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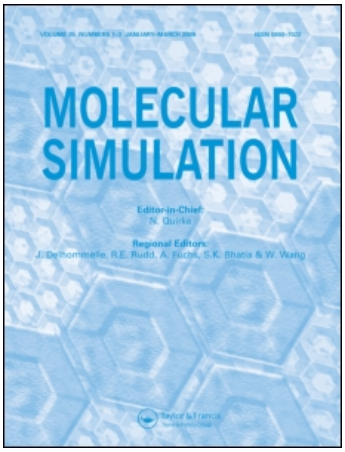


## Molecular Simulation

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# *Litchi chinensis*-derived terpenoid as anti-HIV-1 protease agent: structural design from molecular dynamics simulations

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# *Litchi chinensis*-derived terpenoid as anti-HIV-1 protease agent: structural design from molecular dynamics simulations

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The molecular structures of the binding between human immunodeficiency virus-1 protease (HIV-1PR) and various inhibitors including existing extensive natural products extracts have been investigated for anti-HIV drug development. In this study, the binding of HIV-1PR and a terpenoid from *Litchi chinensis* extracts (3-oxotrirucalla-7,24-dien-21-oic acid) was investigated in order to clarify the inhibition effectiveness of this compound. Molecular dynamics (MD) simulations of HIV-1PR complex with 3-oxotrirucalla-7,24-dien-21-oic acid were performed including water molecules. The MD simulation results indicated the formation of hydrogen bonds between the oxygen atoms of the inhibitor and the catalytic aspartates, which are commonly found in inhibitors–protease complexes. On the other hand, no hydrogen bonding of this particular inhibitor to the flap region was found. In addition, the radial distribution function of water oxygens around the catalytic carboxylate nitrogens of Asp29 and Asp30 suggests that at least one or two water molecules are in the active site region whereas direct interaction of the inhibitor was found for catalytic carboxylate oxygen of Asp25. The results of this simulation, in comparison with the structures of other HIV-PR inhibitor complexes, could lead to a better understanding of the activity of 3-oxotrirucalla-7,24-dien-21-oic acid.

**Keywords:** 3-oxotirucalla-7,24-dien-21-oic acid; HIV-1 protease; MD simulation; natural product

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## 1. Introduction

Human immunodeficiency virus-1 protease (HIV-1PR) is an important enzyme due to its role in the replication of HIV-1 by processing two precursor polyproteins, Pr55gag and Pr160gag-pol, into structural proteins and replication enzymes. Inactivation of this enzyme results in the formation of immature, non-infectious viral particles. Therefore, this enzyme is an attractive target in intensively focused anti-AIDS drug design research [1]. HIV-1 PR is an aspartic protease, and a homodimer with *C*2. Each monomer consists of 99 amino acid residues that possess a loop structure containing the active site triad Asp25(25′)–Thr26(26′)–Gly27(27′). A cavity for the insertion of the substrate is formed by these loop structures containing the active site triads and the flap regions which are presumably related to the entry and affinity of the substrate to the enzyme [1–7].

Terpenoids are widespread natural products with a high diversity of biological and pharmacological activities. 3-Oxotrirucalla-7,24-dien-21-oic acid, a triterpene in the extracts of *Litchi chinensis* seeds isolated by Tu et al. [8], was found to have activity against HIV-1PR. The inhibitory activity of this triterpene was reported to be $IC_{50} = 20$ mg/l (42.9 μM) by Ma et al. [9]. In general, this potency against HIV-1PR is in the middle of the range for triterpenes, which have a range from 230 to 4 μM [10,11]. Even though the extracts from such a natural product still have a relatively high $IC_{50}$ value, chemical modification of the anti-HIV protease triterpenes were shown to improve the potency of the natural product two- to five-fold [12]. In order to use 3-oxotrirucalla-7,24-dien-21-oic acid as a lead compound for possible drug candidates, computer-aided modelling is a very useful tool in the chemical design of modifications based on the understanding of the interaction of the lead with the respective enzyme.

