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Research Article

Discovery of Novel Dengue NS2B/NS3 Protease Inhibitors Using Pharmacophore Modeling and Molecular Docking Based Virtual Screening of the ZINC Database

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Abstract

Background: Dengue, a vector borne disease has become a crucial health concern globally. The search for a suitable dengue vaccine has been going on for the last few decades due to unavailability of any effective treatment. An efficient medication strategy is required to overcome the devastating effects of dengue. Now, computational biology has emerged as a novel tool to improve the domain of computer aided drug designing. The present study reports a complex-based pharmacophore computational modeling that elucidates important pharmacophoric features helpful for the inhibition of protease activity of NS2B/NS3 protein of Dengue Virus (DV). **Materials and Methods:** A seven featured pharmacophore model of DV NS2B/NS3 protease has been developed via crystal structure of NS2B/NS3 protease and its inhibitor complex in Molecular Operating Environment (MOE) pharmacophore constructing tool. The developed pharmacophore model was validated by a test database of the published inhibitors. Validated pharmacophore model was then used to virtually screen the potential compounds from ZINC database. The screened compounds were filtered by Lipinski's rule of five and further evaluated through molecular docking studies. The results of docking and interaction studies were validated through binding affinity analysis and ADMET profiling. **Results:** Six hits (ZINC ID's: 75163069, 59170698, 06395655, 32933073, 13728171 and 65395833) of different scaffolds having interactions with important active site residues (His51, Asp75, Ser135) were predicted. **Conclusion:** It can be concluded from the finding of the present study that predicted hits could serve as potential candidates to act as starting point in the development of novel and potent NS2B/NS3 protease inhibitors. The present modeling explores the significant role of the predicted hits towards blocking the replication of DV.

Key words: Dengue, ZINC database, pharmacophore modeling, molecular docking, drug profiling

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to unavailability of any effective treatment, Dengue, a vector borne disease has become a crucial health concern globally. Dengue related health disorders such as fever and acute joint pains are caused as result of mosquito-borne viral infections[1,2]. This rapidly spreading and debilitating disease has been reported to affect almost 2.5 billion people worldwide[3] with approximately 25,000 deaths per year[4]. According to a latest study approximately 50-100 million people from more than 100 countries are suffering from the devastating effects of this viral infection. Several countries and especially the tropical regions of Asia, Central America, South America and Africa are at a higher risk of dengue infections[5,6]. As investigated by immunological and virological studies, Dengue infection is caused by Dengue Virus (DV), a *Flaviviradae* virus with four serotypes (DV-1, DV-2, DV-3 and DV-4) reported[7]. Dengue virus belongs to viral genus *Flavivirus* that consists of pathogenic RNA viruses. These viruses are mostly colonized in temperate and tropical regions of the world and cause several infections in humans including dengue fever, west-nile fever, tick-borne encephalitis and yellow fever[8].

In humans, DV infection spreads via the bite of two carrier mosquitoes namely *Aedes aegypti* and *Aedes albopictus*[9]. The genomic size of DV is reported to extending over 10.7 kb and encodes a total 10 structural and nonstructural proteins[10]. The three structural proteins include core/capsid protein, membrane associated protein and an envelope protein. The remaining seven proteins namely NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 are non-structural proteins. These proteins are located in the order of 5′-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′ on viral RNA[11,12] (Fig. 1) and play a vibrant role in the structural organization of virus and viral genome entry into the host cell, while non-structural proteins help the DV to control the host synthetic machinery for its own replication and other cellular functions[13]. Recently, it has been investigated that the potential of NS3 depends upon its interaction with cofactor (NS2B). The NS2B is a serine protease domain which is located at the N-terminal region of NS3[14]. As a result of NS3 and NS2B interaction, NS2B/NS3 pro-complex is formed, which helps to cleave viral proteins to perform their functions. Any disturbance in functional behavior of this region is reported to inhibit viral replication. Therefore, NS2B/NS3 complex is believed to be an effective target for the screening and assessment of the effective antiviral drug candidates[1,4,12,14].

Recent advances in computational biology have noticeably impacted and improved the domain of computer aided drug designing. Due to high cost and low hit rates practiced during wet lab testing, computational processes and virtual screening have largely aided the drug designing. Computational methods are supportive in taking decision and virtually stimulating various aspects of drug discovery and development. By the last few decades, computationally generated virtual High Throughput Screening (vHTS) has gained sufficient maturity to becoming an alternative to the counterpart experimental high-throughput virtual screening (HTS). Being a knowledge driven approach, vHTS has proved to be an integral part of drug designing process[15].

