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VIRTUAL SCREENING OF COMMERCIAL CYCLIC PEPTIDES AS β - OG POCKET BINDER INHIBITOR IN DENGUE VIRUS SEROTYPE 2

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ABSTRACT: Dengue virus (DENV) has caused infectious disease which puts roughly 40% of world population at risk. An antiviral drug against DENV infection remains unavailable up until now. This research aims to find a drug candidate, which can inhibit β-OG binding site by a screening of 308 commercial cyclic peptides virtually. Through molecular docking and molecular dynamics simulation, it is discovered that cyclo (-D-Trp-Tyr) ligand has good affinity with β-OG binding pocket. Ligand forms a stable complex with envelope protein in 310 K and 312 K. Cyclo(-D-Trp-Tyr) ligand is revealed to be a potential inhibitor of β-OG binding pocket. Thus, it is feasible for further development as an antiviral drug against DENV infection.

Keywords: Dengue, β-OG pocket binder, Fusion inhibitor, Cyclic peptide, Docking

1. INTRODUCTION

Dengue virus (DENV) still becomes a major health problem worldwide. According to the World Health Organization (WHO), the exposure to DENV increases in a recent decade. Approximately more than 2.5 billion people or 40% of the world's population is at risk of DENV. Before the 1970s, only 9 countries were experiencing the dengue epidemic, but now more than 100 countries in Africa, Americas, Eastern Mediterranean, Southeast Asia and the Western Pacific are exposed to this deadly disease [1].

The efforts from the researchers and scientists are being made to prevent the transmission of DENV. One of them is focused on the eradication of Aedes sp. as DENV vector [2]. Nowadays, the research is concentrated on finding an antiviral drug candidate that able to inhibit the DENV pathways, such as replication path, the path of synthesis of RNA, viral maturation pathway, and lane fusion with host cells [3].

The peptide is an amino acid based molecule which able to inhibit the enzyme's activity with good specificity. Furthermore, it is also has a tendency of non-accumulate in the body [4]. However, the peptide can also be easily degraded in our body. To prevent the peptide degradation by protease enzymes, the peptide molecules need to be cyclized into cyclic peptide [5]. Several previous studies were conducted in an effort to inhibit β-OG pocket binders: Kampmann et al., (2009) have obtained five molecules (namely A1-A5) out of 135,000 small molecules selected. However, A1-A5 molecules need further analysis and biological assay. The results showed that A5 can inhibit the fusion process of DENV [3]. Li et al., (2008) have obtained the thiazoles-modified compounds for inhibiting the DENV envelope protein in the β-OG binding site [6]. Wang et al., (2009) have screened 586.829 compounds by using in silico approach into 111 compounds to be tested further in order to obtain six compounds that can inhibit the DENV effectively based on immunofluorescence study [7]. Finally, Zhou et al., (2008) have screened compounds of the National Cancer Institute (NCI) library and obtained the compounds that can inhibit the viral reproduction at μmol concentrations, and NMR spectroscopy proves PO_2 compound bound to the virus and may compete with the β-OG natural ligand in its binding site with greater affinity [8].

This research aims to screen commercial cyclic peptides to be used as an inhibitor of β-OG pocket binder on DENV serotype 2 based on molecular docking and dynamics simulations to obtain the novel antiviral drug. We also deployed computational ADMET test, such as health effect and toxicity prediction, in order to eliminate the remaining compounds from molecular docking simulation to be selected into molecular dynamics simulation.

2. RESEARCH METHODOLOGY

2.1 Tools and Material

This research used some online and offline softwares such as Accelrys Discovery Studio 2.5, Accelrys Discovery Studio 4.1 Visualizer [9], GROMACS 4.6.5 [10], ChemDraw Ultra 12.0, OSIRIS Property Explorer [11], ACD-iLabs [12], VegaZZ 2.4.0 and Toxtree 3.5.0 softwares [13]. DENV envelope protein sequence database could be obtained from the National Center for Biotechnology

Information (NCBI) (http://www.ncbi.nlm.nih.gov/), and for the three-dimensional structure could be searched by using SWISS-MODEL then downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB) (http://www.rcsb.org/pdb/home/home.do) in .pdb format. The structure of commercial cyclic peptide ligands can be obtained from these following chemical company databases: BaChem, Mimotopes, PolyPeptide Group, AnaSpec and the American Peptide.

