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ISSN : 2231- 3176
CODEN (USA): JCMMDA

Identification of Drug Lead Molecules against Ebola Virus: an *In Silico* Approach

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ABSTRACT

Ebola virus identified is an envelope, single-stranded, negative-sense RNA virus that causes severe hemorrhagic fever in humans and nonhuman primates. This virus is naturally resistant to various antibiotics, and there is no proper treatment for infection caused by this pathogen. And hence there is a need for new drugs and approaches to combat the life threatening infection caused by Ebola virus. Computer aided drug design is one of the powerful tools for discovering new drug leads against important targets. The various proteins that are essential for pathogenesis of organism are selected as targets. The viral proteins vp35 and vp40 are capable of eliciting protective immune responses to EBOV. The various functions of these proteins in pathogenesis suggested them as potential drug targets to control EBOV infections. After the proteins were selected as target, new leads were chosen from a subset of small molecules that scored well when docked in silico against targets. The drug lead molecules were evaluated for their drug likeness using "Lipinski rule of five". The identification of appropriate drug lead molecules against these proteins has led to a successful drug candidate against Ebola virus infection.

Key words: Ebola virus, Hemorrhagic fever, Computer aided drug design, vp35, vp40, Lipinski rule of five, Drug lead.

INTRODUCTION

Ebola virus is a deadly pathogenic virus. It is classified under filoviridae family. It was first identified in Africa in 1976 in democratic republic of Congo. Hemorrhagic fever is the major infection caused by ebola virus which takes place in two phases, incubation period and late phase. Incubation period shows symptoms like arthritis, fever, fatigue, nausea which can last for one week and late symptoms include depression, eye inflammation, and hemorrhagic rash over the entire body [6, 27].

Ebola virus has thread like structure which may be informed of 'u', coil or circle. It has negative stranded genome which contains seven structural genes. Four structural proteins (VP30, VP35, and nucleoprotein and polymerase protein) and three membrane associated protein (VP40, glycoprotein, VP 24) are coded by ebola virus genome [8, 9, 10, 14, 17, 25].

Ebola virus infection was observed in carcasses of gorilla, chimpanzee during outbreaks in 2001, which became the source of human infection. Bats are also considered as a possible reservoir of infection [1]. The main targets of infection are endothelial cells, mononuclear phagocytes and hepatocytes. In secreted glycoprotein (sGP) the Ebola virus glycoprotein (GP) is synthesized after infection. The signaling of neutrophils is interfered by sGP a diametric protein, inhibiting early steps of neutrophils activation and allow the virus to evade the immune system. Budding of ebola virus causes cell damage and the presence of viral particles affects release of cytokines. Cytokines are signaling molecules for fever and inflammation. Further synthesis of GP leads to loss of vascular integrity which leads to coagulopathy and it also reduces the specific integrins which has role in cellular adhesion to intracellular structure. With loss of vascular integrity and improper coagulation blood leaks out through blood vessels leading to hypovolemic shock [2, 24].

As such there is no drug available for treatment of ebola virus infection. *In silico* approach is useful for discovering drug lead candidate against ebola virus fatal infection. The use of computer and computational methods allows, using all aspects of drug discovery, forming core of structure based drug design and has advantage of delivering drug more quickly and at economic cost. Classical drug discovery takes much time which may be 10-14 yrs or more to undergo target discovery, lead generation, optimization, preclinical development, clinical trial, and FDA approval and finally bring to market [12].

Structure based drug design depends on three dimensional structure of biological targets which are obtained from x-ray crystallography and NMR spectroscopy. Structure based drug design is powerful with combinational techniques which can help to get better compounds. Structural based drug design follows with the drug target identification, preparation of target protein, virtual screening of the drug compound which increase sophisticated level of filtration of potential compound, then molecular docking of the drug candidates and then to find out the best lead like compound with further optimization of the compounds to finalize the lead [5, 19, 20].

MATERIALS AND METHODS

Selection of target proteins:

Initially the target proteins were selected which are involved in the pathogenesis. The structural information of the target proteins was obtained from PDB and their active sites were determined using PYMOL.

Screening of lead molecules:

After choosing the target protein the inhibitory drug compounds were chosen from pubchem and virtual screening was done by creating database of these compounds in CHIMERA. These databases were fed to ARGUSLAB for screening the best ligands with the target protein. The best ligands were chosen with low energy values, and virtual screening was done using ZINC DATABASE, from which class of similar compounds were obtained, and again were fed to ARGUSLAB and best compounds obtained were passed for docking studies.

