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Phenolic Lignan (PL) Derivatives For The Inhibition Of Dengue Viral Protease Using *In-Silico* Screening Studies.

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Abstract
Dengue infection has become a worldwide problem and infection rate is increasing each year. Dengue virus carries a positive single strand RNA, belongs to Flaviviridae family consists of four serotypes, give rise to undifferentiated fever(DF), dengue hemorrhagic fever(DHF) or dengue shock syndrome (DSS).Currently there is no licensed drug available for all serotypes and till date several vaccine candidates are still under development. Therefore, there is an urgent need of development of alternative solution for dengue. Dengue virus protease (NS2B-NS3pro) is essential for dengue virus infection and is thus a target of therapeutic interest. Therefore present study was designed to investigate Phenolic lignan derivatives based inhibitors against the dengue virus NS2/NS3 protease of serotype 1 and serotype 4 by in-silico docking approach. Study reveals that most of the Phenolic lignan derivatives bound to active site of dengue viral protease and had potential interactions with the catalytic residues. PL-4 showed potential interactions in both serotype 1(Binding energy-9.1kcal/mol) and serotype 4 (Binding energy-9.8kcal/mol) and hence these can serve as a potential drug candidates to stop viral replication. This finding has provided useful understanding of these compounds in developing potential inhibitor candidates for future studies in developing anti-dengue agents.

Keywords: DENV, Serine protease, molecular docking , Phenolic lignan derivatives

INTRODUCTION

Dengue virus (DENV), the causative agent of the disease dengue fever, is endemic in more than 110 countries, with approximately 390 million people infected yearly, leading to about 20,000 deaths [1-3]. Currently, no direct-acting antiviral drugs are available either in clinics or in development to combat dengue virus infections. Thus, a better understanding of the causative virus and potential viral drug targets is needed to develop effective therapies. DENV is a member of the family *Flaviviridae* and is an enveloped virus with a positive single-stranded RNA genome. There are four different serotypes (DENV1 to DENV4), and each serotype shares 65 to 70% sequence identity of the genome [4]. The dengue virus RNA genome encodes a single polyprotein, which needs to get processed at the cytoplasmic side of host cell rough endoplasmic reticulum membrane by dengue virus NS2B/NS3 protease and at the luminal side by the host cell peptidase [5]. Dengue virus NS2B/NS3 protease is a serine protease that belongs to the chymotrypsin family with a classic Ser-His-Asp catalytic triad [6]. NS2B (amino acids 1394 to 1440), which is referred to as a cofactor

(cNS2B), is required for the proper function of NS3 protease (NS3pro185; amino acids 1476 to 1660) [7] and participates in substrate recognition [8]. Dengue virus protease is responsible for the cleavage at 8 of the 13 polyprotein cleavage sites [9]. These cleavage steps are required for maturation of the viral particle, making dengue virus NS2B/NS3 protease a promising target for drug development.

The DENV genome contains a single open reading frame, which encodes the structural proteins capsid, membrane precursor (prM), and envelope and the nonstructural proteins NS1, NS2, NS3, NS4, and NS5 [10]. Cellular proteases and the viral serine protease (PR) are responsible for cleaving the viral precursor polyprotein into functional proteins. The DENV PR consists of the amino-terminal domain of the NS3 protein and requires NS2B, a 14-kDa protein, as a cofactor to form a stable complex. This heterodimeric PR cleaves at the capsid-prM, NS2A/NS2B, NS2B/NS3, NS3/NS4A, NS4B/NS5, internal NS2A, NS3, and NS4A cleavage sites [10, 11]. PR inhibitors (PIs) have been shown to be valuable antiviral drugs, especially in the treatment of HIV-1 and hepatitis C virus (HCV) [12–17]. In the case of DENV, some already published substances showed inhibition of 50% of DENV replication in a minireplicon system at a submicromolar range [18] or inhibition to 10% of DENV replication with wild-type virus at micromolar concentrations [19]. Despite these promising developments, antiviral DENV drugs are still not available.

Phenolic lignan (PL) is a hypolipidemic agent with antioxidant and anti-inflammatory properties. The antiviral effect of PL and its derivatives has been reported in several studies involving RNA viruses (HCV, HIV, SIV and Influenza virus [20] and DNA viruses (poxvirus, HSV and HPV) [21]. Currently, neither anti- viral treatments nor vaccines against DENV are available. Therefore, efforts directed to search new therapeutic targets and molecules that inhibit viral infection are required.