2.2 Preparation of Cyclic Peptide Ligands

The cyclic peptide sequences were drawn manually by using the ChemBioDraw Ultra 14.0 with .mol file format. All ligands that have been drawn then converted into a 3D structure with VegaZZ 2.4.0 software, then imported into Accelrys Discovery Studio 2.5 software for ligand optimization in .sd format, CHARMm forcefield and the addition of partial charge MMFF94 ligands were conducted as well.

2.3 Preparation of Envelope Protein

DENV envelope protein sequence data was obtained from NCBI (National Center for Biotechnology Information) database in FASTA format, which was accessible on http://www.ncbi.nlm.nih.gov/. DENV envelope protein structure prediction was performed by homology modeling. Homology modeling step was accessed online using SWISS-MODEL server (http://swissmodel.expasy.org/).

The homology modeling results were used to obtain the 3D structure of DENV envelope protein as a template. The 3D structure of proteins could be downloaded from the PDB database by RCSB-PDB database and was stored in .pdb format. Then validated the 3D structure of proteins using RAMPAGE server for generating the Ramachandran plot (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php)[14].

Furthermore, the DENV envelope protein was prepared and optimized by eliminating the unnecessary ligand and water molecules in the protein sequence. Then, the CHARMm Force Field was applied, along with the energy minimization.

2.4 Molecular docking simulations

The whole docking process was done by using Accelrys Discovery Studio 2.5 software, with LibDock module applied in binding free energy calculation. First, we set the parameters to determine the amount of Docking 'Hotspot' and 'Tolerance', other parameters were set according to the default of Accelrys Discovery Studio 2.5 software. The results of the molecular docking simulation process were identified by the 'Calculate Binding Energy' module in Accelrys Discovery Studio 2.5 software. Finally, 2D visualization and molecular interaction of the best ligand-receptor complex from molecular docking simulation could be seen by using Accelrys Discovery Studio 4.1 Visualizer software.

2.5 Computational ADMET test

In this study, we deployed two kinds of ADMET test by using OSIRIS Property Explorer, Toxtree 3.5.0 and ACD-iLabs softwares to determine the ligand's drug-likeness, mutagenicity/carcinogenicity prediction, and the health effect prediction, respectively. These tests were conducted to eliminate the remaining ligands from the previous simulation, in order to get the best ligand, based on its pharmacological properties and drug-likeness, to be selected for molecular dynamics simulation.

2.6 Molecular dynamics simulation

Molecular dynamics simulation was utilized to look at the protein-ligand complexes stability based on the RMSD (Root Mean Square Deviation) graph. The preparation of protein-ligand complexes, such as geometry optimization and energy minimization, was required before performing molecular dynamics simulation. The molecular dynamics simulation was performed using GROMACS 4.6.5 software. The GROMOS43a1 force field and TIP3P explicit solvent models parameters were utilized while the rest of the parameters were set by default.

The molecular dynamics simulation process was performed on the best cyclic peptide ligands which have the lowest $\Delta G_{binding}$ value, the best affinity, and interaction with the target cavity, as well as having the best pharmacological properties, based on computational ADMET test. Molecular dynamics simulations were performed in 20.000 ps (20ns) twice, at 310K and 312K, respectively. The result of molecular dynamics simulation could be seen in GROMACS viewer. Protein-ligand interactions between molecular dynamics during the process could be viewed by using Accelrys Discovery Studio 4.1 Visualizer.

