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Research Article

ZIKA VIRUS SERINE PROTEASE COMPLEX (NS2B-NS3) INHIBITION BY 2-AMINO-5-{[(1Z) -AMINO ({[(Z) -BENZOYL] IMINO}) METHYL] AMINO} -N-(5-AMINO-7-{[CARBAMOYL (PHENYL) METHYL] AMINO} -6-OXOHEPTYL) PENTANAMIDE, *IN SILICO* STUDIES

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ABSTRACT

Objective: The present *in silico* study is taken to report 2-amino-5-{[(1Z) -amino ({[(Z) -benzoyl] imino}) methyl] amino} -N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino} -6-oxoheptyl) pentanamide as Zika virus (ZIKV) NS2B-NS3 protease inhibitor.

Methods: *In silico* studies performed on online docking servers. NS2B-NS3 serine protease from ZIKV with PDB ID: 5GJ4 a hydrolase with total structure weight of 102878.54 is selected as the target. Docking server is used for carrying out docking calculations. Lamarckian genetic algorithm and the Solis and Wets local search methods are used for performing docking simulations. Free energy calculations, hydrogen bond (HB) formation, polar and hydrophobic interactions and HB plot are studied in this study.

Results: Binding pocket is found on a serine protease NS2B chain A. Binding site predictions propose NKK as the suitable ligand for binding, which has structure closely related to the proposed ligand2-amino-5-{[(1Z) -amino ({[(Z) -benzoyl] imino}) methyl] amino} -N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino} -6-oxoheptyl) pentanamide. Free energy of binding is - 4.08 kcal/Mol and inhibition constant (Ki) is very less 1.02 mm. The ligand binds to chain A of NS2B and chain B of NS3 serine protease. The legend is bound to serine protease complex through strong HB, formed between THR 60 (A) and N6 of ligand, GLU62 (A) and N8 of ligand, ARG 55 (A) and N3 of ligand and ASN108 (B) and N7 of ligand apart from polar and hydrophobic interactions.

Conclusion: Docking studies performed establishes the proposed ligand2-amino-5-{[(1Z)-amino ({[(Z)-benzoyl] imino}) methyl] amino} -N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino}-6-oxoheptyl) pentanamide as a molecule which can be used for the inhibition of ZIKV NS2B-NS3 serine protease.

Keywords: Zika virus, NS2B-NS3 protease, Inhibition, *In silico.*

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INTRODUCTION

Zika virus (ZIKV) is a member of genus *Flavivirus* belonging to the family *Flaviviridae* of ribonucleic acid (RNA) viruses. Previously ZIKV was considered as a rare and mild pathogen for humans [1]. Recently, this mosquito-borne *Flavivirus* has triggered global public health crisis, linking ZIKV infection to fetal microcephaly and Guillain-Barre syndrome and other neurological complications in adults like acute myelitis and meningoencephalitis [2-5]. The World Health Organization has declared ZIKA a Public Health Emergency of International Concern [6], as there are no vaccines or antiviral drugs available for treatment or protection from the virus. Dengue virus (DENV), West Nile virus (WNV), yellow fever virus, tick-borne encephalitis virus, and Japanese encephalitis virus (JEV) are other flaviviruses that are important human pathogens besides ZIKV [7,8]. ZIKV has ~10.7-kB single- stranded RNA genome of positive polarity and encodes a single polyprotein of about 3,000 amino acids. During viral replication, this polyprotein is cleaved by host cell proteases (Signals, furin) into three structural proteins (Capsid, membrane, and envelope proteins) that are involved in viral particle assembly and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) responsible for viral replication, virion assembly and evasion from the host defense mechanism [7].

All cytoplasmic cleavages, inclusive of junctions between NS2A/NS2B, NS2B/NS3, NS3/NS4A, and NS4B/NS5 proteins and within the capsid, NS2A and NS4A proteins are carried out with NS2B-NS3 protease [7,8]. The *Flavivirus* NS2B-NS3 protease is essential for the virus replicated and thus constitutes an important and ideal target for antiviral drug development [9]. Suppression of immune responses by cleavage of a stimulator of interferon genes by NS2B-NS3 protease is also observed in DENV [10,11], it also triggers apoptosis via activating caspases in WNV [12] and starts neurotropic pathogenesis by inhibition of activator protein 1 in JEV [13]. Similar to other *Flavivirus* proteases, ZIKV consists of genome size of around 11 KB [14]. With one open reading frame which codes a polyprotein that further cleaved into structural proteins and nonstructural (NS) proteins, i.e., NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [15]. NS3 is a multi-domain protein with RNA triphosphatase (NS3 RTpase) and RNA helicase (NS3 hell) activities while NS5 consist of an N-terminal methyltransferase domain and RNA polymerase (NS5 RdRp) [16-18]. According to Potapova *et al.* (2012) and Shiryaev *et al.* (2011) [19,20], the pathogenic properties of encephalitis virus are due to NS3 polymerase. ZIKV protease consists of the N-terminal domain of NS3, which is a chymotrypsin-like serine protease carrying an absolutely conserved triad His51, Asp75 and Ser135, NS2B is membrane bound and serves as a cofactor essential for folding and catalysis [21,22]. It has been reported that a construct comprising of ~ 185 residues of NS3 and ~ 40 hydrophilic residues of NS2B, covalently linked via a Gly4-Ser-Gly4 sequence, displays strong peptidolytic activity [23]. Potential therapeutic approach is to inhibit NS2B-NS3 protease, responsible for viral replication.

