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IDENTIFICATION OF INHIBITORS OF DENGUE VIRUS (DENV1, DENV2 AND DENV3) NS2B/ NS3 SERINE PROTEASE: A MOLICULAR DOCKING AND SIMULATION APPROACH

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ABSTRACT

Dengue is one of the fatal diseases, which are becoming a global health burden from few decades. Dengue fever, dengue hemorrhagic fever and dengue shock syndrome, caused by dengue virus (DENV), which completes its life cycle in mosquito i.e. *Aedes aegyti*, and human (DENV), and infect about various individuals every year. The objective of this study is to find a potent inhibitor of DENV (DENV1, DENV2 and DENV3). In the present study, NS2b/NS3 serine protease complex in targeted for the screening of the suitable inhibitors for DENV (DENV1, DENV2 and DENV 3). Therefore, the NS2b/NS3 serine protease complex structures were retrieved from the RCSB Protein Databank. The unliganded protein structures were docked, and best three selected and analyzed. A molecular dynamic simulation is also performed to investigate the conformational and positional changes of ligand that provide insights into the binding stability. It was observed that three of screened compounds have the maximum potential against the protein. The analysis was performed on the basis of scoring and binding ability and one of them indicated minimum energy score with high number of interactions with active site residues and the simulation study revealed that this selected ligand could efficiently bind to the NS2b/NS3 protease. These findings conclude that this selected ligand could be a promising inhibitor of all three serotypes of DENV as drug targets.

Keywords: Dengue virus, Aedes aegyti, Flaviviridae, Serine protease, Docking.

INTRODUCTION

The dengue virus (DENV) belongs to the family Flaviviridae and is closely related to the West Nile virus, the yellow fever virus and the hepatitis C virus [1]. DENV is ssRNA positive-strand virus consists of 11,000 bases in its genome that code for three structural proteins; coat protein C, membrane protein prM and envelope protein E, seven nonstructural proteins; NS 1, NS 2a, NS 2b, NS 3, NS 4a, NS 4b, NS 5 and short non-coding regions on both the 5' and 3' end [1,2]. The U.S. Center for Disease Control and Prevention (CDC) estimated that over 2.5 billion peoples at threat for epidemic transmission [3-5]. It is estimated that 100 million cases of dengue fever (DF) and about half a million cases of dengue hemorrhagic fever/dengue shock syndrome occur worldwide, which cause 25,000 deaths, annually (WHO, 2002). DF is an acute febrile disease, which is characterized by sudden onset fever of 3-5 days, intense headache, myglia, anthragic retroornital pain, anorexia, gastrointestinal disturbance and rash [6]. Currently, there is no specific drug or prevention for this disease [2]. This virus contains a Type I cap structure at the 5'-end and codes for single polyprotein precursor (3391 amino acid residues for DEN2) which is arranged in NH2-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH order (WHO, 2007). For the maturation of the DENV, an optimal activity of the NS3 serine protease is essential, and the optimal catalytic activity of NS3 requisite the presence of NS2 [7]. This serine protease binds to NS2B cofactor that is required to cleave the polyprotein. That NS2B-NS3 protease complex is requisite for replication of the virus [8]. Thus, it serves as a promising target for antiviral drug development against the infection of DENV [9,10], in the present study the ligands are screened against the SN2b/NS3 serine protease of DENV (DENV1, DENV2 and DENV3) to find a common putative drug candidate against the NS2b/NS3 serine protease of DENV1, DENV2, DENV3 for treatment or development of drug against DENV.

METHODS

Search for sequence similarity

Sequence similarities of NS2b/NS3 serine protease of DENV are calculated by CLUSTAL-W [11].

Retrieval of protein structure

The protein structures of NS2b/NS3 Serine Protease of DENVs were retrieved from RCSB Protein Data Bank (PDB) [12] in Brookhaven's PDB format and protein cleaning (removal of ligand and water molecules) was done using Autodock 4.2.1 and UCSF Chimera [13].

Binding site prediction

Binding sites, active sites, surface structural pockets (accessible), interior cavities (inaccessible), shape (alpha complex and triangulation), area and volume (solvent and molecular accessible surface) of each pocket and cavities of proteins were found by using CASTp [14].

