Original Article

Identification of Possible Inhibitor Molecule against NS5 MTase and RdRp Protein of Dengue Virus in Saudi Arabia

Afaf S. Alwabli[1,2,*], Ishtiaq Qadri[1]

[1]Department of Biological Sciences, Rabigh College of Science and Arts, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[2]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, SAUDI ARABIA.

ABSTRACT

Background: Non-structural protein 5 (NS5) is considered as an important protein in Dengue viruses (DENVs). It contains N-terminal methyltransferase (MTase) and C-terminal RNA-dependent RNA polymerase (RdRp) domains. NS5 plays a crucial role in the replication of Dengue viruses. Therefore it is considered as an attractive candidate for the development of therapeutics in anti-viral infections and diseases. **Aim:** The aim of proposed *in-silico* study was to to screen and identify potential lead molecules with drug like properties to inhibit the activity of NS5 protein in Dengue virus infections. **Materials and Methods:** Computational bioinformatics analysis was implemented to identify the lead molecules with inhibition activity against MTase and RdRp domains of Dengue virus protein NS5. Phytochemicals and active bioassay compounds were screened based on their reported antiviral mactivities. A total of fifty-one natural compounds and one hundred active compounds were selected by evaluating eighty one bioassay studies. **Results:** Results of the current study revealed galactomannan, galactan, hyperside, carrageenan, tetrahydroxy, lamdacarragenon, zosteric acid, trihydroxy, quercetin and sulfoximine as top inhibitors of the Dengue virus NS5 protein. Lead molecules namely lamdacarragenon, carrageenan, balsacanea, galactan, trihydroxy, hyperside, myricetin, glycyrrhiza, isosilandrin, rhodiola and silyhermin showed the utmost hydrogen bonding. Overall, we observed that the bioassay active compounds showed less interaction with the MTase and RdRp domains of NS5 protein as compared to natural ligands. **Conclusion:** Based on the findings of current study, we concluded that the phytochemicals are the most favourable among the docked molecules that showed a significant inhibitory activity against the MTase and RdRp domains of NS5 protein of dengue viruses. We suggest that these lead molecules can be validated experimentally and implemented for the development of therapeutics in viral infections like Dengue.

Key words: Dengue virus, Molecular docking, Molecular structure, MTase, NS5, Non-structural viral proteins, RdRp.

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Correspondence:
Dr. Afaf S. Alwabli
Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah-21589, SAUDI ARABIA.
Phone: +966 966 531937700
E-mail: afafalwabli@yahoo.com

INTRODUCTION

Flaviviridae is a virus family responsible to cause viral infections and diseases in mammals including humans. Arthropods namely mosquitoes and ticks are the main vectors. There are more than 70 different viruses in Flaviviridae family. These viruses contain positive single-stranded RNA and are enveloped. These viruses cause various types of the infections such as dengue infection, Japanese encephalitis infection, tick-borne encephalitis infection, West Nile infection, yellow fever infection and Zika infection. The greater part of these infections is far-reaching morbidity and mortality.[1,2]The infections caused by Dengue viruses in tropical and subtropical areas are natural. The adverse effects caused by these Dengue virus infections on human health are rising exponentially throughout the globe.[3] It has been estimated that

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globally around 390 million individuals are infected every year by these dengue viruses. Out of these cases around 100 million individuals are associated with clinical dengue reflecting varying degree of illness.[3] Initially the Dengue virus infected person will be having dengue fever. Then along the time scale the person will develop haemorrhagic fever and then dengue shock syndrome. DENVs have five serotypes namely DENV-1, DENV-2, DENV-3, DENV-4 and DENV-5.[4,5] Not with standing the massive burden of DENV infection in humans, neither antibody nor any antiviral medication is available to treat the infection.

The genome of DENV encodes three structural proteins that are called as capsid, layer and envelope proteins. These viruses contain seven non-structural proteins named as NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.[6] Among all DENV proteins, NS5 is the large stand most conserved protein with 65% amino acids sequence similarity among DENV serotypes. NS5 of Dengue virus is comprised of two domains namely N-terminal MTase and C-terminal RdRp. Both domains are crucial for life cycle of dengue viruses.[7] These two domains are connected with a flexible linker.[8] DENV-RdRp during its polymerase activity betray a conformational change from a 'closed' initiation complex attached to single-stranded RNA to an 'open' elongation complex attached to double-stranded RNA. The RdRp domain consists of seven conserved motifs, in which the sections of motifs F and G are absent from the apo-structure of RdRp. Many dengue RdRp structures have been resolved, although only in closed conformation bound to a ligand.[9-11] Current investigation used bioinformatics analysis tools to generate a thorough structure of dengue RNA-dependent RNA polymerase. Moreover, docking studies were performed for screening of effective DENV inhibitors. We hope that present study will provide the basis for the structure based identification and development of target-explicit inhibitors against DENV-RdRp.

