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In Silico Molecular Docking Studies and Design of Dengue Virus Inhibitors

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Abstract: *Dengue fever is an infectious tropical disease which is caused by the dengue virus. Dengue infection is one of the most significant mosquito-transmitted infections which is common in >100 tropical and subtropical countries. Dengue fever is becoming a serious health risk these days and it shortly needs treatment against the increasing problems around the globe and the existence of resisting mutants of dengue virus. In this study the problem of designing an anti-dengue drug with more effectiveness has been solved by using computer aided drug designing. Computer-aided drug design (CADD) entails the use of biochemical information of ligand-receptor interaction sequentially to hypothesize the drugs refinements. Docking of selected ligands, having anti-dengue activity with the active-site of protein 2FOM, was done to find the biochemical information. Docking interactions were interpreted in the form of hydrogen bonding, hydrophobic and ionic interactions. On the basis of this interaction analysis and IC50 value, one of the ligand was recognized as 'lead compound'. Seven analogues of the lead compound were designed and docked with the active site of protein. Interactions of the analogues with the active site of 2FOM protein were analyzed. On the basis of activity and high binding interactions these compounds will be suggested for clinical testing and synthesis in laboratory as a future plan.*
Keywords: *Anti-Dengue Agents; Lead Compound; Computer Aided Drug Design(CADD); IC50; Ligand-Receptor Interactions; Analogs; DENV-proteases like NS3/NS2B protease receptor (2FOM); Quantitative Structure Activity Relationship (QSAR).*
Abbreviations: *DENV=Dengue Virus; ADE=Antibody-Dependent Enhancement; CADD= Computer Aided Drug Design; QSAR= Quantitative Structure Activity Relationship; IC50= The half maximal inhibitory concentration.*

I. Introduction

Dengue infection is one of the most significant mosquito-transmitted infections which is common in >100 tropical and subtropical countries. Dengue viral infection is ever-increasing with an annual estimate of 50 to 100 million cases globally [1]. According to World Health Organization (WHO) approximations, about two fifths of the world population is at risk of DENV. Dengue fever occurrences have been reported from Pakistan in 1994, 1995, and 1997. In Pakistan dengue is emerging as one of the most important public-health dilemma, above all since 2005 threatening the millions of people due to widespread irregular socio-economic conditions and epidemiological circumstances. Evidence suggested that the overall load of disease, on top of its severity getting higher in Pakistan [2]. Dengue virus belongs to the *Flaviviridae* family, which is a well-known human pathogen, with the purpose of causing diseases ranging from a harmless flulike illness to a brutal hemorrhagic fever with the high death rate; particularly occur in children [3]. The genus *flavivirus* consist of over 70 viruses in addition to DENV [4]. There are four serotypes of DENV in approximation to cause 50 to 100 million human infections worldwide annually [5]. Dengue virus contains four antigenically distinct viral serotypes named as DEN-1, DEN-2, DEN-3 and DEN-4) [6]. All the serotypes usually transmitted from one human host to the other by mosquitoes of the *Aedes* genus, primarily *Aedes aegypti* and *albopictus* [7]. Dengue virus possesses a unique human-to-human cycle like distinct arboviruses without the need for an intermediary wild mammalian reservoir. Serological investigations in mammals recommend that the circulation of dengue follows epidemiological patterns, without any noticeable clinical signs in hosts [8].

Challenges countenance in the progress of DENV-specific antiviral treatment are frequent and include the development of low expenditure with the rapid diagnostic procedure that is analytical of infection severity and identifying a compound which should safe, targets numerous serotypes and is more useful even after the onset of severe clinical infection [9]. While there is no licensed dengue drug and vaccine currently exists. Several candidates are currently undergoing either clinical evaluation or preclinical development [10]. As a result there is a need to design antiviral agents to fight dengue infection. One of the antiviral agent approaches involves the blocking and inhibiting the viral enzyme activity, hence preventing virus replication by the bioactivity of these antiviral agents. In addition, new research efforts are needed to identify therapeutic approaches that would be able to achieve DENV suppression or at least blood loss [6].

