ORIGINAL RESEARCH

In silico fragment-based design and pharmacophore modelling of therapeutics against dengue virus envelope protein

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Abstract

Dengue virus, an arbovirus of genus Flavivirus, is an infectious disease causing organisms in the tropical environment leading to numerous deaths every year. No therapeutic is available against the virus till date with only symptomatic relief available. Here, we have tried to design therapeutic compounds from scratch by fragment based method followed by pharmacophore based modelling to find suitable similar structure molecules and validated the same by MD simulation, followed by binding energy calculations and ADMET analysis. The receptor binding region of the dengue envelope protein was considered as the target for prevention of viral host cell entry and thus infection. This resulted in the final selection of kanamycin as a stable binding molecule against the Dengue virus envelope protein receptor binding domain. This study results in selection of a single molecule having high binding energy and prominent stable interactions as determined by post simulation analyses. This study aims to provide a direction for development of small molecule therapeutics against the dengue virus in order to control infection. This study may open a new avenue in the arena of structure based and fragment based therapeutic design to obtain novel molecules with therapeutic potential.

Keywords Dengue virus envelope protein · Fragment based de novo drug design · Pharmacophore modelling · MD simulation and energy analysis · ADMET

Introduction

The Dengue virus (DENV), an arbovirus, or virus carried by arthropods, is the cause of Dengue Shock Syndrome and Dengue Haemorrhagic fever. It belongs to the Flaviviridae family and genus Flavivirus. Dengue is an enveloped virus with a single-stranded, positive-polarity genome and icosahedral symmetry (Salles et al. 2018). The virus has a considerably higher rate of infection between 100 and 400 million infections annually, and its fatality rate is 10% for hospitalized patients and 30% for those who are non-hospitalized. If appropriate treatment is available, the death rate can be as low as 1%. At present, there is no particular, very effective medication accessible, and the form of treatment is solely symptomatic (Salles et al. 2018). To yet, none of the small molecule inhibitors created to combat dengue have progressed past the early stages of clinical trials. This

indicates the necessity of ongoing attempts to formulate novel therapeutic strategies to combat dengue and stop the high number of deaths linked to the disease. The lack of highly efficient treatment options has made the virus a major source of severe illness in several Asian and Latin American countries although a vaccine has been approved for use in recent times.

The most prevalent abnormalities linked to dengue virus sickness include increase of liver enzymes, thrombocytopenia, and leukopenia (Bhatt et al. 2013). Four distinct serotypes of the dengue virus exist, which are distinguished by structural antigens that, after infection, cause the host to produce antibodies unique to the virus (Wilder-Smith et al. 2019). Recently, a fifth serotype has also been discovered and reported in multiple studies (Joob and Wiwanitkit 2016; Mustafa et al. 2015). While heterotypic immunity against the other viral serotypes only lasts temporarily, infection with any virus serotype confers long-term immunity against that serotype (homotypic immunity) (Montoya et al. 2013). The phenomenon known as antibody-dependent enhancement (ADE) indicates that exposure to one DENV serotype increases the probability of severe dengue illness in the event

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of a subsequent secondary infection with a different DENV serotype (Guzman et al. 2010). Because non-neutralizing cross-reactive antibodies bind to heterologous DENV and enhance viral entry through Fc receptors expressed on target cells, such as dendritic cells, monocytes, and macrophages, this accounts for the increased disease severity seen during secondary infections. As previously noted, ADE causes enhanced viral uptake and subsequent replication in the virus target cells (Halstead et al. 1970; Halstead and O'Rourke 1977; Chan et al. 2014; Ong et al. 2017). A mix of pro- and anti-inflammatory reactions caused by ADE results in increased viral load and vascular leakage, which in turn causes severe dengue shock syndrome (Rothman 2011; Yacoub et al. 2015). Epidemic potential, transmission efficiency, disease severity, and host immunity correlations are among the inter-serotypic differences (Rico-Hesse 2003; Halstead 2008). The main explanation for the variations in epidemic potential has been linked to genetic alterations that alter the amino acids in non-structural (NS) proteins (Guzman et al. 2016).

Along with the M protein, the envelope protein, a 53 kDa dimer, makes up the virion surface. The E protein assumes the post-fusion trimeric configuration, where the buried fusion peptide is now accessible and can fuse to the endosomal membrane, after it has become sufficient for binding to the cell surface, fusion, and viral host cell entry in its dimeric form (Sampath and Padmanabhan 2009; Perera et al. 2008).

Here, we have attempted to target the Dengue virus envelope protein which plays a crucial role in virus entry into the host cell and thus its pathogenesis. We have utilized ab initio fragment based drug designing approach to design a new compound from scratch against the dengue virus envelope receptor binding domain which is also the probabilistically most druggable pocket present in the protein. The compounds were designed through structure based and fragment based drug design methods. The designed compounds were selected based on their docking score obtained from the AutoDock Vina algorithm. The compounds' interaction stability was determined from the MD simulation trajectories followed by MM/PBSA binding energy calculations. Water clustering analysis was utilized for optimizing the compounds to achieve a more negative binding energy thus pointing to more stability. The optimized compound was used for pharmacophore modelling study to find already approved drug molecules which share a structural similarity with the designed and optimized drug molecule. These molecules may be able to behave in a similar way to our designed molecule but a positive side of these screened molecule/s is that these have already been approved by FDA and hence no further toxicity assessment for the same is needed. This study could pave the way for development of novel therapeutics against dengue virus and play a huge role in preventing the innumerable deaths caused by the viral infection every year.

