

**HOMOLOGY MODELING AND DOCKING STUDIES OF JAPANESE ENCEPHALITIS VIRUS  
PROTEINS LIKE ENVELOPE PROTEIN AND NS1 PROTEIN**

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**ABSTRACT:** Japanese Encephalitis (JE) is a vector-borne, viral zoonosis that may affect humans. The disease periodically becomes endemic in areas such as northern India, parts of central and southern India. Japanese Encephalitis virus belongs to the mostly vector-borne flaviviridae, which are single stranded RNA viruses. The envelope glycoprotein of JE Viruses contain specific as well as cross relative, neutralizing epitopes. The objective of this research to find out the best ligand molecule each for the two drug targeting protein present in the JEV. This will be done by studying the complete structure of JEV drug targeting proteins and their interaction with their respective ligand. The envelope protein and NS1 protein have been studied. The minimum energies were recorded after the docking studies for all the inhibitors docked with the protein. After comparison of the minimum energies recorded, the ligand with the least minimum docking energy has been considered as the best ligand. The entire study indicates that the inhibitor Mycophenolate with minimum energy -5.00605kJ/mol is the most effective against Envelope protein. However in case of NS1 protein, the inhibitor Deoxynojirimycin with the minimum energy of -6.75932kJ/mol is found to be the most effective.

**Key words-**Envelope protein, NS1 protein, Molecular Docking, Japanese Encephalitis Virus, Homology modelling

**INTRODUCTION**

Japanese Encephalitis is an important mosquito-borne viral disease and one of the leading causes of viral encephalitis and neurological infection in Asia and is one of the important arboviral encephalitis in tropical and subtropical region in Asia. The mortality is as high as 20% during epidemic periods, especially in young children and those over 65 years old. Although severely under-reported, 550000 cases are annually recorded throughout Asia, with the 15,000 death (5-35% cases fatality rate) and a 75% JE-related disability rate. The disease was first recognized in India in 1955, when cases of Japanese encephalitis from North Arcot district of Tamil Nadu and neighbouring districts of Andhra Pradesh. Since 1972, JE has spread to newer areas and epidemics/outbreaks have been reported from West Bengal, Uttar Pradesh, Assam, Manipur, Bihar, Andhra Pradesh, Pondicherry, Karnataka, and Goa and recently from Kerala and Maharashtra. Japanese encephalitis virus (JEV) is a positive-sense single-stranded RNA virus belonging to the Flavivirus genus of the family Flaviviridae along with several other viruses including West Nile virus (WNV), Murray valley encephalitis virus (MVEV) and St. Louis encephalitis (SLEV), Tick borne encephalitis (TBEV), Yellow fever virus (YFV) and Dengue virus (DENV). Japanese encephalitis virus is the prototypic member of JEV serocomplex of flaviviruses. The symptoms of Japanese Encephalitis typically include fever and headache, but other incapacitating manifestations also usually results and frequently involve in neurological problem, including brain damage. The JE Virus genome is single-stranded positive-sense RNA of approximately 11 kb in length and contains both 5' and 3' untranslated regions (Vrati, 2000).

The envelope proteins are the major structural protein, responsible for cellular attachment and possess a hydrophobic loop that mediates fusion of viral and host membranes. Infected by Japanese encephalitis virus is initiated by fusion between the viral membrane and the host membrane. The fusion process is mediated by the Japanese encephalitis virus Envelope protein in a pH-dependent manner (Stiasny and Heinz 2006).

In the present time, there is no treatment against JEV strains. Some vaccines are available but they cannot treat all strains of JEV disease, so further studies are required for the proper evaluation and investigation their use in treating JEV infections. The available data open up a new avenue for identifying antiviral agents active against early steps of JEV infection.

## METHODOLOGY

### Sequence Retrieval and secondary structure prediction

The amino acid sequence of JEV Envelope protein (Accession no ADN27987), NS1 protein (Accession no AAL68990) was retrieved from NCBI database. Secondary structure was analysed by using PSIPRED.

