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Research Article

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 order NH2-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH (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, THR77, VAL78, MET84, GLN89, TRP89, THR118, THR119, THR120, GLY121, GLU122, ILE123, GLY124, VAL147, ASN152, GLY164, ILE165, LA166, GLN167, THR168 and ASN169 for 3U1I.

Table 1: Serotype and PDB ID of serotypes of DENV

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

Table 2: Pocket information by CastP

Protein	Pocket area (Å^2)	Pocket volume (Å^3)
3L6P	424.9	649.8
2FOM	473.2	766.5
3U1I	493.8	752.2

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.

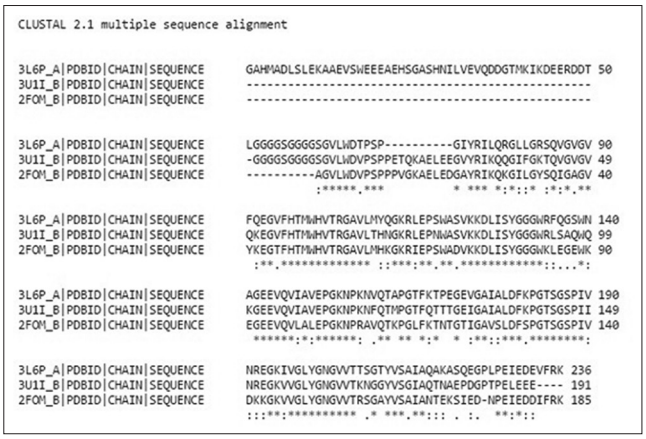

Fig. 1: ClustelW result shows the alignment of 3L6P, 3U1I and 2FOM

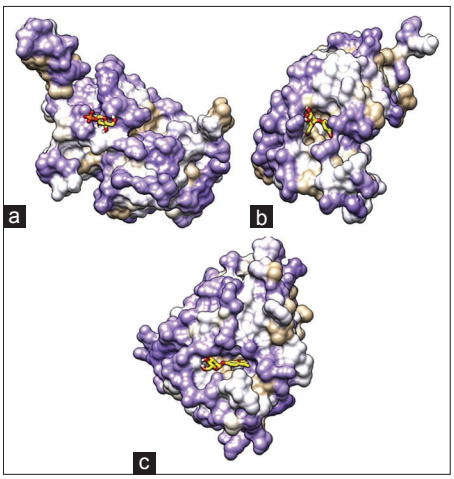

Fig. 2: Ligand ZINC4282211 in the cavity of protein, (a) 3L6P, (b) 2FOM, (c) 3U1I

Table 3- ZINC ID of ligands downloaded from database

1793	265133	1074260	2046841	2554951
19968	338040	1078624	2124579	2572064
58284	485468	1081065	2133555	2575039
134310	488422	1081233	2169830	2583634
134312	488469	1203477	2384668	2586053
134323	517478	1529564	2387029	2596997
155675	518554	1529643	2522476	3156362
155886	518973	1532857	2539694	3212716
156947	1069091	1550030	2539732	3212719
158666	1070097	1672137	2545105	3590892
241780	1074256	1760052	2545107	3653374
3788703	3897841	4028438	4261922	4831392
3814314	3897842	4028439	4261923	4878658
3830179	3900052	4073375	4268259	4878666
3830524	3956712	4073951	4268262	4878672
3830982	3956715	4082020	4268352	5037495
3830983	3956716	4082270	4268354	5037497
3830984	3956720	4095477	4279275	5037498
3830985	3956731	4095490	4282153	5103281
3831470	3977952	4095529	4282211	5126682
3832269	3978744	4095569	4282228	5157172
3847505	3984172	4095664	4303674	5163030
3869396	3984173	4095713	4304316	5178951
3869397	3985065	4095714	4350160	5222064
3869398	3999102	4095786	4513768	5222071
3869520	3999103	4095787	4513770	5224791
3869521	3999104	4096022	4513773	5227195
3869664	3999187	4096145	4521724	5227213
3869665	3999202	4096188	4521723	5239480
3869795	3999203	4096594	4521728	5239485
3869796	3999214	4096638	4544899	5273762
3869810	3999215	4096704	4544905	5273763
3869811	3999312	4096931	4556538	5273764
3869812	3999605	4097029	4556539	5273765
3869910	3999697	4097443	4556540	5341026
3870078	3999698	4097444	4556541	5341027
3870959	3999699	4097529	4556551	5344125
3873956	3999700	4098646	4556852	5420865
3874928	4010856	4098840	4556941	5438604
3875283	4010857	4099041	4557136	5566519
3875284	4010933	4104676	4557391	5566520
3875375	4010934	4104678	4721317	5700054
3881790	4010947	4104822	4721319	5733445
3882070	4015531	4175578	4721320	5736909
3894278	4025052	4217475	4721322	5752331
3897399	4026310	4217548	4787865	5765081
3897400	4026537	4228250	4820559	5765311
3897446	4027427	4228251	4831390	5781539
3897447	4027791	4228295	4831391	5842327
5923622	8551508	13376214	13544222	15251264
5934041	8586495	13376215	13544387	15251267
5954934	8586497	13413424	13544561	15251270
5954938	8628009	13436049	13545523	15262728
5954941	8738281	13436057	13545525	15657731
5954945	8738375	13440025	13547742	17328339
6069213	8738376	13481491	13547790	18033592
6069529	8782776	13481492	13550855	18045874
6069530	8855117	13481493	13551958	18140538
6090974	8869285	13481561	13816232	18166302
6092865	9008779	13507640	14418234	18179993
6184878	11592522	13507867	14418525	18275505
6184881	11592523	13507879	14422035	19312755
6184893	11592524	13508703	14504473	19312821
6360512	11680913	13508877	14504476	19312824
6444474	12358758	13511397	14504479	19312827
6444476	12358840	13512564	14504482	19312830
6444478	12359966	13514113	14504497	19321826
6474030	12359967	13514886	14504500	19322388
6562443	12362075	13515580	14504503	19322865
6562444	12376523	13516052	14512189	19322868
6567584	12377745	13518145	14512194	19322873
6585262	12377746	13520415	14642425	19323012