Docking studies:

In docking studies the interaction between target and ligand was studied. The best ligands screened were loaded in to auto dock and docking studies were carried out. Based on the binding energies and details from the histogram, the drug lead compounds were determined.

Optimization of lead molecules:

The drug lead molecules selected were reevaluated for their drug likeliness by using "Lipinski Rule of Five" to predict which drug molecules would fail because of poor pharmacokinetics.

RESULTS

Antiviral Targets in Ebola Virus:

The Ebola virus proteins evade the immune system and have important role in pathogenesis of the virus. This makes their potential as drug target to combat Ebola virus infection. The viral proteins selected as a target are vp35 and vp40.

Information about the targets:

1) VP40

VP40 is matrix protein and is key particle for maturation of virion. VP40 is known to possess three domains M, I, and L domain. For interaction with specific cellular proteins and for virus host interaction viral L domain is thought to serve as docking site and it facilitates virus budding. Homo-oligomerization is the key feature of viral matrix protein required for efficient release of virus like particles and virions and also to interact directly with lipid membrane or host protein for efficient budding. They bud and assemble from specialized domains known as lipid rafts which are present within the plasma membrane. Thus, the matrix protein, Ebola virus VP40, plays a key role in viral assembly, budding, and virion formation [3, 11, 13, 14, 15, 16, 21, 22, 23, 28].

2) VP35

The Ebola VP35 protein is multifunctional, acting as a component of the viral RNA polymerase complex, a viral assembly factor, and an inhibitor of host interferon (IFN) production. Mutation of selected basic residues within the C-terminal half of VP35 abrogates its dsRNA-binding activity, impairs VP35-mediated IFN antagonism, and attenuates EBOV growth in vitro and in vivo. The dsRNA binding activity mediated by the C terminus of VP35 is critical for viral suppression of innate immunity and for virulence. The dsRNA binding cluster is centered on Arg-312, a highly conserved residue required for IFN inhibition. Mutation of residues within this cluster significantly changes the surface electrostatic potential and diminishes dsRNA binding activity. Knockdown of VP35 leads to reduced viral amplification and reduced lethality in infected mice. Therefore, a functional VP35 is required for efficient viral replication and pathogenesis [4, 18, 26].

DETERMINATION OF ACTIVE SITE OF THE TARGETS:**Table 1: Active sites of the target proteins**

Proteins	Major Active site Residues
VP40	LEU304 , ILE305 ,THR306 ,GLN307
VP35	LYS309, ARG312, LYS339, LYS319, ARG322, ARG305

SCREENING OF LIGANDS:

