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Research Article

Anti-dengue Leads From *Caesalpinia bonduc* - An *In-silico* Approach

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ABSTRACT

Dengue (breakbone fever) is a rapidly spreading arboviral infection transmitted by *Aedes* mosquitoes with major public health implications in more than 100 tropical and subtropical countries mostly in Southeast Asia, South and Central America and the Western Pacific. As the virus spreads to new geographic areas, more frequent dengue outbreaks occur in different parts of the world. Fifty million cases of dengue occur worldwide each year, of which 10% require hospitalization for dengue hemorrhagic fever (DHF). It is a shocking truth that more than 90% of these are children under the age of five. The mortality rate is also significant as 2.5% die from dengue. Currently, there is no effective vaccine or specific drug for Dengue/DHF. Pharmaceutical manufacturers have turned their attention to plant-based drug candidates to produce effective drugs. Following the study investigated the active phytochemicals in the medicinal plant *Caesalpinia bonduc* (L.) Roxb. through docking simulation. Dengue virus non-structural protein five (NS5) and human IMPDH-II were used here as targets for docking with plant compounds. Docking results revealed that 33 compounds out of 82 phytochemicals showed better binding affinity than the native ligands of the targets. Compounds exhibiting the lowest free energy levels were further screened after studying their pharmacokinetics, medicinal chemistry friendliness, lead-likeness, and toxicity prediction to identify lead molecules. At the end of the study, three compounds, Caesaldekarin A, Caesalpinin F and Taepeenin D, which potently inhibited both targets, were selected here for further *'in-vitro' and 'in-vivo'* studies.

Introduction

Dengue is one of the most common mosquito-borne viral infections caused by dengue virus (DENV). It is common in the tropics and subtropics of the world and mostly affects people living in urban and semi-urban areas. DENV is one of the 53 species of the genus *Flavivirus* and family Flaviviridae.[1] This virus exists in four distinct but closely related serotypes. The virus won't be transmitted from man to man. The main vectors of the virus are the female urban mosquitoes *Aedes aegypti* and *Aedes albopictus.* They are also transmitting Zika virus, yellow fever virus, Japanese encephalitis virus and West Nile virus of the same genus and chikungunya, Venezuelan and equine encephalitis virus of the genus *Alphavirus* that belongs to the family Togaviridae. Vertical transmission of the virus is also recorded to a lesser extent. Once infected, dengue can range from asymptomatic to influenza-like and develop multisystem failure. According to an estimate in the World Health Organization's fact sheet severe dengue was first observed in the Philippines and Thailand during the dengue epidemics in 1950s. It is one of the leading causes of hospitalization and mortality among people in Asian and Latin American countries. Although the risk of infection exists in 129 countries, more than 70% of infections are in Asia Pacific. India is one of Asia's most vulnerable endemic countries in terms of dengue. Usually, outbreaks of dengue are caused by the dominance of one serotype, but rarely in many countries, the four serotypes together cause hyper-endemic conditions that become more destructive. The highest number of dengue cases ever reported globally was in 2019, when it affected all endemic regions, including India and was reported in Afghanistan for the first time.[2] Dengue is devastatingly affecting human health and

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the global and national economy. There is no specific treatment for severe dengue other than vector control measures and supportive medical care to lower the fatality rate. The rapid spread of COVID-19 has put a sprain on the world after the dengue outbreak in 2019. A concerted effort is needed from all quarters to fight the viruses that are coming one after the other.

In Ayurveda and traditional medicine, there are many treatments for epidemics based on plant resources. Modern medicine seeks to understand basic principles of the traditional healing system and to identify the effective components contained in plants for the development of medicine. Plants are the renewable resource of a series of metabolites developed in living systems for various purposes, including protection and defense. Identifying new chemical entities (NCEs) with the required pharmaceutical properties from synthetic or natural sources is crucial in the discovery of new drugs. Salicylic acid was the first natural compound produced by chemical synthesis in 1852.[3] It is the most popular antipyretic and analgesic medicine derived from the willow tree still in use. Since then, many safe and effective herbal remedies have been developed, marketed and used effectively. A lion share of the modern drugs used in India is derived from natural products.[4] Pharmacologists are now shifting their focus to the search for natural compounds for new medicines. The main reason for this is the belief that natural products can be safer than artificial ones. With the advent of computer technology and bioinformatics, finding suitable combinations from an extensive collection of chemical compounds becomes easier, time-saving and economically feasible. The current study aims to identify bioactive metabolites from the library of chemical compounds derived from the medicinal plant *Ceasalpinia bunduc* (L.) Roxb. through *in-silico* screening method. The plant is famous for its anti-inflammatory[5] and antiviral[6] properties.

