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Remdesivir Derivatives as Potential Inhibitors or Ebola Virus Matrix Protein VP40

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Abstract: ***Ebola virus 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 Remdesivir against matrix protein VP40 (PDB Id: 1H2C) of Ebola virus using Autodock 4.2 tool. It has shown binding affinity of -2.55 kcal/mol and it is binding around the active site region of the protein. Active site region of matrix protein VP40 was predicted by CASTp Server. The docked complex was analyzed through Python Molecular Viewer software for their interaction studies. Based on the properties of Remdesivir, more similar compounds were retrieved from PubChem database and a structure-based virtual screening was carried out by AutoDock4.2. Best 2 potent drug-like compounds CID70649275 and CID134502627 were identified and amongst them were found to interact with VP40 protein with binding energy of --8.29 and -8.2 kcal/mol, respectively, which were lower than Arenosclerin E (-2.55 kcal/mol). The docked complex structures were optimized by molecular dynamics simulation for 5 ps with the CHARMM-22 force field using NAMD incorporated in VMD 1.9.2 and then evaluating the stability of complex structures by calculating RMSD. Observations made in this study may extend an assuring platform for developing anti-viral competitive inhibitors against Ebola virus infection.***
Keywords: Remdesivir, Ebola virus, Matrix protein VP40, CASTp, Autodock, ArgusLab

I. INTRODUCTION

Ebola infection is a causative specialist of viral hemorrhagic fever which is related with death rates as high as 90% in people (Feldmann and Geisbert, 2011; Paessler and Walker, 2013). Starting clinical indications of EVD incorporate non-particular manifestations, such as, fever, disquietude, and gastrointestinal contribution, trailed by a fast movement to stun, organ disappointment, and passing (Kortepeter et al., 2011). Ebola infection is class A bioweapon creatures. 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 (Borio et al., 2002; Bray, 2003). Case fatalities extend truly near 53 and 90% (Towner et al., 2008). 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 (Mari Saez et al., 2015).

Ebola infection contamination in human causes extreme ailment for which there is directly no antibody or other treatm. The negative-sense single stranded RNA genome is around 19 kb in size, and comprises of a straight, non-sectioned RNA beng to viral family Filoviridae (Casillas et al., 2003). The linear viral genome encodes for seven proteins: nucleoprotein (NP), polymerase cofactor VP35, grid proteins VP40 and VP24, glycoprotein (GP), translation activator VP30, and RNA-subordinate RNA polymerase (L).Matrix protein VP40 assumes an imperative part in infection get together and growing, VP40 is the bottomless protein in viral particles; it is situated under the viral bilayer to make basic honesty of the particles. VP40 framework protein collects and maturing process happen at the plasma film, which requires lipid pontoon miniaturized scale areas (Geisbert et al., 1995; Bavari et al., 2002).

Drug discovery process is a serious issue in the pharmaceutical industry since it is a very costly and time consuming process to produce new drug potentials and enlarge the scope of diseases incurred (Rao and Srinivas, 2011). The process of drug development targets towards the identification of compounds with pharmacological interest to assist in the treatment of diseases. Drug development for Ebola infection (EBOV) has been in advance for quite a few years. In this study, we docked Remdesivir with Ebola virus matrix protein VP40 using AutoDock4.2.

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/).

B. Binding Site Analysis

CastP server (Dundas et al., 2006) uses the weighted Delaunay triangulation and the alpha complex for shape measurements. This software allows the identification and measurements of surface accessible pockets as well as interior inaccessible cavities of protein structures and other molecules.

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. Docking of Remdesivir and its derivatives against matrix protein VP40 structure was done using molecular docking program AutoDock 4.2 (Goodsell and Olson, 1990; Morriset al. 1998) and ArgusLab 4.0.1. During the docking procedure a Lamarckian Genetic Algorithm (LGA) were used for flexible ligand rigid protein docking calculation.

D. Molecular Dynamics Simulations

Molecular dynamics simulations were done using the NAMD (NAnoscale Molecular Dynamics program; v2.7) graphical interface module (Phillips et al., 2005) incorporated visual molecular dynamics (VMD 1.9.2) (Humphrey et al., 1996). After the simulations, the results were analyzed in VMD by calculating the Root mean square deviation (RMSD) of the complex and was accessed in Microsoft excel.

III. RESULTS AND DISCUSSION

A. Binding Site Analysis

CASTp Server predicted binding site residues of matrix protein VP40 consist of residues HIS124, PHE125, GLY126, LYS127, ALA128, PRO131, GLN159, GLN167, LEU168, PRO169, GLN170 and TYR17 as shown in figure 1 in red color space fill model. Molecular surface area and volume are 106.140 and 56.019, respectively.