Thus, the aim of this work was to evaluate the anti-DENV effect of the Phenolic lignan (PL) and its derivatives against the active sites of the dengue viral protease 3L6P and 2VBC of serotypes 1 and 4 respectively.

MATERIALS AND METHODS

In the present study PL derivatives have been docked against Dengue virus NS2/NS3 protease 3L6P and 2VBC for serotypes 1 and 4 respectively.

Ligand preparation:

Chemical structures of PL derivatived were downloaded from Pubchem and the 2D structure of each of them selected was drawn using Chem Draw Ultra version 6.0.1 software and was saved in .cdx format. These 2D structures were converted into 3D structures and optimized by minimizing their energy using Chem 3D Ultra version 6.0 software and saved in .pdb format and were used as input file for opening in Auto Dock. The molecule was opened in PyRx software and was converted and saved in .pdbqt format, which was further used for docking.

Refinement of Receptor Protein

The 3D structure of Dengue virus NS2B/NS3 protease of serotypes 1 and 4 was retrieved from Protein Data Bank(PDB) using PDB ID: 3L6P and 2VBC respectively (http:/www.rcsb.org/pdb) in .pdb format. This was opened in Auto Dock and optimized by deleting the water molecule, removing the heteroatoms etc. and was saved in .pdbqt format. This minimized structure was used as receptor for docking studies.

The grid box (having conformation: centre x = -23.516, center y = -17.515, center z = 28.0691) was chosen for the protein 3L6P on their active sites (Ile-75 ,Arg-104, Gly-105,Pro-117,Trp-119, Asp-125, Tyr-129,Gly-130,Gly-131, Trp-133, Phe-135, Trp-139, Pro-152, Gln-160, Pro-163, Gly-174, Asp-179, Gly-183, Ser-185, Tyr-200, Gly-201, Asn-202, Gly-203, Val-212, Ser-213, Ile-215, Gln-217)

And the grid box (having conformation: centre x = -8.5165, center y = 7.8661, center z = -4.3302) was chosen for the protein 2VBC on their active sites(Asp-75, Lys-104, Asn-105, Lys-117, Leu-119, Ala-125, Asp-129, Phe-130, Lys-131, Gly-133, Ser-135, Ile-139, Asn-152, Asp-160, Ser-163, Pro-174, Asp-179, Phe-183, Lys-185, Thr-200, Lys-201, Arg-202, Ile-203, Leu-212, Lys-213, Arg-215, Arg-217)

Molecular Docking

Molecular docking of the PL derivatives with NS2B/NS3 protease was carried out by using AutoDock Tools and AutoDock vina. The best ranked model with low binding energy was analyzed further and visualized using Ligplot software.

RESULTS

PL derivatives were selected which have been showing antiviral effect , and inhibit viral proteases:
The 3D structure of the DENV proteases was retrieved from PDB. The PDB ID of 3D-structure were 3L6P and 2VBC respectively for serotype 1 and serotype 4. Molecular docking was performed for PL derivatives listed with both the serotypes. PL derivatives which showed least binding energy with highest stability were selected and subjected for ligplot analysis.

Table I: PL derivatives used in the study

Sl no	PL derivatives
1	PL-1
2	PL-2
3	PL-3
4	PL-4
5	PL-5
6	PL-6
7	PL-7
8	PL-8
9	PL-9
10	PL-10
11	PL-11

Results of docking study with 3L6P

Table II : PL derivatives library with 3L6P

Sl no	PL derivatives	Bond affinity (-kcal/mol)
1	PL-1	8.2
2	PL-2	9.0
3	PL-3	8.1
4	PL-4	9.1
5	PL-5	7.6
6	PL-6	6.5
7	PL-7	7.7
8	PL-8	8.5
9	PL-9	9.4
10	PL-10	8.1
11	PL-11	6.7

Results of docking study with 2VBC

Table III : PL drivatives library with 2VBC

Sl no	PL derivatives	Bond affinity (-kcal/mol)
1	PL-1	6.8
2	PL-2	8.4
3	PL-3	8.9
4	PL-4	9.8
5	PL-5	6.9
6	PL-6	6.7
7	PL-7	6.3
8	PL-8	7.7
9	PL-9	7.2
10	PL-10	5.7
11	PL-11	7.5

