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Virtual Screening and ADME/Tox Study of Inhibitors against Ebola Virus Matrix Protein VP40

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Abstract: ***Ebola virus (EBOV) belongs to Filoviridae family and is a causative agent of severe viral hemorrhagic fever with high percentage of fatal outcome in humans and nonhuman primates such as monkeys or the great apes of Africa. It has been used as class A agent of bioweapons. EBOV causes severe hemorrhagic fever. In 2014, Ebola virus infections outbreak was found in western Africa, where over 700 people have died. The Ebola virus membrane-associated matrix protein VP40 is a major structural protein that plays a crucial role in virus assembly and budding of virus particles. Currently there is no specific antiviral treatment available for Ebola virus infection. In this study, we performed molecular docking analysis of antiviral drug GS-5734 against matrix protein VP40 (PDB Id: 1H2C) of Ebola virus using Autodock 4.2 tool. GS-5734 is in phase II clinical trials for the treatment of Ebola virus disease. GS-5734 has shown binding affinity of -3.82 kcal/mol with matrix protein VP40. The docked complex was analyzed through Python Molecular Viewer software for their interaction studies. In docked complex, antiviral drug GS-5734 formed one H-bond with GLN159 of matrix protein VP40. Observations made in putative binding site analysis on the protein surface can be very helpful for rational drug design on target protein matrix protein VP40 of Ebola virus.***
Keyword: Bioweapon, Autodock 4.2, Ebola virus, Matrix proteinVP40, ADME.

I. INTRODUCTION

The increased threat of terrorism requires an assessment of the hazard acted by different microorganisms like organic weapons. Particularly, Filoviruses is one of such imperative microorganisms. Ebola infection is class A bio weapon creatures[1][2]. Biowarfare specialists are considered as potential natural weapons since they represent a risk as deadly pathogens and in light of the fact that their utilization by psychological oppressors may bring about outrageous dread and frenzy[2]. Natural weapons represent the most critical fear based oppression risk.

They are moderately simple to deliver and could bring about passing tantamount to atomic weapons. Ebola hemorrhagic fever is an intense viral disorder that prompts fever and a resulting draining diathesis that is set apart by high mortality in human and nonhuman primates[2]. It is caused by Ebola infection, a lipid-encompassed, negative strand RNA infection that has a place with the viral family Filoviridae[3]. Case fatalities extend truly near 53 and 90%**.** Two fundamental methods of transmission into human populaces have been proposed: either guide Contact to a repository or contact to other natural life that likewise contracts EBOV from the store[4]. Epidemiologic perceptions demonstrated that chimpanzees were the wellspring of one human case in two as of late depicted episodes[5].

Transmission of EBOV in human populace occurs by coordinate contact with contaminated blood, or other natural liquids (spit, sweat, semen, drain), and tissues from dead or living tainted people[2][6]. The essential method of transmission in human episodes is human-to-human transmission through direct contact with a symptomatic or dead EVD case or with polluted surfaces and materials[7].

Ebola infection contamination in human causes extreme ailment for which there is directly no antibody or other treatment available[3]. VP40 assumes an imperative part in infection scale areas[8]. VP40 is dynamic not just in the lipid bilayer amid the gathering procedure yet in addition assumes an imperative part either in viral or have cell RNA digestion amid its replication[7]. C-terminal area of VP40 is required for layer affiliation[9].

II. METHODOLOGY

A. Protein Target Structure

The crystal structure of Ebola virus matrix protein VP40 N-terminal domain in complex with RNA (High-resolution VP40 [55-194] variant) (PDB Id: 1H2C) was retrieve from PDB (https://www.rcsb.org/)[10][6].

B. Binding Site Analysis

CASTp server uses the weighted Delaunay triangulation and the alpha complex for shape measurements[9]. This software allows the identification and measurements of surface accessible pockets as well as interior inaccessible cavities of protein structures and other molecules[11].

C. Molecular Docking

Docking is an automated computer algorithm that determines how a compound will bind in the binding site of a protein. The approach includes verifying the orientation of the compound, its conformational geometry, and the docking scores[12].

