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ORIGINAL ARTICLE

In silico targeting of non-structural 4B protein from dengue virus 4 with spiropyrazolopyridone: study of molecular dynamics simulation, ADMET and virtual screening

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Abstract Dengue fever is one of the most prevalent disease in tropical and sub-tropical regions of the world. According to the World Health Organisation (WHO), approximately 3.5 billion people have been affected with dengue fever. Four serotypes of dengue virus (DENV) i.e. DENV1, DENV2, DENV3 and DENV4 have up to 65% genetic variations among themselves. dengue virus 4 (DENV4) was first reported from Amazonas, Brazil and is spreading perilously due to lack of awareness of preventive measures, as it is the least targeted serotype. In this study, non-structural protein 4B of dengue virus 4 (DENV4-NS4B) is computationally characterised and simulations are performed including solvation, energy minimizations and neutralisation for the refinement of predicted model of the protein. The spiropyrazolopyridone is considered as an effective drug against NS4B of DENV2, therefore, a total of 91 different analogues of spiropyrazolopyridone are used to analyse their inhibitory action against DENV4-NS4B. These compounds are docked at the binding site with various binding affinities, representing their efficacy to block the binding pocket of the protein. Pharmacological and pharmacokinetic assessment performed on these inhibitors shows that these are suitable candidates to be used as a drug against the dengue fever. Among all these 91 compounds, Analogue-I and Analogue-II are analysed to be the most effective inhibitor having potential to be used as drugs against dengue virus.

Keywords Dengue virus 4 · Non-structural protein 4B · DENV4-NS4B · Molecular dynamics simulation · Spiropyrazolopyridone · ADMET · Virtual screening

Introduction

Dengue fever is a viral disease which has approximately 50% mortality rate when it is not treated properly [10]. Dengue fever is caused by Dengue virus which belongs to the family *Flaviviridae*, genus *Flavivirus*. Dengue virus (DENV) mostly prevails in tropical and sub-tropical regions of the world. These areas have sustainable places and favourable eco-epidemiological conditions for the reproduction, growth and development of the dengue viruses [8]. Four serotypes of the dengue virus have been reported, namely DENV1, DENV2, DENV3 and DENV4. These four serotypes exhibit homology in their structural proteins, however, they can be differentiated based on their interactions with the antibodies in the human serum. The four serotypes share a similarity of almost 65% in the genome while within each single serotype, there are genetic variations in the polypeptide. Dengue virus 4 (DENV4) was first reported in Amazonas, Brazil and is

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perilously spreading due to the lack of awareness of preventive measures. According to the World Health Organisation (WHO), large number of people have been affected by the dengue virus [10]. DENV4 was not reported for almost 3 decades and has reappeared in 2010 in Roraima, Northern Brazil [36].

The genome of the dengue virus comprises of a single strand RNA, also referred as positive sense RNA because it can be directly translated into proteins. This strand is translated into a single long polypeptide which is then truncated into ten different proteins. Among these ten proteins, there are three structural proteins namely Capsid (C), Envelope (E) and Membrane (M) [20]. The structure of the dengue is spherical having nucleocapsid at the core, which is basically comprised of the C proteins. E and M proteins encapsulate the nucleocapsid, therefore, the entry of the virus into a human cell is controlled by E and M proteins. The remaining seven candidates are non-structural (NS) proteins, named as NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. These seven proteins are responsible for viral replication and assembly of virus in the host cells. The replication of dengue virus is initiated by the attachment of the virus to the skin of the host cell. This attachment leads to the folding of the membrane of skin cells, forming a pouch, which seals around the particle of the virus, named as an endosome [41]. Non-structural proteins NS2A, NS2B and NS4B are the trans-membrane proteins which play important role in the replication process. It has been reported that these proteins contribute to the inhibition of the interferon-α/β (IFN-α/β) response [23].