(*Contd*...)

Table 3: (*Continued*)

6623665	12406255	13520419	14642428	19323015
6623668	12406257	13520422	14685852	19323021
6930964	12406261	13520517	14685854	19323116
6930996	12495053	13521786	14859228	19323119
6932857	12496482	13527007	14859229	19323325
6932870	12502386	13527740	14859230	19331307
7974994	12502388	13536667	14920311	19331311
8197355	12503321	13536768	14920313	19331315
8197360	12503323	13537301	15113270	19331319
8198840	12503731	13538582	15169388	19331330
8198843	12503733	13538585	15218611	19331334
8376402	12658357	13540027	15218618	19331412
8376405	12890051	13542583	15218624	19331417
8376408	12953204	13542645	15218630	19331424
8376412	13348180	13543975	15251259	26252414
27645575	31163896	35457020	39373976	67903182
28536442	31163900	35457139	39373977	67903185
30724100	31169052	35457147	39373978	67913713
31156872	32109487	35458536	49176734	67913716
31156876	33689262	35464718	49176735	67913718
31156880	33689266	35464732	49181950	67913836
31160626	33834036	35464735	49181952	67913897
31160630	34165724	35465273	49181955	70665126
31160634	34165725	35465276	49181958	71404540
31160638	34165726	35465279	49181973	71404541
31160915	34522850	35878009	49181976	72319970
31160919	34965022	36367411	49181978	
31160923	35455284	36367802	49181981	
31160927	35455293	39373975	62001311	

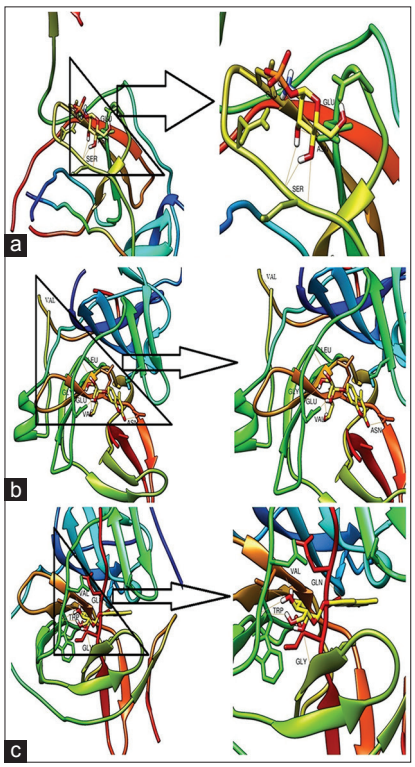

Fig. 3: Molecular visualization of interaction between ligand ZINC2482211 and protein, (a) 3L6P, (b) 2FOM and (c) 3U1I

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].