To date, the detailed inhibition mechanism of triterpenes to HIV-1PR is still not completely understood. Combination of molecular docking and molecular dynamics (MD) simulation of this HIV-1PR triterpenoid complex will allow the designation of new anti-HIV agents from the abundant triterpenes in natural products. In this paper, the orientation and the binding conformation of 3-oxotrirucalla-7,24-dien-21-oic acid including water molecules in the HIV-1PR cavity site were examined using MD simulation results. These results also provide insight into the structural origins of this moderate $IC_{50}$ value in comparison with six food and drug administration (FDA) approved anti-HIV-1PR drugs.

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## 2. Methods

### *2.1 Preparation of the starting structure of HIV-1PR*

The initial HIV-1PR structure was obtained from the HIV-1PR complex with saquinavir at 2.3 Å resolution (1HXB entry in Protein Data Bank (PDB) database). The structural water and the inhibitor of the selected crystal data were then removed for the preparation of the HIV-1PR. Hydrogen atoms were added to this structure using LEaP libraries of AMBER and a minimisation run was performed in order to remove any potentially bad contacts using force field parameter99 within the program package AMBER, version 7 [13,14]. A cut-off distance at 12 Å for van der Waals forces was used in the minimisations (simulations).

### *2.2 Preparation of the initial structure of the inhibitor*

The starting molecular conformation of 3-oxotirucalla-7,24-dien-21-oic acid was built based on the chemical structure reported from NMR and solid-state X-ray crystallographic data [9]. The geometry, as shown in Figure 1, was optimised using AM1 implemented in the program package Spartan'04.

### *2.3 Preparation of HIV-1PR–inhibitor complex by molecular docking and molecular mechanics methods*

The structure of the HIV-1PR–inhibitor was obtained by docking 3-oxotirucalla-7,24-dien-21-oic acid to HIV-1PR, respectively. HIV-1PR was kept rigid and Gasteiger–Marsili charges [15] were used. Grid maps were calculated using the module AutoGrid in AutoDock 3.0 program [16–18] for protease structure. The centre of the grid was assigned at the centre of the cavity, between the two catalytic aspartates. The number of grid points in each direction of Cartesian coordinates was 60 with a spacing of 0.375 Å. This parameter set covered the active site completely letting the ligand move by exploring the enzyme active site without any constraints regarding the box size. The inhibitor was positioned in the active site of HIV-1PR in many different ways using a Lamarckian genetic algorithm. The solvation effect was also included in this docking study.

### *2.4 Molecular dynamic simulations*

The energy minimised conformation of HIV-1PR–inhibitor generated from the previous calculations was used as the starting structure for further analysis. The molecular mechanics potential energy (EP) minimisations and MD simulations were carried out with the program package AMBER, version 7 [13,14]. Calculations were performed using the parameter99 force field reference for HIV-1PR and 3-oxotirucalla-7,24-dien-21-oic acid. The atom types for 3-oxotirucalla-7,24-dien-21-oic acid were assigned by mapping their chemical properties (element, hybridisation and bonding schemes) to the AMBER atom type library and the Gasteiger charges were used.

The enzyme–inhibitor complex was solvated with a TIP3P water model (9298 water molecules) with cell dimensions of $61.06 \times 66.56 \times 75.88$ Å$^3$ and treated in the simulation under periodic boundary conditions. All of the MD simulations reported here were done under an isobaric–isothermal ensemble (NPT) using constant pressure of 1 atm and constant temperature of 298 K. The volume was chosen to maintain a density of 1 g/cm$^3$. A cut-off distance (12 Å) was applied for the non-bonded pair interaction. Three sodium and eight chloride ions were added to neutralise and buffer the system. The EP minimisations holding the HIV-1PR and 3-oxotirucalla-7,24-dien-21-oic acid fixed were performed on the systems using the steepest descent method. After a short minimisation simulation at 298 K with the solvent water molecules and anions for the enzyme and ligand fixed, the temperature of the whole system was gradually increased by heating it to 298 K for the first 60 ps, and then it was kept at 298 K from 61 to 1800 ps. The temperature was kept constant according to the Berendsen algorithm [19]. The trajectories at the temperature (298 K for 1800 ps) were kept and analysed in detail.

