WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 352-361. Research Article ISSN 2277 – 7105

IN SILICO APPROACHES FOR DRUG DESIGNING AND PREDICTING ADMET PROPERTIES OF DRUGS AGAINST DENGUE FEVER

Arpit Trivedi[1,2], Pallavi Gangwar[*2], Ankur Mohan[2], S.M. Dave[1]

[1]Department of Biotechnology, Hemchandracharya North Gujarat University, Patan, Gujarat, India.

[2]Department of Life Sciences, Bio-EGICORE, Lucknow, U.P, India

Article Received on
10 July 2014,

Revised on 04 August 2014,
Accepted on 29 August 2014

***Correspondence for Author**
Pallavi Gangwar
Department of Biotechnology, Hemchandracharya North Gujarat University, Patan, Gujarat, India

ABSTRACT

Dengue fever which is a mosquito-borne tropical disease caused by the dengue virus and also known as breakbone fever. Symptoms include fever, headache, muscle and joint pains, and a characteristic skin rash that is similar to measles. Dengue Virus contains Structural and nonstructural proteins. Among all these proteins Non-Structural protein named is responsible for replication of dengue virus. The 3D structure of NS5 RNA dependent RNA polymerase was downloaded from PDB (2J7U). NS5 RNA dependent RNA Polymerase contains ligand named Tri Ethylene Glycol, which was selected as a drug target candidate. Compounds were downloaded from the various compound databases and screened on the basis related structures and properties with Tri Ethylene Glycol using similarity score. The properties like Common Name, IUPAC name, Melting Point, Boiling Point, pKa Value, Polarizability, Molecular Weight, Solubility, Density etc. were tabulated. Docking was performed on the structure files of the compounds by using docking software named Argus Lab. ADMET properties of chemical compounds were analyzed by Online Database named Danish QSAR database. After reviewing physical, chemical properties, docking and ADMET analysis of all the screened chemical compounds the following chemical compounds were confirmed as drug candidates for Dengue Virus. Ethanol, 2- 2-(2-ethoxyethoxy)ethoxy – (DB02078), Ethanol, 2-butoxy – (CID57448621), Ethanol, 2-(2-methoxyethoxy)- -(CID57999328), Diethylene Glycol – (CID60096730), 1,1'-oxybis- ,Ethoxy Ethane, - (D01772), D-Xylose – (D06346), Ethanol, 2-ethoxy- (DB01749), Ethanol, 2-methoxy- - (DB02806).

KEY WORDS: Dengue, breakbone fever, NS5 RNA dependent RNA polymerase, Tri Ethylene Glycol, ADMET

INTRODUCTION

Dengue fever, a very old disease, has reemerged in the past 20 years with an expanded geographic distribution of the viruses and the mosquito vectors, increased epidemic activity, the development of hyper endemicity and the emergence of dengue hemorrhagic fever in new geographic regions [1], [2]. In 1998 this mosquito-borne disease is the most important tropical infectious disease after malaria, with an estimated 100 million cases of dengue fever, 500,000 cases of dengue hemorrhagic fever, and 25,000 deaths annually. The reasons for this recovery and appearance of dengue hemorrhagic fever in the vanishing years of the 20th century are complex and not fully understood, but demographic, societal, and public health infrastructure changes in the past 30 years have contributed greatly [2], [3].

Dengue viruses replicate in cells of mononuclear phagocyte lineage, and sub neutralizing concentrations of dengue antibody enhance dengue virus infection in these cells [4].This antibody-dependent enhancement of infection regulates dengue disease in human beings, although disease severity may also be controlled genetically, possibly by permitting and restricting the growth of virus in monocytes. [5], [6] Monoclonal antibodies show heterogeneous distribution of antigenic epitopes ondengue viruses. These epitopes serve to regulate disease: when antibodies to shared antigens partially neutralize heterotypic virus, infection and disease are dampened; enhancing antibodies alone result in sensitive disease response. [6] Further knowledge of the structure of dengue genomes should permit rapid advances in understanding the pathogenetic mechanisms of dengue. Dengue virus is an emerging global health threat. Its major envelope glycoprotein, E, mediates viral attachment and entry by membrane fusion. A crystal structure of the soluble ecto domain of E from dengue virus type 2 reveals a hydrophobic pocket lined by residues that influence the pH threshold for fusion. The pocket, which accepts a hydrophobic ligand, opens and closes through a conformational shift in a beta-hairpin at the interface between two domains. [7]

