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Structure Based Virtual Screening and Molecular Docking Studies for Identification of Allosteric Inhibitors against Zika Virus Protease NS2B-NS3

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ABSTRACT

Zika virus (ZIKV) has gained research interests after its recent outbreak in Brazil and America in 2015, where it affected millions of people. Studies have shown its role in developing microcephaly and GBS Syndrome. There is a need to develop drugs against ZIKV. Here we uses pharmacoinformatics techniques to identify potential inhibitors against ZIKV protease NS2B-NS3, which has a well-known role in the viral replication. By using three dimensional structure of the NS2B-NS3, an allosteric site has been identified in the protein. Moreover, the identified site is used for structure-based virtual screening. After screening 100,000 compounds, 14 compounds are selected, which fulfilled different already established drug likeliness parameters such as rule of five and nontoxic nature. These 14 compounds are used for pharmacophore modeling, and Zinc natural compounds database (ZND) is screened against the developed pharmacophore. The compounds with the highest pharmacophore fit scores are screened and docked on the identified allosteric site of NS2B-NS3. Analysis of the data showed two compounds (ZINC00845171, ZINC08782519) as potential inhibitors for NS2B-NS3 of ZIKV.

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Authors' Contribution
FJ designed the study. MF, SK, HR, AM and SK performed the experiments and collected data. FJ and MAR analyzed the data. FJ gathered the data, supervised the study and prepared the manuscript.

Key words
Zika virus, NS2B-NS3, Protease, Inhibitors, Virtual screening, Docking.

INTRODUCTION

Zika virus (ZIKV) belongs to flavivirus genus. The other renowned members of the genus are: Dengue virus (DENV), West Nile virus, Japanese encephalitis virus and Yellow fever virus. ZIKV was isolated in 1947 from the serum of a monkey in Zika Forest (Dick *et al*., 1952). However, it emerged as a disease-causing agent in 2015. ZIKV was spread in Brazil in May 2015, and then it was rapidly spread throughout America (Campos *et al*., 2015). In 2016, 531,000 suspected cases of this virus were reported in different American territories. Moreover, a congenital syndrome has been associated with Zika virus (Ikejezie *et al*., 2017). The main vectors of this virus are *Aedes aegypti* mosquitoes, though other mosquito species can also contribute in its transmission (Colman *et al*., 2012). Additionally, several cases have been reported for sexual transfer of the virus (Ikejezie *et al*., 2017).

ZIKV genome is composed of, positive-sense, single-stranded RNA molecule of approximately 11 kb (Moghadam *et al*., 2016). Studies have shown that the viral genome is translated into a single polyprotein, which is cleaved into 10 mature proteins by cellular and viral proteases. Studies have shown that 7, out of these 10, proteins are non-structural and designated NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Remaining three are structural proteins including Capsid (C), Pre-membrane/ membrane (prM), and Envelope (E) proteins (Kuno and Chang, 2007; Baronti *et al*., 2014; Sironi *et al*., 2016).

ZIKV causes mild illness with symptoms fever, arthralgia, rash, conjunctivitis, muscle and joint pain (Tan *et al*., 2017). The current outbreak of ZIKV in Brazil and Latin America showed a positive correlation between viral infection and rise of microcephaly (Driggers *et al*., 2016). Moreover, studies have shown that ZIKV is also associated with neurological disorders such as Guillain-Barre syndrome (Nowakowski *et al*., 2016).

Studies have highlighted NS2B-NS3, a serine protease, as a key player in ZIKV replication (Mackenzie, 2005; Li *et al*., 2017). The crystal structure of NS2B-NS3 and substrate specificity profile has already been determined (Lei *et al*., 2016; Gruba *et al*., 2016). Analysis showed that the binding pocket of NS2B-NS3 protease is highly hydrophobic like the other similar proteases in other flavivirus (Yildiz *et al*., 2013). A recent study has targeted an allosteric site of NS2B-NS3 complex and they have identified certain compounds for the inhibition

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(hfNPS) of the protease (Shiryaev *et al*., 2017). However, the identified compounds are highly water soluble and host unfavorable pharmacokinetic parameters. The authors have suggested further study to identify inhibitors with favorable pharmacokinetic parameters (Shiryaev *et al*., 2017). In this study, we have screened 100,000 compounds for the inhibitors of NS2B-NS3, perform virtual screening against the allosteric site and determine naturally occurring compounds as inhibitor of the protease. It is a step toward developing a potential drug against ZIKV.