METHODOLOGY

Basic models of dengue infection MTase and RdRp region

The X-ray crystallography structure of proteins MTase and RdRp of dengue virus were retrieved from the PDB database. This incorporates dengue methyltransferase bound to SAM-based inhibitors at a resolution of 1.7 Å (PDB: 3P8Z; UniProt KB AC: P27915)[12] and dengue 3 NS5 protein with inhibitor at 1.99Å resolution (PDB: 5JJR; UniProt KB AC: Q6YMS4). Conserved hydrophobic pockets for both areas have been shown in Figure 1(A) and 1(B).

Protein preparation

The PDB structures of both MTase and RdRp region were imported in CLC Drug discovery work bench. These imported proteins were assigned with polar atomic partial charges, hydrogens and atom types. The assigned structures were named as PDBQT. The PDBQT file was further refined with the hydrogen bond assessment (neutral pH, water orientations) and MMFF94 force field was used as energy minimization. Grid was generated around the selected residues of MTase and RdRp domains of the processed NS5 target protein. Crystal structure of dengue MTase-NS5 bound to a SAM-based inhibitor and RdRp inhibitor are shown in Figure 2 (A) and 2 (B).

Ligand preparation

A total of hundred active bioassays compounds were selected on the basis of eighty one studies submitted in PubChem. These active compounds were selected based on inhibition score of 15 (Primary CPE Inhibition @ 20μM). Compounds explicitly reported as active by ChEMBL or the compounds which showed activity at ≤ 50uM were flagged as active in this PubChem assay presentation. Natural compounds were selected based on best binders from seven studies. The 2D structures of these compounds were retrieved from PubChem. All these compounds were docked against MTase and RdRp domains of NS5 to explore their binding affinity. Ligand preparation was done by using MMFF94 geometry operation and Gasteiger charge was calculated at pH 7.0. Ligand preparation

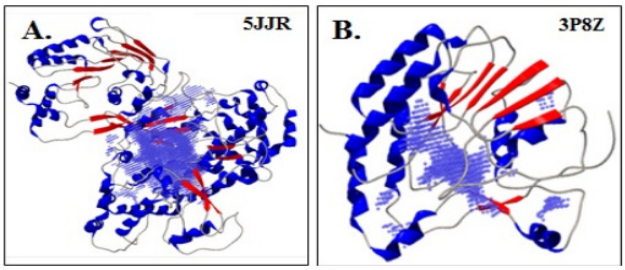

Figure 1: Conserved hydrophobic pockets for both MTase and RdRp region of Dengue virus. A. MTase region (5JJR)- beta sheets is denoted in red colour and helical structure is denoted in blue colour, hydrophobic region is denoted in sphere. B. RdRp region (2P8Z)- beta sheets is denoted in red colour and helical structure id denoted in blue colour, hydrophobic region is denoted in sphere.

was applied using program named "Balloon". This program is primarily used for the generation of 3D structures in the workbench. All the selected structures were imported as SDF format in CLC Drug Discovery Workbench.

Pharmacokinetics structure-activity relationship

All the selected ligands were evaluated for their physiochemical properties and explored for their relationship with biological activities. Absorption, distribution, metabolism and excretion-toxicity (ADMET properties) predict organic expected responses based on chemical structures. Further, the selected compounds were evaluated for the developmental toxicity, mutagenicity model, carcinogenicity model, fast biodegradability and log P physiochemical characteristics. SDF files obtained from the PubChem were used as input formats.

Active site residues

The active binding sites for the MTase-NS5 were explored. The NS5 protein contains catalytic sites such as K61, D146, K180 and E216 in motif positioned in the centre of the □-sheet. The contact residues located in the C-terminal RdRp adopt the canonical right-hand polymerase. Also, the palm subdomain contains a G662-D663-D664 catalytic metal-binding motif.

Docking protocol

Molecular docking was carried out by using CLC Genomic Workbench. PDBQT ligand file generated using AMBER force field. Hydrogen atoms were added to the PDBQT ligand file. Docking wizard implemented with default Molecular Docking optimizer algorithm. The docking parameters for the docking wizard included: (i) maximum iterations of two thousand, (ii) two hundred runs for each ligand, (iii) root-mean square deviation thresholds for similar cluster poses of 1.00, (iv) crossover rate of 0.90 and (v) scaling factor of 0.50. The best lead compounds were selected based on hydrogen interaction and docking score and were visualized by CLC Drug Discovery Visualization tool.