One serotype of Dengue virus (DENV) is the reason of dengue fever. Dengue virus (DENV) has four serotypes. DENV is a mosquito-borne single positive-stranded RNA virus of the family Flaviviridae; genus Flavivirus. All four serotypes can cause the full spectrum of disease. [1], [5] Dengue virus can cause fever of different severity; it can even direct to complex situation such as dengue hemorrhagic fever. When the different strains of dengue

virus interacts with the immune system of different person it can cause complex interaction resulting in conditions such as dengue hemorrhagic fever or dengue shock syndrome. These features point to a structural pathway for the fusion-activating transition and suggest a strategy for finding small-molecule inhibitors of dengue and other flaviviruses. Dengue virus is a single-stranded, enveloped RNA virus that productively infects human dendritic cells (DCs) primarily at the immature stage of their differentiation. All four serotypes of dengue use DC-SIGN (CD209), a C-type lectin, to infect dendritic cells. THP-1 cells become susceptible to dengue infection after transfection of DC-specific ICAM-3 12grabbing non integrin (DC-SIGN), or its homologue L-SIGN, whereas the infection of dendritic cells is blocked by anti-DC-SIGN antibodies and not by antibodies to other molecules on these cells [8]. Viruses produced by dendritic cells are infectious for DC-SIGN- and L-SIGN-bearing THP-1 cells and other permissive cell lines. Therefore, DC-SIGN may be considered as a new target for designing therapies that block dengue infection. Dengue, a major public health problem throughout subtropical and tropical regions, is an acute infectious disease characterized by biphasic fever, headache, and pain in various parts of the body, prostration, rash, lymphadenopathy, and leukopenia. [9] In more severe or complicated dengue, patients present with a severe febrile illness characterized by abnormalities of hemostasis and increased vascular permeability, which in some instances results in a hypovolemic shock. Four distinct serotypes of the dengue virus exist, with numerous virus strains found worldwide. Dengue fever (DF)/dengue haemorrhagic fever (DHF) is the most common arthropod-borne viral infection, where it is now estimated that 2.5-3 billion people world-wide are at risk of infection. [10] Currently there is no available treatment, in the form of vaccine or drug, making eradication of the mosquito vector the only viable control measure, which has proved costly and of limited success.

New potential targets for drugs are emerging. One of the most promising is the dengue non-structural protein 5 (NS5), the largest and most highly conserved of the dengue proteins. [11] Dengue fever, a neglected emerging disease for which no vaccine or antiviral agents exist at present, is caused by dengue virus, a member of the Flavivirus genus, which includes several important human pathogens, such as yellow fever and West Nile viruses. The NS5 protein from dengue virus is bifunctional and contains 900 amino acids. The S-adenosyl methionine transferase activity resides within its N-terminal domain and residues 270 to 900 from the RNA-dependent RNA polymerase (RdRp) catalytic domain. Viral replication begins with the synthesis of minus-strand RNA from the dengue virus positive-strand RNA genome, which is

subsequently used as a template for synthesizing additional plus-strand RNA genomes. [12], [13]. This essential function for the production of new viral particles is catalyzed by the NS5 RdRp. A high-throughput in vitro assay partly recapitulating this activity and the crystallographic structure of an enzymatically active fragment of the dengue virus RdRp refined at 1.85-Å resolution. The NS5 nuclear localization sequences, previously thought to fold into a separate domain, form an integral part of the polymerase subdomains. [13] The structure also tells the presence of two zinc ion binding motifs. In the absence of a template strand, a chain-terminating nucleoside analogue binds to the priming loop site. These results should inform and accelerate the structure-based design of antiviral compounds against dengue virus. [14], [15], [16]