Non-structural protein 4B (NS4B) is a small protein (27 kDa) having 40% similarity among all 4 dengue serotypes and other *flaviviruses* like Yellow Fever virus (YFV) and Tick-borne encephalitis virus (TBEV). NS4B protein is produced by the cleavage of single long polypeptide with the aid of enzyme signalase and serine protease (NS2B/NS3) on N-terminal and C-terminal, respectively [27]. NS4B from dengue virus 4 (DENV4-NS4B) is a non-structural protein which is highly hydrophobic. It is the least targeted protein for drug discovery as compared to other proteins of DENV. It associates with the lumen side membrane of the Endoplasmic Reticulum. N-terminal region of the protein (1–93 residues) is hydrophobic containing three transmembrane domains (TMDs) i.e. TMD-3, TMD-4, and TMD-5. TMD-3 extends through the lumen side membrane of the Endoplasmic Reticulum to cytoplasmic side, while TMD-4 creates distance from cytoplasmic to lumen side. TMD-5 helps in C-terminal cleavage as it spans the membrane from the cytoplasmic side and returns to the lumen of Endoplasmic Reticulum [22]. It works efficiently for phosphorylation of STATI blocking. NS4A and NS4B work in coordination with each other during viral replication and anti-host response [34]. In uncleaved polypeptide of the virus, DENV4-NS4B is situated between the NS4A and NS5. It is not only important for maintaining the NS5 protein in active form, but it is also shares a common 2K-peptide with NS4A [23, 45]. Both NS4A and NS4B proteins in all dengue viruses are highly hydrophobic transmembrane proteins and are known to be responsible for the membrane arrangements leading to the formation of the viral replication complex [25].

The in silico approach for drug discovery helps in finding drugs for diseases by docking mechanism instead of performing the expensive and laborious experimental work. Around 40% of drugs which have been discovered through in silico molecular docking approach fail in clinical trials as the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties are not analysed in these approaches. These failures can lead towards a costly and time consuming procedure for screening and finding new drugs. Accurate prediction of ADMET properties can help in overcoming these failures and can be inexpensive. In a previous study, NS4B from all the four serotypes of DENV4 has been targeted using some bioactive compounds i.e. Catechin, Cianidanol, Epicatechin, Eupatoretin, Glabranin, Laurifolin and DL-Catechin. The binding free energies of the compounds were relatively low for all the four proteins as the maximum binding affinity was − 5.87 kcal/mol. Such low binding energy reflects less inhibitory potential of these compounds against NS4B [26].

The spiropyrazolopyridone is considered as an effective and potent drug against dengue virus, and has been analysed both in vitro and in vivo in various studies against NS4B protein [18, 39, 43, 46]. The in silico targeting of DENV4-NS4B with the spiropyrazolopyridone and its analogue compounds is targeted in this study as it is the least targeted protein for drug discovery as compared to other proteins of DENV. The structure of DENV4-NS4B is computationally characterised and simulations are performed to analyse the structural stability. Four different racemates of spiropyrazolopyridone reported by Zou et al. are evaluated in this study against the DENV4-NS4B. Among these four racemates, Drug-III has shown maximum binding energy and showed effective drug like properties to be used as candidate for drug development. A total of 102 analogue compounds of the most effective and efficient drug, i.e. Drug-III, has been searched out using PubChem compound search and docked against DENV4-NS4B. The ADMET of all these analogues has been calculated for their efficient use in the control of the spread of disease caused by dengue virus 4.

Materials and methods

The present study targets the DENV4-NS4B protein. The method initiates by modelling of the protein and terminates at providing screened compounds, effective and potent against DENV4-NS4B.

Homology modelling

Due to unavailability of the tertiary structure, the homology modelling of DENV4-NS4B was performed using Modeller 9.17 [40]. DENV4-NS4B sequence was retrieved from UniProt using Accession ID: Q6YGE2. The modelling of the structure required template structures similar to DENV4-NS4B and these structures were searched with the help of protein BLAST [14]. The X-ray crystallographic structures were chosen as the template which had the maximum similarity with the DENV4-NS4B. Thus tertiary structure of DENV4-NS4B was predicted using 6 templates of DENV3-NS5 MTase (Protein Databank Identifiers: 5E9Q, 4CTJ, 3P8Z, 2XBM, 5CCV AND 5IKM) [15]. The binding sites were predicted using MetaPocket Server [12]. For refinement of model, energy minimization and geometry optimization was performed in Discovery Studio 2.5.

Posttranslational modifications

Posttranslational modification (PTM) of a protein is used for covalent modification of the protein by non-protein molecule. PTM is the process which is used as a post-process of biosynthesis when a genome is transformed into a proteome. In silico PTM of DENV4-NS4B highlighted phosphorylation, glycosylation, ubiquitination, methylation, acetylation, and palmitoylation sites. Phosphorylation was predicted using NetPhos and KinasePhos while remaining modifications were predicted using BioCUCKOO server by the CUCKOO workgroup. Glycosylation and Phosphorylation synchronisation was performed using Ying O Yang server [9].