METHODOLOGY

Allosteric site identification

The allosteric site of DENV NS2B-NS3 protease (PDB: 2FOM) has already been reported (Mukhametov *et al*., 2014). By superposing ZIKV NS2B-NS3 (5GXJ) on the DENV NS2B-NS3 protease (PDB: 2FOM), we determined the allosteric site in ZIKV NS2B-NS3. Both protein structures are superimposed by using PyMol software (Lill and Danielson, 2011).

Protein preparation for docking

The NS2B-NS3 protein structure (PDB: 5GXJ) was retrieved from Protein Data Bank (https://www.rcsb.org). The chain B and water molecules were deleted using Chimera software for docking analysis. By using AutoDock Vina polar hydrogen and Kollman charges added in the structure (Trott and Olson, 2010). A grid with dimensions 100×100×100 Å with center x = -0.254, y = 11.832 and z = -24.015 was used for targeted docking in the allosteric site of NS2B-NS3.

Virtual screening

Mcule was used to perform the structure-based virtual screening (SBVS) (Kiss *et al*., 2012). It uses a built-in AutoDock Vina tool to perform docking of small ligands against target site. Each ligand of the selected database is docked against the selected allosteric pocket. Ligands were scored according to the minimum binding energy.

Drug-like properties determination of the selected compounds

The best compounds were checked by OSIRIS property explorer (Actelion Pharmaceuticals Ltd., Allschwil, Switzerland) for drug-like properties. Molecular weight, LogP, hydrogen bond donor, hydrogen bond acceptor and polar surface area were determined. Those compounds that fulfill rule of five (Ro5) criteria were further filtered by checking the compound toxicity using Mcule lead optimization tool toxicity checker (Kiss *et al*., 2012). AdmetSar was used to check the ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) properties (Cheng *et al*., 2012), such as blood brain barrier, human intestinal absorption, caco-2 permeability, honey bee toxicity, fish toxicity *etc*.

Pharmacophore modeling

Ligands based pharmacophore modeling performed by Ligand scout (Wolber and Langer, 2005). Ligand scout is a tool that generates three dimensional pharmacophore models from structural data of macromolecules ligands complexes. Zinc Natural Derivatives (ZND) database downloaded from Zinc specialized databases. Each compound of the database is screened against the generated pharmacophore and scored according to matching features.

Compound interactions and toxicity

The compounds with highest pharmacophore fit score were further screened through their different physical properties such as Ro5, toxicity and ADMET parameters. The filtered compounds were docked to the allosteric sites of NS2B-NS3, and their interactions with the allosteric sites residues were checked by using LIGPLOT (Wallace *et al*., 1995).

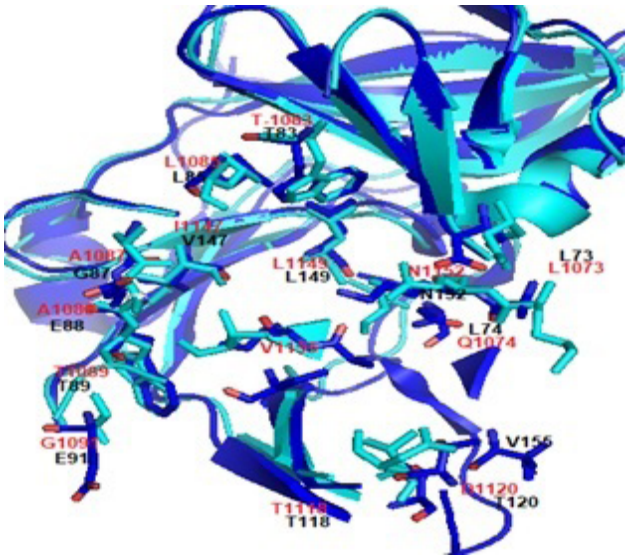

Fig. 1. Superimpose NS2B-NS3 of DENV (blue) on NS2B-NS3 of ZIKV (cyan). It also shows allosteric site residues of DENV (red) and ZIKV (black).

RESULTS AND DISCUSSION

In the post genome era, bioinformatics has emerged a valuable tool for large data analysis. Several applications of different bioinformatics tools have been reported ranging from gene prediction to drug identification (Mukhametov *et al*., 2014; Tariq *et al*., 2016; Rana *et al*., 2017; Jamil *et*

al., 2018). In this study we focus to identify drug against Zika virus which is highly linked with several neurological disorders.

