Research Paper

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Discovery of Novel Drugs for Treatment of Dengue Fever;2'-O-Methyl Transferase as Drug Target.

Medical Science

KEYWORDS : Dengue, Drug, Docking, Pharmacophore.

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ABSTRACT

Dengue fever is an epidemic and the leading cause of illness and death in tropical and subtropical parts of the world (Centres for Disease Control and Prevention [CDC], March 2015). Every year approximately 400 million people are infected (CDC). So there is an urgent need to develop effective drugs for dengue. In this work, an attempt has been made on developing inhibitors for RNA cap (nucleoside-2'-o-)-methyltransferase, an important enzyme required for replication of virus; thus preventing serious effects of infection. The target protein (1L9K) structure was downloaded from PDB and any inconsistencies in the sequence were checked and ruled out by BLAST p. Later we have screened ligands from Pub chem based on IC_{50}(http://en.wikipedia.org/wiki/IC50)values and Lipinski rule of five (http://en.wikipedia.org/wiki/Lipinski%27s_rule_of_five). Their binding site on target was identified and binding energies have been calculated by AUTO DOCK .This resulted in different models having lowest binding energies for which pharmacophore models were designed by Discovery Studio 4.0 client. Later they were overlapped to build a robust pharmacon. Our results show that RNA cap (nucleoside-2'-o-)-methyltransferase can be used as a potent target and our procedure may serve as a reference protocol for developing therapeutic strategies for emerging diseases.

INTRODUCTION:

Dengue fever is an arthropod-borne viral malady occuring in South-east Asia, the Pacific and the Americas (http://en.wikipedia.org/wiki/Dengue_fever). Illness is caused by dengue virus which is a member of genus *Flavivirus* and family *Flaviviridae*. It is transmitted by *Aedes aegypti* mosquito which commonly breeds in artificial water holders. It is typically characterized by fever, rash, and muscle and joint pains and may lead to severe forms like Dengue Hemorhagic fever and Dengue Shock Syndrome which can be lethal. Therefore it is pivotal to develop effective drugs that can cure this in initial stages itself. Although there are no specific drugs available for its treatment, a number of drug targets are being investigated.

TOOLS & TECHNIQUES:

Retrieval of data:

The target protein RNA cap (nucleoside-2'-o-)-methyltransferase (PDB Id:1L9K) was selected from PDB (Protein Data Bank) based on certain criteria like function, presence of complete domain structure, Molecular weight, Resolution and R values and its Fasta format was downloaded. Metal ions were removed manually from the file. BLASTp was performed and then the missing amino-acids were added by SWISS-PDBViewer; the resultant protein structure was energy minimized using Gromos96 and was validated by Ramachandran plot. Active site prediction was also done using 3Dligandsite.

Ligands were screened from Pub Chem database and those having IC_{50} (inhibitory constant) values less than 50μM were chosen and they are further filtered based on Lipinski rule of five.

The following table(Table 1) shows ten ligands that were selected:

Feature	ZINC 02911543	ZINC 03287466	ZINC 01312283	ZINC 01667812	ZINC 00633950	ZINC 00632055	ZINC08264240	ZINC 01226903	ZINC03183875	ZINC 03174529
Mol.wt. (g/mol)	356.41	424.44	397.89	389.90	432.41	420.43	432.41	464.51	466.20	490.52
Mol.Formula	$C_{20}H_{20}N_4O_5$	$C_{24}H_{24}N_4O_4$	$C_{21}H_{23}ClN_4O_3$	$C_{21}H_{21}N_3O_4S$	$C_{25}H_{24}N_4O_4$	$C_{24}H_{24}N_4O_4$	$C_{25}H_{24}N_4O_4S$	$C_{28}H_{32}N_4O_4$	$C_{24}H_{26}N_4O_5$	$C_{26}H_{26}N_4O_5$
XlogP3	4.3	5	5	5	4.3	2.8	2.1	2.9	4	4.2
H-bond donor	4	2	2	2	1	4	2	2	2	2
H-bond acceptor	6	5	5	3	6	6	6	7	6	6
Kia (μM)	7.54	6.01	13.16	37.46	17.04	20.32	16.09	21.31	11.30	6.03

Table 1: list of ligands downloaded from PubChem database

Pre-installation of software:

Different software required for docking are pre-installed in the computer like Python, MGL tools, Autodock 4.2v, and Pymol. Like wise, Discovery Studio 4.2 was installed for Pharmacophore modelling.