Modern drug discovery is differentiated by the production of enormous quantities of compounds which necessitate to store, manage and analyze these quickly growing resources has given rise to the field of computer-

aided drug design (CADD). Digital repositories which contain detailed information on drugs and other useful compounds are the goldmines for the study of chemical reactions capabilities. Virtual *in silico* screening has frequently confirmed to be useful to assemble the special challenges of antiviral drug discovery. Various virtual compound libraries are filtered by different computational screening methods such as docking, ligand-based similarity searches or pharmacophore-based screening, reducing the number of candidate molecules to a smaller set of promising candidates that are then tested biologically [11].

Bioinformatics tools are majorly facilitating *in silico* drug discovery. The current work aimed at the *in silico* development and design of anti-dengue virus drug against the selected target protein, that could inhibit the viral replication. The main purpose of the project was to recommend such compounds which have inhibitory activity against DENV-proteases like NS3/NS2B protease receptor (2FOM) using molecular docking studies. The lead compound was identified on the basis of high binding interactions and low binding energies and its analogs were constructed. Optimistically the results from this study will provide insight about the design of effective antiviral drugs against dengue virus which will prove to be a valuable resource for further research. Different softwares such as ChemBioDraw® Ultra 13.0 [34], HyperChem professional 8.0.10 [35], AutoDock [14], LigandScout [15] and VMD [16] were selected and used to get the results. The complete drug designing scheme has been demonstrated in Figure 2.

II. Methodology

In silico drug discovery process is a source full and beneficial way to design novel drugs by utilizing the knowledge about the biological processes, biochemical activities of chemical compounds and targets. Bioinformatics tools are majorly facilitating in silico drug discovery.

2.1 Selection of target protein:

Identifying and selecting the most appropriate drug target or receptor is the initial step in the drug designing procedure. Excellent drug targets can be identified with the help of bioinformatics. For a drug target to be ideal, it must be linked closely to the selected human disease. Mostly proteins act as good targets for the drugs. In some cases, enzymes can also serve as excellent drug targets [17]. The polyprotein NS3-protein of Dengue virus-2 was selected for the present study. The three dimensional structure of dengue virus NS3/NS2A protease is available in the Protein Data Bank (accession code: 2FOM) and is shown in Figure 1. Once the target has been is identified, the candidate ligand of drugs can be selected either by analyzing previously known drugs having potent effects against DENV or by designing the novel inhibiting compounds.

2.2 Anti-dengue inhibitors selection:

For the *in silico* design and molecular docking studies a number of inhibitors of NS3 and NS5 proteases were identified and selected. There were 26 compounds [6, 18-27] which were selected for molecular studies having drug like properties against dengue virus. These drugs were selected through the analysis of previous and current research on the dengue virus according to their efficiency and potency against virus.

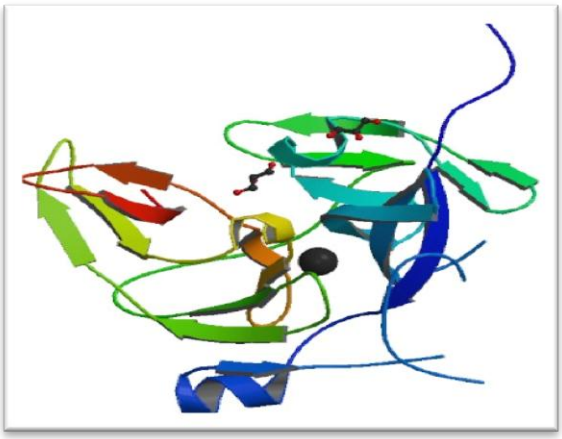

Figure 1. Crystal Structure of dengue virus NS2B/NS3 protease (2FOM)