3. RESULTS AND DISCUSSIONS

3.1 Geometry optimization and energy minimization of DENV envelope protein

In this study, we used PDB file 1OKE as the 3D structure of DENV envelope protein [15]. This 3D structure was opened by using Accelrys Discovery Studio 2.5 software. Furthermore, this PDB contains β-OG, the natural ligand of DENV envelope protein, and it appears in dimer form. First, we prepared the structure by eliminating one envelope protein chain,

removing water, unnecessary molecules, and protonating the structure. After that, the minimization of the structure was conducted by using CHARMm forcefield. Finally, we determined the binding cavity of the DENV envelope protein by using 'Find Receptor Sites Cavity' feature in Accelrys Discovery Studio 2.5. In this study, we selected the residues that bind β-OG. The residues around the cavity are: His27, Leu45, Ile46, Lys47, Thr48, Glu49, Ala50, Lys51, Gln52, Pro53, Val130, Leu135, Glu136, Tyr137, Phe193, Leu198, Leu199, Gln200, Met201, Lys202, Asp203, Lys204, Ala205, Trp206, Leu207, Thr268, Glu269, Ile270, Gln271, Met272, Ser273, Ser274, Gly275, Asn276, Leu277, Leu278, Phe279, Thr280, and Gly281.

3.2. Molecular docking simulation

The molecular docking simulation was done by using LibDock module in Accelrys Discovery Studio 2.5. LibDock module offers a rapid process of docking with the precision of the docking's position [16]. The suitable interaction, that can be expected to inhibit the DENV envelope protein, can be achieved if the ligands capable of binding to a target receptor of the β-OG binding site. The purpose of this study is to deploy 308 commercial cyclic peptide ligands that went into molecular docking simulation. In the end, 4129 poses of the ligands were obtained. Furthermore, we selected 487 poses, that have better binding value than the others, and proceed into 'Calculate Binding Free Energy' process. The free binding energy value ($\Delta G_{binding}$) of the commercial cyclic peptide was expected to have a negative value and lower than the standard ligands (the β-OG compound, Yennamalli R1, and Kampmann A5).

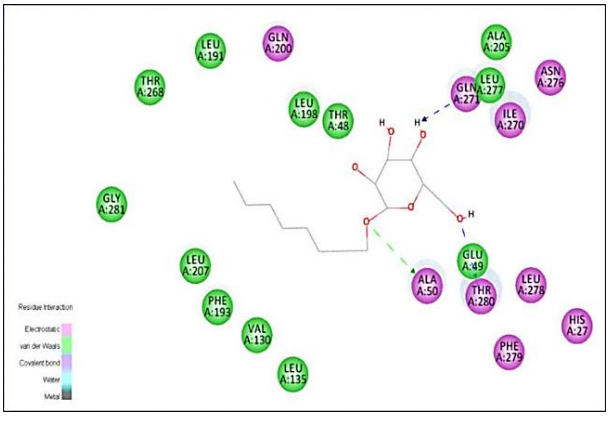

Fig. 1 Visualization of interaction between β-OG with DENV envelope protein. According to the legend from Fig.2, the ligand underwent the electrostatic and van der Waals interaction with the purple-colored and green-colored amino acid residue, respectively.

Free binding energy is associated with the binding affinity between ligand and receptor and can be determined the stability of ligand-protein complexes. To find a ligand that able to inhibit β-OG pocket binder, this ligand must have a better interaction with the binding site of β-OG pocket, in terms of binding energy and molecular interaction. So, this ligand can be utilized as a novel compound to inhibit the DENV envelope protein.

From the molecular docking simulations results, it showed that β-OG ligand can interact with 3 residues (Ala50, Gln271, and Thr280) in the β-OG pocket binder. It can be concluded that the three residues are the main residues of the β-OG binding site, the visualization of the β-OG ligand with its binding sites can be seen in Figure 1. The interaction can be shown by using Accelrys Discovery Studio 4.1 Visualizer. The virtual screening of 308 commercial cyclic peptide ligands yields ten best ligands that have a better interaction and binding affinity than the standard ligands. The results of the $\Delta G_{binding}$ calculations and the inhibition constant of the ligands can be seen in Table 1.