Plant-derived compounds have been shown to inhibit multiple targets simultaneously. Such compounds can inhibit various stages of the disease even if the pathogen mutates. Moreover, many phytomedicines have shown long-term stability and safety. Instead of a drug acting on a single target of a disease, the concept of a drug acting on multiple targets gained more importance. The current drug development strategy of pharmaceutical companies is an integrated approach to identigy drug candidates or drug template molecules from natural sources. Viruses are rapidly mutating organisms that often change the translates (proteins) of the pathogen. Finding chemical compounds that react well with viruses is difficult because viruses have minimal reactivity with chemicals. However, rapid and cost-effective screening and discovery of compounds against biological targets will be crucial in the discovery of antivirals in pharmacology. Here the pharmacologically potential compounds expected to be obtained from the experimental plant will be direct information for antiviral drug candidates for the drug industry.

Materials and Methods

Target Preparation and Active Site Determination

Human IMPDH-II (Inosine monophosphate dehydrogenase-II), a major rate-limiting enzyme up-regulated in the viral replication site catalyze guanosine and deoxyguanosine biosynthesis[7] and the methyltransferase (Mtase) domain of non-structural protein NS5 that is involved in the methylation and capping of viral RNA genome during replication[8] have been selected as two targets for the study. Both targets are vital in the replication and maturation of the viral genome. The 3D structures of the targets were retrieved from the protein databank (PDB ID: 1NF7 (IMPDH-II) and 4V0Q (NS5)). UCSF Chimera software's DockPrep tool was used to prepare the target proteins in which the native ligands and hetero atoms were removed, and the imperfect side chains were repaired with the help of an inbuilt Dunbrack 2010 rotamer library. Subsequently, polar hydrogen atoms were added, assigned partial charges followed by energy minimization of the targets. The reformatted target molecules were then saved in pdb format.

The binding site of the targets was determined based on the binding site of native ligands co-crystallized in the targets. Ribavirin monophosphate (RVP) is the inhibitor small molecule of IMPDH-II co-crystalized with the binding pocket of the natural substrate, Ionosine monophosphate (IMP). Using protein-ligand interaction profiler (PLIP) active site residues were visualised. Residues interacting with RVP include Ser68, Asn303, Gly366, Gly387, Ser388, Met414, Gly415 and Gln441. The recent IMPDH2 (6U8E) PDB deposit, co-crystallized with ATP, IMP, and NAD^+[9] gives an overview of IMP's highly accurate binding site. In addition to the RVP-linked residues, these include Arg322, Ser329, Asp364, Gly365 and Ser416. Also, it contains two highly stereo-specific mobile regions, the active site loop with Cys331 and a flap with Tyr 411.[10] The structure of IMPDH-II and the enzyme mechanism of monomers are well established.[11] MTase domain (1-262) of NS5 is bound with S-adenosyl homocysteine (SAH) in the PDB structure 4V0Q and the active residues that SAH occupies include Ser56, Gly85, Gly86, Trp87, Lys105, His110, Glu111, Asp131, Val132 and Asp146. The MTase domain composed of four helices surrounding a central 7-stranded beta sheet and the active site containing a catalytic Lys61, Asp146, Lys180, Glu216 motif positioned in the centre of the beta sheet.[12]

Phytochemical Selection and Ligand Preparation

A library of phytochemicals so far reported from the well-known medicinal plant *Caesalpinia bonduc* (L.) Roxb.[13-18] has been compiled and 82 small compounds have been

selected as ligands for the current docking simulation study. Native ligands of targets (SAH and RVP) were also included for a comparative account. 3D Structures of all the compounds except two were retrieved from PubChem database. OpenBabel software converts 2D coordinates of two compounds (PubChem CID: 538523 and 71440416) to 3D coordinates. Using the 'Ligand' option of Autodock Tools (ADT) the compounds were added gasteiger charges, merged non polar hydrogen atoms, detect rotatable bonds and set torsional degree of freedom to the ligands. The torsion root of the ligand was detected and made desired bonds between atoms rotatable to make the ligand or the functional groups of the ligand flexible and finally set the number of torsions. The prepared ligands were saved in pdbqt format.

