Rational design of cyclic tetra and pentapeptides as therapeutic agents for dengue NS2B/NS3 protease using structure-based molecular docking (MOE and AutoDock 4.2)

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ABSTRACT

Dengue virus infection is one of the health problems in tropical and subtropical countries. Although this disease is common, unfortunately, until now no licensed vaccine or relevant drugs available in the market. The first objective of this study is to design potent and selective peptidic inhibitors by studying the interactions between the designed peptides and the dengue NS2B/NS3 protease using computational docking technique and secondly to compare the quantitative and qualitative docking results using two independent docking programs (MOE and AutoDock 4.2). The proposed peptides were designed based on literature reviews and previous findings on the interaction between dengue NS2B/NS3 protease and reported peptides, thus, we designed ten cyclic tetrapeptides and twenty cyclic pentapeptides. The reported 3D structure of Wichapong and co-workers on the dengue NS2B/NS3 protease homology model was used in this study. The designed peptides were docked using MOE and AutoDock 4.2 softwares targeting dengue NS2B/NS3 protease. The results demonstrated that most of the proposed peptides were connected to the protease binding pocket and made interactions with the protease catalytic triad residues (His51, Asp75 and Ser135). Based on quantitative and qualitative docking results from the two docking programs, it showed that two cyclic tetrapeptides (1-C4 and 4-C4) and four cyclic pentapeptides (4-C5, 16-C5, 20-C5, and 6-C5) are the best potential inhibitors with the lowest free energy of binding and the high number of interactions with protease. In conclusion, the two independent docking programs could give almost the same results based on its quantitative and qualitative docking results. Thus, these potential peptides could serve as promising inhibitors for dengue virus. These findings will be further continued for the synthesis of these cyclic peptides and in vitro biological assays to confirm their activity.

INTRODUCTION

Dengue is caused by a mosquito-borne dengue virus (DENV) with approximately 390 million dengue cases have been reported every year, with 96 million of them are clinically reported (Bhatt et al., 2013). Currently there are five serotypes of dengue virus (DENV1-5) and the disease symptoms show a similar pattern of disease manifestations (Mustafa et al., 2015). DENV infection can be symptomless or lead to a multitude of clinical manifestations span-
ning from mild dengue fever (DF) to serious diseases such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). The dengue virus is closely related to the West Nile virus, yellow fever virus, and also the hepatitis C virus and is a member of the Flaviviridae family (Steuer et al., 2011). The dengue virion itself is a spherical with surface proteins, the envelope (E) and precursor (prM)/membrane (M), and capsid (C) proteins located in the lipid bilayer, which encapsulates the genome of the virus (Rossmann et al., 2002). The DENV genome encodes three structural proteins (C, prM/M and E) that are taken after seven non-structural (NS) proteins (NS1, NS2A, 2B, NS3, NS4A, 4B and NS5). Structural proteins constitute the viral particle, while non-structural proteins participate in immune system replication (Lindenbach and Rice, 2003).

Peptides are considered to be a rapidly established field of drug discovery for beneficial antiviral medicines. Peptides have low toxicity and tissue accumulation and suggested to be potential for dengue inhibitor drug development (Uhlig et al., 2014). Apparently, viral proteases are great inhibitory targets. Protease inhibition, for example, has been demonstrated as a successful HIV infection treatment strategy (Clercq, 2009). The proteins NS5, NS3 and NS2B (co-factor) play an essential role in DENV enzyme activity, thus become the potential antiviral targets (Egloff, 2002). The full-length NS3 is a wide, multifunctional protein, one-third being a classic serine catalytic triad (His51, Asp75, and Ser135), which is like a trypsin protease. The NS2B contains 40 amino acid domain function as a cofactor which has caused NS3 protease enzyme activity (Falgout et al., 1993). NS2B/NS3 protease enzymes are the primary targets for the development of antiviral drugs because they are required for viral replication (Ismail and Jusoh, 2017). Therefore, in this study, we used dengue NS2B/NS3 protease as our target to study the interactions of our proposed peptides.

