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Full Length Research Paper

Computational design of disulfide cyclic peptide as potential inhibitor of complex NS2B-NS3 dengue virus protease

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Development of genomic and proteomic studies coupled with computational sciences could facilitate the discovery of various target proteins and potential inhibitor to be developed as drugs. Several researches by molecular docking method have been conducted to design disulfide cyclic peptide ligand as potential inhibitors for NS2B-NS3 protease (NS2B-NS3 pro) of dengue virus serotype DENV-2 in order to inhibit replication of dengue virus. This research studied and evaluated the interaction of ligands and the enzyme in the hydrate state using molecular dynamics simulations at two different temperatures. Simulations were performed using two disulfide cyclic peptide inhibitors KRK and RKR, along with one linear peptide Bz-Nle-K-R-R-H as standard ligand. The result shows that dynamic movement of three proposed ligand in hydrated state affects ligand interactions. RKR ligand has the best affinity with the enzyme than KRK and standard ligand. This is shown by the ligand interaction with enzyme active site which remains stable during the simulation. At the end of simulation 300 K, RKR formed a hydrogen bond with Asp75 and at the end of simulation 312 K, RKR also maintained hydrogen bond with Asp75

Key words: Dengue virus (DENV), serine protease NS2B and NS3, molecular docking, molecular dynamics.

INTRODUCTION

The development of genomic study is very helpful in the process of designing new drugs. Human genome did not turn out to offer a direct source for drug development, but the proteins which they encode are the usual targets of drugs. Today, the field of drug development might seem more fertile than ever before, with vast amounts of information from genomic and proteomic studies facilitating the finding of new targets of drug design (Alonso et al., 2006).

Dengue virus belongs to genus Flavivirus (family *Flaviviridae*), which has four antigenically distinct dengue virus serotypes; DENV-1, DENV-2, DENV-3 and DENV-4 (Lescar et al., 2008). Currently, there is no available treatment for a flavivirus infection. No vaccines against DENV effective to cure the dengue disease have reached the market yet, despite several decades of intensive efforts. The main issue is the inability of vaccines to protect simultaneously against all four antigenically distinct serotypes. There is need therefore for antiviral compounds that are able to halt flaviviral infections and critical in light of the significant worldwide mortality and morbidity because of flavivirus infection (Geiss et al., 2009).

Dengue virus consists of seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Yap et al., 2007). These nonstructural proteins are responsible for replicating the viral genome and altering the host cell environment such that replication is efficient and that the host innate immune response does not interfere with replication (Khan et al., 2008). The NS3 viral protease is a potential target for antiviral drugs since it is required for virus replication (Tomlinson et al., 2009). For NS3protease to be active, it must be in a complex with its cofactor NS2B. This protease (NS2B/NS3Pro) plays an essential role in the cleavage of the viral precursor polyprotein and disruption of this function is lethal to viral replication (Geiss et al., 2009). The NS3 protease has three catalytic triad residues; His51, Asp75 and Ser135

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(Brinkworth et al., 1999).

Yin et al. (2005) had developed a potential and selective NS2B-NS3 pro inhibitor based on evaluation of the linear tetra peptide aldehyde sequence synthesis; Bz-Nle-Lys-Arg-Arg-H was the best inhibitor and active in NS3 protease of dengue virus. Tambunan et al. (2010) had conducted molecular docking method and designed seven disulfide cyclic peptide ligands which are potential to inhibit NS2B-NS3 pro dengue virus and obtained KRK as the best ligand. In molecular docking method, enzyme was set in a rigid state, while ligand in a flexible condition. But actually, biomolecules such as proteins is in a dynamic system. Solvent is included in sthe ystem, which will trigger dynamic behavior of enzyme-ligand complex. Computational methods that could provide flexible state of the enzyme and ligand and consider the influence of solvent are molecular dynamics simulation method.

The aim of this research was to study the interaction of three potential inhibitors with NS3-NS2B protease of dengue virus in a solute condition on different time scale and temperatures by molecular dynamics simulation and to evaluate the interaction of three proposed ligand in a solute condition.

MATERIALS AND METHODS

Ligands design and preparation

Determination of amino acid sequence was based on natural substrate NS3-NS2B protease that is recognized by the active site. Amino acids that were used are arginine, lysine, glycine, serine, alanine, and threonine. This study used peptides with amino acid sequence Bz-Nle-CKRRC KRRH as comparison standard. Peptides were designed as cyclic peptides, which are joined by disulfide bonding at each terminal cysteine and modeled into three-dimensional structure using ACDlabs. Peptide optimization was carried out by choosing wash option, partial charge and energy minimization using MMFF94 force field, gas phase solvation and RMS gradient 0.001 kcal / Å mol (Manavalan et al., 2010).

NS2B-NS3 protease preparation

Three-dimensional structure of DENV-2 NS2B-NS3 protease with code2FOM was obtained from PDB and was then loaded into Molecular Operating Environment (MOE) 2008.10. Geometry optimization and energy minimization process of the three dimensional structure of NS2B-NS3 protease was proceeded by removing water molecules and chlorine ion. Enzyme structure was first repaired and properly protonated using the Protonate3D option in MOE (Feher et al., 2009). Hydrogen atoms in the enzyme structure were added by choosing partial charge option, with hydrogen partial fix and regulation load of enzymes. Energy minimization was performed by employing MMFF94x force field, gas phase solvation and RMS gradient 0.05 kcal / Å mol (Manavalan et al., 2010).

Molecular docking

Molecular docking was performed by choosing MOE-dock option. Triangle matcher was generated as placement method with the total number of rotation of 2.5×10^6. Triangle matcher method generates poses in a systematic manner and more accurate way than the alpha triangle method by aligning the ligand triplet of atoms with the triplet of alpha spheres in cavities of tight atomic packing (Feher et al., 2009). A London dG scoring function, ΔG was used to rank candidate poses. In refinement, repetition was set to 100, with only one best pose to be retained.