## 3. Results and discussion

### *3.1 Structural flexibility of 3-oxotirucalla-7,24-dien-21-oic acid in active site of HIV-1PR*

The optimised structure of 3-oxotirucalla-7,24-dien-21-oic acid is shown in Figure 1(a). This molecular structure agrees well with that reported from NMR and solid-state X-ray crystallographic data [9]. The preliminary complex structure was deduced from the molecular docking. The 10-run docking calculations were used in a prior prediction of binding affinities and to simulate crystal geometry as a candidate of the ligand/protein complex and re-docking in order to improve the clustering results. The candidate structure from molecular docking (Figure 1(b)) shows that the molecular torsion angles of flexible part labelled as tor1–8 mentioned in Figure 1 and Table 1 totally changed, resulting in the flipping of carboxylic group of the flexible part. To include the flexibility of the enzyme structure, an MD simulation was performed starting from this docking structure. The conformation of the inhibitor changed into the new equilibrium shown in Figure 1(c). As shown in Table 1, the averaged conformation from MD simulation after reaching equilibrium is almost the same as that found in the X-ray structure except tor5 and tor6. In addition, according to Figure 2, the distribution of tor6 is relatively broad (SD = 38.71°) indicating the highest flexibility of this part. The transitions of tor5 and tor6 are considered to be in relationship with the binding affinity and will be further discussed.

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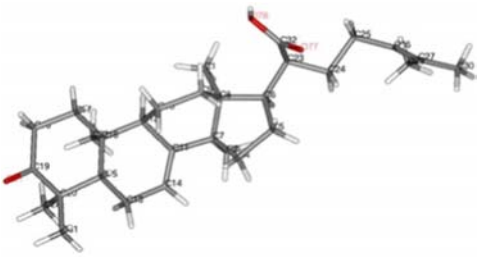
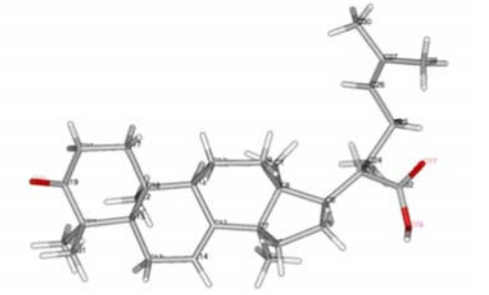
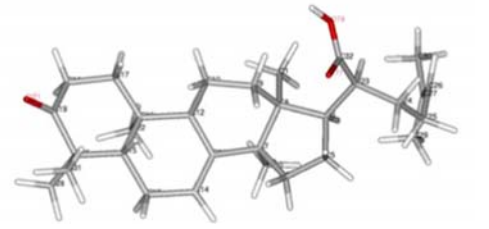
|---|---|---|
| (a) **Structure I** | −168.11 kcal/mol |  |
| (b) **Structure II** | −168.51 kcal/mol |  |
| (c) **Structure III** | −173.79 kcal/mol |  |

