

ORIGINAL ARTICLE

Identification of Zika Virus NS5 Novel Inhibitors through Virtual Screening and Docking Studies

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ABSTRACT

Objective: Screening of ZINC inhibitors library for Zika virus (ZIKV) Non-Structural 5 (NS5) protein as potential drug target.

Study Design: Cross sectional.

Place and Duration of Study: The study was carried out at Department of Biological Sciences of National University of Medical Sciences, Rawalpindi, Pakistan from December 2018 to March 2019.

Materials and Methods: NS5 protein was obtained from Protein databank (PDB ID: 5TMH) and screened against ZINC library of 11,193 drug-like molecules for NS5 and 3 ligands were identified based on optimum binding energy. MOE, PyMOL and CLUSTALW were used for docking studies and structural analysis.

Results: Out of 11, 193 compounds, three ligands were observed to interact with residues of the Methyl Transferase (MT) domain of NS5. These ligands fit in the MT domain by making hydrogen and hydrophobic interactions in the active site and S-adenosyl-methionine (SAM) binding pocket.

Conclusion: Hence, upon experimental validation, these ligands can be utilized as potential inhibitors against NS5 MT activity to control ZIKV viral replication and ultimately control the disease.

Key Words: *Docking, Ligands, Protein, Zika Virus.*

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Introduction

Zika virus is transmitted through mosquitoes (*Aedes aegypti* and *Aedes albopictus*). According to WHO, ZIKV has been reported from a total of 86 countries from Americas, Africa, Pacific and Asia.¹ It was reported from Brazil that ZIKV infection in pregnant women leads to birth defects with microcephaly and also causes Guillain-Barre´ Syndrome.^{1,2} There is no effective medication or vaccine available against ZIKV. ZIKV belongs to family Flaviviridae, which also includes other notorious human pathogens like Hepatitis C virus (HCV), Dengue virus (DENV), Yellow Fever virus (YFW) and Japanese Encephalitis virus

(JEV).³ The genome of ZIKV like other flaviviruses is composed of a single stranded positive-sense RNA of ~11kb. The genome is a single open reading frame (ORF) with a capped 5´- untranslated region (UTR) with the 3´- UTR lacking a poly-A tail.⁴ The ORF is replicated and translated in the host cells to encode a single polyprotein, which is cleaved, by host and viral proteases releasing its three structural proteins (Capsid [C], Premembrane [prM] and Envelope [E]) and 7 non-structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5).^{3,5}

NS5 of the ZIKV is ~100kDa protein, which is the most conserved protein of ZIKV. There is 94% sequence identity amongst the two main ZIKV lineages: African and Asian.⁶ This protein is composed of two domains; the first domain at the N-terminal has methyl transferase activity (MT) while the second large domain at the C-terminus carries RNA-dependent RNA polymerase (RdRp) role.⁷ MT is important for the 5´- end capping which is necessary for the stabilization of the viral RNA genome and translation.⁸ The RdRp domains of NS5 perform the

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function of genome replication.⁷ The ZIKV-NS5 structure shows prominent similarities with JEV and differences in orientation as compared to DENV.³ The MT structure of NS5 forms classical $\alpha/\beta/\alpha$ sandwich.⁹ This domain is composed of three sites, Guanosine triphosphate (GTP) binding site (GBS), active site to catalyze methyl transfer and S-adenosyl-methionine (SAM) binding site (SBS). The active site (AS) is composed of 4 residues (Lys61-Asp146-Lys182-Glu218) forming catalytic tetrad and are arranged in the center of MT.^{3,7} The MT domain mediates methylation of the cap guanine and also performs guanylyl-transferase activity to transfer GMP to 5'-end of RNA.¹⁰

The capping of the ZIKV RNA is important for its genome stability and protein translation. Blocking the activity of the MT domain of NS5 abolishes this capping and thus viral replication can be abrogated to achieve effective antiviral therapy. In this research communication, we have screened the ZINC database of ligands against the ZIKV NS5 protein. Out of 11,193; we are reporting three ligands that can bind to the active site of the MT domain. Structural analysis of the identified ligands to the active site residues, their bonding interaction and energies shows that these ligands bind strongly to the AS. Therefore, these ligands can be considered for in vitro assays to design novel and potential drugs for ZIKV.