1) *Autodock 4. 2:* Autodock is docking program that designed to predict how small molecules bind to a receptor of known 3D structure. AutoDock is actually consisting of two main programs[12]. AutoDock performs the docking of the ligand to a set of grids that are describing the target protein; Auto Grid pre-calculates these grids. In additions to using them for docking, the atomic affinity grids can be envisioned.
2) *AutoDock Tools:* AutoDock Tools is the free GUI for AutoDock program developed by the same laboratory that develops AutoDock. We can use it to set up, run and analyze AutoDock dockings and isocontour AutoGrid affinity maps, as well as compute molecular surfaces, display secondary structure ribbons, compute hydrogen-bonds, and do many more useful things[12].
3) *Cygwin:* Cygwin is a collection of free software tools originally developed by Cygnus Solutions to allow various versions of Microsoft Windows to act similar to a Unix system. It targets mainly at porting software that will run on POSIX systems (such as Linux, BSD, and Unix systems) to run on Windows with little more than a recompilation**.**

D. ARGUSLAB

ArgusLab is a molecular modeling, drug design and graphics program for Windows operating systems.

E. Molecular Dynamics Simulations

Molecular dynamics simulations were done using the NAMD (NAnoscale Molecular Dynamics program; v2.7) graphical interface module incorporated visual molecular dynamics (VMD 1.9.2) [2]**.** The protein-ligand complex was submerged in the center of a 50 Å box of water molecules where the water molecule atoms (H-O-H) were near than 1.5 Å and a CHARMM 22 framework file for proteins and lipids was used in the force field for complexes. The psf has been created from the start of pdb and topology files using psfgen package of VMD. After running psfgen, two new files have been generated i.e. protein psf and protein pdb and by retrieving PSF and PDB files; NAMD generated the trajectory DCD file. After the simulations, the results are examine in VMD by calculating the Root mean square deviation of the complex[13].

F. Prediction Of Pharmacokinetic And Toxicological Properties Of The Compounds

A drug that consist good oral absorption must fulfill the parameters following: molecular weight should be less than 500 Da, logP (lipophilicity) less than five (5); top most of five (5) hydrogen donor groups and utmost of ten (10) groups acceptors binding intestinal permeability and constitute the first step to good oral bioavailability. To avoid the failure at the development a set of in vitro ADME/Tox screens has been performed with the aim of discarding compounds in the discovery stage that are likely to fail further down the line. The preADMET server calculates parameters such as human intestinal absorption, cellular permeability Caco-2 in vitro, cell permeability Maden Darby Canine Kidney (MDCK), skin permeability, plasma protein binding, and penetration of the blood-brain barrier, carcinogenicity and mutagenicity[14].

III. RESULTS AND DISCUSSION

A. Binding Site Analysis

CASTp Server predicted binding site residues of Mca model structure .Molecular surface area and volume are 106.140 and 56.019, respectively.

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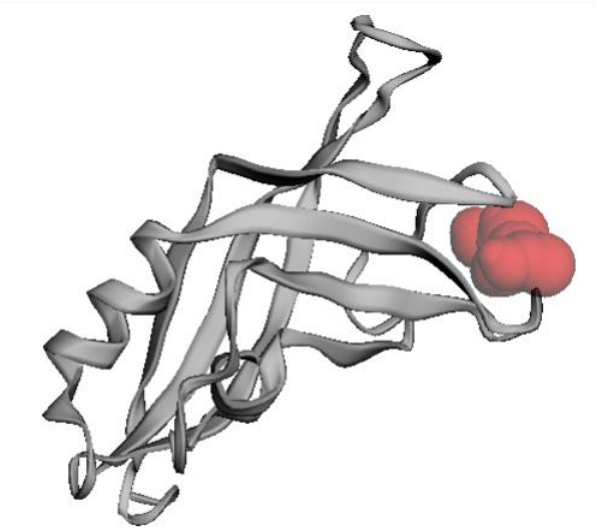

Fig.1: The predicted binding site residues were shown with red color in space fill model and backbone of protein is represented by gray color in ribbon model.

B. Molecular Docking By Autodock

Docking studies prophesied the interaction of ligands with protein and residues involved in binding. After that, the ligands were allowed to run using GA algorithm and A Score scoring function complex. For such interaction studies, the most important requirement was the proper orientation and conformation of ligand which fitted to the enzyme binding site appropriately and formed protein- ligand complex. Therefore, optimal interactions and the best AutoDock score were used as criteria to interpret the best conformation among the 5 conformations, generated by AutoDock program. The docking results of 5 compounds with Mca model.

Table 1: Docking result of Remdesivir and its derivatives with Ebola virus matrix protein VP40.

