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Modification of Kampmann A5 as Potential Fusion Inhibitor of Dengue Virus using Molecular Docking and Molecular Dynamics Approach

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Dengue fever which is caused by dengue virus infection has become a major health problem in the world. New antiviral treatment which inhibits the activity of enzymes or proteins that play a role in replication in the host cell is required at this time. Envelope protein is a structural protein that plays a role in fusion process between virion membrane and host cell membrane. In envelope protein, there is a cavity between domain 1 and domain 2 which is occupied by n-octyl-β-D glucoside (BOG) molecule. BOG was surfactan agent used to break the cell membrane when the envelope protein was crystalized. The cavity is called BOG which known for playing a role in activation of fusion process. Several researches have proven that docking of a molecule which has stronger affinity with BOG pocket can inhibit viral replication. One of the compound which can inhibit the replication of dengue virus replication is kampmann A5. The aim of this study is to design Kampmann A5 derivative that can inhibit the fusion process of dengue virus targeting the BOG cavity. Virtual screening of 10.341 ligands obtained 3 best ligands based on free binding energy (ΔG) and toxicity prediction. The stability of the complex was observed using molecular dynamics simulation. The result showed that ligand 1 and ligand 6 complexes have better stability at 312 K, meanwhile the ligand 7 complex showed insignificant difference at both temperature. Those three ligands could lead to inhibitor candidate against dengue virus fusion process.

Key words: Dengue, envelope, fusion inhibitor, molecular docking, molecular dynamics

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INTRODUCTION

Dengue fever is a disease caused by the dengue virus (Perera and Kuhn, 2008). Dengue viruses are transmitted into the human body through the bite of the genus *Aedes* mosquitoes, mainly *Aedes aegypti* (Huang *et al*., 2010). About 100 countries are in a dengue endemic areas and more than 2,5 billion people who live in the tropics and subtropics have possibility to be exposed for dengue fever (WHO 2012). Dengue virus (DENV) is divided into four serotypes based on the produced antigene by the body, namely: the DENV-1, DENV-2, DENV-3 and DENV-4 (Austin *et al*., 2012). Current research on dengue serotypes is focused on the relationship between the different level of its clinical manifestation. The results show that the following primary infection of both DENV-1 or DENV-3 levels are having clinically more severe effects than both DENV-2 and DENV-4 (Martina *et al*., 2009). Meanwhile, other studies have shown that secondary infection by DENV-2 was associated with more severe clinical levels rather than the primary one (Geiss *et al*., 2009; Martina *et al*., 2009). Antiviral treatments for dengue infection have been studied and some of them that have been reported are viral RNA synthesis inhibitors, NS3 protease inhibitors, DENV maturation inhibitors and monoclonal and polianion antibodies that prevent the bond with host cell receptors (Noble *et al*., 2010). Proteins that can be used as targets for inhibiting virus replication are proteins envelope that play a role in the process of fusion with the cell membrane of the virus particle (Bui *et al*., 2000). Mode of binding of the virion to the cell membrane has identified several important receptors and binding by the receptors are being studied as a target to inhibit the viral replication (Hambleton *et al*., 2007). Challenges in studying the inhibition of the virus through the method of virion binding to cell membranes are the different types of receptors (O'Doherty *et al*., 2000). Envelope protein crystallization that was done by Modis *et al*. (2003), eventually found that there is a gap between domain I and domain II that occupied by the detergent molecules, namely, n-octyl-β-D-glucoside (BOG). The functionality feature of BOG is to break down the cell membrane during the envelope protein crystallization (Poh *et al*., 2009; Modis *et al*., 2003). Gaps are involved in conformational changes of the inactive envelope protein fusion form into the active one (Ivanovic *et al*., 2013). Designing molecule inhibitors based on the structure and properties of cavity or through virtual or high-throughput screening has resulted in several candidate inhibitor compounds (Noble *et al*., 2010; Noble *et al*., 2013). Several compounds are known to have a thiazole ring, but it was found that compounds with tiopen rings (with group (-CH =) that replaces group (-N =) in the thiazole ring) also had inhibitory activity (Wang *et al*., 2009). Kampmann *et al*. (2009) have done the virtual screening of small organic molecules with the target of BOG gap and the result is some of the compounds have inhibitory activity against DENV fusion, including the A5 molecule. In this study, we wanted to see if the modifications of the A5 Kampmann compounds can reduce its toxic properties and improving its activity, so it can be used as a fusion inhibitor of dengue virus during molecular docking and molecular dynamics simulations. The purpose of this research is to design derivative compounds from A5 Kampmann that could inhibit the dengue virus envelope protein fusion with molecular docking and molecular dynamics methods.

MATERIALS AND METHODS

Preparation of denv envelope protein

The search of denv envelope protein sequence: DENV envelope protein sequence data in FASTA format was downloaded from the NCBI database (National Center of Biotechnology Information) http://www.ncbi.nlm.nih.gov.

Multiple sequence alignment: DENV envelope protein sequence alignments was performed using FASTA program online through the website www.ebi.ac.uk/Tools/sss/fasta.

Cavity visualization: 3D structure and DENV envelope cavity was visualized by using the software Molecular Operating Environment 2008. 10.

Geometry optimization and energy minimization of the 3D structure of denv envelope protein: Geometry optimization and energy minimization of DENV envelope protein was made by removing the water molecules. The utilized parameters are the current force field. Furthermore, the energy minimization was performed with AMBER99 force field, solvation of the gas phase and the RMS gradient of 0.05 cal moL^{-1} Å (Vilar *et al*., 2008). Other parameters were using the default standard of MOE.

Ligand preparation

The design of ligand structure: The tested ligands in this study were drawn in 3D by using software ACD Labs 12.0. Ligands used in this study is a modified version of the A5 Kampmann compound (Kampmann *et al*., 2009). The drawn ligands were optimized with options drawn Clean Structure and 3D structure optimization.