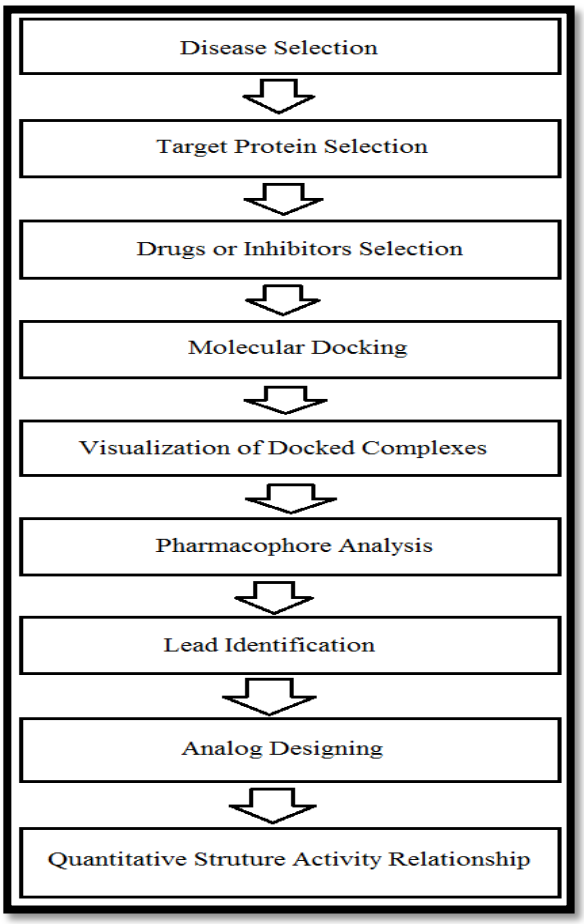

Figure 2. Schematic representation of drug designing

2.3 Molecular Docking:

Structures of 26 selected Anti-Dengue agents were drawn and optimized using ChemBioDraw® Ultra 13.0 [34] and HyperChem professional 8.0.10 [35] respectively. The docking of the ligands i.e. inhibitors and proposed drugs, with the receptor 2FOM structure were performed using AutoDock 4.2 [29]. It gives the active conformation of ligand and the binding modes of 26 selected compounds with the active site of the target protein 2FOM. AutoDock will try a set of different conformers of the ligand in order to obtain the best disposition of the atoms of the molecule for maximizing the scoring function that quantifies ligand-receptor interaction [33]. The structure of target protein 2FOM was obtained from Protein Data Bank (Protein Data Bank ID: 3GGE). For using the receptor or target protein in the AutoDock, all the missing side chain atoms were checked and then repaired and also hydrogen atoms were added using the graphical user interface of AutoDockTools (ADT) [29]. Water molecules were removed before docking experiments. Then for the ligand preparation, input the ligand and Gasteiger charges were merged with non-polar hydrogens of ligand. Detecting root, torsions were selected with most of the ligand atoms and making bonds active (rotatable) or inactive (non-rotatable). The grid box within the dimension of 90x90x90 points using Autogrid and 0.547 Å spacing was used to cover the entire receptor molecule so that the ligand can easily lodge with it making the binding pocket.

For rigid docking the docking parameter file was then prepared by specifying rigid molecule. Then the ligand was specified. The calculations for rigid protein-ligand dockings were done using the Lamarckian Genetic Algorithm (LGA) method. A population size of 150 and 2500000 energy evaluations were used for 10 search runs. After the docking, the docked complex was analyzed to obtain all the conformations. The conformation having the lowest binding energy value was selected as docked complex and saved in pdb format. This docking procedure was applied for all 26 ligands with receptor having 10 different conformations. After the docking searches were completed, docked complexes were analyzed.

The docked complexes were analyzed closely using VMD and interactions between the ligand and the receptor were studied. The binding interactions are of less than 3.5 of length. The protein-ligand conformations including hydrogen bonds, ionic bonds, and covalent bonds with the distances in a pocket were analyzed using VMD. This scheme for molecular docking is mentioned for only single ligand. Therefore all the compounds were docked against the receptor molecule to get the complex of each compound and then analyzed in VMD.

The lead compound was identified on the basis of good binding interactions, least energy values and the good IC50 values. The best interacting compound with least IC50 value was considered as 'Lead'.

2.4 Analog Designing:

After lead identification the analogs of the lead compound were designed. Each of the analogs was designed by the replacement, addition or the removal of side chain atoms in the main structure of the selected lead compound. Analogs were docked against the receptor in AutoDock using the same procedure. Then the analog-protein complex was visualized and analyzed. The analog of an existing drug molecule shares structural and pharmacological similarities with the lead compound. These analogs were designed using the existing drugs, to gain functional drug with more effectiveness and efficiency. Seven designed Analogs are shown in Figure 3.