### Homology modeling

The sequence of envelope protein and NS1 protein were searched against the protein database using BLAST-P. The protein having PDB Id: 3UAJ and 4O6C was selected to be used as template for 3D modelling of envelope protein and NS1 protein respectively. Homology modelling structure prediction was carried out using Automated Swiss model server and RaptorX. The modelled PDB file was visualized using UCSF Chimera and Discovery Studio 4.0 and validated using PROCHECK. 3D models were validated on the basis of Ramachandran plot statistics. From the generated models, the one with highest number of residues in the allowed region and minimum number of residues in the disallowed region were considered as the suitable model for each of the protein. The best model was then considered for further analysis.

### Ligand Selection

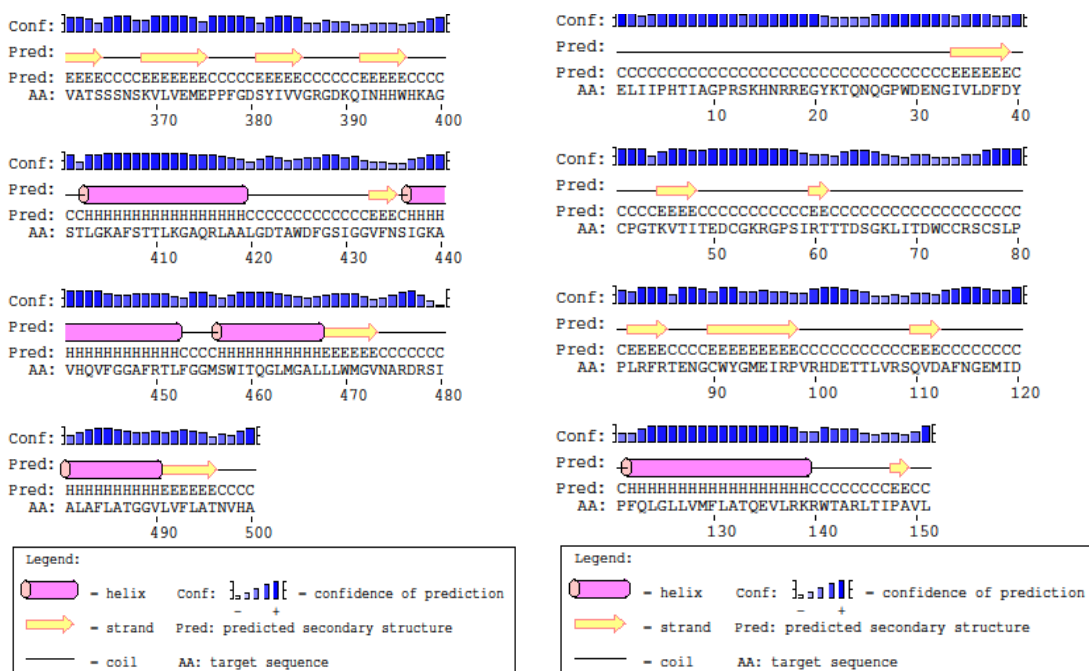
Chemical compounds were taken from the National Centre for Biotechnology Information (NCBI) Pub-Chem database. These molecules were downloaded in Structure Data File (SDF) format and converted to Protein Data Bank (PDB) coordinates file using Discovery Studio 4.0. The selected ligand molecules were passed through the Lipinski filter (<http://www.scfbioitd.res.in/software/utility/LipinskiFilters.jsp>) for identifying their drug-likeness properties and only the molecules that passed through this filter were used for further analysis.

### Molecular Docking

Swiss Dock was used for docking of selected antiviral molecules with Envelope protein and NS1 protein of JEV. Docking studies helps in prediction of the preferred orientation of a ligand with the binding site on a protein. Molecular docking was used to determine appropriate binding orientations and conformations of various chemical compounds at the target site. After docking, all the legend confirmations were ranked on the basis of their binding energy. To check the accuracy of docking result, Patch dock tools (Schneidman et al. 2005) were also used. Protein and ligand interactions were determined using UCSF Chimera and Discovery Studio 4.0, which explained the active binding sites in receptor protein and show best docking confirmation.