Advanced computational approaches such as multifarious pharmacophore modeling, virtual screening and molecular docking can be applied to identify and recognize new and effective inhibitors to minimizing the activity of NS2B/NS3 protease from DV. A pharmacophore is the 3D arrangements of structural or chemical features of small organic compounds of a drug that might be essential for its interaction with the target and drug's bioactivity. In drug designing process, the use of pharmacophore approach can be supportive in several ways: (a) It could be used as a 3D query in virtual screening to

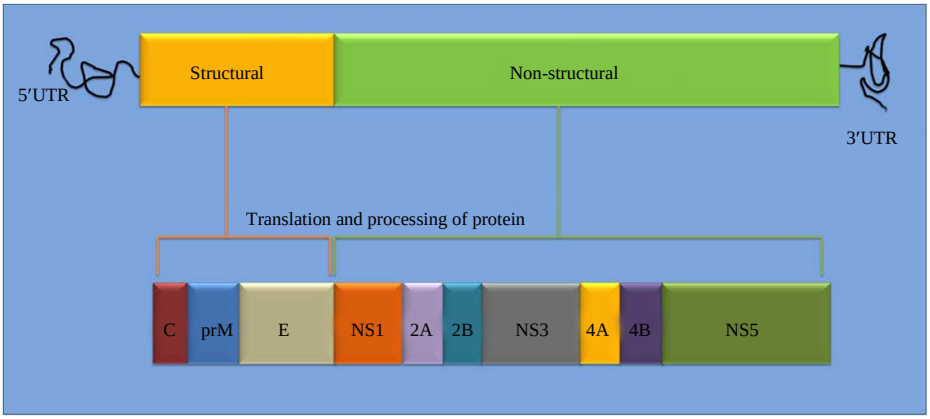

Fig. 1: Schematic diagram of Dengue Virus (DV) RNA gene organization and their translation

recognize novel and effective hits with an arrangement different from the previously reported structures from the databases of drug-like molecules, (b) To identify the possible mode of actions and (c) To predict the mode of action of new compounds prior to synthesis[16,17]. Recent advances in virtual screening technologies have prompted the researchers to rapidly screen drug libraries and inhibitors databases to find out therapeutic activities against different new targets with cost effectiveness[18]. One of the similar approaches, namely molecular docking involves the use of multiple computational methods to predict the true binding orientations of small molecules within their protein targets[19].

The plan of the current study was to design and develop a complex based pharmacophore model and to find out the hits which have important pharmacophore features by computational screening of effective compounds against all subsets of a previously developed ZINC database[20] coupled with molecular docking, protein ligand binding interactions, binding affinity predictions and drug likeness predictions. Virtual screening is supposed to provide us an insight of various features of compounds essential for the inhibition of protease activity of NS2B/NS3 complex. The ZINC is a free database of commercially available small organic compounds for virtual screening and drug discovery. It has over 35 million potential compounds in ready-to-dock, 3D formats[21]. For example, compound-1 of natural product like subset of ZINC database is merely the second immunomodulatory inhibitor to date which has successfully inhibited TLR1-TLR2 heterodimerization *in vitro* and proved as a potential drug candidate against different fungal and inflammatory/immune disorders (atherosclerosis, sepsis syndrome, rheumatoid arthritis, asthma) and viral diseases (human cytomegalovirus, lymphocytic choriomeningitis virus, herpex simplex virus)[22]. Compound-1 also exhibited anti-proliferative activity against cancer cells *in vitro* by inhibiting STAT3 dimerization[23]. Optimization and virtual screening of Janus Kinase 2 (JAK2) Type-II compounds from ZINC database has explored potential inhibitors of Hepatitis C Virus (HCV) translation and replication[24]. Increased resistance of *Plasmodium falciparum* against most of the available drugs is a challenging task towards controlling malaria. According to researchers, previously, 19 potential anti-malarial compounds were reported from ZINC database which successfully inhibited the growth of *Plasmodium falciparum*[25]. The compound 6,6``-biapigenin has been only identified as the second inhibitor of NEDD8-activiating enzyme as well as reported to be anti-cancer agent by virtual screening of ZINC database[26]. There are also many other examples of different compounds from ZINC database which successfully proved as a potential drug compounds against various diseases.

To date, no any other study as such screened whole ZINC database against DV NS2B/NS3 protease. Therefore, in the present study this database have selected for computational screening of potent lead inhibitors which could serve as drug compounds against DV. The identification of six novel and potent lead compounds as NS2B/NS3 protease inhibitors clearly reflects the significance of this study. The results of this study provide valuable information about computer aided drug screening and development of drugs against DV infection.

MATERIALS AND METHODS

Pharmacophore modeling and molecular docking was accomplished via the Molecular Operating Environment (MOE) software package. The MOE is a comprehensive suite developed by Chemical Computing Group (CCG) Incorporation. It has different tools and computational methods that are applied for structure and fragment based drug designing, pharmacophore discovery, protein structure analysis, data processing and molecular docking and simulations[27].