2.5 Quantitative Structure Activity Relationship (QSAR):

QSAR represent an attempt to correlate structural or property descriptors of compounds with activities. These physicochemical descriptors, which include parameters to account for hydrophobicity, topology, electronic properties, and steric effects, are determined empirically or, more recently, by computational methods. Activities used in QSAR include chemical measurements and biological assays. QSAR currently are being applied in many disciplines, with many pertaining to drug design and environmental risk assessment. Quantitative Structure Activity Relationship has been deduced from the calculated chemical properties using ChemDraw and HyperChem.

III. Results and Discussion

3.1 Molecular Docking:

A usually smaller molecule which binds to a larger molecule such as enzyme or protein initiates the replication process. On the basis of docking of anti-Dengue drugs with the receptor protein, the 2FOM was searched for its active site. On the basis of docking, the amino acids within 5 Å were identified i.e. the active site. The study discovered that the residues ALA49, ALA52 and GLN27 were major determinant of binding pocket. The active site within protein 2FOM is shown in Figure 4. Then dockings were carried out using 2FOM target protein on the test set compounds.

Once all the compounds were docked against the receptor protein 2FOM, these docked complexes were visualized in VMD. Interactions of the complexes i.e. Hydrogen bonds, hydrophobic interactions and ionic interactions with QSAR descriptors were analyzed and shown in Table 1.

1.7 Lead Identification:

On the basis of binding interactions of the 26 compounds, one lead compound was discovered. The information in Table 1 showed that compounds like S-adenosyl methionine, Quinazolin-4-amine; Quercetin, Guanosine-5-triphosphate and 6-O-butanoyl castanospermine had good hydrogen and Ribavirin, 4-hydroxypanduratin A, S-adenosyl-l-homocysteine, Myricetin, Guanosine-5-triphosphate and Quercetin ionic bondings with the amino acids within the 5 Å of active site of 2FOM. According to the binding interactions of these compounds and IC50 values, Quercetin was considered as the lead compound. Figure 4 shows the hydrogen bonds and hydrophobic interactions of the lead compound with the amino acids within the active site of 2FOM.

Quercetin forms 12 hydrogen bonds with the receptor protein i.e. 2FOM. Some common reported amino acids were LYS145, VAL59, ASP20, PHE46, THR45, LYS63, GLU19 and GLY44 with the distance of 2.67Å, 2.04Å, 2.08Å, 1.59Å, 1.80Å, 2.60Å, 2.93Å and 2.23Å respectively. The hydrogen bond interactions of Quercetin (lead compound) with the active site of protein 2FOM are shown in figure 5(A).

Quercetin forms 15 carbon bonds with the receptor protein i.e. 2FOM. Some common reported amino acids were LYS145, LYS42, VAL59, TYR23, PHE46, GLU19, GLU43 and ILE65 with the distance of 2.32Å, 2.50Å, 3.26Å, 3.41Å, 2.64Å, 3.10Å, 3.19Å and 3.05Å respectively. The hydrophobic interactions of Quercetin (lead compound) with the active site of protein 2FOM are shown in figure 5(B).

Quercetin forms 3 nitrogen bonds and 1 oxygen bond forming ionic interactions with the receptor protein i.e. 2FOM. One oxygen form bond with PHE46 with the distance of 2.80 and two nitrogen atoms form ionic bond with PHE46, LEU53 and LYS42 at the distance of 1.25Å, 3.15 Å and 2.49 Å respectively. The ionic interactions of Quercetin (lead compound) with the active site of protein 2FOM are shown in figure 5(C).