Docking:

Autogrid and Autodock were performed for all the ligands against drug target and minimum binding energies were calculated. Later different models were constructed based on these values and stored under unique files which were ranked according to their global minimization energy values, the first ranked one having lowest energy. These models were opened using PyMol and orientation of each ligand with target is visualized and interactions of different Amino Acids were noted.

Active site Prediction:

The active site of the drug target was predicted by using 3Dligand site, an online web server which accepts our query and the residues taking part were shown. Alternatively, Oxygen maps built can also be used to predict.

Pharmacophore Modelling:

These models were drawn manually for top two ranked models by Discovery Studio 4.0. The three features selected were Hydrogen bong donor, H bond acceptor and Aromatic Rings. Active Pharmaceutical Ingredient was found by overlapping the above 2 models. A robust pharmacon was built which contains desired

chemical entities and rest were removed.

RESUL5TS & DISCUSSION:

In the current endeavour, an effort has been made to find novel and potent inhibitors for target protein RNA cap (nucleoside-2'-o-)-methyltransferase, which is an important enzyme of DENV required during its transcription.

The target protein's FASTA format downloaded from PDB is

>gi|29726395|pdb|1L9K|A Chain A, Dengue Methyltransferase

MRGSHHHHHHGSNIGETLGEKWKSRLNALGKSEFQIYKKS-GIQEVDRTLAKEGIKRGETDHHAVSRGSAKLRWFVERNLVT-PEGKVVDLGCGRGGWSYYCGGLKNVREVKGLTKGGPGHEE-PIPMSTYGWNLVRLQSGVDVFFIPPERCDTLLCDIGESSP-NPTVEAGRTLRVLNLVENWLSNNTQFCVKVLNPYMSSVIEK-MEALQRKHGGALVRNPLSRNSTHEMYWVSNASGNIVSSVN-MISRMLINRFTMRHKKATYEPDVDLGSGTRNIGIESETPNLDIIG-KRIEKIKQEHETSWHYDQ

BLASTp's result (figure 1) predicts that the protein didn't undergo any changes or variations as the complete graphical result is in red.



Figure 1: BLASTp result.

Missing amino-acids were added using swiss pdb viewer and the amino acids affected were shown in characteristic pink color in the following figure(Figure 2),

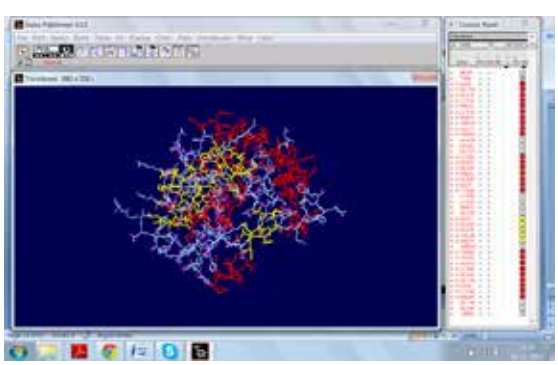

Figure 2: structure of Protein in Deep View after adding missing Amino-acids

Addition of missing Amino acids makes the structure unstable. Hence energy was minimized using GROMOS96 and Ramachandran plot(Figure 3) was plotted to validate the protein structure. It is observed that amino acids forming right handed alpha-helix were present in fourth quadrant and those forming beta sheets were present in the second quadrant indicating that most of them were present in allowable regions proving it is a very good model and has a stable protein structure.