Methods

Dengue envelope structure preparation

The dengue virus envelope protein monomeric structure was retrieved from PDB ID: 1K4R.pdb and the C terminal receptor binding domain taken as the drug binding site (Watterson et al. 2012). The C terminal domain is the main receptor binding domain present in the protein. The dengue virus full protein structure was subjected to 100 ns MD simulation to assess the stability of the full protein. The MD simulation was performed using GROMACS 2021.5 suite (Lindahl 2022). Similar protein conformational studies have previously been conducted in multiple studies where MD simulations have been used to study the conformational dynamics of proteins (Ahamad et al. 2021; Ahamad et al. 2021). The protein force field AMBER99sb-ILDN was employed. TIP3P water model was used to solve the protein in a Dodecahedral simulation box with 10 Å edge length. Na^+ and Cl^- counterions were added in sufficient amounts to neutralize the solvated protein. Utilizing the steepest descent algorithm with a maximum of 50,000 steps, the neutralized system's energy was minimized. Following 500 ps NVT equilibration at 300 K temperature and 1 atm pressure, the energy-minimized systems were then exposed to 500 ps NPT equilibration at 300 K temperature and pressure. A force constraint of 1 kcal/mol/$Å^2$ was applied during the equilibration process. Then, without any constraints, 100 ns production runs with 2 fs time steps were performed on NPT-equilibrated systems. Long-range electrostatic interactions were calculated using the smooth Particle-Mesh Ewald method (Fischer et al. 2015), with a 12 Å limit applied to both PME and van der Waals interactions. GROMACS commands that were pre-built were used for all of the simulated system investigations, including the RMSD computation. The complete script can be seen in Supplementary materials.

Drug binding pocket analysis

PockDrug, CASTp and DogSiteScorer servers were employed to predict the most probable binding site and matched with the data available from literature i.e. the receptor binding domain of the dengue envelope protein (Hussein et al. 2015; Tian et al. 2018; Volkamer et al. 2012).

Drug designing

The receptor binding domain of the dengue virus envelope protein was taken as the binding site so as to prevent the

binding of the dengue virus envelope protein to the host cell receptors and the coordinates of the domain was determined using Biovia Discovery Studio. Drug design techniques based on structure and fragmentation were utilized to design the compounds. Whereas the fragment-based approach employs many fragment molecules in the binding pocket that are subsequently united to form the final drug molecule, structure-based drug design makes use of the protein binding pocket where the designed small molecule is intended to engage. By arranging the different fragments in the protein binding pocket and choosing the most effective binding ones, we have combined the two ways here. The optimal binding fragments are expanded by incorporating additional functional groups until they achieve the highest binding energy achievable in the designated binding pocket. At that point, the growth process achieves a plateau. Small molecules were prepared using AutoGrow4 tool utilizing in-built fragment libraries of various molecular weights and the best compounds from the same selected based on their score (kcal/mol) Docking was performed using the Auto-Dock Vina algorithm and fragment growth continued for 30 generations or till the binding energy plateau is reached. In all the cases it was found that the plateau was reached within the first 20 generations itself (Spiegel and Durrant 2020). The ADMET properties of the designed molecules was checked using SwissADME web server (Daina et al. 2017).

FTMap analysis

16 different sized and chemically different probe molecules are used by FTMap to examine the binding preferences of different protein residues. The dengue envelope protein domain III residues, which serve to bind different receptors and therefore facilitate host cell entrance, were examined in this instance (Kozakov et al. 2015). There is a higher likelihood that residues with strong binding propensities may engage in interactions. The compounds' likelihood of binding can be further increased if these residues are used by the compounds in their binding sites.

Water clustering analysis

From the protein simulation, based on the close contact residues of the protein for the 3 compounds in bound conformation, water clustering analysis was done using the WATCLUST plugin present in VMD to determine the water molecules present in the binding pockets after small molecule binding (López et al. 2015). The clusters were selected based on their Water Footprint Perception (WFP) scores (water finding probability score) and water clusters with WFP ≥ 0.5 were taken into consideration for further analyses. These water molecules' orientations with respect to the bound molecules would be utilized for molecule optimization in the following steps of the study.

Protein-small molecule complex MD simulation

The complexes were simulated in triplicate for 100 ns each and their binding energies determined by MM/PBSA and MM/GBSA calculations using g_mmpbsa and FastDRH web server respectively for the last 10 ns of the MD simulation trajectories (Kumari and Kumar 2014; Wang et al. 2022). Similar protein–ligand complex conformational studies have previously been conducted in multiple studies where MD simulations have been used to study the conformational dynamics of protein–ligand interaction specificity and strength of interaction (Ahamad et al. 2023; Yadav et al. 2023). The small molecules were parametrized using PRODRG tool (Schüttelkopf and Aalten 2004). MD simulation was conducted by the same method and same parameters as stated before except for the duration of NVT and NPT equilibration where the protein–ligand systems were equilibrated for 200 ps each. Post simulation analyses were performed using in-built GROMACS command-line tools which includes RMSD, RMSF, Radius of gyration and solvent accessible surface area calculations. The 3 trajectories were concatenated and Principal Component Analysis (PCA) of the protein backbone was done. The conformations were plotted against the first 2 principal components and from that the representative structures were taken which represent the highest cluster density. The inter-cluster ligand RMSDs were calculated. The RMSD between the cluster representatives and initial structure was calculated using PyMOL. Clusters were selected as the same if they share a RMSD of less than 2 Å. The closest protein residues with respect to the small molecules were determined as well. The complete script can be seen in Supplementary materials.

Small molecule optimization

The compound 1 with two nearby bridging water molecules was optimized by adding polar groups to the same, which can mimic the water molecule and construct additional protein backbone-ligand interactions, based on the results of the water clustering study. interactions involving hydrogen bonds that allow the tiny chemical to bind to the protein structure very specifically. The goal of this optimization was to strengthen the interaction between the protein and the suggested medication molecule. The selectivity of the ligand molecule for its intended target, in this case the dengue virus envelope protein receptor binding domain, may also be significantly influenced by this increase in binding affinity.

