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Molecular docking based screening of predicted potential inhibitors for VP40 from Ebola virus

Danya Abazari[1], Mehrad Moghtadaei[1], Ali Behvarmanesh[1], Bahareh Ghannadi[1], Monireh Aghaei[1], Mahboobeh Behruznia[1] & Garshasb Rigi[1, 2*]

[1]Vira Vigene research institute, Tehran, Iran; [2]Department of Biology, Faculty of Science, Behbahan Khatam Alanbia University of Technology, Behbahan, Iran; Garshasb Rigi - Email: garshasbbiotech@gmail.com; *Corresponding author

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Abstract:
Ebola virus is a member of Filoviridae and cause severe human disease with 90 percent mortality. The life cycle of Ebola contains an assembly stage which is mediated by VP40 proteins. VP40 subunits oligomerize and form ring-structures which are either octamers or hexamers. Prevention of VP40 matrix protein assembly prevents virus particle formation as well as virus budding. In the present study we simulated the biological condition for a single VP40 subunit. Then a library containing 120.000 drugs like chemicals was used as the virtual screening database. Top 10 successive hits were then analyzed regarding absorption, distribution, metabolism, and excretion properties. Moreover probable accessorial human protein targets and toxicity properties of successive hits were analyzed by *in silico* tools. We found 4 chemicals that could bind VP40 subunits in a manner that by making an interfering steric condense prevents matrix protein oligomerization. The pharmacokinetic and toxicity studies also validated the potential of 4 finlay successive hits to be considered as a new anti-Ebola drugs.

Keywords: VP40, Ebola Virus, Molecular docking, AutoDock, Virtual screening

Background:
Ebola virus is a dreadful, horrible term for those who exposure to this deadly agent or at least the possibly risk of pandemic distribution might make them frighten of this catastrophic disaster. The current outbreak of Ebola virus in West Africa with more than 23000 cases and 9000 deaths possess a severe situation of human-being threat **[1].**

The Ebola Virus (EBOV) belongs to Filoviridae family with Marburg virus. So far, 4 species of the virus including: Zaire, Sudan, Ivory Coast and Reston EBOV have been identified, with the highest human lethality of Zaire species **[2].** The EBOV glycoproteins are considered to play major role in pathogenesis. The fourth gene from the 3′ end of the Ebola virus genome encodes two glycoproteins and the envelope glycoprotein (GP), which is held responsibility of receptor binding and fusion of the virus with host cells **[3].** Currently GP protein is a potential drug target, because inhibition of GP, prevents virus entrance to the cell. The non-structural secretory glycoprotein (sGP), which is secreted from infected cells **[4, 5]** and the matrix protein, usually encoded by the most enveloped RNA virus genomes, has a key role in efficient assembly and virus particles budding. The matrix protein is another target for anti-Ebola drugs. Targeting this protein results prevention of virus assembly and budding. This proteins interact with membrane proteins **[6].** Structural information on viral matrix proteins shows four different matrix protein structures including VP40 from Ebola virus, M from Vesicular stomatitis virus, a fragment of M1 from influenza virus and a number of retroviral matrix proteins **[7].** The Ebola virus matrix protein VP40 is an elongated, two-domain monomeric assembly, composed of two structurally related b-sandwich domains connected by a flexible linker. In fact, the unique fold of both domains suggests that the two domains probably arose from a common ancestor