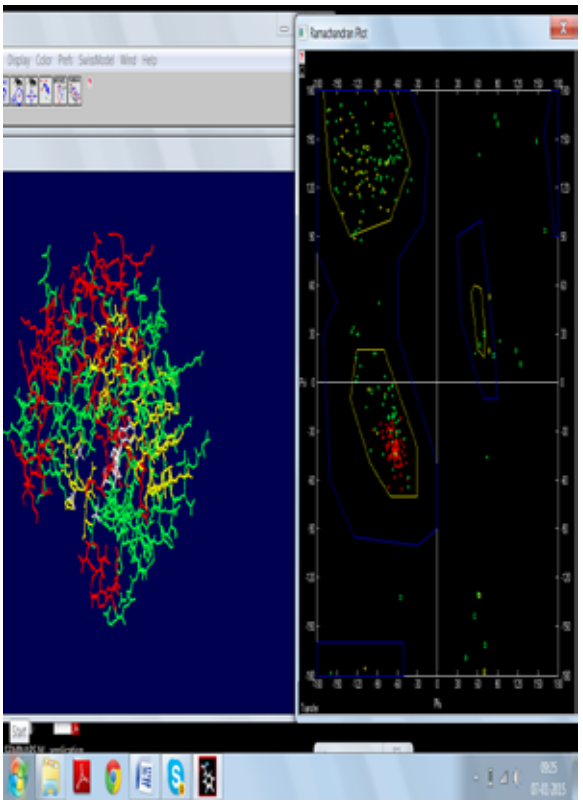

Figure 3: Ramachandran Plot

Active site was predicted using 3Dligandsite server and the result is shown below (Figure 4). The residues Serine, Glycine, Arginine, Tryptophan, Lysine, Histidine, Valine, Threonine, Aspartate, Phenylalanine, Isoleucine and heteroatoms like SAM and SAH were forming binding site.



Figure 4: Result of Active site prediction by 3dligandsite.

Docking was done with all the ten ligands and their pose and interactions were observed. The following table (table 2) shows the list of ligands with their binding energies in docking.

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S.No	Ligand	Binding energy (KCal/Mol)
1	ZINC 02911543 (Alig)	-6.03
2	ZINC 03287966 (Blig)	-5.97
3	ZINC 01112283 (Clig)	-3.98
4	ZINC 01467812 (Dlig)	-4.89
5	**ZINC 00633950 (Klig)**	**-9.65**
6	ZINC 00632055 (LLIGND)	-4.16
7	**ZINC 02642996 (HLIG)**	**-7.28**
8	ZINC 01226983 (GLIG)	-5.08
9	ZINC 01138375 (xlig)	-6.32
10	ZINC 01174529 (ylig)	-6.77

Table 2:List of ligands and their binding energies.
It can be seen that ZINC 00633950 and ZINC 02642996 had least values i.e., -9.65 Kcal/Mol and -7.28 Kcal/Mol respectively. So they were selected for studying Aminoacid interactions. The following figure (Figure 5) shows binding of ZINC00633950 with target and amino acids interacting were Glutamic acid, Glycine, Histidine. Hetero atoms SAH and SO_4 and hydrogen bond were also seen.

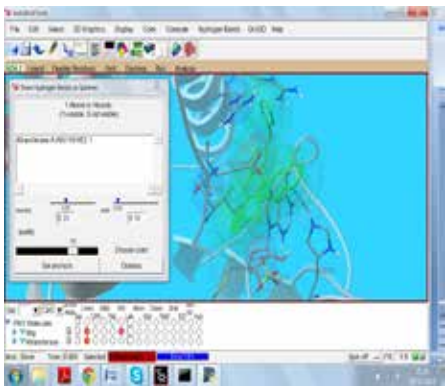

Figure 5: Interactions of ZINC00633950 with drug target.
The following figure (Figure 6) shows binding of ZINC 02642996 and the amino acids Proline, phenylalanine, Asparagine, Serine, Lysine, Leucine were seen interacting with the ligand.

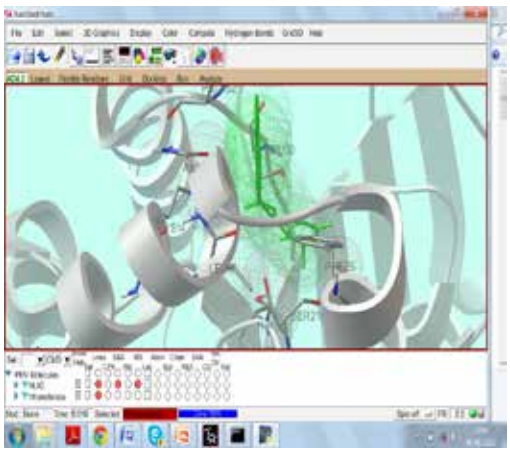

Figure 6: Interactions of ZINC 02642996 with drug target.

Pharmacophore model was built for those ligands which have got the least binding energies.

