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LETTERS

Homology modeling and docking studies of VP24 protein of Ebola virus with Oseltamivir and its derivatives

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ABSTRACT

Homology model of VP24 protein

Ebola hemorrhagic fever (EHF) is a highly contagious disease of high mortality and can affect humans as well as nonhuman primates. Oseltamivir is the only frontline drug available for the treatment of patients infected by Ebola virus. Oseltamivir only alleviates the symptoms of EHF that is closely similar to those of influenza. The viral protein 24 (VP24) of virus helps in formation of nucleocapsids and plays an important role in virulence. Therefore, in the present *in-silico* study, an attempt was made to construct the protein model of VP24 using homology modeling to study potential inhibitory action of oseltamivir against VP24. The protein PDB validation was done by Ramachandran Plot. The validated PDB of the VP24 protein was used for docking with Oseltamivir related derivatives (zinc database). Twenty new inhibitors identified based on better docking score and proposed for further in vitro and in vivo studies.

Keywords: VP24 protein, Homology modeling, Ramachandran plot, Oseltamivir, Docking

INTRODUCTION

Ebola virus is a single stranded RNA virus which has a filamentous structure and belongs to family of RNA virus called Filoviridae.[1,2] The genus Ebola has five species- Bundibugyo Ebolavirus (BDBV), Zaire Ebolavirus (EBOV), Reston Ebolavirus (RESTV), Sudan Ebolavirus (SUDV) and Taiforest Ebolavirus (TAFV). The genes of Ebolavirus are arranged linearly on a negative stranded RNA molecule. These genes encodes for seven structural proteins- nucleoproteins (NP), virion structural proteins (VP) VP35, VP40, Glycoprotein (GP), VP30, VP24 and RNA- dependent RNA polymerase (L).[3] It first appeared in Sudan and Democratic republic of Congo simultaneously in 1976. Ebola virus causes severe hemorrhagic fever and is therefore a fatal disease in humans and non-human primates.[1, 2] Ebola virus has a fatality rate of 90% in humans.[4] The recent epidemic occurred in 2014 and affected multiple countries in West Africa and also a new case was reported on January 14, 2015 in Sierra Leone.[5] Ebola virus can be transmitted by contact with infected person, fomites and also through sexual contact.[6,7]

Ebola viral protein (VP24) is a secondary matrix protein which has various roles in virulence of virus. VP24 interferes with the interferon signaling pathway by binding to karyopherin-α and blocking the Signal Transducers and Activators of Transcription (STAT-1) signalling pathway.[8,9] Infection with Ebola virus blocks the production of alpha and beta interferon in the cell and thereby blocks the cells' response.[10] Together with other viral proteins- Nucleoprotein (NP) and VP35, it is very necessary for the correct assembly and formation of functional nucleocapsids. It is thus involved in packaging of virus and in turn plays an important role in virulence.[9,11] Since VP24 is present on the surface of viral envelope that has an affinity for plasma membrane.[12] This protein is also essential for the replication of other proteins of virus as was suggested by Mateo *et al* and because of these reasons VP24 protein was selected for this study.[9]

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Oseltamivir, commonly known as Tamiflu®, is used to treat viral fever. It is an acetamido cyclohexene that is a structural homolog of sialic acid and inhibits neuraminidase. It is a competitive inhibitor of viral neuraminidase (NA) enzyme. By blocking its activity, it prevents the release of new viral particles through the cleaving of terminal sialic acid on glycosylated hemagglutinin.[13] Due to its wide acceptance/ high prescription rate, this drug is selected for our study.

The FASTA format of VP24 (GenBank: ALG02124.1) was taken from National Centre for Biotechnology Information (NCBI). Various templates were obtained from PHYRE2 which is a protein structure prediction server. Then, homology modeling was done using EasyModeller4.0 software. The validation of modeled protein structure was done by Ramachandran plot Assessment (RAMPAGE). After that, the drug Oseltamivir and its derivatives were retrieved from the Drug Bank and Zinc Database, respectively. Then, docking was performed of the drug and its derivatives with the modeled protein structure.