Generation and validation of complex based pharmacophore model: The assembly of electronic and steric characteristics of pharmacophore is compulsory to ensure finest supra-molecular interactions with a particular biological target for inhibiting its biological response[28,29]. If 3D structure of the target protein is available, complex based pharmacophore system can be used to modify the drug advancement process. In the present study, the crystal structure of DV NS2B/NS3 protease in complex with a substrate based inhibitor benzoyl-norleucine (P4)-lysine (P3)-arginine (P2)-arginine (P1)-aldehyde (Bz-Nle-Lys-Arg-Arg-H) was used (PDB ID: 2FOM) for the creation of complex based pharmacophore system[30]. The inhibitor had strong interaction with catalytic triad residues of DV NS2B/NS3 protease and further used as a reference in molecular docking (Fig. 2). The MOE pharmacophore constructing tool was employed for the creation and visualization of three dimensional (3D) pharmacophore from protein structure ligand complex. The generated pharmacophore system was validated through a test database of 20 known inhibitors of DV NS2B/NS3 protease. These inhibitors of the database and their inhibitory potential were taken from the reported literature[31,32]. The 3D structures of the test database were created in MOE builder tool. Energy minimization of 3D structures was achieved by MOE energy minimization algorithm with parameters; Gradient: 0.05 and Force Field: MMFF94X.

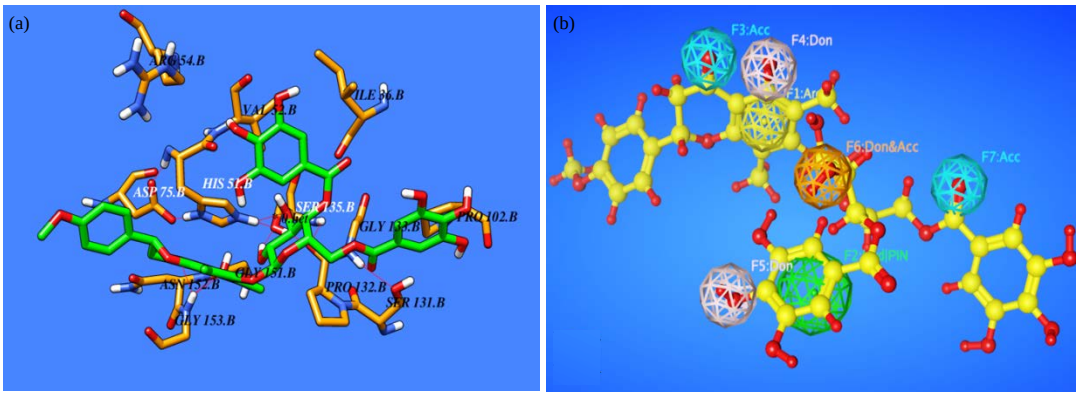

Fig. 2(a-b): Complex structure of reference ligand (Bz-Nle-Lys-Arg-Arg-H) and DV NS2B/NS3 protease. (a) Three dimensional representation of the interaction of the reference ligand and receptor and (b) Three dimensional pharmacophore model generated from DV NS2B/NS3 protease complex map view

Pharmacophore-based database screening: The next step was to identify different hits with multiple chemical natures. This was achieved by using *in silico* pharmacophore based visual screening of the ZINC database with 3D query of the validated pharmacophore model. The MOE software was used for this virtual screening of various hits in the ZINC database[20]. This type of screening was adopted due to a couple of reasons: firstly, the validated pharmacophore model was successfully used for appropriate identification of compounds having known inhibitory potential against dengue protease and secondly, the importance of this technique to recognize new and effective drug-like items for further assessment[29,33]. Similarly, Lipinski's rule of five was used to determine drug-like attributes of the compounds retrieved from the ZINC database. The rule illustrates molecular properties that are significant for a drug's pharmacokinetics in the human body[34]. To check the appropriate molecular properties of compounds, their drug scan was executed using the ligand properties checking tool molinspiration server[35]. Drug scan will tell us the potential and effectiveness of these compounds.

Molecular docking: For further evaluation of hit compounds, all the retrieved compounds were docked into the binding site of 3D structure of DV NS2B/NS3 protease (PDB ID: 2FOM). Original substrate based inhibitor was also test-docked into the binding site of DNV NS2B/NS3 protease as reference ligand. Removal of water molecules, 3D protonation and energy minimization was carried out using MOE[27] with parameters, force field: MMFF94X+solvation, Gradient: 0.05, Chiral constraint and current geometry. This minimized structure was used as receptor for docking analysis. Binding pocket with catalytic triad (His51, Asp75, Ser135) was selected with the help of MOE site finder tool. The selected parameters that have been used to calculate the score and interaction of ligand molecules with catalytic triad of dengue virus NS2B/NS3 were Rescoring function: London dG, Placement: Triangle matcher, Retain: 10, Refinement: Force field, Rescoring 2: London dG. Most appropriate docked ligand target structure was selected on the basis of higher S-score than reference inhibitor S-score and Root Mean Square Deviation (RMSD) values. The S-score is the value calculated by built-in scoring functions of MOE on the basis of ligand binding affinity with receptor protein after docking. While, RMSD value is generally used to compare the docked conformation with the reference conformation or with other docked conformation. The only compounds that have higher S-score and lower RMSD value than its natural substrates can be developed as potential inhibitors[29]. Top hit compounds were preferred and kept in separate database for further evaluation of interactions.

