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Identification of novel inhibitor against dengue NS5

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Abstract

Dengue fever, a neglected emerging disease for which no vaccine or antiviral agents exist at present, is caused by dengue virus, a member of the *Flavivirus* genus, which includes several important human pathogens, such as yellow fever and West Nile viruses. The NS5 protein from dengue virus is bifunctional and contains 900 amino acids. The *S*-adenosyl methionine transferase activity resides within its N-terminal domain, and residues 270 to 900 form the RNA-dependent RNA polymerase (RdRp) catalytic domain. Viral replication begins with the synthesis of minus-strand RNA from the dengue virus positive-strand RNA genome, which is subsequently used as a template for synthesizing additional plus-strand RNA genomes. This essential function for the production of new viral particles is catalyzed by the NS5 RdRp**.**

In this scenario, the present study aims to identify new molecules which could block or suppress the activity of RNA dependent RNA polymerase enzyme by molecular docking studies using AutodockVina. In this study we used *in silico* approach by modeling *NS5* protein, Optimization of the structure was done by adding polar hydrogens and Kollman charges using Pyrx. We further carried out docking studies by means of Autodock Vina, with various phytochemicals. Based on binding energy, phytochemicals were screened and their interaction with *NS5* was identified. Thus, we report Rohitukine alkaloid that has successfully satisfied all *in silico* parameters, necessitating further *in vitro* and *in vivo* studies. The improved conditions and new structural information should accelerate structure-based design of antiviral compounds against Dengue virus.

Keywords: Flavivirus; dengue virus; NS5 polymerase, Molecular Docking, AutoDock/Vina, Medicinal Plants, Alkaloid.

1. Introduction

Dengue fever is an ancient disease that was first recorded in a Chinese encyclopedia of diseases and symptoms published during the Chin Dynasty (265 to 420 AD)[1]. Infection by DENV can be asymptomatic or result in a variety of clinical manifestations ranging from comparatively mild dengue fever (DF) to severe disease such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2,3].

DENV poses a major risk for human health. It is estimated that half of the population of the world is at risk of becoming infected with one serotype of DENV or another [4]. Recent data indicated that in 2013, DENV caused 40–58 million symptomatic infections, including 13,586 fatal cases, with an associated financial cost of US$ 8.9 billion [5]. DENV infection in humans develops differently in each case. Globally, in 2013, 18% of DENV-infected patients were admitted to hospital, 48% received medical attention or advice outside a hospital, and 34% did not need, find, or seek medical attention [5].

The genome is single-stranded, positive-sense RNA about 11 kbp, including a 5'type I cap (m7 Gppp), and a 3' terminus lacking a poly-A tail [6]. It contains a single open reading frame encoding 10 proteins: 3 structural proteins (C, prM and E) and 7 non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) (Figure 1). This single polyprotein is subsequently cleaved by host and viral enzymes.

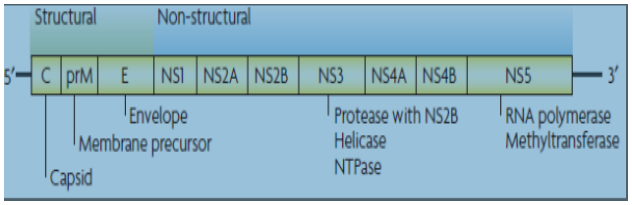


Figure 1: **Dengue viral genome [7]**

NS5 is the largest (102 kDa) and the most conserved protein (with ~70% sequence identity among the four serotypes, see Figure 2 a) [8] expressed during infection by dengue virus. It carries two domains: a methyltransferase domain (MTase) at its N-terminal end and a RNA-dependent RNA polymerase (RdRp) at its C-terminus (Figure 2b). The architecture of the NS5 protein is well conserved across flaviviruses as confirmed by the recent crystal structure determination of the ZIKVNS5 protein [9-12]. This high level of structure conservation suggests that it is possible to design compounds targeting NS5 with broad activity against several flaviviruses. The C-terminal domain (276–900) contains the RdRp enzymatic motifs. Because the RdRp activity is absent in the host cell, NS5 represents a promising antiviral target to design specific inhibitors with low toxicity. In addition to its rolein replicating the viral genome, NS5 can also down-regulate the host immune interferon response, via its interaction with the signal transducer and activator of transcription 2 (STAT2) proteins [13] or, as proposed recently, by modulating RNA splicing within the host cell [14].