Molecular dynamics

Constant temperature MD simulations were performed to study the thermodynamic and structure dynamic characteristics of the DENV4-NS4B using Groningen Machine for Chemical Simulations (GROMACS) v 5.0 [1]. Initially, the DENV4-NS4B was subjected to all atom force field for the optimized potential for liquid simulation (OPLS-AA). The setup of the whole simulations for DENV4-NS4B was set as a cubic box while DENV4-NS4B was kept exactly in the centre of that box with 1.0 Å distance from the cube walls. Continuing the process, the protein was solvated in spc216 water molecules. After the solvation, the protein was subjected to neutralisation with the addition of 8 counter ions of $Na+$ and Cl^- whereas verlet cut off scheme was used at this phase, and energy minimization (EM) simulation of whole system was performed using the steepest descent algorithm with 50,000 maximum number of steps [28].

Constant number volume and temperature (NVT), and constant number pressure and temperature (NPT) were performed under 1 atm atmospheric pressure and three different temperatures i.e. 300, 350, and 400 K. The duration for both the equilibrations was 100 ps whereas the force field used in both equilibrations was particle mesh Ewald (PME) with a cubic interpolation implementation and re-adjustment of hydrogen bonds was performed with the help of linear constraint solver (LINCS) technique [5, 11].

The final Production MD simulation was performed for 1 ns and analysis of the results was performed using *rms* and *gyrate* utilities of the GROMACS. On the basis of RMSD, the graph was plotted using Graphing Advanced Computation and Exploration (GRACE) of data. The molecular dynamics simulations were visualised using Visual Molecular Dynamics (VMD) [13, 38].

In silico evaluation of four racemates of spiropyrazolopyridone

The evaluation of the four racemates of spiropyrazolopyridone was performed on the basis of molecular docking and the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of these four racemates. These four racemates were selected out of the 26 different racemates of spiropyrazolopyridone from the study reported by Zou et al. and were observed to be effective in both in vitro and in vivo analysis [46]. For this purpose, four racemates from spiropyrazolopyridone class were used. The four racemates were docked against DENV4-NS4B and the binding energies were estimated to analyse the inhibition role of spiropyrazolopyridone class inhibitors against DENV4-NS4B.

The ligand and protein preparation was performed in AutoDock Tools while docking was performed using AutoDock Vina [24, 37]. Initially, polar hydrogen bonds were added in DENV4-NS4B. The polar charges helped to enhance the process of interactions. Later on, a three-dimensional grid was designed for DENV4-NS4B, focusing the binding pocket, and the interactions of the all compounds were analysed along with the estimation of binding energies using Autodock Vina [29]. The focused docking approach is reported to have higher accuracy as compared to the blind docking approach [7].

After docking, the ADMET properties of this four racemates were determined using PreADMET webserver [16]. The program also helped in predicting the drug-likeness of these compounds. The predictions were made using the molecular structure file. Physically significant descriptors such as the molecular weight, estimated solubility (ESOL), lipophilicity (logP), and the number of donor–acceptor hydrogen bonds were also analysed. Moreover, pharmaceutically relevant properties i.e. blood brain barrier (BBB) penetration and gastro intestinal absorption associated with the ligand molecules were calculated [4].

Docking of analogues of Drug-III and their ADMET

Both in vitro study [46] and the present in silico evaluation determined that the Drug-III is the most effective drug among all four racemates. On the basis of these results, analogue compounds of the Drug-III were searched using PubChem compound search. A total of 102 compounds were found to be analogue with Drug-III. All these compounds were docked with DENV4-NS4B while the docking protocol used was same as mentioned previously.

After docking, ADMET and drug likeness properties of these 102 compounds were analysed using the PreADMET webserver [16]. For determination of most effective drug, further screening of these compounds was performed on the basis of pharmacological properties i.e. solubility, Lipinski's rule of five, BBB penetration and the gastrointestinal absorption. Constant temperature MD simulations were performed to study the thermodynamic and structure dynamic characteristics of DENV4-NS4B and analogue compounds complex. This helped in analysing the stability of receptor-ligand binding.

Results and discussion

Homology modelling

As mentioned in methodology section, no protein model of NS4B from any serotype was available which could have been used as template for homology modelling of DENV4-NS4B. On performing protein BLAST, DENV4-NS4B has shown its maximum similarity (74%) with methyl transferase domain of DENV3-NS5. The model was built on the basis of 6 templates retrieved from Protein Data Bank demonstrating higher similarities with DENV4-NS4B. The prime major reason for this similarity is that few residues of C-terminus of NS4B and N-terminus of NS5 are the same. Moreover, the rest of the residues of both the proteins also show great similarity. The model of DENV4-NS4B was comprised of 6 α-helices and 4 β-sheets. It was identified that the binding pocket is composed of Gly_{35}, Ile_{36}, Glu_{51}, Thr_{93}, Pro_{95}, Ala_{128}, Phe_{132}, Leu_{134}, and Ile_{135} residues (Fig. 1). The overall environment of the binding pocket is hydrophobic which facilitates the binding of non-polar compounds. In DENV4-NS4B, three transmembrane 3 α-helices were found on C-terminal which helps in the anchorage of the protein in the lumen of endoplasmic reticulum and it has been previously reported [22].