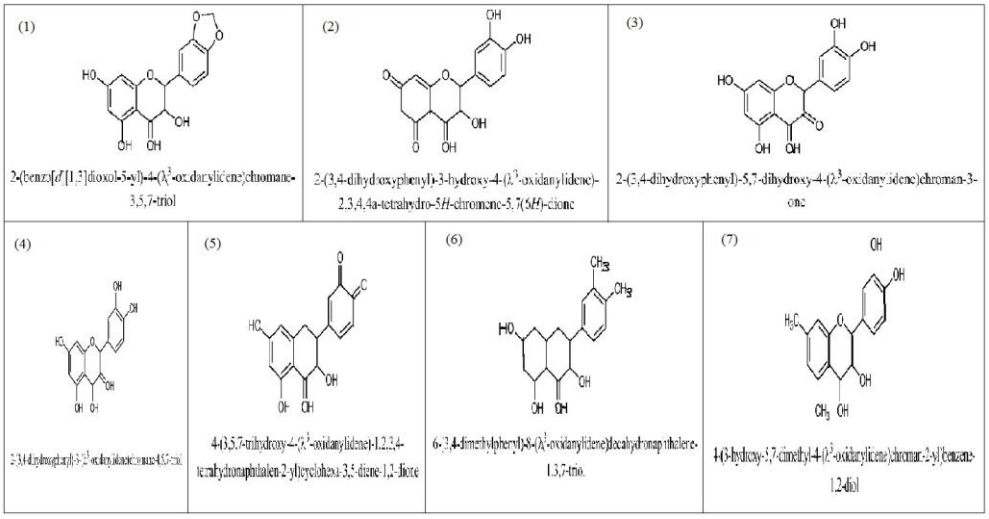

Figure 3. Seven designed analogs of lead compound

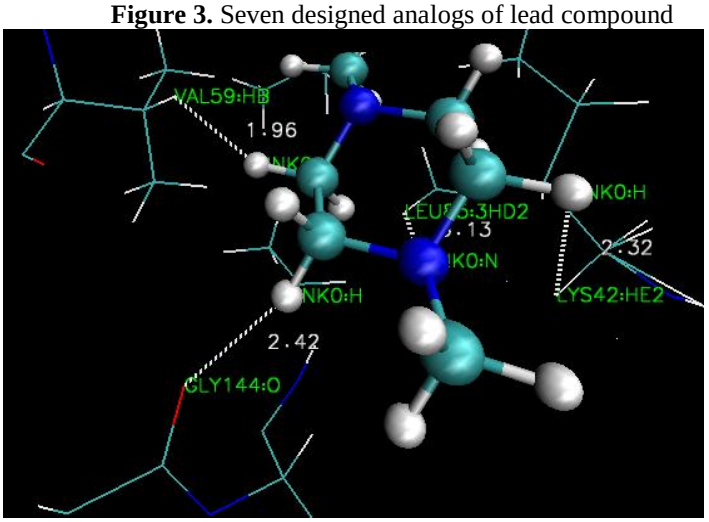

Figure 4. Binding interactions of complex of Quercetin and the target protein 2FOM

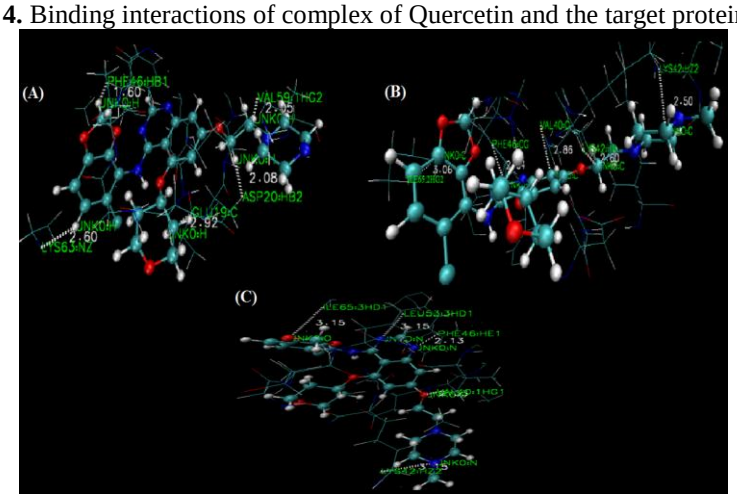

Figure 5. (A) Hydrogen bond interactions of Quercetin (lead compound) with the active site of protein 2FOM (B) Hydrophobic interactions of Quercetin (lead compound) with the active site of protein 2FOM (C) Ionic interactions of Quercetin (lead compound) with the active site of protein 2FOM

