# Identification of Inhibitor against Zika Virus NS3 Helicase

Kirti Singh
M.Tech Graduate
Department of Biotechnology,
Madhav Institute of Technology and Sciences, Gwalior, India

***Abstract*: Zika virus belongs to falvivirus family, which is an arbovirus. Mosquito *Aedes aegypti* is the primary vector of Zika virus. It was first isolated in year 1947 the Zika Forest of Uganda. Zika virus have had instances in past years, but it has recently been declared as a health emergency by World Health Organization. Symptoms of Zika virus share the resemblance of other flavivirus, Dengue virus. Non structural (NS) proteins of Zika virus have a key role in infection, amongst them NS3 helicase has been chosen as an area of interest in the following paper. NS3 helicase is required in unwinding of the RNA secondary structure in the template RNAs. Hence, knowing its essential role in genome replication, NS3 helicase could be an attractive target for drug development against Zika virus. In the following research bioinformatics approach of molecular docking has been selected, for the identification of potential drug against NS3 helicase. Four compounds, Benzoxazole, Suramin, Ivermectin and Ribavirin, which were already reported against other flaviviruses, viz Dengue and West nile virus, were docked against NS3 helicase. Two flavonoids were also docked with NS3 helicase, which are luteolin and catechin. The PDB structure of NS3 helicase was downloaded from RCSB Protein Data Bank and ligand were downloaded from ZINC and PubChem databases. Dock6 software was used for molecular docking which is based on geometric algorithm. Amongst all, Ribavirin was shown to bind successfully with NS3 helicase site 1 whereas catechin and luteolin was found to bind at site 2.**

***Keywords:* Zika virus, flavivirus, molecular docking, NS3 Helicase, Dengue Virus, inhibitor, ligand.**

## I. INTRODUCTION

Zika virus (ZIKV) is an arbovirus belongs to the genus *Flavivirus*, family *Flaviviridae*. It was first isolated in 1947 from a febrile rhesus macaque monkey in the Zika Forest of Uganda **(Dick GWA *et al.*, 1952)**. It is a mosquito-borne flavivirus distributed in Africa, Asia and South America. Mosquito *Aedes aegypti* is the primary vector of Zika virus.

As per the World Health Organization (WHO), there are an expected 200,000 instances of yellow fever every year, bringing on 30,000 passing. Contamination with the infection may bring about intense febrile sickness that clinically looks like dengue fever.

There have been reports of increase in microcephaly and other neurologic complications associated with ZIKV infection, as well as a 20-fold increase in the incidence of Guillain-Barre Syndrome during ZIKV outbreaks **(Darko R *et al* 2016)**. Currently there is no drug or vaccine for the virus wherein antibodies against ZIKV have been detected in numerous other animal species including water buffalo, elephants, goats, hippos, impala, lions, sheep, rodents, and zebras. **(Haddow AD, *et al* 2012).** And number of drugs had been tested and potentially found inhibiting Zika virus, which are: bortezomib and mycophenolic acid (antiviral) and others that had no previously known antiviral activity e.g., daptomycin **(Barrows *et al.* 2016).**

There has been a growing interest in discovering drugs against flavivirus. Since a single amino acid substitution can determine resistance to a given drug, systematic benchmarking of starting genetic material and resulting data is very important to study. To develop a potential antiviral against ZIKV, structural analogy or inhibiting infection and replication approach could be pursued. Small molecule inhibitors and antibodies that could be developed should be selected that has minimal side effects. For that purpose target-based design of flavivirus replication inhibitors may prove as a promising strategy.

The 3-dimensional structure of an enzyme of its active site will be used to identify possible inhibitors of target protein. Molecular docking will be used to identify the ligands with high binding affinity to the protein active site. In addition to the flaviviral replicase complex, the protease and helicase are the centre interest as a target for new antiviral drug.

As ZIKV has been declared as a public health emergency, 20 countries have been reported local transmission of virus during 2016. And there is no clinically approved therapy for Zika virus.