Posttranslational modifications

A total of 41 modifications were predicted in the DENV4-NS4B at different residues (Table S1). The highlighted residues i.e. Leu_{30}, Thr_{32}, and Leu_{58} represent the residues which are involved in ligand interactions at binding site of the protein. Methylation was predicted on Leu_{30} along with seven other arginine residues and phosphorylation was predicted on Thr_{32}. Two modifications i.e. sumoylation and ubiquitination were predicted on Leu_{58}. These three modifications, occurring at binding pocket residues, can alter the mechanism of interaction of drug. Sumoylation and ubiquitination are small modifications in proteins however, addition of methyl group at Leucine can lead to reduction of interactions [17].

NetPhos and KinasePhos, both different predictors for phosphorylation, were used to predict phosphorylation sites on the exterior of the protein where NetPhos has almost 20 kinases while KinasePhos has 7–8 kinases [3, 42]. Phosphorylation was predicted on twenty-three various serine, threonine, and tyrosine residues by NetPhos while only three residues were predicted to be phosphorylated by KinasePhos. The predicted glycosylation sites were Thr_{140}, Thr_{145}, Thr_{147}, Thr_{148} and Thr_{151} of DENV4-NS4B. Palmitoylation was predicted on Cys_{72} whereas

Fig. 1 Predicted model of dengue virus 4—non-structural protein 4B (DENV4-NS4B). Red represents helices, Purple is for strands and white represents random coil

Ubiquitination was also predicted on ten lysine residues. No acetylated residues were predicted in DENV4-NS4B. Sumoylation was predicted on 8 lysine residues which is a reversible posttranslational modification process that actually monitors a wide range of cellular processes for numerous viruses during infection. It is a key regulator of the DENV-2 life cycle. It is also known to be involved in the regulation of replication of dengue viruses host cells [33].

Molecular dynamics simulation and the effect of temperature

Molecular dynamics simulations study of the DENV4-NS4B was performed to determine the structural dynamics and the thermodynamic properties. Quality of the predicted model was analysed through this approach. Dynamics simulations were performed at three different temperatures i.e. 300, 350 and 400 K. After these three simulations at various temperatures, the models were compared with the predicted model using the technique of Template Match Score (TM-Score) by superimposing the structures. The TM-Scores were observed as 0.67, 0.49 and 0.5 for the simulation performed at the 300, 350 and 400 K, respectively. Root mean square deviation (RMSD) observed was 1.8, 2.0 and 2.3 Å for 300, 350 and 400 K, respectively. The instability in RMSD graph, under the high-temperature constraints, is due to the instability of this protein at high temperatures (Fig. 2). The radius of gyration (Rg) helps in determining the compactness of the structure. The instability in gyration was due to the mesophilic nature of this protein and unsteadiness of Rg for DENV4-NS4B represents that the predicted model is not rigid hence showing flexibility which is essential for its proper function inside the host cell (Fig. 2). Enzymes are dynamic molecules so that these can work according to the induced fit theory of enzyme actions. Same results have been reported for DENV3-NS5, homologue of DENV4-NS4B, when this molecule was subjected to molecular dynamics simulations [44].