The best model was selected by validating the structure by Ramachandran plot. The docking between the ligands and modeled protein gives the docking score of more than 5.7 Kcal/mol. This implied that the ligands interact with the protein strongly and the hydrogen bonding and hydrophobic interactions are the dominant forces involved in binding.

EXPERIMENTAL PROTOCOLS

Bioinformatics Analyses

By utilizing the protocol involved in Computer Aided Drug Designing, the amino acid sequence of VP24 of Ebola virus with accession number ALG02124.1 was retrieved from Protein Database, PDB (www.rcsb.org) from National Centre for Biotechnology Information, NCBI (www.ncbi.nlm.nih.gov). The most suitable template was identified using protein BLAST (BLASTP) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and selected for homology modeling. Through Protein Homology Recognition Engine Server, PHYRE2 (www.sbg.bio.ic.ac.uk/phyre2), the most widely used method for protein structure prediction and its analysis[15], the FASTA format of VP24 protein was uploaded, which gave many templates. Out of which, eight templates were selected on the basis of their percentage identity.

Homology Modelling

Modeller is software that is used for the generation of homologous models of protein structure. For generation of structure of VP24 (accession number ALG02124.1), matrix protein of Ebola virus, the selected templates were analysed comparatively through EasyModeller 4.0 (http://easymodeller-40-new-gui-to-modeller.html).[16] General features were evaluated on the basis of Modeller's energy and DOPE score. The generated models were then minimized by YASARA Energy Minimization Server.[17] The validation of generated model was evaluated using Assessment of Ramachandran Plot (RAMPAGE)server (http://mordred.boc.cam.ac.uk/~rapper/rampage.php), which is used to identify the best protein for homology modeling.[18]

Molecular Docking

The Drug Bank database is a unique resource that provides the information about drugs and its targets. The antiviral drug 'Oseltamivir' was retrieved from Drug Bank (www.drugbank.ca) on the basis of "Lipinski's Rule of Five" for docking analysis. The derivatives of this drug were obtained from Zinc Database (www.zinc.docking.org), which contains a collection of commercially available chemicals compounds. The docking analysis of Oseltamivir and its derivatives with Ebola virus matrix protein VP24 was carried out using Schrodinger suit with an automated docking tool called Glide which is designed to predict the size of a molecule, such as drug where it is binding to a receptor, and their images was visualized by Discovery Studio Visualizer (accelrys-discovery-studio-visualizer.software.informer.com/4.5), a viewer that can be used to open data generated by other software in the Discovery Studio.[19-21]

RESULTS AND DISCUSSION

The spread of Ebola virus and its fusion with host cell membrane can be controlled by inhibiting the protein that is involved in virulence of virus. VP24 is a protein that plays an important role in transcription of other proteins of Ebola virus, packaging of virion particles and hence in virulence.VP24 is the viral matrix protein which involves in the packaging of virion particles and virulence. VP24 sequence c3ee1A has been retrieved from NCBI and numbers of structures were generated by homology modeling using Easy modeler software. The best model has been selected keeping the factors like maximum core region, minimum disallowed region and minimum energy in the consideration. Thereafter, RAMPAGE server was used to verify the structure of VP24 protein. The newly formed model of protein VP24 consist 250 amino acids with alpha helix, beta sheets and loops as clearly shown in figure1.

