International Journal of GEOMATE, Sept., 2018 Vol.15, Issue 49, pp. 65 -71
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2018.49.3535
Special Issue on Science, Engineering & Environment

FRAGMENT-BASED LEAD COMPOUND DESIGN TO INHIBIT EBOLA VP35 THROUGH COMPUTATIONAL STUDIES

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ABSTRACT: Ebola virus (EBOV) is a virus that is classified under *Filoviridae* family as a pathogenic organism. On March 2016, World Health Organization (WHO) reported that 28.646 cases caused by EBOV. Thus, it is important to find the antiviral drug for this disease because it can create the epidemic around the world. EBOV VP35 is a potential drug target because it has the component of the viral RNA polymerase complex that will hamper the host interferon (IFN) production. In this research, about 6.662 fragments were obtained from ZINC15 Biogenic Database after the Rules of Three, and pharmacological properties parameters were applied. After that, these fragments were docked into the active side of EBOV VP35 using MOE 2014.09 software. The potential fragments from previous docking simulations were linked each other, resulting 91 ligands in the process. Furthermore, the docking simulation was conducted again and discovered the best three ligands that have lower Gibbs free binding energy than the standards. Moreover, the pharmacological prediction tests were also done to find the ligand with excellent molecular properties. The best three ligands from these tests were continued into molecular dynamics simulation. In the end, we conclude that the LEB 31 ligand can be the new drug candidate as EBOV VP35 inhibitor based on molecular docking, pharmacological prediction test, and molecular dynamic.

Keywords: Fragment-Based Drug Design, Ebola Virus, VP35, Molecular Docking, Pharmacological Prediction

1. INTRODUCTION

The Ebola virus (EBOV) is pathogenic, single-stranded RNA virus of *Filoviridae* family causing fatal hemorrhagic fever in human and non-human primates [1],[2]. Ebola virus was found in Africa for the first time in 1976. On July 2015, there are 11,268 people deaths from 27,621 cases reported. In Africa, this case is an epidemic, but Ebola virus may develop and infected populations in other parts of the world [3].

There are seven codes of protein for EBOV such as RNA-polymerase (L), nucleoprotein (NP), glycoprotein (GP), VP24, VP30, VP35 and VP40 [2]. In this study, we focused on VP35. Viral protein (VP) 35 is the crucial host for EBOV infection that has functions in the filoviral replication cycle and an essential cofactor of the viral RNA polymerase complex [4]-[6]. The EBOV VP35 is a potential drug target because it has the component of the viral RNA polymerase complex that will hamper the transcription of type I interferon (IFN) and the production of IFN will decrease [2],[7]. Initial studies said that the virus target the RIG-I signal pathway inhibit the activation of IRF-3 and production of interferon from infected cells [6].

Raj and Varadwaj [2] used the flavonoid as an inhibitor of EBOV. In this study, we designed EBOV VP35 inhibitor based on in silico biogenic fragment database. Fragment-based has been widely used in the pharmaceuticals and biotechnology industry as it is easy to synthesize and show good results in chemical properties analysis [8]. We used the molecular docking to know the VP35-ligand interaction [9]. To docking fragment database, we used MOE 2014.09 software [10]. In this research, we tried to the design a potential VP35 inhibitor using fragment-based drug methods. The outcome of this study could provide a promising drug candidate for Ebola virus infection.

2. METHODS

2.1 Protein Structure

In this study, we used the three-dimensional structure of EBOV VP35 with PDB ID: 3FKE [2] that available in Protein Data Bank (http://www.rcsb.org/pdb). Wherein the default protocol of protein preparation was conducted according to our validated methods, such as removing the water molecules [11]–[13]. In this present study, we used MOE 2014.09 software to minimized and optimized the 3D structure of EBOV VP35 protein [10].

2.2 Construction of Fragment Database

Biogenic compounds database was downloaded from ZINC15 database [8]. All

fragments were created based on Rule of Three and toxicity prediction using Osiris DataWarrior [5]. The energy minimized and partial charge of both fragments used the MOE 2014.09 with MMFF94x as a forcefield [11].

