

Computational Drug Design against Ebola Virus Targeting Viral Matrix Protein VP30

Arthi Venkatesan 

Lavanya Ravichandran 

Febin Prabhu Dass J* 

Department of Integrative Biology,
Vellore Institute of Technology, Vellore,
Tamil Nadu, India

*email: mail2febin@gmail.com

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Abstract

Ebola viral disease (EVD) is a deadly infectious hemorrhagic viral fever caused by the Ebola virus with a high mortality rate. Until date, there is no effective drug or vaccination available to combat this condition. This study focuses on designing an effective antiviral drug for Ebola viral disease targeting viral protein 30 (VP30) of Ebola virus, highly required for transcription initiation. The lead molecules were screened for Lipinski rule of five, ADMET study following which molecular docking and bioactivity prediction was carried out. The compounds with the least binding energy were analyzed using interaction software. The results revealed that 6-Hydroxyluteolin and (-)-Arctigenin represent active lead compounds that inhibit the activity of VP30 protein and exhibits efficient pharmacokinetics. Both these compounds are plant-derived flavonoids and possess no known adverse effects on human health. In addition, they bind strongly to the predicted binding site centered on Lys180, suggesting that these two lead molecules can be imperative in designing a potential drug for EVD.

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INTRODUCTION

Ebola virus (EBOV) is the deadly etiological agent of Ebola viral disease (EVD), earlier known as Ebola hemorrhagic fever. It belongs to the Filovirus family and order Mononegavirales, which contains negative-sense single-stranded RNA with a protective envelope (Geisbert & Jahrling, 1995). The first outbreak was recorded in 1976, following which EVD outbreaks have proved to be persistently pandemic while the mortality rate is 90% (Feldmann & Geisbert, 2011). According to WHO, five strains of EBOV have been identified, causing a total of 25 outbreaks and differentiated based on virulence properties and geographical patterns observed (Picazo & Giordanetto, 2015). Zaire Ebola virus (ZEBOV), BundibugyoEbola virus (BDBV), Reston Ebola virus (RESTV), Sudan Ebola virus (SUDV), and Tai Forest

Ebola virus (TAFV) are the five strains named after the places affected by the outbreaks. The latest outbreak in 2014 was caused by the most lethal Zaire strain in West Africa, resulting in 11,000 deaths (Baize *et al.*, 2014). Though the reservoirs are yet to be confirmed, fruit bats, gorilla, antelopes and chimpanzees are considered to be potential hosts for serious transmission (Ascenzi *et al.*, 2008). Followed by this, in humans, it is spread through blood and contact with fluid body secretions. The infection projects itself in two phases with symptoms ranging from fever, fatigue, rashes, vomiting in the early phase, whereas in the late phase hemorrhagic shock occurs eventually resulting in death (Chiappelli *et al.*, 2015). Several diagnostic and laboratory tests are used to examine this deadly virus. Some of the investigations are

CBC profile, ELISA, RT-PCR and cell culture techniques (Broadhurst *et al.*, 2016).

The filamentous EBOV is composed of approximately 19kb genome (Yu *et al.*, 2017; Zhao, 2016) with genes coding for 4 structural proteins such as VP30, VP35, nucleoprotein (NP), polymerase protein and three membrane-associated proteins such as VP40, glycoprotein and VP24 (Mühlberger, 2007; Easton *et al.*, 2018). The two molecular forms of viral glycoprotein (GP1 & GP2) in combination with host proteins like Niemann-Pick C1 (NPC1) mediate viral entry (Lee & Saphire, 2009). Once the viral attachment is complete through the GP1 – host cellular receptor interaction, the virions enter the cell through endocytosis. The three-domain matrix protein VP40 facilitates virion maturation and viral assembly (Hoenen *et al.*, 2005). VP40, which is present profusely, also maintains viral integrity (Passi *et al.*, 2015). VP35 is bi-functional by both being a part of viral replication and also inhibiting IFNs production in the host cell (Basler, 2015).

