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Screening of Promising Lead Molecules against Two Drug Targets in Ebola Virus: An Effort to Eradicate Ebola Infection

Sachin Rahangdale[1*], Neelesh Kumar Malviya[2], Ahana Singh Baghel[2], Pushpanjali Sharma[2], Ankit Kale[3]
[1]*Govt. Madhav Science College, Ujjain, Madhya Pradesh, INDIA*
[2]*Department of chemistry IPS Academy, Indore, Madhya Pradesh, INDIA*
[3]*Department of Biotechnology SGBAU Amravati. Maharashtra INDIA*

Abstract: *Ebola virus belongs to Filoviridae family. Recently, Ebola outbreaks have appeared drastically in West Africa. The 2014 Ebola epidemic was lethal which was found to be affecting multiple countries in West Africa. Till date there is no reported host for Ebola infection but it is most likely spread through bats. Still, mankind is struggling to combat this pandemic infection. There are no reported drug targets in Ebola virus. Therefore there are no reported inhibitors for the same. In this present work, two drug targets have been proposed based on its essentiality to Ebola infection and non-homology to human proteome. Further, docking and ADMET analysis have been done to report promising lead molecules for these two drug targets.*
Keywords: *Inhibitors, Docking, Proteome, ADMET analysis.*

I. Introduction

Ebola virus disease brings the most frightening of infectious disease worldwide [8]. In the late 1970s, the discovery of Ebola virus was reported to be the causative agent of major outbreaks of hemorrhagic fever in the Democratic Republic of the Congo (DRC) and Sudan [9]. In March 2014, the World Health Organization (WHO) reported a major Ebola outbreak in Guinea that led to the extensive therapeutic research on Ebola virus [7]. It is highly fatal with huge mass of death rate, but can be prevented. Body fluids like blood, saliva, urine, sperm, etc. of an infected person are the prominent cause for its spread. Signs and symptoms of Ebola includes fatigue, fever, headaches, joint, muscle, abdominal pain, vomiting, diarrhea and loss of appetite [10]. With the used of heat, alcohol-based products, and sodium hypochlorite (bleach) or calcium hypochlorite (bleaching powder) at appropriate concentrations, Ebola virus can be eliminated.

There known treatments for Ebola in humans are hydration, oxygen and treatment of complicating infections, but due to unavailability of specific cure, mortality rate is 90% [13]. Recently, Researchers from the National Microbiology Laboratory identified antibody against Ebola virus protein shell [11][12]. In order to have the severity of this pandemic disease, in this present work, two drug targets have been proposed based on its essentiality to ebola infection and non-homology to human proteome. Further, docking and ADMET analysis have been done to report promising lead molecules for these two drug targets. These are nucleoprotein and membrane associated protein.

II. Material And Methodology

Protein and Ligand Preparation

Referring the various literatures and ZINC database [6] we found that probably inhibition of two target protein effectively reduces the Ebola infection. The NP gene (accession number NP_066243) [4] which encodes the protein nucleoprotein was observed and retrieved from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) with PDB id **4QAZ** [1]. And VP24 gene accession number NP_066250) [4] which encodes the protein membrane-associated protein was observed and retrieved from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) with PDB id **4U2X.[1]**The active sites of protein were identified by Molegro Virtual Docker [3].
All ligands were collected from ZINC database **[**6**].** These ligands are drug like ligands.

Optimization of Protein and Ligand

Two Target proteins, Nucleoprotein (PDBID: 4QAZ) and Membrane-associated protein (PDBID: 4U2X) downloaded from PDB [1]. This protein was prepared for docking by Deleting all hetero atoms, ligands and water molecules. In the crystal structure of protein was taken as active site was chosen for grid generation as there was already a crystal inhibitor. Grid was made using "define site" module of MVD.

50 Ligands were downloaded from ZINC database [6]. All the collected ligands were checked for bad valencies and subsequently isomers and tautomers were generated under "Ligand preparation" module of Discovery studio.

Docking Studies: All the 50 ligands, docked with nucleoprotein and membrane-associated protein in the Software MVD [3] using a genetic algorithm. It predicts Catalyst generated ligand conformations in the protein active site. Standard default parameter settings were used to evaluate the protein- ligand Interactions. We find interactions from MVD in terms of good scoring function and search space. We find active sites from MVD and cross checked with pocket-finder [5].

Visualization of Docked Complex: The docked complex was visualized in MVD [3] showing how the ligand interacts with the nucleoprotein and membrane-associated protein.

Best Ligand Proposal: On the basis of non-bonded and bonded interactions, ADME properties and scoring functions, best drug like ligands as potent inhibitors proposed. OSIRIS PROPERTY EXPLORER **[2]** was used to know the mutagenicity, carcinogenicity, reproductively and toxicity.