Molecular Docking and Analysis

Pre-formatted ligands were docked with the targets using Autodock 4.2.6. The graphical user interface ADT 1.5.6 is used for setting up and running AutoDock. Save the already prepared target molecule in 'pdbqt' format and then set up a grid map around the active site. The grid output file will be saved in grid parameter file (gpf) format and run the 'Autogrid' inorder to generate grid map files. Default Genetic algorithm parameters and docking run options were accepted and generated the docking parameter file (dpf) for each ligand. Run the AutoDock using each dpf file and record the free energy of binding. Interpretation of the docking result and the visual examination could also be done using the analysis option of the ADT. Discovery studio visualizer was used to study the molecular interaction between active site residues and best-docked ligands.

Validation of Docking Procedure

The docking procedure was evaluated by re-docking the co-crystallized native ligands into the active site of both the targets and understanding the root mean square deviation (RMSD) obtained by superimposing the re-docked and co-crystallized native ligands. PyMOL molecular visualizer is used here to notice the RMSD.

Analysis of Pharmacokinetics, Toxicity and Drug likeness

The pharmacological activity of a new chemical entity is primarily determined based on its physicochemical properties, absorption, distribution, metabolism, excretion, toxicity (ADME/Tox) and drug analogy evaluation. It increases the success rate of drug development by eliminating poor drug-like compounds in advance and avoids more research and development expenses. Important molecular properties (logP, topological polar surface area, number of hydrogen bond donors and acceptors), as well as prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors, enzyme inhibitors) were studied using molinspiration cheminformatics software. Drug likeness score based on molecular properties is predicted using the online server MolSoft. The pharmacokinetics and toxicity analysis of the hits were carried out in the quantitative structure-activity relationship (QSAR) server, 'admetSAR'.

Results and Discussion

The study aims to find putative lead compounds for the treatment of dengue from the active chemical entities contained in the medicinal plant *Cesalpinia bonduc*. For this, the PDB structure of dengue active targets, human IMPDH-II (PDB ID: 1NF7) and a non-structural protein (NS5) of dengue virus (PDB ID: 4V0Q) were used. 1NF7 is complex with ribavirin monophosphate (RVP), and 4V0Q is complex with S-adenosyl homocysteine (SAH). The consistency of the docking procedure and the algorithm was primarily confirmed. The RMSD of the examined native ligands RVP and SAH against the crystal structure was found to be 1.635 and 1.165 Å, respectively. It is an indication that the docking software (AutoDock) used here is reliable for further studying the binding poses of the novel hits. The alignment of the co-crystallized ligand and re-docked ligand is shown in Fig. 1.

A total of 82 small plant compounds have been subjected for screening along with the native ligands. The experimental free energy of binding (ΔG_{bind}) value of native ligands RVP and SAH against the parent proteins are -7.83 and -9.37 kcal/mol, respectively. Binding affinity of 23 phytochemicals showed free energy of binding less than that of the native ligand of IMPDH-II (-7.83). Meanwhile nine compounds with free energy of binding lower than that of the native ligand (-9.37) of the Mtase domain of NS5 (Table 1). In practice, evaluating drug similarity is manifested as rules, the most real and best known of which is Rule of five (Ro5). The law states that a compound is likely to exhibit poor absorption or permeability upon complying the following two or more physicochemical criteria: molecular weight (MW) > 500 Da; Lipophilicity (ClogP) > 5; hydrogen bond donors > 5 or hydrogen-bond recipients (nitrogen and oxygen atoms) >10. Although this law generally does not apply to biological transporters or natural compounds, it is still used as a guideline for evaluating compounds as they are conceptually simple

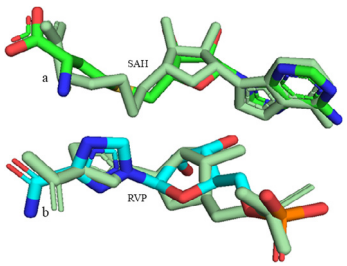

Fig. 1: The superimposed alignment of both co-crystalized and re-docked native ligand poses. a. S-adenosylhomocysteine (SAH) and b. Ribavirin monophosphate (RVP)

and easy to implement. However, 33 compounds with a free energy of binding ≤ -8 Kcal/mol against any one or both of the targets and complying Ro5 were further optimized. Table 1 gives the name of the hit compounds and their compound ID (CID), but only the compound ID is given in the rest of the tables. Hence the first table should be referred to understand the names of the compounds while reading further.