## RESULT AND DISCUSSION

The Envelope protein, most important structural protein present on the surface of mature JEV, mediates the receptor binding and membrane fusion is a possible target for drug designing. Envelope protein and NS1 protein has significant sequence information in NCBI virus database. The secondary structure prediction indicated the difference in the structure of these two proteins. The Envelop protein has almost equal proportion of helix and strands, whereas NS1 protein has only one helix and more strands as shown in the figure 1.



(A) (B)  
**Figure 1-Secondary Structure of (A) Envelope Protein (B) NS1 Protein**

### Tertiary structure of Envelope protein and NS1 protein by Homology modeling

The complete protein sequence of JEV envelope protein (ADN27987) and NS1 protein (AAL68990) was used in the study. The length of E protein was 500 amino acid residues and NS1 protein was 151 amino acid residues. The Protein BLAST program for envelop protein and NS1 protein sequence were executed and hits provided 46.94% and 76.11% similarity respectively. Envelope protein and NS1 protein sequence was modelled using suitable template with pdb id: 3UAJ and 4O6C respectively. The models were generated by Swiss model server and Raptor X, and free energy of 3-D structures of E protein and NS1 protein were evaluated. Only the model which was thermodynamically stable was selected for further refinement and validation. The 3D models for both proteins are shown in figure 2.

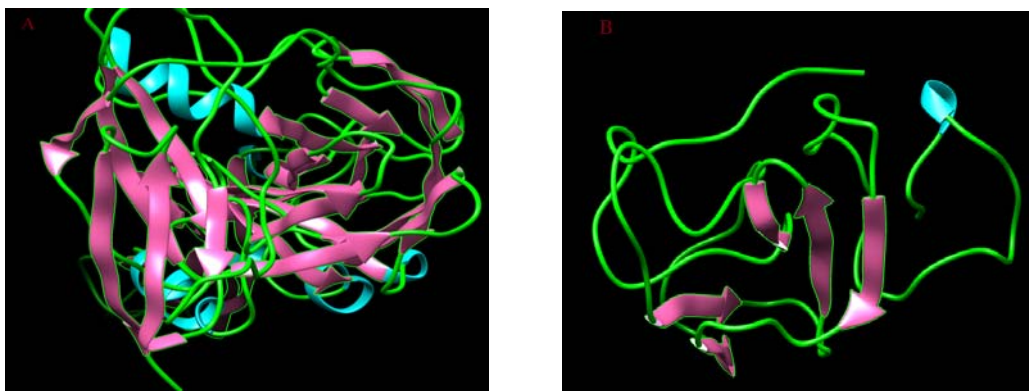


Figure 2-Homology Model of (A) Envelope protein (B) NS1 Protein

### Validation of predicted structure

The models were subjected to validation using PROCHECK server. According to Ramachandran plot, a good quality model will be expected to have over 90% residues in core region. Ramachandran plot for Envelope protein shows that 90.0% residues are in most favoured region, 8.7% are in additional allowed region and only 0.4 % residues are present in disallowed region as shown in figure 3A, and NS1 protein shows that 91.0% residues are in most favoured region as shown in figure 3B. Thus, the final model was validated as good quality model whose 3-D coordinates were viewed via Discovery Studio and UCSF Chimera tool and it depicts beta sheets rich structures.