Optimized molecule analysis

The optimized molecule-protein complex was simulated in triplicate for 100 ns and their RMSD profiles and binding free energy using MM/PBSA calculation. MD simulation was conducted by the same method and parameters as stated before. PCA-based clustering analysis was performed using ligand heavy atoms to construct the covariance matrix.

Pharmacophore modelling and pharmacophore based screening of ligand molecules

Biovia Discovery Studio was used for pharmacophore modelling against the designed and optimized ligand. The modelled pharmacophore was screened against the drug database present with the Discovery Studio suite. The selected molecules were docked with the Dengue virus envelope protein receptor binding domain independently using the AutoDock Vina algorithm and the best binding poses taken for further studies.

MD simulation analyses of the screened ligand molecules against Dengue virus envelope protein

The generated protein–ligand complexes were simulated using the same protocol as has been stated above. MD simulation was conducted in 2 steps, first a 50 ns MD simulation production run was performed. Using the 50 ns trajectory ligand pose clustering analyses was performed using the linkage method of clustering and a cut-off of 2 Å was taken for determining the clusters. Structure from the most populous cluster was taken and further simulated for 20 ns using the same protocol. 100 snapshots were taken from the last 10 ns of the 20 ns MD simulation trajectory to perform MM/PBSA binding energy calculation of the generated complex between the Dengue virus envelope protein and screened ligand molecules.

Results

Dengue envelope receptor binding domain structure analysis

The starting structure i.e. 1K4R.pdb was matched with other available structures taken from PDB to assess the quality of the starting structure. RMSD with 1OKE.pdb, another structure of Dengue virus envelope protein in the pre-fusion conformation was seen to be 1.7 Å. RMSD with 3J2P.pdb and 1TG8 were seen to be 2.5 Å and 1.7 Å. This shows that all the structures have minimal difference between them. So, working with 1K4R.pdb will not create any problem in the study. MD simulation shows the

receptor binding domain to be highly stable in nature as determined from the RMSD profile (2–3 Å) of the same which reached the plateau very early in the simulation trajectory (20 ns onwards). The RMSD profile can be seen in the Fig. 1. The average structure of the envelope protein was prepared and used for further analysis. The low RMSD points to the highly stable nature of the envelope protein in the pre-fusion conformation i.e. the initial stage of the viral entry process.

Druggable pocket analysis

PockDrug showed the receptor binding domain to be the most druggable site with a probability score of 0.83 and a volume of 455.13 $Å^3$. CASTp was also used to validate the same result and it showed that the only highly probable binding site is located in the DIII domain of the envelope protein with volume of 137.5 $Å^3$ and surface area of 230.7 $Å^2$. The DogSiteScorer also showed the most probable drug binding site to be the receptor binding site itself with druggability probability being more than 0.8. So, the receptor binding domain is the most probable small molecule binding site based on the results of these 3 independent algorithms. The predicted druggable sites for all the three algorithms can be seen in Supplementary Fig. S1. All three algorithms covered the common region on the receptor binding domain of the Dengue virus envelope protein ranging from residues 334–339 and 347–356 indicating the high confidence of the generated results.

Drugs designing by fragment growth and screening for best binding compounds

3 compounds with the highest AutoDock Vina energy (more negative than − 10 kcal/mol) designed by fragment growth method of AutoGrow4 were selected for further analysis. The 3 compounds had energies of − 10.8 kcal/mol, − 10.8 kcal/mol, and − 10.3 kcal/mol.

FTMap analysis for bonding intensity of the residues that interact with the designed molecules

FTMap data shows the involvement of the domain III residues in interactions as these residues have high propensities to not only participate in non-bonding interactions but the Hydrogen bond interactions as well. Particularly if the closest residues of the protein to the compounds are considered it can be seen that all the residues have very high bonding and non-bonding propensities as can be seen from Supplementary Table S1.

Fig. 1 RMSD profile and 3D structure of dengue envelope protein along with position of water molecules close to the protein receptor binding domain (water molecules coloured based on conservancy. Red indicates high conservancy, blue indicates low conservancy). The circled region is the receptor binding domain present on the Dengue virus envelope protein
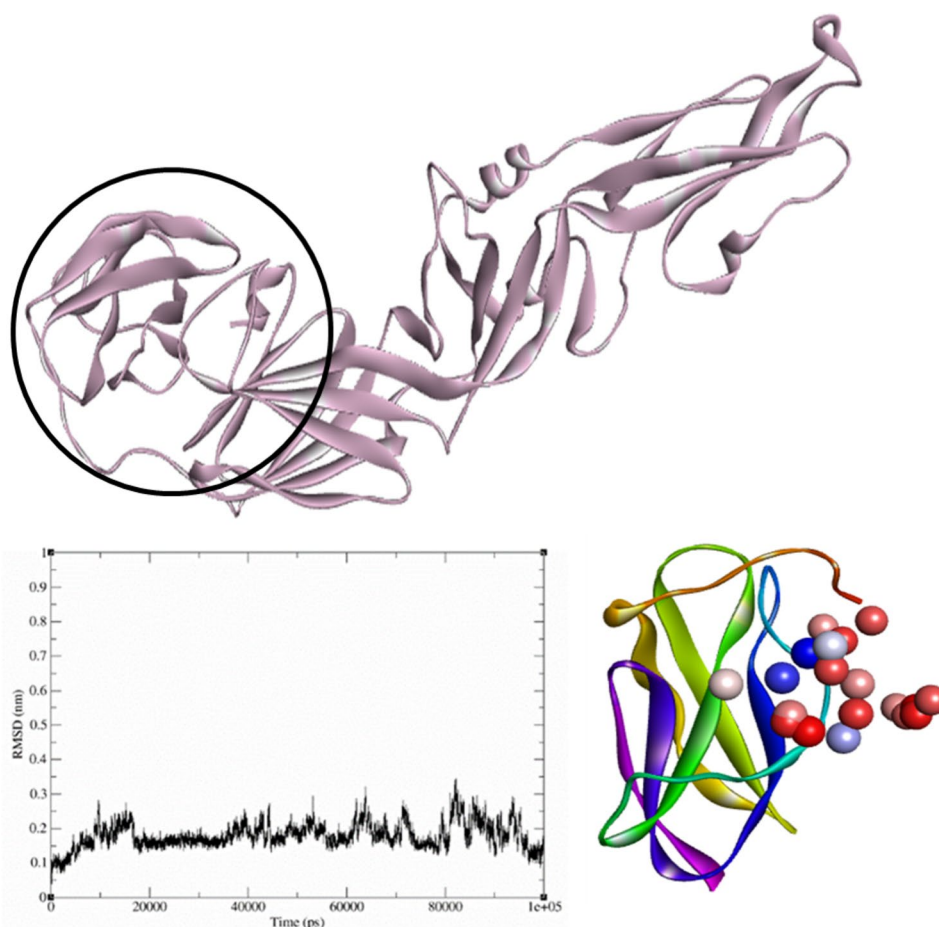
Water clustering analysis of protein receptor binding domain