Molecular dynamics simulation

Molecular dynamic simulation was performed by executing MOE-dynamic option. Born solvation was used in MD simulation with RMS gradient 0.05. Complex NS2B-NS3 pro with ligand and NS2B-NS3 pro without ligand were geometry optimized and energy minimized. Energy minimization was performed using MMFF94x force field, and Nosé-Poincaré-Andersen (NPA) algorithm was employed in this simulation along with NVT ensemble. MD simulation in MOE 2008.10 has three main processes; initialization, main simulation (include with equilibration) and production.

MD simulation was performed in two different temperatures. At 300 K simulation, initialization process was performed for 50 ps, main simulation for 5 ns and after main simulation had finished, it continued with cooling stage for 20 ps until the temperature reached 1 K. At 312 K simulation; heating stage for 20 ps was needed after initialization process to increase temperature 300 to 312 K, and remaining parameters were equal to 300 K simulation. The result of both temperature, including position, velocity and acceleration were saved every 0.5 ps.

Drug scan

Drug scan analysis was carried out according to Lipinsky's Rule of Five: molecular weight of about 500 g / mol, value of log P < 5, H donor < 5, and H acceptor < 10.

RESULTS AND DISCUSSION

Molecular docking analysis

Docking process was performed using 49-candidate ligands, seven comparator ligands and a standard ligand against NS3-NS2B protease enzyme. Ligands were arranged to interact only with the selected enzyme active site residues, His51, Asp75 and Ser135. Placement method that was used was the triangle matcher, which indicates random movement of ligands to produce optimal binding orientation (Cook, et al., 2009). Binding free energy values ($\Delta G_{binding}$) was quantified by KA biological activity constant with the assumption of thermodynamic equilibrium conditions for the formation of protein-ligand complex [EI] (Kitchen, et al., 2004). The relationship between the value of bond energy (ΔG^o) with KA and [EI] is directly proportional. $\Delta G_{binding}$ relatively small or negative indicates that the ligand conformations formed are in the most stable conformation.

Our data showed that there were two ligand candidates who have the relatively small binding free energy (RKR and ARR), compared to standard ligand and ligand CRA. Ligand RKR $\Delta G_{binding}$ value was 22.6955 kcal / mol and ligand ARR $\Delta G_{binding}$ value was 21.3025 kcal / mol, while

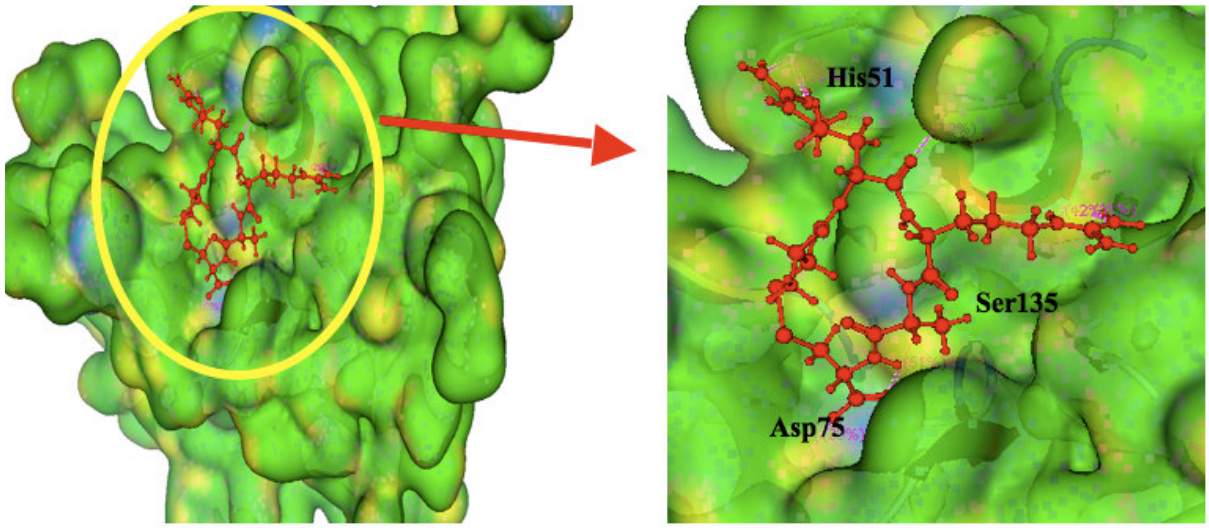


Figure 1. Hydrogen bond between RKR ligand and enzyme.

the standard ligand had $\Delta G_{binding}$ value of 20.4984 kcal / mol and CRA ligand value of 20.6022 kcal / mol. Based on the thermodynamic functions described earlier, relationship between $\Delta G_{binding}$ with constant activity of KA and [EI] was directly proportional. Ligand RKR and ARR could form better enzyme-inhibitor complex conformation compared to standard ligand and CRA. Hydrogen bonding that occurs in enzyme-ligand complexes were also identified and analyzed.

The CRA and ARR ligands formed more hydrogen bonds with target enzyme compared to standard ligand and ligand RKR. However, CRA only form hydrogen bond with His51 at the active site of enzyme. As for RKR, hydrogen bonds with His51 and Asp75 at the active site occurred. ARR formed hydrogen bonds with Asp75 and Ser135, and the standard ligand also formed hydrogen bonds with His51 and Ser135 (Figure 1). This hydrogen bonding contributes to ligand-enzyme affinity due to electrostatic interaction between oxygen atoms or nitrogen atoms of ligands with hydrogen atoms of amino acid residues of the enzyme or vice versa.

