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EBOLA VIRAL PROTEIN 24 (VP24) INHIBITOR DISCOVERY BY IN SILICO FRAGMENT-BASED DESIGN

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ABSTRACT: Ebola hemorrhagic fever (EHF) is a fatal disease caused by *Ebolavirus* that can potentially lead to death. The number of fatalities reached 11.000 of the 28.000 reported cases. A serious concern should be taken because neither drug nor treatment to cure this disease has been found until now. Recent studies show that viral protein 24 (VP24) is one of the non-structural protein that plays a key role in EBOV proliferation and viral life cycle. This study tried to find the potential inhibitor for EBOV VP24 through in silico experiment. About 242.520 compounds from ZINC15 In Vitro Database were obtained and screened according to the Rules of Three and pharmacological properties to get a proper lead-like fragment compounds. These compounds were docked into the active site of VP24 using MOE 2014.09 software. The potential fragment compounds were linked to generate potential inhibitor ligands. These ligands were screened earlier based on Lipinski's Rule of Five and toxicity prediction, then they were docked once again to obtain the favorable ligand. Furthermore, the dynamics simulation of best ligand, namely L833, L217, and L595, were performed to predict the ligand-enzyme complex stability. This research concludes that L595 is the best ligand. Moreover, the pharmacological and toxicity prediction also confirm that L595 can be developed as the potential inhibitor for EBOV VP24.

Keywords: Ebolavirus, Viral Protein 24, Molecular Docking, Fragment-based, Toxicity Prediction

1. INTRODUCTION

Ebolavirus is a part of *Filoviridae* family that can cause a fatal disease like Ebola hemorrhagic fever [1]. The virus is endemic and deadly in humans and non-human primates [2]. Fever, sore throat, muscle aches, and headache are symptoms of Ebola hemorrhagic fever [3]. The spread of this virus has occurred from one country to another such as in West Africa. Liberia, Guinea, Sierra Leone was attacked by *Ebolavirus* in 2014-2015. This disease causes up to 11.297 deaths as reported by World Health Organization (WHO) [4].

Ebolavirus genome encodes seven structural protein and one non-structural protein [5]. The encoded structural proteins are transmembrane glycoprotein (GP), nucleoprotein (NP), four viral protein (VP24, VP30, VP35, and VP40) and RNA polymerase (L) [4]-[5]. VP40 is known to be the principal protein matrix and the most abundant viral protein. It contributes to the proliferation process of EBOV. The viral maturation involves VP40 that joins the cell membrane. The process of viral maturation of infected cells by inducing virion assembly in the plasma membrane [7]. VP24 is the second protein matrix and a minor portion of the viral protein [8]. The VP24 function as viral protein is not known certainly [6]-[7]. However, as a structural protein, VP24 may play a role in the virion assembly process [8]. Other supporting statements are put forward by Watanabe in 2007. They reported that VP24 can reduce transcription and replication process of the *Ebolavirus* genome. The direct association of the ribonucleoprotein complex in the infected cells occurs in both processes [9].

In silico studies have been used for several years by researchers. These studies provide efficient and effective results, as it provides accelerated identification and optimization of drug discovery. Not only researchers but also the pharmaceutical industry has also utilized in silico analysis in their drug design pipeline because it can determine the affinity and selectivity to a small number of compounds. Furthermore, the in silico study utilizes the three-dimensional (3D) structure of the protein as the starting point of the pharmacological test, and this structure provides a visualization of the interaction occurring in proteins. Zoeta [10] reported that there are more than 50 compounds of in silico study results that have entered the clinical trial stage and some of them have been approved by The Food and Drug Administration (FDA) [10].

The fragment-based design is one of the drug discovery approaches in silico study. In a fragment-based approach, the small-molecule formed from

low molecular fragments from the molecule period is different from the high throughput screening (HTS) approach. HTS is not yet clear which part of the molecule is heavily involved in the binding energy even though HTS hit binding is obtained by this approach [11].