Binding affinity calculations and ADMET profiling: To recognize the most effective ligands, MOE Generalized Born/Volume Integral (GB/VI) implicit solvent method was used for the determination of binding affinities of drug-like compounds with NS2B/NS3 protease complexes. In general, the generalized born interaction energy is the non-bonded interaction energy connecting the ligand with the receptor molecule. This energy is found between several binding interactions like implicit solvent interaction, Coulomb electrostatic interaction and van der Waals[29,36]. Nonetheless, the strain energies between the hit compounds and receptor molecules were not considered. According to the computations, the receptor atoms were kept stiff which

situated away from the ligand. However, the receptor atoms falling closely to ligand were kept supple. The selected compounds were further studied for the binding pocket. For this purpose, in the selected compounds, energy minimization of binding pocket was carried out in NS2B/NS3 protease-ligand complex before going for the evaluation of their binding affinity which was measured in kcal mol^{-1}. The selection criteria for the most capable compounds were: (a) Visualization of binding of each hit inside the catalytic cavity of receptor protein, (b) Compounds having binding energy and affinity equal or higher than that calculated for the reference ligand in the complex structure and (c) Hits showing interactions with important residues (His51, Asp75, Ser135) in binding cavity of DV NS2B/NS3 protease[29]. The basis of selecting compounds with binding affinities higher than that of reference ligand is that only compounds that can bind to DV NS2B/NS3 protease with a higher affinity than its natural substrates can be developed as possible inhibitors. Applying the above mentioned criteria, most appropriate potential compounds were selected and saved in separate database for further evaluation of ADMET properties. For *in silico* screening of the ADMET profiles of the potential compounds, admetSAR server[34] was used which predicted the ADMET-associated properties of the active compounds for various kinds of models, all of which explained significant results.

RESULTS AND DISCUSSION

Enormous research study has been carried out to develop therapeutic vaccines against DV. However, no any effective drug/vaccination is available to contest dengue virus till now. Thus, there is a strong need to search cost-effective treatment strategy that can target and control DV[3]. Different strategies have been used so far to search out potent inhibitors for DV NS2B/NS3 protease[37,38] including rational discovery of potential agents based on non-competitive binding[39], structure based virtual screening[40-42], ligand based virtual screening[43,44], synthesizing rationally designed substrate-based cyclopeptide[45] or peptidomimetics[46-48], virtual screening and scaffold hopping[49], screening natural products[50-52] and small compound libraries[53]. The conventional drug discovery techniques are mostly inefficient and therefore unproductive to tackle the emerging threats to public health worldwide[54]. Thus, there is need to introduce most efficient methods which could promptly handle this adverse situation.

However, to date, only few peptide or small molecule (natural, synthetic) inhibitors of DV NS2B/NS3 protease have been reported[37,38]. Although, extensive study has been performed and several peptides, cyclopeptides, natural inhibitors, synthetic small molecules have been reported so far but undesirable features, weak bonding with receptor protein and high toxicity may hinder their future development into the drug compounds[46-49]. Just recently, Lima *et al.*[55] reported a novel peptide-hybrids based on 2,4-thiazolidinedione scaffolds containing non-polar groups. The most promising compound has an IC_{50} of 0.75 μM against WNV protease, which represents a seventy fold improvement in activity compared to previously reported compounds (Kalta B1 analogues)[45]. Idrees and Ashfaq[56] designed different cyclic peptides in combination with positively charged amino acids. They docked their peptides against DV NS2B/NS3 protease. Only seven of them showed potential interactions with DV NS2B/NS3 protease. Their results need further optimization and *in vitro* investigation to confirm their efficacy. Tomlinson *et al.*[40] reported 2 compounds with potential inhibitory activity against the DV protease *in vitro*. Vernekar *et al.*[57] reported 5'-Silylated 3'-1,2,3-triazolyl thymidine analogues as DV inhibitor *in vitro*. Meanwhile, this study also reported 12 medicinal plant phytochemicals with strong inhibitory activities against DV NS2B/NS3 protease *in silico*[4,31]. But, all mentioned and other published compounds[58] need further optimization to be therapeutically useful.

Keeping in view of above discussion, current study focuses on the pharmacophore based virtual screening followed by molecular docking and drug likeness prediction. This is the novel methodology and revealed potential compounds which strongly bind with DV NS2B/NS3 protease active site by hindering its replication and can be further used as drug compounds.