2.3 Molecular Docking of Fragment Database and Standart Compound

Three-dimensional structure of chloroquine, the standard ligand of EBOV VP35 protein, was obtained from ChemSpider. Molecular docking simulation for chloroquine and fragment database was performed using MOE 2014.09 software. In the present study, Triangle Matcher and London dG parameters were applied as 'Placement' and 'Scoring' functions, respectively. Furthermore, the simulations were conducted twice, with retain the value of 30 and 100 was utilized in the first and second docking. The other parameters were set according to default parameters of docking protocol in MOE 2014.09 software, with AMBER10: EHT was applied as the forcefield [11].

2.4 Fragment Linking

Potential fragments were linked in MOE 2014.09 software to generated new ligands. These new ligands were docked with the EBOV VP35. The best three ligands that created in this study which have low binding energy were selected for the pharmacological prediction test.

2.5 Analysis of Ligand Interactions and Pharmacological Properties

Three new ligand and standard ligand were analyzed their interaction with the active side of EBOV VP35. In this study, we used Osiris DataWarrior [14], VEGA-QSAR [15] and Toxtree [16] to predict the drug-likeness and toxicity predictions of the selected ligands.

2.6 Molecular Dynamics Simulation

In this study, the best three ligands from molecular docking and pharmacological properties prediction were selected for molecular dynamics simulations to determine the stability of the ligand-protein complex that formed during the docking simulations. First, the heating process was conducted for ten picoseconds (ps) at 300 K and 312 K in the first step. After that, equilibrium step simulation was conducted for 100 ps at 312 K and production in 20000 ps at 312 K. For the last step, cooling simulation is conducted in 10 ps at 300 K [17]. Finally, the result of this simulation was analyzed.

3. RESULT AND DISCUSSION

3.1 Protein Structure

In this study, we used EBOV VP35 chain A (Fig. 1) as protein target because this protein has a function to protective immune responses to EBOV [18]. This protein has the interaction with Gln244, one of the critical amino acid residues in the protein [2]. We used LigX in MOE 2014.09 to prepare the protein with AMBER 10: EHT as a forcefield.

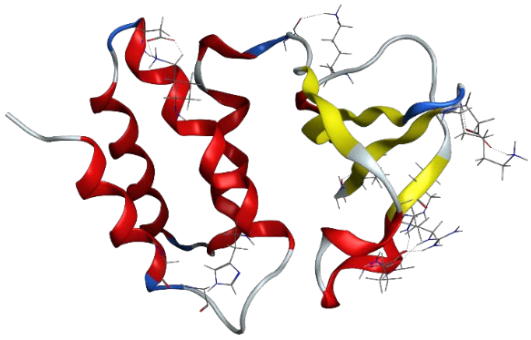


Fig.1 Structure of EBOV VP35

3.2 Construction of Fragment Database

In this present study, we obtained 246.244 compounds from ZINC Biogenic database. We used the Rule of Three to reduce the amount of the compounds to find the potential fragment in Osiris DataWarrior. Three things must be followed in Rule of Three: (1) molecules have a mass less than 300Da, (2) molecules have the hydrogen donor, and acceptor up to three, and (3) the value of clogP is 3 [19]. Toxicity screening could also be used to predict the risk of toxicity or metabolic instability [11]. We used mutagenic, tumorigenic, irritant and reproductive prediction as a parameter in Osiris DataWarrior. After the screening process was conducted, about 6.662 fragments after toxicity and Rule of Three screening. The energy minimizes, and partial charges of both fragments were applied using the MOE 2014.09 software with MMFF94x as a force field.

3.3 Molecular Docking and Linking Process

Molecular docking is the way to predict the position, orientation, and conformation of the protein target binding the ligand [20]. We used the MOE 2014.09 software to performed molecular docking of 6.662 fragments into the active site of EBOV VP35. After docking, we search two potential candidates of the fragment for the linker. Fragment 1 is the fragment that has interaction with Gln244 residue [2].