So, developing an antiviral agent with the use of structural biology and inhibition mechanisms using computer aided drug designing we may develop antiviral agent that may halt spread of this potential devastating virus.

Aim of the research is: 1.Analysis of mechanism of ZIKV virus to cause disease. 2 To identify virus targets and selection of suitable drug target. 3. Searching the database for compound similar to target agonist. 4. Selection from database hits by molecular Docking.

## II. METHODS

### 2.1. Retrieval of NS3 Helicase Zika Virus PDB Structure:

PDB structure of NS3 helicase of Zika virus French Polynesia strain (PDB_ID- 5JRZ) (**J Rinku *et al*., 2016**) was retrieved from Protein Data Bank (**Kongsaeree *et al*., 2005**).

### 2.2. Structural Validation using PROCHECK 3.4.

PROCHECK was downloaded from EMBL-EBI home page. A version of PROCHECK programs running under Windows NT was prepared by Bernard Rupp of Lawrence Livermore national laboratory, http://www.ruppweb.org/ftp. Zip file was then extracted. (**Laskowski *et al*., 1993**, **1996**). File name was entered i.e., 5JRZ on cmd_prompt to get the plot.

### 2.3 Ligand searching:

Zika virus helicase shares structural similarity with Dengue virus helicase (**J Rinku *et al*., 2016**) hence compounds which have been reported as potential inhibitors for flavivirus helicase were retrieved from ZINC database and PubChem.

Table 1. Ligands found to bind against flaviruses helicase through literature reviewed

| S.no. | Research Paper | Target | Ligand | Binding site | Domain | Whether clinically approved? |
|---|---|---|---|---|---|---|
| **1** | Chelsea M. Byrd *et al* 2013 | NS3 helicase of DENV | Benzoxazole | A263 | Domain I | **Yes** |
| **2** | Chandrakala Basavannacharya *et al* 2014 | NS3 helicase of DENV | Suramin | K199 | Domain I | **Yes** |
| **3** | Mastrangelo *et al* 2016 | NS3 helicase of west nile virus | Ivermectin | D409 | Domain II and III | **Yes** |
| **4** | Jordan I *et al* 2000 | NS3 helicase of west nile virus | Ribavirin | - | - | **Yes** |

Table2: Ligands identifiers and chemical properties

| S.no | Compound Name | Compound ID | Formula | Chemical Name |
|---|---|---|---|---|
| **1** | Benzoxazole | ZINC00404297 | $C_7H_5NO$ | 1-Oxa-3-aza-1H-indene |
| **2** | Suramin | PubChem_CID 5361 | $C_{51}H_{40}N_6O_{23}S_6$ | 8-[[4-methyl-3-[[3-[[3-[[2-methyl-5-[(4,6,8-trisulfonaphthalen-1-yl)carbamoyl]phenyl]carbamoyl]phenyl]carbamoylamino]benzoyl]amino]benzoyl]amino]naphthalene-1,3,5-trisulfonic acid |
| **3** | Ivermectin | PubChem_CID 9812710 | $C_{48}H_{74}O_{14}$ | - |
| **4** | Ribavirin | ZINC01035331 | $C_8H_{12}N_4O_5$ | 1-(β-D-Ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide |

Two flavonoids were also docked against NS3 Helicase, which are as follows:

Table3. Ligands identifiers and chemical properties.

| S.no | Compound Name | Compound ID | Formula | Chemical Name |
|---|---|---|---|---|
| 1 | Luteolin | ZINC18185774 | $C_{15}H_{10}O_6$ | 2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone |
| 2 | Catechin | ZINC00119983 | $C_{15}H_{14}O_6$ | (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol |

**2.4 Molecular Docking of ligands with NS3 helicase using DOCK6:**

Geometric algorithm of macromolecular-ligand interactions was used for molecular docking. Dock6 is written in C++ and is functionally separated into independent components, allowing a high degree of program flexibility (**Kuntz *et al.*, 1982**). Dock divides the problem into three parts:

1) Representation of the receptor and ligand structures, which includes identification of the possible binding sites on the receptor molecule.