The hit selection was performed according to their least free energy of binding (ΔG_{bind}), permissible physicochemical properties, bioactivity, and drug-analogy score. Lipid dissolution is a direct measure of the transport potential of a compound through biological membranes. Topological polar surface area (TPSA) is another important factor that was considered to be less than 140 Å. It is recommended that the number of existing atoms between 10 and 40 and the number of rotating bonds are less than 10. Out of 33 hit compounds selected, 16 can block both targets simultaneously (ΔG_{bind} ≤ 8 Kcal/mol). At the same time 7 hit compounds have an enhanced independent inhibitory effect against target IMPDH-II and 10 compounds against NS5. 13 hit compounds are less soluble in lipids (mLogP > 5) and exceed the maximum permissible molecular volume of 400Å which reduce the intestinal absorption and bioavailability of the compounds when administered orally. 19 compounds that are fully compliant with 'Rule of Five' and do not exceed the limit of TPSA and the limit of rotatable bonds were subjected for further analysis (Table 2).

The bioactivity of an investigational drug candidate is based on their activity score. Score >0.00 is Active; -0.50 to 0.00 is moderate and <-0.50 is inactive. The compounds Coumarine,3[2-[1-methyl-2-imidazolylthio]-1-oxoethyl]; 2',4',4-trihydroxychalcone; 2',4-dihydroxy-4'-methoxy-chalcone; Bonducellin and 8-methoxybonducellin are inactive and rest of them are moderately bioactive. Drug likeness score is another criterion for selecting hits. It states that the good drug candidate is the compound that possesses the key physicochemical properties of approved drugs. Drug-like compound refers to compounds that have acceptable ADME/Tox properties and are capable of surviving phase I clinical trials.[19] The drug likeness score is measured by MolSoft and a compound with a score between zero and 1.5 is considered more similar to known drugs. Compounds including caesaldekarin A, caesaldekarins F, caesalpinin F, quercetin, neocaesalpin H, taepeenin B, taepeenin C and taepeenin D (Table 3) were chosen for further pharmacokinetic and toxicity analysis as they are moderately bioactive and more analogous to known drugs.

ADME/Tox validation allows selecting more accurate hits. The promiscuous inhibitory activity of a novel compound with the isoforms of cytochrome P450 (1A2, 2C9, 2C19, 2D6, 3A4) is indicative of its drug-drug interaction (DDI) possibility that is a major problem in drug discovery**.** If a given small molecule inhibits more CYP isoforms, it will more likely interact with many other drugs. Recent history reveals that CYP450 inhibitory promiscuity (a compound inhibiting multiple CYP isoforms) led to the withdrawal of many drugs from the market. Examples include zeldine, posicor, hismanal, propulside, lotronex, bacol, and serone.[20] In the same way, when two or more drugs are given at the same time, if one is a potent P450 inhibitor, it can disrupt the other drug's metabolism, causing its increase in plasma and leading to undesirable toxic effects.[21] Powerful sorting models are in the AdmetSAR to predict the high or low P450 inhibitory promiscuity of new chemicals, which can be used as a filter to explore potential drug-drug interaction (DDI) problems in the early stages of drug detection. CYP450 inhibitory promiscuity of all the experimental hits except quercetin is low promiscuous. P-glycoprotein (P-GP) limits cellular absorption and supplies xenobiotics and toxins. Therefore, it reduces the absorption, oral bioavailability and shortens the retention time of the drug.[22] It is expressed in many pharmacokinetic related organs and physical barriers such as the gastrointerstinal tract, blood-brain barrier (BBB), kidney, liver, endothelium and placenta.[23] During drug discovery, the FDA recommends screening to ensure that the investigational compounds are P-gp inhibitors, non-inhibitor or inducer for further clinical trial. Like enzymes involved in drug metabolism, substrates of P-gp can act as inhibitors or stimulants of its action. Inhibition of P-gp may increase the bioavailability of the drug while P-gp induction reduces bioavailability.[24] The selected investigative hits here are substrate (S) of P-gp. Compounds Caesaldekarin A, caesaldekarins F, caesalpinin F and taepeenin D are real inhibitors (I) and taepeenin C and taepeenin B are partial inhibitors (NI/I) while others are non-inhibitors (NI). Both the native ligands are non-inhibiting substrates of P-gp (Table-4).