RKR ligands formed hydrogen bonds with the enzyme active site residues, His51 and Asp75. From its conformation, RKR were fit in the area of enzyme binding sites. Therefore, ligand RKR was proposed as a potential competitive inhibitor because it can bind to the enzyme binding sites and disrupt the activity of the active site of the NS3-NS2B protease enzyme. Non-covalent inter-actions that occur between enzymes and ligands can increase ligand-enzyme affinity. Contact residues of the enzyme-ligand complex docking results were identified and visualized. From our results, ligand RKR and ARR interacted with Tyr residues, both Tyr161 and Tyr150 while CRA did not have this interaction. Phi (Π) electrons in aromatic Tyr150 and Tyr161 might interact with arginine residues of RKR and ARR who has conjugated π bond to form π-π interaction.

Comparison with KRK

Another molecular docking was then performed to compare RKR with previously proposed KRK ligand (Tambunan et al., 2010). Results show that after been superimposed, RKR had a better conformation compared to KRK. Figure 2 shows that RKR (red) were located closer to the enzyme active site compared to KRK (yellow). RKR also formed hydrogen bonds with residues His51 and Asp75 in a closer distance compared to KRK. Ligand RKR was also better than standard ligand (Figure 3). RKR has lower binding energy than standard and KRK ligand (Table 1). Interaction between ligands and enzyme can be seen in Figure 4.

Molecular dynamics analysis

Dynamic behavior of enzyme and enzyme-ligand complex in hydrated state were observed in initializing process for 6 frames, heating stage for 3 frames, cooling stage for 5 frames, generated at each 10 ps of MD simulation. Main simulation process was at 300 K for 11 frames, at 312 K for 12 frames, generated at each 500 ps. At 300 K, total of 22 frames for each ligand was obtained, and at 312 K, 26 frames for each ligand were obtained includes the heating stage.

Born solvation that were used in molecular dynamics simulation means that the solvent was included during the simulation, so that E_{sol} is also calculated in the system. The position, velocity and acceleration were saved every 0.5 ps. Simulation at 300 K and 101 kPa was performed to examine enzyme-ligand complex

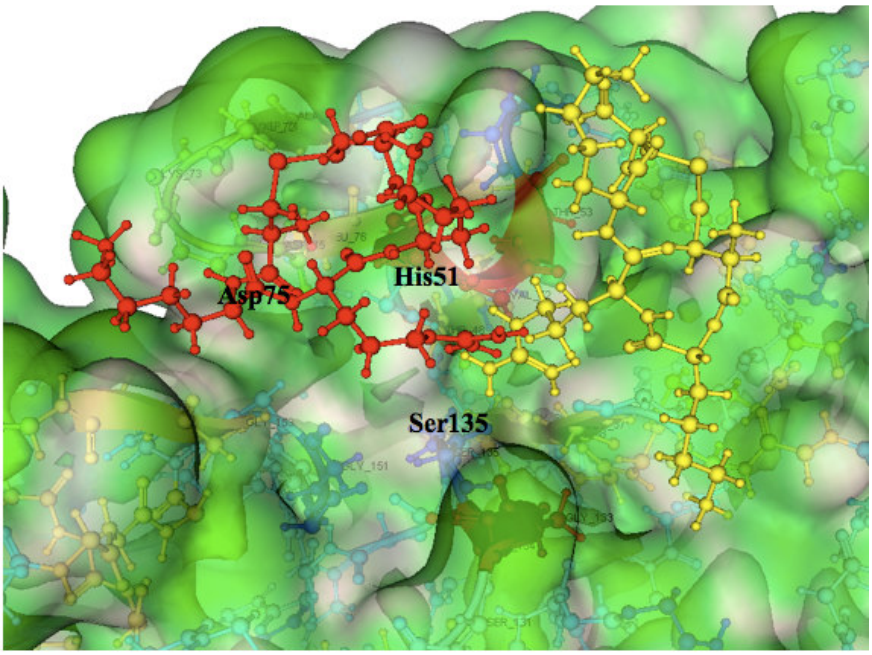


Figure 2. Superimpose result of RKR ligand (red) with KRK Ligand (yellow)

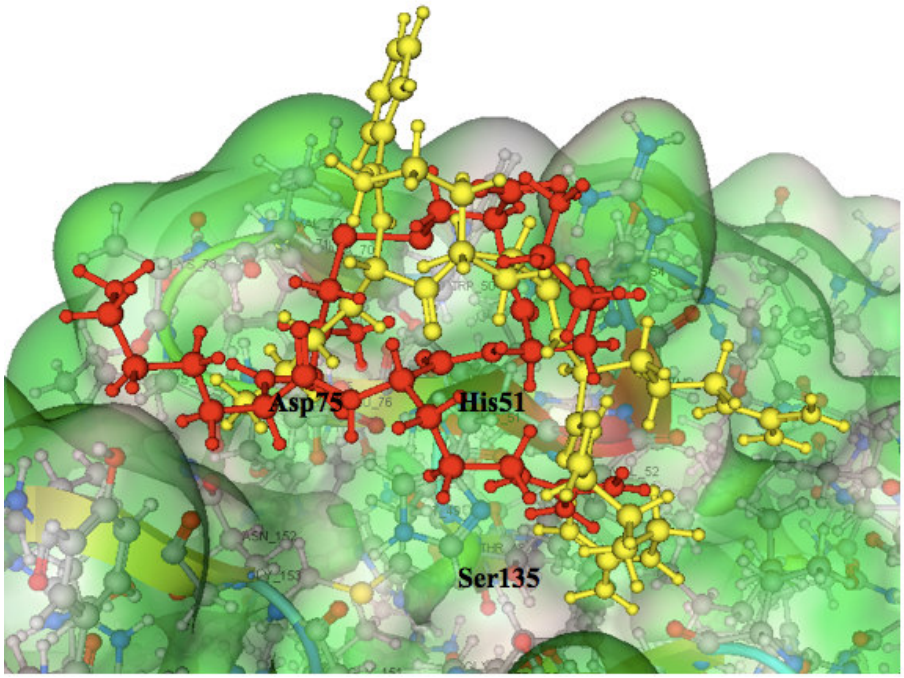


Figure 3. Superimpose result of RKR ligand (red) with standard ligand (yellow)

Table 1. RKR, KRK and Standard ligand binding energy.

