, the possibility of three curcumin derivatives was investigated to inhibit the w*ild-type and mutant HIV-1 protease.* Molecular docking via AutoDock version 4.2 program was applied to evaluate the binding interactions of three curcumin derivatives (CP-1, CP-2 and CP-3) with w*ild type and six single mutant HIV-1 proteases (V32I, I50V, I54M, I54V I84V and L90M).* The docking results exhibited structural differences of curcumin derivatives in all complexes. The best conformations of ligands were analyzed for binding interactions with the residues of binding cavity of *HIV-1 protease*. The binding interactions and docking scores were observed. As expected, the interactions from CP-3 to w*ild type* HIV-1 protease are stronger than those from CP-1 and CP-2. In the case of mutant HIV-1PR, CP-1 has stronger interaction than CP-2 and CP-3.
Keywords: HIV-1 Protease; Molecular Docking; Curcumin Derivatives

1. Introduction

The human immunodeficiency virus type 1 protease (HIV-1 PR) is a virus-specific aspartic protease which is catalyzes the conversion of a polyprotein precursor encoded by *gag* and *pol* genes to mature proteins needed for the production of infectious HIV partic1es. The activity of HIV-1 PR enzyme is essential for virus infectivity, thus it is one of the major targets for the treatment of the pandemic disease HIV/AIDS. The structure of the HIV-1 PR was recently resolved by X-ray crystallographic methods. HIV-1 PR is a homodimeric protein that consists of two identical polypeptides of 99 amino acids. Each monomer contains an extended β-sheet region (a glycine-rich loop) known as the flap. The flexible flap regions consisting of residues 48–50 and residue 50 lay at the tip of the HIV-1 PR flap. The flap region of both monomers constitutes in part of the substrate-binding site and plays an important role in substrate binding, and one of the two essential aspartyl residues, Asp25 and Asp25' which lay on the bottom of the cavity.[1] To date, many HIV-1 PR inhibitors have been approved by the United Stated Food and Drug Administration (FDA), and are in clinical use. However, the emergence of drug resistance to HIV-1 PR inhibitors has compromised the effectiveness of treatment of HIV infections, thus drug resistance has become a severe challenge for treatment of HIV infections. Accumulation of 10–20 mutations in PR may be required to evolve high levels of resistance.[2]

Curcumin is one of three curcuminoids present in turmeric (Zingiberaceae) or Curcuma longa Linn which is a medicinal plant widely cultivated in tropical regions of Asia. Many proteins and enzymes have been reported to be targets of curcumin.[3] From previous research has demonstrated that curcumin is a potent anti-inflammatory agent that can reduce

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inflammation and may even play a role in cancer treatment. More recently, curcumin has been reported to inhibit HIV replication and was claimed for anti-HIV-1 and HIV-2 activities in a recent patent application.[4] In addition, curcumin and related compounds have also been reported for inhibition of HIV-1 and HIV-2 proteases with IC_{50} of 100 and 250 M, respectively.[5]

The objectives of this research are to predict the preferred orientation of curcumin derivative when bound to wild-type and six single mutant HIV-1 PR and investigate the interaction in molecular level of wild-type and six single mutant HIV-1 PR as well as their binding with three curcumin derivatives. Thus, the molecular docking was carried out for wild-type and six single mutant HIV-1 PR with three curcumin derivatives to obtain the best docking conformation of complex.

2. Materials and Methods

2.1 Starting Structure

A set of wild-type and six single mutant HIV-1 PR - amprenavir (APV) complexes with experimentally determined structures chosen from the Protein Data Bank (PDB), were used in this study.[6] All complexes were determined by x-ray crystallography with resolution and R-factor less than 1.85 and 0.236, respectively. The PDB codes of all complexes are listed in Table 1. For all system, the structure of amprenavir was removed in the preparation of the protease to be docked with the curcumin derivatives.

In this study we use three curcumin derivatives as ligand which were selected from the literature.[7] The 2D structures of all ligands were drawn using chemical structure drawing package, ChemDraw Ultra 12.0[8] as shown in Figure 2 that are CP1, CP2 and CP3 compounds. Energy of the molecules was minimized using Chem3D pro 12.0 program. The energy minimized compounds were then read as input for AutoDock 4.2.[9]

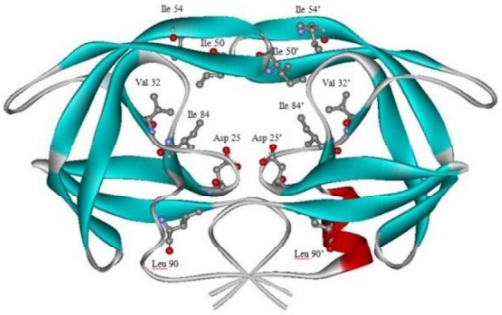

Figure 1. Schematic representation of the crystal structure of wild-type HIV-1 PR and demonstrate the mutation position that chose as single mutation system.

