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Identification of Potent Inhibitors against NS3 Protease of West Nile Virus using in Silico Approaches

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Abstract: ***West Nile Virus (WNV) belongs to* Flavivirus *genus and transmitted by Culex mosquitoes between its avian hosts and occasionally in mammalian hosts. It was first isolated in 1937 from Uganda's West Nile area. The outbreak of WNV into New York in 1999, and its continued spread throughout the north America contaminating more than 19,000 individuals and causing more than 700 fatalities. Presently, there is no effective antiviral treatment available for human WNV contamination. NS3 protease has potential to work as drug target protein since it was involved in fundamental of viral replication. Inhibition of protease could be considered as a strategy for treatment of WNV infection. In this study, we performed molecular docking analysis of antiviral drug Favipiravir against NS3 Protease (PDB Id: 3E90) of WNV using Autodock 4.2 tool. Favipiravir is an antiviral drug, which shown to protect mice against experimental infection with a lethal dose of West Nile virus. Favipiravir has shown binding affinity of -4.63 kcal/mol with NS3 protease. The docked complex was analyzed through Python Molecular Viewer software for their interaction studies. In docked complex, Favipiravir formed two H-bonds with GLY124 and TRP89 of NS3 protease. Observations made in putative binding site analysis on the protein surface can be very helpful for rational drug design on target protein NS3 Protease of West Nile Virus.***
Keywords: NS3 protease; AutoDock4.2; Favipiravir; West Nile Virus.

I. INTRODUCTION

West Nile infection (WNV) belong to *Flavivirus* genus, which contains numerous noteworthy human pathogens, including dengue infection, Japanese encephalitis infection and yellow fever infection, and was first isolated in 1937 from Uganda's West Nile area[1]. WNV has thusly been found in areas of Africa, the Middle East, Europe, Russia, western Asia, and Australia and most as of late in North America[2]. WNV is transmitted by Culex mosquitoes from avian supply hosts to vertebrate deadlock has, including people and stallions[3]. Human contamination is by and large asymptomatic or causes a gentle febrile malady, West Nile fever[4]. Notwithstanding, later contaminations of WNV have additionally been related with higher rates of serious neurological sickness and fatalities, especially among the elderly. Since the presentation of WNV into New York in 1999, the infection has spread quickly all through North America, contaminating more than 19,000 individuals and causing more than 700 fatalities[1][2]. Right now there is no immunization or antiviral treatment for the aversion or treatment of human WNV contamination. WNV is a little, wrapped infection with a solitary stranded, positive sense 11-kb RNA genome, which encodes a solitary polyprotein forerunner[5]. This polyprotein must be cut co-and post-translationally to create 10 utilitarian proteins: three basic (C, prM, and E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). NS3 is a multifunctional protein, the protease containing the N-terminal third and nucleotide triphosphatase, RNA triphosphatase, and helicase parts involving the rest of NS3 protease is a potential helpful target since it is fundamental for viral replication[6].

II. METHODOLOGY

A. Target Protein Structure

The 3D coordinates of the West Nile virus NS2B-NS3 protease in complexed with inhibitor Naph-KKR-H (PDB Id: 3E90) was retrieved from Protein Databank. This is used as a target model for flexible docking. The structure was optimized using the chimera tool[2][7].

B. Binding Site Analysis

CASTp server is used for shape measurements. This software provides the identification and measurements of surface accessible pockets as well as interior inaccessible cavities of protein structures and other molecules[8].

C. Inhibitor Dataset

The 3D structure of Favipiravir was downloaded in .sdf format from pubchem compound database. That was later converted in .mol format with the help of open babel tool[6][3].

D. Molecular Docking

Docking is an automated computer algorithm that decides how a compound will bind in the active site of a protein[9]. This includes determining the orientation of the compound, its conformational geometry, and the scoring. The scoring may be a binding energy, free energy, or a qualitative numerical measure[10].

1) *Autodock 4.2:* Autodock 4.2 is a docking program that is designed to predict how small moleculesbind to a receptor of known 3D structure. AutoDock 4.2 actually consists of two main programs: AutoDock performs the docking of the ligand to a set of grids depicting the target protein; Auto Grid pre-calculates these grids. In additions to using them for docking, the atomic affinity grids can be visualized[11].
2) *Autodock Tool:* AutoDock Tool is the free GUI for AutoDock program .We can use it to set up, run and analyse AutoDock dockings and isocontour AutoGrid affinity maps, as well as compute molecular surfaces, display secondary structure ribbons, count 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 permit various versions of Microsoft Windows to act similar to a Unix system. It aims mainly at porting software that runs on POSIX systems (such as Linux, BSD, and Unix systems) to run on Windows with little more than a recompilation[13].

E. Molecular Dynamics Simulation

Molecular dynamics simulations were done by using the NAMD (NAnoscale Molecular Dynamics program; v2.7) graphical interface module incorporated visual molecular dynamics (VMD 1.9.2)[14]. A protein structure file (psf) stores structural information of the protein, such as different types of bonding interactions. The psf was created from the initial pdb and topology files using psfgen package of VMD. After running psfgen, two new files were generated protein pdb and protein psf and after accessing PSF and PDB files; NAMD generated the trajectory DCD file[15]. After the simulations, the results were analysed in VMD by calculating the Root mean square deviation 28 (RMSD) of the complex by using rmsdtcl source file[14].

