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Virtual Screening for Natural Products with Potential Inhibitory Effect on Ebola Virus Glycoprotein

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Abstract: **The latest Ebola virus disease outbreak in West Africa caused over ten thousand deaths. Due to its high fatality rate, patients have only about ten to fifty percent survival rate. According to World Health Organization (WHO), there is no approved drug for Ebola virus disease yet. Thus the need to find the curing drug for Ebola virus disease still persists. Natural products are invaluable in term of drug development. Thus in this study, natural products from ZINC database were used for virtual screening against Ebola virus glycoprotein responsible for the virus infectivity. Result analysis shows that a natural product could potentially inhibit Ebola virus glycoprotein inhibitor.**

Keywords: **Ebola virus, natural products, glycoprotein.**

I. INTRODUCTION

Ebola virus disease is a hemorrhagic fever disease caused by Ebola virus. The disease is highly fatal [1]. Ebola virus belongs to Filoviridae family consisting of three genera: Ebola virus, Marburgvirus, and Cuevavirus [2]. There are five Ebola virus species: Zaire Ebola virus, Bundibugyo Ebola virus, Sudan Ebola virus, Tai forest Ebola virus (formerly referred to as Côte d'Ivoire Ebola virus) and Reston Ebola virus [2]. The most virulent Ebola virus is Zaire Ebola virus which caused the latest Ebola virus disease outbreak in Africa [2].

Currently World Health Organization (WHO) announces that there is still no approved vaccine or drug to cure Ebola virus disease. Thus the search for a drug that can effectively cure Ebola virus disease is still highly needed. Without an effective cure, future outbreak of Ebola virus would still cause large problems to the infected countries as already experienced in the latest outbreak [3]. The resulting deaths, weaken healthcare systems, and economic disruptions would badly affect the infected countries.

Ebola virus genome is single-stranded negative sense RNA genome. The genome is filamentous and non-segmented with the size of 19 kilobases [3]. The genome encodes proteins vital to Ebola virus life cycle as follow: nucleoprotein (NP), polymerase cofactor VP35 and VP40, glycoprotein (GP), transcription activator VP30 and VP24, and RNA-dependent RNA polymerase (L). Glycoproteins on its membrane are responsible for the entry of Ebola virus into the host cell [4]. A secreted form of Ebola virus glycoprotein is also reported but its role in Ebola virus pathogenesis is still unclear [4]. The host cell cysteine proteases cathepsin B and L activate the virus glycoprotein thus enables cellular entry of the virus [5].

Ebola virus has broad cell tropism and can infect many cell types. The reason may be that Ebola virus cell entry involves several cell surface molecules including the receptor-type tyrosine kinases (RTKs), calcium-dependent lectins, and β1 integrin [6]. VP30 of Ebola virus acts as its transcription initiator [7]. Its polymerase cofactor VP35 and viral RNA polymerase L are involved in new genome synthesis of Ebola virus [7]. Its VP24 protein acts as a type I interferon (IFN)

antagonists and an important virulence factor [7]. The VP40 is its major matrix protein [8]. It plays a vital role in Ebola virus budding from plasma membrane of the host cell [9].

If contacted with body fluids or feces from infected patients, healthy people can be infected with Ebola virus. These body fluids include such as blood, saliva, sweat, urine, semen, mucus, vaginal fluids, and vomit [10]. Incubation time of the disease could take about 2 days to 21 days [11]. The disease symptoms include sudden onset of fever, chills, myalgia, and malaise. Then nasal discharge, cough, breath shortness, nausea, vomiting, diarrhea, and abdominal pain would occur [12]. Hemorrhagic symptoms would then follow in severe cases [12].

The latest Ebola virus disease epidemic occurred mainly in West-Africa. However, many cases of the disease were found in other continents [13]. However, these patients contracted the disease in Africa then went to the other continents including North America and Europe.

To detect Ebola virus RNA, reverse transcriptase polymerase chain reaction (RT-PCR) technique can be used [14]. Host antibodies against Ebola virus using serologic tests detects can be used to detect Ebola virus infection [15]. Tests to detect Ebola virus antigen proteins can also be used [15]. Ebola virus RNA still persisted in many patients long after their recovery and was even suggested to be able to persist for a period of time after the dead of its host organism [16].