2) Matching of the receptor and ligand representations.

3) Optimization of ligand position within the binding site.

**Steps of Molecular docking via Dock 6:**

**2.4.1 Structural preparation:**

NS3 Helicase (5jrz) and ligand molecules were structurally prepared as inputs for DOCK calculations that predict orientations of a ligand in a receptor active site.

The program Chimera was used for the following procedure:

Extraneous atoms, such as alternate conformations, ligands, ions, solvent molecules, cofactors, etc. were removed from NS3 Helicase (5jrz). Missing atoms, such as, hydrogens, incomplete side chains, etc. were added. Atom types and partial charges were assigned. A final mol2 file and a pdb file without hydrogens were created. For assigning atom types and partial charges, Dock uses Sybyl atom type labels but Amber force field parameters. For a ligand, the general procedure is simpler but similar.

**2.4.2 Molecular spheres were generated using Write DMS tool in chimera.**

To generate the surface: Actions > Surface > Show. File was saved then and spheres were generated through -Tools > Structure Editing > Write DMS

**2.4.3(a). A box around the spheres/ active site was created**.

The interactive program showbox was used to visualize, define the location and size of the grid to be calculated using grid.

Command used: ./ showbox < box.in

**2.4.3(b). Grid Generation:**

Grid creates the grid files necessary for rapid score evaluation in DOCK. Two types of scoring are available: contact and energy scoring. The scoring grids were stored in files ending in *. cnt and *. nrg respectively. The program grid that is distributed as an accessory to DOCK was used to generate grid.

$$E = \sum_{i=1}^{lig} \sum_{j=1}^{rec} \left( \frac{A_{ij}}{r_{ij}^{a}} - \frac{B_{ij}}{r_{ij}^{b}} + 332 \frac{q_i q_j}{Dr_{ij}} \right)$$ equation 1

Command used: grid -i grid.in -o grid.out

The program generated separate grid files for the contact, energy and bump calculations, with .cnt, .nrg, and .bmp extensions respectively.

**2.4.4 Docking:**

Flexible ligand-docking was done, that allows ligand to be flexible. It allows the ligand to structurally rearrange in response to the receptor. The program dock6 that is distributed with DOCK in the /bin directory was used for anchor and grow algorithm.

Command used: dock6 -i dock.in -o dock.out

**2.5. Analysis of docking results:**

The obtained docking results of different ligands were analyzed through LIGPLOT (**Wallace AC *et al.*, 1996**) program, to view hydrogen bond and hydrophobic interactions in protein and ligand. The lowest internal energy conformations were considered as the most favorable docking.

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## III. RESULTS AND DISCUSSION

**3.1Retrieval of NS3 Helicase Zika Virus PDB Structure:**

PDB structure of NS3 Helicase of Zika Virus was retrieved from RCSB protein databank.

Figure1. PDB structure of NS3 Helicase (PDB_ID 5JRZ) of Zika virus French Polynesia

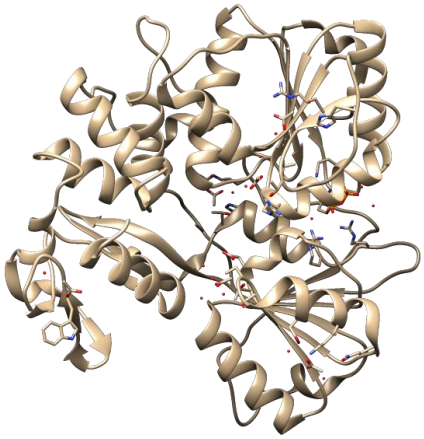


Table4. NS3 Helicase Experimental data (**J Rinku et al., 2016**)

| | Classification | Total Structure Weight | Method | Resolution | R-Value Work | R-Value Free |
|---|---|---|---|---|---|---|
| 5JRZ | HYDROLASE | 51075.78 | X-ray diffraction | 1.62 Å | 0.161 | 0.193 |

ZIKV NS3-Helicase is composed of three domains of roughly similar size, domains 1 and 2 (residues 182–327 and 328–480, respectively). The refined model has 175–617 residues, one pyrophosphate, six acetate ions and 552 solvent molecules.