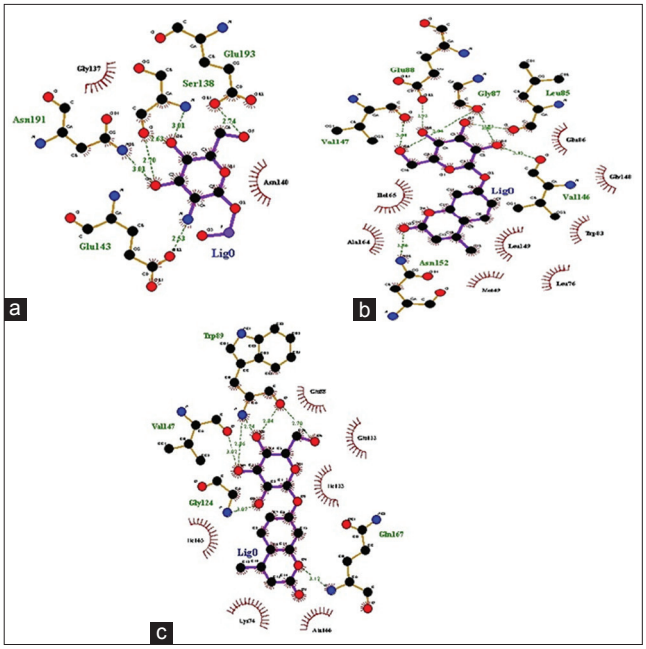

Fig. 4: Ligplot showing hydrogen bonds between ligand ZINC4282211 and protein, (a) 3L6P, (b) 2FOM, (c) 3U1I

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)	340.284	258.143	338.312
	XlogP	0.99	−0.06	0.10
DENV2 (2FOM)	Binding energy (K cal/mol)	−8.38	−5.49	−8.66
	K_i (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_i (μm)	882.26	152.28	664.24
	H Bonds	6	7	6
	MW (g/mol)	340.284	258.143	338.312
	XlogP	0.99	−0.06	0.10