The relevant x-ray structure of West Nile virus NS2B/NS3 protease in a complex with benzoylnorleucine-lysine-arginine-arginine-aldehyde (Bz-Nle-Lys-Arg-Arg-H) inhibitor (1.68Å; PDB ID: 2FP7) has reported by Erbel and co-workers (Erbel et al., 2006). In another study, a crystal structure of the DENV NS2B/NS3 protease has also been published; however, they reported in an apo-form (1.5Å; PDB ID: 2FOM). Based on the experimental data available, it has been suggested that the effective inhibitor of DENV NS2B/NS3 protease should be closed to the WNV protease. The sequence identity between WNV and DENV NS2B/NS3 demonstrated a high similarity between these two structures (Erbel et al., 2006).

A decade ago, Wichapong and co-workers developed several homology models of DENV NS2B/NS3 protease complexed with a peptide inhibitor, Bz-Nle-Lys-Arg-Arg-H. The first DENV protease model (DENV-1) was made from the available apo DENV NS2B/NS3 protease crystal structure, while the second model (DENV-2) was constructed with the WNV NS3/NS2B protease as a template structure in the inhibitor complex form. They found that the DENV-2 model provided results that were well agreed with experimental data and this model represent the DENV NS2B/NS3 in the productive conformation and can be used for structural design purposes.

The peptide ligands in this study were designed and identified based on dengue NS2B/NS3 protease specificity to the substrate and a binding pocket analysis of the NS2B/NS3 catalytic protease site. Proteases of different serotypes seem functionally uniform based on the likeness of the target binding patterns. A strong suggested preference was established in P1 site for fundamental amino acid components (Arg/Lys), whereas the criteria for P2-4 sites were in the order of (Arg > Thr > Gln/Asn/Lys) for P2, (Lys > Arg > Asn) for P3 and (Nle > Leu > Lys > Xaa) for P4 position. Small and polar amino acids in P1’ and P3’ are the primary substrate specificity. In comparison, there was little or no activity on the P2’ and P4’ roles (Li et al., 2005). The proposed of P1-P4 positions of the amino acids by Jun Li and co-workers have been used by Tambunan and Alamudi (Tambunan and Alamudi, 2010), (Usman et al., 2013) as well as by Sobia I., Usman A. Ashfaq (Idrees and Ashfaq, 2014) in the designing of cyclic peptide through disulfide linkage as dengue virus NS2B/NS3 protease inhibitor. The biological availability and metabolic stability challenges must be addressed when designing linear peptides as an inhibitor. These problems were solved through the design and production of cyclic peptides with significant advantages of these peptides through a better permeability and good metabolic stability over linear peptides (Horton et al., 2002). Although the peptides have low stability, their high activity and specificity make peptides are ideal for the design of drug inhibitors (ligands) and the cyclization of ligands by S-S disulfide bridge could improve their stability (van Hell et al., 2009).

In another study, the substrate’s P1 and P2 subsites have been shown to play an essential role in the binding with DENV2 NS2B/NS3 protease through an analysis of molecular dynamics simulation (Yot-
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Figure 1: Our proposed cyclic tetrapeptides, C4 (A) and cyclic pentapeptides, C5 (B) based on the reported findings.

|   | A)            | B)             |
|---|---------------|----------------|
| 1 | CKRC          | CRRRC          |
| 2 | CRKC          | CKKRC          |
| 3 | CRRC          | CTRC           |
| 4 | CQRC          | CNKC           |
| 5 | CTRC          | CNRC           |
| 6 | 6-C4          | 6-C5           |
| 7 | 7-C4          | 7-C5           |
| 8 | 8-C4          | 8-C5           |
| 9 | 9-C4          | 9-C5           |
| 10| 10-C4         | 10-C5          |
| 11| CRRRC         | CGKRC          |
| 12| 2-C5          | CNKRC          |
| 13| 3-C5          | CNTKRC         |
| 14| 4-C5          | CGRKRC         |
| 15| 5-C5          | CRRKRC         |
| 16| 11-C5         | CTRKRC         |
| 17| 12-C5         | CGKKC          |
| 18| 13-C5         | CKKKC          |
| 19| 14-C5         | CNKKC          |
| 20| 15-C5         | CNNRC          |
|   | 16-C5         | CRTKRC         |
|   | 17-C5         | CNNKC          |
|   | 18-C5         | CKTRC          |
|   | 19-C5         |                 |
|   | 20-C5         |                 |

Figure 2: An overview of the control ligand before docking (yellow), its docked structure from MOE (red) and AutoDock 4.2 (blue) at the binding site of dengue NS2B/NS3 protease.

