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Research Article

# RNA POLYMERASE INHIBITORS AGAINST PROTEASE NS3 HELICASE (DENGUE VIRUS) AS WELL AS DERIVED NOVEL SCAFFOLD AND VALIDATED BINDING AFFINITY FOLLOWED BY MOLECULAR PROPERTIES

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**Abstract:** The dengue virus is the most significant arthropod-borne human pathogen, and rising quantities of cases have been report over the preceding few decades. At this time neither vaccines nor drugs aligned with the dengue virus are available. The NS5 polymerase and NS3 helicase have a key role in flavivirus RNA replication in dengue virus NS3 helicase have several stages in catalytic pathway which exhibited bound relation to single-stranded (ss) RNA, an ATP analogue, a transition state analogue and ATP hydrolysis products. Certainly, on ssRNA binding site, the NS3 enzyme switches to a catalytic competent state imparted by an inward movement of P-loop; the NS3 could exert an effect as RNA anchoring device and thus participated both in flavivirus RNA replication and assembly. Protease NS3 which assists in viral attachment to the host cell and it fallows potential antiviral objective. In order to explored novel inhibitors of NS3 RNA helicase site by performed a computer-aided virtual screening on available RNA helicase inhibitors, with structure-based screening using crystal structure of Protease NS3 and also predicted novel scaffold's by LigBuilder v2.0 software package. These compounds are validated through binding affinity and ADMET molecular properties.

**Keywords:** Dengue Virus, Flavi Virus, Protease NS3, RNA Polymerase Inhibitor (RPI)

## INTRODUCTION

Dengue, an endemic disease transmitted by *Aedes aegypti* mosquitoes, causes B 25000 deaths per year. There is no precise remedy exist to care for illness caused by flaviviruses, a family with the intention of comprise yellow fever, Japanese encephalitis and West Nile virus, all considerable human pathogens. Currently, dengue fever is the most important tropical infectious disease subsequent to malaria, as well as more than 100 countries include reported infections, particularly countries in tropical and subtropical regions [1]. An approximate 100 million cases of dengue fever occur annually. In general, the dengue virus (DV) is a plus-strand RNA virus of the Flavivirus genus of the Flaviviridae family [2]. The DV has an approximately 50 nm covering and contain a 10.7 kb single strand RNA that is translate into a single polyprotein followed by co-translational cleavage into 10 established proteins. These 10 developed proteins consist of three structural proteins (capsid (c), premembrane (prM), envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5)(Figure 1) [3]. The non-structural proteins are involved in evading innate immune responses, a complete virus particle gathering, and the duplication of the genome.

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The structural proteins take part in the formation of the viral particles [3-5]. To date the enzymatic activities of NS3 and NS5 are the best characterized among the non-structural DV proteins.

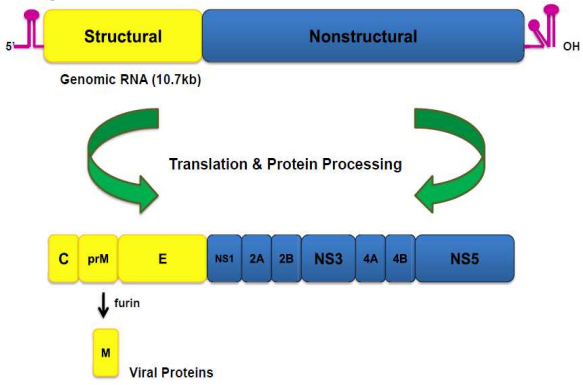


**Figure.1:** Schematic figure of Flavivirus RNA and its translation into proteins involved in the DV lifecycle.

Based on a number of studies for the various enzymatic events involved in viral RNA replication and packaging is scant for flaviviruses. Their genome a single-stranded (ss) plus RNA segment of about 11 kb—has several functions during the virus life cycle: on capsid (C) disassembly, it is translated keen on a