2. METHOD

2.1 Preparation of EBOV VP24

The 3D structure of the target protein become a new approach to structure-based drug design [12]. In this study, the 3D structure of EBOV VP24 was obtained from the Protein Data Bank (http://www.rcsb.org/pdb), water molecules, and heteromolecules that attached to the protein structures were removed [13]. LigX function in MOE 2014.09 software was applied to viral protein for designing optimized process of EBOV VP24.

2.2 Preparation of Fragment

Fragment database was obtained by downloading the molecules from ZINC15. ZINC15 is a database developed by combining molecular biology and chemoinformatics fields together. With this database, researchers can access ready-made compounds for virtual screening, ligand discovery [14]. Compounds from the database then screened using RO3 (rule of three). The main purpose of the screening process is to get molecules that have the desired properties so that it becomes a more efficient sample [15]. The rule of three was applied by using Osiris DataWarrior software,(www.openmolecules.org/datawarrior/). Moreover, MOE 2014.09 software was carried out to minimize the ligands and also apply the partial charge, with MMFF94x was used as the ligand force field.

2.3 Molecular Docking Simulation Of Standard Compounds and Fragment Database

In the present study, the molecular docking simulation was performed to prophesy the natural position, orientation, and confirmation of the small-molecule ligand within the binding site of a targeted molecule [10]. In this step, the molecular docking simulation was done by utilizing MOE 2014.09 software [16] in oleuropein, ouabain and fragment database. Oleuropein is one of phenolic compound in olive leaves. It has been proved by Pleśko 2015 that oleuropein can act as an inhibitor of EBOV VP24 [6]. Moreover, Picazo and Giordanetto [17] reported that ouabain has the possibility to inhibit the activity of EBOV VP24 as well [17]. The first molecular docking use retains the value of 30, which means 30 times of placement pose was done for each compound.

In addition, redocking also to validate the results from the previous docking simulation**.** To validate the ligand pose from the first docking, we continued to dock the ligand once more with 'Placement's Retain' value of 100 instead of 30. The ligands with root mean square deviation (RMSD) values lower than 2.0 and Gibbs binding energy ($\Delta G_{binding}$) lower than standards will be selected as the best ligand [10].

2.4 Hits Determination, Fragment Linking, and Screening

In the initial stage, we determined the fragment that bound to an amino acid. Then, we searched for a fragment that did not overlap to the first fragment. Both of these fragments were linked by using linker database in MOE 2014.09 software to generate potential inhibitor ligands. The results are screened using Lipinski's Rule of Five (RO5). RO5 was applied because it can predict drug-like feature closely related to the efficacy of the drug in clinical trials [18]. Then we performed molecular docking and redocking with the same retain as the previous molecular docking parameter. The results obtained from the simulation were saved in .mdb format.

2.5 Analysis Of Protein-Ligand Interaction, Pharmacological and Toxicity Prediction

We determined the protein-ligand interaction by finding the active site of VP24 that bound with low binding energies ligand. At this step, we also applied Toxtree, Osiris DataWarrior, and VEGA to predict pharmacological and toxicity characteristic.

2.6 Molecular Dynamics

In this study, we performed dynamics simulation using MOE 2014.09 software as the final step. The first stage in the simulation is the ligand preparation. Energy minimization and geometry optimization were applied in dynamics simulation. The process of minimizing energy and optimization is almost similar to the docking simulation stage as well as the parameter except for Generalized Born solvation is applied to dynamics simulation consisting of initialization, equilibration, production. At the initialization, the heating process was performed for 10 ps from 300 K to 312 K. At the equilibration stage was done for 100 ps at 312 K, while in the production phase was set to 20,000 ps (picosecond) at 312 K.

3. RESULTS AND DISCUSSION

3.1 Set Up VP24

The protein used in this study was obtained from Protein Data Bank (PDB ID 4U2X) as reported by Pleśko 2015 [6] and Xu [19] with some modification. The interaction between ligand and one of the amino acid residues was considered as a potential inhibitor candidate for EBOV VP24. We minimized the structure of EBOV VP24 when RMS Gradient structure of this protein is 0.05 kcal/mol.Å^2 by using LigX in MOE 2014.09 in Amber10: EHT force field.