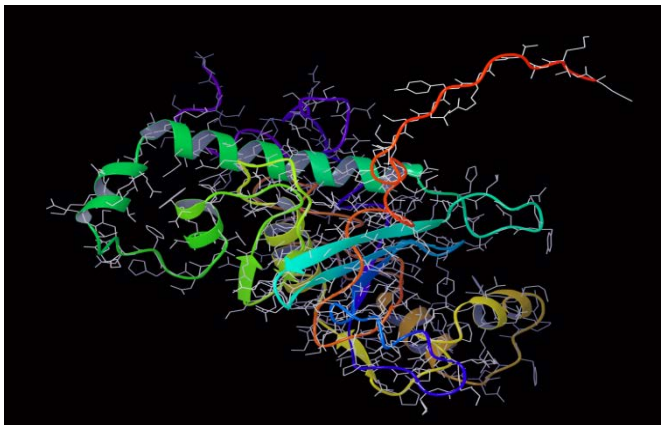


Figure 1. Secondary structure of predicted VP24 protein

The validation of the best generated model was done by analysis of Ramachandran plot generated using RAMPAGE server. Homology model of VP24 was identified by the analysis of Ramachandran plot which shows that 224 amino acid residues (90%) lies in the favoured region, 19 residues (7.6%) lies in allowed region and 6 residues (2.4%) lies in the outlier

region as shown in Figure 2. This model was selected because the residues lying in the outlier region were less in comparison to the other models.[2]

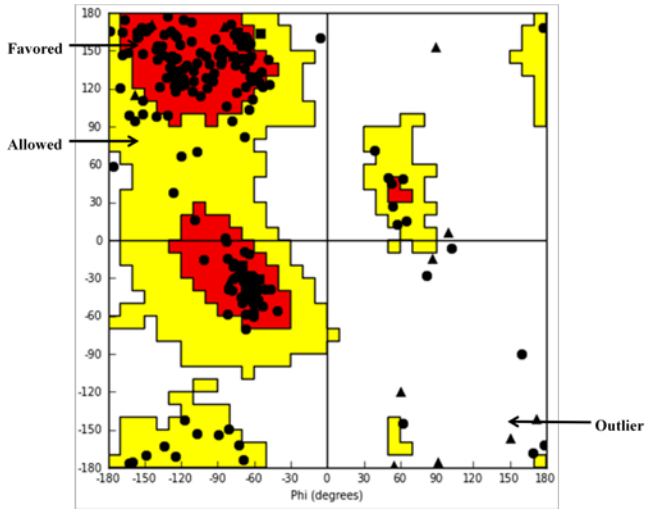


Figure 2. Ramachandran plot of predicted VP24 protein

Gupta *et al*[14] has predicted the active sites of Oseltamivir: Tyr (406), Ser (294), Arg (224). After this, Oseltamivir and its derivatives were docked with the modeled VP24 protein using Autodock. Figure 3 showed the three dimensional docked model of Oseltamivir for VP24. The descriptive teo dimensional interaction map for docking of Oseltamivir with VP24 was shown in figure 4.

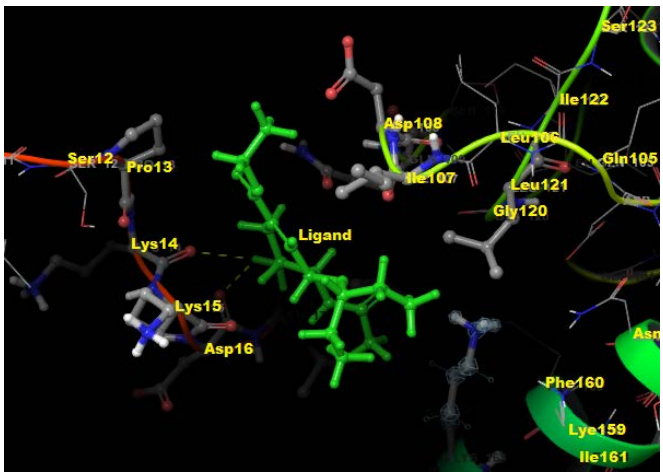


Figure 3. 3D docked model of Oseltamivir with VP24 protein

Both figure 3 and figure 4 show tight binding of Oseltamivir in the active site of VP24 protein. Figure 4 clearly shows the interacting amino acid residues inside the binding site within 4Å resolution for Oseltamivir. Residue Asp 16 and Lys 14 forms hydrogen bond with hydrogen atoms of amine group attached with cyclohexene ring of Oseltamivir. Besides, Ile 181, Val 21, Pro13, Leu121, Ile107 showed hydrophobic interactions with the ligand. Other residues as shown contributes toward binding via other non-covalent binding interactions.