Generation and validation of complex based pharmacophore model: Complex based pharmacophore model of 3D structure of NS2B/NS3 protease in complex with a reported inhibitor was generated by using MOE pharmacophore constructing tool. Binding interactions induce significant chemical features which were taken into account for the creation of pharmacophore model. Significant binding interactions were observed in the protein ligand complex via MOE LigPlot tool. By using default factors of MOE, seven key features including two hydrogen bond donor (Don), two hydrogen bond acceptors (Acc), one donor and accepter (Don and Acc), one aromatic (Aro) and one hydrophobic (Hyd) were generated in the resulting pharmacophore model (Fig. 3). The created pharmacophore system was assessed via a test database of twenty known inhibitors of DV NS2B/NS3 protease. All inhibitors of the test database along with their mapping modes were evaluated on the basis of seven featured complex pharmacophore model. The evaluation

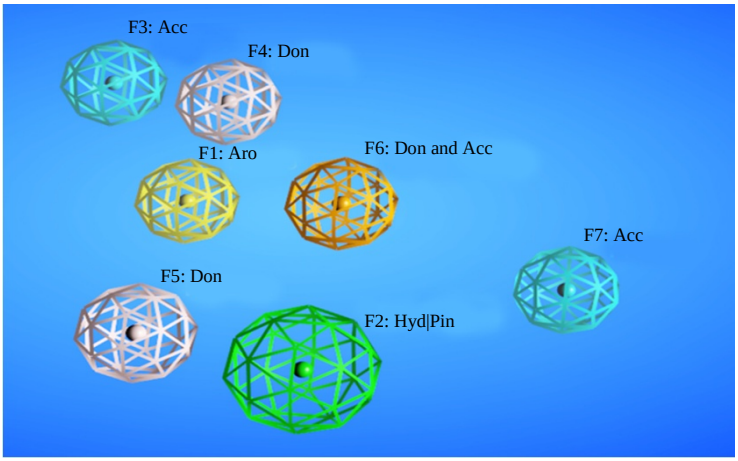

Fig. 3: Three dimensional pharmacophoric features generated from complex structure of DV NS2B/NS3 protease. Green, Orange, Yellow, Purple and Cyan represent hydrophobic, hydrogen bond donor and acceptor, aromatic, hydrogen bond donor and hydrogen bond acceptor features, respectively

resulted in 14 out of 15 most active compounds as hits. These hits showed mapping of 100 various conformations of seven features created pharmacophore system. None of the inactive was mapped to any feature of complex based pharmacophore system. The results from the test database revealed the precision of generated pharmacophore model.

Pharmacophore-based database screening: The best hit compounds with similar features and novel structural conformations were screened from the ZINC database using the validated pharmacophore model. In Computer Aided Drug Designing (CADD), virtual screening is one of the time saving methods for the discovery of novel, potent and drug-like compounds[29]. Pharmacophore model based screening resulted in 900 structurally diverse hits from ZINC database, strengthening seven featured concept of generated pharmacophore model. For further evolution of drug ability of these hit compounds, Lipinski's rule of five was used. These rules state that drug-like molecules should contain log p-value <5, molecular weight <500 Da, hydrogen bond acceptors <10 and hydrogen bond donors <5. A deviation from these rules results in poor permeation or absorption of the compounds[59]. Only 400 hits of protease inhibitors were able to pass the criteria of Lipinski's rule of five and were further analyzed.

Molecular docking: Algorithms of molecular docking technique as compared to pharmacophore modeling are more advanced, composite and computationally challenging. Thus, molecular docking has more potential to accurately predict binding affinities of screening hits as well as potentially elucidate lead structures with novel modes of binding[60]. Together with the pharmacohore modeling and virtual screening, it is believed that molecular docking has great promise in drug discovery. Hence, for further improvement of the hit inhibitors, all the retrieved compounds were docked into the binding site of DV NS2B/NS3 protease using the MOE docking tool to discover the binding pattern of small molecules against their targets. The best docked results of compounds were saved in a separate database. On the basis of docking score, top 100 compounds were taken for further assessment. The MOE LigPlot tool was used to visually observe the binding interactions between 100 inhibitor's hits and NS2B/NS3 protease. The molecules having vital interactions with most of the significant catalytic triad of DV NS2B/NS3 protease (His51, Asp75, Ser135) were selected as capable hits. From docked structures, 40 out of 100 hits showed essential interactions with the catalytic triad of target protein. These 40 hits were further subjected to binding energy, binding affinity calculation and ADMET profiling analysis.