Table 1 The Results of Binding Free Energy Calculation and Inhibition Constants

Ligands	**ΔG binding (Kcal/mol)**	**Inhibition Constant (Psi)**
Acetyl-(N1e4, Asp5, D-2Na17, Lys10-cyclo-αMSH (4-10) amide	-29.7963	21.7062
Cyclo(-Arg-Gly-Asp-D-Phe-Val)	-27.0986	19.7409
Acetyl(n1e4, Asp5, Dtyr7, Lys10)-cyclo-α-MSH (4-10) amide	-26.8260	19.5423
(D-Cys6, Asn7, D-Ala11, Cys14)-Bombesin (6-14	-26.2073	19.0916
(Arg8)-Deamino Vasopressin Desglycinamide	-24.4771	17.8312
Cyclo(-D-Trp-Tyr)	-23.7543	17.3046
Cyclo(-Arg-Gly-Asp-D-Phe-Lys)	-23.0906	16.8212
Cyclo(-Gly-Tyr(PO3H2)-Val-Pro-Met-Leu	-19.3360	14.0860
Bremelanotide	-19.1494	13.9500
Felypressin	-17.7451	12.9270
β-OG	-14.2837	10.4055
Kampmann	-7.8441	5.7143
Yennamalli R1	-2.6863	1.9569

*Note: **Bold** ligand indicates standard ligand

3.3. In silico prediction of pharmacological and toxicity properties of ligands

This study aims to find new drug candidates, so it is very important to know whether ligands that have passed the screening has good pharmacological properties. This can be reached by obeying into Lipinski's Rules of Five. This rule specifically

evaluates the oral drug administered into the body [17]. The virtual prediction of pharmacological properties can be done either by offline or online softwares. One of the software that is frequently used in this field is Osiris Property Explorer, which can be accessible online at http://www.organic-chemistry.org/prog/peo/. The results of these tests can be seen in Table 2.

Cyclo(-Gly-Tyr(PO3H2)-Val-Pro-Met-Leu) and Felypressin ligands cannot be tested by the Osiris Property Explorer software because it cannot read the ligand structure perfectly. Based on the results in Table 2, only cyclo(D-Trp-Tyr) that has drug score above 0.70, while the rest of commercial cyclic peptide ligands and standard ligands have a drug score below 0.70. Besides, the molecular weight of the cyclic peptides tends to be above 500 Da and relatively have a large size, except for cyclo (-D-Trp-Tyr), which led to poor drug score results based on the molecular weight indicators and Total Polar Surface Area (TPSA).

Table 2 Pharmacological property prediction by using OSIRIS Property Explorer

Ligand	LogP	Solubility	MW	TPSA	Drug-likeness	Drug score
Acetyl-(Nle4, Asp5, D-2-Nal7, Lys10)-cyclo-α-MSH (4-10) amide	-0.56	-6.88	1073	382.20	-3.99	0.09
Cyclo(-Arg-Gly-Asp-D-Phe-Val)	-2.30	-1.94	574	244.70	7.09	0.63
Acetyl-(Nle4, Asp5, DTyr7, Lys10)-cyclo-α-MSH (4-10) amide	-2.99	-4.81	1039	402.40	-2.60	0.12
(D-Cys6, Asn7, D-Ala11, Cys14)-Bombesin (6-14)	-3.97	-5.08	1012	440.00	5.78	0.37
(Arg8)-Deamino Vasopressin Desglycinamide	-2.59	-4.25	1012	451.10	-0.70	0.28
Cyclo(-D-Trp-Tyr)	1.53	-3.32	349	94.22	6.98	0.84
Cyclo(-Arg-Gly-Asp-D-Phe-Lys)	-4.66	-1.89	603	270.70	4.86	0.60
Cyclo(-Gly-Tyr(PO3H2)-Val-Pro-Met-Leu)	ND					
Bremelanotide	-0.95	-5.20	1024	376.40	-3.69	0.19
Felypressin	ND					
β-OG	1.28	-2.07	306	99.38	-23.03	0.46
Kampmann A5	7.97	-7.58	468	87.64	1.80	0.12
Yennamalli R1	5.03	-6.25	414	72.95	5.58	0.32

*Note: ND = not determined, MW = molecular weight, **Bold** ligand indicates standard ligand