In silico evaluation of four racemates of spiropyrazolopyridone

The docking of four racemates of spiropyrazolopyridone i.e. Drug-I ($C_{20}H_{16}Cl_2N_4O_2$), Drug-II ($C_{21}H_{14}ClF_3N_4O_2$), Drug-III ($C_{19}H_{13}Cl_2N_5O_2$) and Drug-IV ($C_{21}H_{16}Cl_2N_4O_2$) was performed with DENV4-NS4B. These compounds are basically the R-enantiomers of the spiropyrazolopyridone. Different residues from binding pocket were involved in making interactions with these four compounds (Figs. 3, 4, Table 1). The Drug-I formed bonds with the Lys_{30}, $Glu_{51}$and Leu_{63}, having bond lengths of 4.87, 3.62 and 2.10 Å respectively. The binding affinity for docking of Drug-I with DENV4-NS4B was − 8.0 kcal/mol. The Drug-II formed a bond with the Gly_{35} with the bond length of 3.53 Å, while six more bonds formed with Ile_{36}, Asn_{39}, Ala_{92}, Phe_{132}, Asn_{137}, and Gln_{139} with the bond lengths of 3.53, 2.36, 4.57, 4.84, 2.63 and 2.96 Å respectively. The binding affinity for docking of Drug-II with DENV4-NS4B was − 9.7 kcal/mol. The Drug-III interacted with the Thr_{32}, Glu_{51}, Lys_{58}, Phe_{59}, Glu_{60} and Ala_{92} residues with the bond lengths of 2.06, 3.45, 4.13, 2.29, 1.93 and 3.71 Å respectively. Drug-III had the highest binding affinity with DENV4-NS4B which was − 10.1 kcal/mol. The Drug-IV formed bonds with Gly_{35}, Ile_{36}, Thr_{93}, Pro_{95}, Ala_{128}, Phe_{132}, Leu_{134}, and Ile_{135} with the bond lengths of 3.67, 4.77, 2.55, 4.64, 5.13, 4.94, 4.92 and 5.01 Å respectively. The binding affinity was calculated − 8.2 kcal/mol for Drug-IV with DENV4-NS4B. The docking with Drug-III has been observed to be best among all the inhibitors, in terms of binding affinity at the binding site. These results are in agreement with those reported by Zou et al. They reported that Drug-III was effectively bound with DENV2-NS4B, having good in vivo mouse efficacy when it was administrated orally at 50 mg/kg twice daily (bid) for 3 days, compared to vehicle control group, significant viremia reduction (about 1.9 log, P value < 0.05) was achieved. Similar efficacy was observed when Drug-III was orally dosed at 100 mg/kg once daily (QD) for 3 days. [46].

While determining the ADEMT properties of the four enantiomers, Drug-III has demonstrated high suitability among all four compounds based upon the ADMET and drug likeness properties (Table 2). Drug III was observed to have less values of Blood Brain Barrier permeability (0.0476391) and Skin permeability (− 4.43325) while the solubility of Drug-III was highest among all four drugs (6.31412 mg/L). The results were in accordance with the in vivo study on Drug III which demonstrated that Drug III was effectively used against dengue virus 4 in mouse [46].

The Drug-likeness study of all the four inhibitors was performed on the basis of multiple factors such as CMC like rules, Lead like rules, MDDR like rules, Lipinski's Rule of Five and WDI like rules(Table 2). The first column in Table 2 represents the parameters considered in ADMET study. Due to higher molecular weight, the compounds violated the Lead like rule however, these compounds fell within permissible limits for remaining parameters. It has been reported that the compound which usually obeys these rules can be used as a therapeutic agent in humans [6]. In ADME study, the values of Plasma Protein Binding, Blood–Brain Barrier Penetration, Skin Permeability, Human Intestinal Absorption, and $CaCO_2$ cell permeability were determined for all the four compounds. Toxicity of these compounds was predicted using