Ligand	$\Delta G_{binding}$ (Kcal/mol)
RKR	-21.0946
KRK	-19.9133
Standard	-19.4025

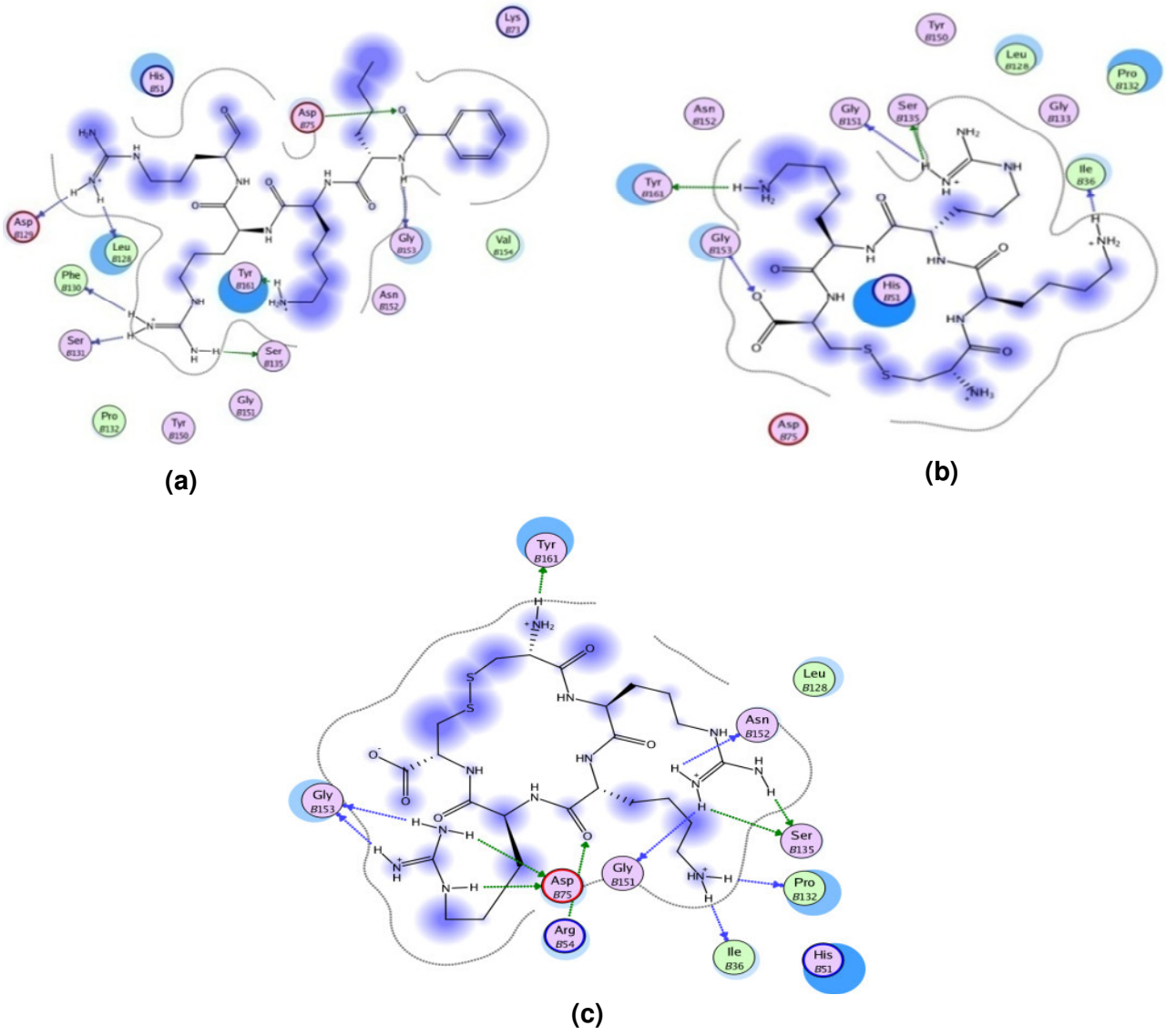


Figure 4. a) KRK; (b) RKR; (c) Standard ligand (Bz-Nle-K-R-R-H).

model in dynamic processes. The stability of enzyme-ligand complex can be observed from the position of RKR (red ball and stick). It can be seen that there were changes in enzyme-ligand complex structures at various stages of time (0, 25, 50, 75 and 100ps). Conformation of the ligand-enzyme was stable during the molecular dynamics shown by the position of ligand (0, 25, 50, 75 and 100 ps), which remained close to the active site and also indicated by the interaction between ligand and active site residues (Figures 5 to 7). Interaction with contact residues that occurred during the simulation was different in each time range and movement. Data showed that at least 50 ps until 75 ps were needed to optimize ligand-enzyme complex, so interaction with 11 amino acid residues occurred. This showed the time between 50 ps and 75 ps was the time when the conformation of the ligand-enzyme achieved the best circumstances.