Water clustering analysis was performed using the 100 ns simulation trajectory of the dengue virus envelope protein. Water clustering showed the presence of 5 water molecules with a WFP score of more than 0.5 in the binding site of compound 1. Similarly, for compound 2 binding site the number was 3 and for compound 3 it was 8 but the water clusters were much more diffused in the last case. Of these water clusters, those which were located close to the designed compound and also participated in Hydrogen bond formation with the protein molecule were taken as the basis for small molecule optimization so that the added polar groups can utilize the interaction that the water molecule shares with the protein in order to form more Hydrogen bonds in turn contributing to higher interaction strength and specificity. Based on these optimizations, it was seen that for compounds 2 and 3 no such optimized molecules were obtained owing to the lack of bridging waters, while in the case of compound 1, one such compound was obtained where a hydroxyl linkage was added. This optimized compound was further subjected to MD simulation analysis in order to ascertain the stability of the same in the binding pocket. The water clusters close to the binding site can be seen in Fig. 1.

Protein–ligand complex simulation to study structural dynamics of ligand binding in protein binding pocket

The complex structures were simulated for 100 ns each in sets of three independent runs. RMSD profiles show a convergence in the later part of the simulations during plateau formation. After optimization of the first compound it could be seen that they reached RMSD convergence (0.7 ± 0.1 nm) quite early in the simulation trajectory as is indicated by the low standard deviation of RMSD through the course of the MD simulation. The complex also was seen to have very low values of RMSD following an initial region of gross destabilization which can be considered as the equilibration phase of the molecule, binding to the protein binding site.

Hydrogen bond analysis showed the involvement of 4–5 Hydrogen bonds throughout the course of the simulation for compound 1 and 2–3 Hydrogen bonds were formed for compounds 2 and 3. From the last 10 ns of the trajectory,

MM/PBSA binding energy calculations were performed. The binding energies showed high negative values indicative of the highly stable nature of the complexes. Binding energy for the last 10 ns for compound 1 is − 104.45 kJ/mol while for compound 2 it is − 124.51 kJ/mol and for compound 3 it is − 124.57 kJ/mol. The MM/GBSA binding energies were found to be − 132.2 kJ/mol, 167.4 kJ/mol and 164.3 kJ/mol respectively for molecules one, two and three. The small molecules in bound form throughout the simulation trajectories were clustered in order to find the most probable binding poses. It showed that for all the 3 compounds, the first 3 clusters represent more than 50–60% of the conformations in which the ligand exists with the first cluster itself covering more than 20–30% of the cases. RMSD values were calculated between the clusters and between each cluster and the starting structure. It was found that the RMSD for all the cases is below 2 Å so it can be said that the ligand deviation is within the acceptable range and thus the starting structure can be considered for all further analyses. The RMSD values are given in Supplementary Table S2.

The closest residues for each of the 3 designed compounds are as follows: for Compound 1 the residues are 339, 340, 342, 357, 360, 361, 362, for Compound 2 the residues are 306, 339, 356, 357, 358 and for Compound 3 the residues

are 342, 353, 355, 356, 357, 358. The small molecule bound protein structures and the 2D ligand interaction diagrams can be seen in Fig. 2. The designed molecules were also seen to be abiding by the various physico-chemical characteristics needed for the molecule to be considered as a potential drug molecule (Fig. 3).

Optimization of designed compounds for better binding in the receptor binding domain