In addition to the pharmacological properties, it is very important to know the toxicity properties of the ligand, such as mutagenic and carcinogenic properties. In drug design and development, mutagenic and carcinogenic properties should be avoided because it will give bad side-effects of the drugs. One of the approach that can be taken in the toxicity test is QSAR (Quantitative Structure-Activity Relationship). In this study, we determined the ligand's toxicity properties by using two kinds of software, which were Osiris Property Explorer and Toxtree. The former one, besides from predicting the drug-likeness properties, can be also deployed to determine the toxicity properties of the ligand such as tumorigenic, mutagenic, irritant and reproduction effect. The prediction results of this test can be seen in Table 3. Based on the results, we observed that Acetyl-(Nle4, Asp5, D-2-Nal7, Lys10)-cyclo-α-MSH (4-10) amide ligand has a tumorigenic risk while Acetyl-(Nle4, Asp5, DTyr7, Lys10)-cyclo-α-MSH (4-10) amide ligand has a risk to be an irritant. It can be seen also that the Kampmann A5 ligand has a tumorigenic risk, and Yennamalli R1 ligand tends to be irritants. Furthermore, the rest of the ligands, including the β-OG ligand, have good pharmacological properties due to lack of tumorigenic, mutagenic, irritant and reproduction effect properties.

Toxicity properties of these ligands can be searched further by using Toxtree v.2.6.6 software, this toxicity test is based on Benigni-Bossa rule, which stated that the ligand may potentially mutagen or carcinogen if this ligand has the fragments that can cause mutagenic or carcinogenic, such as acyl halides, haloalkane, epoxides, aldehyde, hydrazine, alkyl/aromatic nitro, isocyanates and polyaromatic hydrocarbons. Furthermore, there are two carcinogenic parameters that can be used, they are genotoxic and non-genotoxic carcinogenicity. While the former one is based on the potential of the compound to induce cancer by using the irreversible mechanism in the genetic material, the latter one is based on the potential of the compound to induce cancer by using a different mechanism other than the genotoxic carcinogenicity. Along with the carcinogenicity test, mutagenicity test was also conducted, using the *Salmonella typhimurium* bacteria as the indicator [18]. The result of this test can be seen in Table 4.

Table 3 Toxicity prediction by using OSIRIS Property Explorer.

Ligand	Toxicity Risk			
	Mutagenic	**Tumorigenic**	**Irritant**	**Reproductive Effect**
Acetyl-(Nle4, Asp5, D-2-Nal7, Lys10)-cyclo-α-MSH (4-10) amide	No	High Risk	No	No
Cyclo(-Arg-Gly-Asp-D-Phe-Val)	No	No	No	No
Acetyl-(Nle4, Asp5, DTyr7, Lys10)-cyclo-α-MSH (4-10) amide	No	No	High Risk	No
(D-Cys6, Asn7, D-Ala11, Cys14)-Bombesin (6-14)	No	No	No	No
(Arg8)-Deamino Vasopressin Desglycinamide	No	No	No	No
Cyclo(-D-Trp-Tyr)	No	No	No	No
Cyclo(-Arg-Gly-Asp-D-Phe-Lys)	No	No	No	No
Cyclo(-Gly-Tyr(PO3H2)-Val-Pro-Met-Leu)	ND			
Bremelanotide	No	No	No	No
Felypressin	ND			
β-OG	No	No	No	No
Kampmann A5	No	High Risk	No	No
Yennamalli R1	No	No	Low Risk	No

*Note: ND = not determined, **Bold** ligand indicates standard ligand

From this test, we can see that all ligands, including the standard ligands, are not mutagenic, whereas four out of ten ligands are predicted to be a carcinogen. The reason why Acetyl-(Nle4, Asp5, D-2-Nal7, Lys10)- cyclo-α-MSH (4-10) amide, Acetyl-(Nle4, Asp5, DTyr7, Lys10)-cyclo-α-MSH (4-10) amide, (D-Cys6, Asn7, D-Ala11, Cys14)-Bombesin (6-14), and bremelanotide ligands are predicted so because they have imidazole or benzimidazole fragments in their chemical structure. In addition, Acetyl-(Nle4, Asp5, DTyr7, Lys10)-cyclo-α-MSH (4-10) amide ligand also predicted as a genotoxic carcinogen. This is due to the aldehyde functional group in the structure of Acetyl-(Nle4, Asp5, DTyr7, Lys10)-cyclo-α-MSH (4-10) amide ligand.