Ligand-enzyme interaction

After initialization process at 300 K, standard ligand could still interact with one active site residue of the enzyme; Ser135. But at 500 ps until the end of the simulation, the standard ligand showed no interaction with the active site residues of the enzyme. For KRK, interaction with active site residues of the enzyme did not occurred after 4000 ps of simulation. As for RKR, interactions with enzyme active site were still maintained along the simulation. At the end of simulation, RKR formed hydrogen bonds with Asp75 (Figure 8). Throughout the simulation, RKR and KRK showed interactions with Asp129 of enzyme, but the

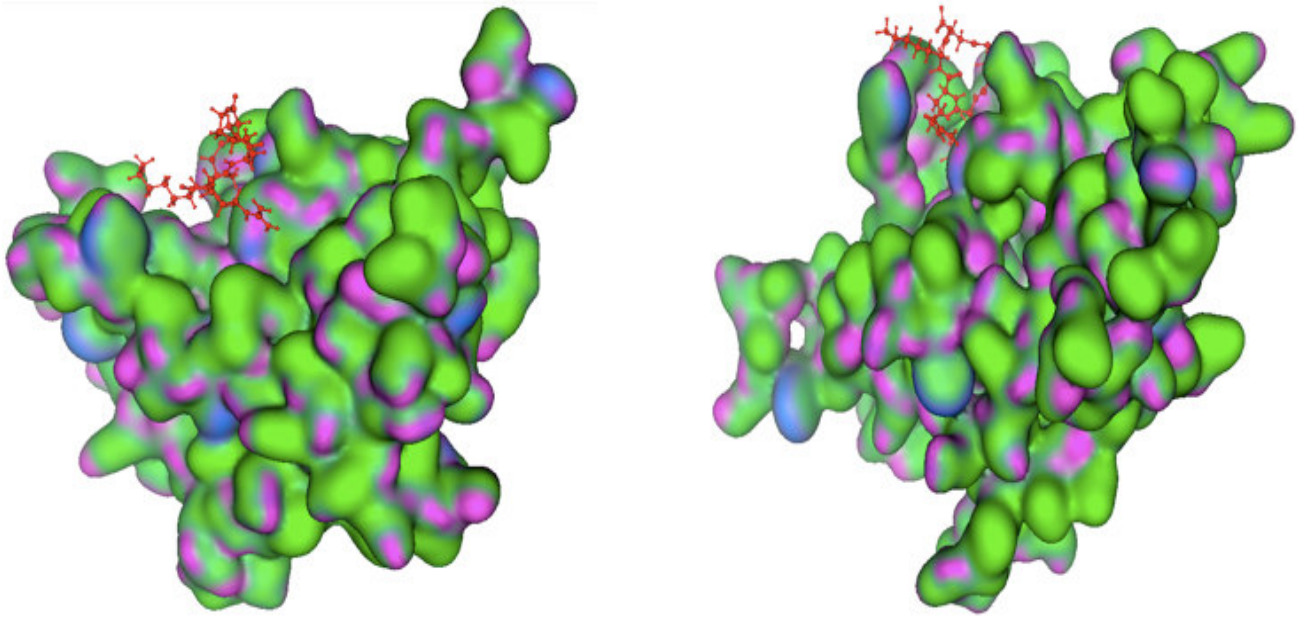


Figure 5. Protein-ligand conformation 0 and 25 ps.

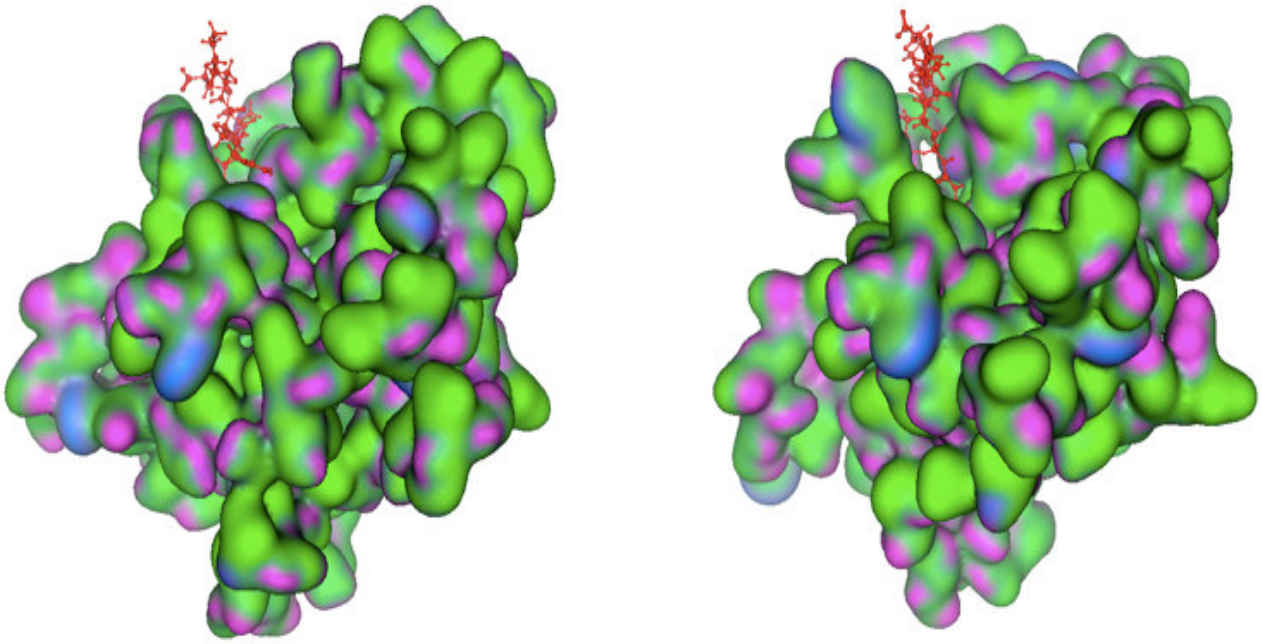


Figure 6. Protein-ligand conformation 50 and 75 ps.

standard ligand had no interaction with Asp129 during simulation. Asp129 of NS3 protease has an important role in stabilizing the base amino acids such as arginine or lysine at an inhibitor (Li et al., 2005).