The closest water molecules that form the bridge between the Compound 1 and the dengue virus envelope protein were used for compound optimization so that more Hydrogen bonds could be formed thus further stabilizing the interaction. MD simulation followed by cluster analysis showed the molecule to be stabilizing in the required binding pocket (Fig. 2). Binding energy calculation showed the binding energy to be highly negative in nature, the value of which for the last 10 ns of the trajectory is − 140.69 kJ/mol (MM/GBSA binding energy − 185.4 kJ/mol), thus more negative than the original molecules in turn pointing to the higher binding stability of the optimized compound when compared to the original compounds. This increased binding energy is due to the fact that more H bonds are being formed between
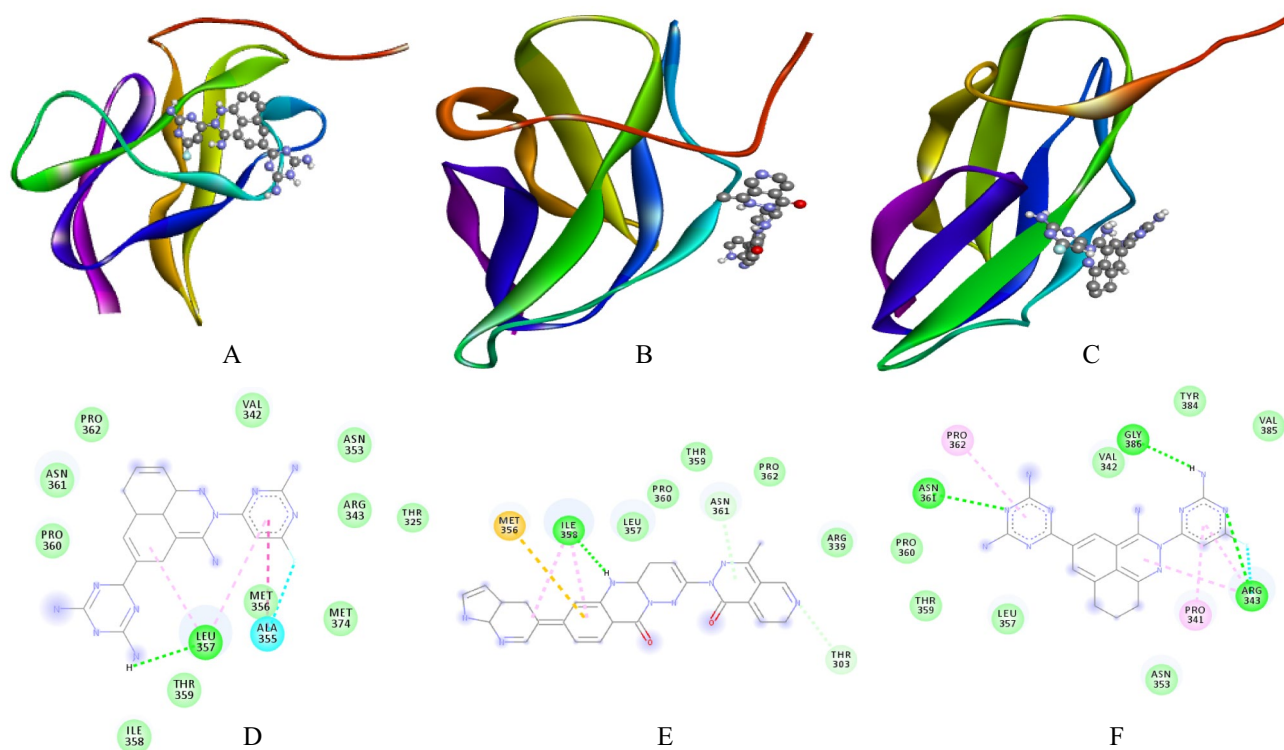

Fig. 2 Designed compounds in complex with dengue envelope following MD simulation. **A** Dengue envelope protein- Compound 1 complex. **B** Dengue envelope protein- Compound 2 complex. **C** Dengue envelope protein- Compound 3 complex. **D** 2D interaction diagram of Compound 1 in protein binding pocket. **E** 2D interaction diagram of Compound 2 in protein binding pocket. **F** 2D interaction diagram of Compound 3 in protein binding pocket
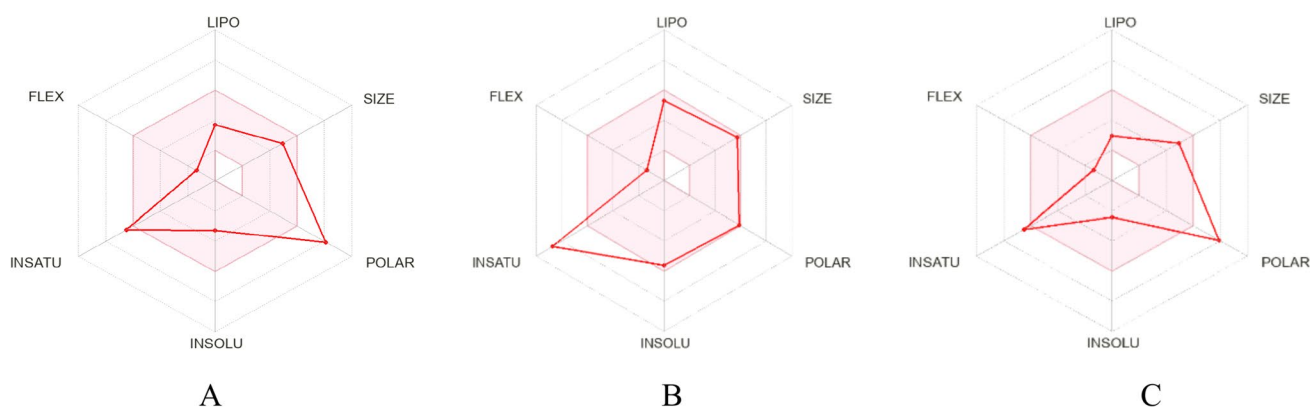
Fig. 3 ADMET characterization of designed compounds. **A** Compound 1. **B** Compound 2. **C** Compound 3

the Dengue virus envelope protein receptor binding domain and the designed and optimized compound. These additional interactions are performed by the added polar group which was added during the molecular optimization process. In case of the optimized compound the number of formed Hydrogen bonds for all 3 runs ranged from 5 to 6 Hydrogen bonds thus showing an increase in the Hydrogen bond forming propensity after optimization which is again caused due to the increase in the polarity of the molecule leading to more Hydrogen bonds being formed in the process. The optimized molecule in the protein binding pocket along with its 2D ligand interaction diagram can be seen in Fig. 4.