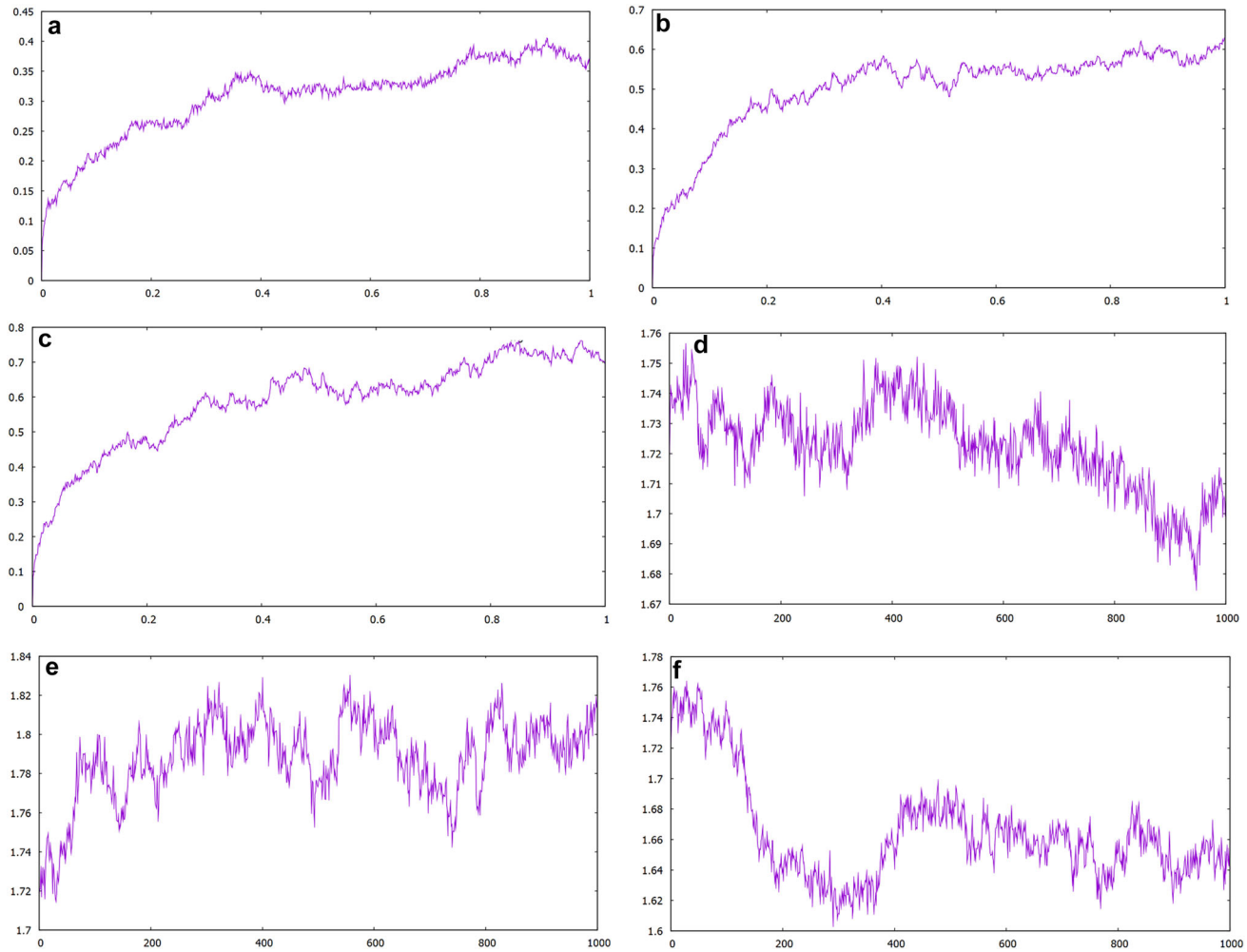

Fig. 2 RMSD and gyration plots of DENV4-NS4B. **a** RMSD at 300 k, **b** RMSD at 350 k and **c** RMSD at 400 k. **a** Gyration plot at 300 k, **b**: Gyration plot 350 k and **c** Gyration plot 400 k respectively

Ames test and Rodent Carcinogenicity. The results showed that all the compounds were non-toxic, non-mutagenic and non-carcinogenic for humans. Therefore, all the four derivatives of spiropyrazolopyridone can be used as drug against dengue virus 4 in human beings. Moreover, the safety of these drugs has been established by Zou et al., when these compounds were used in mouse to treat the dengue virus [46].

Docking of analogues of Drug-III and their ADMET

As Drug-III has shown maximum affinity for DENV4-NS4B therefore a total of 102 analogue compounds of Drug-III were docked with DENV4-NS4B and after performing ADMET analysis and drug-likeness properties, it is found that 91 compounds could be used as drugs against dengue virus in human. The most suitable analogues, having high binding affinities, were further filtered on the basis of Blood Brain Barrier (BBB) penetration behaviour. The BBB does not usually allows the drugs reaching the brain and central nervous system and it is preferred that a drug should not usually approach the central nervous system. Prediction based upon Rodent and Ames test revealed that the selected analogues were also nontoxic and non-carcinogenic. The top seven compounds with non-BBB penetration behaviour and high binding affinities interacted with various residues at the binding site of DENV4-NS4B i.e. Thr_{32}, Gly_{35}, Ile_{36}, Glu_{51}, Thr_{90}, Ala_{92}, Pro_{95}, Ala_{131}, Ile_{135}, Gln_{139} and Pro_{141}. Among these, Analogue-I and Analogue-II were observed to be docked with highest binding affinities (− 9.5 kcal/mol) making interactions with residues Arg_{31}, Ile_{36}, Glu_{51}, Ala_{92}, Leu_{130}, Ala_{131} and Gln_{139} at the binding site of DENV4-NS4B (Table S2).

The interactions of the both analogues with the protein were stronger than those of Drug-III. Analogue-I and Analogue-II blocks the binding pocket by making interactions with more residues (Arg_{31}, Ile_{36}, Glu_{51}, Ala_{92}, Leu_{130}, Ala_{131} and Gln_{139}) as compared to Drug-III (Thr_{32},