Sl. No.	Compounds	BE	IME	IE	TorE	VdwE	EE
1	Remdesivir	-2.55	-7.62	-4.82	5.07	-7.46	-0.16
2	58527341	-6.32	-8.41	-2.64	2.09	-8.38	-0.03
3	70649275	-8.29	-11.57	-2.61	3.28	-11.56	-0.02
4	90048786	-7.57	-10.26	-2.98	2.68	-10.28	-0.02
5	117913880	-7.8	-10.79	-3.04	2.98	-10.71	-0.08

BE: Binding Energy; IME: Intermolecular Energy; IE: Internal Energy; TorE: Torsional Energy; VdwE: Vdw-lb Dissolve Energy; EE: Electrostatic Energy.

C. Docking Studies By Arguslab

The model structure of Mca protein was downloaded into ArgusLab program and binding site was made by choosing “Make binding site for this protein” option. The inhibitors were chosen, centered and added hydrogens. In next step, the ligands were allowed to run using Genetic algorithm and Alignment Score scoring functions. ArgusLab 4.0.1 program has two options for docking algorithm which are GA (*Genetic Algorithm*) dock and Argusdock (shape-based search algorithm). We chose GA dock only to compare with AutoDock 4.2. For GA parameters of ArgusLab, population size 50, grid resolution 0.35 Å, maximum generation 1,000, crossover rate 0.8, mutation rate 0.2 and dock engine used Lamarckian Genetic Algorithm. In Argus lab software, Docking calculation type was set to “Dock” and “Flexible” ligand docking mode and used for each docking run.

Table 2: Docking result of Arenosclerin E derivatives with Ebola virus matrix protein VP40.using ArgusLab.

Sl. No.	Compounds	Binding Energy (Kcal/mol)
1	Remdesivir	-2.85
2	58527341	-6.62
3	70649275	-8.54
4	90048786	-7.59
5	117913880	-7.9

Table 3: Binding energy of Remdesivir and its derivatives from AutoDock 4.2 and ArgusLab 4.0.1.

Compounds	Binding Energy (Kcal/mol) AutoDock	Binding Energy (Kcal/mol) ArgusLab
Remdesivir	-2.55	-2.85
58527341	-6.32	-6.62
70649275	-8.29	-8.54
90048786	-7.57	-7.59
117913880	-7.8	-7.9

From the study, the docking results with AutoDock 4.2 and ArgusLab 4.0.1 were compared in table 3. Both programs show almost similar results.

Docking poses of the best conformation of Remdesivir and its four derivatives were analysed by Python molecular viewer in modeled Mca protein were shown in figure 2 to 5. The docking gives proteins the ability to develop or inhibit chemical reactions and to accelerate or stop the process that keep cells alive and maintain a balanced environment. However the specific outcome of a drug could depend on the structure of the molecular aggregates formed. Arenosclerin E derivatives are screened from pubchem compound database have been further dock and verified by ArgusLab with Mca model.

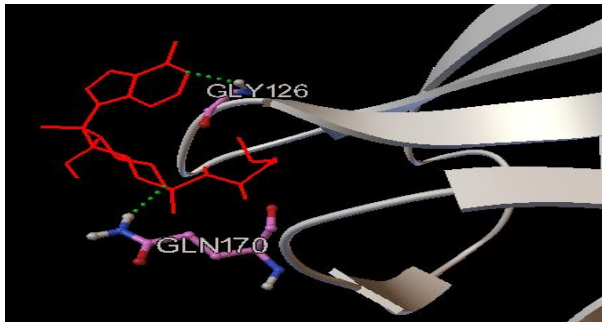

Figure 2: Docking orientation of compound CID58527341 with Ebola virus matrix protein VP40.

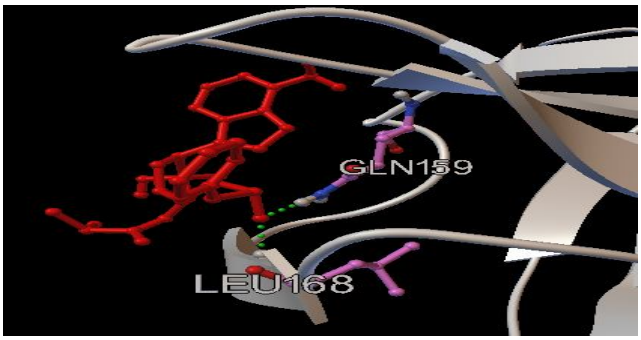

Figure 3: Docking orientation of compound CID70649275 with Ebola virus matrix protein VP40.