Materials and Methods

Molecular Docking

In this study, docking of drug like molecules retrieved from ZINC database into ZIKA NS5 protein (PDB ID: 5TMH) was carried out. Molecular Operating Environment (MOE) software package (<http://www.chemcomp.com/>) was used for docking. MOE is a software system designed by the Chemical Computing Group to support Molecular Modeling, Cheminformatics, Bioinformatics, Structure-Based-Design, Virtual Screening, and Dynamic Simulation, QSAR and can be used to build new applications based on SVL (Scientific Vector Language). To analyze the interaction between NS5 Protein and ligands, ligX implementation; MOE was used.

Ligand Preparation

The ligand molecules used in our study were retrieved from ZINC database. The energies of all the

molecules were minimized using energy minimization algorithm of MOE tool. Following parameters were used for energy minimization; gradient: 0.05, Force Field: MMFF94X, Chiral Constraint: Current Geometry. All the minimized molecules were saved in the separate file in .mdb format. In the next step, the prepared file was used as input for MOE-Dock.¹¹

Protein Preparation

The NS5 (PDB ID: 5TMH) protein molecule used in our study was obtained from Protein Data Bank (PDB). The 3D protonation of the protein molecule was carried out. The energy of the protein molecule was minimized using following energy minimization parameters; gradient: 0.05, Force Field: MMFF94X+Solvation, Chiral Constraint: Current Geometry. Energy minimization was terminated when the root mean square gradient drops below the 0.05. The minimized protein structure was subjected to docking.¹²

Docking

Binding of the ligand molecules with ZIKA NS5 protein molecule was analyzed using ligX application in MOE docking program to find the correct conformation of the ligand, to obtain minimum energy structure.

Sequence and Structural Alignment's:

The sequence alignment was carried out using CLUSTALW (<https://www.ebi.ac.uk/Tools/msa/clustalw2/>).

The 3D protein-ligand complex analysis and alignments were performed in PyMOL (<http://www.pymol.org>).

Results

Once the docking was completed, the ligands that had shown active binding to NS5 were selected. From the screened ZINC database of 11,193 ligands with Tanimoto cutoff level of 60%, the top 1000 inhibitors were selected on the basis of docking score and binding energy with NS5. These 1000 were redocked and we obtained 3 ligands (Table 1) that have shown optimal binding to MT domain of NS5 (Figure 1A). All the 3 inhibitors (Figure 1) were observed to bind to the AS of MT domains and extend to the SBS.

Discussion

Ligand1 (L1) (ZINC05386955) was observed to occupy both the active site and SAM binding site of MT (Figure 1B). It forms hydrogen bonds (H-Bond) via

its amine group with amine of Lys182 of the active site catalytic tetrad located on β 14. This ligand also extends to form another hydrogen bond with His110 in the SAM binding site, while a third hydrogen bond is made with Ser56 at the junction of SAM and active site. It was also observed that SA-Adenosyl-L-homocysteine (SAH) in 5TMH show the same consistent H-bond to Ser56 and His110 and the same hydrophobic interactions as observed for L1. Hydrophobic interactions were also observed for L1 with Lys61, Thr104, Gly81, Asp146 and Glu218. This ligand completely occupies the catalytic tetrad of Lys61-Asp146-Lys182-Glu218 via hydrophobic and hydrogen bonding.