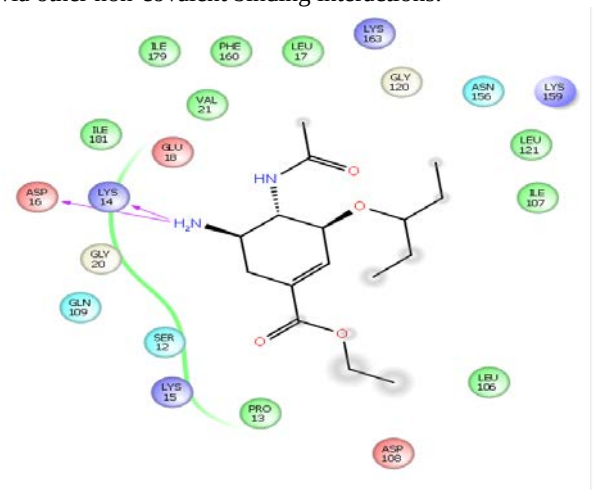


Figure 4. Two dimensional interaction map for docking of Oseltamivir with VP24 protein.

Similarly to Oseltamivir, other derivatives of Oseltamivir from zinc database were docked with modelled VP24 protein. The top docking scores for 20 Oseltamivir derivatives with their interacting residues are listed in Table 1.

From Table 1, it is evident that VP24 protein and oseltamivir bind with dock score -5.8 Kcal/mol. On the other hand, among the top 20 derivatives of oseltamivir retrieved from Zinc database, various derivatives have been predicted as better inhibitor of viral protein because they have a docking score of more than -5.8 Kcal/mol.

For representation, Oseltamivir derivative Zinc_77287098 which showed best docking with VP24 protein is shown as three dimensional docked model in figure 5.

Oseltamivir, the only standard drug present for the treatment of Ebola fever, binds with the modeled protein. But its derivatives also show binding interactions with the modeled proteins with a good docking score implying that these derivatives may also inhibit the protein. But, the 13 derivatives given in table 2, display even better inhibition of the modeled protein because they have a docking score of more than -5.8 Kcal/mol.