Compounds selection and preparation

According to Lipinski rule of five several natural derivative compounds were filtered from the Zinc Database [15], and then selected compounds were screened against three serotypes using AutoDock 4.2.1. The legends were retrieved in SDF format from the database and then converted to PDB format by using Open Babel GUI [16]. All values (molecular weight and XlogP) for selection of ligand for docking were taken using Zinc Database [15]. Ligand preparation includes the addition of hydrogen atoms, neutralization of the charge groups and removal of any miscellaneous structures from the ligand by Autodock 4.2.1. Prepared and optimized structures of ligand and protein were ultimately used for molecular docking.

Molecular docking

Virtual screening of the ligand-protein interaction for their binding affinity was carried out using AutoDock 4.2.1 [17] and the results that include the understanding of the association that involves H-bonding and hydrophobic interactions were analyzed using LIGPLOT1.4.5 [18], a program to generate schematic diagrams of protein-ligand interactions.

The search for the best ways is to fit ligand molecules into structure, using Autodock 4.2.1 resulted in docking files that contained detailed records of docking. The obtained log files were read in auto dock tool to analyze the results of docking. The similarity of docked structures was measured by computing the root mean square deviation (RMSD)

between the coordinates of the atoms and creating clusters of the conformations based on the RMSD values [19]. The lowest binding energy conformation in all clusters was considered as the most favorable docking pose [19]. Binding energies that are reported represent the sum of the total intermolecular energy, total internal energy and tensional free energy minus the energy of the unbound system [19]. The top three ligands were selected based on the energy score after virtual screening.

Molecular dynamic simulation

On the basis of docking result molecular dynamic simulation of NS2b/NS3 serine protease of DENV1, DENV2 and DENV3 protein with selected ligand were carried out with software GORMACS 4.5.5 Using gromos force field [20,21]. The protein-ligand complexes were placed in the center of a cubic box of dimension 90 Å × 90 Å × 90 Å and solved by SPCE/E water molecule. The GROMACS topology files for proteins and ligand were generated by command pdb2gmx (reads PDB formats and generate GROMACS topology file.gro) and PRODRG server [22] (http://davapc1.bioch.dundee.ac.uk/prodrg) respectively. These coordinates were used to build the protein-ligand complex. The environment was set to 300 K and 1 bar. 100 Pico second. position restraining simulations were carried out to restrict the movement of the proteins in the simulation. The cutoff for coulomb interaction and Vander Waal interaction were set to 1.0 nm and 1.4 nm, respectively, of all proteins and the LINCS algorithm, was used for all bond constraints.

Absorption, distribution, metabolism, excretion and toxicity (ADMET) prediction

The various properties of the best ligand were predicted by using Online ACD/I Lab tool (https://ilab.acdlabs.com/iLab2/), and Ames test result predicted by Online Chemical Database (https://ochem.eu/home/show.do) showing.

RESULTS AND DISCUSSION

Search for sequence similarity

The result of CLUSTAL-W shows 73.82, 67.57 and 65.41 scores between 3L6P and 3U1I, 3L6P and 2FOM and 3U1I and 2FOM respectively (Fig. 1).

Retrieval of protein structure

Structures of DENV NS2b/NS3 serine protease were downloaded from PDB (Table 1).

Binding site prediction

Binding pockets were calculated by CastP server and selected according to maximum pocket area and pocket volume (Table 2). These pockets contains TRP17, GLU19, ALA21, HIS23, HIS28, ASN29, ILE30, LEU31, ILE42, LYS43, SER138, TRP139, ASN140, GLY142, GLU143, GLU144, VAL145, GLN160, ASN191, ARG192, GLU193 and VAL197 for 3L6P, MET49, LYS73, LYS17, LEU76, TRP83, LEU85, GLU86, GLY87, GLU88, TRP89, THR118 and THR120 for 2FOM and LYS73, LYS74, LEU76,

Serotype	PDB ID	Length (aa)	Resolution (Å)
DENV1	3L6P	236	2.20
DENV2	2FOM	185	1.50
DENV3	3U1I	191	2.30

PDB: Protein data bank, DENV: Dengue virus

Tabl	le 2:	Poc	ket	infor	mat	ion	by	CastP
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Protein	Pocket area (Ų)	Pocket volume (Å ³)
3L6P	424.9	649.8
2FOM	473.2	766.5
3U1I	493.8	752.2

THR77, VAL78, MET84, GLN89, TRP89, THR118, THR119, THR120, GLY121, GLU122, ILE123, GLY124, VAL147, ASN152, GLY164, ILE165, LA166, GLN167, THR168 and ASN169 for 3U1I.