Docking between envelope protein and the ligand: The docking process was performed using the software MOE 2008.10. The utilized Placement setting method is the triangle matcher. The scoring function is the London dG that displays the 100 best poses Tambunan and Alamudi, 2010.

The data analisis of the molecular docking results: The identification of the contact residues of protein-ligand complex and the hydrogen bonds were performed by using LigX-interaction in MOE 2008.10 (Tambunan *et al*., 2012). The visualization of ligand-protein complex was employed using the software MOE 2008.10. ÄG values were indicated by S on the output data from the docking.

The prediction of the adme-tox ligand: The prediction of ADME-Tox properties (Adsorption, Distribution, Metabolism, Excretion and Toxicity) was performed with ACD software iLabs online (https://ilab.acdlabs.com/iLab2/index.php). The Toxtree v2.1.0 and Osiris Property Explorer (www.organic-chemistry.org/prog/peo/) (Geerts and Vander Heyden, 2011).

Molecular dynamics: The Preparation of protein-ligand complex is required before performing molecular dynamics simulations (Zhang *et al*., 2012). The partial charge setting of the protein-ligand complexes was performed with AMBER99 parameters. Moreover, the born solvation settings were used for the solvation system and the energy minimization was performed with RMS gradient 0,05 kcal moL^{-1} Å (Tambunan and Parikesit, 2011). The parameters used are the NVT ensemble and NPA algorithms (Tambunan *et al*., 2011a). AMBER99 force field was used.

The determination of initialization time: Molecular dynamics simulations for protein-ligand complexes were carried out for 100 ps to determine the initialization before running the main simulation (Zhang *et al*., 2012).

Molecular dynamics simulation at 310 K: The molecular dynamics simulations were performed for protein-ligand complexes with a temperature of 310 K, a time of major simulation for 5.000 ps, cooling for 10 ps; until the temperature reached 1 K (Kang *et al*., 2012). The data of position, velocity and acceleration were saved every 0.5 ps. The other parameters are in accordance with MOE-default.

Molecular dynamics simulation at 312 K: The molecular dynamics simulations were performed for protein-ligand complexes with a temperature of 312 K, a time of major simulation for 5.000 ps, heating for 10 ps; until the temperature reached 1 K (Bayas *et al*., 2003). The data of position, velocity and acceleration were saved every 0.5 ps. The other parameters are in accordance with MOE-default.

Data analysis of molecular dynamics simulation: The results of molecular dynamics simulation can be seen in the output of the MOE database viewer. Interactions between protein-ligands for molecular dynamics simulation process can be viewed using LigX-Interaction (Stacklies *et al*., 2011).

RESULTS

Inhibitor design: The entire structure of the designed inhibitor and compounds was generated by using Chemsketch 12.0 software. The utilized hit compound (Kampmann A5) is derived from the studies of Kampmann *et al*. (2009). The compound was not screened from Maybridge database. All screened candidates by Kampmann come from that database.

Lipinski Rule states that the drug has good oral bioavailability if the LogP is less than or equal to 5 (Pajouhesh and Lenz, 2005). Therefore, these compounds have violated the rule because of the cLogP value of 6,40. However, the compound is still in a good range of medicinal properties since the violated parameters are only one. The breach of the Lipinski rules is still within the acceptable limits (Sabitha and Rajkumar, 2012). The designed ligands also have detergent properties, which has hydrophobic and hydrophilic parts in a single molecule (Hong *et al*., 2010). The included modifications are the substitution of hydrogen atoms that attached to the benzene ring, pyridine or thiazole with a group that can improve the hydrophobicity of these ligands. H atoms may be replaced with halogen atoms such as F and Cl or replaced with methyl functional group ($-CH_3$), hydroxyl (-OH), hydroxymethyl ($-CH_2OH$), amyl ($-CONH_2$), formyl (-CHO) or methoxy ($-OCH_3$) (Lednicer, 2008). Group that containing electronegative atoms (F, Cl, O) can be hydrogen bond acceptor and increasing the affinity of the target protein, while the methyl group can push the electron distribution towards benzene ring that has more electronegativity and increasing the hydrophobic interaction. Substituting the atomic level was based on the concept of classical isoster (Meanwell, 2011).

Protein sequence and template determination: The obtained sequence was the accession code AAA17506.1. The retrieved sequence was in FASTA format that forwarded to the Multiple Sequences Alignment (MSA)

pipelines. Uniprot recommended the 3D structure of the Protein Model Portal with ID Q66396 GDP that refers to the PDBID 1OK8. It is the 3D structure of the envelope protein after fusion, while for a suitable template for a simulation model is the conformation prior to the fusion protein envelope and contain the molecules of BOG (Zhang *et al*., 2006). RCSB PDB (http://www.pdb.org) shows that there are several PDB entries associated with 1OK8, including the 1OAM which is a complex of protein envelope with BOG. Later, it was stated that the entry has replaced the 1OKE with PDBID 1OK8. The Entry of 1OKE is also an envelope protein complex with BOG, so 1OKE template is a suitable template for molecular docking.

Protein preparation: Protonate 3D was the first thing to` be done in the preparation stage of the protein, which also serves to show the position of hydrogen atoms in the crystal structure (Davis *et al*., 2007). Protonation state was set at 300 K, pH 7 and 0.1 M salt concentration. The temperature of the simulation system was 300 K (Tambunan *et al*., 2011b). The appropriate force field for protein geometry optimization is AMBER99. The utilized solvation method is the gas phase. The solvation was done in a vacuum so that there will be no solvent effect and solvation energy does not need to be taken into account. The energy minimization was performed until RMS gradient 0.05 cal/Å (MacKerell *et al*., 1998). The purpose of the energy minimization is to obtain a protein with the lowest energy state and to avoid the bad contact interactions. Other parameters will follow the MOE standard parameters.