More also, the heating stage at 312 K influenced ligand-enzyme interaction greatly if compared with simulation at 300 K. After the heating stage, the standard and KRK lost interaction with active site. Standard ligand lost interaction with Asp75 and His51, while KRK lost interaction with Ser135. But RKR could still maintain

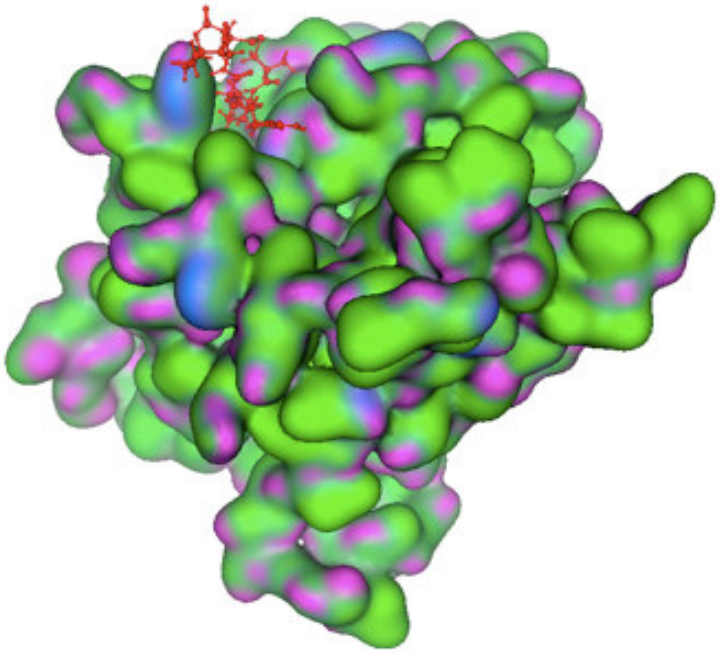


Figure 7. Protein-ligand conformation 100 ps.

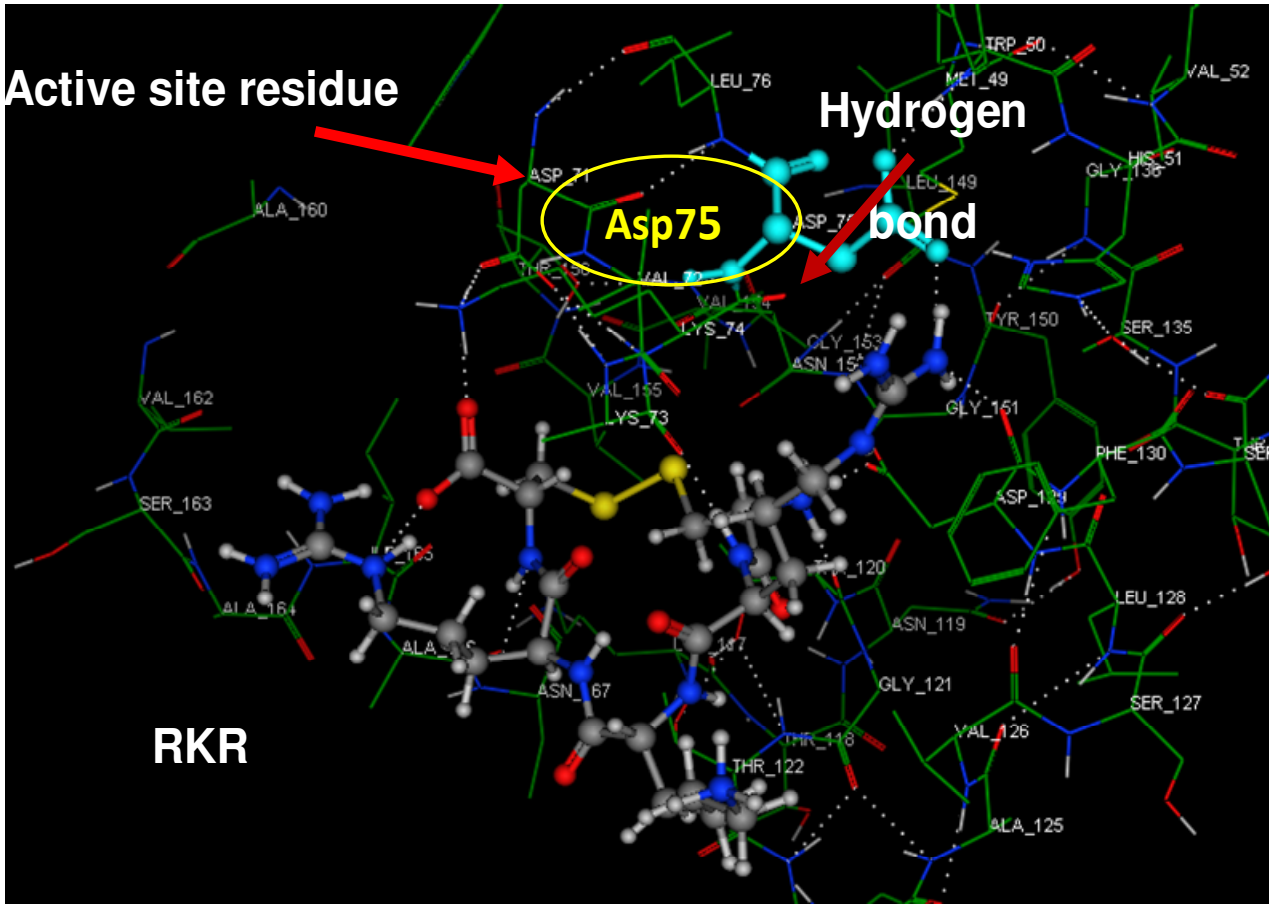


Figure 8. RKR-enzyme interaction after molecular dynamics at 300 K.