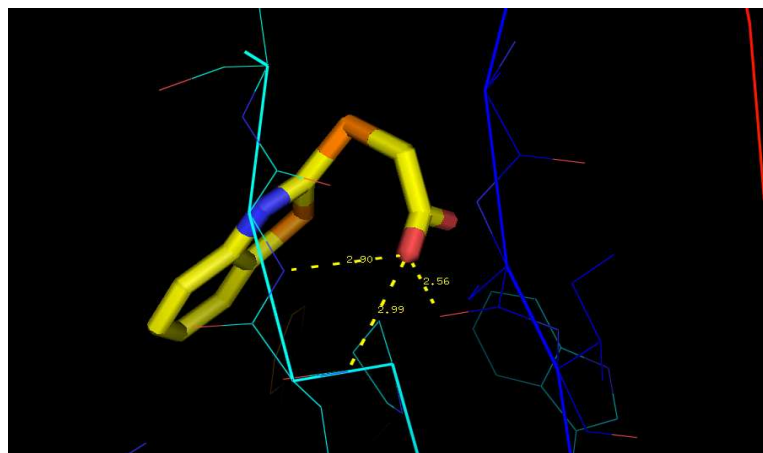
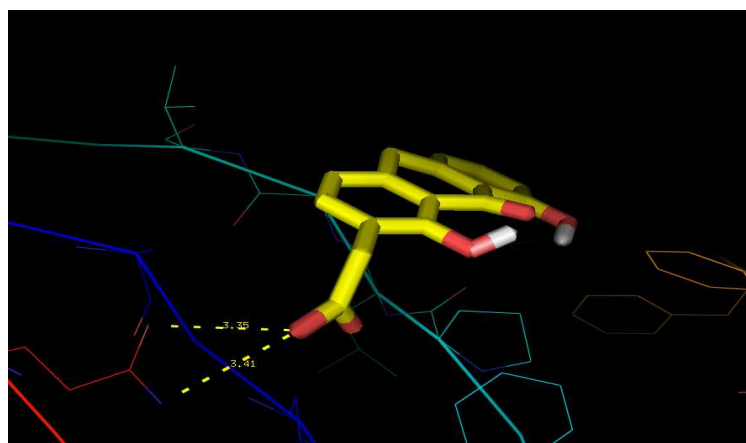
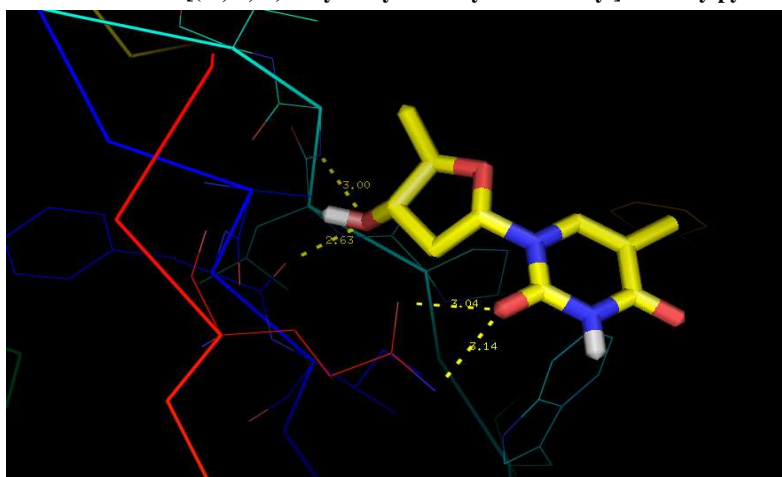
Around 371 antiviral compounds, 138 nucleic acid synthesis inhibitory compounds and 46 reverse transcriptase were taken from PUBCHEM. Using these compounds new databases were made with the help of CHIMERA. These compound database was screened in ARGUSLAB and 200 ligands were obtained having proper interaction with the target protein. By drawing structure of these obtained compounds in zinc database, similar compounds were obtained and were again fed in arguslab. Compounds with low binding energies from ARGUSLAB were subjected to docking studies.

DOCKING STUDIES:1) **For vp40 with selected leads:**

Nearly fifty ligands were obtained after virtual screening in ARGUSLAB and were docked in auto dock. Docking results with VP40 with the best hits arrived four ligands, which could possibly inhibit the target protein. The ligands obtained are:

- a) 2-(1,3-benzothiazol-2-ylsulfanyl)acetate
- b) 2-(1,8-dihydroxy-9-oxo-10h-anthracen-2yl)acetic acid
- c) 1-[(2s,4s,5r)-4-hydroxy-5-methyloxolan-2-yl]-5-methylpyrimidine,2,4 dione
- d) 1-[(2r, 4s, 5s)-5-(hydroxymethyl)-4-methyloxolan-2-yl]-1,2,4-triazole-3-carboxamide.

The docking results are shown in Figure 1-4.

Figure 1: Interaction of vp40 with 2-(1,3-benzothiazol-2-ylsulfanyl)acetate**Figure 2: Interaction of vp40 with 2-(1,8-dihydroxy-9-oxo-10hanthracen-2yl) acetic acid****Figure 3: Interaction of VP40 with 1-[(2s,4s,5r)-4-hydroxy-5-methyloxoloan-2-yl]-5-methylpyrimidine, 2,4-dione**