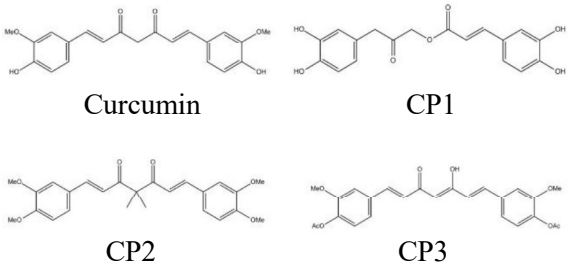

Figure 2. Molecular structure of curcumin and curcumin derivatives.

2.2 Molecular Docking

Three curcumin derivatives were docked with wild-type and six single mutant HIV-1 PR using AutoDock 4.2 software[9] in order to find the most favorable binding interaction and to identify the potent inhibitor against the enzyme. The Graphical User Interface program ‘‘Auto-Dock Tools’’ was used to prepare, run, and analyze the molecular docking. Kollman united atom charges, solvation parameters and polar hydrogen’s were added to the receptor for the preparation of protein in molecular docking. For ligands, Gasteiger charge was assigned and then non-polar hydrogens were merged. AutoDock requires calculated grid maps that representing the proteins were calculated using AutoGrid 4.0, this grid must surround the region of interest (active site) in HIV-1 protease. The grid box size was set at 70Å x 52Å x 62Å for x, y and z respectively, and the grid center was

set to 16.440, 22.922 and 19.125 for x, y and z respectively. The spacing between grid points was 0.375Å. Docking of three ligands to all HIV-1 PR were evaluated by standard docking protocol on the basis a population size of 150 randomly placed individuals, crossover rate of 0.80, an elitism value of 1, a mutation rate of 0.02 and a maximum number of 2.5 x 10^7 energy evaluations. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered for each compound. Finally, twenty-one of complex structures were constructed as shown in Figure 3 (three complex structures from one system of HIV-1 PR).

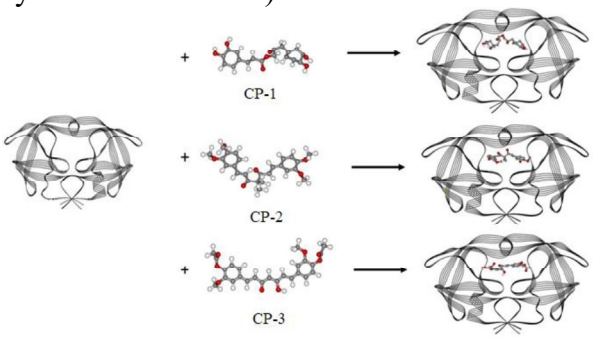

Figure 3. The complex structures of HIV-1 protease and curcumin derivatives present the docking process.

3. Results & Discussion

Previous experiments targeting HIV-1 protease using curcumin have failed due to the curcumin are flexible and move toward closing the flap region. Its move away from the catalytic residue and does not form hydrogen bonds with the catalytic residue. Thus, we opted to test curcumin derivatives that have *different functional groups* in the *middle* of the *molecule*. For non-peptide inhibitors, a common feature observed is the two catalytic aspartates Asp 25 and Asp 25' interact through hydrogen bonds with hydroxyl group in the *middle* of the inhibitor *molecule.*[10]

Docking results indicated that most of these ligands in twenty-one complexes could not fit into the active site of HIV-1 PR and are located close to the flap region. The comparison of ligand positions between APV from crystal structure and the CP1 from the docking complex is shown in Figure 4. In addition, we found only four complexes (WT-CP3, V32I-CP2, I54M-CP1 and L90M-CP1) that ligand can form hydrogen bonds with the Asp25 or Asp25' of PR. To find the best binding pose of each ligand at the active site of protein, the binding energy and the number of hydrogen bonds between ligand and protein were shown in Table1.

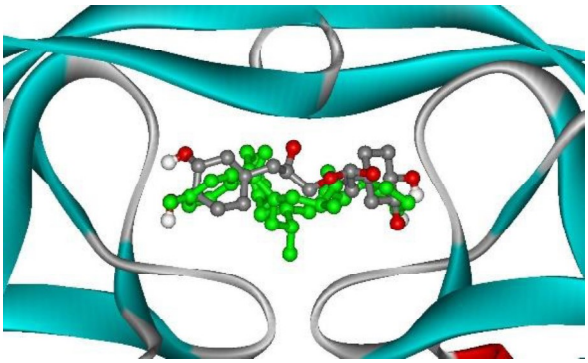

Figure 4. Structural alignment between APV from crystal structure (green) and the CP1 from I84V-CP1 complex.

Considering binding energy in Table 1, the CP1 compound has least binding energy with all wild-type and mutant HIV-1 PR but it can binds with amino acids in both *active site* cavity and flap region as can be seen from the *number* of hydrogen bonds in Table 1. The *larger number* of hydrogen bonds found in most of CP1 complex. Nonetheless, all ligands showed low *binding affinities* towards I50V, I84V and I54V mutant HIV-1 PR due to these mutation changes the original amino acid to *Valine.* It should be note that the mutation of I50V, I84V and I54V to a shorter side chain is expected to reduce the steric effect between protein and inhibitor and generated more free space in the binding site, leading to the conformational of ligand in binding pocket

that cannot directly interacted with active site residues.