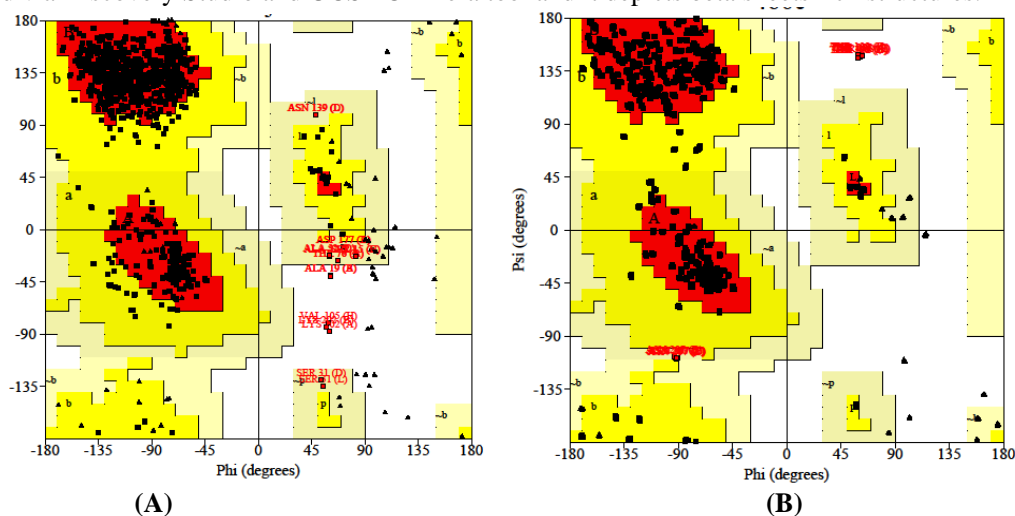


Figure 3- (A) Ramachandran plot of JEV Envelope protein, (B) Ramachandran plot of NS1 protein.

### Screening and optimization of inhibitors

The envelope protein of JEV has been reported to play a major role in the virus life cycle. Therefore, antiviral molecules were required to block the virus infection. 50 lead molecules were selected from the PubChem compound database as ligand molecules. Out of 50 molecule, 44 lead molecules did not follow the 5 Lipinski rules, i.e., not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight not more than 500 kDa, and an octanol– water partition coefficient, log P not more than 5 (Oprea et al. 2001) or those that had a polar surface area of 140 Å<sup>2</sup> as suggested by Ghose et al. (1999) were not considered. After this filtration step, only 6 lead molecules remained that were used for further analysis. The PDB coordinates file of the Envelope and NS1 proteins and selected lead molecules (as ligand) were subjected to docking study using SwissDock and PatchDock tools.

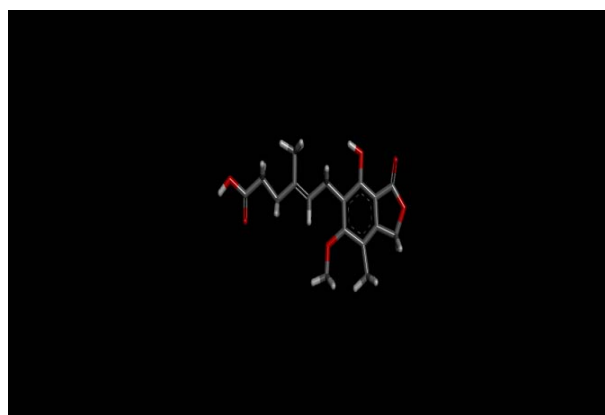
### Docking Study

Binding energy for each docking was calculated using a semi-empirical free energy force field. Out of these 6 docked molecules with each receptor, top three molecules were filtered out on the basis of binding energy. The binding modes and geometrical orientation of all compounds were almost identical, suggesting that all the inhibitors occupied a common cavity in the receptor. The binding energy of top three inhibitor molecules with an active site of receptor protein is given in Table 1.

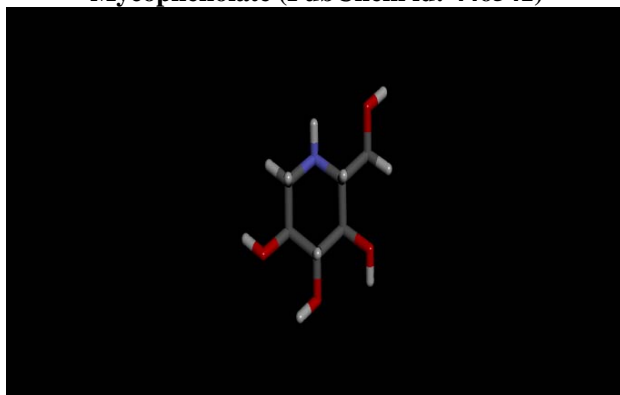
**Table 1 - Docking Energies for selected Ligand molecules with different tools.**