The nucleoprotein, along with RNA, forms the helical complex whereas VP24 inhibits host STAT1 signaling pathway and mediates nucleocapsid formation. The phosphoprotein VP30, 30 KDa in size, is a major component for viral RNA synthesis and initiates transcription (Cantoni & Rossman, 2018). The interaction of VP30 with nucleocapsid (NP) brought by the phosphorylation of the N-terminal region, directly affects the transcription process (Biedenkopf *et al.*, 2013). Thus, the VP30 bound NP complex is required for transcription initiation (Weik *et al.*, 2002). The ribonucleoprotein is released from the virion and serves as a template for transcription. Mutation studies reveal that the Phosphorylation sites of VP30 at serine residues is the core requirement for transcription (Martínez *et al.*, 2008). At present, there is no licenced anti-viral treatment available for EVD (Schuler *et al.*, 2017). Factors such as

high resistance, the requirement of advanced biosafety levels in clinical trials and the need for further exploration of pathophysiology and complex molecular mechanism makes it highly challenging to combat EBOV (Dhama *et al.*, 2018). Several *in silico* drug design approaches prove potentially useful for developing anti-viral drugs against EBOV disease as computational biology provides various tools to obtain inherent knowledge of protein structure and interactions to identify potential lead molecules (Schuler *et al.*, 2017). Also, they aim to simulate models of the real world that requires validation through wet-lab experiments.

Most of the Ebola drug design research focuses on targeting VP35 and VP40 because of their importance in viral replication and assembly (Nasution *et al.*, 2018; Roca *et al.*, 2015) but the recent findings describe that intermolecular interactions between VP30 and NP regulate viral mRNA synthesis. NP binds to VP30 at a strongly conserved region in a narrow groove (Xu *et al.*, 2017). This indicates that VP30 is a promising drug target to block viral replication at the initial stage. Hence, this computational approach identified and analyzes the properties of drug-likeness to filter compounds and were subjected to molecular docking identifying potential inhibitors based on binding energy and interaction properties.

MATERIALS AND METHODS

Identification of lead molecules

One hundred and twenty lead molecules with known antiviral properties were selected from literature search (De Clercq, 2015; Huggins *et al.*, 1999; Kouznetsova *et al.*, 2014; Schuler *et al.*, 2017; Shen *et al.*, 2015; Vlietinck *et al.*, 1998). These 120 compounds were from both synthetic and non-synthetic (plant) sources. The SMILES file format of the compounds were retrieved from PubChem database (Kim *et al.*, 2016). SMILES are line notations

distinct for different structures that are required to obtain two-dimensional and three-dimensional models of the molecules. Later the SMILES format was converted into PDB format using online CORINA server that generates 3D structures (Sadowski *et al.*, 1994).

Protein structure preparation

The protein crystal structure of Ebola VP30 protein (PDB code: 5DVW) with the resolution of 1.75Å was retrieved from RCSB Protein Data Bank (PDB), a repository providing crystal structures of biotic macromolecules (Berman *et al.*, 2000). The data in PDB is deposited from various researches based on the results of X-ray crystallography and NMR spectroscopy and is validated using defined standards of stereochemistry (Gore *et al.*, 2017). The water molecules and heteroatoms were removed, and the protein molecule was subjected to energy minimization using Swiss PDB Viewer, a tool for comparative protein analysis (Guex & Peitsch, 1997).

Analyzing properties of drug-likeness

All the 120 lead compounds were analyzed for Lipinski rule satisfaction and subjected to ADMET analysis using pkCSM online server (Pires *et al.*, 2015). Lipinski rule of five is the primary criteria to evaluate small lead molecules. This aids in discerning capable drug-like molecules using filters and thus enabling to predict clinical success with higher probability. The five parameters of this rule (Lipinski, 2004), demands the molecular weight to be higher than 500, more than five hydrogen donors and greater than 10 Hydrogen acceptors. Also, the Log P- value should be greater than 5.0 and with ten or more than ten rotatable bonds. The first three parameters correlate with 90% clinical success. Rotational bonds higher than ten indicate low bioavailability and the ideal value of log P or log D indicating lipophilicity is between 0-3. Compounds that met these criteria were subjected to ADMET analysis.

ADMET analysis allows to quickly scan through 140 properties to identify lead compounds that are potential with high binding affinity and non-toxic to the human body while absorption and distribution rate are other key factors (Venkatesan *et al.*, 2018). Performing ADMET tests *in vivo* are highly time-consuming and are not cost-effective. Computational tools serve to provide quick screening of toxicity and pharmacokinetic properties. One such tool is pkCSM that represent chemical entities mathematically based on graphical modeling. Some parameters are drug-specific, and in our study parameters such as water solubility, intestinal permeability, not less than 30%, CaCO-2 permeability that predicts absorption of orally administered drugs, AMES toxicity to test mutagenicity, hepatotoxicity and inhibition of hERG potassium channels which indicates the drug can lead to QT syndrome were considered. Finally, the filtered compounds were subjected to molecular docking study.