MATERIALS AND METHODOLOGY

Target Identification

Dengue Virus contains Structural and nonstructural proteins. Among all these proteins Non-Structural protein named NS5 RNA dependent RNA polymerase is responsible for replication of dengue virus. [5] Hence NS5 RNA dependent RNA polymerase was selected for the drug designing of Dengue Virus from Protein Data Bank (Figure: 1) which is submitted through x-ray diffraction method. The experimental detail shows, Resolution [Å]: 1.85, R-Value: 0.201 (obs.) and R-Free: 0.234. NS5 RNA dependent RNA Polymerase contains ligand named Tri Ethylene Glycol. Tri Ethylene Glycol was selected as a drug target candidate.

Drug Library

Chemical databases across the web were used to obtain compounds. Compounds were screened which had related structures and properties with Tri Ethylene Glycol using similarity score. The properties like Common Name, IUPAC name, Melting Point, Boiling Point, pKa Value, Polarizability, Molecular Weight, Solubility, Density etc. were tabulated. [17], [18]

Docking

Structure files of these chemical compounds were downloaded from the Chemical compound database. Docking was performed on the structure files of the compounds by using docking software named Argus Lab. Binding energy of almost forty compounds was calculated by the software. These chemical compounds were used for the ADMET analysis. [19]

ADMET

ADMET properties of docked chemical compounds were analyzed in dry lab experiment. ADMET properties of chemical compounds were analyzed by Online Database named Danish QSAR database. [20] ADMET properties include Carcinogenicity, Mutagenicity, Arylhydroxylase Activity, Estrogenicity, Multicase Acute Aquatic Toxicity, Lethal Body Burden, Bioconcentration, Biodegradation, Environmental Partitioning and General Properties etc. Thirty compounds have confirmed ADMET properties using software but out of these thirty compounds only eight compounds lastly fulfill maximum of the characteristics of drug target candidates. Hence Physico Chemical Properties and ADMET of those eight compounds have been shown in the result tables (Table:1 & Table:2).

RESULTS AND DISCUSSION

In this work total 50 chemical compounds were screened as Drug Target Candidate for Dengue Virus. Chemical compounds analyzed and screened for drug target candidates but some compounds do not fulfill any of the characteristics of drug target candidates. Hence chemical compounds were screened for their similarity with Tri Ethylene Glycol, the drug candidate for the Target Protein. Chemical and physical properties were taken as per chemical database (shown in Table 1). These properties were analyzed as for each and every drug, chemical properties like pka value, Molecular weight, state, Polarizibility, Melting Point, and Boiling Point etc. play role in selecting drug candidate chemicals.

A weak acid has a pKa value in the approximate range between -2 to 12. And pKa values more than 12 are alkaline drug. Acid with a pKa value of less about -2 are said to be a strong acid. Similarity Score indicates relevance of the new chemical with the chemical which can fit to the drug target pocket.

Polarizability shows the ability of a molecule to be polar, which depends upon the Charge distribution and effects the bond formation in a chemical compound.Docking results show Lower binding energy show the higher affinity of the Drug target candidate. The normal range of Binding energy should be between –5 to -9 Kcal/Mol. [13]. Each and Every drug must have been passed through its ADMET properties. Among all their properties Mutagenicity, Toxicity, In vitro Test and Carcinogenicity are valuable properties of drug target candidates to be analyzed. Ideally these properties are analyzed by certain tests like Ames test, FDA-CDER properties on RAT and Mouse, Lethal Concentration, Lethal Dose in human, HGPRT test etc. These properties can be predicted using ADMET software.

Severe skin irritation is caused by chemical substances that directly irritate the skin and leads to the symptoms like slight burn, redness, itching, pain etc. In severe skin irritation CID57448621, CID57999328, CID60096730, D01772 D06346, DB01749, DB02806, DB02078 show negative effect in skin irritation, it means they do not acquire skin irritation properties hence these compounds can be considered as drug target candidates.