Ligand preparation: Wash operation was done to improve the structure of the ligand and the position of hydrogen atom (Aparoy *et al*., 2012). The energy minimization was using the force field MMFF94x with RMS gradient of 0.05 that suitable for small organic molecules. Other parameters was using default settings of MOE. Elimination of bad contact will result in geometric structures that in accordance with the actual conditions.

Molecular docking: The designed 10.341 ligands were having virtual screening by molecular docking. The selected contact residues are based on the results of several research studies with the same goal (Tomlinson and Watowich, 2012). Some amino acids that can be targeted as the binding sites are summarized in Table 1.

It can be seen from the table above which the residues that make up the cavity and could be the target bond. The table explains why the target cavity is hydrophobic, which is due to the nonpolar and hydrophobic residues. The triangle matcher placement was based on the charge group and spacial fit to produce an optimal bonding orientation (Tambunan *et al*., 2011b). The employed rescoring was the London dG. The selected number of retain is as much as 30 repetitions and without duplication. Refinement using the force field is more accurate than using GridMin because it uses Generalized Born solvation model at the final evaluation stage (Tambunan *et al*., 2013). The more posing is chosen, then the chance of the emergence with lower energy will be greater (Meslamani *et al*., 2012). Of every 100 ligand screening, the best ligand were taken to the final stage. After five times ligand screening, it produce the 23 best ligands and twice screening of the final stage produced 8 best ligand. The eight best ligand then screened based on the prediction of toxicity before molecular dynamics session.

Table 1: The binding site of the BOG cavity in the envelope protein DENV

No. charge polar	Thr 48, Gln 52, Gln 200, Gln 271, Ser 274, Thr 280, Gly 281
Acid polar asam	Glu 49, Asp 203
Basic polar basa	Lys 128
Nonpolar and hydrophobic	Val 130, Pro 132, Leu 135, pro 187, Leu 191, Phe 193, Leu 207, Ile 270, Leu 277, Phe 279

Table 2: The data of binding free energy, partition coefficient and the best eight ligand inhibition constant

Ligand	ÄG (Kkal moL^{-1})	K_i	Log P
1	-21.4537	$2.0448.10^{-16}$	4.41
2	-21.1199	$3.5875.10^{-16}$	4.28
3	-21.0235	$4.2196.10^{-16}$	4.23
4	-18.7721	$1.8696.10^{-14}$	3.69
5	-18.6839	$2.1704.10^{-14}$	4.18
6	-18.1539	$5.2947.10^{14}$	5.18
7	-17.8512	$8.8148.10^{-14}$	4.41
8	-17.1729	$2.7622.10^{-13}$	4.31
Kampmann standard	-16.8965	$4.3994.10^{-13}$	6.00
Mayhoub standard	-16. 5453	$7.9475.10^{-13}$	4.63
Li 11 standard	-16. 3446	$1.1143.10^{-12}$	6.38
Wang 6 standard	-15. 4308	$5.1913.10^{-12}$	6.00
Li 36 standard	-15. 0991	$9.0752.10^{-12}$	4.63
Zhou p02 standard	-14. 1376	$4.5815.10^{-11}$	1.08
BOG standard	-8. 6846	$4.4550.10^{-7}$	

Docking results analysis: There are several things that can be seen from the results of docking, namely the orientation of the ligand to its target protein, the identification of compounds that have an affinity for the target protein and prediction of the affinity of a molecule to the target protein (Sunaryo and Rachmania, 2011).

Binding free energy and inhibition constant: Ligand conformation states with a lower free energy are more stable. The smaller the value of Ki, the affinity of ligand for the target protein are working more effective as an inhibitor (Smyth and Collins, 2009). The data of the binding free energy and partition coefficient (LogP) of the eight best ligand are summarized in Table 2.

Based on the data above, it can be concluded that the value of the eight ligand binding free energy has no significant difference, although it is lower than the hit compound. Moreover, we can conclude that the modified compound has better hit interaction of the cavity target. The differences of each modified ligand with compound hit was the number and location of the donor/acceptor atoms of hydrogen bonds, as the isoster abide by the rules.

Hydrogen bond and residue contact: Hydrogen bonds are not the only interactions between ligands to proteins, but it is the most dominant contribution to the ligands' affinity for protein targets. It is because the hydrogen bonding interaction is stronger than the possible interaction between the ligand with its target protein cavity, provided that ionic interactions did not take place (Bissantz *et al*., 2010). The data molecular docking visual results can be seen in Fig. 1.

Based on the data in Table 3, it appears that the ligand 1 is forming five hydrogen bonds interaction within cavity target. Ligand 2 form four hydrogen bond interactions, ligand 3 form 3 hydrogen bonds, ligand 4 is forming two hydrogen bonds and the ligand 5 to ligand 8 is forming one hydrogen bond with the target cavity. The whole best ligands have thiazole group, except ligand 8. Several studies on the inhibition of the dengue virus envelope protein fusion showed that the active compounds has thiazole ring and the study by Wang *et al.* (2009) showed that compounds with tiopen ring (with group (-N =) that replaced by group (-CH_2 =) also has activity. It appears that the binding free energy is proportional to the number of hydrogen bonds. The degree of bonding that can be rotated (rotatable bonds) is 10 by Veber's Rule. It is a threshold value for a compound to have good oral bioavailability (Veber *et al*., 2002).