polypeptide precursor containing three structural and seven non-structural (NS) proteins. Subsequent polyprotein cleavage via the viral protease activity here in the N-terminal domain of NS3 and in addition by host cell proteases, the NS5 RNA-dependent RNA polymerase (RdRp) is released and alliance with NS3, synthesizes a genome piece minus strand RNA. Sequentially, this strand serves the same as a stencil designed for the production of an intemperance of plus genomic RNA. The latter is distinctively packed in hopeful viral particles in association with capsid proteins [6].The C-terminal section of NS3 possesses RNA-motivated nucleoside triphosphatase activity that provides chemical energy to slow down the viral RNA replication intermediates interested in willing to amplification by the RdRp[7-8]. As a product of its RNA triphosphatase activity, the 50 last part of viral genomic RNA is dephosphorylated, prior to cap accumulation by the N-terminal methyl transferase region of NS5. Owing to their excellent role in the virus life cycle, both NS3 and NS5 form antiviral target of leading consequence.[9-12]. The existence of a position of conserved sequence motifs within its two N-terminal Rec-A-like subdomains qualifies the flavivirus NS3 as a superfamily 2 (SF2) helicase [13-14]. RNA helicase synthesis site is displayed (Figure 2 & 3) in which make-up a complete set of ligand-bound sites for a RNA helicase.

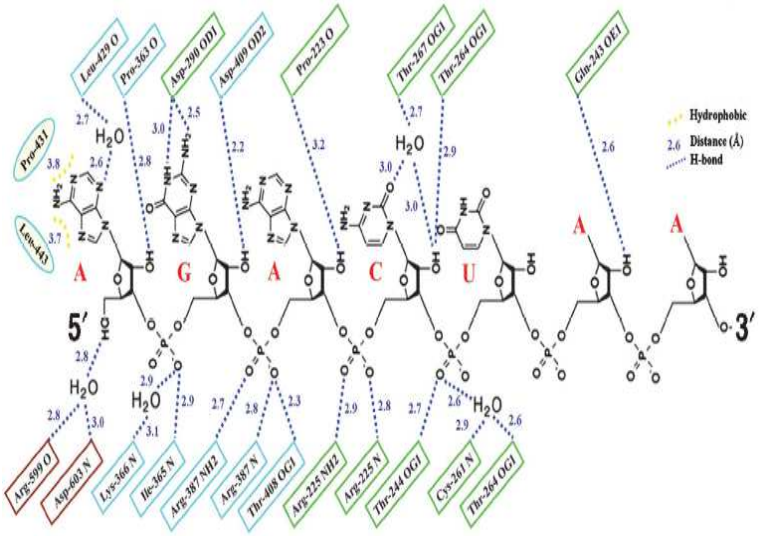


**Figure.2:** The flavivirus NS3h–RNA–AMPPNP ternary complex. Diagram representation of NS3h bound to ssRNA and AMPPNP, a nonhydrolysable ATP analogue shown as sticks. Schematic representation of the interactions observed between NS3h and ssRNA (RNA12). Notice several contacts established with the 20-hydroxyl groups of the ribose moieties.

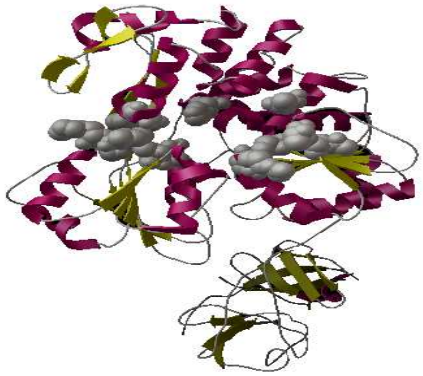

**Figure.3:** Displaying secondary structure of Protease NS3 with a RNA helicase site, highlighted in white surfaces, based on highlighted amino acids in figure 2.

Contacts established by the ssRNA with NS3h in its different nucleotide-bound states does not reveal any leading diversity, and RNA detection appears like despite of the ATP-bound state, the ssRNA is accommodated in an extended conformation in the tunnel that separates the ATP-binding subdomains of NS3h from subdomain. Both the ssRNA location and polarity follow that of a deoxyuridine octamer bound to the HCV helicase [15]. The exact path of the sugar phosphate backbone, however, differs markedly between these two structures, among the ssRNA in compliance to one strand of A-form dsRNA in the flavivirus helicase, while it is further extensive in the HCV helicase. In this outcome ssRNA was identified by DENV NS3h. It is noted that the HCV helicase is more active on DNA templates [16], whereas the DENV helicase exhibits higher activity on RNA duplexes [11]. The sugar phosphate backbone is well ordered for nucleotides (Figure 2) and weak electron density is evident for the consequent bases. As seen in the HCV helicase complexed with a multiple functions of the DENV NS3 helicase domain (NS3h), crystal structures was retrieved from RCSB PDB database for identifying effective RNA polymerase inhibitors from ligand databases to fallowing structure based virtual screening as well as finding out novel scaffold on RNA helicase site (Figure 3) to inhibition DENV replication process does not leads to Dengue fever.