Compounds selection and preparation

18,000 natural compounds were filtered according to Lipinski rule of five and then 500 filtered compounds were selected for docking (Table 3).

Molecular docking

Analysis of ligand protein complex by ligplot shows hydrogen bonds between ligand ZINC4282211 and protein A) 3L6P, B) 2FOM and C) 3U1I at Glu193, Glu143 and Ser138, at Glu88, Leu85, Val146, Asn152 and val147 and at Trp89, Gln167, Gly124 and Val147 respectively (Figs. 2-4 and Table 4).

ADMET prediction

Analysis of ligand protein complex by ligplot shows hydrogen bonds between ligand ZINC4282211 and protein A) 3L6P, B) 2FOM and C) 3U1I at Glu193, Glu143 and Ser138, at Glu88, Leu85, Val146, Asn152 and val147 and at Trp89, Gln167, Gly124 and Val147 respectively (Figs. 2-4 and Tables 4-6).

Molecular dynamic simulation

This investigation revealed that ligand ZINC4282211 could efficiently bind to the NS2b/NS3 protease without changing the conformation of the protein [23]. To evaluate the stabilities the RMSD and other parameters (Table 7) were calculated with respect to initial structures.

CLUSTAL 2.1 multiple sequence	alignment	
3L6P_A PDBID CHAIN SEQUENCE	GAHMADLSLEKAAEVSWEEEAEHSGASHNILVEVQDDGTMKIKDEERDDT	50
3U11 B PDBID CHAIN SEQUENCE		
2FOM_B PDBID CHAIN SEQUENCE		
3L6P_A PDBID CHAIN SEQUENCE	LGGGGSGGGGGGGVLWDTPSPGIYRILQRGLLGRSQVGVGV	90
BUII_B PDBID CHAIN SEQUENCE	-GGGGSGGGGSGVLWDVPSPPETOKAELEEGVYRIKOOGIFGKTOVGVGV	
FOM_B PDBID CHAIN SEQUENCE	AGVLWDVPSPPPVGKAELEDGAYRIKOKGILGYSOIGAGV	
	***** *** * *** *.*.* ****	
BL6P_A PDBID CHAIN SEQUENCE	FQEGVFHTMWHVTRGAVLMYQGKRLEPSWASVKKDLISYGGGWRFQGSWN	140
BUII_B PDBID CHAIN SEQUENCE	QKEGVFHTMWHVTRGAVLTHNGKRLEPNWASVKKDLISYGGGWRLSAQWQ	99
2FOM_B PDBID CHAIN SEQUENCE	YKEGTFHTMWHVTRGAVLMHKGKRIEPSWADVKKDLISYGGGWKLEGEWK	90
3L6P_A PDBID CHAIN SEQUENCE	AGEEVQVIAVEPGKNPKNVQTAPGTFKTPEGEVGAIALDFKPGTSGSPIV	190
BUII B PDBID CHAIN SEQUENCE	KGEEVQVIAVEPGKNPKNFQTMPGTFQTTTGEIGAIALDFKPGTSGSPII	149
2FOM_B PDBID CHAIN SEQUENCE	EGEEVQVLALEPGKNPRAVQTKPGLFKTNTGTIGAVSLDFSPGTSGSPIV	
3L6P_A PDBID CHAIN SEQUENCE	NREGKIVGLYGNGVVTTSGTYVSAIAQAKASQEGPLPEIEDEVFRK 236	
BU11_B PDBID CHAIN SEQUENCE	NREGKVVGLYGNGVVTKNGGYVSGIAQTNAEPDGPTPELEEE 191	
2FOM_B PDBID CHAIN SEQUENCE	DKKGKVVGLYGNGVVTRSGAYVSAIANTEKSIED-NPEIEDDIFRK 185	

Fig. 1: ClustelW result shows the alignment of 3L6P, 3U1I and 2FOM

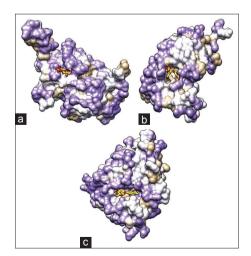


Fig. 2: Ligand ZINC4282211 in the cavity of protein, (a) 3L6P, (b) 2FOM, (c) 3U1I

Table 3- ZINC ID of ligands downloaded from database

Table 3: (Continued)
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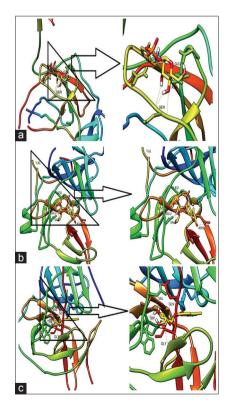