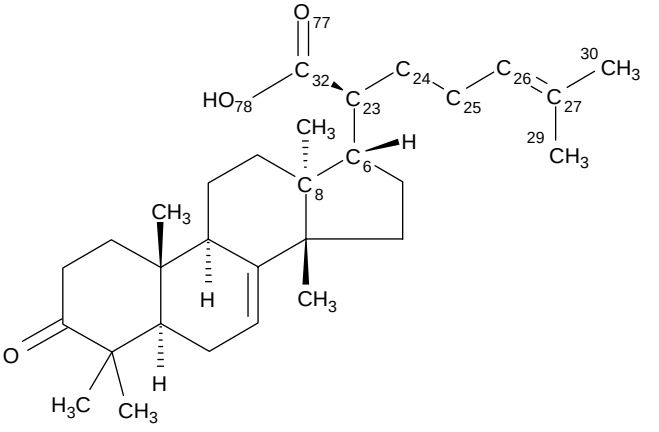



Figure 1. Chemical structure of 3-oxotirucalla-7,24-dien-21-oic acid and molecular geometries after (a) optimisation, (b) molecular docking and (c) explicit MD simulation.

Table 1. Average torsion angles in 3-oxotirucalla-7,24-dien-21-oic acid after optimisation, molecular docking and explicit MD simulation.

| Torsion | Abbreviation | Opt | Dock | MD |
|---|---|---|---|---|
| C8–C6–C23–C32 | tor1 | 58.6 | 159.3 | 64.0 |
| C6–C23–C32–O77 | tor2 | 94.7 | −144.7 | 60.0 |
| C6–C23–C32–O78 | tor3 | −86.7 | 33.8 | −119.0 |
| C8–C6–C23–C24 | tor4 | 179.5 | −79.7 | −171.0 |
| C23–C24–C25–C26 | tor5 | 164.3 | −70.3 | 62.0 |
| C24–C25–C26–C27 | tor6 | 82.3 | 126.3 | −126.0 |
| C25–C26–C27–C30 | tor7 | 179.8 | 179.8 | 180.0 |
| C25–C26–C27–C29 | tor8 | 0.2 | 0.2 | 0.0 |

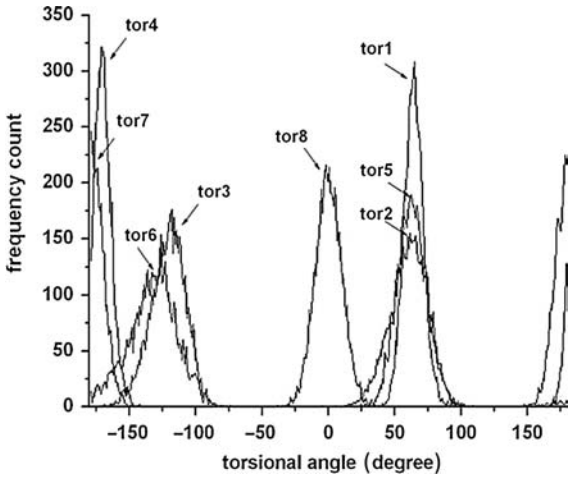



Figure 2. Eight bond torsions distributions of 3-oxotirucalla-7,24-dien-21-oic acid after 1200 ps of explicit MD simulation.

### 3.2 *Binding structure of HIV-1PR–terpenoid complex*

The minimised structure (Figure 3(a)) from molecular docking shows the direction of OH group in inhibitor points to the catalytic site of enzyme (N:Asp29 and N:Asp29′) as a crude complex structure. In the MD simulation, the total energy, EP and kinetic energy over simulations from 0 to 1800 ps were investigated. After the equilibrium stage, 3-oxotirucalla-7,24-dien-21-oic acid was found to bind to the enzyme at catalytic site Asp25 and Asp29 (Figure 3(b)). As shown in Table 2, the energy minimised structure obtained from MD simulations directs CO and OH group of the inhibitor to the catalytic site of enzyme, O:Asp25 and N:Asp29, respectively, with more than 89% hydrogen bond formation. The transition of the binding structure during molecular dynamic simulation can be observed from the interatomic distance plot against simulation time in Figure 4, the transition of the enzyme residue binding position from N:Asp29′ to O:Asp25 after 300 ps. According to Figure 4, N:Asp29′ is too far from the inhibitor, so this residue cannot form a strong hydrogen bond interaction with the triterpene inhibitor. On the other hand, O:Asp25′ shows two possible equilibrium stages that indicate a 15% possibility of a hydrogen bond to O78 of 3-oxotirucalla-7,24-dien-21-oic acid (Table 2).