**3.2 Structural Validation using PROCHECK 3.4.**

Figure 2. Ramachandran plot. 98.4% (439/446) of all residues were in favored (98%) regions. 99.8% (445/446) of all residues were in allowed (>99.8%) regions

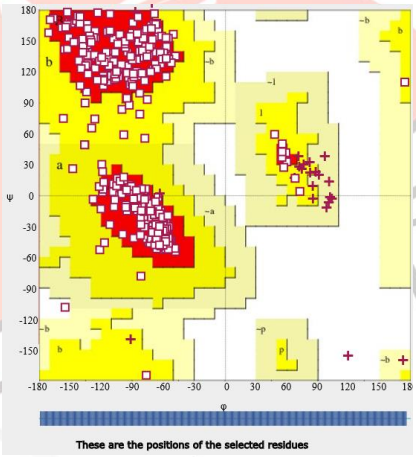



Figure3: Plot statistics

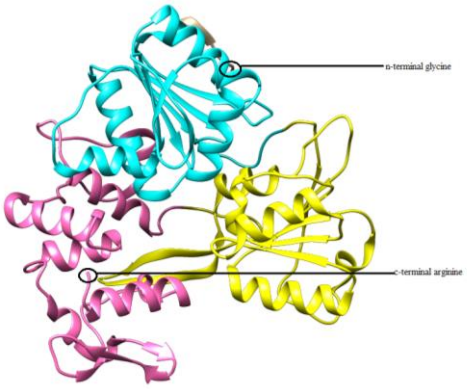
| | | No. of residues | %-tage |
|---|---|---|---|
| Most favoured regions | [A,B,L] | 362 | 92.6% |
| Additional allowed regions | [a,b,l,p] | 29 | 7.4% |
| Generously allowed regions | [~a,~b,~l,~p] | 0 | .0% |
| Disallowed regions | [XX] | 0 | .0% |
| Non-glycine and non-proline residues | | 391 | 100.0% |
| End-residues (excl. Gly and Pro) | | 1 | |
| Glycine residues | | 27 | |
| Proline residues | | 24 | |
| Total number of residues | | 443 | |

**3.3 Molecular Docking**

**3.4.1 Structural preparation**

One pyrophosphate, six acetate ions and 552 solvent molecules have been deleted from PDB file using Chimera.

Figure4: Three domains of NS3 Helicase of ZIKV: cyan- domain I, yellow- domain II, pink- domainIII.



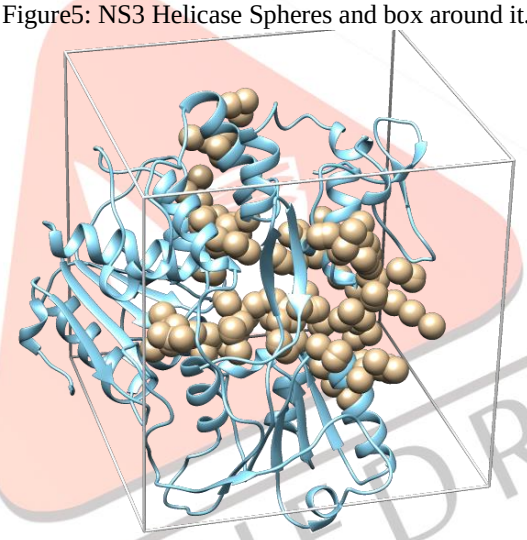

Ligands were prepared in the similar manner, and saved into mol2 format.

**3.4.2 Dock Outputs:**

**Sphere generation:**

NS3 helicase spheres fall into three major clusters of 147, 93 and 35 of total 86 clusters. The largest cluster of 147 spheres was chosen as a binding pocket of ligands. Minimum radius for clusters was 1.400Å.