DENV: Dengue virus

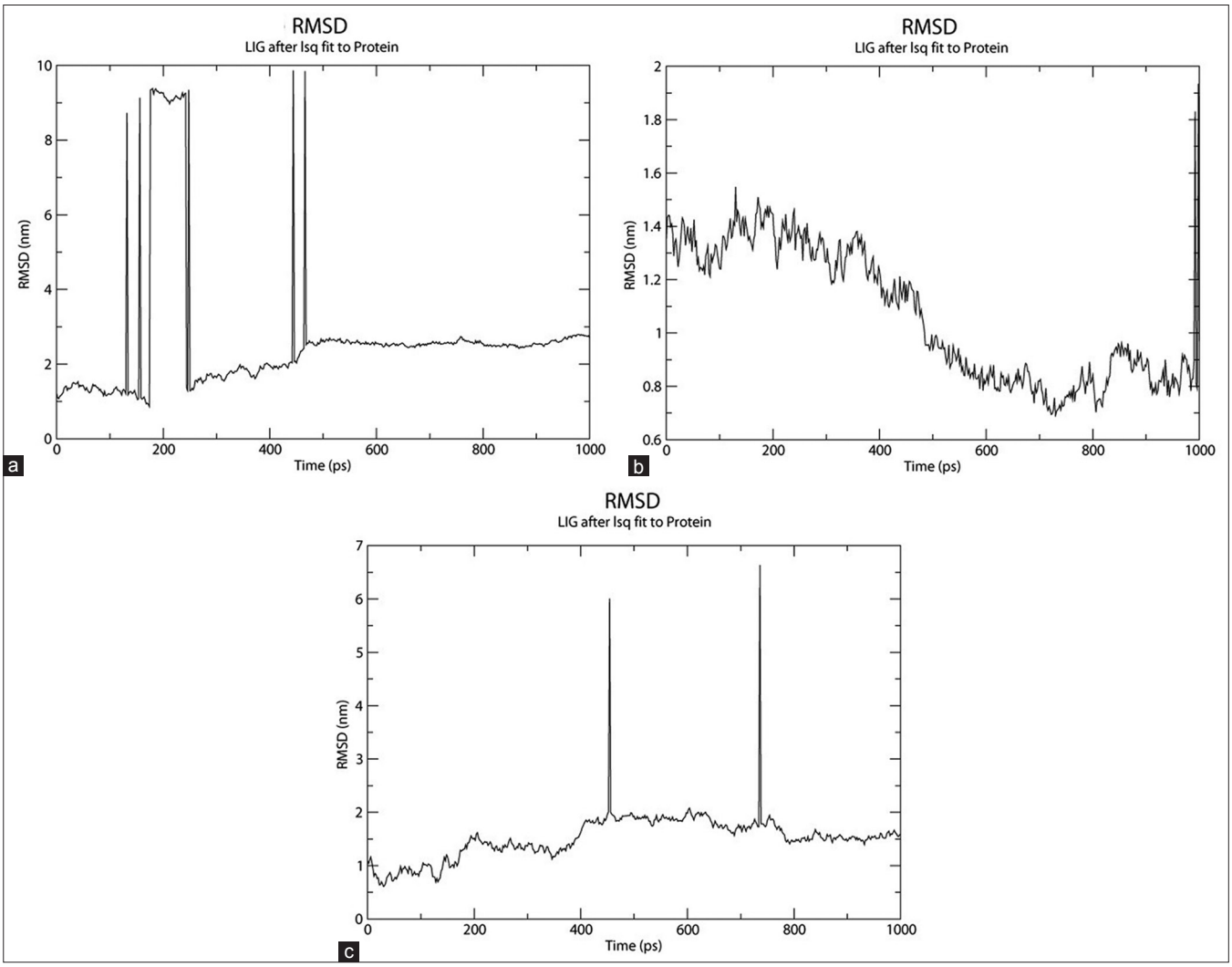

Fig. 5: Root mean square deviation graph by MD simulation, (a) 3L6P, (b) 2FOM and (c) 3U1I

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 proteins 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 as common drug candidate or development of drug against DENV1, DENV2 & DENV3 for treatment of dengue.

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
pK			
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 energy (Kj/mol)	−8.5104688e+05	−5.1585806e+05	−5.1585806e+05
Radius of gyration (nm)	1.819	4.085	3.325