Fig. 3: Molecular visualization of interaction between ligand ZINC2482211 and protein, (a) 3L6P, (b) 2FOM and (c) 3U11

Molecular dynamic simulation showed the stabilization of the proteins after 1ns in system with maximum RMSD values of 9.891, 1.805 and 5.981 nm for 3L6P, 2FOM and 3U1I respectively (Figs. 5 and 6). The stability of system proves the stabilization of protein and credential of docking results (Figs. 7-9) [23].

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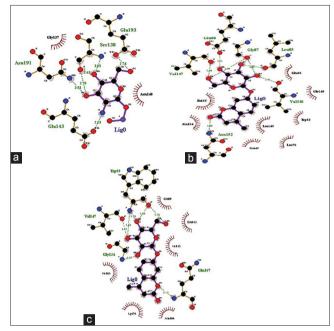


Fig. 4: Ligplot showing hydrogen bonds between ligand ZINC4282211 and protein, (a) 3L6P, (b) 2FOM, (c) 3U1I

Table 4: Binding energy and other parameters of the ligands

Serotype	Parameters	Ligand ID		
		1069091	4097029	4282211
DENV1 (3L6P)	Binding energy (K cal/mol)	-6.14	-4.36	-6
	K _i (μm)	31.67	633.61	39.79
	H Bonds	8	6	6
	MW (g/mol) XlogP	340.284 0.99	258.143 -0.06	338.312 0.10
DENV2 (2FOM)	Binding energy (K cal/mol)	-8.38	-5.49	-8.66
	Κ. (μm)	724.24	251.09	499.67
	H Bonds	5	5	7
	MW (g/mol)	340.284	258.143	338.312
	XlogP	0.99	-0.06	0.10
DENV3 (3U1I)	Binding energy (K cal/mol)	-8.26	-5.21	-8.43
	K, (μm)	882.26	152.28	664.24
	H Bonds	6	7	6
	MW (g/mol) XlogP	340.284 0.99	258.143 -0.06	338.312 0.10

DENV: Dengue virus

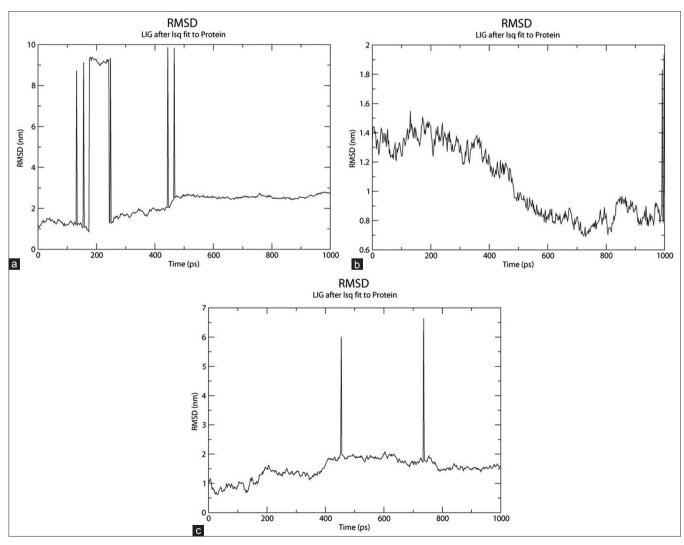


Fig. 5: Root mean square deviation graph by MD simulation, (a) 3L6P, (b) 2FOM and (c) 3U1I

CONCLUSION

In this study 500 ligands obtained from Zinc database were docked against NS2b/NS3 serine protease of above three serotypes of DENV, whose infection results in DF using Autodock 4.2.1 resulted in three ligands ZINC1069091, ZINC4097029, ZINC4282211 (Table 4) obtained as best compounds. Simulation was done to investigate the conformational and positional changes of ligand that provide insights in to the binding stability. This investigation revealed that ligand ZINC4282211 could efficiently bind to the NS2b/NS3 protease without changing the conformation of the protein. To evaluate the stabilities the RMSD and other parameters (Table 7) were calculated with respect to initial structures. Molecular dynamic simulation shows the stabilization of the protein after 1 ns in system. The stability of system proves the stabilization of protein ligand complex.