RESULTS

Pharmacokinetics Structure-activity relationship of selected ligands

Results evaluated by ADMET properties were enough to determine the chemical properties of the selected lead compounds. The ADMET properties for natural compounds and active bioassay compounds are represented in Supplementary Table 1.

Molecular interaction of natural dengue antivirals with MTase protein

In our computational docking analysis, fifty natural dengue antivirals were docked with MTase protein. From these natural antiviral molecules, only ten natural ligands showed multiple hydrogen bonds and electrostatic interactions with the active binding residues. The top inhibitors included galactomannan, galactan, hyperside, carrageenan, tetrahydroxy, lamdacarragenon, zosteric acid, trihydroxy, quercetin and sulfoxycin. Further, the five best binding confirmations with MTase protein (PDB ID: 5JJR) are shown in Figure 3. Galactomannan emerged as the best binder by interacting with active side residues of MTase extending 13 hydrogen bonds characterized by interaction energy of -35.39 Kcal/mol. The residues that participate in interactions are LYS357, THR539,

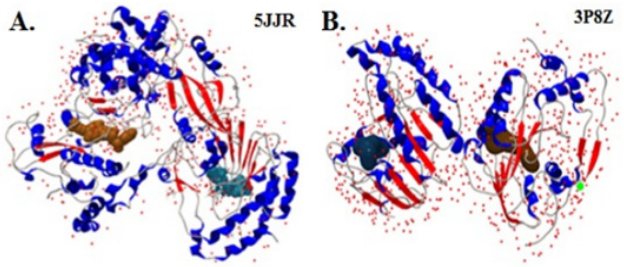

Figure 2: Crystal structure of Dengue MTase NS5 bound to a SAM-based inhibitor and RdRp inhibitors of Dengue virus. A. MTase region (5JJR)-beta sheets is denoted in red colour and helical structure id denoted in denoted in blue colour, inhibitor are brown and dark blue sphere shape. B. RdRp region (2P8Z)-beta sheets is denoted in red colour and helical structure id denoted in blue colour. Crystal structure of dengue MTase-NS5 bound to a SAM-based inhibitor and RdRp.

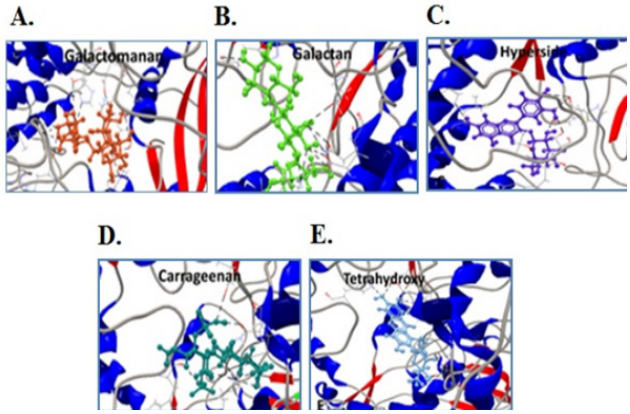

Figure 3: A-E. The five best binding confirmation with MTase protein.

GLU356 (2), ASP256, HIS52, THR51, ARG540, ALA259, ILE691 (3) and ARG688).

Likewise, galactan formed second best binder with 10 hydrogen bonds extending interaction towards residues such as GLU507, ASN492 (3), GLU493 (2), LYS401 (3) and HIS800. The interaction energy for second best binder was obtained as -35.22 Kcal/mol. Other binder among top ten formed an average of best interaction energy of -34.22 Kcal/mol, which includes natural extract ligands such as hyperside, carrageenan, tetrahydroxy and lamdacarragenon. Zosteric acid and trihydroxy formed equal number of seven hydrogen bonds with an interaction energy -24.40 Kcal/mol and -31.39 Kcal/mol respectively. The information on hydrogen bonding, interaction energy and number of amino acid residues involved in interaction of ligand with MTase domain of Ns5 is presented in Table 2.