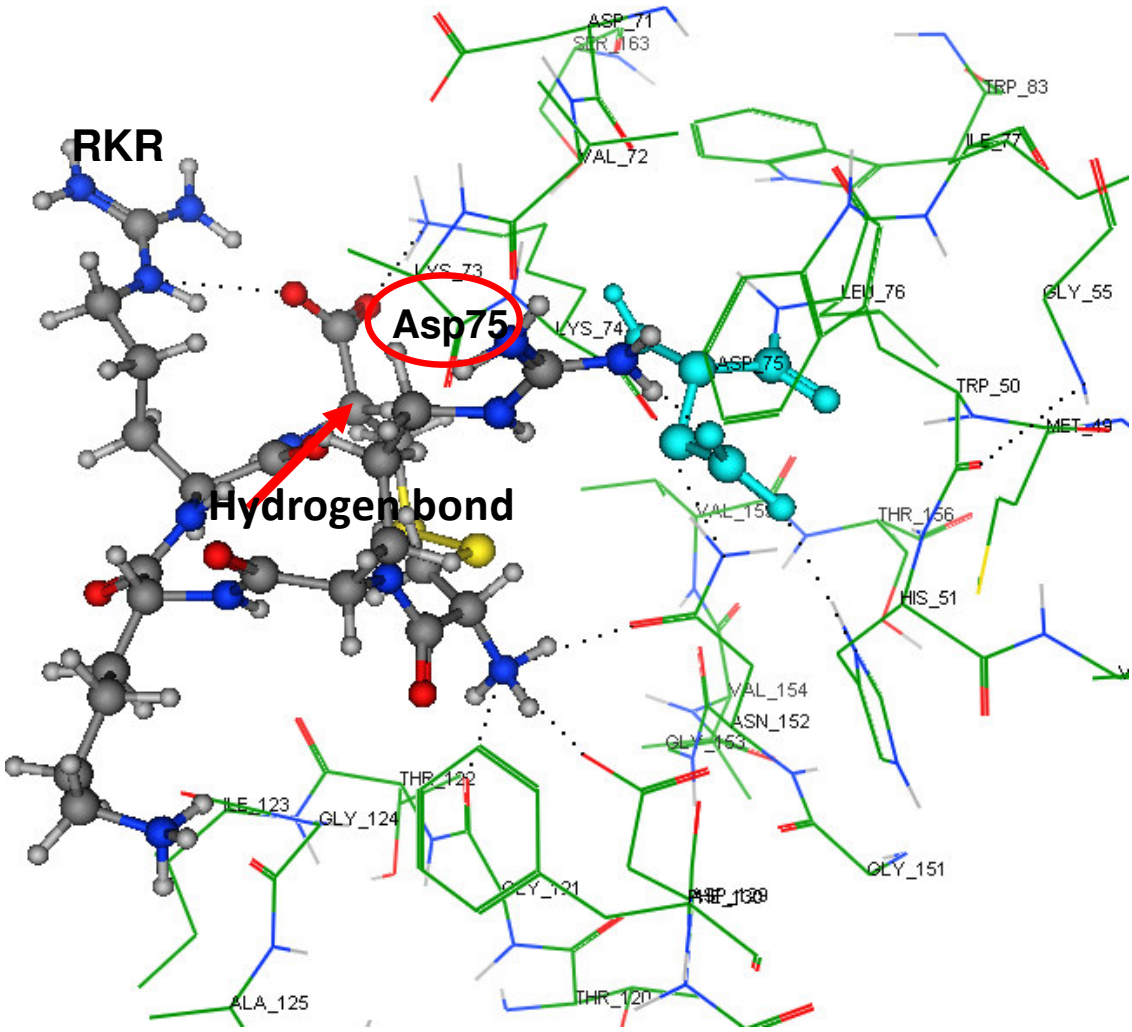


Figure 9. RKR-enzyme interaction after molecular dynamics at 312 K.

stable hydrogen bonds with Asp75 from the beginning until the end of simulation (Figure 9). Ligand interaction at the end of 300 K and 312 K simulation is shown in Figure 10.

Conformation

Displacement of ligand positions that occurred on ligand conformation during the simulation showed that ligands moved away from the binding site area. KRK had slightly displacement position of conformation from the binding site area and could still maintain several interaction with the binding site; Asp129, Asn152, Gly133 and Tyr150, but KRK could not interact with the active site residues of the enzyme. Dynamic movement in the hydrated state of enzyme-RKR complex did not influence the position of RKR at the binding site area. Structure of the enzyme and enzyme-ligand remained stable. Each constituent amino acid residues had distinct movement in solvent and the presence of inhibitor had different effect, and therefore generated different conformation.