Figure5: NS3 Helicase Spheres and box around it.



**Grid generation:**

Total charge on 5jrz.pdb : -0.000
Box center of mass : 28.105 26.580 33.340
Box dimensions : 49.129 49.351 49.696
Number of grid points per side [x y z]: 165 166 167
Total number of grid points: 4574130

**NS3 Helicase docking with ligands:**

**Benzoxazole**: Molecule: ZINC00404297

| | |
|---|---|
| Elapsed time for docking: | 1 second |
| Anchors: | 1 |
| Orientations: | 500 |
| Conformations: | 97 |
| Grid Score: | -21.700558 |
| Grid_vdw: | -20.503685 |
| Grid_es: | -1.196873 |
| Int_energy: | 0.000000 |

**Ribavirin:** Molecule: ZINC01035331

| | |
|---|---|
| Elapsed time for docking: | 51 seconds |
| Anchors: | 1 |
| Orientations: | 500 |
| Conformations: | 92 |
| Grid Score: | -34.836975 |
| Grid_vdw: | -31.169493 |
| Grid_es: | -3.667482 |
| Int_energy: | 1.673606 |

**Luteolin:** Molecule: ZINC18185774

| | |
|---|---|
| Elapsed time for docking: | 17 seconds |
| Anchors: | 1 |
| Orientations: | 1000 |
| Conformations: | 136 |
| Grid Score: | -35.961929 |
| Grid_vdw: | -33.132271 |
| Grid_es: | -2.829658 |
| Int_energy: | 8.717237 |

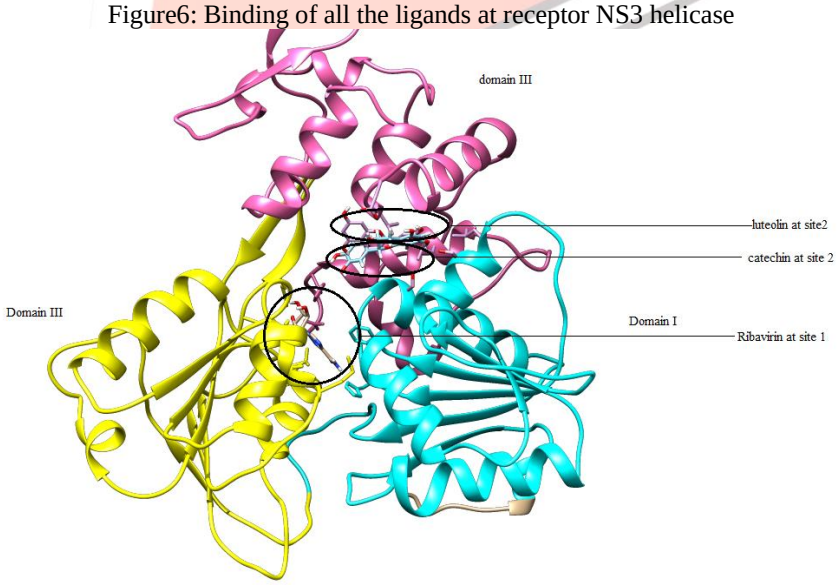
**Catechin:** Molecule: ZINC00119983

| | |
|---|---|
| Elapsed time for docking: | 42 seconds |
| Anchors: | 1 |
| Orientations: | 1000 |
| Conformations: | 129 |
| Grid Score: | -35.220440 |
| Grid_vdw: | -30.911760 |
| Grid_es: | -4.308680 |
| Int_energy: | 3.007876 |

**3.5 Analysis of docking results:**

Ribavirin (ZINC01035331), luteolin (ZINC18185774) and catechin (ZINC00119983) amongst 6 ligands bound to the receptor NS3 helicase. Benzoxazole did not bind at all.