III. Result And Discussion

Target Nucleoprotein (pdb id 4U2X) and Membrane-associated protein (pdb id 4QAZ) was downloaded from Protein data bank (PDB). 50 ligands were proposed form ZINC database to inhibit ebola infection. We here by performed Molecular docking studies of the two target protein with these proposed 50 ligands. The coordinates for the active sites in Nucleoprotein (4U2X) was X=-15.04; Y=-46.99; Z=-28.39(Volume of cavity = 1140.192). The coordinates for the active sites in Membrane-associated protein (4QAZ) was X=-11.04; Y=26.53; Z=16.90 (volume of cavity = 24.57).
The scoring function and hydrogen bond interactions of ligand-protein are given in Table 1.

Table 1: Molecular docking Results

S.No	Ligand	Nucleoprotein(4U2X)			Membrane-associated protein(4QAZ)		
		Moldock Score	RMSD	Interaction	Moldock score	RMSD	Interaction
1	Zinc_633944	-133.65	3.83	0	-113.5	1.7	4
2	Zinc_633946	-128.38	3.0	3	-107.8	3.6	2
3	Zinc_633953	-122.2	3.9	3	-104.48	1.35	3
4	Zinc_633955	-120.5	3.6	4	-95.22	4.16	4
5	Zinc_633958	-92.7	5.3	3	-88.57	2.11	7
6	Zinc-1019824	-73.1	0.01	0	-57.4	0.1	0
7	Zinc_982962	-108.1	0.7	1	-73.10	3.8	2
8	Zinc_1700294	-68.5	0.5	3	-58.6	1.1	0
9	Zinc_3901268	-73.8	2.8	2	-78.9	2.5	0
10	Zinc_730699	-115	1.20	6	-89.3	6.9	3
11	Zinc_984053	-81.4	0.03	3	-61.59	0.10	2
12	Zinc_633962	-108	8.2	5	-89.56	2.5	4
13	Zinc_633984	-100	1.8	5	-87.33	1.53	1
14	Zinc_633992	-119.7	1.8	5	-107.65	1.82	5
15	Zinc_633997	-110.6	2.1	4	-118.35	5.25	3
16	Zinc_634115	-94.9	0.04	3	-87.37	0.7	2
17	Zinc_666987	-89.4	3.2	3	-85.4	0.6	4
18	Zinc_1240782	-79.8	6.7	7	-68.8	4.5	5
19	Zinc_1700294	-49.2	3.9	1	-56.5	7.5	2
20	Zinc_3901268	-80.2	6.8	2	-65.5	3.7	3
21	Zinc_5286115	-97.5	5.6	3	-87.5	2.9	0
22	Zinc_5519407	-58.7	1.5	4	-67.7	5.8	1
23	Zinc_5556455	-80.1	0.4	3	-71.6	0.4	6
24	Zinc_6182368	-56.1	6.1	2	-56.8	6.7	2
25	Zinc_20031600	-71.98	1.88	2	-77.54	1.0	2
26	Zinc_19990070	-88.74	0.977	2	-87.9	0.5	1
27	Zinc_19990034	-69.09	0.02	2	-88.8	6.5	4
28	Zinc_19799526	-81.98	1.01	3	-71.55	3.17	2
29	Zinc_19794473	-96.52	2.34	2	-35.5	4.7	0
30	Zinc_19166762	-98.41	0.19	3	-53.19	1.04	2
31	Zinc_18141403	-97.82	0.03	0	-82.66	0.01	2
32	Zinc_12378847	-96.96	2.11	2	-95.08	1.99	7
33	Zinc_8442293	-97.84	1.53	1	-102.81	1.98	2
34	Zinc_8442272	-105.592	7.71	3	-118.4	2.1	3
35	Zinc_8442270	-94.72	3.16	1	-56.8	4.3	0
36	Zinc_8442214	-93.54	2.79	0	-98.8	2.9	5
37	Zinc_8442186	-69.20	0.67	1	-89.8	4.1	1
38	Zinc_8442171	-120.23	4.54	1	-88.8	5.2	4
39	Zinc_8442163	-91.36	3.35	2	-87.6	4.5	2

40	Zinc_9365179	-72.08	0.06	0	-70.31	0.09	3
41	Zinc_8575396	-98.43	1.01	2	-79.43	2.06	4
42	Zinc_8442278	-110.57	2.01	1	-103.30	0.93	6
43	Zinc_8442277	-114.26	7.8	3	-98.17	1.9	3
44	Zinc_8442271	-83.44	1.5	2	-98.5	2.3	2
45	Zinc_8442219	-64.07	0.06	0	-78.8	4.5	1
46	Zinc_8442218	-102.82	2.24	4	-98.4	2.3	2
47	Zinc_8442210	-72.81	0.18	3	-77.8	0.1	3
48	Zinc_8442204	-126.42	4.82	4	-98.5	5.2	2
49	Zinc_8442174	-111.25	2.05	3	-98.7	6.5	9
50	Zinc_8442165	-118.0	2.7	2	-89.6	7.5	8

On the bases of scoring function and hydrogen bond interactions of ligand-protein we selected 5 ligands compound which are interact with both target protein.
The proposed best 5 ligands interact with both target protein are given in Table 2.