Figure 3: An overview of the standard ligand (yellow), its docked conformation (red) and two cyclic peptides, 1-C4 (green) and 4-C5 (black) at dengue protease using MOE.
The arginine residue was found at these two subsites and became a preferential binding at the active site with stabilization energy less than -10 kcal/mol. Furthermore, the subsites P3, P1’, P2’ and P4’ had a smaller binding contribution with an approximately around -2.00 kcal/mol. The peptide substrate from the cleavage region of the capsid shows that the highest number of hydrogen bonds formed by this peptide substrate (NRRRSAGMI) (Yotmanee et al., 2015). Thus, according to the previous findings, several cyclic tetra and pentapeptides through disulfide linkage have been proposed in this study as shown in Figure 1 (A) and (B) and were further docked against DENV NS2B/NS3 protease using two independent docking programs, Molecular Operating Environment (MOE) and AutoDock 4.2.

**MATERIALS AND METHODS**

**Preparation of peptide ligands**

The peptide ligand structures were built and converted into their respective 3D structures using PerkinElmer ChemDraw Ultra 16.0 software. Modelled cyclic peptides were added to form a disulfide bond, which produces cyclic tetra and pentapeptides by disulfide linkage at each end of cysteine.
residues. The energy of proposed peptides minimized by the same program (MM2 force field minimization). For MOE, all the designed peptides were optimized using the same platform and saved in mdb (mole2) for further docking studies, while for AutoDock, the peptides were optimized by using AutoDock 1.5.6 tools. The Gasteiger charges and rotatable number were assigned to the peptides and then saved in pdbqt format for docking. The peptide Bz-Nle-Lys-Arg-Arg-H has been chosen in this study as a control ligand due to its activity and anti-dengue strength (Yin et al., 2006). This control ligand was retrieved from the PubChem database and optimized using the same procedure as above.

**Preparation of the dengue NS2B/NS3 protease structure**

The dengue NS2B/NS3 protease homology model used for docking was downloaded from the literature of Wichapong and co-workers (Wichapong et al., 2009). The MOE software was used to optimize the protease structure geometrically. This was first done by eliminating all the water molecules, performed the protonation of the NS2B/NS3 protease, followed by the optimization of the partial charge and energy minimization. By using AutoDock, the protein was also prepared using AutoDock tools 1.5.6. All water and unwanted molecules were removed, the polar hydrogen atoms and Kollman charges were assigned to the protein structure. The optimized protein structure was saved as pdbqt format for further docking.

**Molecular docking by MOE software**

The docking score was used to determine the ligand-receptor binding affinity and the refinement to the corresponding force field of the ligand-receptor complex. With a help of the docking algorithm of MOE software, the proposed peptides were docked onto the catalytic triad of dengue protease which consists of His51, Asp75, and Ser135 as well as with other close contact residues compared to the control ligand. The docking parameters were set as published by (Idrees and Ashfaq, 2014) with some mod-

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Figure 6: The docked conformations of 1-C4 (A) and 4-C5 (B) using AutoDock 4.2. Left: 2D interaction using LigPlot+. Right: 3D visualization using BIOVIA Discovery Studio.
Table 1: The S score and the interaction of best proposed cyclic tetra and pentapeptides with NS2B/NS3 protease using MOE program.