Docking technique predicts the orientation of one molecule to second during binding to form a stable complex. Docking studies identify different parameters and structural features important for binding predictions during *in silico* screening, which helps in

the identification of suitable binding species. Binding interactions are analyzed through different parameters such as free energy of binding ΔG, hydrogen bond (HB) formation [24,25], hydrophobic interactions, polar interactions through different plots such as 2D plot and HB plot. This study focuses on *in silico* studies of inhibition of ZIKV NS2B-NS3 serine protease complex by 2-amino-N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino} -6-oxoheptyl)-5-[(Z)-N"-(1-phenylethenyl) carbamimidamido] pentanamide. Potential inhibition activities of carbamimidamido complex have previously been reported in case of WSV [23] and influenza A and B virus [26-29]. Carbamimidoylphenyl containing molecules has also been reported to inhibit Matriptase a type II transmembrane serine protease expressed in most human epithelia [30]. Hence, in this study, we have docked the NS2B-NS3 protein, which plays a key role in capping and replication of the virus. There is no specific medicine or vaccine reported till now for ZIKA. Most of the therapies available rely on symptomatic treatments only. The present *in silico* studies establish 2-amino-5-{[(1Z)-amino ({[(Z)-benzoyl] imino}) methyl] amino}-N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino}-6-oxoheptyl) pentanamide as an effective inhibitor for ZIKV NS2B-NS3 serine protease complex, the molecule is fit for wet lab synthesis and can be taken up for *in vitro* studies, as per results obtained from *in silico* analysis.

METHODS

Inhibition of NS2B-NS3 serine protease from ZIKV by 2-amino-5-{[(1Z)-amino ({[(Z)-benzoyl] imino}) methyl] amino}-N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino} -6-oxoheptyl) pentanamide (Chemical structure Fig. 2) is studied through *in silico* studies performed on online servers. NS2B-NS3 serine protease from ZIKV with PDB ID: 5GJ4 a hydrolase with total structure height of 102878.54 (Table 1 and Fig. 3) is selected as the target. Docking server is used for carrying out docking calculations [31]. Gasteiger partial charges are added to the ligand atoms by the server during docking, non-polar hydrogen atoms are merged, and rotatable bonds are defined. As per server notification AutoDock tools [32] are used for adding essential hydrogen atoms, Kollman united atom type charges and solvation parameters. Autogrid program [32] generated affinity grid maps of ×× Å and 0.375. AutoDock parameter set- and distance-dependent dielectric functions are used in the calculation of van der Waals and electrostatic terms, respectively. Lamarckian genetic algorithm and the Solis and Wets local search methods [33] are used for performing docking simulations. Initial position, orientation, and torsions of the ligand molecule are set randomly. All rotatable torsions are released during docking. 10 different runs, terminating after a maximum of 2,50,000 energy evaluations is used for each docking experiment. Population size is 150, a translational step of 0.2Å, quaternion and torsion step of 5 are applied. The Raptor X binding server is used for binding site predictions on 5GJ4 serine protease complex.