Toxicity prediction: Today, the *in silico* toxicology modeling for the toxicity prediction of a compound has demonstrated its role in providing information to the pharmaceutical industry in the design phase to identify the lead compounds with low toxicity, to facilitate the selection process of the lead compounds candidate and drug development potential (Valerio, 2009). In addition, toxicity prediction computing approach also allows a testing of data acquisition toxicity towards the compounds (Judson *et al*., 2009). Based on the prediction of toxicity, it can be seen that the ligand 1 and 3 does not have a red mark on each criterion. Other ligands have two red marks, unless 5 which has 4. It can be concluded that the ligand 1 and 3 has not been proven to be toxic by Benigni-Bossa Rulebase (Benigni *et al*., 2008; Benigni *et al*., 2007).

Based on data from Osiris Explorer, the nature of the toxicity was caused by the fenilhidrazon cluster. Ligands that have the same toxicity values with hit compound on the mutagenicity and tumorigenicity category have not been modified (Jonsdottir *et al*., 2005). Meanwhile, the substitution trial of nitrogen atom has a double bond (imine group,-N =) shows a dramatic decline in the value of toxicity, it also increases the value of drug compound score. In the example below, the imine group (-N =) was substituted by isoster groups, namely alkene group (-CH =).

Drug screening: Drug screening was based on parameters that have been known to affect the characteristics of drug bioavailability (Forgue *et al*., 2006).

Based on Table 4, it appears that the eight ligands are eligible to Lipinski rules, except the number 6 that has a value of clogP more than 5. The Lipinski rules convey the molecular weight of not more than 500 dA, the number of hydrogen bond donor atoms of not more than 5, the number of hydrogen bond acceptor atoms of not more than 10, the partition coefficient (logP) is not more than 5 (Lipinski *et al*., 2001). Some tuberculosis and antibacterial drug are not even follow this rule (Koul *et al*., 2011). Some antibiotics are also more hydrophilic properties that not in accordance to the Lipinski's rule (Payne *et al*., 2007). Modifications could be made to Lipinski rules because three of the four parameters can be determined based on its structure, namely the molecular weight, the number of atoms of hydrogen bond donor and acceptor atoms (Pajouhesh and Lenz, 2005).

In addition to Lipinski's rules, Veber and Egan rule describe the characteristics-characteristics of the drug with good oral bioavailability (Veber *et al*., 2002; Egan *et al*., 2000). It appears that these two rules are different in TPSA, which is the rule of Veber value is less than or equal to 140 Å, while the rule of Egan value is less than or equal to 132 Å. The value of 132 Å was chosen because compounds with TPSA less than or equal to 132 Å will comply with the second rule. Table 5 shows the values of each parameter of the best eight ligands.

Based on the above data, it can be concluded that the ligand 4 was breaking the Egan rules, while the ligand 3 was against the rules of Egan and Veber. Ligand 6 only violates one parameter of Lipinski's Rule. Therefore ligand 6 can be inferred to follow the rules of Lipinski.

The combination of toxicity prediction data, lipinski's rule, egan's rule and veber'srule: Based on the prediction of toxicity using the Osiris Property Explorer, it can be concluded that the ligand 1 and 6 were having the lowest toxicity, while the ligands 7 has the highest score among the eight best ligand. Meanwhile, the prediction of