Phytochemicals are found abundantly in Medicinal Plants [15]. These phytochemicals act as a strong defense mechanism forplants and also safeguard human bodies and animals against various contagious viruses and epidemics [16]. A broad range of phytochemicals can be traced in medicinal plants including organosulfur compounds, limonoids, furyl compounds, alkaloids, polyines, coumarins, thiophenes, peptides, flavonoids, terpenoids, polyphenolics and saponins. These phytochemicals serves their remedial function by scavenging and hindering viral entry and DNA\RNA replication against a wide range of viruses [17]. Treatment of Dengue Virus with medicinal plant costs less as compared to good old traditional methods [18]. It may also be preferred because of the multiple target activities, little probable to cause resistance and nominal side-effects [19]. In this context, we performed an *in silico* study to find new potential NS5 RdRp efficient agonists from a chemical library of South African Natural Compound Database (SANCDB), Maps database, Super Natural database. Our *in silico* approach provides the basis for subsequent in vitro and in vivo studies to test these newly identified DENV NS5 RdRp inhibitor candidates.

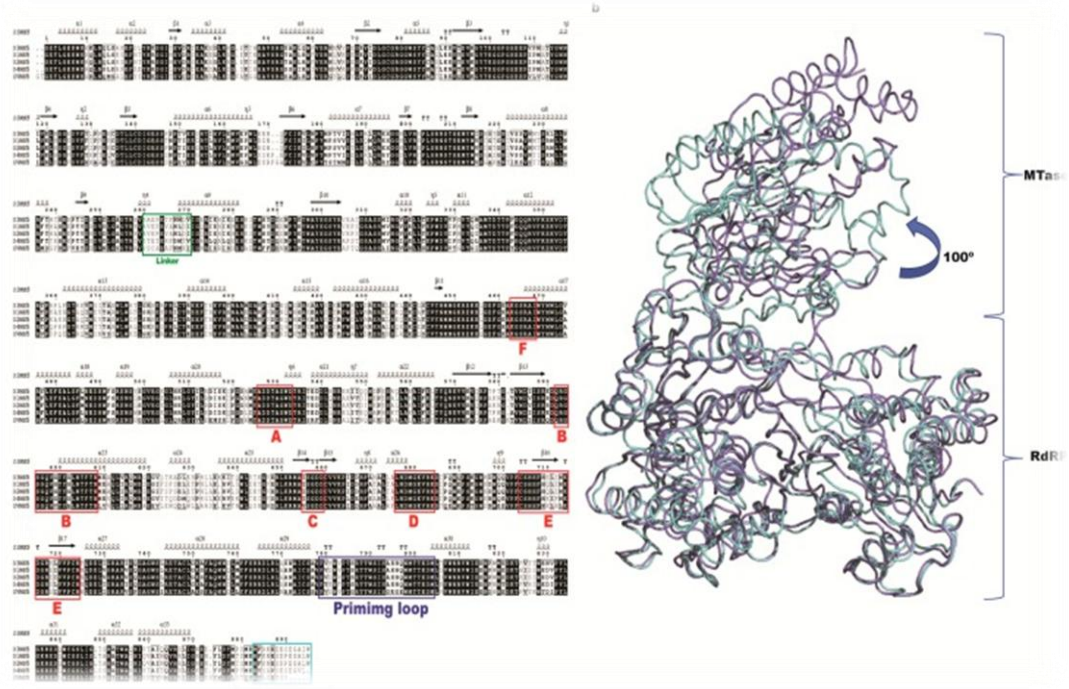


Figure 2: a). Sequence alignment of dengue virus (DENV) NS5 proteins from the four serotypes. Sequence numbering is according to DENV2 NS5. Secondary structure assignment follows the DENV3 NS5 full-length protein structure (Protein Data Bank (PDB) access code 4V0Q). Specific sequence motifs (A–F) are labeled in red. The linker region is indicated in green. The recently characterized nuclear localization signal is colored in light blue and the priming loop in blue.
(**b**). Superimposition between DENV 3 NS5 (4V0Q, light blue) and Zika virus (ZIKV) NS5 (5TFR, purple) full-length protein structure is represented as carbon traces. MTase: methyltransferase; RdRP: RNA-dependent RNA polymerase.

2. Material and methods

This study involves the docking of 1371 phytochemicals of antiviral medicinal plants against Dengue virus NS5 protein. This data has been taken from South African Natural Compound Database (SANCDB), Maps database, Super Natural database. Autodockvina in Pyrx software package was used to carry out docking.