### 3.3 *Roles of water molecule in active site*

The catalytic mechanism of the protease is most likely a combination of favourable binding of the inhibitor with the enzyme and the role of the water molecules in the enzyme pocket. With the optimal configuration of the enzyme–inhibitor complex, a water molecule was needed

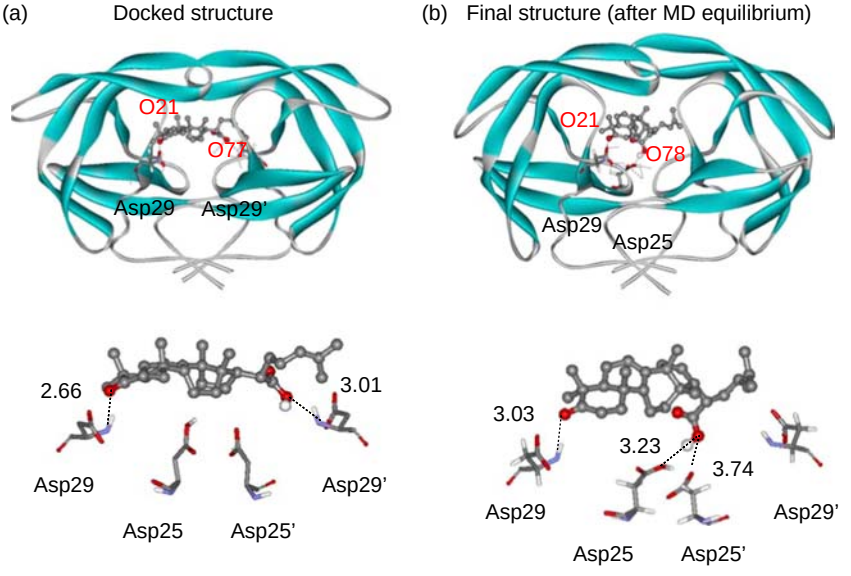



Figure 3. The structure of HIV-1PR–inhibitor (a) docking method and (b) the final structure after explicit MD simulation. The binding residues were shown in sticks.

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Table 2. Possible hydrogen bonds between 3-oxotirucalla-7,24-dien-21-oic acid and HIV-1PR after reach equilibrium in explicit MD simulation.

| | H-bond formation (%) | Average distance (Å) |
|---|---|---|
| Case 1: HIV-1PR as donor and Inh as acceptor | | |
| ASH25:OD2HD2–Inh:O78 | 1.98 | 3.07 |
| ASP29:NH–Ihn:O21 | 94.71 | 2.97 |
| ASP30:NH–Inh:O21 | 1.73 | 3.21 |
| ALA28′:NH–Inh:O78 | 3.27 | 3.05 |
| Case 2: Inh as donor and HIV-1PR as acceptor | | |
| Inh:O78H79–ASH25:OD1 | 0.04 | 3.17 |
| Inh:O78H79–ASH25:OD2 | 89.18 | 2.95 |
| Inh:O78H79–ASP25′:OD1 | 12.98 | 2.93 |
| Inh:O78H79–ASP25′:OD2 | 2.00 | 2.95 |

to facilitate proton transfer in the catalytic process. In this study, radial distribution functions to the oxygen atom of water molecule were evaluated centred at the hydrogen bond forming atoms (amide oxygens and nitrogen) of the all possible amino residues in Table 2. There is no water involved in the binding of Asp25, Asp25′ and Ala28′ meanwhile the solvation shells including coordination numbers of water bound Asp29 and Asp30 are given in Figure 5. In the first shell (about 2.7 Å), there are two water molecules close to Asp29. A water molecule moving in and out of the second shell (about 5 Å) of Asp29 and Asp30 was also observed here.