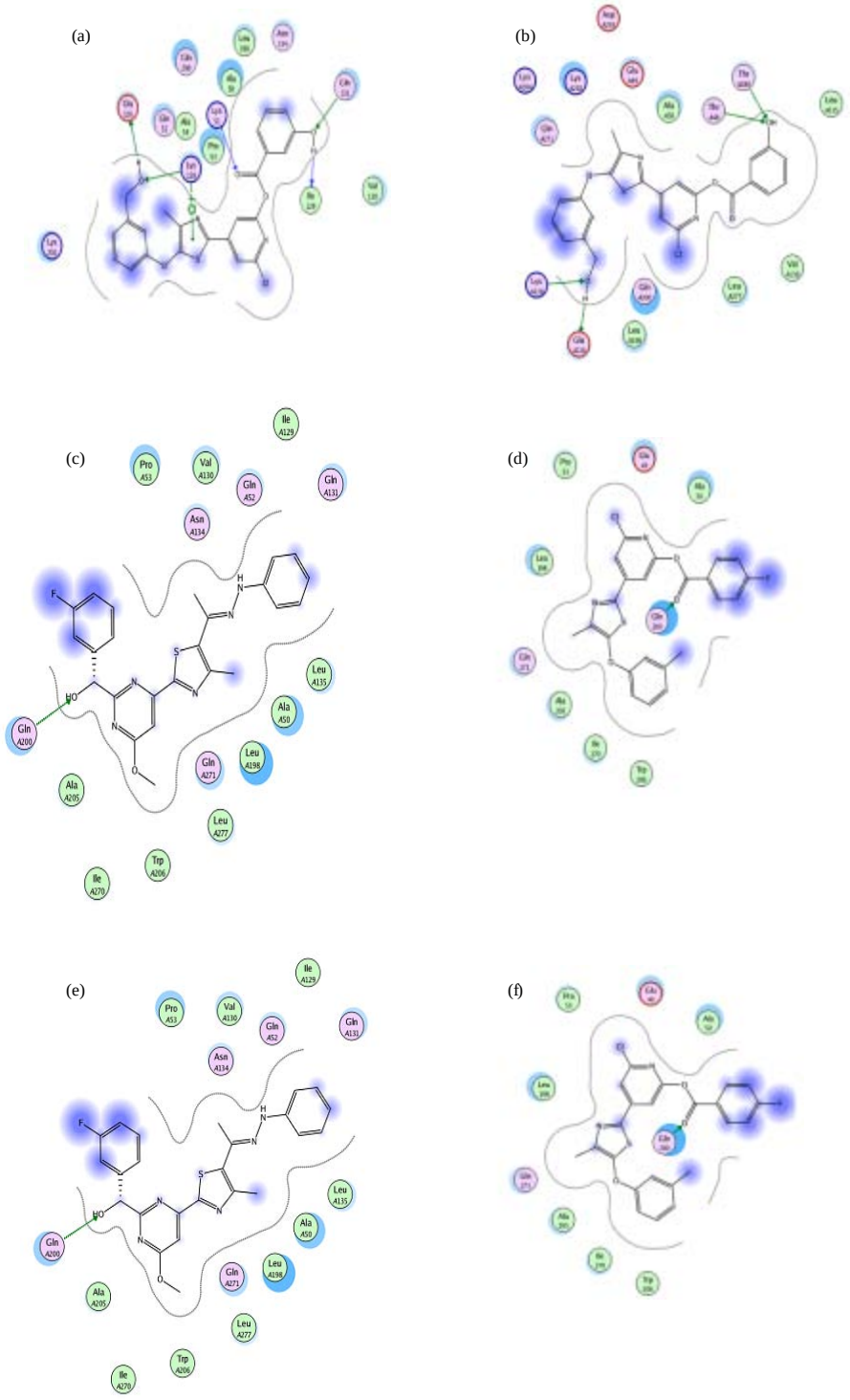

Fig. 1(a-h): Continue

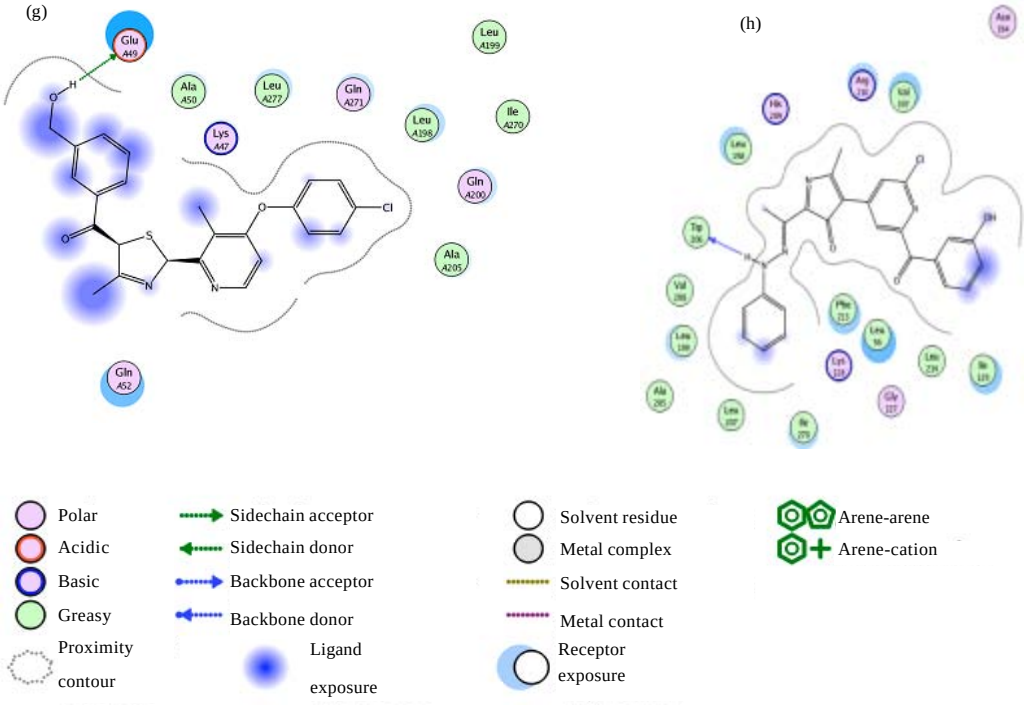

Fig. 1(a-h): Data of two-dimensional visualization of molecular docking result. Ligand 1-8 are a-h

Table 3: Residue contacts between the ligand with the protein envelope

Ligand	ÄG (Kcal moL^{-1})	Residue contact
1	-21.4537	Glu 126*, Lys 128, Lys 51*, Gln 131*, Ile 129*
2	-21.1199	Thr 48, Glu 126*, Lys 128, Thr 280
3	-21.0235	Gln 200, Lys 204*, Leu 199*
4	-18.7721	Trp 206*, Leu 199*
5	-18.6839	Gln 200
6	-18.1539	Gln 200
7	-17.8512	Gln 52
8	-17.1729	Trp 206*
Kampmann A5 standard	-16.8965	-
Mayhoub standard	-16.5453	Leu 199*, Met 201*, Lys 204*,
Li 11 standard	-15.9945	Ala 50
Wang 6 standard	-15.4308	-
Li 36 standard	-15.0991	-
Zhou p02 standard	-14.1376	Thr 48, Asp 203
BOG standard	-8.6846	Gln 52 (2), Glu 49

*Not bond target

Table 4: Parameter data of lipinski rule for the eight best ligand

Ligand No.	Molecular weight	H-bond donor	H-bond acceptor	cLogP
1	468.91	2	7	4.41
2	458.32	0	6	4.28
3	489.4	2	6	4.23
4	446.52	2	8	3.69
5	463.5	2	7	4.18
6	454.9	0	5	5.18
7	452.95	1	5	4.41
8	458.9	2	7	4.31

toxicity based on Benigni-bossa Rulebase (Toxtree) also showed that the ligand 1 has a low toxicity level. Thus,

Table 5: Data of egan and veber rules of the eight best ligand

Ligand No.	Rotatable bonds	tPSA	Total no. of acceptor atom of hydrogen bond donor	logP
1	7	130.01	9	4.41
2	6	102.44	6	4.28
3	6	144.53	8	4.23
4	7	133.65	10	3.69
5	7	120.76	9	4.18
6	6	89.55	5	5.18
7	6	97.08	6	4.41
8	6	104.01	9	4.31

only the ligand 1 that do not show the potential toxicity. Ligand 3 does not show the potential toxicity data on toxicity prediction using Toxtree, but the toxicity prediction using Osiris Property Explorer shown that it has red alerts on mutagenicity. The same thing occurs in the ligand 6 and 7. Although both ligands show a low potential toxicity of Osiris Property Explorer parameters, but each ligand showed carcinogenic potential non-genotoxic on Toxtree.