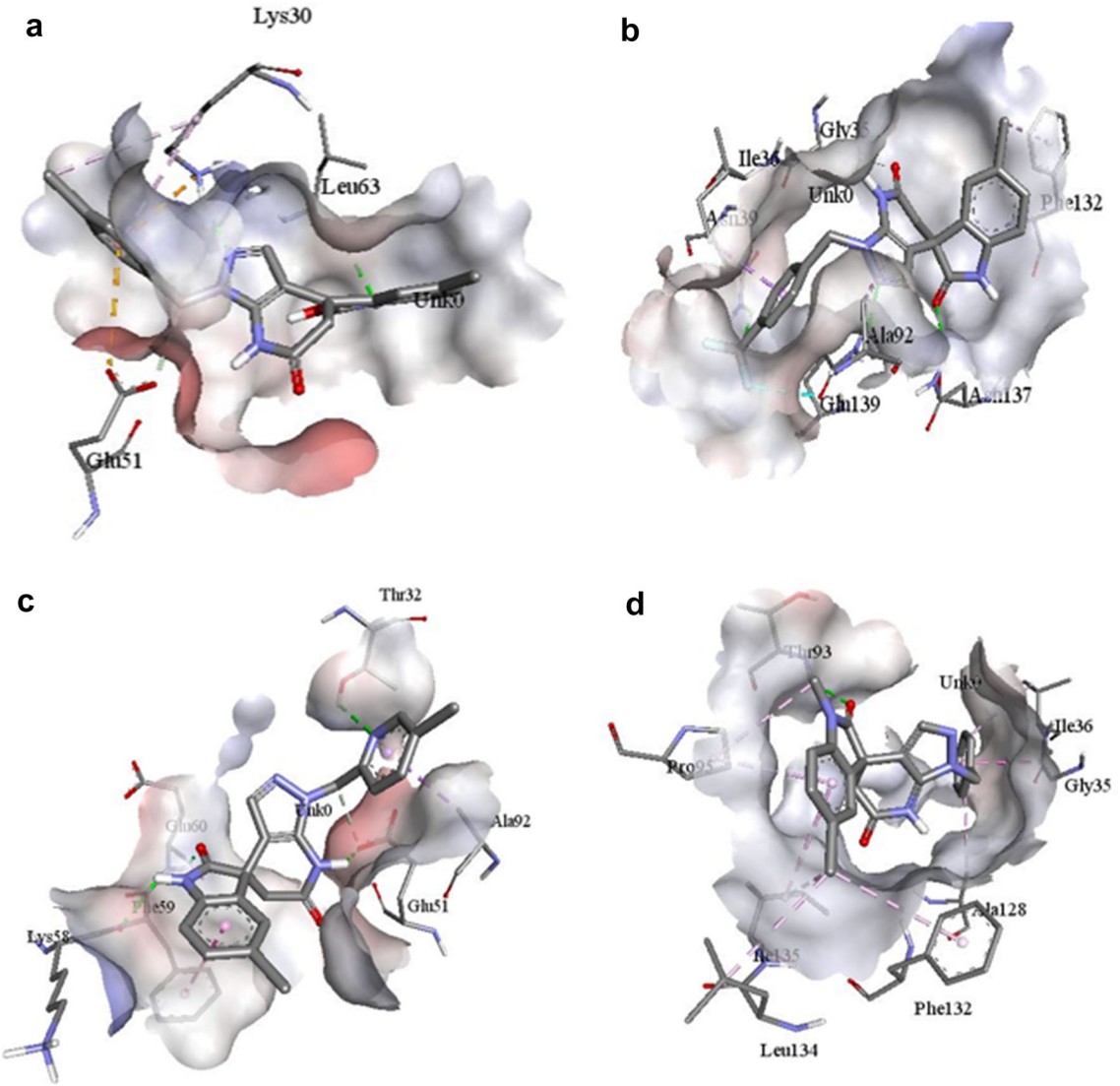

Fig. 3 Docking of DENV4-NS4B with Spiropyrazolopyridone Drugs (**a**) Docking with Drug-I (**b**) (Docking with Drug-II (**c**) Docking with Drug-III (**d**) Docking with Drug-IV

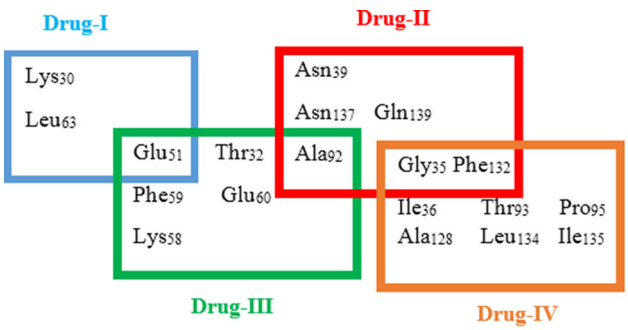

Fig. 4 Venn diagram showing binding site residues of DENV4-NS4B making interactions with four racemates of Spiropyrazolopyridone