The present study concludes that the ZINC4282211was found to be most active against above three serotypes of DENV and it could be used

Table 5: ADMET and other parameters of ligands

Parameters	Ligand ID			
	1069091	4097029	4282211	
Absorption (passive)	19%	2%	72%	
BBB (LogPS)	-0.45	-8.2	-3.7	
Bio availability (oral)	<30%	<30%	Between 30 and 70	
рК				
Acid	7.8±0.8	0±0.5	12.5±1.0	
Base	NA	7.8±0.5	NA	
Ames test	Neg (76%)	Neg (78%)	Neg (89%)	
Density (g/cm ³)	1.679±0.06	1.81±0.1	31.78±0.5 10 ⁻²⁴	

BBB: Blood-brain barrier, ADMET: Absorption, distribution, metabolism, excretion and toxicity

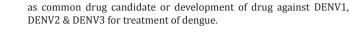


Table 6: Predicted qualitative absorbance of ligands by ACD/I lab online tool

Ligand ID	Threshold	Probability	Reliability
1069091	10 mg/ml	0.63	Borderline (0.43)
	1 mg/ml	0.9	Borderline (0.57)
	0.1 mg/ml	0.99	High (0.83)
	0.01 mg/ml	1	High (0.79)
4097029	10 mg/ml	1	Moderate (0.74)
	1 mg/ml	1	High (0.82)
	0.1 mg/ml	1	Moderate (0.73)
	0.01 mg/ml	1	Moderate (0.72)
4282211	10 mg/ml	0.67	Not reliable (0.27)
	1 mg/ml	0.82	Borderline (0.33)
	0.1 mg/ml	0.98	Moderate (0.73)
	0.01 mg/ml	0.99	Moderate (0.65)

Table 7: Parameters by molecular dynamic simulation studies

Parameters	3L6P	2FOM	3U1I
RMSD (nm)	9.891	1.805	5.981
RMSF (nm)	0.9464	0.802	0.790
Potential	-8.5104688e+05	-5.1585806e+05	-5.1585806e+05
energy			
(Kj/mol)			
Radius of	1.819	4.085	3.325
gyration (nm)			

RMSD: Root mean square deviation, RMSF: Root mean square fluctuation

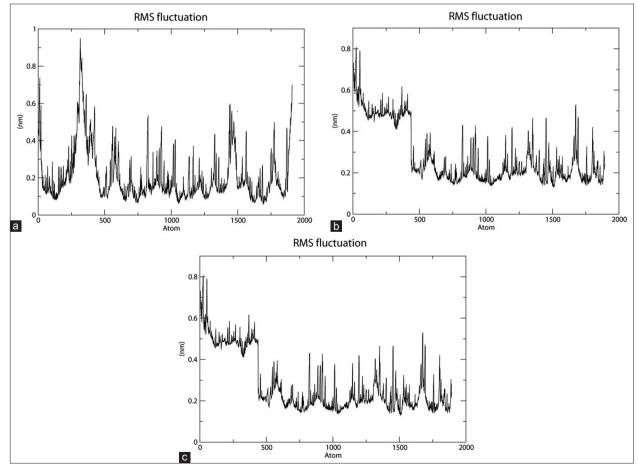


Fig. 6: Root mean square fluctuation graph by MD simulation, (a) 3L6P, (b) 2FOM, (c) 3U1I

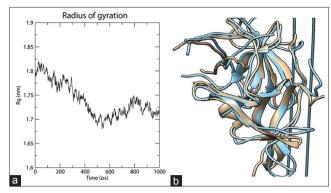


Fig. 7: (a) Radius of gyration graph, (b) Superimposition of structure (opaque before simulation and cyan after simulation) of 3L6P

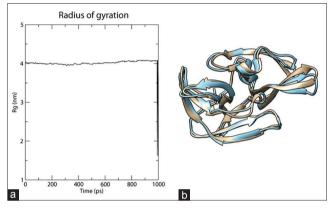


Fig. 8: (a) Radius of gyration graph, (b) Superimposition of structure (opaque before simulation and cyan after simulation) of 2FOM

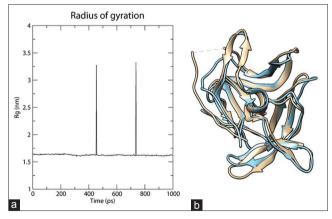


Fig. 9: (a) Radius of gyration graph, (b) Superimposition of structure (opaque before simulation and cyan after simulation) of 3U1I

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