2.1 Receptor refinement

Three dimensional structure of NS5 was retrieved from the database of Protein Data Bank (PDB) using PDB id 2j7w (figure 3). PDB ID was converted into required PDBQT format .Optimization of the structure was done by adding polar hydrogens and Kollman charges using Pyrx. To optimize the structure H2O molecules were removed from the structure and 3D protonation was done to change the state into ionization level.

2.2 Alkaloid refinement

All the alkaloids were optimized by adding hydrogens using Openbabel in Pyrx software. All these alkaloid were saved in PDBQT which was further used for docking studies.

2.3 Molecular docking

The docking algorithem of Autodockvina software was used to dock these phytochemicals with catalytic site of NS5. Generation of 3D structure, energy minimization, and conversion into PDBQT format was carried out using OPEN BABEL in PYRX. All single bonds were allowed to have free rotation. Grid parameters were optimized, dimensions (Angstrom) of vina search space were set to default of 25x25x25 with center X: 32.49 Y:60.86 Z:16.01 and exhaustiveness 8. For molecular visualization, docking poses generated by AutoDockVina were directly loaded into PyMol through PyMol Autodock/Vina Plugin. Pictures of the modeled protein-ligands complex were produced by PyMol. Docking programme of Autodockvina provides correct conformation of the ligand so as to obtain minimum energy structure. Top conformation of each phytochemical was selected on the basis of binding energy and were further evaluated to study the hydrogen bonding interactions.

3. Results

The 3D xray crystallographic structure of DENV NS5 was retrieved using PDB id 2j7w which was having resolution of 1.85 Angstrom (20). All phytochemicals were docked with the catalytic site of NS5 RNA dependent RNA polymerase domain.

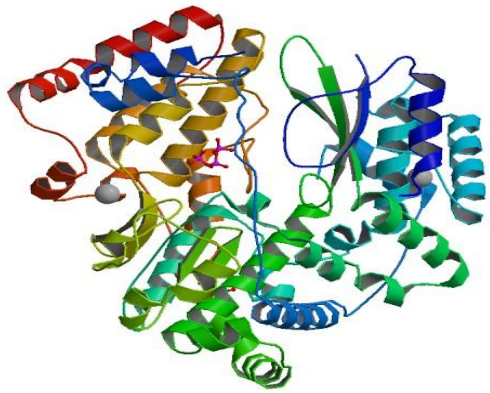
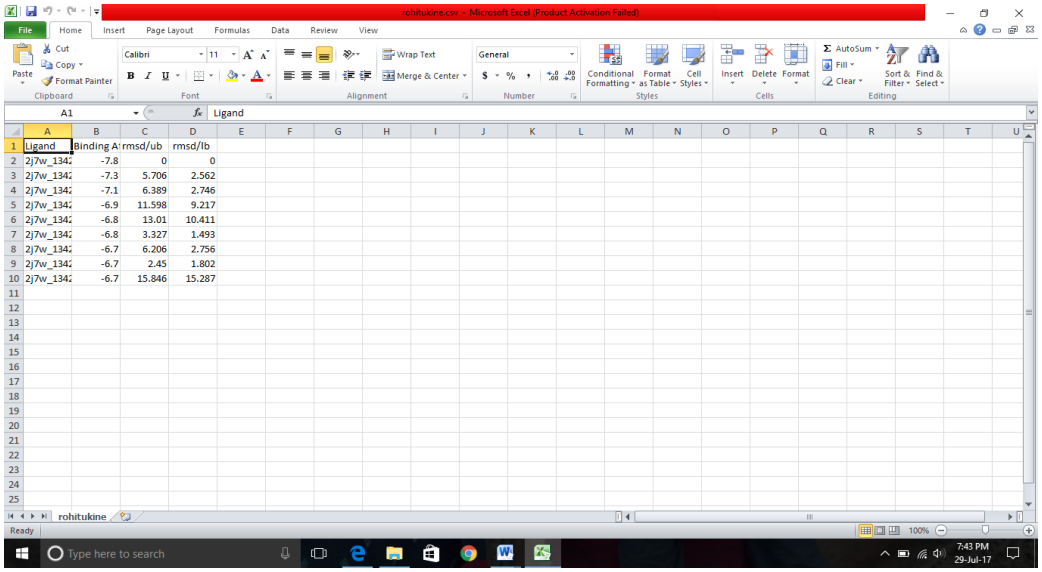


Figure 3-Crystal Structure of the Dengue Virus RNA-Dependent RNA Polymerase Catalytic Domain at 1.85 Angstrom Resolution (20).

3.1 Molecular Docking

Autodockvina docking programe provided conformation of each phytochemicals. All the conformations were sorted according to binding energy and top ranking conformation with minimum binding energy were further analyzed. Alkaloid Rohitukine was ranked as top conformation (Table 1).