Fragment 1 has the root mean square deviation (RMSD) 1.4186 Å (Fig. 2). Fragment 2 is another ligand that not overlaps with Fragment 1. Fragment 2 has the value of RMSD 1.5146 Å (Fig. 3**)**. In general, the binding interaction of essential amino acid residues and ligands, especially through hydrogen bonds, is crucial to inhibit the protein activity.

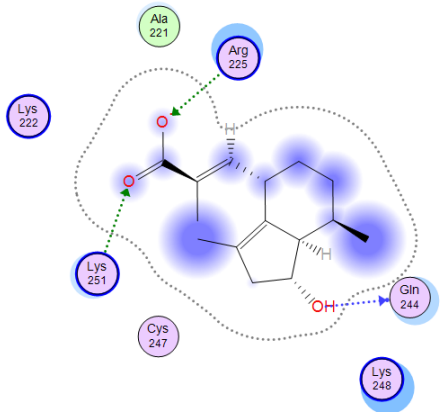


Fig. 2 The interaction between Fragment 1 with the binding site of EBOV VP35

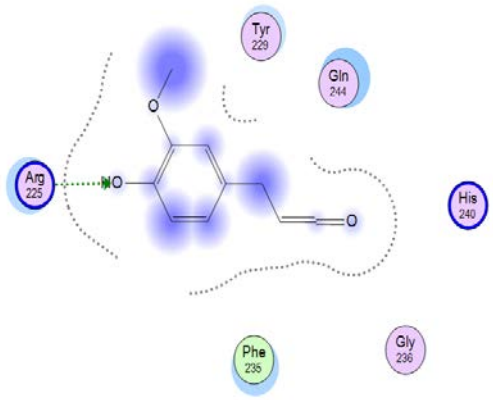


Fig. 3 The interaction between Fragment 2 with EBOV VP35 at its active site

In the present study, we linked two fragments using 'Link Multiple Fragments' feature in MOE 2014.09 software to generate higher affinity binding compounds that have interaction with the active site of EBOV VP35. Through this step, we acquired 91 ligands as result of linked, and then we docked these ligands. After docking simulation with rescoring 30, we found ligands with the low $\Delta G_{binding}$ value, but the RMSD score is higher than 2.0 Å in LEB 31 (3.26920 Å). In general, a docking pose would be acceptable if the value of RMSD that generated during the docking simulation is lower than 2.0 Å [21]. Because of this reason, we re-docked the fragment into rescoring 100, and the result showed that the RMSD of LEB 31 decrease to 1.0642 Å.

In this step, we found three best ligands that have potential as a drug candidate for EBOV VP35 based on the value of $\Delta G_{binding}$, inhibition constant (pKi) and RMSD. The ligand with the code LEB 31 is the best ligand that showed the $\Delta G_{binding}$ -50.5453 kcal/mol with an inhibition constant (pKi) of 36.8215 and RMSD is 1.0642 Å. The value of $\Delta G_{binding}$, inhibition constant (pKi) and RMSD of the three best ligands and standard ligands that used in our work showed in Table 1.