Binding affinity calculations and ADMET profiling: To distinguish the most effective ligands, binding affinities for all the 40 retrieved compounds were measured with Generalized Born/Volume Integral (GB/VI) feature of MOE. However, before executing this step, energy minimization of binding pocket in NS2B/NS3 protease-ligand complex was done in each case. The binding affinity was reported in unit of kcal mol^{-1}. A selection criterion of hit compounds was defined and strictly followed. Only those hit compounds were selected that not only visualized in the binding pocket but also shown interactions with catalytic triad residues of DV NS2B/NS3 protease. Thirty out of 40 compounds met the above mentioned criteria. Moreover, we also used admetSAR server[34]

for the *in silico* screening of ADMET profiles of the potent lead compounds. The barrier is formed between endothelial cells of brain capillaries by the occurrence of high resistance tight junctions which avoids the brain uptake of nearly all pharmaceuticals[61]. Blood Brain Barrier (BBB) is measured as the ratio of the compound concentration in the brain to that in the blood. Information of the dissemination of drugs through BBB is one of the main factors to be optimized in drug discovery[62]. Oral bioavailability frequently considered as a significant factor to find out the drug likeness of active compounds as remedial agents[63]. In addition, oral drug bioavailability can be noticeably influenced by physiological, physiochemical and certain biopharmaceutical parameters[64]. High penetration of blood brain barrier is requisite for Central Nervous System (CNS) active drugs, while for non-CNS, low penetration is enviable to lessen CNS-related side effects. In many studies, dengue infection has been reported in patients due to involvement of CNS[65]. In a recent study, two cases involving oligosymptomatic dengue which caused meningitis, has been reported in the city of Kolkata, West Bengal, India[66]. Lipophilicity, hydrogen-bond desolvation potential, molecular size and pK_a/charge are the various parameters on which BBB permeability of compound depends[67,68]. The ADMET associated properties of the potential compounds for several types of models such as P-glycoprotein substrate, BBB penetration, human intestinal absorption, renal organic cation transporter and $CaCO_2$ permeability showed positive results which strongly supports the ability of compounds to work as drug candidates. Cytochrome P450 (CYP) is cluster of isozymes involved in the metabolism of drugs, steroids, fatty acids, bile acids and carcinogens. Fifty-seven CYP enzymes are encoded by human genome of which fifteen are involved in the xenobiotic chemicals and other metabolism of drugs[69]. Approximately, 75% of phase I drug metabolism depends on the association of CYP enzymes[70]. The selection criteria for ADMET profiling were: (a) Compounds must pass through blood brain barrier, can absorb in human intestine and $CaCO_2$ permeable, (b) Non inhibitor to CYP enzymes, (c) Non AMES toxic and (d) Non carcinogenic. Only six hit compounds were accepted through ADMET test. The details of finally selected compounds i.e., ZINC ID, docking score, RMSD value, binding affinity values, interacting residues of the DV NS2B/NS3 protease and Lipiniski's screening results are shown in Table 1. Chemical structures of the finally selected compounds are shown in Fig. 4. The pharmacophore mapping, binding mode, binding affinity and energy, visual prediction and ADMET results (Table 2) showed that these predicted potent compounds might act as novel effective and structurally diverse inhibitors against DV NS2B/NS3 protease.

Table 1: Docking score, Binding affinity calculations and Drug scan results of selected hits

ZINC ID	S-score	RMSD value	Binding affinity (kcal mol^{-1})	Residues Interacting with ligand	Lipinski's drug like screening
75163069	-12.63	1.80	-19.98	His51, Asp75, Ser135, Gly153, Gly151, Pro132, Val154 and Leu128	MW: 373 g/mol, LogP: -1.42, TPSA: 93.69, Don: 4, Acc: 6 and Violations: 0
59170698	-12.46	1.75	-18.26	His51, Asp75, Ser135, Lyc73, Gly153, Pro132 and Arg54	MW: 454 g/mol, LogP: 0.38, TPSA: 136, Don: 4, Acc: 6 and Violations: 0
06395655	-12.02	1.34	-20.08	His51, Asp75, Gly153, Gly151, Pro132 and Tyr161	MW: 488 g/mol, LogP: 2.17, TPSA: 88.74, Don: 4, Acc: 6 and Violations: 0
32933073	-11.63	1.34	-22.34	His51, Asp75, Ser135, Pro132, Gly153 and Lie36	MW: 405 g/mol, LogP: 4.81, TPSA: 99.68, Don: 4, Acc: 7 and Violations: 0
13728171	-11.43	1.01	-10.22	His51, Asp75, Tyr161, Gly153, Pro132, Lie36, Leu128 and Gly151	MW: 498 g/mol, LogP: 0.19, TPSA: 116.91, Don: 3, Acc: 10 and Violations: 0
65395833	-11.27	1.78	-19.89	His51, Asp75, Gly151, Ley128 and Gly153	MW: 312 g/mol, LogP: -0.06, TPSA: 47.87, Don: 3, Acc: 4 and Violations: 0
Reference ligand (Bz-Nle-Lys-Arg-Arg-H)	-10.28	2.98	-19.29	His75, Asp75, Ser135, Ser131, Gly153,Arg154 and Asn152	MW: 780.68 g/mol, LogP: 2.96, TPSA: 288.66, Don: 9, Acc: 16 and Violations: 4