Table 2. Best 5 ligands which interact with both target protein and ADMET Property of Ligands: OSIRIS Property Explorer

S.No	Ligand		clogP	Solubility	DrugLikeness	Drugscore	Molweight
1	Zinc_984053	1-(3-Chloro-quinoxalin-2-yl)-piperidin-3-ol	2.13	-3.04	3.13	0.7	263.0
2	Zinc_634115	4-{4-[(5-bromo-3-methyl-1-benzofuran-2-yl)carbonyl]-1-piperazinyl}phenyl methyl ether	4.44	-5.05	4.02	0.31	426.0
3	Zinc_5556455	Ethyl	0.98	-0.98	-2.36	0.53	29.0
4	Zinc_19166762	3-(1,2-diazabicyclo[2.2.2]oct-2-yl)-1-phenylpropyl acetate	0.99	-1.93	6.08	0.75	288.0
5	Zinc_8442210	4-bromo-2-(1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro-9-acridinyl)phenoxy]acetic acid	3.32	-4.75	-3.47	0.18	445.0

The proposed best 3 ligands interact with Nucleoprotein **(4U2X)** are given in Table 3.

Table 3. Best 3 ligands which interact with Nucleoprotein (4U2X) and ADMET Property of Ligands: OSIRIS Property Explorer

S.No	Ligand		clogP	Solubility	DrugLikeness	Drugscore	Molweight
1	Zinc_1700294	4-(dimethylamino)-3-methyl-2,2-diphenylbutanenitrile	2.77	-2.59	1.93	0.83	278.0
2	Zinc_19990070	2-[(4,6-dioxo-2-thioxo-1-[3-(trifluoromethyl)phenyl]tetrahydro-5(2H)-pyrimidinylidene)methyl]hydrazinecarboxamide	-0.72	-3.97	-1.42	0.17	373.0
3	Zinc_19990034	4-tert-butyl-N-(2-sulfanylphenyl)benzamide	4.33	-5.93	-7.48	0.22	285.0

The proposed best 3 ligands which interact with **Membrane-associated protein (4QAZ)** are given in Table 4.

Table 4. Best 3 ligands which interact with Membrane-associated protein (4QAZ) and ADMET Property of Ligands: OSIRIS Property Explorer