| Cyclic peptide | S score (kcal/mol) | Hydrogen bond interaction | Close contact residues |
|----------------|-------------------|---------------------------|-----------------------|
| 1-C4*          | -17.41            | His51, Asp75, Gly82, Ser135, Asn152 | Asp129, Ser131, Pro132, Val155, Tyr161 |
| 4-C4*          | -15.08            | His51, Asp75, Asp129, Phe130, Tyr150 | Ser131, Ser135, Pro132, Val155, Tyr161 |
| 4-C5 *         | -17.82            | His51, Asp75, Ser83, Ser135 | Asp81, Gly82, Tyr150, Gly151, Asn152, Tyr161 |
| 11-C5          | -17.75            | His51, Asp75, Met84, Ser135, Asn152, Gly153 | Ile36, Val52, Arg54, Val72, Asp81, Gly82, Ser83, Pro132, Gly151, Tyr161 |
| 16-C5 *        | -17.74            | Asp75, Gly82, Ser135, Gly151 | His51, Phe130, Ser131, Pro132, Asn152, Gly153, Val154, Val155, Tyr161 |
| 20-C5*         | -17.70            | His51, Asp75, Gly82, Ser83, Met84, Ser135, Phe130, Tyr150, Asn152, Tyr161 | Ile86, Asp129, Ser131, Pro132, Gly151, Gly153, Val154, Val155 |
| 6-C5*          | -17.13            | His51, Asp75, Thr134 | Asp129, Phe130, Ser135, Tyr150, Gly151, Tyr161 |

*indicate the potential peptides that have been observed from two independent docking programs.

ifications. The MOE docking program provides the correct conformation of the ligand to obtain a minimum energy structure. In this study, the confirmation of every peptide was chosen based on the minimum S score and further evaluated and analyzed their hydrogen bonding, van der Waals and hydrophobic interactions with dengue NS2B/NS3 protease.

Molecular docking by AutoDock 4.2 software

The molecular docking of the proposed peptides ligands with dengue NS2B/NS3 protease were performed using AutoDock 4.2 software. A grid box with a dimension of 60 x 60 x 60 points and 0.375Å of grid spacing were used to cover the entire enzyme binding site in order to accommodate ligands to move freely. All calculation for docking was done using Lamarckian Genetic Algorithm (LGA) method and the parameters were set as default for 100 search runs. Following the completion of the docking search, clustering histogram analysis was performed, and the best conformation with the lowest estimated binding energy and estimated inhibition constant, Ki were selected for further analysis data (Tambunan and Alamudi, 2010). The interactions between the enzyme and ligand conformations including hydrogen bonding, van der Waals and hydrophobic interactions were evaluated.

The 2D visualization of the selected best peptides with the dengue NS2B/NS3 protease is presented using Ligplot v.2.1 program (Wallace et al., 1995). Meanwhile, the 3D visualization was prepared by using discovery studio visualizer v.17.2 (www.accelrys.com).

RESULTS AND DISCUSSION

Validation of the standard ligand

Figure 2 shows the docked conformations of the control ligand, Bz-Nle-Lys-Arg-Arg-H from both MOE and AutoDock 4.2 programs bound at the same binding region of the protease as its conformation before docking. From the results obtained from MOE, the standard ligand has S score with a value of -15.6426 kcal/mol and formed hydrogen interactions with residues His51, Asp75, Met84, Ser135, Gly151, Gly153 as well as hydrophobic and van der Waals interactions with close contact residues Ser83, Phe130, Pro132, Tyr150, Val154, Val155 and Tyr161. On the other docking using
AutoDock 4.2, the standard ligand showed estimated free energy of binding (\(\Delta G_{\text{bind}}\)) of -5.96 kcal/mol and it showed hydrogen bond interactions with residues His51, Asp75, Gly82, Phe130, Ser135 as well as Tyr161, and also performed a network of hydrophobic and van der Waals interactions with close contact residues particularly with Met84, Asp129, Tyr150, Gly151, Gly153 and Val155. It shows that the standard ligand demonstrated and shared the same binding region and binding interactions with dengue protease, although two independent docking softwares were used.