### *3.4 Comparison of HIV-1PR–terpenoid and HIV-1PR–commercial drugs complexes*

The X-ray crystallographic structures of HIV-1PR complexed with six commercial anti-HIV-1PR drugs; amprenavir (1HPV), lopinavir (1MUI), ritronavir (1HXW), indinavir (1HSG), nelfinavir (1OHR) and saquinavir (1HXB) were used here. The orientations of six drugs in the active site were compared with 3-oxotirucalla-7,24-dien-21-oic acid as shown in Figure 6. The essential subsites of anti-HIV-1PR drugs and 3-oxotrirucalla-7,24-dien-21-oic acid were superimposed and shown in the right figure of Figure 7. In comparing with HIV-six commercial drugs complexes, the anti-HIV activity of 3-oxotrirucalla-7,24-dien-21-oic acid would be resulted from the presence of P2 and P2′. The distribution of the energy decomposition analysis for the interacting residue pairs of HIV-1PR with 3-oxotrirucalla-7,24-dien-21-oic acid were also illustrated in Figure 7. It has a peak close to zero range from −2.0 to 0.5 kcal/mol. There is some unfavourable interaction, indicated by the positive energy in the P1 subsite region, which interacts with the flap region at Gly48–Ile50

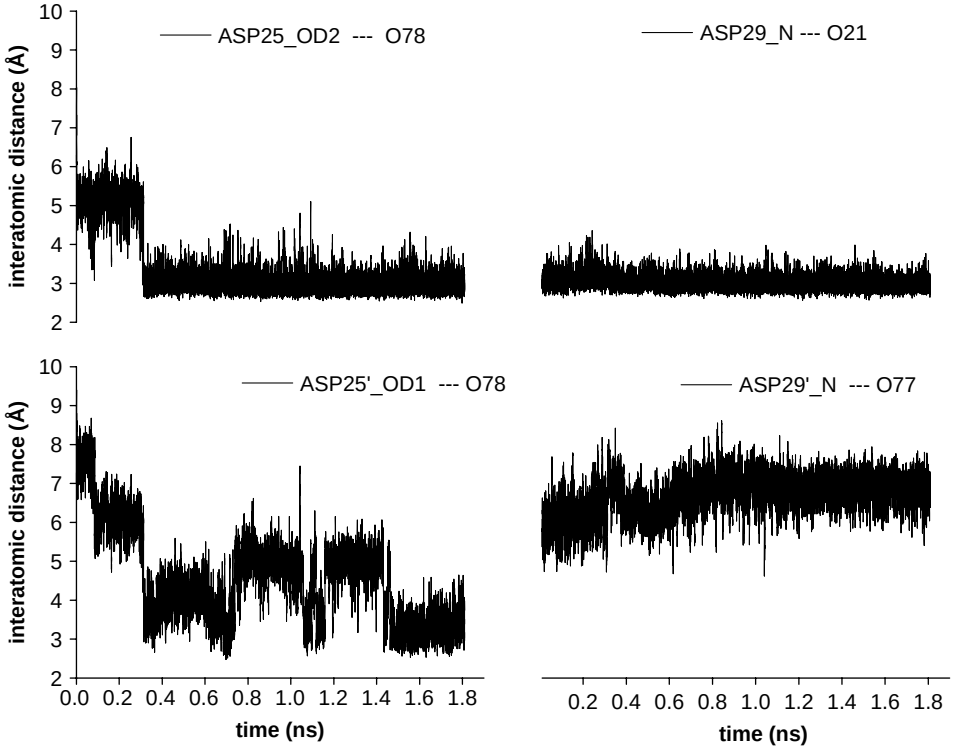


Figure 4. Changes of the distances from the O atom of the inhibitor to the H-bond binding group of the Asp25, Asp25′, Asp29 and Asp29′ at the catalytic site.