The Osiris Property Explorer is a system that works quantitatively because it can predict the quantitative parameters and also provide information about the intensity of toxicity with color coding (Simon-Hettich *et al*., 2006). Ligand 6 and 7 exhibit carcinogenic potential of non-genotoxictoxicity on Toxtree, but the amount is totally unknown. Compared with other ligands (except ligand 1) that show the

potential toxicity on both tools, both ligands have advantages. Based on the prediction of toxicity, three best ligands were earned that are going through stages of molecular dynamics, namely ligand 1, 6 and 7.

The eight best ligands comply with Lipinski's rule (ligand 6 has only one violation and is considered to meet the rule). Meanwhile, the ligand 3 and 4 are against the rules because it has TPSA Veber of more than 132 Å (ligand 3 also violate TPSA Egan as having more than 140 Å). There are six ligands that satisfy both the rule, namely ligand 1, 2, 4, 5, 6, 7 and 8. Only three ligands that have green markings on the four categories of toxicity in Osiris Property Explorer out of six ligands that meet the Lipinski's, Egan's and Veber's rules, namely ligand 1, 6 and 7. Based on the final stage of toxicity predictions, ligands that are going through the molecular dynamics simulation are 1, 6 and 7.

ADME Prediction of ligand 1, 6 and 7: ADME prediction tools are not used as a screening test in the ligand because they are more complex than the toxicity and three rules (Lipinski, Veber and Egan) data. The utilized ADME data comes from the ACD iLabs (Moroy *et al*., 2012; Van de Waterbeemd and Gifford, 2003). The three ligands have been predicted to have good bioavailability by three rules and the bioavailability of the three ligands can be expressed quantitatively and found to bediffering from each other through ADME prediction.

Based on these three data the, only the ligand 7 that does not have a red mark on the available six parameters. This is consistent with the predictions of Osiris Property Explorer toxicity that ligand 7 is having the highest score among the eight ligands even though the value is not so satisfactory. Ligand 1 is the most water soluble, marked in green on the solubility. Ligand 6 is having a partition coefficient of 5,18 which is a violation of the Lipinski's Rule. Therefore, the ligand 6 tends to be hydrophobic and its solubility values are marked in red. Although ligand 1 and 7 are having exactly the same LogP value of 4,41, but the ligand 1 is more water soluble because it has a greater TPSA. Ligand 6 and 7 have the oral bioavailability values between 30-70% and the ligand 1 has the lowest oral bioavailability, ie, less than 30%. The third prediction of ADME data ligand are summarized in Table 6.

Oligopeptides Transporter 1 (PepT1) and Apical Sodium-dependent Bile acid Transporter (ASBT) is the part of active transport in intestinal absorption. P-Glycoprotein (PGP) is a transporter molecule in the active transport that carries foreign molecules out from the cell (Klaassen and Aleksunes, 2010). PGP has a broad substrate specificity that can recognize different molecules either charged or neutral, linear and cyclic and aromatic or non-aromatic (Pajeva and Wiese, 2009). All

Table 6: The prediction data of ADME ligand 1, 6 and 7

	Ligand 1	Ligand 6	Ligand 7
Oral bioavailability	less than 30%	30-70%	30-70%
Active transport:			
PepT1	Not transported	Not transported	Not transported
ASBT	Not transported	Not transported	Not transported
PGP Inhibitor	Probability: 0.25 Reliability: 0.33 (borderline)	Probability:0.6 Reliability:0.17 (not reliable)	Probability:0.91 Reliability:0.29 (not reliable)
CNS active	Sufficient	Sufficient	Sufficient
Probability of effect on:			
Blood	0.85	0.91	0.36
Cardiovascular	0.95	0.96	0.75
Gastrointestinal	0.98	0.95	0.3
Kidney	0.23	0.41	0.37
Liver	0.19	0.1	0.05
Lungs	0.91	0.92	0.24

three ligands also have the potential to penetrate the blood-brain barrier and potentially cause interference with the Central Nervous System (CNS).

Each xenobiotic (foreign substances on living systems) has the potential to accumulate in certain body parts because of the fitness between its characteristics with the target environment (Van Der Oost *et al*., 2003). Among the three ligands, ligand 7 has the lowest probability of the effect towards the blood, gastrointestinal and pulmonary systems. Compared with ligand 1 and 6, the accessibility of ligand 7 is high due to the low probability of side effect. These data are consistent with the drug-likeness features on Osiris Property Explorer.

Molecular dynamics: After docking simulation, next three ligand were getting through the molecular dynamics simulations. In molecular dynamics simulations, both the ligand and protein are in a state of flexibility, so that conformational changes in the protein-ligand complex during the simulation time can be studied (Nabuurs *et al*., 2007). Molecular dynamics can also study the effect of the solvent in the system. It could be applied to explore the conformation of the receptor protein to improve the process of drug design (Alonso *et al*., 2006).