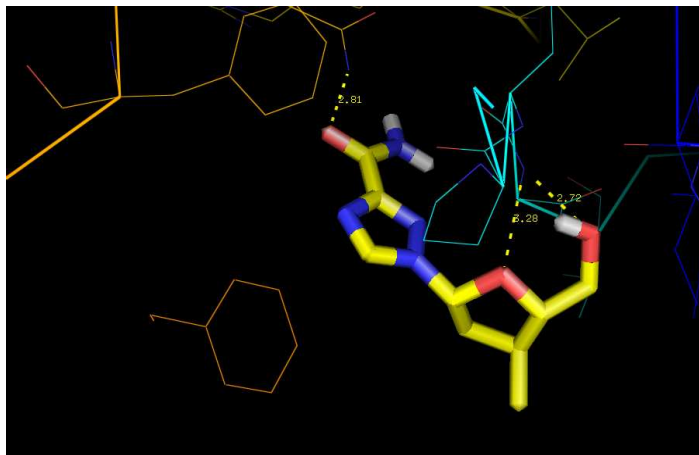


Figure 4: Interaction of VP40 with 1-[(2r, 4s,5s)-5-(hydroxymethyl)-4-methyloxolan-2-y]1,2,4-triazole-3-carboxamine.

2) For vp35 with selected Leads:

Seventy ligands obtained after virtual screening with vp35 were docked in auto dock. Docking results of vp35 with the best hits arrived at four ligands, which may inhibit the target vp35 activities. The ligands obtained are:

- 2-(2,3-diamino-3-oxopropyl)sulfynyl acetic acid
- 5-cyclohexypyridine 2-carboxylic acid
- Copper carboxymethoxyanamide dihydrate
- 2, 3-dihydroxy-3-[(4-methylphenyl)carbamoyl]propanoic acid

The docking results are shown in figure 5-8.

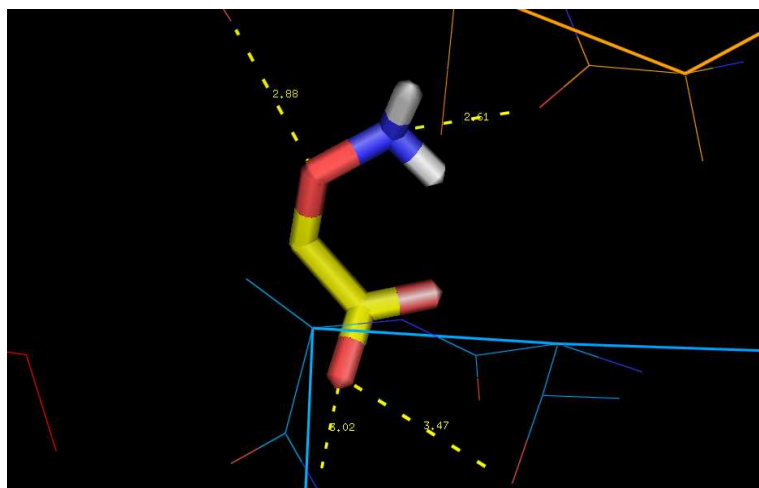


Figure 5: Interaction of vp35 with 2-(2,3-diamino-3-oxopropyl)sulfynyl acetic acid