Molecular interaction of natural dengue antivirals with RdRp protein

Next, we evaluated the interaction of these ligands with RdRp domain of Ns5 target protein. Results revealed lamdacarragenon, carrageenan, balsacanea, galactan, trihydroxy, hyperside, myricetin, glycyrrhiso, isosilandrin, rhodiolin and silyhermin as top ten binders of RdRp domain reflecting maximum number of hydrogen bonds. The five best binding confirmations with RdRp protein are shown in Figure 4. Natural ligand lamda carragenon exhibited 14 hydrogen bonds with RdRp protein with minimum interaction energy of -15.49 Kcal/mol with active residues namely VAL55, ARG38, GLU111, LYS42, LYS29 (2), GLY148 (2), LYS180 (2), SER150 (2) and ARG211 (2). Next, the compound carrageenan potentially formed 13 hydrogen bonds with residues namely GLY81, CYS82, GLY85, GLY86, SER56, LYS180, GLY148, GLU216, TYR218 (3) and LYS61 (2). The interaction energy for this complex was -13.44 Kcal/mol. Further, balsacanea, galactan and trihydroxy formed 9 hydrogen bonds with an average interaction energy of 11.35 Kcal/mol. Eight hydrogen bonds were formed by hyperside with interaction energy of -9.43 Kcal/mol and myricetin with interaction energy of -10.48Kcal/mol. isosilandrin, rhodiolin, glycyrrhiso and silyhermin equally formed seven hydrogen bonds, however the interaction energy of isosilandrin was slightly higher (-7.33 Kcal/mol) compared to average interaction energy of others (-9.34 Kcal/mol). The information on hydrogen bonding, interaction energy and number of amino acid residues involved in interaction of ligand with RdRp domain of Ns5 is presented in Supplementary Table 1.

Molecular interaction of active bioassay compounds with MTase and RdRp region

Results of the current study revealed that active bioassay compounds showed less molecular interaction with target protein as compared to natural ligands. Molecular docking study between bioassay active compounds and MTase region and RdRp region is shown in Table 3. Only bioassay active compound CID49799036 formed nine hydrogen bonds with MTase region with an interaction energy of -34.34 Kcal/mol with residues namely SER56(2), GLY86, GLY85, GLY148(2), LYS180, ASP131 and PHE133. It was followed by CID54681617 that formed six hydrogen bonds interacting with residues ASP146 (2), GLY81, LYS180 and ARG163, with interaction energy of -23.44 Kcal/mol. CID3062316 and CID129069 extended four hydrogen bonds with an interaction energy of -22.44 Kcal/mol and -19.44 Kcal/mol, respectively.

Further we observed a very few interactions of active bioassay compounds with RdRp domain as compared to MTase domain of Ns5 protein. The top two binders include CID54710332 extending five hydrogen bonds with residues CYS709 (2), SER710 (2), ARG729, with an interaction energy of -29.42 Kcal/mol and CID49799036 extending four hydrogen bonds with residues MET342, MET340, GLN339 and ALA316, with an interaction energy of -24.39 Kcal/mol. Rest of the bioassay hit binders targeted towards RdRp region formed only two or less hydrogen bonds with an average interaction energy of -15.47 Kcal/mol. The information on hydrogen bonding, interaction energy and number of amino acid residues involved in interaction of natural ligand with MTase domain

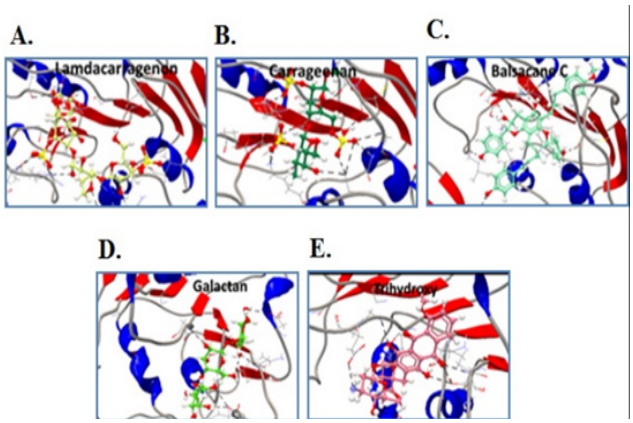

Figure 4: A-E. The five best binding confirmation with RdRp protein.

Table 1: Molecular docking study between natural dengue antiviral and RdRp region and their intermolecular docking values presented with interaction energy, number of hydrogen bonds and the interacting residues.