3.2 Set Up Fragment Libraries

From ZINC15, we obtained 242.520 compounds. Osiris DataWarrior was used to screen and create the fragment libraries. The rule of three (RO3) was applied for ensuring that fragment libraries contained fragment-like properties and only consisted of simple molecules that can be easy to elaborate [9]-[12]. The molecule properties in the Rule of Three were applied to screen compounds, and it resulted in 6,662 fragments. The criteria of Rule of Three (RO3) follow rules such as the value of molecular weight are less than 300g/mol; the amount of hydrogen bond acceptor and donor are less than or equal three; the value of cLogP is 3, and have less than or equal with three hydrogen bond donor [20].

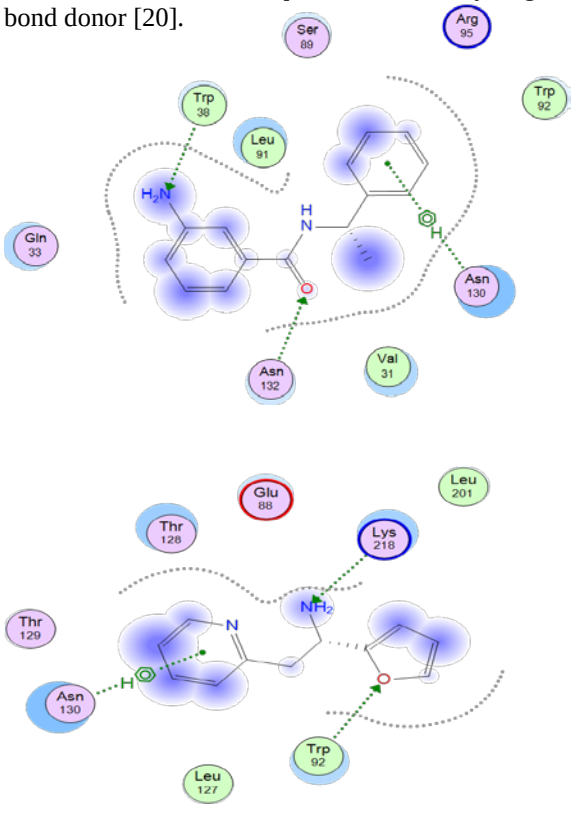


Fig.1 Molecular interaction of the protein EBOV VP24 with fragment 1266 (top) and fragment 440 (bottom)

Besides that, some rules are added in RO3, which is the number of rotatable bonds is less than or equal to three and the value of the polar surface area is 60 Å^2. The construction of fragment libraries used Osiris DataWarrior. Toxicity parameters, such as mutagenic, tumorigenic, irritant and developmental toxicity were applied. Finally, we obtained 1,285 ligands after toxicity screening assay.

3.3 Molecular Docking Simulation Of Oleuropein, Ouabain, and Fragment Database

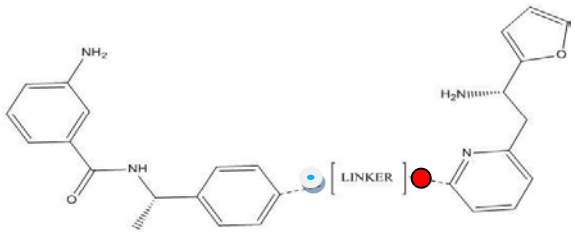
Molecular docking simulation to oleuropein, ouabain, as ligand standards and the fragment database as a sample by using MOE 2014.09 software, which docked about 1,285 ligands and obtained a $\Delta G_{binding}$ score from each ligand. The $\Delta g_{binding}$ score is used to predict which ligand-protein conformation that binds stronger. We also obtained the active site of VP24 at asparagine and tryptophan (Asn130, Asn132, and Trp38). Moreover, we acquired interactions among VP24 with ligands. The first, among asparagine (N130) with fragment number 1266 as shown in Figure 1. This residue in line with what has been reported by Pleśko in 2015 [21]. The other with fragment number 440 as shown in Figure 1. These fragments are the candidate that would be linked to the next phase and each fragment has RMSD value of 1.2275 and 0.8195, respectively.