Finally, the ADMETox test was conducted to determine the oral bioavailability, health effect, maximum passive adsorption and Central Nervous System (CNS) activity of the ligand. This test was performed by using ACD/I-Lab software. We

selected four ligands ((Arg8)-Deamino Vasopressin Desglycinamide, cyclo(-D-Trp-Tyr), cyclo(-Gly-Tyr(PO_3H_2)-Val-Pro-Met-Leu), and Felypressin) that have better results than the rest of the ligands from our previous tests. The results of this test can be seen in Table 5.

Oral bioavailability can be defined as the rate and extent of a compound/substance that is absorbed by our body through oral delivery [19,20]. In this test, the cyclo(-D-Trp-Tyr) ligand has the highest oral bioavailability (between 30% and 70%) while the rest of the ligands have a low oral bioavailability (lower than 30%). The similar results were occurred as well in the health effect prediction, other than cyclo(-D-Trp-Tyr) ligand, the other three ligands have a high probability to affect our internal organs. Hence, these ligands may have an undesirable side effect to our body when it is consumed.

The active transport prediction can be also done in this test, based on the chance of the ligand can be carried by peptide transporter 1 (PepT 1) and apical sodium-dependent bile acid transporter (ASBT) protein. These proteins are the carrier protein that has an important role in the active transport process. From the result of this test, we discovered that there is no ligand that can be carried by these proteins. In addition to active transport, there is also another way for the drug to be absorbed into our body by passing through cell membranes, such as passive adsorption. Passive adsorption is a diffusion movement through a semi-permeable membrane from the high concentration gradient into a low one. Unlike the active transport, which requires energy or a carrier protein, the passive adsorption does not require energy nor carrier to be able to penetrate the cell membrane [21]. In this test, we can also see that the cyclo(-D-Trp-Tyr) ligand have a good passive adsorption while the rest were completely unable to passively adsorbed into our body. Finally, based on this test, all the ligands cannot penetrate the blood-brain barrier, thus, they cannot interfere with the CNS.

Based on all tests that we were conducted in this phase, we discovered that the cyclo(-D-Trp-Tyr) ligand has the best results among all. Thus, this ligand will enter the final step of this study; molecular dynamics simulation.

Table 4 Mutagenicity and Carcinogenicity Prediction by Toxtree software

Ligand	**QSAR-based Carcinogenicity**	**Genotoxic Carcinogenicity**	**Non-Genotoxic Carcinogenicity**	**Potential Mutagenicity (on *S.typhimurium*)**
Acetyl-(Nle4, Asp5, D-2-Nal7, Lys10)-cyclo-α-MSH (4-10) amide	No	Negative	Positive	No
Cyclo(-Arg-Gly-Asp-D-Phe-Val)	No	Negative	Negative	No
Acetyl-(Nle4, Asp5, DTyr7, Lys10)-cyclo-α-MSH (4-10) amide	No	Positive	Positive	No
(D-Cys6, Asn7, D-Ala11, Cys14)-Bombesin (6-14)	No	Negative	Positive	No
(Arg8)-Deamino Vasopressin Desglycinamide	No	Negative	Negative	No
Cyclo(-D-Trp-Tyr)	No	Negative	Negative	No
Cyclo(-Arg-Gly-Asp-D-Phe-Lys)	No	Negative	Negative	No
Cyclo(-Gly-Tyr(PO3H2)-Val-Pro-Met-Leu)	No	Negative	Negative	No
Bremelanotide	No	Negative	Positive	No
Felypressin	No	Negative	Negative	No
β-OG	No	Negative	Negative	No
Kampmann A5	No	Negative	Negative	No
Yennamalli R1	No	Negative	Positive	No