**Criteria of choosing the best peptides**

In this study, two essential criteria have been used in choosing the potential peptide ligands after docking based on their quantitative and qualitative results. The first criterion is choosing the lowest binding free energy (\(\Delta G_{\text{bind}}\)) of the docked ligand, represented the quantitative result. After the ligand has been docked with the receptor, the ligand-binding affinity was assessed by measuring the free binding energy, thus, demonstrating the interactions between the ligand and the receptor protein in the lower score for any ligand and indicates a favourable ligand-receptor protein interaction (Kapetanovic, 2008). The choosing of potential candidate of the best peptides in this study are applicable for both MOE and AutoDock 4.2 softwares. However, it has been suggested that the predicted \(\Delta G_{\text{bind}}\) possess a typical error of \(\pm 2.00\) kcal/mol. Therefore, the estimated \(\Delta G_{\text{bind}}\) values should not be the exclusive criteria to choose the potential peptide when predicting free energy of binding (Cosconati et al., 2010). The second criterion is the binding interactions of the peptide ligand with the dengue NS2B/NS3 cat-alytic triad (His51, Asp75, and Ser135), considered as qualitative results. Since the catalytic triad residues are significant for viral replication, therefore, by targeting at this site, it could wedge the viral replication (van Hell et al., 2009). For this reason, we have chosen the best conformation based on the interaction, including hydrogen bonding between ligand with minimum two interactions especially with the catalytic triad residues. Taken together, the interaction between the dengue NS2B/NS3 protease and our proposed peptides may occur not only with hydrogen bond interaction but also with other non-covalent interactions such as van der Waals and hydrophobic interactions, which may also affect the binding affinity (Arunan et al., 2011).

Based on these criteria, all the conformations of each proposed peptides in this study have been sorted based on the lowest S score energy for MOE and free energy of binding (\(\Delta G_{\text{bind}}\)) for AutoDock. The top peptides with the minimum S score and \(\Delta G_{\text{bind}}\) have been further analyzed for protease interaction. The best proposed peptides from both docking softwares were chosen based on the lowest energy and the high number of interactions with dengue protease’s catalytic triad.

**Docking studies**

Table 1 shows the best proposed peptides with the lowest binding energy and the high number of interactions with dengue NS2B/NS3 protease from MOE docking result. Based on our criteria, two cyclic tetrapeptides (1-C4 and 4-C4) and five cyclic pentapeptides (4-C5, 11-C5, 16-C5, 20-C5 and 6-C5) have been identified as the potential designed peptides targeting dengue NS2B/NS3 protease. Their S score values were lower than the standard ligand except for 4-C4. The observation of the qualitative results revealed that most of the potential cyclic peptides made a network of interaction as shown by standard ligand. Among of all the potential cyclic peptides, 1-C4 and 4-C5 of cyclic tetra and pentapeptides have been chosen to overview their binding region and it showed that these two cyclic peptides bound at the same binding region as original and docked conformations of the standard ligand (Figure 3). The 2D and 3D visualization of 1-C4 and 4-C5 using MOE are shown in Figure 4 and it indicates that most of their hydrogen bond and close contact residues were almost same with the standard ligand.

For AutoDock results, the best proposed peptides having the lowest estimated \(\Delta G_{\text{bind}}\) and the high number of interactions with dengue protease catalytic triad are shown in Table 2. Based on our criteria, three cyclic tetrapeptides (1-C4, 3-C4 and 4-C4) and six cyclic pentapeptides (4-C5, 16-C5, 20-C5, 7-C5, 6-C5 and 17-C5) have been found as potential designed peptides targeting dengue NS2B/NS3 protease. It has been observed that their \(\Delta G_{\text{bind}}\) values were also lower than the standard ligand except for one cyclic pentapeptide, 17-C5. Based on the qualitative results of the binding interaction, the potential cyclic peptides from AutoDock results were also formed hydrogen bond and shared close contact residues as the standard ligand. This indicates that these proposed cyclic peptides bound at the same binding region as the standard ligand. This has been shown in Figure 5 by choosing 1-C4 and 4-C5 of cyclic tetra and pentapeptides to verify their binding region at the dengue NS2B/NS3 protease. This finding has been shown in the 2D and 3D visualization of 1-C4 and 4-C5, which also demonstrates that these potential peptides contacted with the same residues of dengue protease as shown by standard ligand (Figure 6).
Table 2: The estimated free binding energy, estimated inhibition constant and the interaction of potential cyclic peptides with dengue protease using AutoDock 4.2.