Natural products are interesting chemical molecules with nature origins and many are valuable resource for drug discovery [17]. For example, more than half of cancer drugs are derive natural products [18]. However, natural products are of diverse properties and many are fully studied in term of the possible use. Thus in term of searching for a new drug, natural products are also interesting choices.

ZINC database is a database containing chemical molecules including natural products [19]. Molecules can be downloaded in various chemical formats. Vendors selling these chemical molecules are also listed. Many of its molecules are linked to PubChem database which is a public chemical database by the National Center for Biotechnology Information (NCBI), National Institutes of Health (NIH) [20].

In PubChem, chemical molecules can be searched using related keywords. Comprehensive information related to the searched molecules would subsequently be provided. The information include PubChem CID, molecular formula, molecular weight, 2D structure, chemical vendors, related literatures, as well as chemical and physical properties of the molecules [20]. Three-dimensional conformers of most chemical molecules are also available [21]. PubChem data have been used in various researches including Quantitative Structure–Activity relationship (QSAR) studies [22].

II. MATERIALS AND METHODS

A three-dimensional structure of Zaire Ebola virus glycoprotein, 5JQ3 [23], was downloaded from PDB [24]. ZINC database was used as the source of natural product structures. Necessary conversions between chemical formats were done using Open Babel [25]. This was because different cheminformatics programs used in this study accept different formats as their inputs. Virtual screening of natural product structures against Ebola virus glycoprotein was done using AutoDock Vina [26] and PyRx [27]. The resulting docked structures were inspected and analysed using Chimera [28], Discovery Studio 4.5 Visualizer [29], and PyMol [30]. Apart from ZINC database, chemical information of the investigated structures were also explored using PubChem. Data preparation and analysis were done using Microsoft Excel. Notepad++ was used for inspecting and viewing data. Computer programming was done using Python 3.4 and Microsoft Visual Basic for Applications (VBA).

III. RESULTS AND DISCUSSION

From virtual screening results, a natural product (Fig. 1) registered as ZINC04147782 in ZINC database was found to interact with Ebola virus glycoprotein with binding energy value of -11.9 kcal/mol (Fig. 2). Its name is 3-[2-[5-(3-morpholinophenoxy)tetrazol-1-yl]-4,8-dioxabicyclo[3.3.0]oct-6-yl]-1-(4-phenoxyphenyl)-ure. From the information in ZINC database, the molecule has apolar desolvation value of 5.9 kcal/mole. Its polar desolvation value is -16.45 kcal/mol. The molecular weight of the molecule is 586.621 g/mol.

Apart from ZINc database, the molecule is also deposited in PubChem database. Its PubChem CID is 11879610 while its PubChem SID is 51342610. The molecular formula displayed is $C_{30}H_{31}N_7O_6$. Its isomeric SMILES is C1COCC N1C2=CC(=CC=C2)OC3=NN=NN3[C@H]4CO[C@H]5[C@@H]4OC[C@@H]5NC(=O)NC6=CC=C(C=C6)OC7=CC

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=CC=C7. InChI Key of the molecule is GXSJPJRAMOLEIB-YVHASNINSA-N. The number of hydrogen bond acceptor is 10 while the nnumber of hydrogen bond donor is 2. Its xlogP value is 2.8. It has 4 defined atom stereo centers. Its topological polar surface area is 134 angstrom^2.

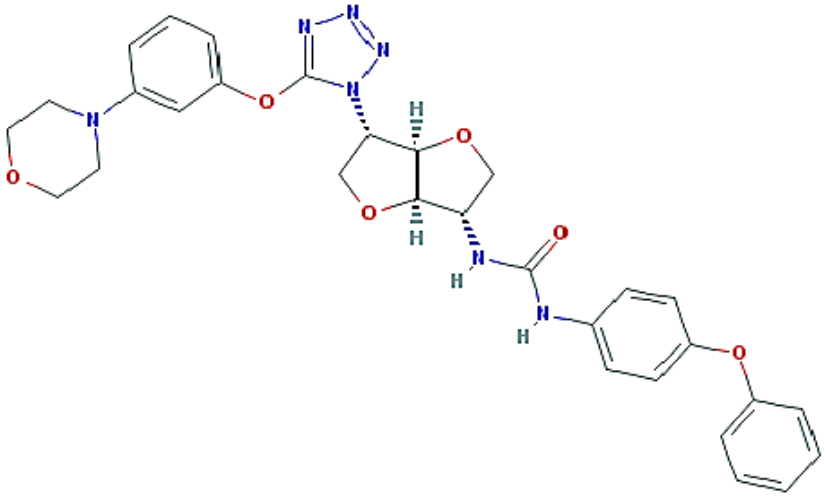

Fig. 1. Natural product ZINC04147782 was found to interact with Ebola virus glycoprotein. The structure shown was taken from its PubChem entry