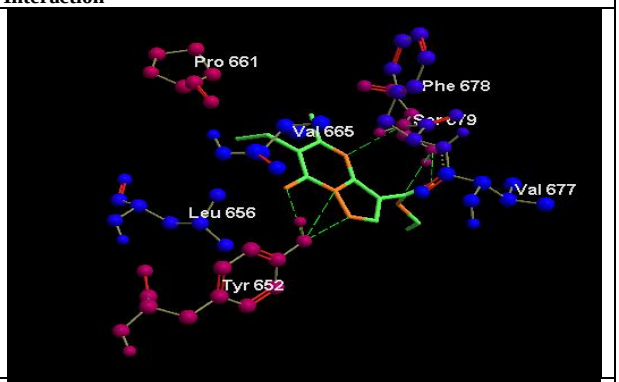
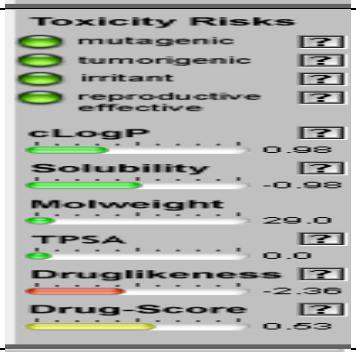
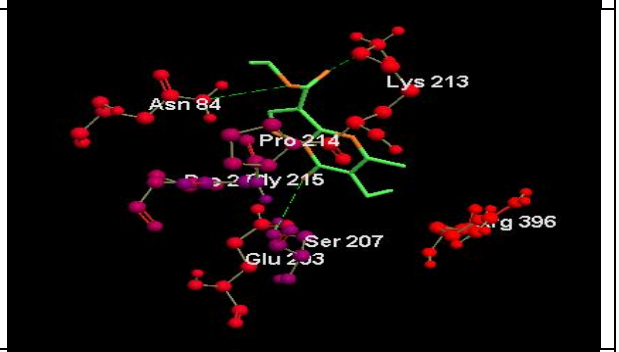
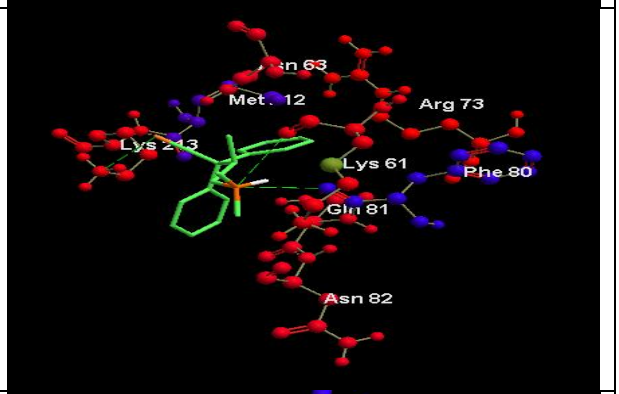
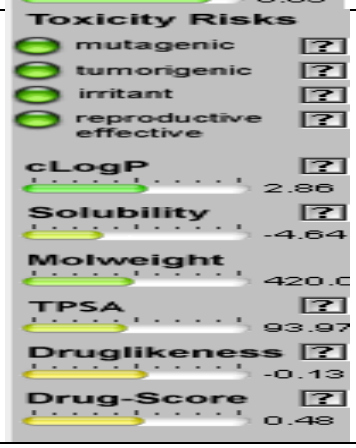
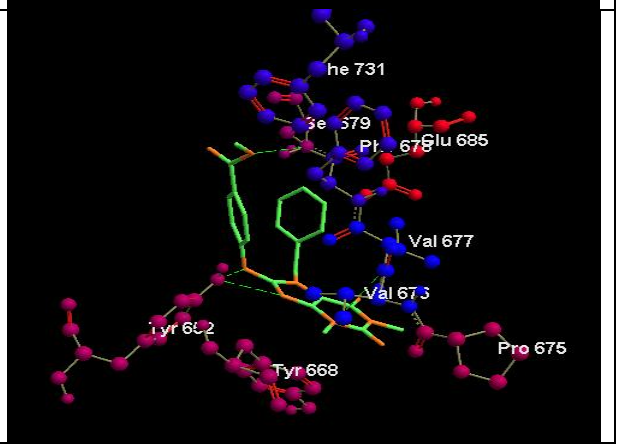
S.No	Ligand		clogP	Solubility	DrugLikeness	Drugscore	Molweight
1	Zinc_9365179	N-(4-methyl-2-pyridinyl)-2-(1-naphthyl)acetamide	3.69	-4.77	0.64	0.33	276.0
2	Zinc_666987	methyl 4-[(7-benzyl-1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)oxy]benzoate	2.86	-4.64	-0.13	0.48	420.0
3	Zinc_8442278	4-methyl-N-(2-methyl-5-{[(4methylphenyl) sulfonyl]amino}phenyl)benzenesulfonamide	3.29	-5.31	-6.69	0.28	430.0

These three ligands were checked in ADMET study.

Zinc_5556455, Zinc_1700294 and Zinc_666987 follow ADME properties well. The Electrostatic**,** Hydrophobic, H-bond interaction and Toxicity Properties of 3 ligands in human body is shown in table 5. Out of 5 ligands, Zinc_5556455 is best for Nucleoprotein (4U2X) and Membrane-associated protein (4QAZ),

Zinc_1700294 is best for Nucleoprotein (4U2X) and Zinc_666987 is best for Membrane-associated protein (4QAZ).

Table 5: Electrostatic, Hydrophobic, H-bond interaction and Toxicity Property of 3 best ligands with target proteins.

Ligand	Protein	ADMET Properties	Interaction
Zinc_5556455	4U2X		
Zinc_5556455	4QAZ		
Zinc_1700294	4QAZ		
Zinc_666987	4U2X		

IV. Conclusion

The interactions between Ebola infected target protein and the ligands were studied by using Molecular docking method. Based on binding energy and hydrogen bond formation, docking results were analyzed. The result were compared to find out the best ligand and Analog of Zinc_5556455, Zinc_1700294 and Zinc_666987

is the most potent ligand which can inhibit the property of the Nucleoprotein (4U2X) and Membrane-associated protein (4QAZ). Thus the in silico method adopted in the present study helped in identifying the ligands using the Docking software and online tools for the treatment of Ebola infection.

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