*Note: **Bold** ligand indicates standard ligand

Table 5 The result of the ADMETox test by ACD/I-Labs software

Parameters/ Ligands	(Arg8)-Deamino Vasopressin Desglycinamide	Cyclo(-D-Trp-Tyr)	Cyclo(-Gly-Tyr(PO3H2)-Val-Pro-Met-Leu)	Felypressin	**β-OG**	**Kampmann A5**	**Yennamalli R1**
Oral Bioavailability	<30%	30%-70%	<30%	<30%	30%-70%	30%-70%	30%-70%
Blood	100%	56%	98%	100%	36%	47%	31%
Cardiovascular System	0%	84%	94%	0%	36%	86%	70%
Gastrointestinal System	68%	84%	60%	89%	1%	97%	84%
Kidneys	100%	87%	92%	99%	10%	57%	52%
Liver	100%	89%	100%	99%	7%	15%	62%
Lung	16%	40%	93%	63%	8%	94%	47%
Active Transport	No	No	No	No	No	No	No
Passive Adsorption (Maximum)	0%	100%	0%	0%	100%	100%	100%
CNS *Active*	Inactive	Inactive	Inactive	Inactive	Active	Active	Inactive

*Note: **Bold** ligand indicates standard ligand

3.4. Molecular Dynamics Simulation

In general, molecular dynamics simulation consist of three phases: initialization, equilibration, and production [22]. In the initialization phase, the solute-solute interaction is simulated to determine the coordinate system of the complex, then, we proceed into the equilibration phase, which is influenced by the temperature (this step may be included with the heating process). Finally, the production phase is performed to see the stability and molecular interaction of the ligand-protein complexes under the influence of solvent and temperature at the desirable intervals, at this phase, the RMSD graph will be obtained, this graph is necessary needed to observe the stability of ligand-protein complexes. Furthermore, the molecular interaction from the docking and dynamics simulation can be compared each other to determine their stability without and under the influence of temperature, solvent, and time, respectively.

In Figure 2, it can be observed that the interaction of hydrogen bonds from cyclo(-D-Trp-Tyr) ligand and DENV envelope protein were formed at two different temperatures, at the normal body temperature (310 K) and at the fever body temperature (312 K), respectively. Furthermore, the cyclo(-D-Trp-Tyr) ligand still able to maintain the hydrogen bonds at the binding site residues (Gln271 and Gly275) in the both temperature. The intervention of explicit solvent and temperature can change the overall interaction of the ligand-protein complex, including the hydrogen bonds. As we can see in Table 6, the cyclo(-D-Trp-Tyr) ligand was able to maintain its hydrogen bonds with Gln271 and Gly275 at both temperatures on molecular dynamics simulation. However, it can be also seen at molecular docking simulation that cyclo(-D-Trp-Tyr) ligand interacts with the Ala50, Gln200 and Lys202 residues of DENV envelope protein. Although that was slightly different, this indicates that the cyclo(-D-Trp-Tyr) ligand was able to inhibit binding sites of DENV envelope protein at either normal or fever body temperature.

Table 6 Comparison of hydrogen bonding between the Cyclo(-D-Trp-Tyr) ligand with DENV envelope protein in molecular docking and molecular dynamics simulation

Docking simulations	Dynamics simulation (310K)	Dynamics simulations (312K)
Ala 50	**Gln271**	**Gln271**
Gln 200	**Gly275**	**Gly275**
Lys 202		