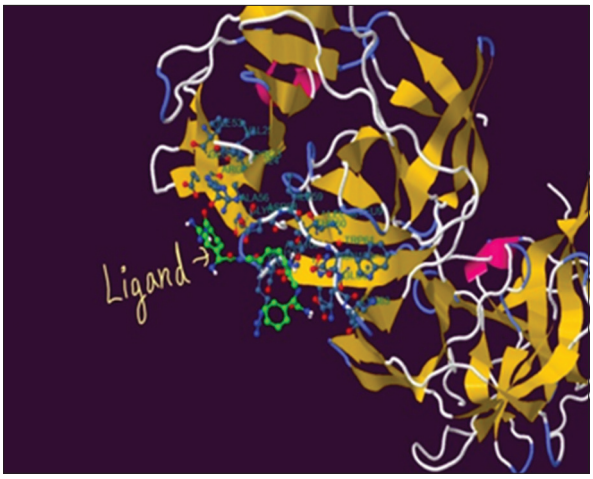

Figure 1: Ligand binding with serene protease

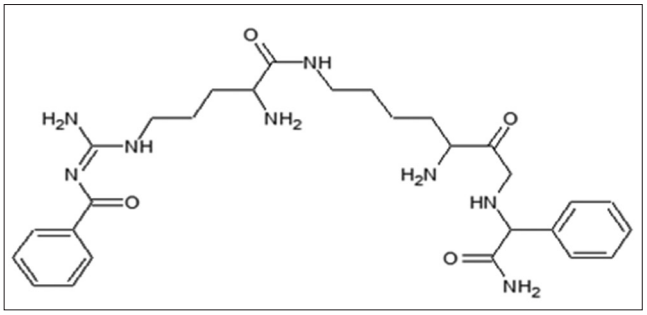

Figure2: Chemical structure of docking Ligand 2-amino-5-{[(1Z) -amino ({[(Z) -benzoyl] imino}) methyl] amino} -N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino} -6-oxoheptyl) pentanamide

RESULTS

One binding pocket with multiplicity of 5 is found on serine protease NS2B chain A with binding residues located at S41, G42, D43, F44 and S45 as per binding site prediction studies performed at RaptorX binding server [34] Fig. 4. Binding site predictions propose NKK as the suitable ligand for binding, which has structure closely related to the proposed ligand2-amino-5-{[(1Z)-amino ({[(Z)-benzoyl] imino})

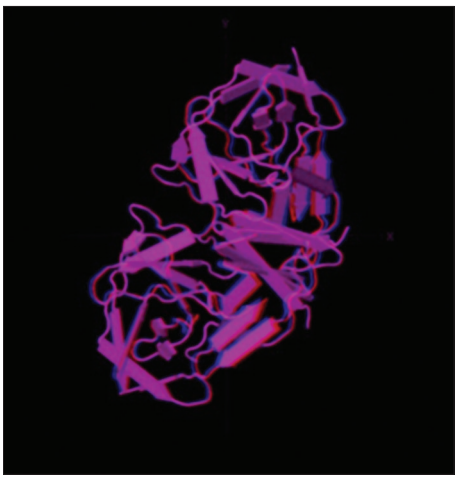

Figure 3: Structure of serine protease NS2B NS3 from ZIKA virus PDB ID 5GJ4

Figure 4: Binding site and binding residue location On NS2B Serine protease chain A

methyl] amino}-N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino}-6-oxoheptyl) pentanamide. Fig. 1 shows ligand binding with serine protease. Free energy of binding is - 4.08 kcal/Mol and inhibition constant Ki is very less 1.02 mm (Table 2). The ligand binds to chain A of NS2B and chain B of NS3 serine protease as shown by 2D plot showing HB and their bond length between serine protease and ligand (Fig. 5) and interaction table. The ligand is bound to serine protease complex through strong HB, formed between THR 60 (A) and N6 of ligand, GLU62 (A) and N8 of ligand, ARG 55 (A) and N3 of ligand and ASN108 (B) and N7 of ligand apart from polar and hydrophobic interactions (Table 3). Ligand fits into the binding pocket found on serine protease NS2B chain A. Interaction of ligand and protein is depicted in HB Plot Fig. 6.

DISCUSSION

Energy values are one of the important parameters used for evaluation of binding between ligand and target protein. Binding energy is the amount of energy released when the ligand is associated with the target. Negative lesser values of binding energy measure the stability of the ligand-protein complex. The greater the energy released on binding of a ligand to the protein, great is the propensity of the ligand to associate with that protein. Molecular docking methods use binding energy values to evaluate the stability of binding complex formed [35]. 4.08 kcal/Mol of binding is indicative of strong and stable bond formed between protein and ligand. The total energy of - 7.07 kcal/Mol is inclusive of all energy terms included in ligand or protein binding scoring function and the changes on binding. Negative values indicate stable complex formed between ZIKV NS2B-NS3 serine protease and 2-amino-5-{[(1Z)-amino ({[(Z)-benzoyl] imino}) methyl] amino}-N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino} -6-oxoheptyl) pentanamide. Electrostatic energy values of - 0.16 kcal/Mol. again support the binding between ligand and protein complex. Weak intermolecular interactions like HB and hydrophobic interactions play a key role in stabilizing energetically favored legends, in an open conformation environment of protein structures [36]. Different workers have used HB and hydrophobic interactions to establish the stability of ligand-protein complex [36,37]. In this study, HB are formed between amino acids of chain A of subunit NS2B, chain B of subunit NS3 and N3, N6, N7 and N8 nitrogen atoms of ligand. The presence of a large number of HB with good bond lengths indicates the stability of binding complex formed. Hydrophobic interactions provide an important driving force in complex formation and although individually small their contribution to ligand-protein interaction is substantial. Excellent polar and hydrophobic interactions are also observed during the present docking studies, as depicted in Table 2. Inhibition constant K_ip gives inhibition capacity of a particular ligand of the target protein and reflects the binding capacity. K_ip values can be applied for tests of docking functions [38] Smaller values of K_i 1.02 mm in this study indicate that a small quantity of ligand is required to inhibit the serine protease complex and ligand has the inhibitory potential to be developed as clinically relevant drug candidate against ZIKV. HB Plot generated confirms HBnetwork with amino acid residues on chain A and chain B of the serine protease complex.