According to the mutation, these three mutations generated more free space in the binding site *that make* the ligands are much *more flexible* than other mutation and has a strong influence on the conformation of ligand as show in Figure 5. The detected data suggest us to conclude that CP1, CP2 and CP3 compound could not possible to inhibit I50V, I84V and I54V mutant HIV-1 protease.

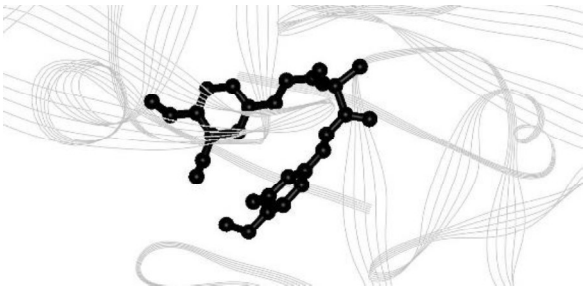

Figure 5. The CP2 structure of I54M-CP2 docking complex.

Table 1. Molecular docking results which are details of each system, the binding energy and the number of hydrogen bonds between curcumin derivatives and HIV-1 PR.

PDB code	Protease	Ligand	Complex	Binding Energy (kcal/mol)	H-bond (number)
3NU3	WT	CP1	WT-CP1	-8.61	7
		CP2	WT-CP2	-10.86	3
		CP3	WT-CP3	-9.35	3
3NU4	V32I	CP1	V32I-CP1	-7.35	5
		CP2	V32I-CP2	-10.45	4
		CP3	V32I-CP3	-9.37	3
3NU5	I50V	CP1	I50V-CP1	-7.73	6
		CP2	I50V-CP2	-9.93	3
		CP3	I50V-CP3	-8.25	3
3NU6	I54M	CP1	I54M-CP1	-7.74	8
		CP2	I54M-CP2	-10.37	5
		CP3	I54M-CP3	-9.55	5
3NU9	I84V	CP1	I84V-CP1	-6.57	5
		CP2	I84V-CP2	-9.53	4
		CP3	I84V-CP3	-9.60	7
3NUJ	I54V	CP1	I54V-CP1	-8.69	6
		CP2	I54V-CP2	-10.62	4
		CP3	I54V-CP3	-10.72	6
3NUO	L90M	CP1	L90M-CP1	-7.76	9
		CP2	L90M-CP2	-10.23	4
		CP3	L90M-CP3	-10.01	3

In case of four docking complexes (WT-CP3, V32I-CP2, I54V-CP1 and L90M-CP1) that ligand can forms hydrogen bonds

with the Asp25 or Asp25' of protease, the observed differences in hydrogen bond formation can be of valuable use in the design of further derivatives with better binding affinities for HIV-1 PR. Correspondingly, from the current results, the complex of I54M-CP1 and L90M-CP1 are the best docking complex due to CP1 has two carbonyl group which from hydrogen bonds active site and flap residue as show in Figure 6. It indicates that the CP1 compound has the tendency to interact with mutant HIV-1 PR with efficient binding and emerges out as a potential candidate inhibitor of mutant HIV-1 PR for further experimentation.

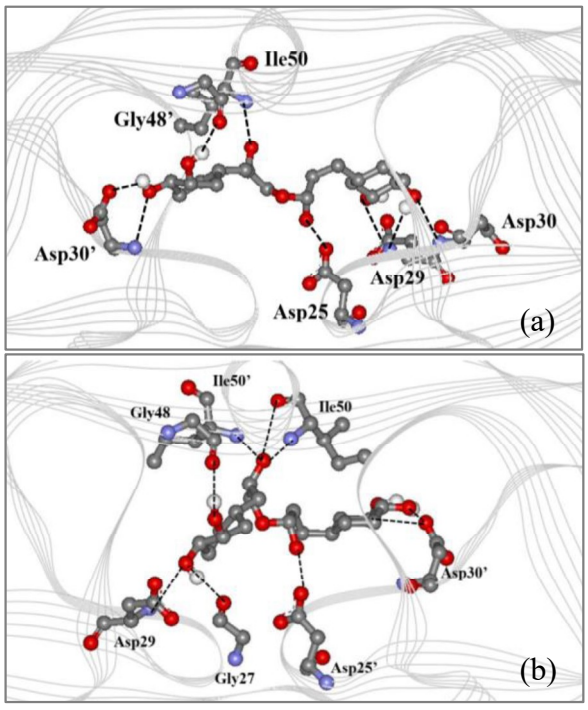

Figure 6. Hydrogen bonding between CP1 compound to I54V (a) and L90M (b) mutant HIV-1 PR.

4. Conclusion

Molecular docking studies were performed on the wild-type and mutant HIV-1 PR to identify the binding modes of curcumin derivative to the receptor. The best conformations of ligands were analyzed for binding interactions with the residues of binding cavity and the flap region of *HIV-1 PR*. The binding interactions and docking scores were observed. As expected, the interactions from CP3 to w*ild-type* HIV-1 protease are stronger than those of CP1 and CP2. In the case of mutant HIV-1 PR, CP1 has stronger interaction than CP2 and CP3.

Acknowledgements

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