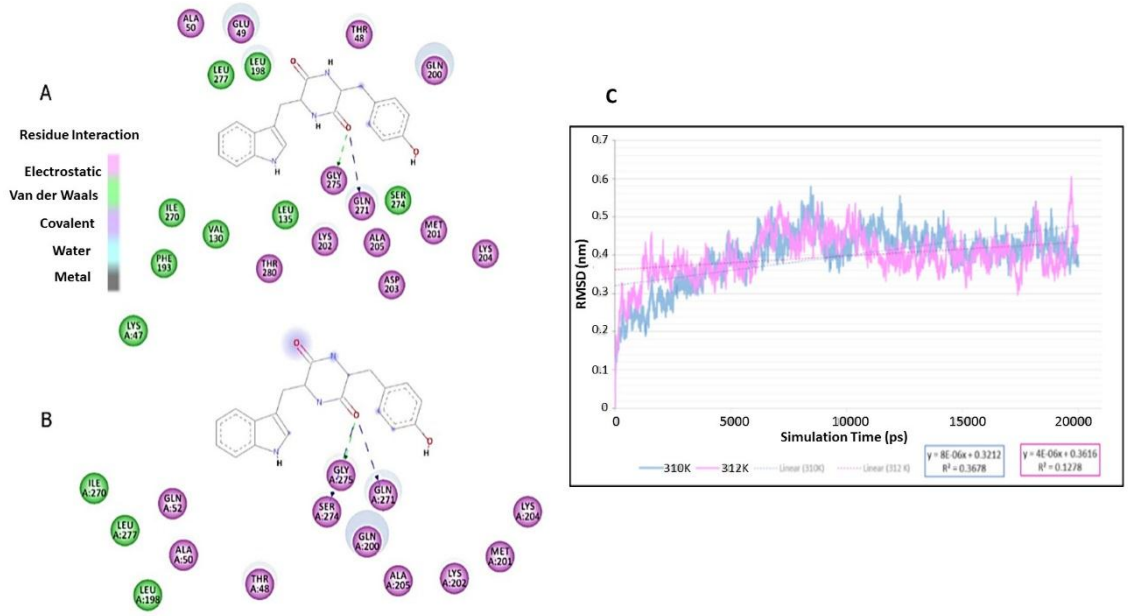

Fig. 2 2D visualization of molecular interaction between Cyclo(-D-Trp-Tyr) ligand with DENV envelope protein in 310 K (2a) and 312 K (2b). Visualization of interaction between β-OG with DENV envelope protein. According to the legend from Fig.2, the ligand underwent the electrostatic and van der Waals interaction with the purple-colored and green-colored amino acid residue, respectively. Moreover, the Figure 2c shows the RMSD graph of Cyclo(-D-Trp-Tyr) ligand with DENV envelope protein. The X-axis shows the time (in ps) on the 20 ps MD simulation, while the Y-axis graph shows the RMSD change (in nm) during the simulation.

In the molecular dynamics simulation, the interaction between the protein and ligand can occur in a solvent, then they can move dynamically. This may lead to the changes in the complex conformation. These changes can be observed from the RMSD graph. From the Figure 2c, we can see that there is no significant difference between the two complex conformation in the both temperature (310 K and 312 K), although the protein-ligand complex tends to be more linear or stable at 310 K. This means that the cyclo(-D-Trp-Tyr) ligand is more likely to form a ligand-protein complex in the normal body temperature, but it can still able to maintain its interactions in the fever body temperature (due to slight RMSD difference between the two temperatures). Additionally, the absence of significant RMSD change between the two temperatures indicate that the conformation of the DENV envelope protein does not change dramatically, so that the cyclo(-D-Trp-Tyr) ligand may disrupt the conformational change in the DENV envelope protein that holds the key factor to the fusion process and DENV attachment to the host cells.

4. CONCLUSIONS

The virtual screening of commercial cyclic peptide compounds was made by in silico method to find a novel inhibitor of β-OG pocket binder in DENV-2 envelope protein. This research has screened 308 commercial cyclic peptide ligands through molecular docking, computational ADMET test, and molecular dynamics simulation. From the first phase, we discovered that ten out of 308 ligands have good binding affinity on the β-OG pocket binder residues, such as Ala50, Gln271, and Thr280. Moreover, the computational ADMET test uncovered that cyclo(-D-Trp-Tyr) ligand is the best ligand, based on its pharmacological properties and low toxicity prediction. This ligand was further analyzed for its interaction with DENV envelope protein through 20 ns molecular dynamics simulation. The results are the cyclo(-D-Trp-Tyr) ligand formed a stable complex with DENV envelope protein at normal (310 K) and fever body temperature (312 K), and able to maintain its interaction with the binding site of the β-OG pocket binder residues. Therefore, we conclude that the cyclo(-D-Trp-Tyr) ligand is the best ligand among all the commercial cyclic peptides to inhibit DENV envelope protein. Thus, it can be viable to be developed into a novel antiviral drug candidate through in-vitro and in-vivo experiment.

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