The interacting area between ZINC04147782 and Ebola virus glycoprotein (Fig. 2) was similar to the area found in the interactions between lead inhibitor molecule 8a and Ebola virus glycoprotein (Fig. 3). Lead inhibitor molecule 8a was reported to specifically inhibit Ebola virus glycoprotein and affect the virus infectivity [31]. Thus this natural product could potentially inhibit Ebola virus glycoprotein in similar manner to that of lead inhibitor molecule 8a. By investigating residues involved in the binding between the natural product and Ebola virus glycoprotein, the interactions found seem to be strong with many interacting points (Fig. 4). Thus the binding should be strong enough to facilitate its effect on Ebola virus glycoprotein.

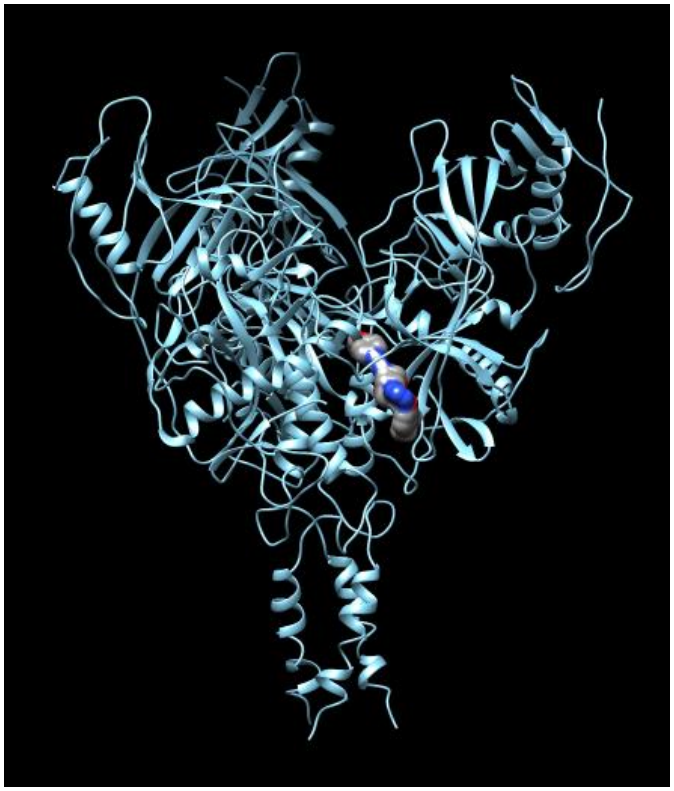

Fig. 2. The interacting area between natural product ZINC04147782 and Ebola virus glycoprotein

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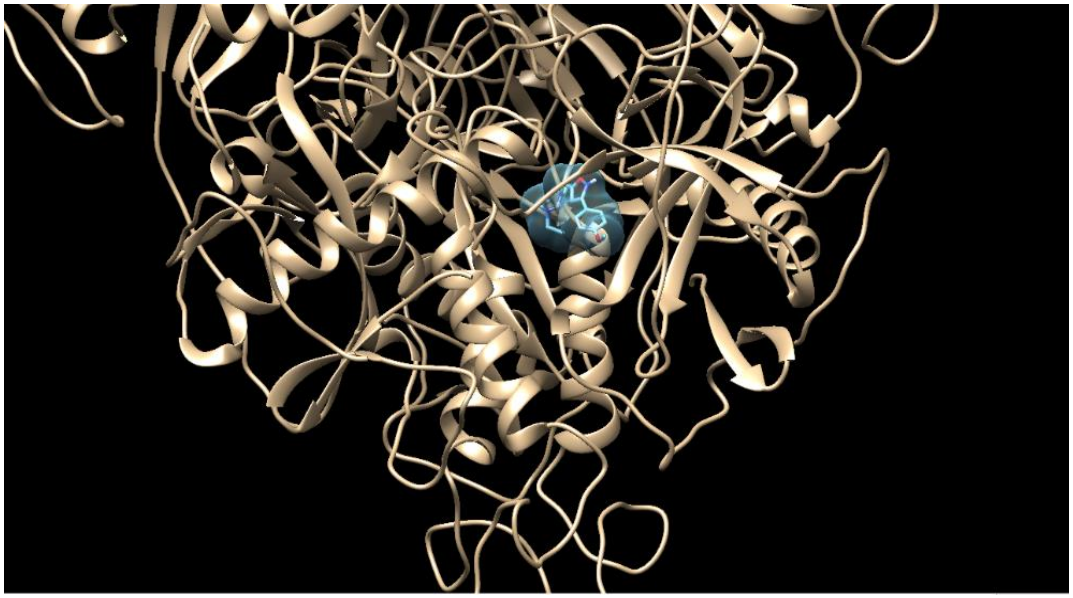