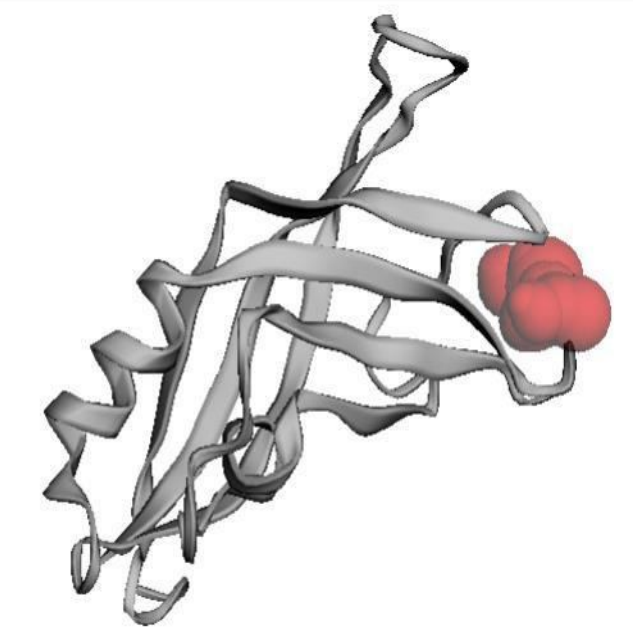

Figure 1: Predicted ligand-binding site. The predicted binding site residues were shown with red color in space fill model and backbone of protein is represented by gray color in ribbon model.

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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 score were used as criteria to interpret the best conformation among the 10 conformations, generated by AutoDock program. The docking results of Remdesivir and its derivatives with matrix protein VP40 was shown in table 1.

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
6	126693021	-7.43	-11.31	-2.97	3.88	-11.25	-0.06
7	126719084	-6.61	-9.3	-2.81	2.68	-9.19	-0.11
8	130312728	-6.53	-9.22	-2.32	2.68	-9.18	-0.04
9	134502618	-6.1	-8.78	-3.26	2.68	-8.69	-0.09
10	134502627	-8.2	-10.88	-2.04	2.68	-10.73	-0.15

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

C. Docking Studies By Arguslab

The Ebola virus matrix protein VP40 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: Binding energy of Remdesivir and its derivatives from AutoDock 4.2 and ArgusLab 4.0.1.

Compound Name/ Pubchem CID	Binding Energy (Kcal/mol) from AutoDock	Binding Energy (Kcal/mol) from 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
126693021	-7.43	-7.41
126719084	-6.61	-6.65
130312728	-6.53	-6.58
134502618	-6.1	-6.28
134502627	-8.2	-8.60

From the study, the docking results with AutoDock 4.2 and ArgusLab 4.0.1 were compared in table 2. Both programs show almost similar results. Docking poses of the best conformation of Remdesivir and its derivative CID134502627 and CID70649275 with Ebola virus matrix protein VP40 were analysed by Python molecular viewer were shown in figure 2 to 4.

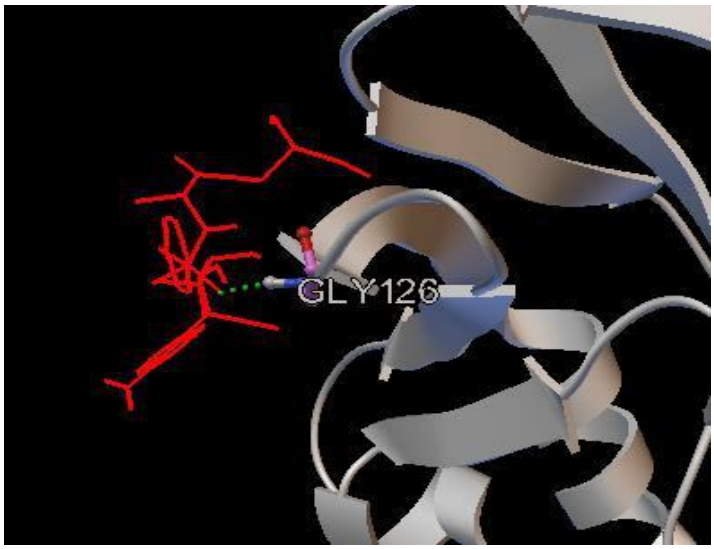

Figure 2: Docking orientation of Remdesivir with Ebola virus matrix protein VP40. Complex depicting compound formed one H-bond with GLY126 of protein. CID134502627 is represented as lines and colored as red.

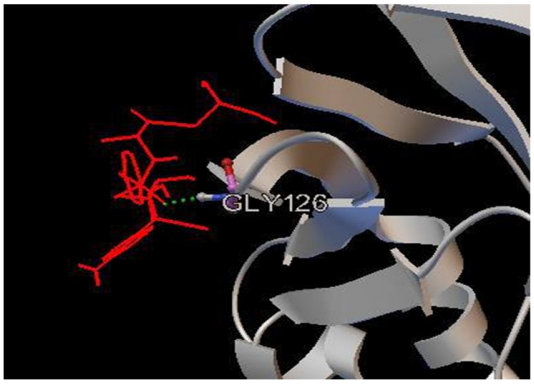

Figure 3: Docking orientation of Compound CID134502627 with Ebola virus matrix protein VP40. Complex depicting compound formed one H-bond with GLY126 of protein. CID134502627 is represented as lines and colored as red.