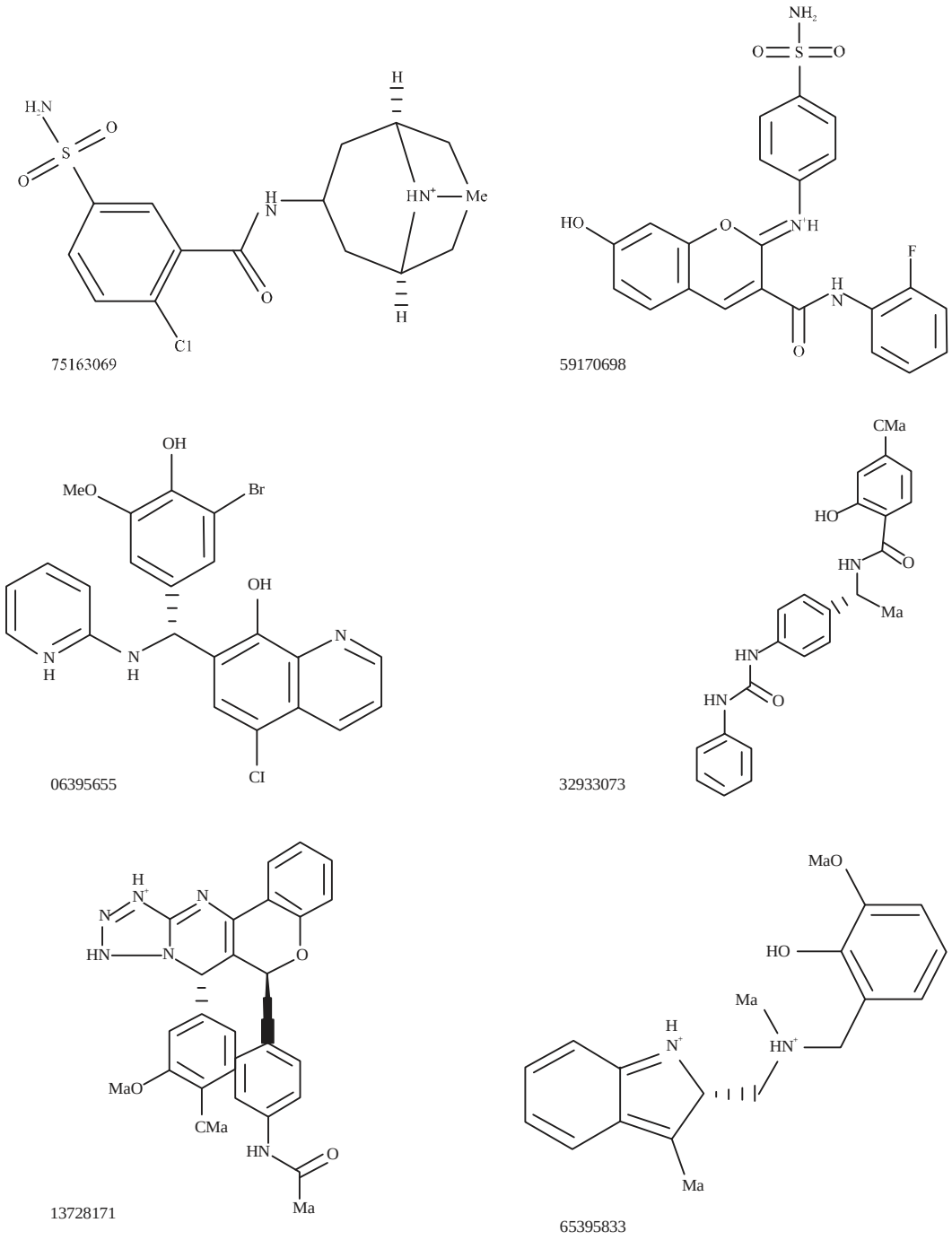

Fig. 4: 2D structures of finally retrieved hits from ZINC database. The numbers are representing their ZINC database IDs

Interaction analysis of finally selected hit compounds: The S-score is the mathematical method used to calculate the strength of interaction between receptor protein and ligand after they have been docked[27]. On the basis of S-score, binding energy and affinity, it is possible to conclude that these finally selected compounds have potential interaction with catalytic triad residues and fulfill the requirements to be drug-able. The compound having ZINC ID 75163069 was placed at the top as it had potential interactions with His51, Asp75, Ser135, Gly153, Gly151, Pro132, Val154 and Leu128 residues of binding pocket and bears best docking score. Docking conformations and pharmacophore mapping of top three selected compounds are given in Fig. 5.