1.8 Analog Designing:

Seven analogs were made of Quercetin, lead compound, in order to get the possible active compounds to use as anti-dengue drug. Analogs of lead compound with their structures designed by using ChemBioDraw® Ultra 13.0. Analogs were designed by changing the functional groups in order to get an active compound on the basis of effectiveness. All the analogs were docked within the active site of 2FOM with the same procedure as

discussed earlier. The best confirmation was selected up on the least binding energy of analog in 10 conformations. These energies are shown in Table in 2. Each selected analog conformation was saved and then analyzed. The PDB file of protein with the best confirmation of the analog is opened in VMD software in order to get the interactions. The interactions of 7 analogs shown in Table 3.

1st analog of the lead compound consists of 3 hydrogen interactions, 6 hydrophobic interactions and 3 ionic interactions were found. 2nd analog of the lead compound consists of only a single hydrogen interaction, 6 hydrophobic interactions and 3 ionic interactions were found. 3rd analog of the lead compound consists of 2 hydrogen interactions, 2 hydrophobic interactions and 5 ionic interactions were found. 4th analog of the lead compound consists of 2 hydrogen interactions, 1 ionic interaction and 6 hydrophobic interactions were found. 5th analog of the lead compound consists of 1 hydrogen interaction, 4 hydrophobic interactions and 4 ionic interactions were found. 6th analog of the lead compound consists of 3 hydrogen interactions, 4 hydrophobic interactions and 3 ionic interactions were found. 7th analog of the lead compound consists of 3 hydrogen interactions, 3 hydrophobic interactions and 4 ionic interactions were found.

On the basis of interactions with the target protein 2FOM, analog 1 showed the maximum interactions with the target. Although all analogs show good interactions with the target, but high binding affinity is shown with the analog 1. Analog 1 showed three hydrogen interactions and ionic interactions and six hydrophobic interactions with lower distances within the 3.5 Å range of the binding pocket interactions. Therefore, analog 1 showed the good interactions with the target protein 2FOM.

1.9 ESTABLISHING QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP (QSAR):

QSAR studies were performed on the data of 28 compounds. In order to know that why some compounds show better signs of action and others do not, QSAR model was built and a set of descriptors were chosen. These were assumed to control whether a given compound will succeed or fail in binding to a given target. The descriptors of the data of the ligands chosen for QSAR studies include Molar Refractivity (MR) and hydrophobicity (LogP) (steric descriptors) and Hydration Energy (H_E). Molar Refractivity (MR) and hydrophobicity (LogP) were calculated by using ChemDraw. Refractivity and Hydration Energy (H_E) were calculated by using HyperChem. The values of the calculated descriptors were illustrated in Table 1.

In order to check the correlation of the ligand's activity with the chosen descriptor, some of calculated descriptors have been plotted against the IC50 value of the ligands. In each plot IC50 value has been taken along X-axis as independent variable while the particular descriptor has been taken along Y-axis as dependent entity. Regression value R2 was obtained for each plot in order to find out the correlation. In order to have the good correlation of activity with these descriptors the regression coefficient value should be greater than 0.6. If the value is less than 0.6, then there will be no correlation.

Results showed that if the regression correlation was 0.6 then there is no correlation between these descriptors i.e., the value is less than 0.6. The regression value was calculated upon the steric descriptors like LogP, Hydration Energy, Refractivity and MR against IC50 values of the compounds. The regression value of steric descriptor LogP is 0.0021, which has been shown in Figure 6(A). The regression value of steric descriptor Refractivity is 0.0013, which has been shown in Figure 6(B). The regression value of steric descriptor MR is 0.0046, which has been shown in Figure 6(C). The regression value of steric descriptor Hydration Energy is 0.0054, which has been shown in Figure 6(D).