S.No	Natural drugs	Number of HBonds	Interaction energy (Kcal/mol)	RESIDUES (Rdrp region)
1	Galactomanan	13	-35.39	LYS357, THR539, GLU356(2), ASP256, HIS52, THR51, ARG540, ALA259, ILE691(3), ARG688
2	Galactan	10	-35.22	GLU507, ASN492(3), GLU493(2), LYS401(3), HIS800
3	Hyperside	9	-35.94	SYS709, HIS798(2), SER796, THR794(2), TRP795, TRP762, LEU734
4	Carrageenan	9	-34.78	LYS401(5), GLU493, ASN492, GLU507, TRP795
5	Tetrahydroxy	8	-33.45	HIS798, TYR606, SER661, ASP663, TYR606, GLY604, GLN602, SER600
6	Lamdacarragenon	8	-34.34	THR793, THR794(2), CYS709, SER710, ASP663(3)
7	Zosteric acid	7	-24.40	GLN602, THR605, TYR606(2), SYR796(2), ILE797
8	Trihydroxy	7	-31.39	MET342, ALA316, ARG352, LYS253, ASP254, GLU356(2)
9	Quercetin	6	-23.44	GLU356(2), ASP254, ASP344(2), ILE251
10	Sulfoxycinn	5	-32.44	TYR766(2), ARG729, HIS711, GLN802,
11	Methylglycrr	5	-21.33	GLN602, SER600, ILE797, SER661(2)
12	Kaempfrol	5	-13.39	ASP690, LYS689, GLY536, THR539, LYS357
13	Flemiflavone	5	-20.39	GLU733, ARG737, THR794, TYR766, SER796
14	Baicalein	4	-20.44	GLY601, GLN602(2), SER600
15	Solophenol	4	-17.33	SER710(2), ARG729, THR794
16	Myricetin	4	-9.43	ASP344(2), ARG352, ILE251
17	Fucoidan	4	-9.33	GLU356, ASP344, MET342, ARG352
18	Dehydro- 5-Carboxy	4	-8.43	SER710, SER661(2), ASP663
19	Balsacanea	3	-8.30	SER796(2), CYS709
20	Triterpene	3	-8.43	GLN339, MET342, LYS253
21	Silyhermin	3	-2.44	TRP795, GLY604, LYS401
22	Glycyrrhiso	3	-4.33	SER600, GLU493, GLY489
23	Glabranine	3	-5.32	TYR606, LYS401, GLU493
24	Castanospermine	3	-3.59	LYS253(2), MET 342
25	Balcone B	3	-3.24	LYS401, TRP795, ISN452
26	Euchrestaflavone	2	-2.44	ASN609, SER661
27	Aminoglycoside	2	-2.44	MET453, ASN452
28	Trigonoste	2	-2.55	SER710, ARG729
29	Aglycone	2	-2.94	TYR606, LYS401
30	Rhodiolin	2	-2.74	TRP795, SER796
31	Isosilandrin	2	-2.85	MET342, ASP344
32	Andrographiode	1	-2.49	ASP663
33	Umbelliprenin	1	-2.40	TRP418
34	Turmerone	1	-2.45	GLY607
35	Prenylmucronu	1	-2.84	LYS253
36	Pentamethoxy	1	-2.44	LYS401
37	Palmaline	1	-1.38	LYS253
38	Myrsellinol	1	-2.49	ASN 492
39	Methylneobava	1	-2.43	TRP795
40	Acetoxy	1	-1.38	LYS253
41	Licobenzofuran	1	-1.32	GLN693
42	Kanzonol	1	-1.33	TYR606

43	4-Hydroxypanduratin	1	-1.93	GLN602
44	Euchrestaflav	1	-1.21	ASP334
45	Chartaceone	1	-1.23	SER710
46	Balcone C	1	-1.11	THR794
47	7-0-Methylglabranine	1	-1.13	MET342
48	amentoflavone	1	-1.23	TYR606
49	ladanein	1	-1.24	ASP663
50	Nobelitin	1	-1.47	GLY607

Table 2: Molecular docking study between natural dengue antiviral and MTase region and their intermolecular docking values presented with interaction energy, number of hydrogen bonds and the interacting residues.