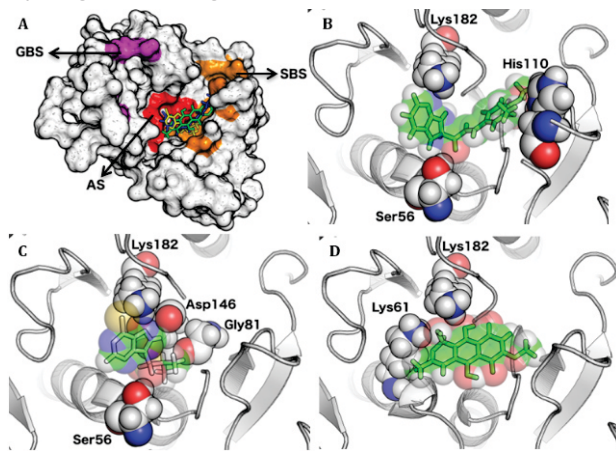


Fig 1: MT Domain of ZIKV NS5 Protein (PDB ID: 5TMH) Bound to the Docked Ligands. (A) MT Domain Structural Organization Representing its GTP Binding Site (GBS) Colored in Magentas, Active Site (AS) Colored in Red and its SAM Binding Site (SBS) Colored Orange. All the Three Docked Ligands [Ligand1 (Blue), Ligand2 (Yellow) and Ligand3 (Green)] can be Observed Bound in the AS and SBS. (B) Ligand1 Bound in the Active Site Showing its H-Bond with the AS Residue to Lys182 and His110 and also Extends to SBS Site. (C) Ligand2 Binding Pattern was Observed to Bind Mainly in the AS, by Making H-Bond to Lys182, Asp146, Gly81 and Ser56. (D) Ligand3 also Occupies the AS via H-Bond to Lys61 and Lys182 Where it doesn't Show any Interaction in the SBS Region.

Ligand 2 (L2) (ZINC05525008) mainly occupies the active site (Figure 1C). This ligand seems to be a good binder of MT, as it makes multiple hydrogen bonds. Though, it does not completely extend to the SBS but it still makes a hydrogen bond with Gly81, and show hydrophobic interaction with Gly83, Cys82. In the active site, L2 binds Asp146 and Lys186 of the active site tetrad. The amine of the sulphonate in the ligand

makes hydrogen bonds both with Gly81 and Asp146, while its oxygen makes hydrogen bonds to the hydroxyl of Ser56. Other residues Gly58, Glu218, Cys82 and Gly83 make the hydrophobic interactions with L2.

As compared to the previous two ligands, the third ligand (L3) (ZINC05767720) also makes two hydrogen bonds with Lys61 and Lys182 and hydrophobic interaction with Asp146 of the catalytic tetrad (Figure 1D). There was no interaction observed in the SBS.

The MT domain of Zika and Japanese Encephalitis virus (JEV) NS5 (PDB ID: 4K6M) are very similar in structural residues and orientation.³ Residues of the AS, SBS and GBS for both ZIKV and JEV are conserved (Figure 2a). Once the structures of both ZIKV and JEV aligned, it was observed that all the three ligands placed to the same site as docked to ZIKV (Figure 2b). Thus, these ligands could also serve to block the AS and SBS of JEV as well.

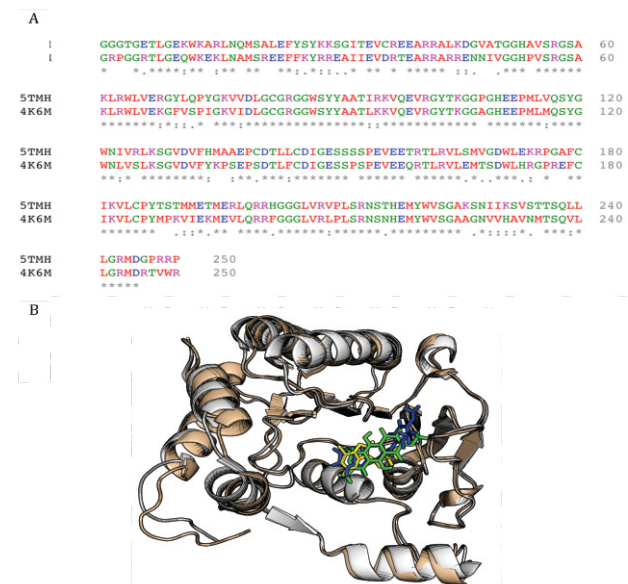
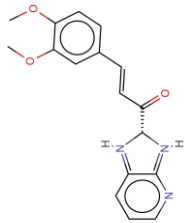
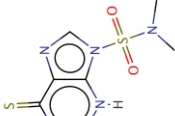
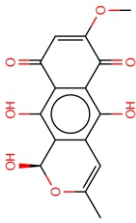