In skin sensitization CID57448621, CID57999328, CID60096730, D01772, D06346, DB01749, DB02806, DB02078 show negative effect in skin sensitization hence can be considered as drug target candidates. Respiratory sensitizer is a substance that will induce respiratory hypersensitivity. In Respiratory Sensitization CID57999328, DB01749, DB02078 show positive effect on respiratory sensitization hence these compounds have to be modified before treating them as drug target candidates while CID57448621, CID57999328, CID60096730, D01772, D06346, DB02806 show negative effect in respiratory sensitization hence can be considered as drug target candidates.

Hypoxanthine Guanine Phospho Ribosyl Transferase (HGPRT) is an In vitro test of the chemical compound which is done in order to know the Mutagenic nature of the chemical compound. CID57448621, CID57999328, CID60096730, D01772, D06346, DB01749, DB02806, DB02078 show negative HGPRT test thus can be taken in lights as drug targets.

Mutagenicity of the Drug can be checked by performing Ames Test. Positive result shows that the drug will be the Mutagenic. DB01749, DB02078 chemical compounds show Positive Ames test, hence they are Mutagenic and can only be considered as drug target candidates after changing their molecular structure.

Compounds other than these CID57448621, CID57999328, CID60096730, D01772 D06346, DB02806 etc. show negative Ames Test, hence they are not mutagenic and can consider as drug target candidates. Carcinogenicity of the drug is determined by certain tests like FDA Cancer Male Rat, FDA Cancer Female Rat, FDA Cancer Male Mouse, FDA Cancer Fem. Mouse, -CDER Proprietary MR, -CDER Proprietary FR, -CDER Proprietary MM, -CDER Proprietary FM. Positive result shows that the drug will be the Carcinogen. CID57448621 chemical compounds show Positive Carcinogenicity hence they can only be considered as drug target candidates after modification in the structure. Compounds other than these DB01749, DB02078, CID57999328, CID60096730, D01772 D06346, DB02806 etc. show negative Carcinogenicity hence they are not Carcinogen and can consider as drug target

candidates. Lethal dose is one way to measure the short term poisoning potential of the material. Lethal concentration values refer to the concentration of the Chemical. This toxicity is settled on Lethal Concentration of the Drug. Lethal Concentration range 100 – 1000mg/L shows moderately toxicity of Compound and on the basis of that concentration Lethal Dose is decided as 0.5-5 gm/kg. If lethal concentration range is 10 -100 mg/L then it shows highly toxicity of the compound and thus Lethal Dose should be 5-50 mg /kg. If lethal concentration range is 1000 – 10000 mg/L then it shows slightly toxicity of the compounds and lethal dose is decided as 5 – 15 gm/ kg.

Here screened all Eight compounds have lethal concentration range 100 – 1000 mg/L hence they are moderately toxic and their lethal dose should be 0.5 – 5 gm/Kg. After reviewing physical, chemical properties, docking and ADMET analysis of all the screened chemical compounds we confirm following chemical compounds as drug candidates for Dengue Virus. Ethanol, 2- 2-(2-ethoxyethoxy)ethoxy – (DB02078), Ethanol, 2-butoxy – (CID57448621), Ethanol, 2-(2-methoxyethoxy)- -(CID57999328), Diethylene Glycol – (CID60096730), 1,1'-oxybis- ,Ethoxy Ethane, - (D01772), D-Xylose – (D06346), Ethanol, 2-ethoxy- (DB 01749), Ethanol, 2-methoxy- - (DB02806), Chemical Compounds other than these are lacking in above criteria. Hence those chemical compounds cannot be used in present form as drug candidate for Dengue Virus.