S.No	Ligand Compound Name and PubChem ID	Receptor Protein Name	Swiss Dock Kcal/mol	PatchDock Kcal/mol
1	Mycophenolate (446541)	Envelope protein	-5.00605	-45.90
2	Castanospermine (54445)	Envelope protein	-4.59304	-28.63
3	Beta-L-Fucose (444863)	Envelope protein	4.34804	-23.13
4	Deoxynojirimycin(29435)	NS1 protein	-6.75932	-20.13
5	N-Acetyl-D-Glucosamine(24139)	NS1 protein	-3.82255	-16.46
6	Triaryl Pyrazoline (11646325)	NS1 protein	26.322	-13.85

The binding energy profile of each ligand molecule indicated that Mycophenolate (PubChem id: 446541) and Deoxynojirimycin (PubChem id: 29435) bind to envelop protein and NS1 protein respectively with the highest efficiency. The structure of these two compounds have been shown in figure 4.



**Mycophenolate (PubChem id: 446541)**



**Deoxynojirimycin (PubChem id: 29435)**

**Figure 4 – Structure of best lead molecules with their compound IDs and name.**

For confirming the accuracy of the predicted molecule, PatchDock tools were also used. The PatchDock tool is a geometry-based molecular docking algorithm to identify docking transformations, molecular shape complementarities, perform clustering and calculates the global binding energy. The binding geometry of Envelope protein with Mycophenolate and NS1 protein with Deoxynojirimycin is shown in figure 5.

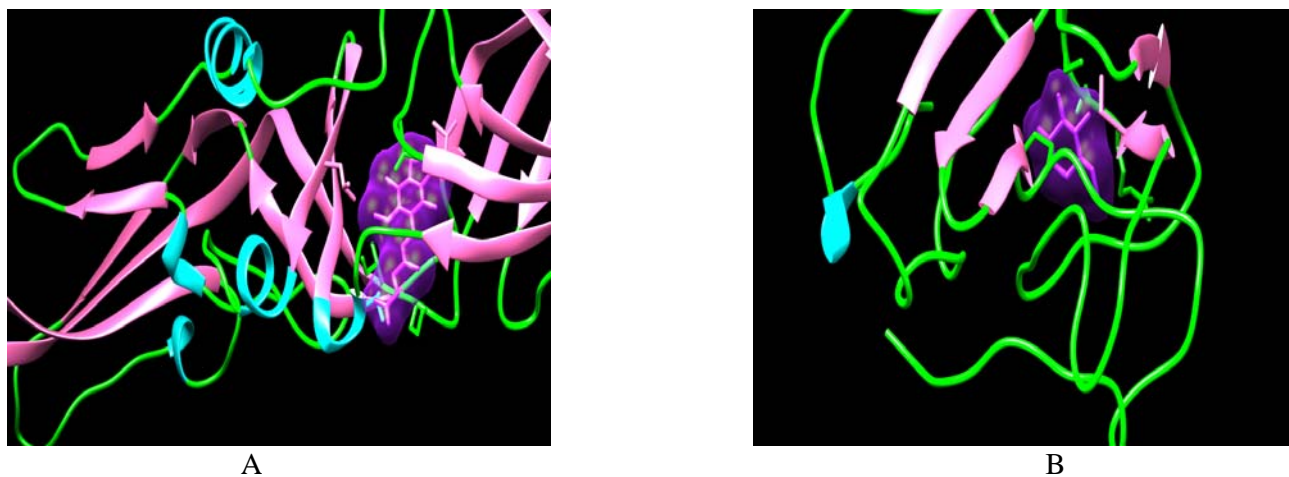


Figure 5 – Binding geometry of (A) Envelope protein with Mycophenolate and (B) NS1 protein with Deoxyojirimycin.