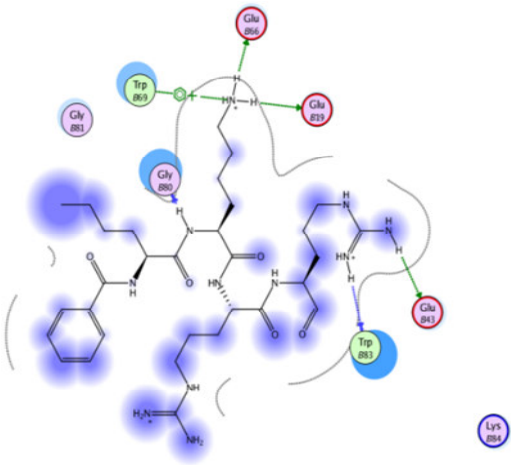
Drug scan analysis

Log P is the partition coefficient defined as the ratio of the concentration of a molecule in octanol and water. Log P describes the hydrophobicity of drug molecules. Drug molecule should not be hydrophobic or hydrophilic due to lining of lipid bilayer. Hydrophobic drug molecules tend to have greater toxicity and more widely distributed in the body. Only RKR ligand fulfilled the criteria according to the Lipinsky rules of good medicine. RKR showed Log P value = -8.05, four H donors and two H acceptor.

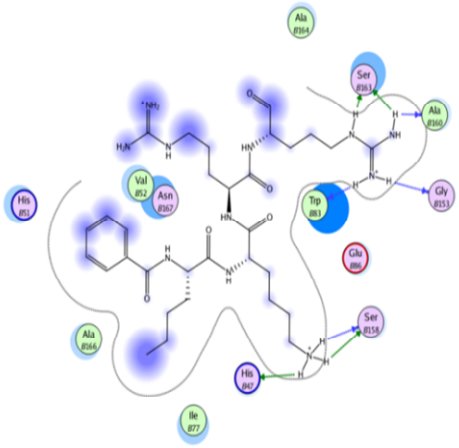
Conclusion

Dynamic movements of three enzymes-ligand complex in

Standard ligand (Bz-Nle-K-R-R-H)

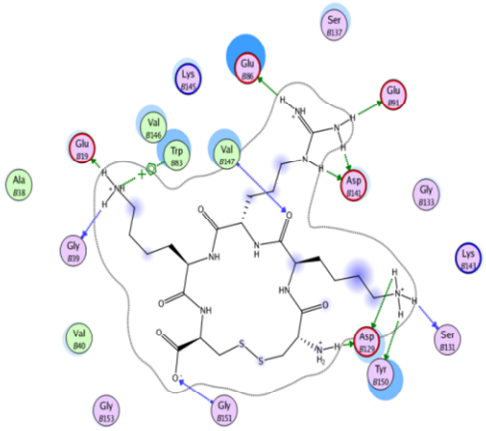


(a) 300 K

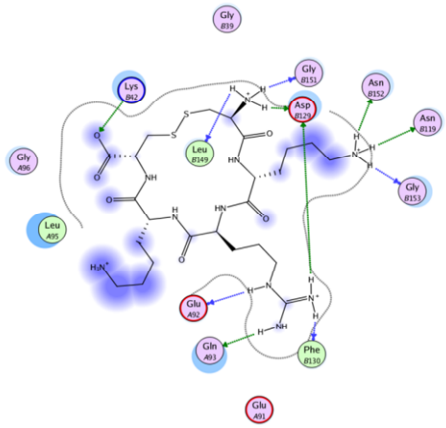


(b) 312 K

KRK

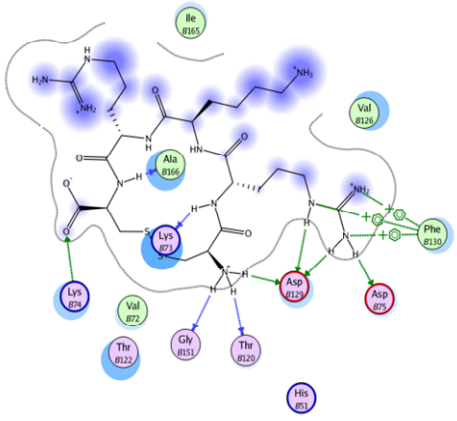


(d) 312 K

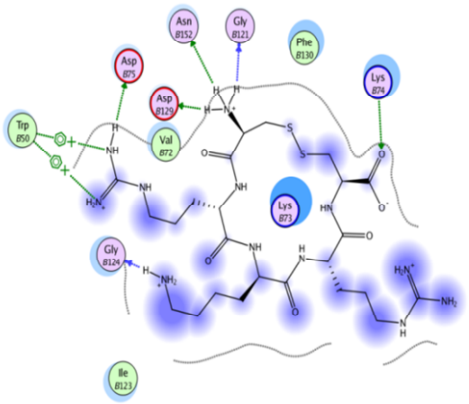


(c) 300 K

RKR



(e) 300 K



(f) 312 K

Figure 10. (a) Standard (Bz-Nle-K-R-R-H) at 300 K; (b) standard (Bz-Nle-K-R-R-H) at 312K; (c) KRK at 300K; (d) KRK at 312 K; (e) RKR at 300K; (f) RKR at 312 K.

the hydrated state at two different temperature conditions showed different effect on ligand interaction with NS2B-NS3 pro dengue virus type 2. Interaction changes occurred in residue contacts and hydrogen bonds between ligand and amino acids residue of the enzyme. Docking results indicate that three ligands could interact with enzyme active site residues. MD simulation showed that only RKR interacted with the active site of the enzyme during simulation. At the end simulation at 300 K, RKR formed hydrogen bond with Asp75, while at the end simulation at 312 K, RKR maintained hydrogen bond with Asp75. RKR conformation was suitable to fit in the binding site of enzyme during the simulation. Ligand-enzyme complex conformation remained stable during simulation, as well as the conformation of the enzyme without ligand. Based on the analysis of ligand interactions and conformational compatibility amongst three proposed ligands from this study, RKR showed potential to bind the active enzyme and had the best affinity to the enzyme. Thus, we proposed RKR as potential inhibitor of NS2B-NS3 pro dengue virus type 2.

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