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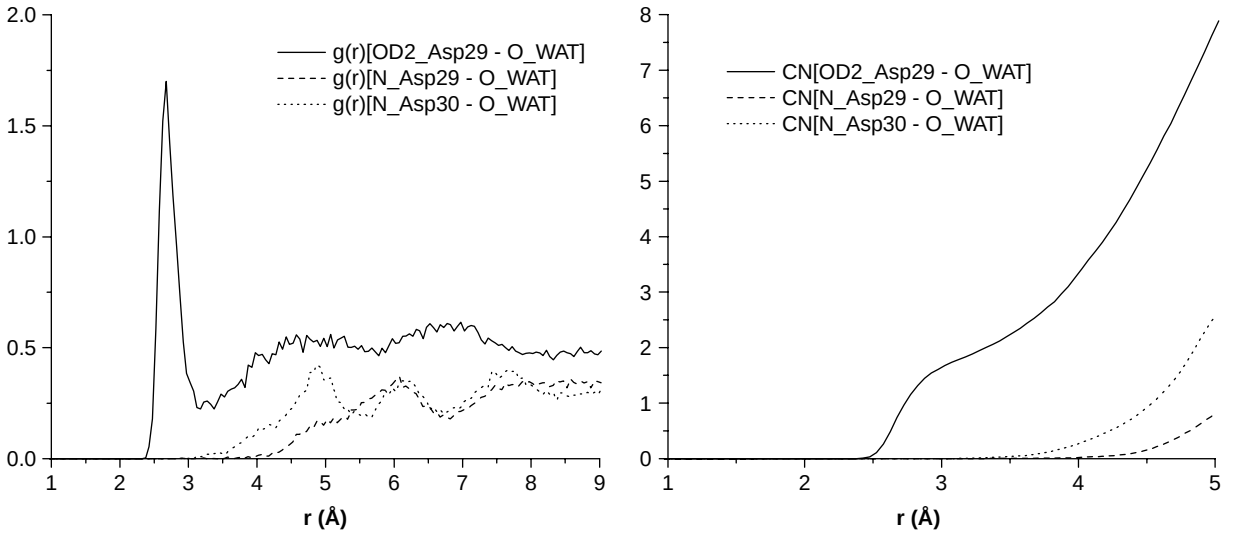


Figure 5. Radial distribution function of water oxygens around the Asp29 and Asp30.

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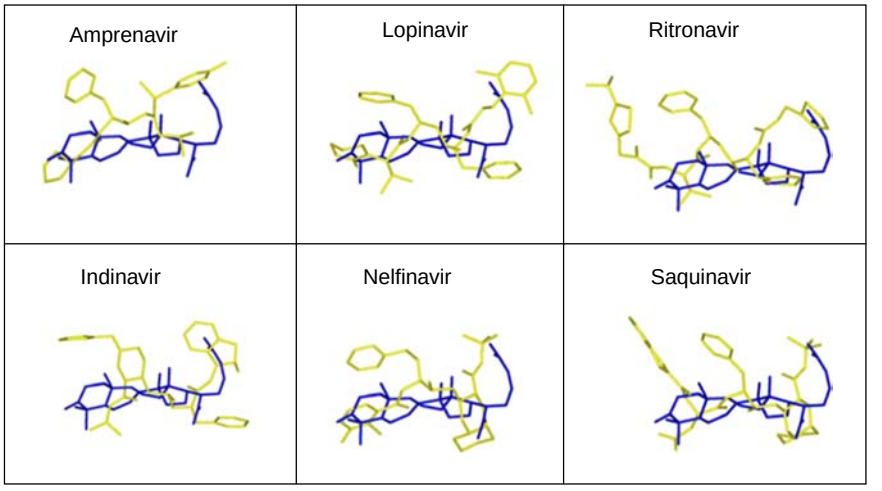


Figure 6. Superimposition of all atoms between 3-oxotirucalla-7,24-dien-21-oic acid and six commercial drugs in enzyme–inhibitor complexes.

of HIV-1PR. The preferable negative free energy of the HIV-1PR flap region interacting with saquinavir P1 was reported by Wittayanarakul et al. [20]. This evidence indicates that the activity of triterpene will be improved by the addition of P1 subsite.