Allosteric site analysis

The allosteric sites of ZIKV NS2B-NS3 comprise 15 residues: Asp71, Lys73, Gln74, Trp83, Leu85, Ala87, Ala88, Trp89, Gly91, Thr118, Asp120, Ile147, Leu149, Asn152 and Val155. These residues are located at topological positions to the residues of allosteric site in DENV NS2B-NS3 (Mukhametov *et al*., 2014). This site comprises 3 polar (Gln74, Thr118, Asn152), 9 nonpolar (Trp83, Leu85, Ala87, Ala88, Trp89, Gly91, Ile147, Leu149, Val155), 1 basic (Lys73) and 2 acidic residues (Asp71, Asp120) residues (Fig. 1). Analysis of the site suggested that it will hinder the interactions of NS3 with its cofactor NS2B, leading the inhibition of protease as reported by Shiryaev *et al*. (2017). Therefore, the site is used as a drug target site in this study.

Structure-based virtual screening

100,000 compounds from Mcule were docked on the allosteric site of the NS2B-NS3. Top 100 compounds were selected based on their lowest binding energy (< -9 kcal/mol) (Table I). We analyzed different features of these selected 100 compounds, such as toxicity and Ro5 as explained by Lipinski *et al*. (2001). According to Lipinski *et al*. (2001), a good permeable drug must have these five features. First, the molecular weight of drug preferably be less than 500 g/mol; second, hydrogen bond donor (HBD) moieties in the drug be less than 5; third, hydrogen bond acceptor (HBA) be less than 10; fourth, its polar surface area (PSA) be less than 140 Å and last is logarithm of partition coefficient between n-octanol and water, $\log(c_{octanol}/c_{water})$ (cLogP) must be less than five. In addition to Ro5, we determined toxicity and interactions of these compounds with NS2B-NS3. Only 14 compounds, out of the 100 selected compounds, passed Ro5 and toxicity test so they were taken for further analysis. Table I summarizes the feature of these compounds and their structures are shown in Figure 2.

Analysis of the data showed that M1 has lowest binding energy (-9 kcal/mol), suggesting its strongest interactions with the allosteric site as compared to the other 13 molecules (Table I). Such interactions are facilitated by its 6 HBA and 1 HBD. Additionally, the cLogP and relative PSA of M1 falls in the allowed reign. On the other hand, M14 pose the maximum binding energy in these 14 molecules (-7.8 kcal/mol). It host 8 HBA whereas HBA are absent in the structure.

Table I.- Features of the 14 inhibitors of the NS2B-NS3.

No	Molecules	Binding energy (kcal/mol)	Molecular weight (g/mol)	cLogP	HBA	HBD	Relative PSA
1	M1	-9	427.55	3	6	1	0.17071
2	M2	-8.6	404.322	4.3022	6	0	0.24966
3	M3	-8.5	446.958	2.7846	7	1	0.22571
4	M4	-8.4	375.349	2.1211	5	0	0.15715
5	M5	-8.2	326.423	1.5393	5	0	0.29039
6	M6	-8.1	396.493	1.7641	8	1	0.23992
7	M7	-8.1	310.4	2.1138	5	2	0.23924
8	M8	-8.1	441.811	3.8176	5	1	0.17553
9	M9	-8	449.477	3.0763	7	0	0.21256
10	M10	-7.9	333.346	3.0529	6	1	0.27512
11	M11	-7.9	410.476	1.5397	9	0	0.26448
12	M12	-7.9	384.459	3.2613	7	2	0.32795
13	M13	-7.9	390.502	1.5855	6	2	0.25924
14	M14	-7.8	355.328	1.6942	8	0	0.32856

The compounds respective molecule ID's are: M1, MCULE-6483787945-0-3; M2, MCULE-2598332550-0-1; M3, MCULE-6717908673-0-1; M4, MCULE-4017669026-0-1; M-5, MCULE-4088032230-0-2; M-6, MCULE-8969381945-0-1; M7, MCULE-8631834817-0-1; M8, MCULE-3248415882-0-2; M9, MCULE-3498445429-0-1; M10, MCULE-3686500192-0-1; M11, MCULE-1165852831-0-1; M12, MCULE-7095580128-0-1; M13, MCULE-7204888140-0-1; M14, MCULE-2760593673-0-1.