## MATERIAL AND METHODS

**Software and program:**

PyMol [17] and DS Visualizer were employed to visualize and modify the receptor and small molecules. A molecular design alteration program, OpenBabelGUI, was used to set up a narrative scaffold based on selected small molecules against the binding pocket, through LigBuilder v2.0. AutoDock Vina [18] was the primary docking program used in this work. The preparation of the Protease NS3 pdbqt file and determination of the grid box size were carried out using Auto Dock Tools version 1.5.4 (The Scripps Research Institute,La Jolla, USA). Selected ligands are

undergone ADMET properties test with Ontamine plat form tool, simultaneously predicted side effects for selected inhibitors.

**Preparation of Protease NS3 structure:**

The three-dimensional structure of Protease NS3 was retrieved from the Protein Data Bank [PDB: 2WHX]. The Protease NS3 structure contains water molecules and Ions are removed with Discovery studio visualize and purified structure was written in PDB format with modified coordinated. Structure is ready to perform multiple docking against ligand database to perform structure based virtual screening.

**Structure-based virtual screening:**

Auto Vina is utilized to search for potential inhibitors against Protease NS3 by matching the binding site map of the receptor against chemical compounds in the database. With the help of multiple ligand docking shell script, for Auto Vina in Linux plat form was performed effectively to analyze best binding affinity conforms chemical interaction and GUI(Graphical User Interface) helps to determine physical property to binding pocket with respective of ligand.

**Novel Scaffold's:**

Generally, LigBuilder v2.0 [20-21] synthesize scaffolds based on chemical and physical properties of protein structures. It builds an active ligands based on binding site, with interactive fragments known as functional groupies, derived from selective lignads. Therefore, this process was carried out using three-dimensional structures of protein as well as ligand for predicting active relationship with binding pocket. Finally, the generated scaffold will satisfies Lipenski rule of five, because it's a primary screening process of LigBuilder v2.0 [20-21].

**Molecular docking:**

Molecular docking was performed using AutoDock Vina[18]. Autodock Vina was used due to its accuracy and free, which is just about two commands of extent faster than its predecessor, AutoDock 4 [19]. AutoDock Tools was utilized to prepare the input pdbqt file for Protease NS3 and to set the size and the center of the grid box. Kollman charges and polar hydrogen atoms were supplementary to the purified Protease NS3 structure, contains RNA helicase site, the grid box cavity size and center were set separately for both sites. The Protease NS3 structure center was predicted at -2.01 × 12.522 × -1.891 in the dimensions of x, y and z using 1.000Å spacing. To perform blind dock in AutoDock Vina requires the pdbqt input files of ligands to be prepared using AutoDock Tools. Owing to the quantity of molecules to be docked, the shell script was utilized to automate the docking process and act as an interface to perform the molecular docking of those compounds using AutoDock Vina. The predicted binding affinity (kcal/mol), which indicates that how powerfully a ligand binds to the receptor, is intended derived from the scoring function used in Auto-Dock Vina. High negative energy scores specify tough binding. The scoring utility in AutoDock Vina is divided into two parts: i) a conformation-dependent part that can be seen as a amount of intramolecular and intermolecular assistance, together with steric, hydrophobic, hydrogen bonding interactions and ii) a conformation independent part that depends on the total of rotatable bonds between heavy atoms in the ligand. Each contribution (steric, hydrophobic, hydrogen bonding and number of rotatable bonds) is specified a different credence in the AutoDock Vina scoring function [18]. The validation of docking was carried out by redocking the Protease NS3 active ligands (RNA polymerase) into their binding sites.