Binding site analysis of VP30

The functional residues of VP30 were analyzed to know the active binding site. Based on mutagenesis analysis ARG179, LYS180, and LYS183 in the secondary nucleoprotein VP30 have been identified as the base residues, which are involved in consorting itself with the nucleocapsid activation of transcription (de La Vega *et al.*, 2015). Mutations in these residues lead to loss of transcription function. The binding site centered on Lys 180 can thus be considered as an expedient target for small inhibitory molecules (Salata *et al.*, 2019). CASTp server (Binkowski *et al.*, 2003) is an online resource for studying surface topography and functional region of proteins used to analysis binding site residues. It depicts concave cavities on protein surfaces and allows the user to explore the interior of the protein providing information about the functional notations.

Molecular docking

Molecular docking is an *in silico* tool that has an increasing significance in drug design (de Ruyck *et al.*, 2016). It allows characterization of protein-ligand interaction at the atomic level and combines the properties of quantum and molecular mechanics (Aminpour *et al.*, 2019). The requisites of docking include the tertiary structure of the protein, pdbqt format of ligand, binding mode and involves four classes of scoring. While docking can be rigid or flexible, we performed rigid docking where the bond length and angles are kept constant. Compounds filtered from ADMET analysis were subjected to docking using AutoDock 4.2 (Forli *et al.*, 2016). Protein preparation is the vital step of docking that requires the removal of water molecules, whereas hydrogen atoms and Kollman charges were added (Xie *et al.*, 2015). The size of the grid box was set to 84 × 90 × 92 to enable docking only within the binding site. A threshold of -6.5 kcal/mol was set to select potential inhibitors. The Lamarckian genetic algorithm was used to allow flexible docking between ligands and the receptor. Out of 10 confirmations obtained, the first two compounds with the least binding energy were taken as the best-docked conformations (Garcia-Godoy *et al.*, 2015). The docking results were analyzed by comparing with the control drug BCX4430, an antiviral adenosine analog from BioCryst Pharmaceuticals. It blocks the function of viral polymerase and acts against a broad range of Filovirus by instigating RNA chain termination (Warren *et al.*, 2014). Intramuscular injection of this drug confers protection in rodents exposed to EBOV, thus, making it a controlled drug for docking studies of EBOV (Raj & Varadwaj, 2016). The control drug was docked with VP30, and its interactions were studied in similar conditions. The docking results were visualized using PyMOL software that delivers high-quality 3D images of the ligand-bound

to its protein (Seeliger & de Groot, 2010). The interacting residues for the control and test compounds were analyzed using LigPlot+, a graphical system that reads 3D coordinates and fosters details of protein-ligand interaction as several two-dimensional figures (Laskowski & Swindells, 2011).

Bioactivity score prediction

In silico screening of structural properties related to the pharmacological activity of the selected molecules can aid in understanding their similarity to the known drugs. Molinspiration, a web-based tool was utilized to calculate the bioactivity score of the best-docked complexes and the control drug. Indispensable properties involved for a lead molecule to bind to its receptors of drug target like GPCRs, nuclear receptors, ion channel modulation, inhibition of kinases, proteases and enzymes were calculated (Khan *et al.*, 2017). The input is imported as SMILES structure, and the predicted bioactivity of the screened compounds is given as a numerical score between -5 and +5.

RESULTS AND DISCUSSION

ADMET results and active site residues

This computational study was initiated with the screening of retrieved 120 antiviral compounds for ADMET properties. The results from pkCSM tool for Lipinski rule and ADMET analysis filtered thirty-six lead molecules fulfilling all five criteria of Lipinski rule with acceptable solubility, absorption and possess no known toxic or mutagenic effects were shown in **Supplementary Table I**. The protein structure of VP30 from PDB is a homodimer with four chains. The binding residues of protein structure from CASTp include ARG168, THR172, ARG179, LYS180, PHE181, SER182, LYS183, HIS215, SER216, ASP217, LYS218, GLY219, GLY220, GLU223, GLU252, SER254, VAL256 and VAL257. These were in par with the earlier studies with

binding residues centred on LYS180, vital for transcription initiation and nucleocapsid interaction suggested by mutagenesis analysis (Hartlieb *et al.*, 2007).

Molecular docking

These thirty-six compounds were subjected to molecular docking to anticipate the protein-ligand binding affinities, modes of interaction and inhibition activity. Compounds with the least binding energy are known to be better hits (Venkatesan & Febin, 2017) and based on the set threshold (binding energy < -6.5 kcal/mol), 10 compounds were selected. Further results from LigPlot+ revealed that two out of ten compounds with PubChemId 5281642 and 64981 are the most effective of all. Both are phytochemicals with previously reported antiviral activity (Vlietinck *et al.*, 1998) and binds at the predicted active binding site forming strong hydrogen bonds. Visualising docking results revealed that ten amino acid residues are in contact with Compound 1 and 13 amino acids with compound 2.