F. Prediction Of Pharmacokinetic And Toxicological Properties Of The Inhibitors

A drug to have good oral absorption must satisfy to these following parameters: molecular weight of less than 500 Da, logP (lipophilicity) less than 5; maximum of 5 hydrogen donor groups and maximum of 10 groups of acceptors binding intestinal permeability and comprise the first steps to good oral bioavailability. The absorption, distribution, metabolism, excretion (ADME) and toxic (Tox) properties were calculated with the aid of online server preADMET[16]. Over 50% of the candidates failed due to ADME/Tox deficiencies during development. To avoid this failure at the development a set of in vitro ADME/Tox screens has been implemented with the target of discarding compounds in the discovery phase that are likely to fail further down the line[17]. The preADMET server calculates parameters such as human intestinal absorption, cellular permeability of Caco-2 in vitro, cell permeability, skin permeability, plasma protein binding, and blood - brain barrier penetration, mutagenicity and carcinogenicity[12][18].

III. RESULTS AND DISCUSSION

A. Analysis Of Binding Site

CASTp Server had predicted binding site residues of NS3 protease structure.
Molecular surface area and volume are 378.834 and 493.576, respectively.

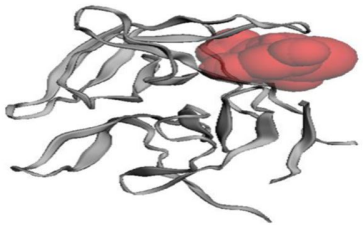

Fig 1: Predicted ligand-binding site. The predicted binding site residues were shown with red colour in space fill model and backbone of protein is represented by grey colour in ribbon model.

B. Molecular Docking

Docking studies prophesied the interaction of ligand with protein and residues involved in binding. After that, the ligand was allowed to run using GA algorithm and a Score scoring function complex. Optimal interactions and the best score is used as criteria to interpret the best conformation among the all conformations, generated by AutoDock program.

Table 1: Docking result of Favipiravir with NS3 protease

Sl no.	Pubchem CID/Drugs	BE	IME	IE	TorE	VdwE	EE
1	Favipiravir	-5.11	-5.41	-0.48	0.3	-5.31	-0.1

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 WNV NS3 Protease protein was downloaded into ArgusLab program and binding site was made by choosing "Make binding site for this protein" option. The inhibitor was chosen, centred and added hydrogens. In next step, the ligand was allowed to run using GA (Genetic Algorithm) dock to compare with AutoDock 4.2. In Argus lab tool, docking calculation type was set to "Dock" and "Flexible" ligand docking mode and used for each docking run.

Table 2: Binding energy of Favipiravir from AutoDock 4.2 and ArgusLab.

Sl no.	Pubchem CID/ Drug	Binding Energy(Kcal/mol) from AutoDock	Binding Energy (Kcal/mol) from ArgusLab
1	Favipiravir	-5.11	-5.02

From the study, the docking result with AutoDock 4.2 and ArgusLab 4.0.1 was compared in table 2. Both programs show almost similar result.

Docking poses of the best conformation of Favipiravir with WNV NS3 Protease were analysed by Python molecular viewer were shown in figure 2.

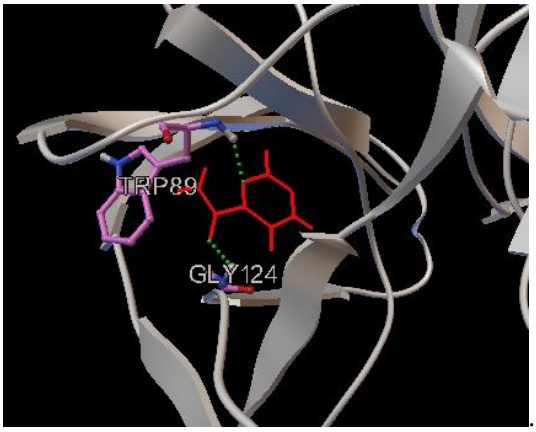

Fig 2: Docking orientation of Favipiravir with NS3 Protease**.** Complex depicting compound formed two H-bond with GLY124 and TRP89 of protein. Compound Favipiravir is represented as lines and coloured as red.

Docking activity provides ability to the protein 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 Favipiravir was screened from pubchem compound database was further dock and verified by ArgusLab with NS3 Protease.

D. Molecular Dynamics Simulations

MD simulation is a well-known theoretical technique and is mainly used for evaluating the stability of any predicted 3D model. RMSD, a crucial parameter to analyse the equilibration of MD trajectories, is estimated for backbone atoms of the compound Favipiravir with NS3 Protease complex. Measurements of the backbone RMSD for the complex provided insights into the conformational stability.