Fig 2: Sequence and Structural Alignments of MT Domains of NS5 Proteins of ZIKV (PDB ID 5TMH) and JEV (PDB ID 4K6M). (A) Aligned Sequences of the ZIKV and JEV MT Domain, Showing that it's Highly Conserved for AS, SBS and GBS. (B) Aligned Structures of ZIKV (Gray) and JEV (Wheat), all the three Ligands [Ligand1 (Blue), Ligand2 (Yellow) and Ligand3 (Green)] are shown to Bind in the Same Region of JEV MT Domain Active Site.

The Generalized-Born Volume Integral/Weighted Surface Area (GBVI/WSA) is a scoring function, which estimates the free energy of binding ligand from a given pose. For all scoring functions, lower scores

Table 1: Table showing Ligands Docked to MT Domain of ZIKV NS5 Protein, their Zinc Library Codes, Chemical Names, Structures and their Protein Interacting Residues along with Binding Energies and Docking Score

S. No	Zinc Code	IUPAC Name	Chemical Structure	Interacting Residues Bonds	Binding Energy (GBVI/WSA)	Docking Score	
1	ZINC05386955	2-[(2E)-3-(3,4-dimethoxyphenyl)-1-(lambda3-oxidanidylidene)prop-2-en-1-yl]-2H-4lambda4-imidazo[4,5-b]pyridine-2,3a-bis(ylium)-4-ide		Hydrogen Ser56, His110, Lys182 Hydrophobic Lys61, Gly81, Thr104, Asp146, Glu218	-20.628 (Kcal/mol)	-10.3696	
2	ZINC05525008	9-[bis(-lambda3oxidanidylidene)[methylidene(methylmethyl)amino]- lambda4-sulfanediiumyl]-6-sulfanyl-9H-1lambda1,3lambda1,7lambda4-purin-7-ide-1,3-diuide		Ser56, Gly81, Asp146, Lys182	-15.958 (Kcal/mol)	-10.9006	
3	ZINC05767720	7-methoxy-3-methyl-1H,6H, 9H-cyclohexa[g]isochromen-1- yium-1,5,6,9,10-pentakis(olate)		Lys61, Lys182	Ser56, Arg57, Gly58, Gly83, The104, His110, Glu111, Asp146	-19.896 (Kcal/mol)	-10.8382

indicate more favorable poses. In molecular docking studies, binding energies and docking score are the primary bases for the selection of active and non-active compounds. Finally selected three compounds had low binding energies and docking scores were in correlation with it (Table 1).

Conclusion

ZIKV is an emergent pathogen with its regular epidemics across the globe. There is no vaccine or medical intervention available for its treatment. NS5 protein of ZIKV is a necessary enzyme required for its replication and also involved in immune perturbation. Thus, the inhibitors that can block the methyltransferase activity of this enzyme can be deemed as a pharmacological approach to block the replication of ZIKV. In this study, we are reporting three ligands that bind in the active site pocket of the MT domain of NS5 and interact with the active site residues. These ligands have also shown to occupy the SAM binding site, therefore, completely blocking the enzyme pocket. Blockage of the MT domains activity can lead to failure of the viral RNA 5'-capping, which would result in unstable viral RNA vulnerable to degradation and loss of translation.

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