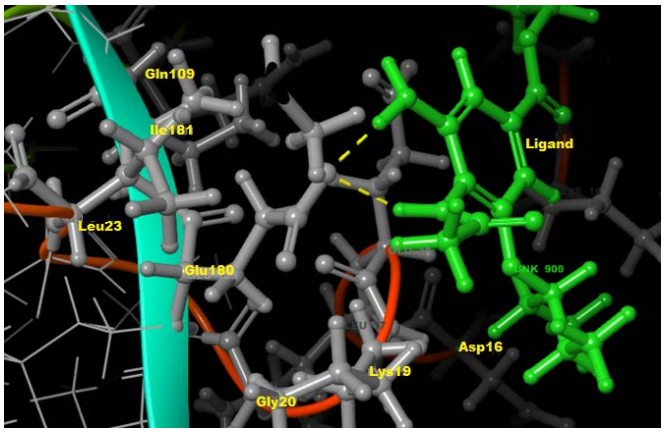


Figure 5. 3D docked model of Zinc_77287098 with VP24 protein

Table 1. Molecular docking interactions and their binding energies

S. No.	Ligand	Interacting Amino Acids	Dock Score (Kcal/mol)
1.	Oseltamivir	Asp 16, Asp 16, Ile 181, Val 21, Pro13, Leu121, Ile107	-5.8
2.	Zinc_77312077	Arg154, phe245, Leu150, Gly44, Ala43, Ile102	-5.7
3.	Zinc_77287101	Val40, Ile157, Arg154, Gly44, Phe245, Leu98	-5.8
4.	Zinc_77287100	Ile102, Leu98, Thr129, Asn130	-5.9
5.	Zinc_77287098	Arg154, Val40, Trp42, Gly44, Ile102, Leu98, Ile45, Tyr41, Ala43, Ile157, Phe230, Leu158	-6.1
6.	Zinc_77287096	Ile102, Ile153, Ala43, Val40, Gly44	-6.0
7.	Zinc_77286930	Val40, Leu98, Arg154, Gly44, Ala43, Leu150	-5.8
8.	Zinc_77286925	Glu180, Leu23, Ile181, Pro13, Leu176	-6.1
9.	Zinc_65748305	Phe245, Ile153, Ile157, Gly44, Ala43, Ile102	-5.9
10.	Zinc_36451498	Arg154, Ile157, Phe245, Leu98, Ala43	-6.0
11.	Zinc_11592802	Ile157, Arg154, Val40, Ala43, Gly44	-6.0
12.	Zinc_6777830	Ile102, Asn130, Leu98, Arg95, Thr129, Asn132	-5.9
13.	Zinc_6777829	Ser178, Leu75, Phe76	-5.8
14.	Zinc_6777828	Val40, Gly44, Ile102, Arg154	-5.9
15.	Zinc_6777826	Phe245, Arg154, Val40, Ile102, Ile153, Gly44	-5.7
16.	Zinc_3929509	Phe245, Ala43, Gly44, Leu158, Phe230	-6.1
17.	Zinc_3929508	Val40, Leu98, Leu156, Ile153, Ala43, Gly44	-5.9
18.	Zinc_3874571	Val40, Leu98, Ile153, Gly44, Ala43, Leu158	-6.1
19.	Zinc_3874570	Leu98, Arg95, Asn130, Thr129, Thr131	-6.0
20.	Zinc_3874569	Asn130, Ile102, Arg95, Leu98, Thr131, Thr129	-5.8
21.	Zinc_3874568	Ile102, Leu98, Asn130, Thr129, Gln103, Phe134	-5.8