Binding mode analysis of control compound BCX4430

Docking VP30 with BCX4430 indicates binding energy of -4.16 kcal/mol with 11 amino acids in contact with three hydrogen bonds GLU191, ALA255 and ASP217. Among these residues, only ASP217 belong to the predicted active site. Two more hydrogens bonds were visualized within BCX4430 atoms stabilizing the complex. Residues involved in hydrophobic interactions were ARG179, LYS183, VAL256, SER253, LEU218, GLY220, GLY219, LEU188. The intermolecular energy was -5.95 kcal/mol, which involves both desolvation and electrostatic energy. This indicates the energy of non-bounded atoms. The total internal energy was -1.93 kcal/mol explaining the total of all energy changes and entropy.

Binding mode analysis of compound 1

Compound 1-6-Hydroxyluteolin (PubChem ID: 5281642) also called as 5,6,7,3',4'-pentahydroxyflavone is a flavonoid isolated from *Valerianella eriocapa* (Italian corn

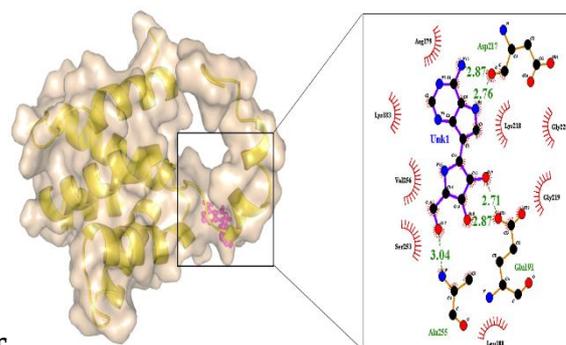
salad) and also found in other herbs and spices (Yannai, 2003). Unlike other antiviral compounds, flavones have a wide range of activity. Though the action of flavones in ebola is not fully explored, it is possibly by binding to a glycoprotein of RNA virus (Vlietinck *et al.*, 1998) blocking VP30 interaction with GP necessary for EBOV transcription. The binding energy of this compound was -7.71 kcal/mol forming one hydrogen bond within itself and five hydrogen bonds with LYS180, GLU252, LYS218, ASP217 and ARG179 residues that were a part of predicted binding site of VP30. The hydrophobic interactions of ligand were with ARG179, LYS180, PHE181, HIS215 and SER216 of VP30. The intermolecular energy was -7.99 kcal/mol with the total internal energy being -1.37 kcal/mol.

Binding mode analysis of compound 2

The second compound (-)-Arctigenin (PubChem ID: 64981) is a lignanoid of the dibenzyl butyrolactone type and is known to block HIV-1 viral replication in human cell line experiments (Vlietinck *et al.*, 1998). It is isolated from certain plants of Asteraceae and possesses antiviral and anticancer effects (Afendi *et al.*, 2012). With a docking score of -7.65 kcal/mol, it formed three hydrogen bonds with active site residues LYS180, GLU252 and LYS218. Hydrophobic interactions with ARG179, HIS215, HIS215, GLU252, SER182, ARG179, PHE181, LYS180, SER182 and PHE181. The intermolecular energy was -10.04 kcal/mol, and the total internal energy change was -1.97 kcal/mol. The docking results for the control compound and the above discussed best hits are summarized as presented in **Table I** followed by **Figure 1** visualizing the interactions of compounds with VP30.

Table I. Molecular docking results of control and the best two test compounds

Compounds	Binding Energy (kcal/mol)	Hydrogen bonds	Hydrogen bond residues	Hydrogen bond distance (Å)	Hydrophobic interaction residues
BCX4430 (Control)	-4.16	5	Glu191	2.71	Arg179
			Ala255	2.87	Lys183
			Asp217	3.04	Val256
				2.76	Ser253
				2.87	Leu218
					Gly220
6-Hydroxyluteolin	-7.71	6	Lys180	2.75	Arg179
			Glu252	2.72	Lys180
			Lys218	3.11	Phe181
			Asp217	2.82	His215
			Arg179	2.88	Ser216
				3.03	
(-)-Arctigenin	-7.65	3	Lys180	3.23	Arg179
			Glu252	2.75	His215
			Lys218	2.76	His215
					Glu252
					Ser182
					Arg179
		Phe181			
		Lys180			
		Ser182			
		Phe181			

**Figure 1.** The ribbon structure of VP30 docked with (a) BCX4430, (b) 6-Hydroxyluteolin and (c) (-)-Arctigenin shown as ball and stick appearance (left) and their respective interacting residues (right).