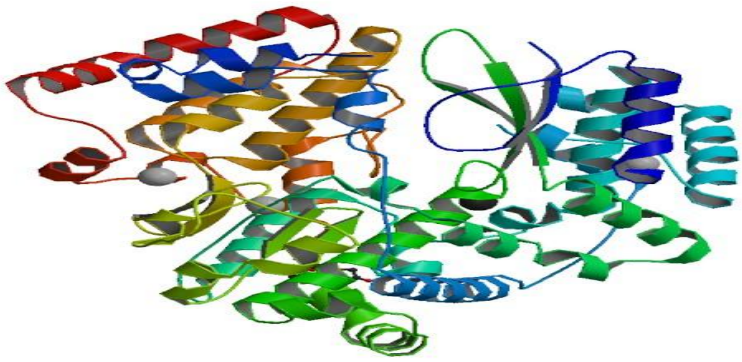

Figure 1: 3D structure of NS5 RNA dependent RNA polymerase (PDB ID: 2J7U)

Table: 1 Physical and Chemical Properties of Chemical Compounds

	Binding Energy	Pka value	Polarizability ($10^{-24}cm^3$)	Mol.Weight (gm/mol)	Boiling Point(°C)	Melting Point(°C)	State	LogP
(DB02078)	-3.91	12.3	20.81	178.22	216	-45	Solid	-0.016
CID57448621)	-5.25	13.14	13.132	118.18	167.689	-79	NA	0.797
(CID57999328)	-4.37	15.24	11.988	120.15	194	-70	Liquid	-1.156
CID60096730)	-4.77	14.26	10.068	106.12	245-246	-10	Solid	-1.513
(D01772)	-4.94	NA	8.851	74.12	34.6	-116.3	NA	1.041

(D06346)	-5.56	12.14	12.454	150.12	415.462	153-154	NA	-2.116
(DB 01749)	-4.4	13.02	10.3	90.12	82-83	-58	Solid	0.078
(DB02806)	-4.73	12.12	8.22	76.09	124.1	-85.1	Solid	-0.57

Table 2: ADMET Properties

	Health End Point			**Mutagenicity**	**In Vitro Tests**	**Carcinogenicity**
	Severe skin irritation	**Skin sensitization**	**Respiratory sensitization**	**Ames test (Salmonella)**	**HGPRT**	
(DB02078)	NEG	NEG	POS	POS	NEG	NEG
(CID57448621)	NEG	NEG	NEG	NEG	NEG	POS
(CID57999328)	NEG	NEG	POS	NEG	NEG	NEG
(CID60096730)	NEG	NEG	NEG	NEG	NEG	NEG
(D01772)	NEG	NEG	NEG	NEG	NEG	NEG
(D06346)	NEG	NEG	NEG	NEG	NEG	NEG
(DB 01749)	NEG	NEG	POS	POS	NEG	NEG
(DB02806)	NEG	NEG	NEG	NEG	NEG	NEG

(* POS = POSITIVE)

(* NEG =NEGATIVE)

SUMMARY AND CONCLUSION

Dengue Virus protein responsible for Virus replication into Host Cell, RNA Dependent RNA Polymerase was identified as Drug Target protein and structure of protein (PDB format) was downloaded from the Protein Data Bank. Ligand Tri Ethylene Glycol of RNA Dependent RNA Polymerase was obtained for further analysis. Similar structure search of different chemical compounds was done which were regards to Tri Ethylene Glycol and fifty structures were obtained with their physical and chemical properties. Further Docking and Screening were performed. ADMET study was done at the end of ADMET analysis only thirty Chemical compounds were found to affect Protein Target. Further all parameters of those thirty chemical compounds were analyzed and following eight chemical compounds confirm their potential as drug target candidates. Ethanol, 2- 2-(2-ethoxyethoxy)ethoxy – (DB02078), Ethanol, 2-butoxy – (CID57448621), Ethanol, 2-(2-methoxyethoxy)- -(CID57999328), Diethylene Glycol – (CID60096730), 1,1'-oxybis- ,Ethoxy Ethane, - (D01772), D-Xylose – (D06346), Ethanol, 2-ethoxy- (DB 01749), Ethanol, 2-methoxy- - (DB02806). Thus it can be concluded that above eight chemical compounds show great affinity against Dengue Virus Protein NS5 RNA Dependent RNA polymerase. Hence these eight chemical compounds can be further analyzed as Drug target candidates for Dengue fever disease in Wet lab for the Production of new synthesis of Drug against Dengue fever Disease.