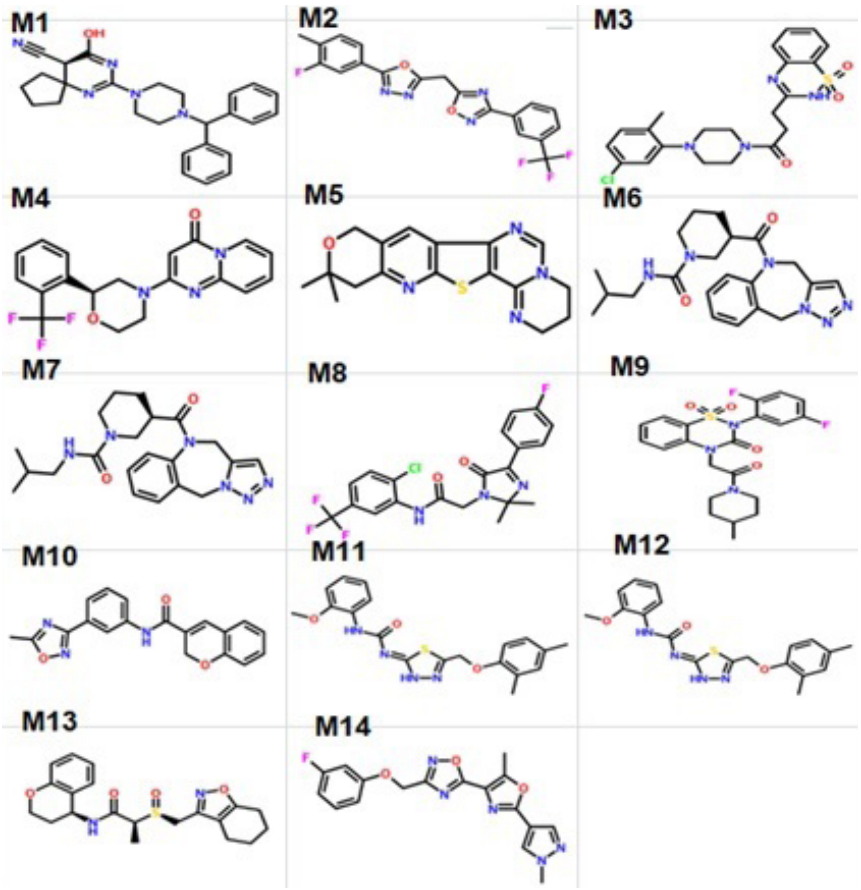

Fig. 2. Structure of selected 14 compounds.

Table II.- Features of 3 pharmacophores.

Drug ID	Fit score	Acceptors	Binding energy (kcal/mol)	Donor	Weight	Log P	TPSA	Toxicity
ZINC00845171	53.33	7	-8.9	0	452.44	3.29	59.73	Nontoxic
ZINC08782519	52.88	6	-8	2	413.4	2.93	75.08	Nontoxic
ZINC15674546	52.65	7	-8.3	2	432.49	1.61	96.53	Nontoxic

Pharmacophore

A pharmacophore is generated by merging the chemical features of the above 14 compounds (Table I; Fig. 2). This pharmacophore host five hydrogen bond acceptors and two hydrophobic rings (Supplementary Fig. S1).

Pharmacophore screening results

The pharmacophore is used to screen 30,793 natural compounds from ZND, and 10 compounds with the maximum pharmacophore fit score (lowest 52.65) has been selected (Table II). A fit score shows the extent of similarity of one compound with the given pharmacophore. Only 3 compounds, out of these 10 compounds, passed the toxicity test. Physical features of these three compounds showed their potential to be a valuable inhibitor for the allosteric site of NS2B-NS3. For example, their HBA, HBD, logP and the topological polar surface area are within the allowed reign. These values are summarized in Table II. Moreover we determine the total no of hydrogen bond between these three ligands and the allosteric site (NS2B-NS3) residues (Fig. 3). Analysis of the structures showed that ZINC08782519, ZINC00845171 and ZINC15674546 forms 3, 1 and 1 hydrogen bonds, respectively, with different residues of allosteric site (Fig. 3). These interactions play a vital role in binding affinity of a ligand.