Table 2: ADMET profiling results for selected hit compounds

ZINC ID	Blood-brain barrier	Human intestinal absorption	$CaCO_2$ permeability	P-glycoprotein inhibitor	Renal organic cation transporter
Absorption					
75163069	BBB+	HIA+	$CaCO_2$-	NI	T
59170698	BBB-	HIA-	$CaCO_2$-	NI	T
06395655	BBB+	HIA+	$CaCO_2$+	NI	T
32933073	BBB+	HIA+	$CaCO_2$-	NI	T
13728171	BBB-	HIA+	$CaCO_2$-	NI	T
65395833	BBB+	HIA+	$CaCO_2$-	NI	T
ZINC ID	CYP450 1A2 inhibitor	CYP450 2C9 inhibitor	CYP450 2D6 inhibitor	CYP450 2C19 inhibitor	CYP450 3A4 inhibitor
Metabolism					
75163069	NI	NI	NI	NI	NI
59170698	NI	NI	NI	NI	NI
06395655	I	NI	NI	I	I
32933073	NI	NI	NI	NI	NI
13728171	NI	I	NI	NI	I
65395833	NI	NI	NI	NI	NI
ZINC ID			AMES toxicity		Carcinogens
Toxicity					
75163069			Non AMES Toxic		Non carcinogenic
59170698			Non AMES Toxic		Non carcinogenic
06395655			Non AMES Toxic		Non carcinogenic
32933073			Non AMES Toxic		Non carcinogenic
13728171			Non AMES Toxic		Non carcinogenic
65395833			Non AMES Toxic		Non carcinogenic

I: Inhibitor, NI: Non inhibitor

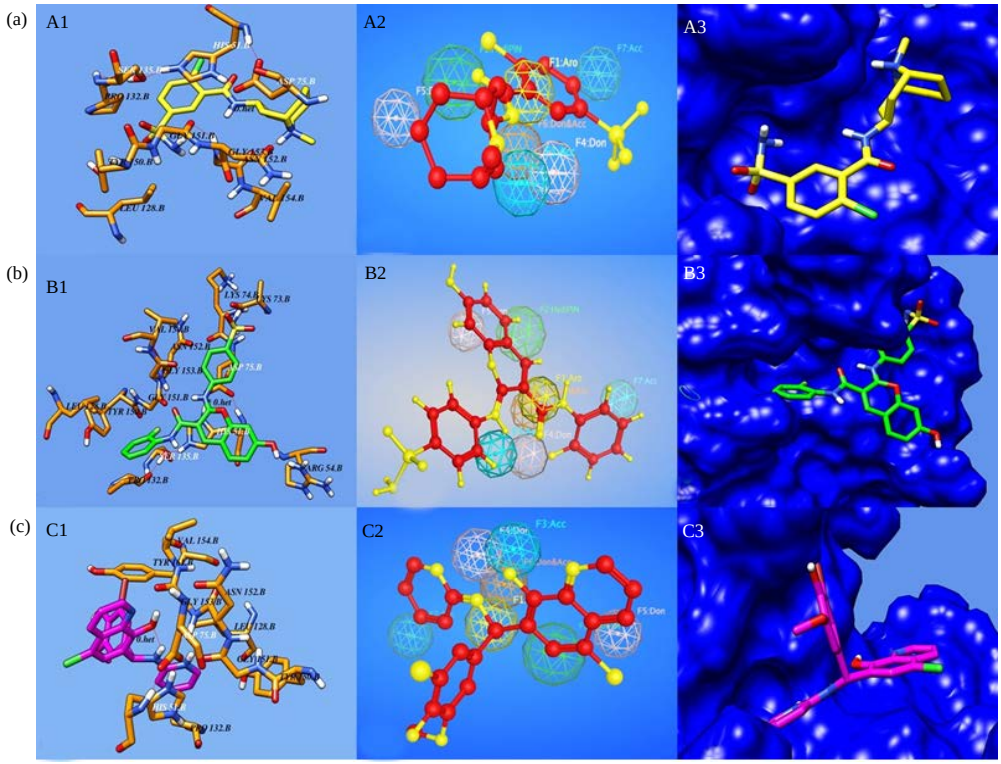

Fig. 5(a-c): Docking conformations and pharmacophore mapping of selected hits, (a) A1, B1 and C1 represent 3D interactions of selected compounds 75163069, 59170698 and 06395655, respectively with DV NS2B/NS3 protease, (b) A2, B2 and C2 represent pharmacophore mapping of selected compounds 75163069, 59170698 and 06395655, respectively, While (c) A3, B3 and C3 represent binding pocket mode of selected compounds 75163069, 59170698 and 06395655, respectively with DV NS2B/NS3 protease

CONCLUSION

The present study focuses on structure based pharmacophore modeling, computational screening and molecular docking of ZINC database compounds against Dengue Virus (DV) NS2B/NS3 protease. As an outcome of the study, six compounds from ZINC database (ZINC IDs: 75163069, 59170698, 06395655, 32933073, 13728171 and 65395833) have shown strong bindings with catalytic triad of DV NS2B/NS3 protease. This study also explores that these compounds can be utilized as potential and strong drug candidates against DV NS2B/NS3 protease on the basis of drug profiling. The findings will be useful as they provide insight for the effectiveness of drug before its manufacturing and testing on pilot scale in the pharmaceutical industry (for *in vivo* drug design and development). Hence, it can be concluded that in future these compounds can serve as a strong and potential drug leads against DV on the basis of significant binding affinity against DV NS2B/NS3 protease.

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