Pharmacophore modelling based on designed and optimized compound and screening of compounds against dengue virus envelope protein

Pharmacophore modelling of the designed and optimized compound reveals presence of 9 prominent pharmacophoric features. Molecule screening was performed based on the presence of these 9 features in the screened molecules. It was found that the pharmacophore model consists of 5 ionizable groups along with 4 Hydrogen bond donor groups. These Hydrogen bond donors may contribute in interacting with
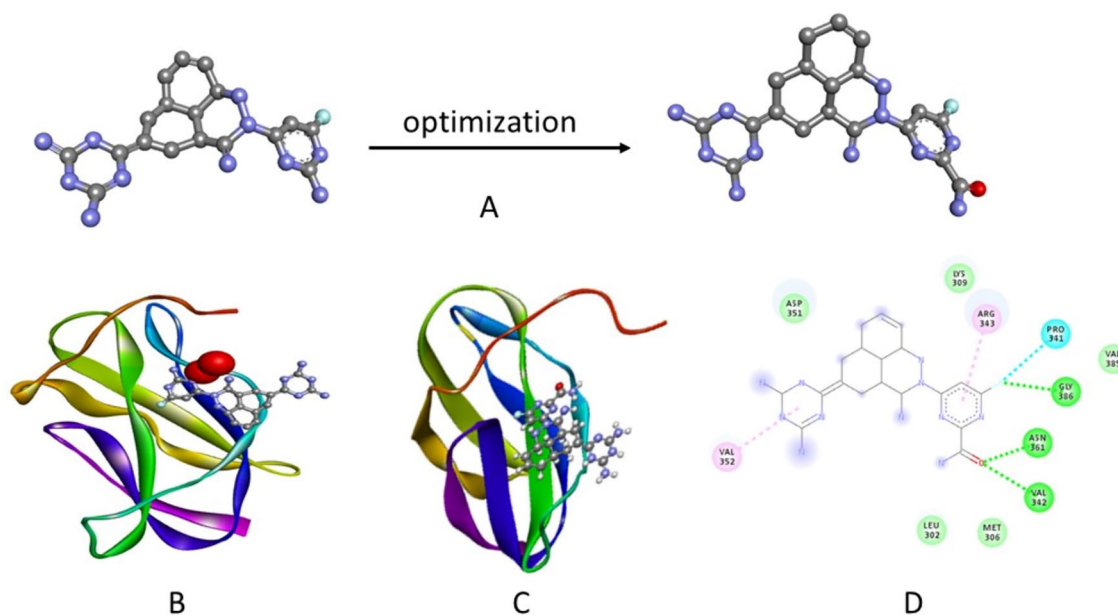

Fig. 4 Properties of optimized compound. **A** Optimization of compound 1 into the final optimized compound. **B** Binding site water molecules used for molecule optimization. The red circles are the water molecules which are capable of forming bridging Hydrogen bonds between the ligand and protein binding site. The ligand was optimized making use of these bridging water molecules so that the optimized molecule can bind more efficiently to the protein binding site. **C** Optimized molecule in the envelope binding site. **D** 2D interaction diagram of optimized molecule

Fig. 5 Pharmacophore model of the designed and optimized compound. Red spheres represent ionizable groups. Purple spheres represent Hydrogen bond donor groups
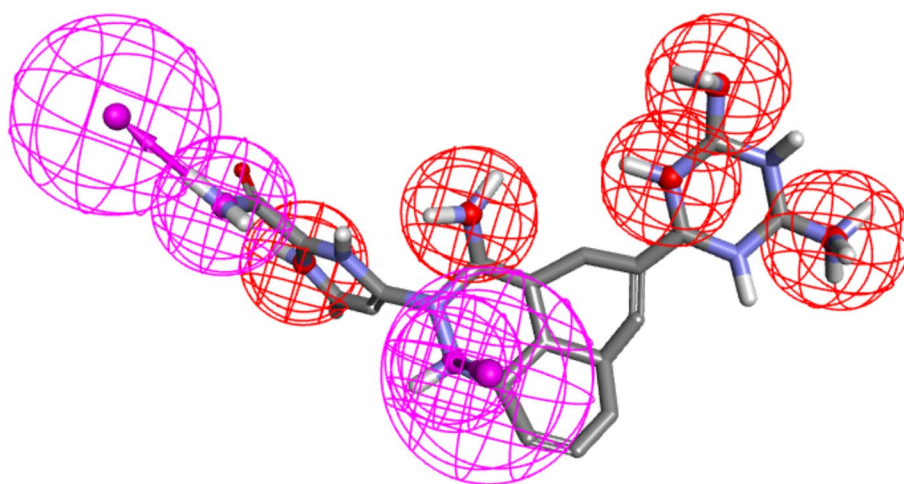
Hydrogen bond acceptor groups present in the Dengue virus envelope protein. The ionizable groups may play crucial role in participating in electrostatic interactions with the Dengue virus envelope protein Fig. 5.

Screening resulted in three potential compounds- kanamycin, vistamycin and tobramycin. Docking of all the three compounds with Dengue virus envelope protein receptor binding domain showed a binding score of in excess of − 6 kcal/mol. The binding score of the best binding pose for the three molecules was found to be − 6.3 kcal/mol for kanamycin, − 6.2 kcal/mol for tobramycin and − 6 kcal/mol for vistamycin. Previous studies have taken − 6.5 kcal/mol as good binding scores for molecule selection prompting us to proceed with these molecules for later parts of the study (Hosseini et al. 2021). All three compounds' binding stability with the Dengue virus envelope protein was further analyzed in later sections of this study.