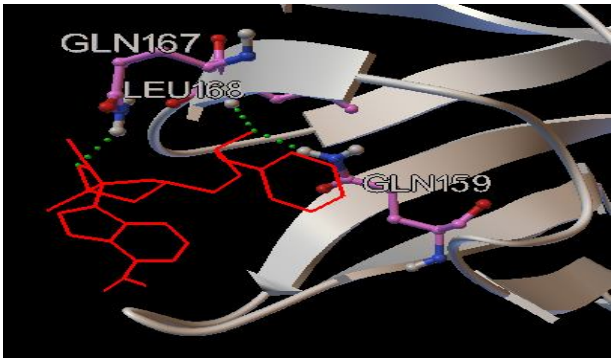

Figure 4: Docking orientation of compound CID90048786 with Ebola virus matrix protein VP40.

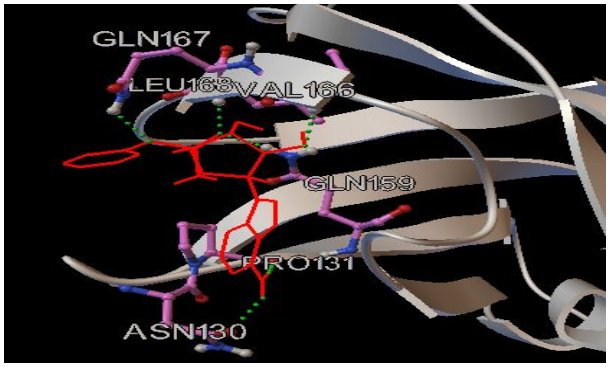

Figure 5: Docking orientation of compound CID117913880 with Ebola virus matrix protein VP40.

D. Molecular Dynamics Simulations

After the simulations, the results were analyzed in VMD by calculating the Root mean square deviation (RMSD) of the complex using rmsd tcl source file from the Tk console and at last file rmsd.dat was saved and accessed in Microsoft office excel. RSMD, a crucial parameter to study the equilibration of MD trajectories, was estimated for backbone atoms of the compound - Mca Measurements of the backbone RMSD for the complex provided insights into the conformational stability.

E. ADME And Toxicological Properties Of Best Predicted Derivatives

In analyzing the parameters of the selected compounds was observed that all had values within Lipinski parameters, except XlogP to evaluate oral absorption .It was observed that the compounds CID 70649275 have human intestinal absorption (HIA) values in the range 98.155672. This compound categorize as in the range of well absorbed compound (HLA: 70-100%). The cell permeability in Caco-2 is an important test to assess intestinal absorption of drugs. It was found that the P_{CaCO2} (nm/s) value were 18.6935 nm/s for compound CID 70649275 .Standard range is P_{CaCO2} >70 nm/sec.

Cell permeability in vitro in MDCK system is used as a tool for the rapid analysis of permeability. This derivative compound CID 70649275 has 0.0526946nm/s as low MDCK. Skin permeability parameter is used in the pharmaceutical industry to assess the risk chemical products in case there is accidental contact with skin. Predicted derivatives compounds 0.290534 showed negative permeability values.

The blood-brain barrier (BBB) has an importance in the pharmacology of drugs, because the compounds are classified as inactive and active compounds. Derivatives bind strongly to plasma proteins. In relation to the penetration of the blood brain barrier the inhibitors analyzed showed penetration values less than 1.The Ames test showed compound was mutagen and carcinogenicity in the mouse and rat showed negative values.

Table 4: Physicochemical properties of best two predicted derivatives.

Pubchem CID	Molecular Weight	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor Count	XLogP
70649275	482.797	1	3	8.4

Table 5: Absorption properties of best predicted two compounds.

Derivative	PubChem CID	Absorption			
		HIA (%)[a]	P_{Caco-2}(nm/sec)[b]	MDCK[c]	Skin Permeability[d]
1	70649275	76.031831	18.6935	0.0526946	-4.83527

Table 7:Distribution properties in percentages of PPB and penetration of the blood brain barrier for two compounds.

Derivative	CID	Distribution	
		PPB (%)	BBB
1	70649275	94.924747	0.0743593

Table 8: Toxicological properties of mutagenicity (Ames test) and carcinogenicity (mouse and rat).