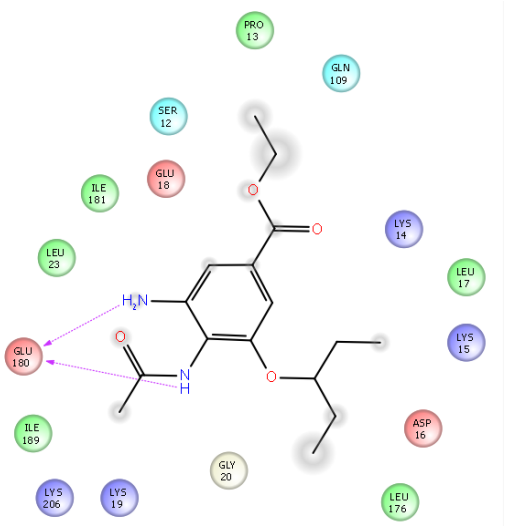
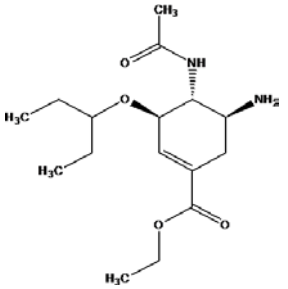
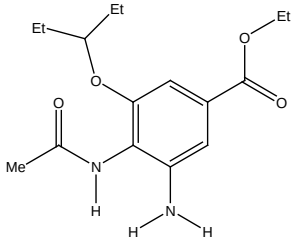
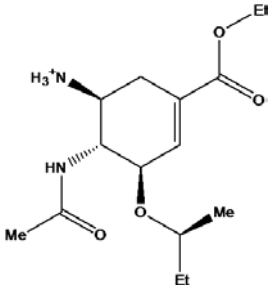
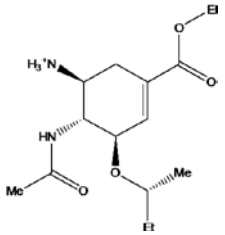
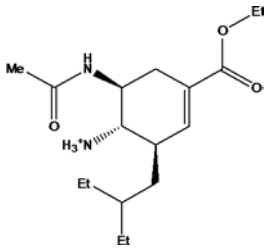
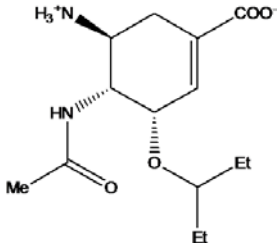
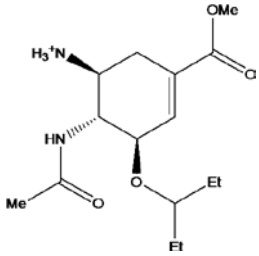
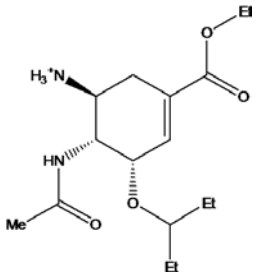
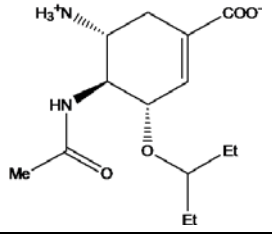
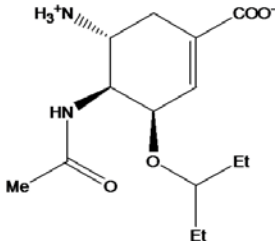
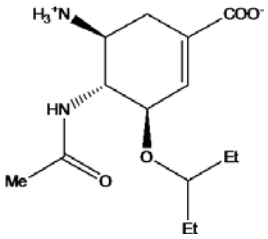
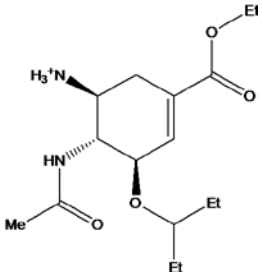
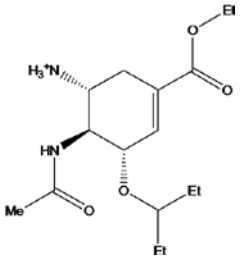
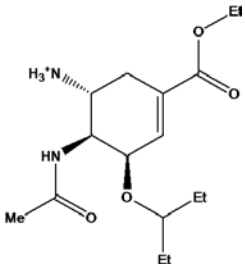


Figure 6. Two dimensional interaction diagram displays interaction of Zinc_3874571 with VP24 modeled protein.

Because of their better inhibition of modeled protein, these derivatives can also be proposed for further preclinical animal studies for enhancing better health preparedness against Ebola hemorrhagic fever.

Table 2. Molecular structure of Oseltamivir and its derivatives with better inhibition of modeled protein

S.No.	Ligand	Structure
1.	Oseltamivir	
2.	Zinc_77287100	
3.	Zinc_77287098	

4.	Zinc_77287096	
5.	Zinc_77286925	
6.	Zinc_65748305	
7.	Zinc_36451498	
8.	Zinc_11592802	
9.	Zinc_6777830	
10.	Zinc_6777828	
11.	Zinc_3929509	
12.	Zinc_3929508	
13.	Zinc_3874571	
14.	Zinc_3874570	

CONCLUSION

Homology modeling and validation of the targeted protein gives the best secondary structure of the protein that was docked with Oseltamivir and its derivatives. The docking has proved that Oseltamivir's derivatives can also inhibit the targeted viral protein and thereby the virulence of Ebola virus. But the predicted compounds has to undergo clinical trials for proving their efficiency in the treatment of disease. The above work will help the researchers working on finding a treatment for Ebola hemorrhagic fever.

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