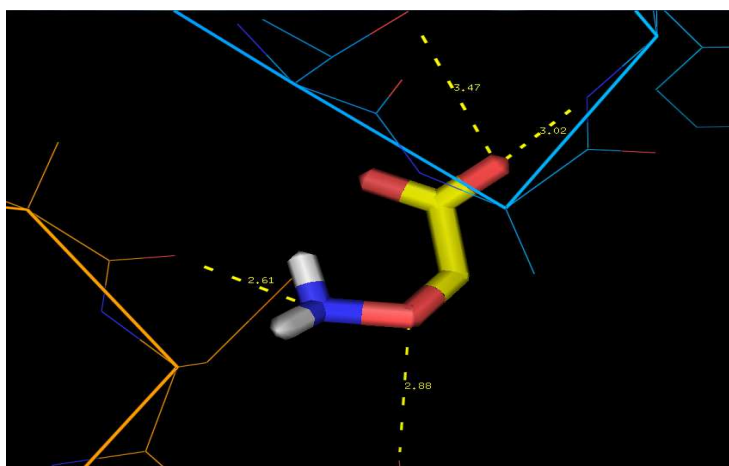


Figure 6: Interaction of vp35 with copper carboxymethoxyanamide dehydrate



Figure 7: Interaction of vp35 with 5-cyclohexypyridine 2-carboxylic acid

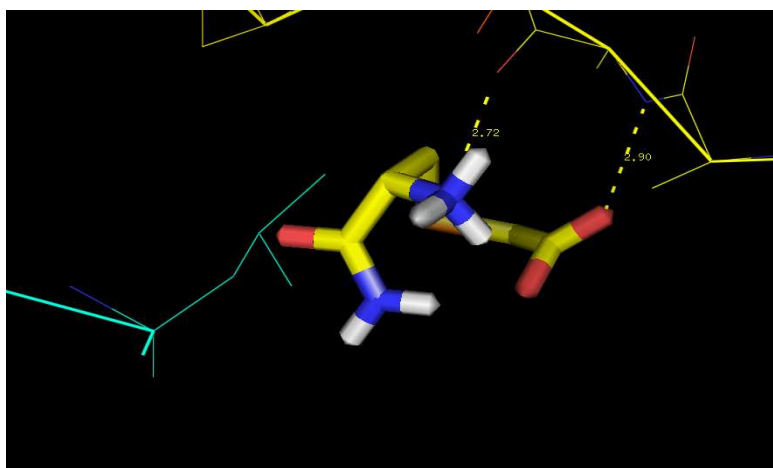


Figure 8: Interaction of vp35 with 2-(2,3-diamino-3-oxopropyl)sulfinyl acetic acid

OPTIMIZATION OF LIGANDS:**Table 2: Properties of leads for VP40**

Compound	Properties			
	Molecular weight [g/mol]	Log p	H-bond donor	H-bond acceptor
2-(1,3-benzothiazol-2-ylsulfanyl)acetate	224.27944	3.3	0	2
2-(1,8-dihydroxy-9-oxo-10h-anthracen-2-yl)acetic acid	284.26348	2.7	3	5
1-[(2s,4s,5r)-4-hydroxy-5-methyloxolan-2-yl]-5-methylpyrimidine,2,4 dione	226.22916	-0.7	2	4
1-[(2r,4s,5s)-5-(hydroxymethyl)-4-methyloxolan-2-yl]-1,2,4-triazole-3-carboxamide	226.2324	-0.6	2	3

Table 3: Properties of leads for VP35

Compound	Properties			
	Molecular weight [g/mol]	Log p	H-bond donor	H-bond acceptor
2,3-dihydroxy-3-[(4-ethylphenyl)carbamoyl]propanoic Acid	239.22462	0.3	4	5
Copper Carboxymethoxyazanide Dihydrate	90.05806	-2.7	1	4
2-(2,3-diamino-3-oxopropyl) sulfanyl acetic acid	178.2095	-3.8	3	4
5-cyclohexylpyridine-2-carboxylic acid	205.253	3.2	1	3

DISCUSSION

Currently the life threatening Ebola hemorrhagic fever caused by Ebola virus is untreatable with high mortality rates and hence there is need to develop a proper treatment. One potential target is the VP40 matrix protein, the key viral protein that drives the budding process, in particular by mediating the specific virus-host interactions to facilitate the efficient release of virions from the infected cells. Key structural and functional domains of VP40 believed to be necessary for efficient budding of virions and virus-like particles. The Ebola VP35 protein is multifunctional, acting as a component of the viral RNA polymerase complex, a viral assembly factor, and an inhibitor of host interferon (IFN) production. Thus targeting these proteins, that are important in growth and pathogenesis, this is appropriate way to treat the infection. Inhibition of these proteins can help to prevent the growth and pathogenesis of ebola virus.

By screening Zinc Database followed by the docking studies, we have identified leads for the three targets. For vp40, four ligands are identified from AUTODOCK out of 50 hits obtained from ARGUSLAB. For vp35 four ligands were obtained out 60 hits obtained from ARGUSLAB. All these molecules follow the Lipinski Rule of five, showing their drug likeness. These molecules may constitute to control multidrug resistant viral infections.

CONCLUSION

Ebola, have the dubious distinction of being associated with some of the highest case-fatality rates of any known infectious disease. Ebola cause severe hemorrhagic fever in humans and nonhuman primates, since it causes high mortality rate and currently no drugs are available, there is an urgent need for novel antiviral against Ebola virus infections.

Computer aided drug design helps in reducing the cost and time for drug discovery process which otherwise takes many years. Virtual screening and docking studies helped to obtain ligand molecules that can inhibit the important proteins involved in the pathogenesis of ebola virus.

The findings of this study are important as there is need for new drug to inhibit ebola virus. The lead found out, could possibly inhibit the infection. However, these leads should undergo various preclinical analysis and optimization process before going into clinical trials.

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