by gene duplication **[8].** The ring-structures are either octamers or hexamer. In both cases, the N-terminal domain of VP40 makes the oligomerisation domain, which forms an anti-parallel dimer and is the building block for oligomerisation **[9].** So the N-terminal domain of VP40 is another potential drug target foe anti-Ebola therapy. The C-terminal domains are flexibly attached to the rings and mediate membrane association in vitro and in vivo. The octamer was found to bind single stranded RNA at the dimer–dimer interface having the sequence 50-UGA-30. RNA binding creates a new dimer–dimer interface and its binding stabilizes the protein– protein interaction generating the octamers **[10].** Preventing octamer-RNA interaction can also inhibit Ebola virus. Based on the crystal structure of the octamer, the complete N-terminal segment from residues 1 to 68 has to unfold in order to create the new ssRNA-binding interface. This interface is highly potential area for drug binding. Interestingly, the SDS resistant octamer is present in Ebola VLPs and in virus particles **[11].** A role for the ring structures in assembly and budding is also evident from the fact that only oligomeric VP40 interacted with WW3 from human Nedd4 in vitro via its N-terminal PPXY motif **[12]**, an interaction which is important in vivo. A number of studies also showed the importance of the PTAP motif present at the N-terminus for budding. This motif binds to the UEV domain of Tsg101 independent of its oligomeric state. Thus, monomeric VP40 recruits Tsg101 to the site of budding **[12, 13]**, which in turn might recruit the complete Vps machinery for efficient budding. The structural studies of Ebola virus VP40 have now firmly established three conformations of Ebola virus VP40, although their role in assembly and budding or additional functions during the life cycle of Ebola virus is far from clear. It is, however, an interesting example of evolution that packs different functional aspects into one relatively small protein probably due to the limiting size of the viral genome **[14].** In the present study we tried to target a key protein of Ebola virus which has the vital role in both virus assembly and budding processes. Prevention of CP40 assembly prevents octamer structure formation and frustrates a critical stage in the virus life cycle. Unoligomerized VP40 proteins are unable to play the role of octamer matrix protein and as the results virus assembly process will be misadjust. In this study we tried to simulate a single VP40 structure in the biological condition, then by high throughput virtual screening we tried to find a pharmacophore model which prevents oligomerization of VP40 structures.

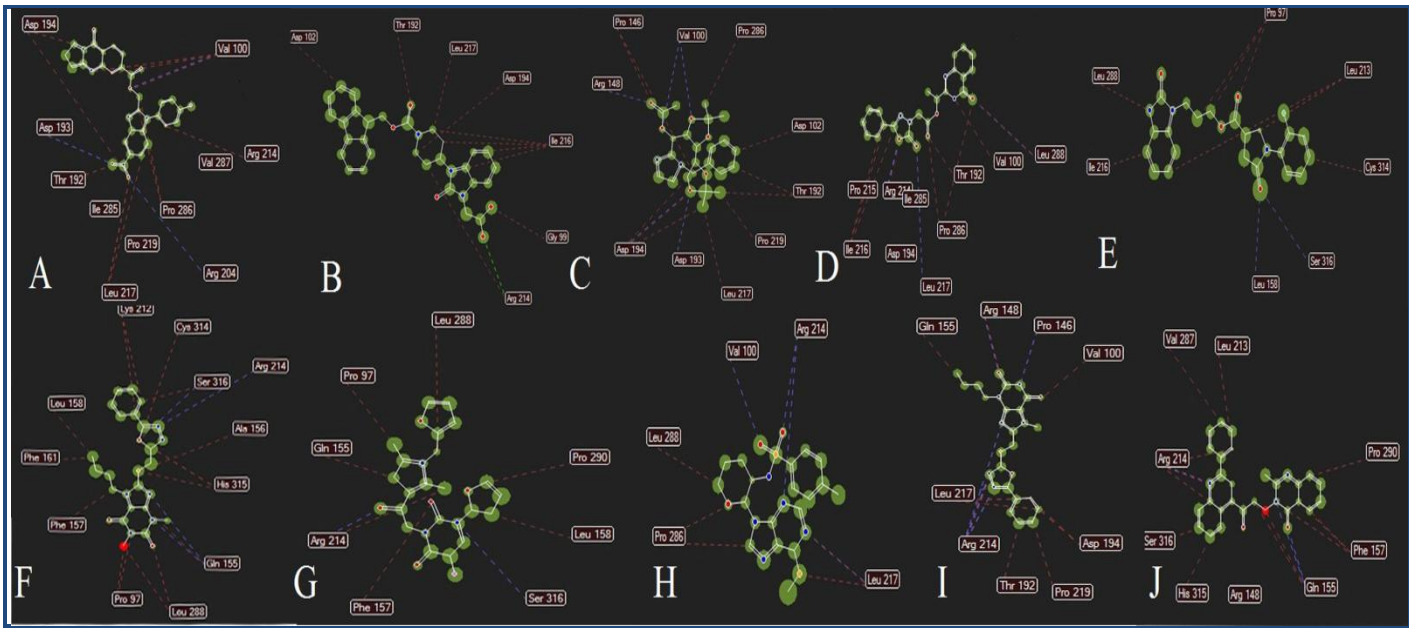

Figure 1: the ligand map of top 10 successive hits. **A:** the top chemical inhibitor, binding affinity: -11.3. **B:** second successive hit, binding affinity: -11.1. **C:** third successive hit, binding affinity: -10.9. **D:** fourth successive hit, binding affinity: -10.1. **E:** fifth successive hit, binding affinity: -9.8. **F:** sixth successive hit, binding affinity: -9.8. **G:** seventh successive hit, binding affinity: -9.4. **H:** eighth successive hit, binding affinity: -9.2. **I:** ninth successive hit, binding affinity: -8.7. **J**: tenth successive hit, binding affinity: -8.2.