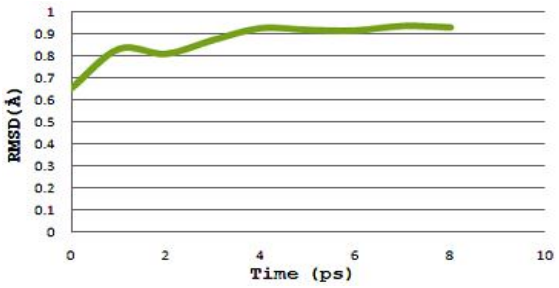

Fig 3: Graph displaying root mean square deviation (RMSD) of the backbone atoms of docked complex (Favipiravir - NS3 Protease) versus time at 310 K, resulted in highest peak at 0.93 Å.

IV. CONCLUSION

We used AutoDock and ArgusLab for docking studies of Favipiravir against WNV NS3 Protease. Both the tools were used Lamarckian Genetic Algorithm for this docking calculation. Thus from this docking study, we got that Favipiravir had optimal binding energy -5.11 kcal/mol with AutoDock and -5.02 kcal/mol with ArgusLab, respectively. Both the docking tool predicts almost same binding energy. Analysis of docked complex using Python Molecular Viewer shows Compound Favipiravir formed two H-bond with GLY124 and TRP89 of protein NS3 Protease. Optimization of the docked protein - inhibitor complex shows its stability.

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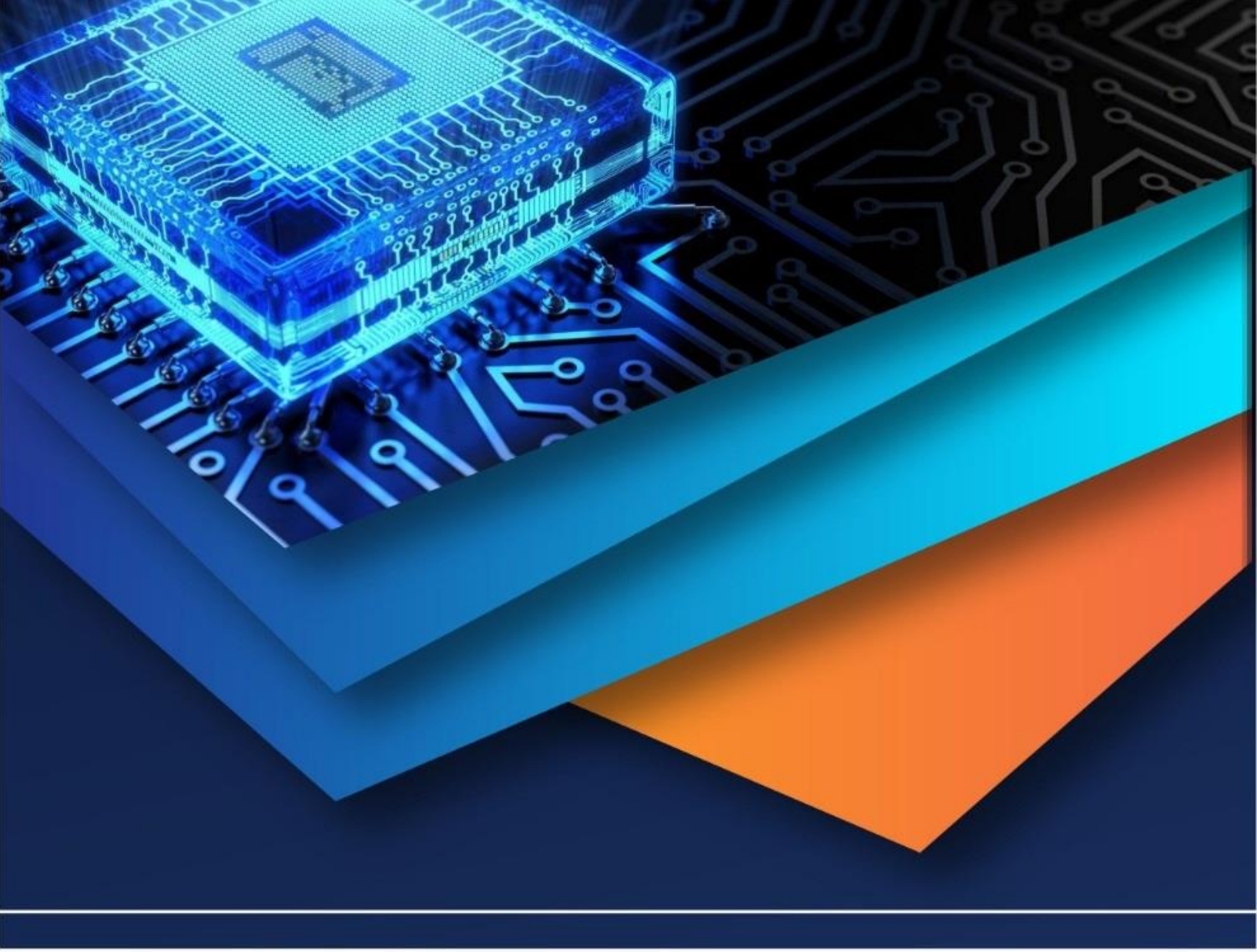

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