Fig. 3. The interacting area between Ebola virus glycoprotein and lead inhibitor molecule 8a. The lead molecule was previously reported to specifically inhibit Ebola virus glycoprotein [31]

Some of the residues involved in the interactions between lead inhibitor molecule 8a and Ebola virus glycoprotein were also involved in the interactions between ZINC04147782 and the glycoprotein (Fig. 5). Although some residues were not directly involved in the interaction, their three-dimensional arrangement around the interactions between ZINC04147782 and the glycoprotein suggested that ZINC04147782 would shield these amino acid residues and consequently would prevent them from functioning.

Natural products are large in number and are of diverse nature. Thus many natural products are left uninvestigated. This is also the case for ZINC04147782 which is just one of many natural products listed in the ZINC database. No previous study ragarded this molecule is found. Thus future work regarding the use of this natural product to inhibit Ebola virus glycoprotein and its other properties including toxicity should be further investigated.

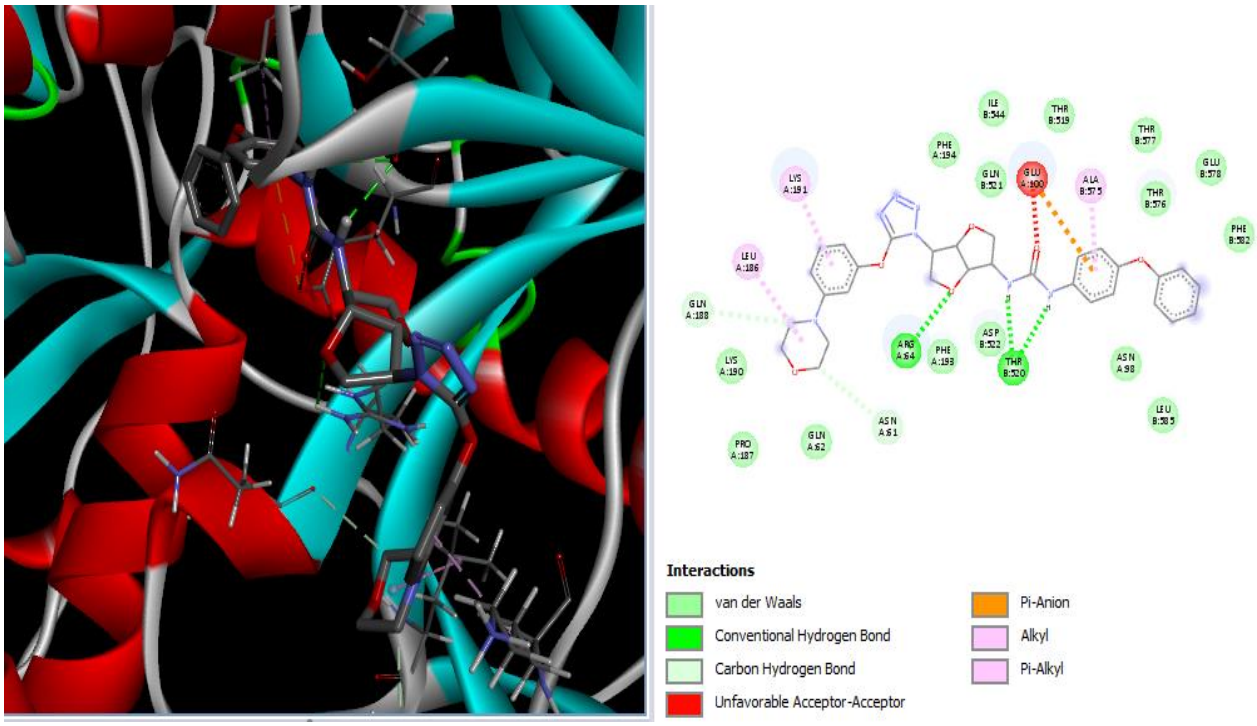