S.No.	Natural drugs	Number of HBonds	Interaction energy (Kcal/mol)	Residues (Mtase region)
1	Lamdacarragenon	14	-15.49	VAL55, ARG38, GLU111, LYS42, LYS29(2), GLY148(2), LYS180(2), SER150(2), ARG211(2)
2	Carrageenan	13	-13.44	GLY81, CYS82, GLY85, GLY86, SER56, LYS180, GLY148, GLU216, TYR218(3), LYS61(2)
3	Balsacanea	9	-12.34	LYS42, LEU209, GLU216(2), LYS61, LYS180, ASP146, CYS82, TRP87
4	Galactan	9	-11.45	ASP131, LYS105, THR104(2), GLY109(2), GLU111, GLY81, GLY148
5	Trihydroxy	9	-11.48	GLY109, GLU149, THR104(2), LYS105, GLY81, GLY148, ASP146(2)
6	Hyperside	8	-9.43	LYS130, ASP131, VAL132(2), ARG163, GLU149(2), GLY148, ASP146
7	Myricetin	8	-10.48	ASP 131, LYS130, LYS105, THR104, ASP146, GLY148(2), LYS180
8	Glycyrrhiso	7	-11.40	ASP131(2), LYS130, GLU111, THR104(2), LYS105
9	Isosilandrin	7	-7.33	ARG84, CYS82, GLY86, SER56, GLY85, LYS130, VAL132
10	Rhodiolin	7	-8.39	ASP131, LYS130, VAL132, GLU149, THR104, GLY148, ASP146
11	Silyhermin	7	-8.33	ASP131, LYS130, LYS105, THR104, GLY109, GLY148, ASP146
12	Aglycone	6	-7.44	ASP131(3), THR104, GLY148, LYS180
13	Castanospermine	6	-5.24	ASP37(2), GLU40(2), VAL255, ASP254
14	Euchrestaflav	6	-4.56	ASP131, ASP130, THR104, LYS105, GLY148, LYS180
15	Fucoidan	6	-7.53	LYS105, THR104(2), ASP146(2), LYS180
16	Kaempfrol	6	-8.43	LYS105, THR104, ASP146(2), ASP131, VAL132
17	Methylglycrr	6	-4.35	THR104, LYS105, ASP131, VAL132, ASP146, LYS180
18	Sulfoxycinn	6	-4.67	LYS130, VAL132, GLY148(2), LYS180(2)
19	Tetrahydroxy	6	-3.65	LYS130, VAL132(2), GLY148(2), LYS180
20	Aminoglycoside	5	-3.77	GLY149, THR104, ARG163(2), GLU149
21	Solophenol	5	-4.56	GLY148(2), LYS180(2), ASP146
22	Pentamethoxy	5	-3.55	THR104, GLY106, LYS105, GLU149, ARG163
23	Quercetin	5	-4.56	LYS130, ASP131, ASP146, GLY148, LYS180
24	Trigonoste	5	-4.44	THR104, ASP146(2), LYS180, GLU216
25	Zosteric acid	5	-3.56	LYS180(2), GLY148, LYS130, VAL132

26	Acetoxy	4	-4.86	ASP131, GLY109, LYS180, GLY148
27	Andrographiode	4	-5.43	GLU149, HIS110, GLY106, THR104
28	Euchrestaflavone	4	-1.55	THR104, LYS105, LYS130, ASP131
29	Flemiflavone	4	-2.14	THR104, LYS105, LYS130, ASP131
30	Galactomanan	4	-1.35	THR104, PHE133, ARG163, ASP146
31	Kanzonol	4	-1.87	THR104(2), GLY81, ASP146
32	Triterpene	4	-1.34	ARG38, SER56, GLY86, GLY85
33	Baicalein	3	-1.35	HIS110, THR104, LYS105
34	Chartaceone	3	-1.34	ARG160(2), GLU149
35	Prenylmucronu	3	-3.24	GLY148, ASP131, VAL132
36	7-0-Methylglabranine	2	-1.30	ASP131, LYS130
37	4-hydroxypandurain	2	1.34	LYS105, THR104
38	Balcone C	2	3.30	GLY109, ARG163
39	Balcone B	2	2.40	ARG163(2)
40	Dehydro- 5-Carboxy	2	-2.32	GLU149(2)
41	Glabranine	2	-3.23	THR104, VAL132
42	Licobenzofuran	2	-2.45	THR104, VAL132
43	Myrsellinol	2	-1.34	ASP131, HIS110
44	Methylneobava	1	-1.33	ASP131
45	Palmaline	1	-1.94	THR104
46	Turmerone	0	0	0
47	Umbelliprenin	0	0	0
48	amentoflavone	0	0	0
49	ladanein	0	0	0
50	Nobelitin	0	0	0
51	luteolin	0	0	0

Table 3: Molecular docking study between bioassay active compounds MTase region and RdRp region. The top five interactions with intermolecular docking values presented with interaction energy, number of hydrogen bonds and the interacting residues.

S. No	Bioassay active compounds	Number of HBonds	Interaction Energy (Kcal/mol)	Residues (Mtase region)
1	CID49799036	9	-34.34	SER56(2), GLY86, GLY85, GLY148(2), LYS180, ASP131, PHE133
2	CID54681617	6	-23.44	ASP146(2), GLY81, LYS180, ARG163
3	CID3062316	4	-22.44	HIS110, THR104(2), LYS105
4	CID129069	4	-19.34	THR104, LYS105, HIS110, VAL132
5	CID371439	3	-18.33	THR104, ARG163(2)
S. No	**Bioassay active compounds**	**Number of HBonds**	**Interaction Energy (Kcal/mol)**	**Residues (Rdrp region)**
1	CID54710332	5	-29.42	CYS709(2), SER710(2), ARG729
2	CID49799036	4	-24.39	MET342, MET340, GLN339, ALA316
3	CID54681617	2	-15.30	TRP795(2)
4	CID4144427	2	-15.93	TYR606(2)
5	CID54715399	2	-15.84	TRP795(2)