The preparation files for the molecular dynamics simulation: Minimization process was done so that the geometry of atoms that do not fit can be improved and the lowest potential energy could be obtained (Nurbaiti *et al*., 2010). The utilized algorithm is NPA (Nose-Poincaré-Anderson), with force field AMBER99, NVT canonical ensemble, the selected temperature is 310 K (normal body temperature) and 312 K (body temperature during fever) and a pressure of 101 kPa (Buhl *et al*., 2011). The selected solvent model is an implicit solvent that simulates the conditions of each atom to move under the influence of

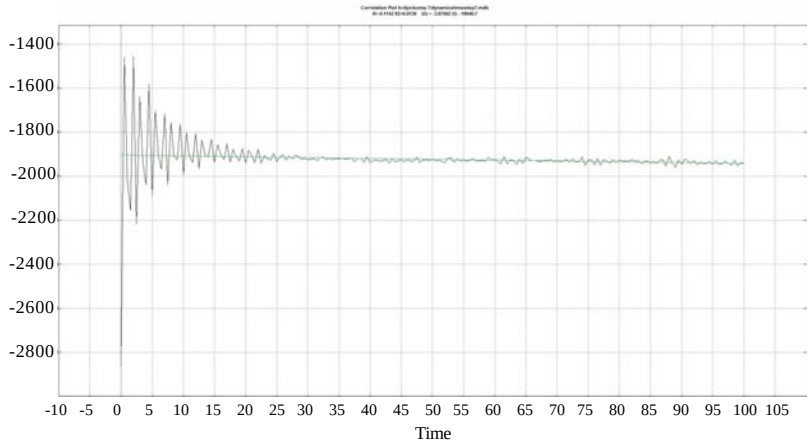

Fig. 2: The timing of curve initialization of the three ligand at 100 ps

Table 7: Contact residues of the ligand during molecular dynamics simulations at 310 K

	Ligand 1	Ligand 6	Ligand 7
Heating 10 ps	-	-	Glu 49
5.000 ps simulation	Lys 128 (2)	Lys 47	Gln 200, Lys 47, Thr 48
10 ps cooling	Lys 128 (1)	Lys 47 (2)	Glu 49

Table 8: Comparison of the contact residues of hydrogen bonds between the simulated docking and dynamics at 310 K

Ligand simulation	Ligand 1	Ligand 6	Ligand 7
Molecular docking	Glu 126, Lys 128, Lys 51, Gln 131, Ile 129	Gln 200	Gln 52
Molecular dynamics	Lys 128 (2)	Lys 47 (2)	Glu 49

the solvent as in explicit solvent model. An ensemble is a collection of all possible and macroscopic characteristics of the same, but differ microscopically, systems (Lawrenz *et al*, 2009; Van Gunsteren *et al*., 2006).

Molecular dynamics simulation process: There are three stages in molecular dynamics simulations, the initialization, heating/equilibration and production (Nurbaiti *et al*., 2010). The new atom positions are determined as a result in the production stage where the system has reached equilibrium (Anderson and Lekkerkerker, 2002). The obtained data at the production stage as 3D coordinate of each atom during the simulation is called trajectory.

Initialization time determination: Initialization is a solvent preparation before entering the heating and equilibration stages. The timing of the initialization was performed for 100 ps. The determination results of the initialization time can be seen by the changes in the potential energy value of protein-ligand complex system (Vedadi *et al*., 2010). At this stage, the initialization phase for is 100 ps (Fig. 2).

The initialization time for 30 ps was selected based on the screening results of the correlation plot of protein-ligand complex energy during the simulation for 100 ps. At the time of 30 ps, the energy of the systems tend to be stable and selected as the first stage of the main simulation for 5.000 ps. The main simulation was run at temperature of 310 and 312 K.

Molecular dynamics simulation at 310 K: The initialization was followed by molecular dynamics simulations at temperatures of 310 K. It is the normal body temperature. The process was carried out for 10 ps heating to raise the temperature of the system towards the equilibrium state. Then do the cooling for 10 ps, to find the lowest energy conformation of the molecule. The process was known as annealing. The cooling was conducted down to 1 K. The results of position, velocity and acceleration were saved every 0.5 ps. Table 7 shows the ligand interaction data during the simulation.

The small number of interactions was due to the nature of the hydrophobic cavity. The target cavity was having many non-polar hydrophobic amino acids, although there are some polar amino acid residues that could act as a good binding site. Based on Table 6, it appears that most interactions of the ligand 7 are occurred during major simulation for 5.000 ps. Unfortunately, after entering the cooling phase for 10 ps interactions, it cannot be maintained. The ligand 1 was experiencing two interaction with the same residue (Lys 128), but after the cooling, it lost one interaction. Both during the main simulation and after the cooling stage, ligand 6 had only one interaction with the same residual phase. During the molecular dynamics, protein and ligand is set as flexible, so that there is a difference between the interaction during molecular docking and molecular dynamics. The difference in the interaction are shown in Table 8.

During the dynamics simulations and docking, ligand 6 and 7 only have one interaction, but with a different

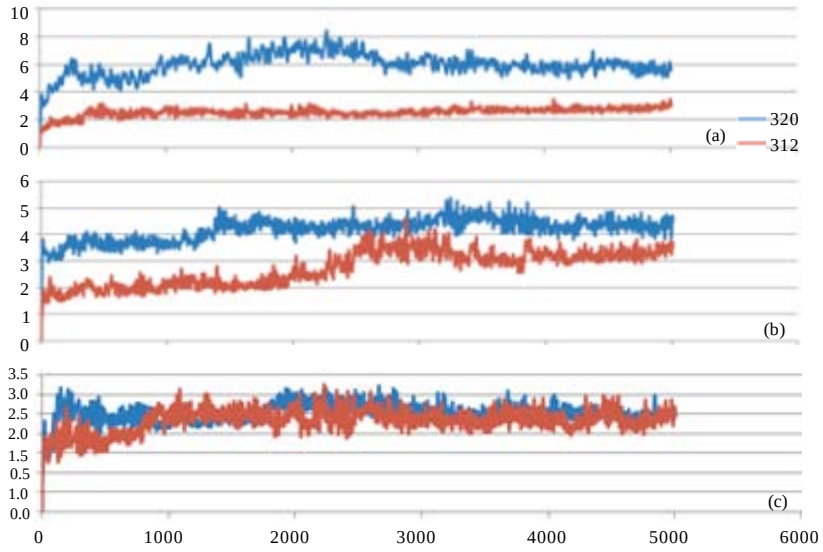

Fig. 3(a-c): The curve of RMSD versus simulating time of the three ligands. X axis is the time in ps and the Y axis is the RSMD, (a) Ligand 1, (b) Ligand 6 and (c) Ligand 7