Ligplot analysis of lead molecules with 3L6P

LIGPLOT 1

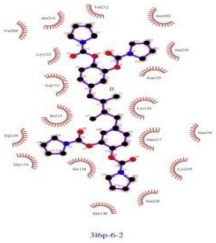

Figure 1: PL-9

LIGPLOT 2

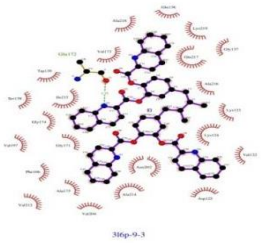

Figure 2: PL-4

LIGPLOT 3

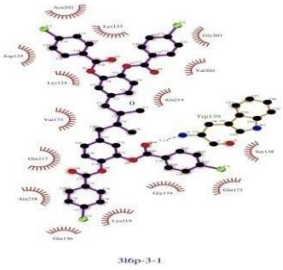

Figure 3: PL-2

Among the PL derivatives chosen and docked against 3L6P, PL-9 showed best interaction (binding energy-9.4 kcal/mol) with Hydrophilic interactions-Glu-172 and Hydrophobic interactions-Gln-136, Lys-219, Gln-217, Gly-137, Ala-216, Lys-123, Lys-124, Val-122, Asp-125, Asn-202, Ala-214, Val-204, Ala-175, Val-212, Ghe-166, gly-171, Val-197, Gly-174, Ser-138, Ile-215, Trp-139, Val-173, Ala-218. This was followed by PL-4 (BE -9.1 kcal/mol) with Hydrophobic interactions: Val-212, Asn-202, Ala-216, Asp-125, Lys-124, Gln-217, Ala-218, Lys-219, Ala-220, Gln-136, Ser-138, Gly-174, Trp-139, Ile-215, Val-173, Lys-123, Val-204, Ala-214. PL-2 occupied third place (BE -9.0 kcal/mol) with Hydrophilic interactions : Trp-139 and Hydrophobic interactions: Asn-202, Lys-123, Gly-203, Asp-125, Lys-124, Val-204, Ala-214, Val-173, Ser-138, Glu-172, Gly-174, Lys-219, Gln-136, Ala-218, Gln-217.

Ligplot analysis of lead molecules with 2VBV

LIGPLOT 4

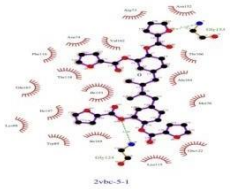

Figure 4: **PL-4**

LIGPLOT 5

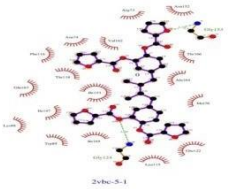

Figure 5: **PL-3**

LIGPLOT 6

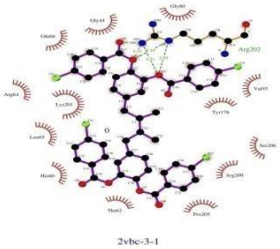

Figure 6: **PL-2**

Similarly among the PL derivatives chosen and docked against 2VBC, PL-4 showed best interaction (binding energy-9.8 kcal/mol) with Hydrophilic interactions : Trp-69, Asp-175 and Hydrophobic interactions:Ile-203, His-194, Pro-174, Glu-173, Gln-327, Ile-171, Glu-169, Ala-70, Pro-331, Ser-328, Asn-329, Gly-82, Ala-197. This was followed by PL-3 (BE -8.9 kcal/mol) with Hydrophilic interactions:Gly-153, Gly-124 and Hydrophobic interactions: Asn-152,Thr-166, Ala-164, Met-76, Glu-122, Leu-115, Ile-165, Trp-89, Lys-88, Ile-149, Gln-167, Thr-118, Phe-116, Asn-74, Val-162, Arg-73, Ile-123 . PL-2 occupied third place (BE -8.4 kcal/mol) with Hydrophilic interactions : Arg-202 and Hydrophobic interactions :Gly-80, Val-45, Try-176, Ser-206, Arg-209, Pro-205, Thr-62, His-60, Leu-65, Lys-201, Arg-64, Glu-66, Gly-44

DISCUSSION

Dengue is an appalling disease and requires urgent attention to develop new inhiditory compounds that could work against it. Like other flaviviruses dengue virus NS3 protease has been declared as significant drug target. The active residues are important in viral replication therefore, any disruption in it may block the replication of virus. Thus, efforts directed to search new therapeutic targets and molecules that inhibit viral infection are required . Thus, the aim of this work was to evaluate the anti-DENV effect of the Phenolic lignan (PL), a hypolipidemic agent with antioxidant and anti-inflammatory properties

In addition, PL also inhibited the replication of hepatitis C virus (HCV), a member of the *Hepacivirus* genus within the *Flaviviridae* family [20], thus becoming an interesting candidate for broad antiviral development against flaviviruses and related viruses.