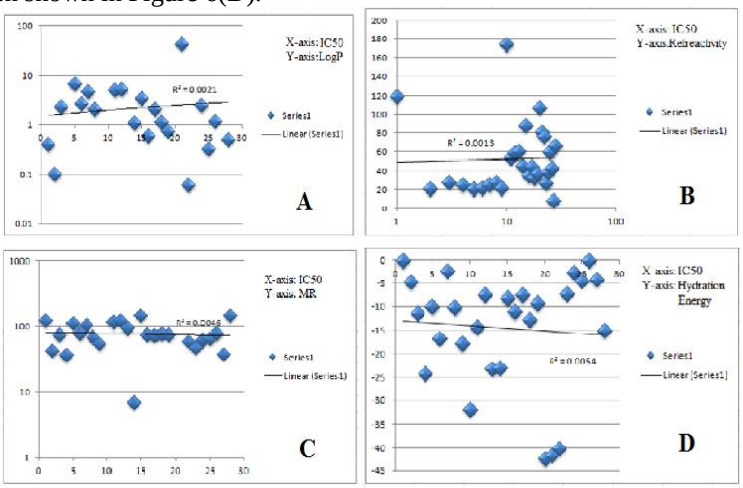

Figure 6. (A)Plot of half inhibitory concentration (IC50) and LogP (B) Plot of half inhibitory concentration (IC50) and Refractivity (C) Plot of half inhibitory concentration (IC50) and Molar Refractivity (MR) (D) Plot of half inhibitory concentration (IC50) and Hydration Energy (HE)

Table 1. Structure and QSAR Descriptors of the 26 test data compounds

NO.	Name	LogP	M_R (Cm^3/mol)	Hydration Energy (H_E) Kcal/mol	Hydrogen Bonding	Hydrophobic Interaction	Ionic Interaction
1.	1RTL	0.4	121.11	-0.17	0	10	0
2.	Acetaminophen	0.1	42	-4.86	0	5	3
3.	Alpinetin	2.3	75.3	-11.43	1	7	1
4.	Ascorbic acid	-3.36	36.61	-24.31	2	1	3
5.	Brequinar	6.58	109.21	-10.05	2	9	1
6.	Cardamonin	2.68	78.3	-16.79	2	5	2
7.	Chloroquine	4.7	104.51	-2.29	0	10	2
8.	Pinostrobin	2.02	69.86	-10.23	1	5	3
9.	Ribavirin	-2.48	54.87	-17.92	0	4	7
10.	Suramin	-0.12	-	-31.92	0	3	5
11.	4-Hydroxypanduratin A	4.96	117.4	-14.44	1	10	2
12.	Panduratin A	5.23	122.84	-7.58	0	9	3
13.	S-Adenosylmethionine	-1.03	95.44	-23.21	13	5	4
14.	S-Adenosyl-1-Homocysteine	1.09	6.88	-23.02	0	11	9
15.	Quinazolin-4-amine	3.33	146.82	-8.32	22	8	1
16.	Quercetin	0.58	75.43	-11.10	12	15	4
17.	Phloretin	2.05	72.82	-7.61	0	16	4
18.	Myricetin	1.12	77.14	-12.81	0	9	7
19.	Kaempferol	0.74	74.7	-9.36	0	7	4
20.	Guanosine-5-triphosphate	0.06	59.31	-40.22	8	0	13
21.	Castanospermine	-2.08	46.48	-7.42	1	8	3
22.	9-10-Anthraquinone	2.42	62.75	-2.66	0	10	0
23.	6-O-butanoyl castanospermine	0.32	65.17	-4.57	12	6	0
24.	N-nonyl-deoxynojirimycin	1.18	79.29	-0.11	0	12	0
25.	N-acetylcysteine	-1	37.7	-4.32	0	6	2
26.	Pyrocyanidin B2	0.5	145.6	-15.11	0	7	5

Table 2. Conformational energy values of analogs

Analog No.	Binding Energy	Ligand Efficiency	Torsional Energy	Intermolecular Energy
1.	-5.3	-0.23	1.19	-6.49
2.	-5.72	-0.26	1.19	-6.91
3.	-4.64	-0.21	1.49	-6.13
4.	-4.82	-0.22	1.19	-6.01
5.	-5.08	-0.23	1.19	-6.27
6.	-4.63	-0.21	1.79	-6.42
7.	-5.28	-0.24	1.19	-6.47

Table 3. Binding interactions of analogues with amino acids present within 5 Å Radius