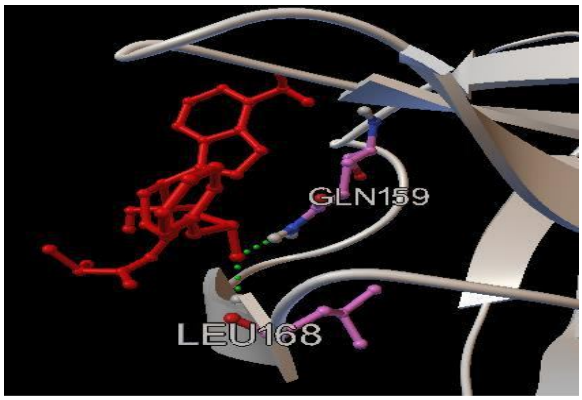

Figure 4: Docking orientation of compound CID70649275 with Ebola virus matrix protein VP40. Complex depicting compound formed two H-bond with GLN159 and LEU168 of protein. CID134502627 is represented as lines and colored as red.

The docking activity gives proteins the ability to promote or inhibit chemical reactions and to accelerate or prevent the processes that keep cells alive and maintain a balanced micro environment. More over the specific effects of a drug could depend on the structure of the molecular aggregates formed. Remdesivir derivatives were screened from pubchem compound database were further dock and verified by ArgusLab with matrix protein VP40.

D. Molecular Dynamics Simulations

RSMD, a crucial parameter to analyze the equilibration of MD trajectories, is estimated for backbone atoms of the compounds CID70649275 and CID134502627 with matrix protein VP40 complex (shown in figure 5 & 6). Measurements of the backbone RMSD for the complex provided insights into the conformational stability.

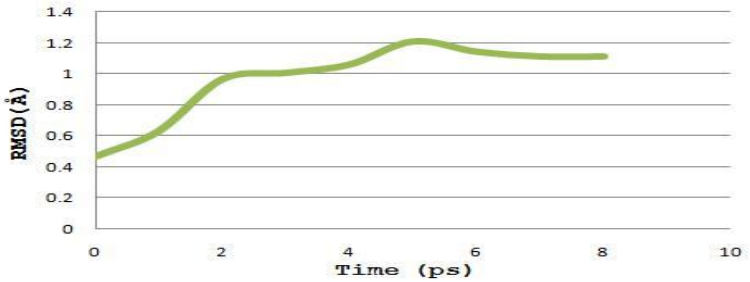

Figure 5**:** Graph displaying root mean square deviation (RMSD) of the backbone atoms of docked complex (CID70649275 - matrix protein VP40) versus time at 310 K, resulted in highest peak at 1.2 Å.

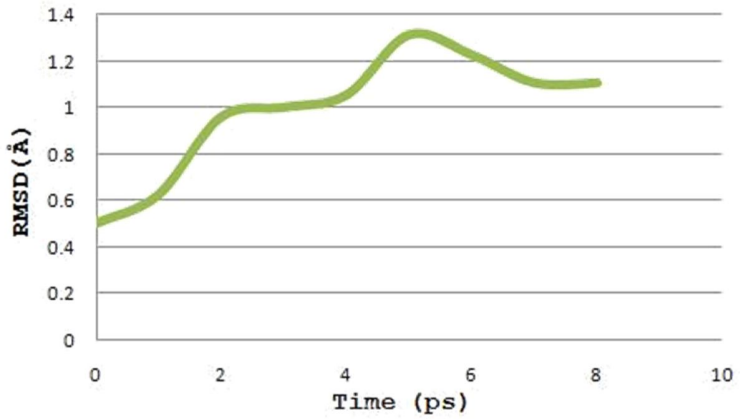

Figure 6: Graph displaying root mean square deviation (RMSD) of the backbone atoms of docked complex (CID134502627 - matrix protein VP40) versus time at 310 K, resulted in highest peak at 1.31 Å.

IV. CONCLUSION

Since chemical synthesis of natural products was problematic and expensive due to their structural complexity, we have used Remdesivir derivatives for docking studies against Ebola virus matrix protein VP40. We used AutoDock and ArgusLab for docking studies in this work because they are convenient for users who are familiar with the Windows operating system. Both the tools were used Lamarckian Genetic Algorithm for docking calculation. Thus from Remdesivir derivatives, which were docked, we got two compound CID70649275 and CID134502627 of them with optimal binding energy -8.29 kcal/mol with AutoDock and -8.2 kcal/mol with ArgusLab. Both the docking tool predicts almost same binding energy. Anaysis of best-docked complex using Python Molecular Viewer shows Compound CID134502627 formed one H-bond with GLY126 of protein. And compound CID70649275 formed two H-bond with GLN159 and LEU168 of protein. Optimizatin of docked protein - inhibitor complexes shows its stability.

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