MD simulation and post simulation analyses of screened compounds against Dengue virus envelope protein

MD simulation of the three protein–ligand complexes showed the very low stability of interaction between the Dengue virus envelope protein and tobramycin and vistamycin, both of which got dissociated through the course of the MD simulation. Only kanamycin was found to remain closely associated with the Dengue virus envelope protein receptor binding domain following an extended initial period of equilibration. Clustering analyses of the 50 ns MD simulation trajectory showed simulation convergence over the course of the trajectory as is deciphered using the cluster counting method where it was seen that no new protein–ligand complex clusters were found for the latter section of the simulation trajectory. Final 20 ns MD simulation of the Dengue-virus envelope protein-kanamycin complex showed the highly stable interaction of

the protein with the ligand molecule as can be seen from the low RMSD of the ligand heavy atoms (0.29 ± 0.1 nm) as also the high negative MM/PBSA binding energy data (-387.96 ± 73.78 kJ/mol). The MM/GBSA binding energy was found to be -270.5 ± 20.5 kJ/mol. It was also seen that this binding energy is predominantly dominated by electrostatic linkages between the ligand molecule and the negatively charged amino acids like Asp and Glu present in the Dengue virus envelope protein receptor binding domain. MD simulation study showed the kanamycin molecule to be interacting with high stability with the Dengue virus envelope protein close to the hinge region encompassing both the receptor binding domain and also parts of the adjacent D-I domain. This interaction may also play a role in restricting the conformational change from the folded pre-fusion state of the envelope protein to the straight post-fusion state by stabilizing the inter-domain interaction further by bridging the interactions between them. The 3D orientation of the kanamycin molecule with respect to the protein binding site and the 2D interaction diagram of the ligand molecule can be seen in Fig. 6. So, this molecule may affect the conformational dynamics of the Dengue virus envelope protein as also the interaction dynamics of the envelope protein with its natural partners in the host cell.

Discussion

One of the main causes of death in tropical regions, such as the Indian subcontinent, Southeast Asia, and Latin American nations, is the dengue virus. As of right now, there is no recognized specific medication to treat the virus-caused illness, which presents as dengue shock syndrome and dengue hemorrhagic fever. The discovery of innovative medicines against viruses has become necessary due to the dearth of available treatments and the increasing number of deaths
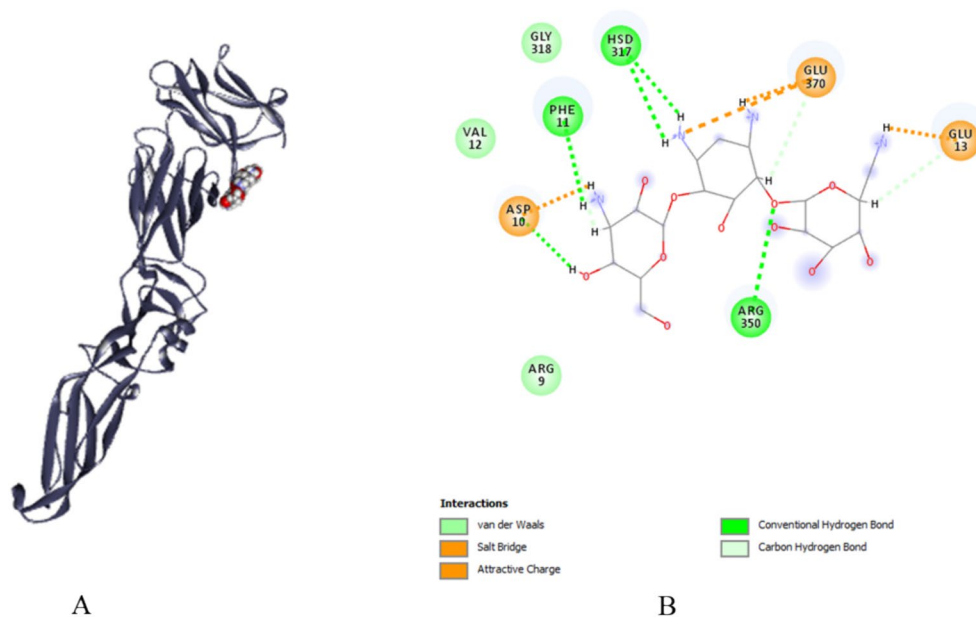
Fig. 6 MD simulation analysis of protein–ligand complex. **A** Protein–ligand complex showing binding of kanamycin molecule to Dengue virus envelope protein. **B** 2D ligand interaction diagram showing different type of interactions between Dengue virus envelope protein and kanamycin

caused by virus infection. The dengue envelope protein is the main protein that binds to the glycosaminoglycans that are expressed on the surface of the host cell, which then causes the virus surface protein to attach to a receptor and enter the host cell (Watterson et al. 2012). The domain III is the major domain responsible for the receptor recognition and interaction of the virus protein with the host receptors (Chin et al. 2007 Jan).

Structure-based and fragment-based drug design (FBDD) approaches are cutting edge methods for creating brand-new, unique drug molecules that may work far more effectively than any naturally occurring substances or their derivatives, which have been the standard of care up until recently. Numerous extremely effective medicinal compounds have already been found as a result of these novel approaches. These include the anti-HIV medications Raltitrexed and Amprenavir, which target the retroviral protease and the thymidylate synthase enzyme, respectively (Anderson 2003 Sep; Wlodawer and Vondrasek 1998). Other therapeutics include isoniazid against tuberculosis, epalrestat against diabetic neuropathy and many others (Marrakchi et al. 2000 Feb; Wang et al. 2013). Among many additional medications that have been approved for use, fragment-based drug design techniques have produced medications like venetoclax, which treats chronic lymphocytic leukemia, and vemurafenib, which treats melanoma (Kim and Cohen 2016; Fairbrother et al. 2019). As more and more compounds generated by FBDD are moving through various phases of clinical trials, this approach to drug discovery is well known. Target-based drug discovery can make use of

fragment-based drug design. Target-based drug development always analyzes the druggability of a target and its binding pocket, as this study has done utilizing FTMap's Bonding and Non-bonding propensity analysis. These analyses are utilized to predict possibility of developing drugs in the binding pocket favoring small-molecule compounds. For most cases, druggable targets are selected for this approach as the probability to develop small molecule drugs is very high in these cases (Dang et al. 2017).