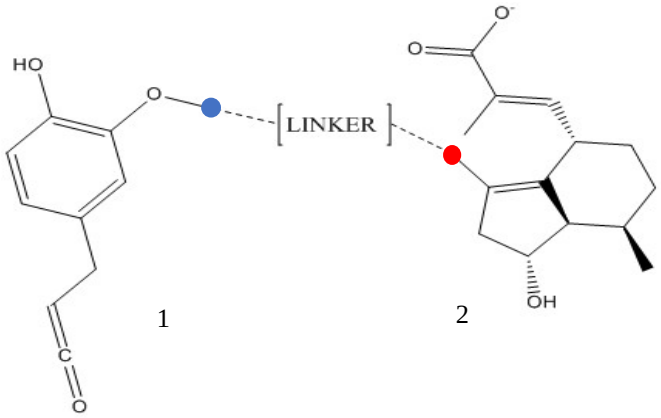
3.4 Pharmacological Analysis

The toxicity and drug-likeness analysis of the best ligands were performed using Osiris DataWarrior, VEGA, and Toxtree [22], [23]. The parameter of toxicity like as mutagenic, tumorigenic, toxicity and irritant was predicted using Osiris DataWarrior, while development or reproductive toxicity using VEGA and potential *S. typhimurium* TA100 mutagen and potential carcinogenic using Toxtree (Table 2). In Table 2., chloroquine as standard ligand shows that it has a high mutagenic and irritant than other ligands when predicted its toxicity using Osiris DataWarrior software. Toxicity prediction test using VEGA and Toxtree show that all of the ligands did not possess toxicity properties in three parameters.

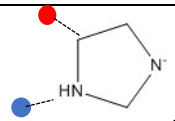
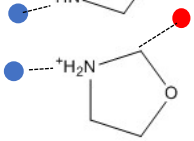
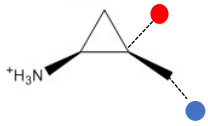
The bioavailability properties prediction based on Lipinski and Veber rules such as rotatable bonds, clogP, hydrogen bond donor and acceptor and also the polar surface area. These parameters were determined using Osiris DataWarrior (Table 3**).**

Table 3 shows that all of the ligands give the proper result based on Lipinski rules: the value of clogP less than 5, have five hydrogen donors and ten hydrogen acceptors and also Veber rules: (1) ligand have a number of rotatable bonds no more than 10 and (2) polar surface area equal or less than 140 Å [24].

Table 1. ΔG energy, pKi, and RMSD value from the selected linked compounds and standard ligands



1 2

Ligands	Linker	ΔG (kcal/mol)	pKi	RMSD (Å)
LEB 31		-50.5453	36.8215	1.0642
LEB 39		-47.8037	34.8243	1.3152
LEB 89		-47.4118	34.5388	1.5913
*Chloroquine	-	-29.0212	21.1415	1.9169

Note: * Standard Ligand; the blue circle in linker is linking with the blue circle in fragment 1 while the red circle is linking with the red circle in another fragment.

Table 2. Toxicity test using Osiris DataWarrior, VEGA and Toxtree

Ligands	Parameters						
	Mutagenic	Tumorigenic	Irritant	Toxicity	Developmental /Reproductive Toxicity	Potential *S. typhimurium* TA100 mutagen	Potential carcinogen
LEB 31	None	None	None	None	NO	NO	NO
LEB 39	None	None	None	None	NO	NO	NO
LEB 89	None	None	None	None	NO	NO	NO
*Chloroquine	High	None	High	None	NO	NO	NO

Note: * Standard Ligand

Table 3. The molecular properties using Osiris DataWarrior

Ligands	Parameters					
	Molecular Weight	Number of H-Donor	Number of H-Acceptor	cLogP	Polar Surface Area	Number of Rotatable bonds
LEB 31	495.594	3	8	0.6552	111.33	9
LEB 39	497.586	3	2	1.0868	120.56	9
LEB 89	495.641	3	7	2.4432	134.53	10
*Chloroquine	321.894	1	3	4.2018	28.16	8

Note: *Standard Ligand

3.5 Molecular Dynamics Simulation

The best three ligands were analyzed with molecular dynamics simulation to observe protein-ligand complex stability. Table 4 showed the amino acid residues that interact with a protein target. The result shows that some of the amino acid residues in molecular docking are also found in molecular dynamics. This result indicated the protein-ligand complex is quite stable. RMSD score is also the parameter that shows the stability of protein-ligand interaction because the distance of the particular atom in the complex can indicate from this value.