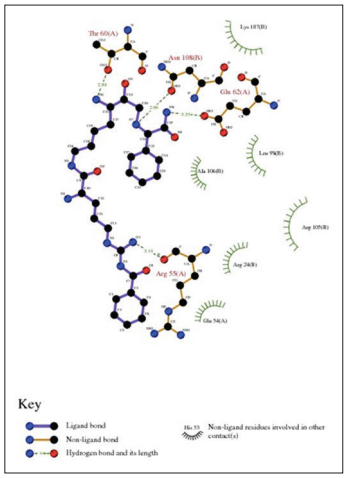

Figure 5: 2D Plot showing hydrogen bonds and their bond length between serine protease and Ligand

Table 1: Specification of macromolecular entities

Molecule	Chain	Length	Organism	Details
Serine protease subunit NS2B	A, C, E, G	61	ZIKA virus	Fragment: UNP residues 1411-1462, UNP residues 1492-1496
Serine protease NS3	B, D, F, H	177	ZIKA virus	Fragment: UNP residues 1497-1673

UNP: Uniprot

Table 2: Energy table for rank 1 docking out of 10 runs

Rank	EST free energy of binding	Inhibition constant, Ki	VdW+Hbond+dissolve Energy	Electrostatic energy	Total intermolec. energy	Frequency	Interact. surface
1	−4.08 kcal/Mol	1.02 mm	−6.91 kcal/Mol	−0.16 kcal/Mol	−7.07 kcal/Mol	10%	927.089

VdW: Van der Waal, H bond: Hydrogen bond, Intermolec: Intermolecular, interact: Interacting

Table 3: Ligand and non-ligand interaction table

Hydrogen bonds	Polar	Hydrophobic
N3-ARG55 [3.16] (O)	O1–GLU54 [2.82] (OE1)	C17-LEU98 [3.70] (CD1)
N6 () [2.89]-THR60 (OG1)	H13 () [2.79]-THR60 (OG1)	
N8 () [3.35]-GLU62 (CD, OE1, OE2)	H14 () [2.45]-THR60 (OG1)	
N7 () [2.65]-GLU62 (CD, OE2)	H27 () [2.37]-GLU62 (OE1, OE2)	
N7 () [2.96]-ASN108 (OD1)	H28 () [3.82]-GLU62 (OE1)	
	O4 () [3.90]-ARG105 (NE)	
	O3 () [3.64]-ASN108 (OD1)	
	H12 () [3.34]-ASN108 (OD1)	
	N6 () [3.66]-ASN108 (OD1)	
	H13 () [3.36]-ASN108 (OD1)	
	H14 () [3.84]-ASN108 (OD1)	

Hydrogen bond: Ligand interactions, Polar: Nonligand interactions, Hydrophobic: Non-ligand interactions

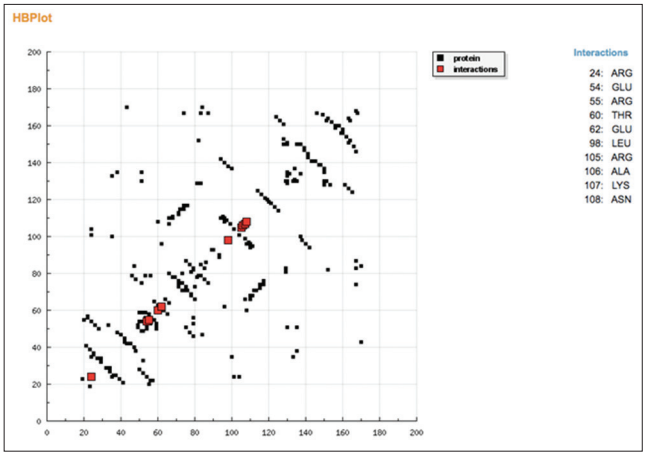

Figure 6: HB Plot for Hydrogen bond network in protein Interactions

CONCLUSION

Docking studies performed establishes the proposed ligand2-amino-5-{[(1Z)-amino({[(Z)-benzoyl] imino})methyl] amino}-N-(5-amino-7-{[carbamoyl (phenyl) methyl] amino}-6-oxoheptyl) pentanamide as a molecule which can be used for the inhibition of ZIKV NS2B-NS3 serine protease thus inhibiting viral replication. Further, *in vitro* studies needs to be carried out to establish the ligand molecule as the prospective drug candidate for ZIKV.

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