The non-bonded interactions of Mycophenolate with Envelope protein active site amino acids and Deoxyojirimycin with NS1 protein active site amino acids were found using PatchDock tool. The different pattern of hydrogen bonding and pi-pi stacking can be observed in the figure 6.

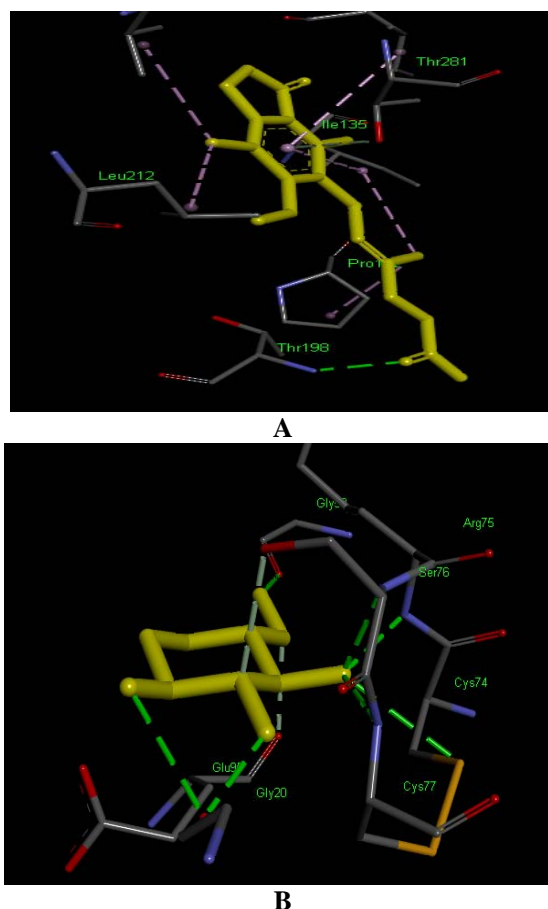


Figure 6- Non-bonded interaction of (A) Envelope protein with Mycophenolate and (B) NS1 protein with Deoxyojirimycin.

Mycophenolate has shown good docking energy with Envelope protein, which is -5.00605 kcal/mol and -45.90 kcal/mol as calculated by Swiss Dock and PatchDock tool respectively. This compound has shown strong binding pattern as well with envelop protein receptor. Hence, in the present study, Mycophenolate was confirmed to be an appropriate molecule, and it might be considered as potential antiviral drug candidate for Envelope protein.



Deoxynojirimycin has shown good docking energy with NS1 protein, which is -6.75932 kcal/mol and -45.90 kcal/mol, as calculated by Swiss Dock and PatchDock tool respectively. This compound has also shown strong binding pattern with NS1 protein receptor. Hence, in the present study, Deoxynojirimycin was confirmed to be an appropriate molecule using and it might be considered as potential antiviral drug candidate for NS1 protein.

## CONCLUSION

The main objective of this study was to identify suitable ligand molecules against envelope protein and NS1 protein JEV. Consequently, the 3-D model of conserved envelope protein NS1 protein of Japanese encephalitis virus was designed and validated. The envelope protein model described that it has 90.0 % residues in core region and NS1 protein model has 91.0% in core region of Ramachandran plot analysis. A computational screening protocol was used to identify small molecular compounds that bind to the active pocket of the Envelope protein and NS1 protein of JEV with the goal of identifying potential lead molecules. The screening was performed over more than one million molecules from PubChem compound database relying on computational docking prediction with Swiss Dock and PatchDock. The comparatively less docking energy of the three lead molecules suggests these novel leads would potentially bind more strongly to active pocket of JEV E protein and NS1 protein. The Mycophenolate (CID 446541) indicated the best interaction with less docking energy and excellent pharmacological properties with JEV Envelope protein where as Deoxynojirimycin (CID 29435) indicated the best interaction with less docking energy and excellent pharmacological properties with JEV NS1 protein.

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ISSN : 0976-4550

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