Protein-ligand complex is stable when the changes in the value of RMSD does not exceed 3 Å in a reach of 1 nanosecond (ns) [17]. According to Fig. 4, all of the ligands have RMSD low than 3 Å. LEB 31 is the most stable than another ligand and standard, with an average RMSD of 0.5 Å, compared to the LEB39 and LEB89, which have an average RMSD of 1.5 Å and 0.8 Å, respectively. Thus, we can safely conclude that all of these ligands have a decent protein-ligand complex stability and can be used as a potential drug candidate for Ebola targeting EBOV VP35 protein.

Table 4. Amino acid residues based on molecular docking and dynamics simulation

Ligand	LEB 31	LEB 39	LEB 89	Chloroquine
Molecular docking	Q241, Q244, K248, K251, **R225**, A221, W229, K222, F235, P304	H240, K248, Q244, G236, F235, **K251**, C247, K222, A221, N226, **R225**, Y229	K248, **Q244**, F235, **R225**, N226, Y229, A221, K222, **K251**,C247	R225, Q241, Q244, V245, I295, K248, P293, L249, Vl294
Molecular dynamics	K222, **R225**, Q244, A221, K251, K248, Cs247	P239, Q244, **K251**, **R225**, A221, L249, Q241, K248, I295,V249	K248, **Q244**, Q241, F328, I295, D302, P304, **R225**, A221, K222, **K251**	K248, PP293, H296, I295, L249, V249

Note: amino acid residues in red color are the residues found in the binding site of the EBOV VP35 protein. Residues in bold font are the residues that can be found in molecular docking and dynamics simulation.

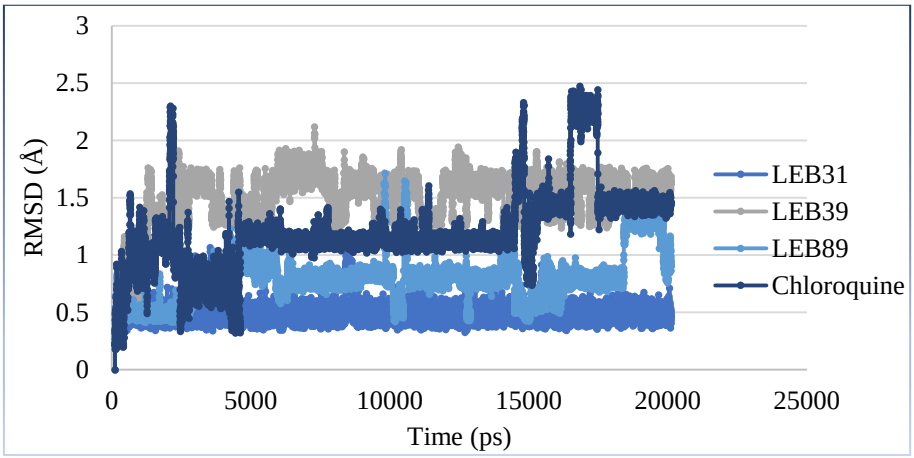


Fig. 4 Graph of RMSD (Å) vs time (ps)

4. CONCLUSION

In this research, we constructed fragment database and docked it into the active site of VP35. After docked, we linked the two-potential fragment and then docked again with VP35. We obtained 91 ligands from this fragment linking process. In the next step of the study, we used three best ligands from molecular docking simulation to analyzed in molecular dynamics simulation. In general, all of the ligands showed the decent pharmacological properties and gave the value of RMSD lower than 3 Å in molecular dynamics. Among all ligands, LEB 31 is the best inhibitor based on the result of molecular docking, pharmacological properties prediction, and molecular dynamics simulation.

5. ACKNOWLEDGEMENTS

This research has been supported and funded by Directorate of Research and Community Engagement (DRPM) Universitas Indonesia through Hibah Publikasi Internasional Terindeks Untuk Tugas Akhir Mahasiswa (PITTA) No: 693/UN2.R3.1/HKP.05.00/2017. We also would like to thank Mochammad Arfin Fardiansyah Nasution for helping proofread this paper.

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