## 4. Conclusions

In this study, the binding of HIV-1 protease and *L. chinensis* extracts (3-oxotirucalla-7,24-dien-21-oic acid) was investigated. MD simulations of HIV-1 protease complex with 3-oxotirucalla-7,24-dien-21-oic acid in water were performed. The initial structure of the enzyme–inhibitor complex was constructed based on docking the 3-oxotirucalla-7,24-dien-21-oic acid structure, optimised by semi-empirical calculation, AM1, with the X-ray crystallographic HIV-1 protease structure (PDB code: 1HXB). The MD calculation results predict the hydrogen bond being formed between the oxygen atoms of the inhibitor and catalytic aspartates, which is common to protease–inhibitor complexes. However, there is no hydrogen bonding of the inhibitor to the flap region. Structural parameters were investigated in order to compare the complex structures in all three systems throughout the MD trajectory. In addition, radial distribution function of water oxygens around the catalytic

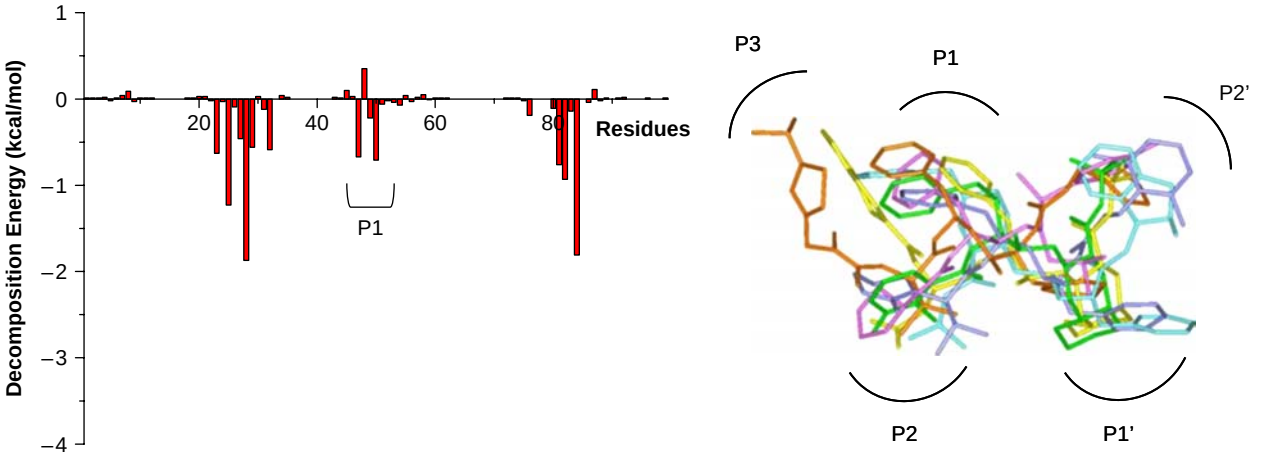


Figure 7. Subsites P1, P1′, P2, P2′ and P3 shown in the superimposition of six commercial drugs.

carboxylate nitrogens of Asp29 and Asp30 suggested that at least one or two water molecules will be in the active site region whereas a direct bound of the inhibitor to the catalytic carboxylate oxygen of ASP25 was found. Our simulation results compared with HIV–six commercial drugs complexes indicate the anti-HIV activity of 3-oxotrirucalla-7,24-dien-21-oic acid will be improved by addition of P1 subsite.

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