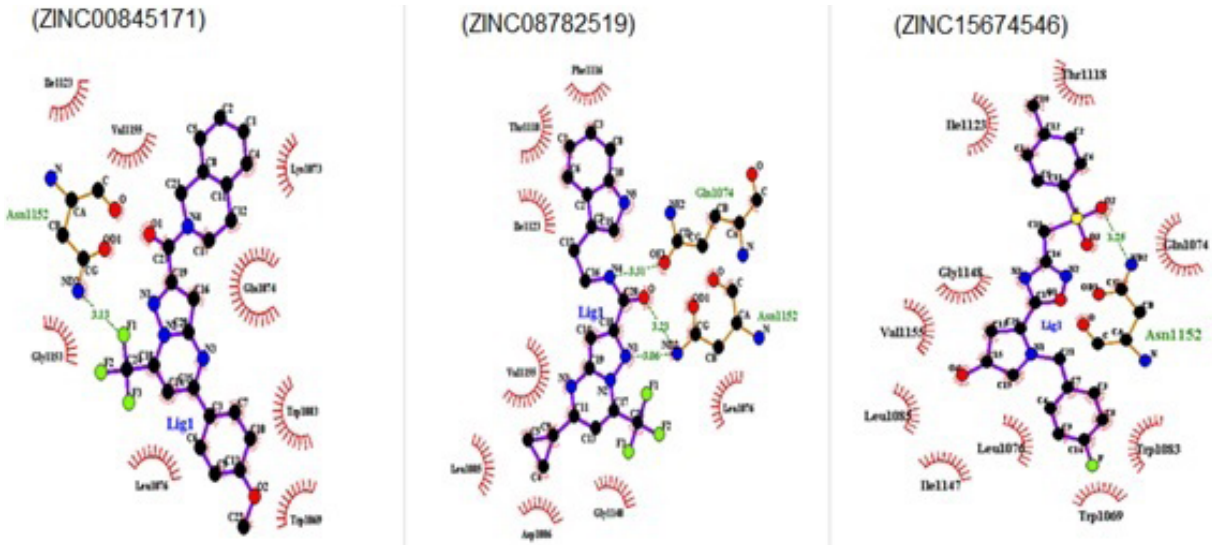

Fig. 3. Hydrogen bonding between ligands and NS2B-NS3 allosteric site residues.

ADMET properties of the novel compounds

For an orally administrated drug, it should fulfill the ADMET parameters. If the properties are weak, the candidate will have a high risk of failure. One of the compound, ZINC15674546, is eliminated from the study because it does not follow the ADMET parameters. The ADMET properties of the two novel compounds are in Table III.

Table III.- ADMET properties of two novel compounds.

Properties	ZINC00845171	ZINC08782519
Blood-brain barrior (BBB+)	0.9834	0.9376
Human intestinal absorption (HIA-)	1	1
Caco2 permeability	0.5	0.6856
CYP450 2D6 inhibitor (non-substrate)	0.571	0.7596
AMES toxicity	NT	NT
Carcinogens	NC	NC
Acute oral toxicity (III)	0.5863	0.5475
Aquous solubility (LogS)	-3.3133	-3.4356
Rat acute toxicity (LD50, mol/kg)	2.8742	2.7787

Analysis of their ADMET properties showed that both the compounds are nontoxic and noncarcinogenic as established from AEMS and carcinogenicity test (Table III). Moreover, we determined Rat Acute Toxicity LD50 for both the compounds. Another interesting feature of these compounds is optimal putative blood brain barrier value (BBB: >0.93), which is essential for penetration through the CNS. On the other hand, analysis of these structures showed these compounds are non-substrate for CYP450 2D6, suggesting a moderate metabolism rate of these compounds. Most importantly, both the compounds are water soluble as evident from their logS and both compounds have logS value (>-3.40).

CONCLUSION

In silico screening against the allosteric pocket of ZIKV protease NS2B/NS3 protease led us to identify new inhibitors for the protease. Fourteen compounds have been selected after a structure-based virtual screening of 100,000 compounds, and a pharmacophore is developed. By screening available natural compounds in the ZND database against pharmcophore, top 10 compounds were selected based on their fit scores and drug properties determined. Analysis showed two (ZINC00845171 and ZINC08782519) compounds as potential inhibitors for ZIKV NS2B/NS3 protease and lead compounds against ZIKV.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.5.1709.1715

Statement of conflict of interest

Authors have declared no conflict of interest.

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