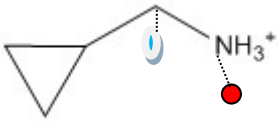
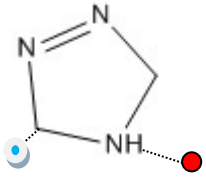
3.4 Prediction Of Linking Fragment and Molecular Docking Of Ligands And Potential Fragment

In this step, we linked fragments that resulted from the previous step. Both of fragments were linked with residue amino acids to form a linking fragment. It was expected that all of the linked fragments will produce higher affinity [20]. The linking process was done by using 'Link Multiple Fragments' feature in MOE 2014.09 software. The result of linked fragments was screening according to 'Energy Minimize' and 'Partial Charge' features.

Molecular docking was applied twice with different retain. The docked ligands were given code L and followed by mseq number. In this study, we chose three ligands for selecting. The redocking process was applied to get root mean square deviation (RMSD) score lower than 2.0 Å [10]. The best ligand is L595, 3-amino-*N*-((*S*)-1-(4-((*E*)-1-(6-((*S*)-2-amino-2-(furan-2-yl)ethyl)pyridin-2-yl)-4-hydoxybut-2-en-2-yl)phenyl)ethyl)benzamide which has $\Delta G_{binding}$ -54,2638 kcal/mol, pKi (inhibition constants) 39,5304 and RMSD 1.5134. The $\Delta G_{binding}$ score, RMSD, pKi and the results from Osiris DataWarrior of three ligands and standard ligands were shown in Table 1 below.

Table 1 $\Delta G_{binding}$ energy, pKi, RMSD score from the selected linked compounds and standard ligands



Ligand	Linker	$\Delta G_{binding}$ (kcal/mol) (RMSD)	pKi	MW	H-D	H-A	cLogP	Polar Surface Area
L833		-54,8520 kcal/mol 1,7644	39,9986	496,633	4	7	2,9530	123,78
L217		-54,8511 kcal/mol 1,9861	39,9582	495,585	3	9	1,8767	135,13
L595		-54,2638 kcal/mol 1,5134	39,5304	496,609	4	7	2,9814	127,40
Oleuropein *		-44,1153 kcal/mol 2,8153	32,1373	540,516	6	13	0,3124	201,67
Ouabain *		-41,2201 kcal/mol 1,1113	30,0282	584,656	8	12	1,4423	206,60

Note: MW (molecular weight), H-D (H-donor), H-A (H-acceptor), the blue circle in linker is linking with the blue circle in fragment 1 (top) while the red circle is linking with the red circle in another fragment (bottom)

3.5 Prediction Drug Scan

In this present study, we also predicted toxicity and drug-likeness for all of the ligands and standard ligands by using Osiris DataWarrior, VEGA and Toxtree software [22]. Parameters mutagenicity, tumorigenic, irritant effect, and developmental toxicity are showed in Osiris DataWarrior analysis. Parameters developmental/reproductive toxicity, potential S Typhimurium TA 100 mutagen based on QSAR, potential carcinogen based on QSAR in VEGA analysis are showed in Toxtree. The results from OSIRIS DataWarrior and VEGA analysis are shown in Table 2 below.