Table 1: Molecular docking analysis for potential inhibitor compounds at the binding site of NS5 RdRp



Ligand	Binding A	rmsd/ub	rmsd/lb
2j7w_1342	-7.8	0	0
2j7w_1342	-7.3	5.706	2.562
2j7w_1342	-7.1	6.389	2.746
2j7w_1342	-6.9	11.598	9.217
2j7w_1342	-6.8	13.01	10.411
2j7w_1342	-6.8	3.327	1.493
2j7w_1342	-6.7	6.206	2.756
2j7w_1342	-6.7	2.45	1.802
2j7w_1342	-6.7	15.846	15.287

3.3 Interaction analysis

Out of 1371 selected phytochemicals used for interaction analysis only 1 alkaloid Rohitukine (other names- 5,7-dihydroxy-8-(3-hydroxy-1-methylpiperidin-4-yl)-2-methylchromen-4-one) has shown significant interactions with Ser 661, Asp 663, Asp 664 and bound deeply inside the binding pocket (figure 5).

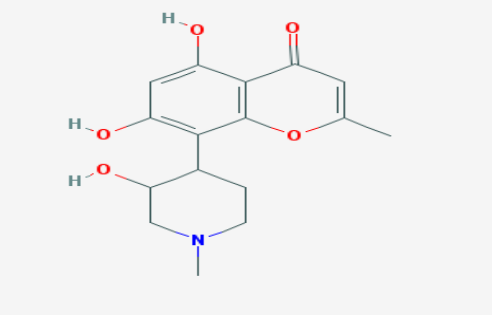


Figure 4: Chemical structure of selected alkaloid is shown in figure (PubChem CID- 5387431)

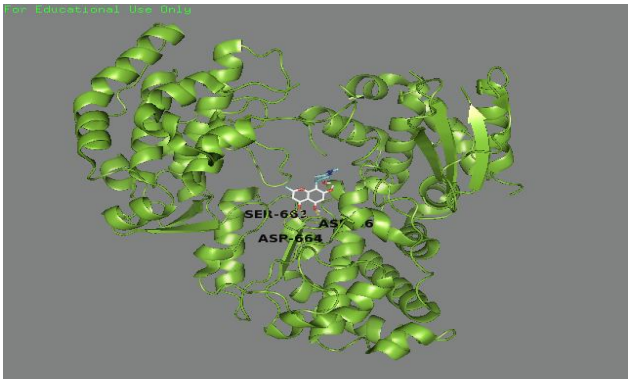


Figure 5: Docked Rohitukine complex with DENV NS5 pocket

3.4 Drug scan

Final selected alkaloid compound was analyzed using the Ligand properties checking tool of MOE which assessed the molecular properties and practicability of these compounds of being drug candidates on the basis of "Lipinski's Rule of Five"[30]. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion. This Compound was examined for their drug-suitableness and the results are shown in Table. Our results showed that the alkaloid compound used in this study fulfill the criteria of being drug candidates (Table 2).

Table 2: Molecular properties of alkaloid assessed through Ligand properties checking tool of MOE

Phytochemical Name	Molecular Formula	Molecular Weight	LogP	TPSA	Hydrogen Bond donor	Hydrogen bond acceptor	Lipinski's rule of five
Rohitukine	$C_{16}H_{19}NO_5$	305.33 g/mol	1.4	90.2A^2	3	6	Suitable

4. Discussion

We performed this study to identify and characterise the inhibitory potential of the Rohitukine alkaloid against DENV NS5. The results of the protein-protein docking study showed that the Rohitukine alkaloid bound to the NS5 by hydrophobic residue interactions with Ser 661, Asp 663, and Asp 664. The binding of Rohitukine to NS5 may effectively inhibit binding of the substrate to the active site. Therefore, inhibition of dengue NS5 may directly lead to inhibition of the replication process of the viral polyprotein and subsequent virus replication.

5. Conclusion

Current study focuses on the assessment of alkaloid Rohitukine from medicinal plants as drug candidate against NS5. Molecular docking of alkaloid against NS5 has revealed strong interactions between alkaloid Rohitukine (5,7-dihydroxy-8-(3-hydroxy-1-methylpiperidin-4-yl)-2-methylchromen-4-one) and the active site of NS5. The information acquired through this study on the binding mode of alkaloid and NS5 will highly facilitate the synthesis and testing of alkaloid Rohitukine as drug against DENV. On a concluding note, this study has suggested that Rohitukine will be strong future drug candidates against DENV.

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