Methodology:

Protein and ligands structure

Crystal structure of VP 40 protein of Ebola virus obtained from protein data bank (www.rcsb.org/) with pdb code: 4LDB. The provided model is in tetrameric form with the quality of X-ray diffraction and resolution of 1.89 Å. The monomeric structure extracted and the further study were performed in one chain. To simulate the biological condition of a single VP 40 protein, we solved the structure in a water box with 10 A border. The system was then neutralized with Na^+ and/or Cl^- by using GROMACS 4.5.4 software. Moreover zinc database **[15]** was used as the ligand database for virtual screening. 120.000 ligands in reference pH and were obtained used as virtual screening library.

Virtual screening:

PyRX software **[16]** was used as the virtual screening software. It includes AutoDock **[17]** and AutoDock vina **[18]** with the Lamarckian genetic algorithm as scoring function. In this research we used AutoDock Vina for molecular docking simulation. Before initiation of docking operation charge assigned to protein and ligand structures by AutoDock Vina. A docking radius with coordinate of X: -22.30 Y: -62.57and Z: 8.61 was used to cover interacting area of monomers.

Pharmacokinetic analysis

FAFDrugs3 **[19]** and admetSAR web servers were used to analyze the absorption, distribution, metabolism, and excretion properties of top 10 Virtual screening hits. ADME properties of top successive hits were checked in optimal descriptors (hydrogen bonds, charge) in pH=7.4. Moreover the oral toxicity properties were analyzed by PROTOX web server **[20].** Also with this server the probable accessorial human protein targets were checked for every successive hits.

Results & Discussion:

To theoretically prevent interaction between VP40 monomers, it is essential to make a steric condense by finding a high affinity ligand. For gaining this purpose, we set the docking radius in the interaction area. The structure of VP40 provided in protein data bank (4LDB) is in tetramer form in a manner that each monomer engages in interaction with 3 other monomers. The monomers are tightly in interaction with hydrogen bonds, electrostatic and steric interactions. Also in this structure inter molecular interactions are mainly created by beta sheets. Moreover in this structure, the interacting area has the volume of 22 Â, but in virtual screening process we used a radius of 26 Â to ensure that the entire intermolecular interaction era between single monomers are assumed as the target for ligand binding. Among 120000 ligands, top 10 virtual screening successive hits were selected for further chemo informatics and pharmacokinetic analysis. **Table 1 (see supplementary material)** describes the binding affinity of top 10 hits. Top 10 hits could reach the binding affinity of -8.2 to -11.3 kcal/mol. Based on binding affinity, the top successive hit (-11.3) is the first drug candidate but further pharmacokinetic analysis were performed on top 10 hits to check a wide range of pharmacophore properties of potential inhibitors. Also the structure of top 10 chemical inhibitors are shown in **Figure 1**. As depicted in this figure, all of the hits have aromatic rings mostly containing carbon atoms. This implies that hydrophobic interactions play a key role in ligand binding. Moreover existence of hydrogen bonds between VP40 and inhibitor makes the ligand more stable into its binding position. The coordinate of hydrogen donor and acceptors in ligand structure makes a specific binding pattern for it. In the step, we checked oral bioavailability properties of ligands by FAFDrugs3 webserver. Interestingly the FAFDrugs3 results indicated that all of the top 10 hits has accepted statues with good oral bioavailability rank. So we assumed that the entire 10 ligands can be used for complementary pharmacokinetic study. The results of FAFDrugs3 with more details is illustrated in **Table 1 (see supplementary material)**. Although all of top 10 chemicals could reach accepted statues in FAFDrugs3, but based on the provided dada in the table, there are only 4 highly specific chemical inhibitors of VP42 assembly. It is probable that this specify (binding affinities: -11.3, -11.1, -10.9 kcal/mol) is due to engaging more pharmacophore properties in interaction with VP40. So we performed the supplementary pharmacokinetic tests on top 4 hits which showed more specific binding pattern. The toxicity of chemicals were then checked by PROTOX webserver and as described in **Table 1 (see supplementary material)**, the first successive hit has the LC50 of 5000mg/kg with the toxicity class of 5 (1 high toxic and 6 nontoxic). The predicted LC50 indicates that it has low toxicity level. Also the distribution of molecular weight of this ligand is under normal distribution curve. The results of preliminary *in silico* analysis indicated that this ligand has high potential for being used as a drug candidate.