In this study we have selected as our target the dengue envelope protein receptor binding domain for fragment based drug designing. PockDrug, CASTp and DogSiteScorer, 3 independent web servers using different algorithms predict the most druggable site in the protein and found the binding site to be located at the same site in the protein itself which functions as the receptor binding domain of the protein and thus this site can act as the drug binding site with very high probability. The binding site was specified for fragment based drug formation and the best results taken for further analysis. For fragment growth, the AutoGrow4 tool was used along with its inbuilt libraries and the energetic calculations were performed using AutoDock Vina thus leading to a robust search of the search space and binding poses of the compounds. It was seen through the bonding and non-bonding analysis in FTMap that the residues participating in bond formation with the 3 selected compounds are those with the highest bond forming propensity. This proves that the binding site for all the 3 compounds which is highly overlapping actually has the propensity to take part in bond formation and is thus a true binding site in the protein.

Water molecules at the protein's binding site were taken into account when doing the water clustering study. Three sets of protein–ligand complexes were simulated, and in every case, the interactions between the ligands and the protein were extremely robust and stable. All through the trajectories it could be seen that never had the ligand molecule left the binding groove in spite of its pose varying slightly. Pose clustering showed the presence of highly dense clusters where the top 3 clusters account for a huge proportion of the trajectory frames. This further validates the interaction stability of the ligands in the protein binding cavity. The ligand poses generated from the MD simulation and clustering were compared to the positioning of the placed water molecules. By adding polar groups, it is possible to increase the formation of polar hydrogen bonds by utilizing the water molecules that act as bridges between the ligand and the protein binding site. This was done in an attempt to optimize the molecule. The purpose of optimizing the first compound was to increase the stability of the formed complex by forming many hydrogen bonds between the protein and the proposed designed ligand. This is demonstrated by the formation of more hydrogen bonds throughout the length of the MD simulation trajectory and by the higher negative binding energy that is determined by applying the MM/PBSA calculation method. The high number of the Hydrogen bonds can be seen from the Fig. 4 showcasing the 2D diagram of the optimized ligand molecule. For the other 2 compounds no such bridging water molecule was found and thus based on Hydrogen bond formation there was no scope for molecule optimization there. ADMET characterization showed that all the 3 molecules are highly soluble with a high drug-likeliness score. The first compound was optimized by adding the polar group which enhanced the molecule's solubility. The great tendency of the proposed and optimized chemical to be used as a treatment is demonstrated by all these analyses. The optimized molecule was further subjected to pharmacophore modelling to find out molecules with a similar structure to our designed molecule but has already been previously approved as a drug by the FDA. It was found that there are three molecules (kanamycin, vistamycin and tobramycin) with a similar structure when compared to our designed molecule. It was found following MD simulation that it is only kanamycin which is capable of remaining bound to the Dengue virus envelope receptor binding domain with high stability as is indicated by the low RMSD value of the ligand heavy atoms with respect to the protein binding pocket as also the high negative binding energy value. Kanamycin was found to remain bound to the receptor binding domain of the Dengue virus envelope protein and this may play a crucial role in preventing the Dengue virus envelope protein from interacting with its specific receptors present on the host cell surface in turn reducing the viral entry into the host cell and infection progression. Previously it has been shown that aminoglycoside molecules like geneticin have antiviral activity and kanamycin being an aminoglycoside as well may play a similar function (Zhang et al. 2009). Another report has shown kanamycin to be having minor inhibitory effect in case of Japanese encephalitis virus infection which is a Flavivirus as well and thus this molecule may also play similar role in case of Dengue virus as well (Rashmee and Siraj 2018). Structural comparison with heparan sulphate, a glycosaminoglycan which plays a crucial role in Dengue virus entry also showed high similarity between the two molecules, i.e. heparan sulphate and kanamycin thus indicating that kanamycin may play a role similar to heparan sulphate in interacting with the Dengue virus envelope protein ultimately leading to inhibition of the entry in the host cell. This study may open the door for novel application of other very effective therapeutic choices to combat the virus disease and the deaths that are linked with it.

Conclusion

In nations with tropical or subtropical climates, dengue virus is a major cause of severe infections and a substantial cause of mortality. The absence of effective, targeted treatments to combat the dengue virus exacerbates this issue, which highlights the significance of our work. Here, our goal has been to create a molecular that selectively targets the receptor binding domain of the dengue virus envelope protein and using the features of this designed molecule to search for similar structure compounds. This substance may bind the protein with extreme stability, preventing it from connecting with its cellular receptors. The main limitation of fragment based drug design approach is the synthesizability of the proposed compound. This limitation has been taken care of in this study through pharmacophore based screening of drugs having similar physico-chemical characteristics with the designed ligand. Further in vitro and in vivo studies need to be performed in the future to shed more light into the efficacy of kanamycin as a Dengue virus entry inhibitor through targeting of the envelope protein receptor binding domain. This would be extremely important in blocking the virus's ability to enter host cells and halting subsequent processes. This research could pave the way for the creation of cutting-edge treatments to fight viral infections and result in the creation of extremely effective medicinal substances.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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