Table 9: The contact residue of the ligand during molecular dynamics simulations at 312 K

00 Ligand simulation	Ligand 1	Ligand 6	Ligand 7
Initialization	Ser 274, Gln 200, Lys 128, Phe 279	-	Lys 47, Thr 48, Ala 50
Heating 10 ps	Ser 274, Lys 128, Phe 279	-	Lys 47, Thr 48, Gln 200
Simulation 5.000 ps	Glu 49, Ala 50, Lys 128, Phe 279	Lys 47	Lys 47, Thr 48, Ala 50
Cooling 10 ps	Gln 200, Lys 128, Glu 49, Ala 50	Lys 47	Lys 47, Lys 128, Thr 48

Table 10: Comparison of the residual contacts of hydrogen bonding between the simulated docking and dynamics at 312 K

0ligand simulation	Ligand 1	Ligand 6	Ligand 7
Molecular docking	Glu 126, Lys 128, Lys 51, Gln 131, Ile 129	Gln 200	Gln 52
Molecular dynamics	Glu 49, Ala 50, Lys 128, Phe 279	Lys 47	Lys 47, Thr 48, Lys 50

residue. Ligand 1 lost some interaction during the dynamics simulation and leaving only one interaction with Lys 128.

Molecular Dynamics Simulation at 312 K: Treatment of molecular dynamics simulations at 312 K is equal to 310 K. Molecular dynamics simulations indicate that there are some changes in the residue contact interactions and hydrogen bonding in both the ligands during the simulation. Table 9 shows the data for the simulations of ligand interaction. Table 10 shows the comparison of residue contacts when simulating the molecular docking and molecular dynamics.

Conformational analysis of the molecular dynamics results: Complex conformational changes in protein-ligand can be seen from the curve of RMSD simulation (root mean square deviation) (Frimurer *et al*., 2003). The magnitude of the conformational changes between the two atoms coordinates are described as the magnitude value of RMSD simulation time (Ai *et al*., 2010). The plot of conformational changes in the protein-ligand complexes for molecular dynamics simulations was revealed in the the RMSD values and can be seen in Fig. 3.

Based on Fig. 3, it appears that the value of RMSD lig and 7 complex did not differ significantly at 310 K and 312 K. Ligand complex 7 at a temperature 312 K has the same number of hydrogen bonds to 310 K, it indicates that complex formation on both the temperature has the same stability. Ligand complex 6 has the same number of bonds at 310 K and 312 K. Ligand complex 1 has more number of bonds in 312 K compared to 310 K. RMSD curve of ligand complexes 1 and 6 shows that the mean RMSD at 312 K is smaller than 310 K, it shows that this conformational change the structure of the ligand complex 1 and 6 at 310 K is much larger than the 312K. Conformational changes during the whole simulations (0-5.000 ps) following the protein structure changes in dimeric to the trimerform. Thus, it can be concluded that the ligand 1 and 6 can make the protein structure more stable at 312 K compared to 310 K.

Structural novelity: Structural similarity results on ZINC is 90 and 80%. Structural similarity used in ChemSpider is exact structure (Muresan *et al*., 2012). The both results show that the negative results, which means that the structure did not exist in the database. Thus, the three ligands have a structure that is completely new and has a novelity value.

DISCUSSION

The efficiency of our pipeline has been improved; by selecting three ligands out of 10.341 lead compounds. The progress of the computational power has eventually enhances the generated information for a solid drug design. The earlier pipeline that rely only upon the molecular docking has eventually extended to cover molecular docking as well, due to the availability of strong computational graphic subsystem. The combined molecular docking and dynamics features of MOE has greatly simplify the whole pipeline, as separated software for each process is not necessary. The feasibility of fusion protein inhibitor could be assessed, although the structural complexity of the interaction are the focal points of the whole computational procedure. Henceforth, no hindrance in the pipeline due to the seamless integration of the MOE toolbox.

As shown by research of the Novartis group, the trends of the drug design are clearly toward the smart molecules for the specific Protein targeting with thorough evaluation of the post-simulation (Ertl and Schuffenhauer, 2009; Wang *et al*., 2009). In this end, Fusion protein would eventually be a ideal target for dengue drug design, as only feasible molecular engineering that could offer an inhibiting features into the target.

The various approach toward Dengue drug design are already in sight. The inhibitor designs on NS (Leung *et al*., 2001; Yin *et al*., 2006a,b), RNA-polymerase (Noble *et al*., 2013), Envelope proteins (Yennamalli *et al*., 2009) have paved the way toward a more complete approach on tackling with Dengue virus. However, some issues are still need to be considered. It should remain to be seen, which ones would eventually passed on the clinical trial and the generation of online drug design library should be devised.

CONCLUSION

Virtual screening against 10.341 ligands eventually produced eight ligand with lower binding free energy value (ÄG) than the standards. The results of Toxicity prediction indicate that the ligand 1, 6 and 7 have the lowest potential toxicity compared to the other ligands. During the molecular dynamics simulation on both the test temperatures of 310 and 312 K, the three ligands showed different interactions in the simulation of 5.000 ps with 10 ps cooling. The conformational changes analysis suggests that at temperatures 312 and 310 K, 7 ligand complex has a level of stability that is not much different on both temperatures, whereas ligand complexes 1 and 6 are more stable at a temperature of 312 K. The molecular dynamics simulations should performed on the hit compound to observe the influence of the modifications on the stability of the complex.

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