Fig. 4. Residues involved in the interactions between Ebola virus glycoprotein and natural product ZINC04147782

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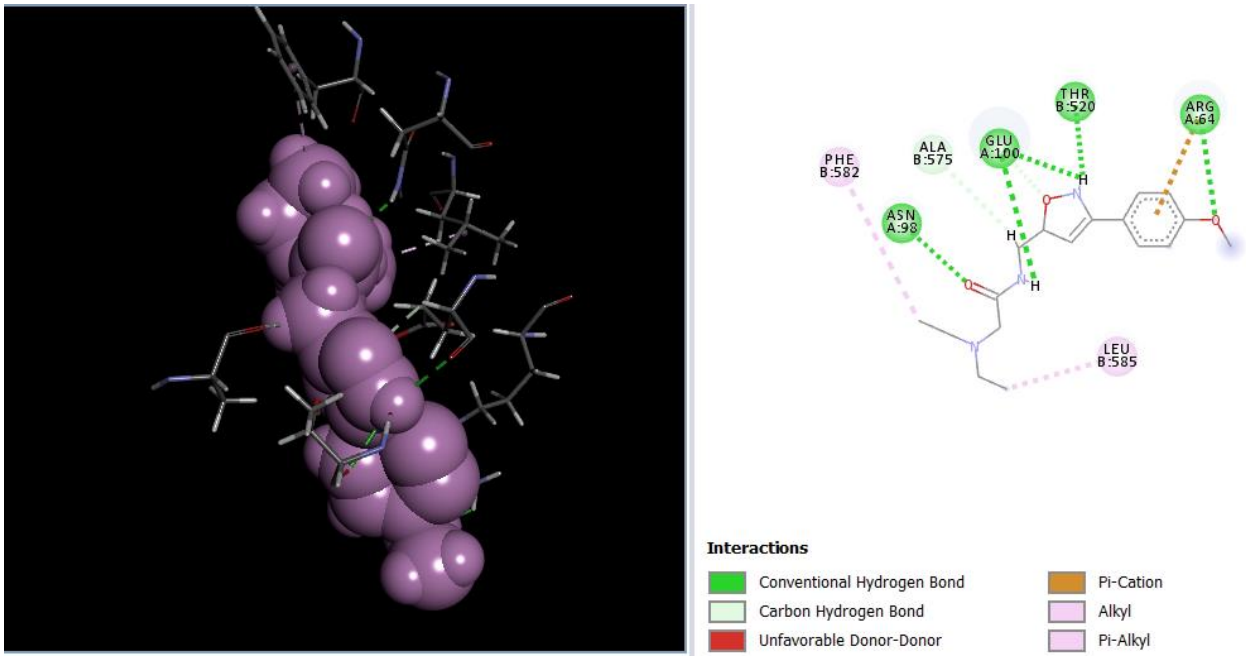

Fig. 5. Residues involved in the interactions between Ebola virus glycoprotein and lead inhibitor molecule 8a

IV. CONCLUSION

As Ebola virus glycoprotein involved in the infection of Ebola virus to its host cell, a chemical molecule that can suitably bind to Ebola virus glycoprotein would inhibit the glycoprotein's functioning. This would result in the inhibition of Ebola virus infection. From this study, a particular natural product, ZINC04147782 from ZINC database, interacts with Ebola virus glycoprotein in similar area to that found in the interaction between lead inhibitor molecule 8a and Ebola virus glycoprotein. Details about residues involving in the interactions of the two cases also suggest that this natural product could bind to and inhibit Ebola virus glycoprotein. Thus it is interesting to further investigate about the inhibiting effect of this natural product toward Ebola virus glycoprotein in details.

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