The second hit reached the LD50 equal to 3322mg/kg in PROTOX webserver, while its toxicity level is 5. This chemical is also predicted to be less toxic. This ligand also has fine potential to be considered as a drug but it is more toxic that first successive hit. The third chemical with the binding affinity equal to -10.9 gained the rank of 4 in toxicity level with the LD50 of 1670mg/kg. It means that because of high toxicity value this ligands need to be modified before consideration as the basic structure of a potential inhibitor of VP40 assembly. The forth theoretical inhibitor also reached class 4 of toxicity level with the LD50 of 1690mg/kg. This ligand is also has accepted toxicity level but it needs modification before consideration as a potential inhibitor.

To reach more details about toxicity, admetSAR webserver also has been used. As described in **Table 2 (see supplementary material)**, none of the ligands indicated the carcinogenic properties. Other characteristics related to toxicity is described in the table. The results of **Table 2 (see supplementary material)** in support of **Table 1 (see supplementary material)** indicates that top found hits generally has high potential to be used as the base structure for structure based drug design but also there are some undesirable characteristics in the pharmocophore model of ligands. It seems that further *in vitro* and *in vivo* studies following chemical modifications are required to achieve a final anti Ebola drug which targets VP40 assembly.

Conclusion:

The presented study introduced 4 drug like chemicals which could specifically bind to VP40 and by making steric condense prevents octamer and hexamer formation and frustrates the virus life cycle. The presented chemicals showed considerable binding affinity and the results of pharmacokinetic studies also confirmed the potential of structures to be considered as a base structure for anti-Ebola treatment.

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Supplementary material:

Table 1: The overall properties of top 10 successive ligand which is derived from virtual screening data.

Ligand	Binding affinity	LD50	Molecular weight	Rotatable bonds	flexibility	H acceptor	H donor	Ring	Carbon atom	Hetero atom	Oral bioavailability
Lig 1	-11.3	5000	497.43	6	0.15	10	0	3	26	11	good
Lig 2	-11.1	3322	498.55	6	0.15	8	6	3	29	8	good
Lig 3	-10.9	1670	430.49	7	0.24	8	0	4	23	8	good
Lig 4	-10.1	1690	416.74	6	0.21	5	1	4	23	7	good
Lig 5	-9.8	-	414.86	7	0.23	7	1	3	21	8	good
Lig 6	-9.8	-	427.50	7	0.23	9	0	3	20	10	good
Lig 7	-9.4	-	414.41	6	0.20	8	0	4	21	9	good
Lig 8	-9.2	-	420.53	5	0.17	8	1	3	18	10	good
Lig 9	-8.7	-	413.47	7	0.23	9	1	3	19	10	good
Lig 10	-8.2	-	420.48	5	0.14	5	0	3	27	5	good

Table 2: The toxicity properties of top 4 successive hits of virtual screening data

Property	Ligand 1	Ligand 2	Ligand 3	Ligand 4
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor Non-inhibitor	Weak inhibitor Inhibitor	Weak inhibitor Non-inhibitor	Weak inhibitor Inhibitor
AMES Toxicity	AMES toxic	Non AMES toxic	Non AMES toxic	Non AMES toxic
Carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens
Fish Toxicity	High FHMT	High FHMT	High FHMT	High FHMT
Tetrahymena Pyriformis Toxicity	High TPT	High TPT	High TPT	High TPT
Honey Bee Toxicity	Low HBT	Low HBT	Low HBT	Low HBT
Biodegradation	Not ready biodegradable	Not ready biodegradable	Not ready biodegradable	Not ready biodegradable
Acute Oral Toxicity	III	III	III	III