Table 2 Toxicity prediction by Toxtree and VEGA

No	Ligand	Mutagenic	Tumorigenic	Irritant	Develop-mental Toxicity	Repro-ductive Toxicity	Mutagenicity (S.Thypium)	QSAR Carcino-genicity
1	L833	None	None	None	None	No	No	No
2	L217	Low	High	None	High	No	No	No
3	L595	None	None	None	None	No	No	No
4	oleuropein*	None	None	None	None	No	No	No
5	ouabain*	None	None	None	None	No	No	No

Note*: standard ligand

The results toxicity prediction by OSIRIS DataWarrior, Toxtree, and VEGA analysis showed, L595 has same characteristics with ligans standard oleuropein, ouabain. The drug-likeness characteristics according to Lipinski's RO5 and Veber rules. Lipinski's RO5 has several parameters [23]. Ligands have these parameters are considered as potential inhibitor candidates.The molecular weight (MW) of the ligands is less than 500 g/mol, clogP is less than 5.0, have less than 5 hydrogen bond donor and no more 10 hydrogen bond donor and no more 10 hydrogen bond acceptor. The parameters of Veber rules are different with Lipinski's RO5. Veber rules only have 2 characteristics for candidate inhibitor. The first parameter is no more 10 rotatable bonds. The last, polar surface area is equal to or less than 140 Å.

3.6 Molecular Dynamics Simulation

The three selected ligands are continued to the dynamics stage. This stage is the last of these research methods that able to predict the stability of a ligand-complex through three successive stages of initiation, equilibration, and production. From the production stage, we obtained interactions with amino acid residues from three selected ligands. Dynamics simulation, another consideration for choosing the best ligand, the change in RMSD value of not more than 3 Å every 1 nanosecond (ns). The meaning of this change value indicates the stability of the ligand [24]. The change of RMSD value of dynamic simulation result is shown in Figure 2. Table 3 shows there are several amino acid residues found both at the simulation docking stage and dynamics simulation. The presence of some amino acid residues at these two stages also shows the stability of the protein-ligand complex.

Table 3 Interaction L833, L217, L595 with amino acid residues

	L833	L217	L595
Molecular docking	Q33, W38, N132, V31, L91, W92, L127, **K218**, L201, **E88**, T128, S89, l87,T129, N130	L201, **K218**,L127, **S89**, L91, W92,T128, T129, **N130**, **E88**,V31, N132, Q33, **W38**	L201, **K218**, L127, S89, L91, W92,T128, T129, N130, E88,V31, **N132**, Q33, **W38**, I87
Dynamics simulation	N130, **E88**, **K218**, T86, T129, T128, l87, S85, P216, K61, P83, N82, N84	**E88, K218**,L201, T128, **S89**, W92,**N130**, T129, L127,L91,**W38**, Q33 V31, N132	E88, W92, L201, L127, **K218**, T128,T129, L91, N130,V31, **N132**,S89, l87**, W38**, Q33

Note: red color is amino acid residues that interact with the binding site of the protein. Bold font are amino acid residues that interact in molecular docking and dynamics simulation

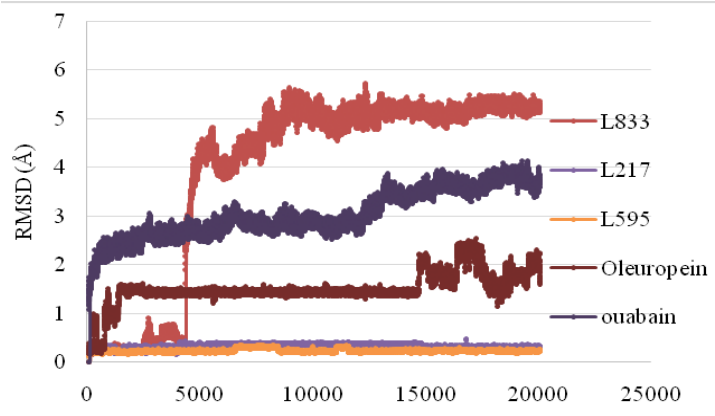


Fig.2 Graph of RMSD (Å) against time (ps)

4. CONCLUSION

This research concluded that L595 is the best ligand from L833 and L217. The L595 has ΔGbinding -54.2638 kcal/mol, and RMSD 1.5134. Toxicity prediction by Osiris Data Warrior, Toxtree, and VEGA showed that L595 can be developed to be a promising lead candidate for inhibiting *Ebolavirus* and more stable than L833 and L217 which have smaller ΔG binding. It is expected that

this research will be continued in vitro and in vivo experiments.

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