REFERENCES

1. Mukhopadhyay, S. Kuhn, R.J. and Rossmann, M.G. (August 2005). "A structural perspective of the Flavivirus life cycle." Nature Reviews Microbiology 3: 13-22.
2. Whitehead, S.S. Blaney, J.E. Durbin, A.P. and Murphy, B.R. (May 2007). "Prospects for a dengue virus vaccine." Nature Reviews Microbiology 5: 518-528.
3. Halstead, S.B. (Jan 2007). Dengue. Lancet 370: 1644-1652.
4. Qi, R.F. Zhang, L. and Chi, C.W. (March 2008). "Biological characteristics of dengue virus and potential targets for drug design." Acta Biochimica at Biophysica Sinica 40: 91-101.
5. Rodenhuis-Zybert, I.A. Wilschut, J. and Smit, J.M. (August 2010). "Dengue virus life cycle: viral and host factors modulating infectivity". Cell. Mol. Life Sci.67 (16): 2773–86.
6. Mazzon, M. M. Jones, A. Davidson, B. Chain, and M. Jacobs. (June 2009). "Dengue virus NS5 inhibits interferon-alpha signaling by blocking signal transducer and activator of transcription 2 phosphorylation. J." Infect. Dis. 200:1261–1270.
7. Ackermann M, Padmanabhan R. et al. (April 2001). "De novo synthesis of RNA by the dengue virus RNA-dependent RNA polymerase exhibits temperature dependence at the initiation but not elongation phase." J Biol Chem; 276(43):39926–39937.
8. Ago, H. Adachi, T. Yoshida, A. et al. (October 1999). "Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus." Structure; 7(11):1417–1426.
9. Alvarez, D.E. De Lella Ezcurra, A.L. Fucito, S. et al. (December 2005). "Role of RNA structures present at the 3'UTR of dengue virus on translation, RNA synthesis, and viral replication." Virology; 339(2):200–212.
10. Richard, J. K. Zhang, W.et. al. (June 2010). "Structure of dengue virus: implications for flavivirus organization, maturation, and fusion.";100 (12):6986-91
11. Gao, L. Tu, H. Shi, S.T. et al. (2003). "Interaction with a ubiquitin-like protein enhances the ubiquitination and degradation of hepatitis C virus RNA-dependent RNA polymerase." J Virol; 77(7):4149–4159.
12. Garcia-Montalvo, B.M. Medina, F. Angel, R.M. (2004). "Protein binds to NS5 and NS3 and to the 5' and 3' ends of Dengue 4 virus RNA." Virus Res; 102(2):141–150.
13. Yorgo, M. Steven, O. (Apr 2003). "A ligand-binding pocket in the dengue virus envelope glycoprotein." 7;197 (7):823-9
14. Grun, J.B. Brinton, M.A. (1987). "Dissociation of NS5 from cell fractions containing West Nile virus-specific polymerase activity." J Virol; 61: 3641–3644.

15. Ago, H. Adachi, T. Yoshida, A. et al. (1999). "Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus." Structure; 7(11):1417–1426.
16. De Azevedo, W.F. (2010). "Structure-based virtual screening." Drug Targets, 11, 261-3.
17. Timmers, L.F. Pauli, I. Caceres, R.A. Azevedo, W.F. (2008). "Drug-Binding Databases." Drug Targets, 9(12):1092-9.
18. Dias, R. De Azevedo, W.F. (2008). "Molecular docking algorithms." Drug Targets, 9, 1040-7.
19. Yang, J.M. Chen, C.C. (2004). "DOCK: a generic evolutionary method for molecular docking." Proteins. 55:288-304.
20. Bissantz, C. Folkers, G. Rognan, D. (2000). "Protein-based virtual screening of chemical databases: Evaluation of different docking/scoring combinations and drug ADMET properties." J. Med. Chem., 43: 4759-67.