In this work 11 PL derivatives were examined for their potential against dengue virus NS2B-NS3 protease 3L6P and 2VBC of serotype 1 and serotype 4. In our study , the PL derivatives (table 1) were docked with the active residues of dengue virus NS2B-NS3 of both serotype 1 and serotype2 to find their affinity as inhibitors .Our results revealed potential and significant binding interactions of PL derivatives with the active residues of the protease (table II and table III)

Among the PL derivatives docked against 3L6P, PL-9, PL-4 and PL-2 showed binding affinity with -9.4, -9.1, and -9.0kcal/mol respectively. Similarly among the PL derivatives docked against 2VBC, PL-4, PL-3 and PL-2 derivative showed binding affinity with -9.8, -8.9 and -8.4kcal/mol respectively.

In the docking study with the protease 3L6P, PL-9 was ranked first (-9.4kcal/mol) interacting with Hydrophilic interactions-Glu-172 and Hydrophobic interactions-Gln-136, Lys-219, Gln-217, Gly-137, Ala-216, Lys-123, Lys-124, Val-122, Asp-125, Asn-202, Ala-214, Val-204, Ala-175, Val-212, Ghe-166, gly-171, Val-197, Gly-174, Ser-138, Ile-215, Trp-139, Val-173, Ala-218.

This was followed with PL-4 at second place (-9.1kcal/mol) with Hydrophobic interactions: Val-212, Asn-202, Ala-216, Asp-125, Lys-124, Gln-217, Ala-218, Lys-219, Ala-220, Gln-136, Ser-138, Gly-174, Trp-139, Ile-215, Val-173, Lys-123, Val-204, Ala-214 .Then PL-2 was ranked third place (-9.0 kcal/mol) with Hydrophilic interactions : Trp-139 and Hydrophobic interactions: Asn-202, Lys-123, Gly-203, Asp-125, Lys-124, Val-204, Ala-214, Val-173, Ser-138, Glu-172, Gly-174, Lys-219, Gln-136, Ala-218, Gln-217.

Similarly the docking study with the protease 2VBC, PL-4 was ranked first(-9.8kcal/mol) with Hydrophilic interactions : Trp-69, Asp-175 and Hydrophobic interactions:Ile-203, His-194, Pro-174, Glu-173, Gln-327, Ile-171, Glu-169, Ala-70, Pro-331, Ser-328, Asn-329, Gly-82, Ala-197.

This was followed with PL-3 at second place (-8.9kcal/mol) with Hydrophilic interactions:Gly-153, Gly-124 and Hydrophobic interactions: Asn-152,Thr-166, Ala-164, Met-76, Glu-122, Leu-115, Ile-165, Trp-89, Lys-88, Ile-149, Gln-167, Thr-118, Phe-116, Asn-74, Val-162, Arg-73, Ile-123 . Then PL-2 was ranked thrd (-8.4kcal/mpl) with Hydrophilic interactions : Arg-202 and

Hydrophobic interactions :Gly-80, Val-45, Try-176, Ser-206, Arg-209, Pro-205, Thr-62, His-60, Leu-65, Lys-201, Arg-64, Glu-66, Gly-44

Through our study it was found that among the PL derivatives docked, PL-4 showed potential interaction and significant hydrophobic contact with active residues of both 3L6P and 2VBC of serotypes 1 and 4 respectively. PL-4 was found interacting with hydrophobic interactions and hydrophilic interactions followed by PL-2. Thus it can be concluded that these compounds could be used as potential drugs against dengue virus NS2B/NS3 protease of both the serotypes 1 and 4. Furthermore studies can also be designed to synthesise these compounds or the chemically modified compounds of these molecules and could be used as potential drugs against dengue virus.

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