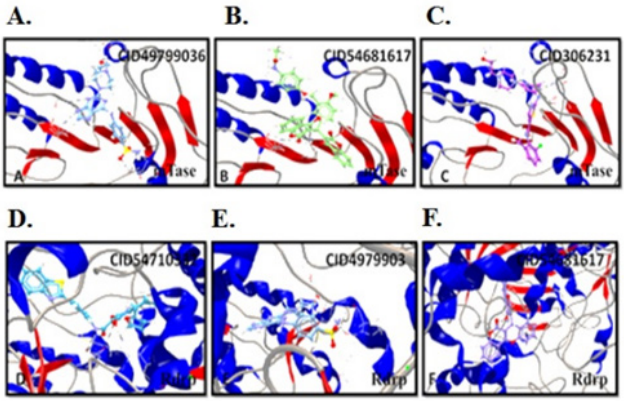

Figure 5: A-F. The five best binding confirmation with MTase and RdRp protein with bioassay hit compounds.

of Ns5 is presented in Table 3. The five best binding confirmations for MTase-NS5 and RdRp protein with bioassay-hit compounds are shown in Figure 5.

DISCUSSION

Non-structural proteins in Dengue viruses play an important role in viral replication and are crucial for the survival of viruses. These properties of non-structural proteins make them an eminent target candidate for the development of therapeutics in dengue virus related infections and diseases and many studies have highlighted them as promising anti-viral targets.[13] Several new functions of DENV non-structural proteins have been revealed by studies, thus opening opportunities for the identification of new anti-viral targets.[14] As large portion of the world population is infected with these viruses, reflecting that there is an urgent need for the identification and development of treatment for these infections. By keeping in mind the notions originated from previous research studies, we have to move on for the discovery of new and reliable treatment and cure for these Dengue virus related infections such as Dengue fever.

In the present *in-silico* study we screened different compounds for their potential to inhibit the NS5 protein of Dengue virus.[15-21] Different computational bioinformatics analysis tools[6] were implemented to identify the lead molecules with inhibition activity against MTase and RdRp domains of Dengue virus protein NS5. Phytochemicals and active bioassay compounds were screened based on their reported antiviral mactivities. A total of fifty-one natural compounds and one hundred active compounds were selected by evaluating eighty one bioassay studies. Results of the current study revealed galactomannan, galactan, hyperside, carrageenan, tetrahydroxy, lamdacarragenon, zosteric acid, trihydroxy, quercetin and sulfoximine as top inhibitors of the MTase protein. Lead molecules namely lamdacarragenon, carrageenan, balsacanea, galactan, trihydroxy, hyperside, myricetin, glycyrrhiza, isosilandrin, rhodiola and silyhermin showed the utmost hydrogen bonding. Overall, we observed that the bioassay active compounds showed less interaction with the MTase and RdRp domains of NS5 protein as compared to natural ligands.

CONCLUSION

Based on the findings of current study, we concluded that the phytochemicals are the most favourabl among the docked molecules that showed a significant inhibitory activity against the MTase and RdRp domains of NS5 protein of dengue viruses. We suggest that these lead molecules can be validated experimentally and implemented for the development of therapeutics in viral infections like Dengue

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

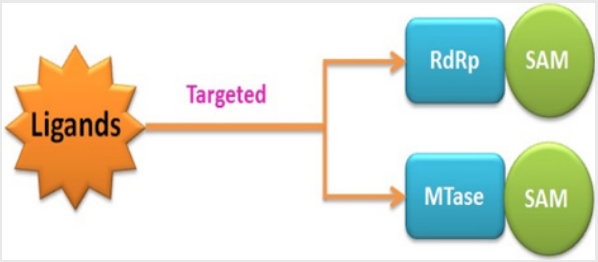
DEV: Dengue virus; **NS5:** Non-structural protein 5; **RdRp:** RNA-dependent RNA polymerase; **MTase:** Methyltransferase; **SAM:** S-Adenosyl Methionine.

REFERENCES

1. Solomon T, Mallewa M. Dengue and other emerging flaviviruses. Journal of Infection. 2001;42(2):104-15.
2. Han VDW, Gifford E. ADMET *in silico* modelling: Towards prediction paradise. Nature Reviews Drug Discovery. 2003;2(3):192-204.
3. Kuno G, Chang GJJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus Flavivirus. Journal of Virology. 1998;72(1):73-83.
4. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 2001;46(1-3):3-25.
5. Powers CN, Setzer WN. An *in-silico* investigation of phytochemicals as antiviral agents against dengue fever. Combinatorial Chemistry and High throughput Screening. 2016;19(7):516-36.