Figure6: Binding of all the ligands at receptor NS3 helicase

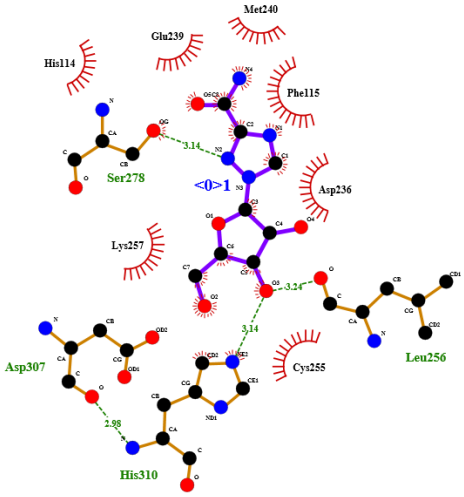



**Ribavirin (ZINC01035331):**

Ribavirin binds at Domain II and III, which shares hydrogen bond with L430, S452 and H484 (fig.8). It has 7 hydrophobic interactions with receptor residues of domain I only. Also, ribavirin had the lowest internal energy of the other two ligands.

*residues number after docking was altered by default, originally it was started with 175 proline and in docking it has started with proline as 1 so consider adding 174 with the residues number shown in ligplot results.

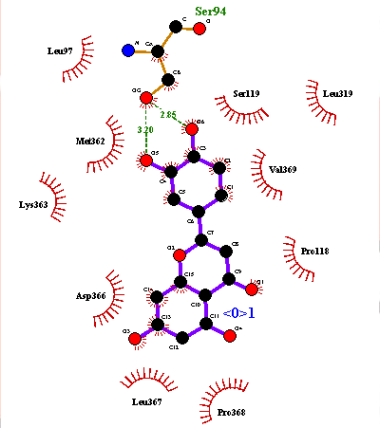
Figure 7: LIGPLOT analysis (**residues numbering was altered during docking instead of 169 numbering starts from 1 in docking results*)




**Luteolin (ZINC18185774):**

Luteolin binds at S268 of domain I, and has 10 hydrophobic interactions with residues of domain I and III both.

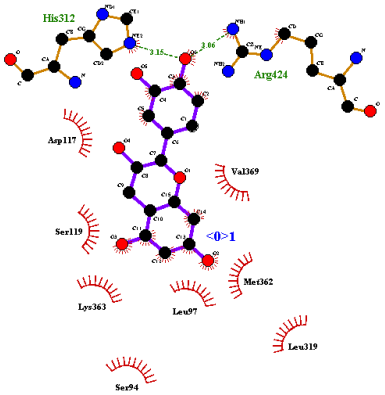
Figure 8: Luteolin docking analysis by LIGPLOT




**Catechin (ZINC00119983):**

Catechin shares two hydrogen bonds with histidine 486 and arginine 598 of domain II but has hydrophobic interactions with the residues of all three domains.

Figure 9: Catechin docking analysis by LIGPLOT.




## IV. CONCLUSIONS

The two most noticeable binding pockets on ZIKV NS3-Hel are between areas 1 and 2 (site 1) and at the intersection of spaces 1 and 3 (site 2). Site 1 is near the additional "pyrophosphate" thickness, while site 2 is inside the RNA-restricting depression, near the putative 3′ end of bound RNA. Both pockets have polar and hydrophobic qualities **(J Rinku *et al.*, 2016).** Researchers have suggested

that P loop and the RNA-binding loop are the most flexible segments in NS3-Hel structures and intermittently sample conformations of the ligand-bound state. Ribavirin was found to bind within domain II and domain III and shown that hydrophobic interactions with domain one, ie., at site 1. Ribavirin may prove as potential inhibitor of NS3 helicase which in result inhibit the viral replication. Luteolin binds at site 2 of NS3 helicase and catechin also binds at site 2. Therefore the present study reveals that phenolic compounds or flavonoids may play a crucial role in inhibiting NS3 helicase of zika virus

## V. ACKNOWLEDGMENT

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