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

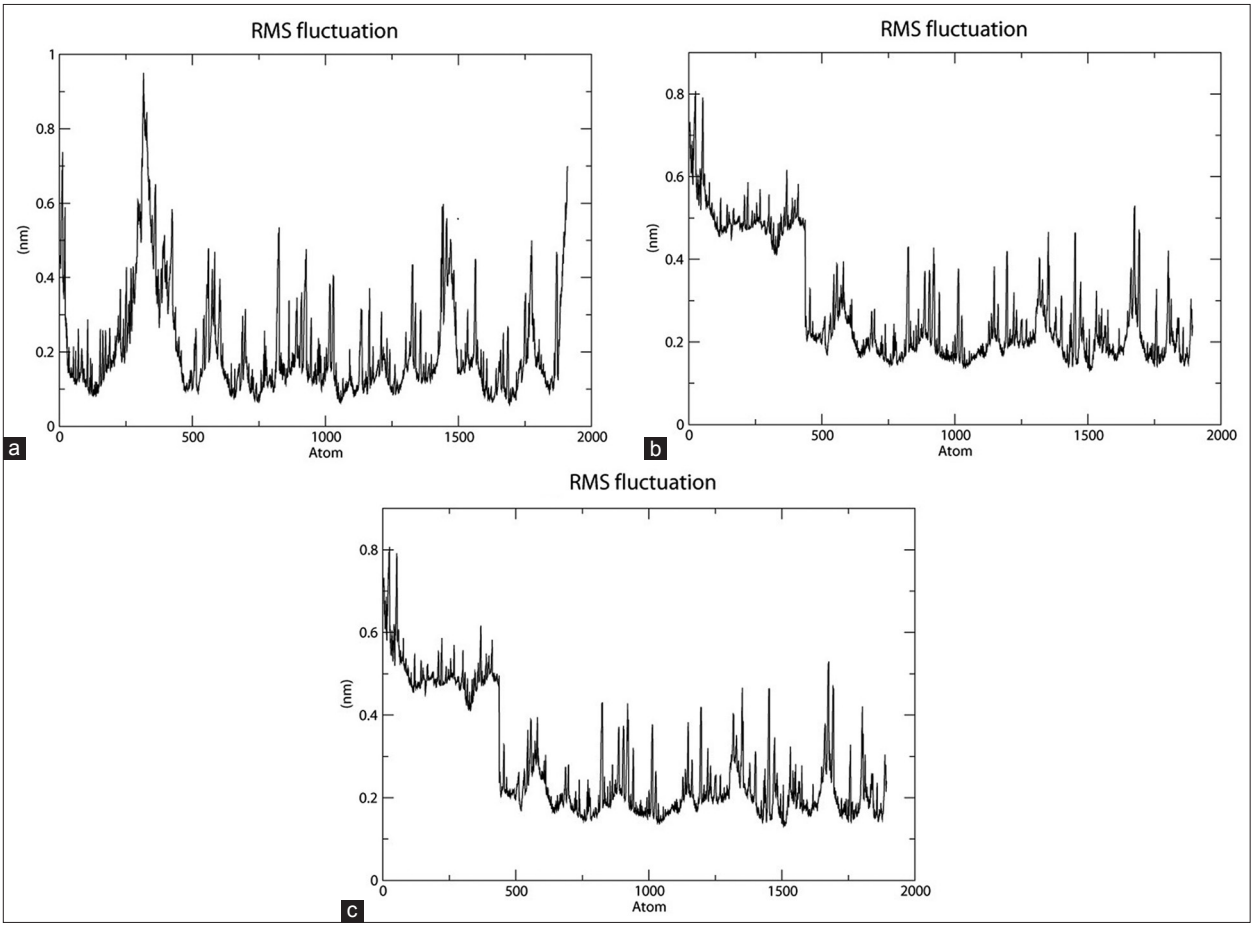

Fig. 6: Root mean square fluctuation graph by MD simulation, (a) 3L6P, (b) 2FOM, (c) 3U1I

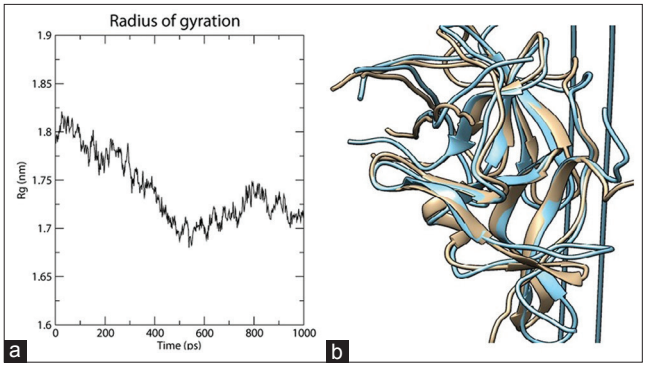

Fig. 7: (a) Radius of gyration graph, (b) Superimposition of structure (opaque before simulation and cyan after simulation) of 3L6P

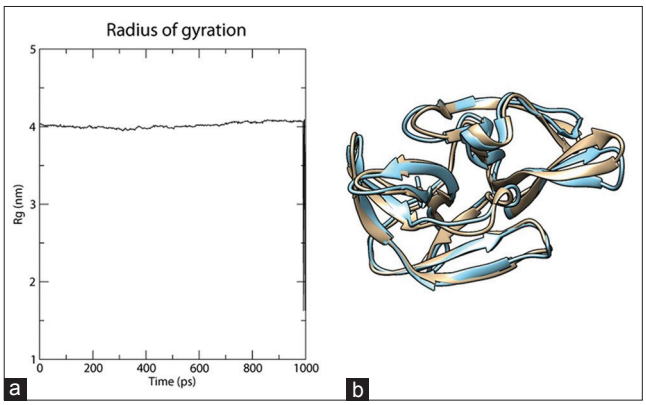

Fig. 8: (a) Radius of gyration graph, (b) Superimposition of structure (opaque before simulation and cyan after simulation) of 2FOM

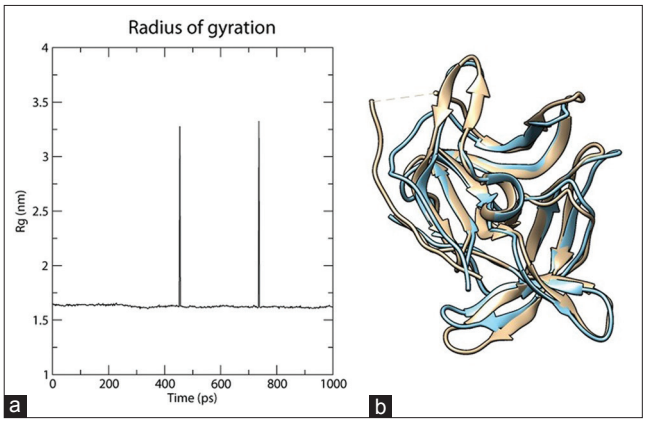

Fig. 9: (a) Radius of gyration graph, (b) Superimposition of structure (opaque before simulation and cyan after simulation) of 3U1I

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