**Molecular Descriptors:**

Five molecular descriptors widely used in ADMET predictions were used. This study consist of octanol-water partitioning coefficient (AlogP) based on the Ghose and Crippen's method, apparent partition coefficient at pH=7.4 (logD derived from the Csizmadia's method, molecular solubility (logS) based on the multiple linear regression model developed by Tetko *et al.,* molecular weight (MW), the number of hydrogen bond donors (nHBD), the number of hydrogen bond acceptors (nHBA), the number of rotatable bonds (nrot), the number of rings (nR), the number of aromatic rings (nAR), the sum of oxygen and nitrogen atoms (nO+N), polar surface area (PSA), molecular fractional polar surface area (MFPSA), and molecular surface area (MSA). All descriptors can be divided into two classes: physiochemical properties (AlogP, logD, logS, MW, nHBD, nHBA, nR, nARandnO+N), and geometry-related descriptors (PSA, MFPSA and MSA). All this above descriptors were calculated by using Ontomine-Platform software novamass [22].

## RESULT AND DISCUSSION

**Ligand selection and retrieval:**

In this work, we employed two different approaches to virtually screen potential inhibitors against dengue virus Protease NS3 RNA helicase site. A structure-based virtual screen was performed using AutoDock Vina to generate a map based on the NS3 RNA helicase binding site. This map was used to screen compounds for those that fit into the binding site map. Compounds screened from databases were consequently ranked based on their binding affinities to NS3 helicase site using AutoDock Vina. The settings used in our experiments are suitable for most laboratories in developing countries with facilities that are inadequate for HTS and limited access to powerful multiprocessor computers to reduce selection time.

Meanwhile, scaffold was generated, using the Ligbuilde 2.4 algorithm performed to search for the similar functional groups from selected compounds which matches binding site with best electron orientation map. Finally, join all the electronic maps together with the functional groups to form an effective ligand with respective binding sites. Formed ligand is a novel scaffold (Figure 4).

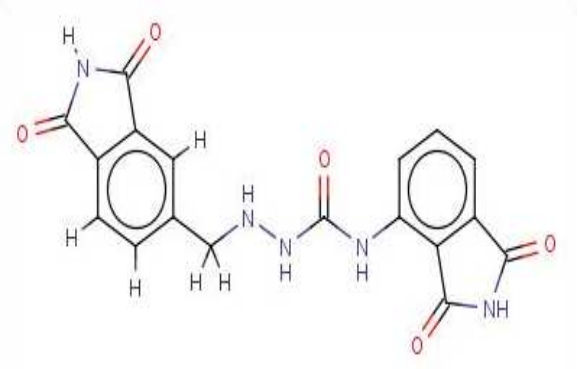

**Figure.4:** Novel scaffold (RPI-1)

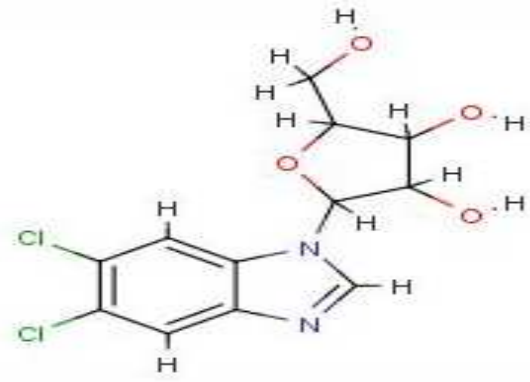

**Figure.5**: RNA polymerase inhibitor (RPI-2)

**Validation of the docking:**

In molecular docking, the size and center of the coordinates of the grid box need to be validated in order to perform blind dock, whether that the ligands bind to the binding pocket in correct conformation explains the efficiency of inhibitor. In this work, docking validation was performed by redocking co-crystallized RNA polymerized inhibitor (Figure 5) along with novel scaffold into their relevant binding sites. We originate that the necessary conformations of redocked RNA helicase site reproduced the binding modes of the co-crystallized ligands with binding affinities of -8.0 to -8.9kcal/mol. The RNA helicase site of Protease NS3 is an open and shallow pocket (Figure 3). The Novel scaffold exhibit effective interaction with binding affinity -8.3(Figure 6) to RNA helicase site Arg 387 along with active site neighboring amino acids Arg 599. Important point is that, interaction are formed With Arg 84 amino acid also, which impacts the structural change; it implies physical effect on active region (49 – 79) amino acid residues along with compound exhibited binding affinity as -8.0(Figure 7) with Arg amino acids 387 and 599; from loop region, are take part actively with selective ligands.