| Cyclic peptide | Estimated free energy of binding (kcal/mol) | Estimated Inhibition constant (μM) | Hydrogen bond interaction | Close contact residues |
|----------------|---------------------------------------------|-----------------------------------|---------------------------|------------------------|
| 1-C4*          | -9.57                                      | 0.10                              | His51, Asp75, Asp129, Phe130, Tyr150, Thr134, Gly151, Asn152, Gly153, Val155, Tyr161 |                        |
| 3-C4           | -8.20                                      | 1.00                              | His51, Asp75, Met84, Asp129, Gly151, Gly153, Tyr161, Phe130, Ser131, Pro132, Ser135, Tyr150 |                        |
| 4-C4*          | -8.18                                      | 1.00                              | His51, Asp75, Gly153, Tyr161 |                        |
| 4-C5*          | -6.94                                      | 7.64                              | Ile36, His51, Asp75, Asp129, Ser135, Gly151, Asn152, Gly153, Tyr161 | Pro132, Val155         |
| 16-C5*         | -6.88                                      | 9.07                              | His51, Asp75, Phe130, Pro132, Ser135, Tyr150, Gly151, Asn152, Gly153 | Ser83, Asp129, Tyr161  |
| 20-C5*         | -6.82                                      | 9.76                              | His51, Asp75, Met84, Ser135, Phe130, Gly151, Asn152, Gly153 | Ser83, Val154, Val155, Tyr161 |
| 7-C5           | -6.34                                      | 22.36                             | His51, Asp75, Asp129, Phe130, Pro132, Gly151, Gly153 | Ser135, Tyr161         |
| 6-C5*          | -6.19                                      | 28.84                             | Ile36, His51, Val52, Asp75, Thr134, Ser135, Gly151, Asn152, Phe130, Ser131, Ser135, Asn152, Gly151, Gly159 | Phe130, Ser131, Pro132, Tyr150, Gly153, Tyr161 |
| 17-C5          | -5.64                                      | 72.86                             | His51, Asp75, Asp129, Ser131, Ser135, Asn152, Gly151, Gly159 | Phe130, Pro132, Tyr150, Val155, Ala160 |

* indicate the potential peptides that have been observed from two independent docking programs.
Based on the binding affinity (quantitative) and binding interaction (qualitative) results that obtained from both MOE and AutoDock 4.2, two of our proposed cyclic tetrapeptides (1-C4 and 4-C4) and four cyclic pentapeptides (4-C5, 16-C5, 20-C5 and 6-C5) have been observed in both independent docking programs. Overall, the S score and the $\Delta G_{\text{bind}}$ values for all potential designed cyclic peptides were scored lower than the standard ligand, Bz-Nle-Lys-Arg-Arg-H, except for 4-C4 from MOE and 17-C5 from AutoDock. The lower and more negative values of the S score and $\Delta G_{\text{bind}}$ energies demonstrate a strong favourable binding between peptide ligands and dengue protease. This might be possible due to the proposed cyclic peptides contain more atoms, increasing the number of rotatable bonds and torsional free energy, thus may lowering their binding affinity (Tambunan and Alamudi, 2010). The $\Delta G_{\text{bind}}$ energies are consistent with the estimated inhibition constant, Ki values, as shown in Table 2. Generally, all the potential proposed cyclic peptides shown a calculated Ki with reasonably small values (within micromolar range), demonstrating a stable formation of enzyme-ligand complexes.

CONCLUSIONS

In this study, we designed two groups of cyclic peptides (ten tetrapeptides and twenty pentapeptides) based on the literature reviews and previous findings on the interaction between the peptide ligands and the DENV protease. The designed peptides were docked using MOE and AutoDock 4.2 softwares. We found that both docking programs produced similar results. The docked conformations of the standard ligand from both programs bound at the same binding pocket as shown by its original structure. The potential peptides have been chosen based on the lowest S score or binding free energy and the most number of interaction with the catalytic triad of dengue NS2B/NS3 protease. Based on that, it showed that two of our proposed cyclic tetrapeptides (1-C4 and 4-C4) and four cyclic pentapeptides (4-C5, 16-C5, 6-C5 and 20-C5) have been observed from both MOE and AutoDock 4.2 and could serve as promising inhibitors for DENV NS2B/NS3 protease. These findings will be further continued with the synthesis of the potential peptides and in vitro biological assay will also be performed to confirm their activity.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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