6. Lim SP, Noble CG, She CC, Soh TS, ElSahili A, Chan GKY, *et al*. Potent allosteric dengue virus NS5 polymerase inhibitors: Mechanism of action and resistance profiling. PLoS Pathogens. 2016;12(8):e1005737.
7. Klema VJ, Ye M, Hindupur A, Teramoto T, Gottipati K, Padmanabhan R, *et al*. Dengue virus non-structural protein 5 (NS5) assembles into a dimer with a unique methyltransferase and polymerase interface. PLoS Pathogens. 2016;12(2):e1005451.
8. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, *et al*. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerging Infectious Diseases. 2008;14(8):1232.
9. Noble CG, She CC, Chao AT, Shi PY. Ligand-bound structures of the dengue virus protease reveal the active conformation. Journal of Virology. 2012;86(1):438-46.
10. Noble CG, Shi PY. Structural biology of dengue virus enzymes: Towards rational design of therapeutics. Antiviral Research. 2012;96(2):115-26.
11. Lim SP, Sonntag LS, Noble C, Nilar SH, Ng RH, Zou G, *et al*. Small molecule inhibitors that selectively block dengue virus methyltransferase. Journal of Biological Chemistry. 2011;286(8):6233-40.
12. Norazharuddin H, Lai NS. Roles and Prospects of Dengue Virus Non-structural Proteins as Antiviral Targets: An Easy Digest. Malays J Med Sci. 2018;25(5):6-15.
13. Abbas EI, Sahili AE, Lescar J. Dengue Virus Non-Structural Protein 5. Viruses. 2017;9(4):91.
14. Sahu M, Sultan A, Barik MR. Molecular docking and high throughput screening of designed potent inhibitor to PTPN11 involved in Peptic Ulcer. South Asian J Exp Biol. 2016;6(4):07.
15. Sultan A. Identification and development of clock-modulating small molecules - an emerging approach to fine-tune the disrupted circadian clocks. Biological Rhythm Research. 2019;50(5):769-86.
16. Byrd CM, Grosenbach DW, Berhanu A, Dai D, Jones KF, Cardwell KB, *et al*. Novel benzoxazole inhibitor of dengue virus replication that targets the NS3 helicase. Antimicrob agents Chemother. 2013;57(4):1902-12.
17. Sampath A, Padmanabhan R. Molecular targets for flavivirus drug discovery. Antiviral Res. 2009;81(1):6-15.
18. Tomlinson SM, Watowich SJ. Anthracene-based inhibitors of dengue virus NS2B-NS3 protease. Antiviral Res. 2011;89(2):127-35.
19. Rothan HA, Han HC, Ramasamy TS, Othman S, Rahman NA, Yusof R. Inhibition of dengue NS2BNS3 protease and viral replication in vero cells by recombinant retrocyclin-1. BMC Infect Dis. 2012;12(1):1.
20. Nasir A, *et al*. Structural Insights into the Zika Virus NS1 Protein Inhibition Using a Computational Approach. Journal of Biomolecular Structure and Dynamics. 2020;38.
21. Nasir A, *et al*. Molecular dynamics simulation of Zika Virus NS5 RNA dependent RNA polymerase with selected novel Non-nucleoside inhibitors. Journal of Molecular Structure. 2019;127428.

PICTORIAL ABSTRACT



SUMMARY

Non-structural protein 5 is deliberated as a binding protein in Dengue viruses. It contains two major domains, such as MTase and RdRp. NS5 involved fruitfully in the replication of Dengue viruses. In this manuscript, we identified probable molecules with drug-like properties by using silico available tools. The basis of the result reveals that the screened molecule has inhibitory activity against the MTase and RdRp domains of the NS5 protein of dengue viruses. This identified molecule would be used in the future for therapeutics in Dengue disease.

About Authors

Dr. Afaf S. Alwabli was born in Jeddah, Saudi Arabia. She received Ph.D. in Microbiology from Department of Biological Sciences, Faculty of Science, King Abdulaziz University. Dissertation title is "The molecular characterization of NS methyltransferase domains of Dengue virus and its applications in the Kingdom of Saudi Arabia". She has published many papers in international refereed journals. Dr. Afaf S. Alwabli interests in Microbiology, .Virology, Bioinformatics, and Mathematical Biology.

Dr. Qadri is a molecular virologst with interests in the pathogenesis of viral diseases, antiviral and vaccine developments. Dr. Qadri is investigating the underlying molecular mechanism(s) that govern the onset of viral-induced diseases. The particular focus is on viral hepatitis (HBV & HCV), vector .borne viral diseases (Dengue), and MERS-CoV.

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