Analog No.	Hydrogen Bonding	Hydrophobic interactions	Ionic bonding
1.	H-ASN105:OD1[1.82] H-LEU74:O[2.12] H-ILE76:CD1[3.15]	C-ARG55:2HH1[3.12] C-ARG55:NH1[3.52] C-GLU19:OE1[3.33] C-ARG55:O[3.48] C-LYS87:CD[3.56] C-ILE76:N[3.19]	O-ARG24:NH2[3.52] O-ILE76:HN[2.95] O-ALA56:O[3.41]
2.	H-ASN105:O[2.14]	C-ASN88:OD1[2.99] C-ASN88:O[3.12] C-ILE73:O[3.32] C-ARG107:N[3.54] C-ASN105:CB[3.53] C-ILE73:N[3.48]	O-ASN105:2HD2[2.03] O-ASN88:CB[3.22] O-LEU74:CA[3.00]
3.	H-VAL52:O[1.97] H-ALA49:O[2.06]	C-ALA56:HN[3.37] C-ALA49:CB[3.17]	O-ALE36:CD1[2.93] O-THR53:O[3.46] O-LEU47:CD2[2.95] O-GLU48:OE1[3.53] O-THR53:CA[3.49]
4.	H-GLY54:O[2.39]	C-ALA70:O[3.09]	O-TYR79:CZ[3.38]

	H-SER68:N[2.58]	C-ALA70:N[3.24] C-ALA70:CB[3.51] C-ALE77:CG1[3.29] C-ALE77:CD1[3.02] C-GLU66:C[3.49]	
5.	H-VAL59:CB[3.06]	C-LYS145:HZ2[3.37] C-ASP20:O[3.46] C-ASP20:CB[3.10] C-ASP20:OD2[3.57]	O-VAL146:CG2[3.06] O-LYS145:CD[2.99] O-VAL59:HN[2.94] O-ARG55:IHH2[2.56]
6.	H-GLU54:OE2[1.95] H-ASP58:OD2[1.89] H-ASP58:CB[3.40]	C-ARG55:O[3.27] C-ILE76:O[3.48] C-ILE76:N[3.32] C-ALA56:CA[3.32]	O-LYS87:NZ[2.79] O-ASP58:CG[3.34] O-ALA56:O[2.82]
7.	H-LEU85:CD2[3.47] H-VAL146:CG2[2.92] H-GLY21:HN[1.85]	C-GLY144:O[3.16] C-VAL59:HN[2.85] C-ARG55:NH2[3.21]	O-VAL146:CG2[3.36] O-VAL140:CG1[3.22] O-ARG55:2HH2[2.94]

IV. Conclusions

The study was intended to find novel drug molecules as anti-dengue compounds using the structure-based drug design technique. 2FOM protein was used as a target for the purpose of finding novel drugs. About 26 drugs were selected as ligands for this study that showed activity against the dengue virus. Each ligand with the target, the complexes formed were analyzed based on their energy values and their binding affinities with the target. Based on the results of this analysis, a lead compound was identified. Seven different analogs were then designed of the selected lead compound by doing chemical modifications in their structures and were docked again for finding the best candidate of anti-DENV agent. These docked analog complexes analyzed upon their interaction such as hydrogen, ionic and hydrophobic interactions. All the analogs showed good interactions with the target protein 2FOM but the analog 1 showed the best interaction with the target. Hence the analog 1 can be proposed as the best out of all analogs and can be suggested as drug for future research.

Quantitative structure activity relationship (QSAR) was established successively to find the dependency trends in anti-dengue inhibitors against the descriptors. It showed the dependency of activity of drugs, against the dengue virus, based on various descriptors. Different steric and electronic descriptors were used to analyze the relationship of drug's activity with its structure. This kind of approach also helps in designing novel drug with the greater efficiency and resisting property against the DENV.

The desired aim of this study was achieved by employing the structure based drug designing which provided the best analog that could serve as a potent agent against dengue virus. The significance of the present study is clearly reflected by the identification of seven highly potent analog compounds, with one best, as dengue virus inhibitors. The lead compound identified and analogs that are constructed to improve the efficacies of the drugs, in combating the problem of resistance acquired by the virus. The suggested compounds can provide valuable information for persistent search, discovery and design of novel potent antiviral agents against dengue virus.

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