Derivative	CID	Ames Test	Carcinogenicity	
			Mouse	Rat
1	70649275	mutagen	out of range	out of range

IV. CONCLUSION

The result obtained from this study would be useful in understanding the inhibitory mode of CID 70649275 with the Ebola virus matrix protein VP40 and accurately predicting the activities of drugs on the basis of docking scores. Best predicted compound CID 70649275 having lower binding energy. Molecular dynamics simulations showed that predicted was stable. In Silico ADME and Toxicological properties of predicted compounds showed satisfactory results. Therefore it is predicted that compound CID 70649275 could be promising inhibitor for matrix protein VP40 as drug target yet experimental studies have to confirm it.

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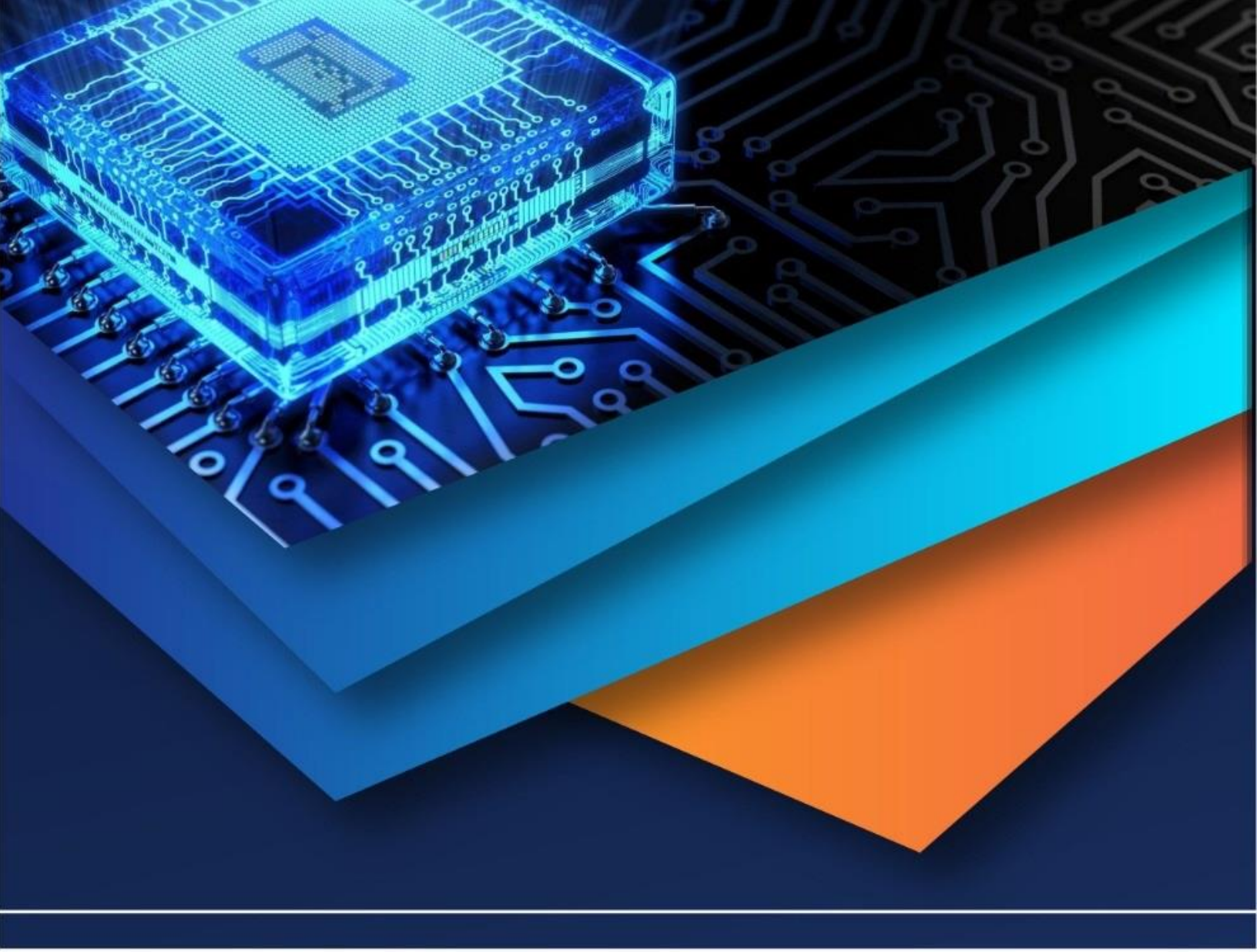

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