Glu_{51}, Lys_{58}, Phe_{59}, Glu_{60} and Ala_{92}). The reason is the difference in chemical structures of Drug-III with the both analogues. The presence of Chlorine in Drug-III increases the weight of this compound and interactions are not strong as compared to both analogues which the chlorine group present. The ADMET properties of Analogue-I and Analogue-II were also better than Drug-III (Table S3). The results suggests that Analogue-I and Analogue–II can be used as safe, effective and potent drugs against dengue virus 4 in humans. The stability of DENV4-NS4B, bound with strongly inhibiting seven analogies of Drug-III, was analyzed using molecular dynamics simulations. It was observed that most stable binding was for the complexes containing Analogue-I and Analogue-II as ligands (Table S4). Singh et al., 2016, used similar approach for docking analogue compounds of *S*-adenosyl-L-homocysteine (SAH), an effective drug against *S*-adenosyl-L-methionine-dependent methyltransferase (MTase) protein.

Table 1 Docking of four racemates of spiropyrazolopyridone against DENV4-NS4B

Drug compounds	Binding sites (DENV4-NS4B)	Binding affinities (kcal/mol)	K_i value (μM)
Drug-I	Lys_{30}, Glu_{51} and Leu_{63}	− 8.0	1.347
Drug-II	Ile_{36}, Asn_{39}, Ala_{92}, Phe_{132}, Asn_{137}, and Gln_{139}	− 9.7	0.076
Drug-III	Thr_{32}, Glu_{51}, Lys_{58}, Phe_{59}, Glu_{60} and Ala_{92}	− 10.1	0.039
Drug-IV	Gly_{35}, Ile_{36}, Thr_{93}, Pro_{95}, Ala_{128}, Phe_{132}, Leu_{134}, and Ile_{135}	− 8.2	0.961

Table 2 ADMET of four racemates of spiropyrazolopyridone

ID	Value			
	Drug-I	Drug-II	Drug-III	Drug-IV
BBB	0.97202	0.153845	0.0476391	0.0840044
Buffer_solubility_mg_L	0.344579	1.2964	2.68907	3.84824
Caco2	20.9962	21.7035	20.6507	24.5805
CYP_2C19_inhibition	Non	Non	Non	Non
CYP_2C9_inhibition	Inhibitor	Inhibitor	Inhibitor	Non
CYP_3A4_substrate	Substrate	Weakly	Substrate	Weakly
Pgp_inhibition	Non	Non	Non	Inhibitor
Plasma_Protein_Binding	90.252538	92.918943	93.762795	95.523918
Pure_water_solubility_mg_L	0.504172	1.37261	6.31412	1.74017
Skin_Permeability	− 4.53806	− 2.9924	− 4.43325	− 3.84887
Algae_at	0.0104108	0.0161804	0.0285677	0.0163192
Ames_test	Non-mutagen	Non-mutagen	Non-mutagen	Non-mutagen
Carcino_Mouse	Negative	Negative	Negative	Negative
Carcino_Rat	Negative	Negative	Negative	Negative

Seventeen analogs found non-carcinogenic, non-mutagenic, as well as having good ADMET properties and good drug-like profile. These SAH analogues were reported as potent inhibitors of MTase of dengue virus [19].

The analogues of Drug-III from spiropyrazolopyridone reported in this study are novel in terms of antiviral efficacy. No such study have also been reported targeting DENV4-NS4B with these compounds. Various studies have been reported for docking analogue compounds of effective drugs against dengue virus, previously. Such compounds when used in vitro or in vivo studies has shown good results to cure dengue fever [35]. Many researchers have reported such compounds including derivatives of quinolone, benzoathiazole, ammonium species BP2109, amidobenzamide, ivermectin, selamactin, methylbenzethonium chloride, tyrothricin, and alexidine hydrochloride [2, 21, 30–32].

In the present study, the structure of DENV4-NS4B is computationally characterised and molecular dynamics simulations are performed. Computational analysis of four racemates of spiropyrazolopyridone revealed that Drug-III ($C_{19}H_{13}Cl_2N_5O_2$) was the best among all four based upon binding affinity and ADMET properties. Various analogues of Drug-III have been docked with the NS4B, binding energies and the ADMET properties of these drugs has been calculated. Based upon these analyses, it is concluded that Analogue-I and Analogue-II are effective and potent therapeutic agents for DENV4-NS4B. These analogues have strong binding affinities efficacy as compared to Drug-III and have effective drug like properties. The computational analysis can be considered as a pre-process of experimental work as it highlights the pros and cons necessary to be considered before administration of any molecule as a drug to living organisms. In future, the potential of Analogue-I and Analogue-II against replication of DENV4 in cell can be analysed targeting NS4B from DENV4.

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