The following figure (Figure 7) shows pharmacophore model of ZINC 00633950,



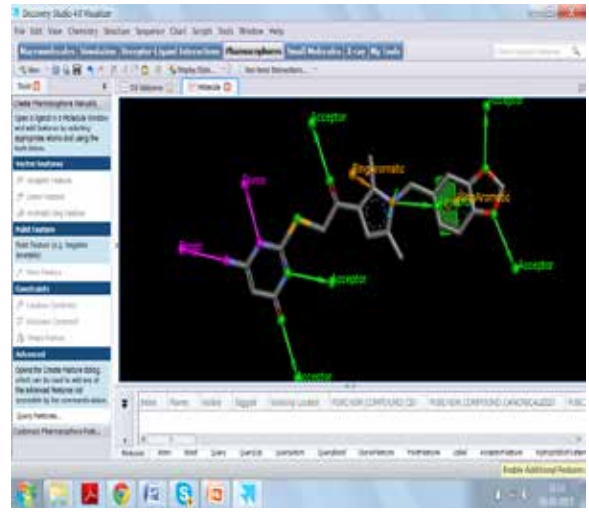

Figure 7: Pharmacophore model of ZINC 00633950.
The following figure (Figure 8) shows pharmacophore model of ZINC 02642996,

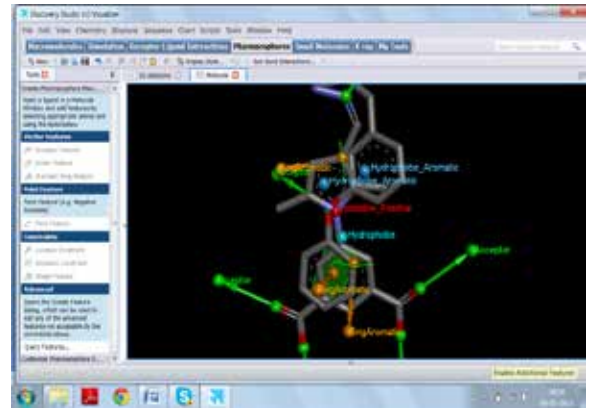

Figure 8: Pharmacophore model of ZINC 02642996.

Finally the two pharmacophore models were superimposed to find out pharmacon (Figure 9) which is actually responsible for biological action.

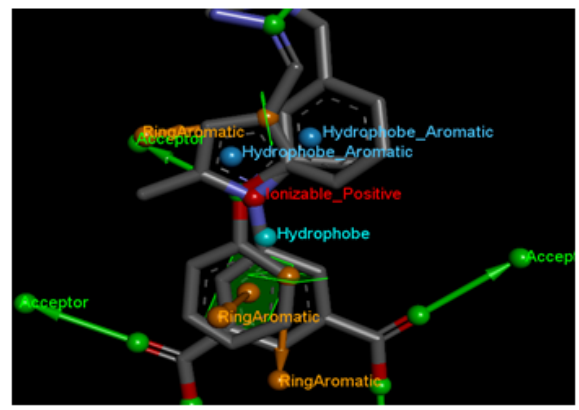

Figure 9: Pharmacon designed against 2'-O-methyltransferase of dengue virus.

CONCLUSION:
Dengue Virus is a global concern due to its epidemic and endemic activities and has conquered more than 100 countries. It is vectored primarily by *Aedes aegypti* and currently there is

no vaccine or treatment.

Viral genome contain RNA dependent RNA polymerases (RdRps) required for replication of ssRNA and often these are highly regulated by RNA elements (Supanee *et al.,* 2014). Likewise, NS5 region of Dengue genome contain 2'-O-methyl-transferase activity in the initial 296 Amino acids (Davidson, A. D. **2009**). This enzyme methylate 5' viral RNA cap at guanine N-7 and ribose 2'-O positions; a dual methylation essential for synthesis of viral cap (Kathryn A Massana, *et al.*2010). In this work, it is shown that the selected ligands (ZINC 00633950 and ZINC 02642996) have the ability to bind to the active site of target protein and disrupt the process of methylation, which is lethal to the virus, thus inhibiting the viral infection. These results provide a structural basis for the rational drug design against not only the dengue fever and but also other emerging flavivirus infections. They may certainly act against other diseases caused by RNA viruses like Hepatitis, etc.

Therapeutic strategies involving Computer aided Drug Design has become a boon in present day scenario of science and technology. These *in silico* models exactly mimic the real models without undergoing elaborate procedures, thus saving a lot of time and money.

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