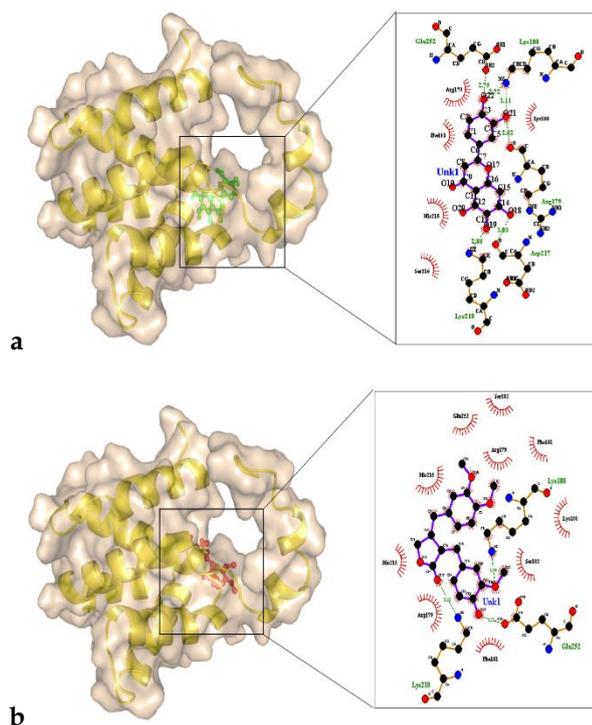
Bioactivity score prediction

In general, bioactivity score of more than 0.0 indicates the complex is active whereas if the score is between -5.0 and 0.0 the compound is moderately active and if the score is less than -5.0 it is probably inactive. The scores for the test compounds are listed in **Table II**, which indicates that both these test compounds possess bioactive properties of potential drugs as all the scores were above -5.0 and 0.0 (Joshi *et al.*, 2018).

Table II. Molecular docking results of control and the best two test compounds

compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
BCX4430	0.58	0.28	0.58	-0.74	0.09	1.39
6-Hydroxyluteolin	-0.07	-0.17	0.26	0.25	-0.27	0.25
(-)-Arctigenin	0.22	-0.02	0.00	0.13	0.10	0.20

In the current scenario, EBOV–host interactions are still being explored where it is challenging to develop effective drugs and vaccines against EVD, and there is no detailed report of pharmacological inhibition against Ebola virus (Yu *et al.*, 2017). The current strategy being symptomatic management emphasizes the need for targeted drug therapy. High throughput drug screening involving lead optimization, ADMET analysis, animal models and clinical trials is time-consuming, cumbersome and cost-ineffective (Sneha & Doss, 2016). Here is where computational studies substantially aid



experimental biology in designing potential drugs within a short period.

Several *in silico* studies for Ebola virus have been reported targeting VP40 and VP35 to inhibit the activity of viral polymerase and viral assembly (Baikerikar, 2017). However, recent advances exploring the role of VP30 in transcription initiation enables it to be an attractive target though there is no much report with this regard. This study aims to explore the drug target potentiality of VP30. Compounds were screened using ADMET analysis and narrowed down for molecular docking. 6-Hydroxyluteolin and (-)-Arctigenin were identified as promising lead molecules since they are permeable, non-toxic, non-mutagenic and possess higher binding affinities than the control compound. Therefore, they can bind to the transcription initiation factor VP30 and block viral proliferation at a much earlier stage than the other targets.

Out of the two investigated compounds, 6-Hydroxyluteolin had the least binding energy of all and with six hydrogen bonds, which is higher in comparison to the control compound. Higher the number of hydrogen atoms greater is the stability (Saleh & Elshemey, 2017). Though the compound (-)-Arctigenin formed a lesser number of hydrogen bonds in comparison to BCX4430, it had lower binding energy pointing to the hydrophobic interactions making the compound more favourable for stable binding. The intermolecular energy for both the test compounds was considerably lower than that of the control. Moreover, the bioactive prediction scores again support the properties of their drug-likeness. Notably, plant-derived compounds, especially flavonoids, are potent inhibitors of Ebola viral disease, as suggested by earlier reports (Raj & Varadwaj, 2016).

CONCLUSION

With persistent periodical outbreaks, it is a high priority to developing a potential drug to resist Ebola viral disease. In an attempt to explore if VP30, transcription initiation protein of EBOV can function as a potential drug target for structure-based drug design. The study revealed that the two plants derived compounds 6-Hydroxyluteolin and (-)-Arctigenin are potential lead molecules with the least binding energy and active biopredictivity scores. However, the solidity and efficiency should be validated using *in vivo* experimentations and clinical trials. These compounds identified as potential inhibitors of VP30 functional activity can be used for extensive drug design studies against EBOV study shortly.

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