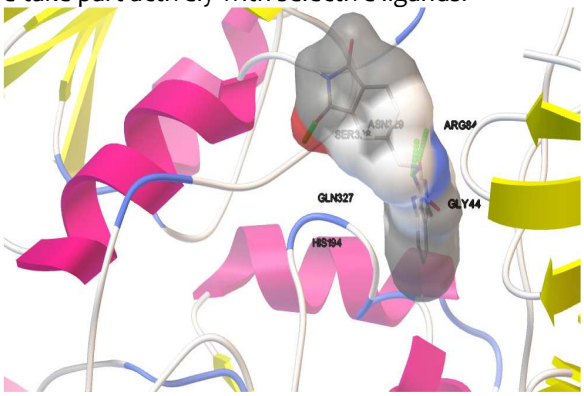


**Figure.6:** Novel scaffold interaction with Protease NS3 protein structure possess effective hydrogen bonds, are representing in green dotted line.

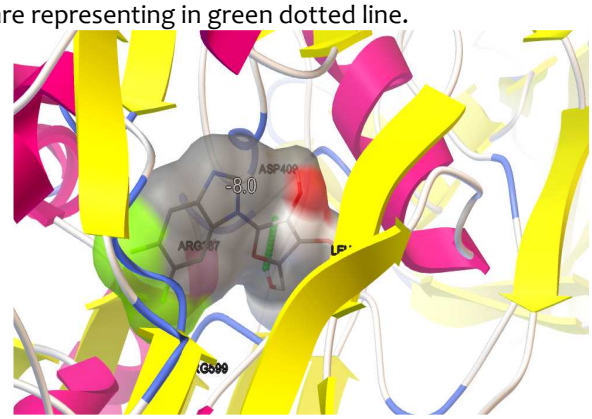


**Figure.7:** Competitive inhibitor interaction with Protease NS3 protein performed effective hydrogen bond formation and possess -8.0 binding affinity.

**ADMET molecular properties:**

Predictions of ADMET properties for selected ligands have been verified to be supportive, and they were hardened to illustrate a lot of molecular properties, such as lipophilicity, hydrogen bonding ability, molecular flexibility, molecular weight, etc. In this study, we thoroughly examined the molecular properties that are extensively used in ADME predictions. Molecular properties include MW, log*S*, log*D*7.4, Alog*P*, PSA, *n*HBA and *n*HBD. Molecular properties considered here are Alog*P*, log*D* and log*S* are allied with hydrophobicity of a molecule. All these properties are represented in (Table 1) for each ligand. It is a general phenomenon that increasing lipophilicity is usually favorable for the binding of the studied molecules to protein receptors.

**Table.1:** Values described about ADMET molecular properties.

| Compound Type | Log S Mol/dm$^3$ | Aqueous Solubility | Log P | Acid pKa Mol/dm$^3$ | Base pKa Mol/dm$^3$ | Log D Mol/dm$^3$ | Toxicity |
|---|---|---|---|---|---|---|---|
| Novel scaffold | -3.887 | Partially soluble | Lipophilic | 0.00 | 8.511 | -0.628 | Low |
| RNA polymerase Inhibitor | -4.169 | Partially Soluble | Lipophilic | 16.154 | 6.443 | 0.77 | Low |

## CONCLUSIONS

Compounds are discovered from several potential inhibitory of dengue virus Protease NS3 helicase through structure based virtual screening and fallowed scaffold hopping to attain a novel scaffold. RPI-1 a novel scaffold was competed with RPI-2 (RNA Polymerase Inhibitor). Both showed effective interaction towards the RNA helicase site, by creating hydrogen bonds and hydrophobic interactions with important residues in the binding pocket with less binding affinity. These compounds performed a detailed analysis of chemical properties such as Adsorpson, Distribution, Metabolism, Excretion and Toxicity (ADMET) were exhibited with acceptable result. Therefore, these lignads take part in developing an effective dengue virus drug.

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