Blood brain barrier limits the penetration of molecules into the central nervous system (CNS). A major barrier to the development of CNS medicine is the inability of drug molecules to penetrate the BBB, but it is best to prevent drugs that target the peripheral organs, from entering the CNS to avoid possible side effects.[25] The current investigation found that all compounds except quercetin are BBB permeable. Since, neurological signs of dengue infections are increasingly reported[26] BBB permeable natural compounds are suggestive for anti-dengue drug development. Human intestinal absorption (HIA) or absorption of a drug in the human gut is one of the most important ADME properties, considered the most important factors influencing the bioavailability of drugs.[27] All the hit compounds selected here are showing positive absorption through human intestine (Table 4).

The toxicity of drugs is predicted mainly based on AMES toxicity, carcinogenicity and HERG inhibition. A positive

Table 1: Hit compounds showing free energy of binding against the targets IMPDH-II (1NF7) and NS5 (4V0Q)

Sl. No.	PupChem CID	Compound name	ΔG_{bind} (1nf7)	ΔG_{bind} (4v0q)
1	538521	17,[1,5-Dimethyl-hexyl]4,4,9,13,14-pentamethylhexadecahydrocyclopenta(a) phenanthren 3-one	-9.99	-10.54
2	11595333	Taepeenin F	-9.67	-9.34
3	11645561	Taepeenin A	-8.95	-8.79
4	5280794	Stigmasterol	-8.86	-10.29
5	10383930	Caesaldekarin A	-8.78	-9.00
6	11419457	Caesalpinin D	-8.73	-8.82
7	11580921	Taepeenin E	-8.58	-8.52
8	581589	Coumarine,3[2-[1-methyl-2-imidazolylthio]-1-oxoethyl]	-8.56	-6.99
9	11165955	Caesalpinin C	-8.53	-8.56
10	5322052	2',4',4-trihydroxychalcone	-8.51	-7.78
11	14985	Alpha tocopherol	-8.44	-8.12
12	44260092	6-methoxypulcherrimin	-8.42	-7.05
13	5280343	Quercetin	-8.41	-8.25
14	11530252	Taepeenin C	-8.33	-8.22
15	11616598	Taepeenin B	-8.26	-8.63
16	5711223	2',4-dihydroxy-4'-methoxy-chalcone	-8.25	-7.46
17	11594457	Taepeenin G	-8.22	-8.45
18	173183	Campesterol	-8.21	-9.51
19	15381600	Caesaldekarins F	-8.18	-8.24
20	14079439	Bonducellin	-8.14	-7.15
21	5326346	Stereochenol A	-8.12	-7.72
22	11235450	Caesalpinin F	-8.01	-9.00
23	73299135	8-methoxybonducellin	-8.00	-7.47
24	11186554	Neocaesalpin I	-7.80	-9.52
25	73145	Beta amyrin	-7.60	-10.50
26	10893432	Neocaesalpin H	-7.41	-9.95
27	11703693	Taepeenin D	-7.07	-8.91
28	222284	Beta sitosterol	-6.58	-9.24
29	5280459	Quercetin 3'O-alpha-rhamnopyranoside	-6.58	-8.35
30	92157	Lupeol acetate	-6.48	-10.24
31	259846	Lupeol	-6.20	-9.25
32	3003908	Cyclopentanone, 2- Cyclopentylidene	-6.16	-10.35
33	73170	Alpha amyrin	-4.54	-9.82
X1	439155	S-Adenosylhomocysteine (SAH)	-	9.37
X2	100252	Ribavirin monophosphate (RVP)	-7.83	-

Legend: ΔG_{bind}- free energy of binding, X1 and X2 Native ligands

result of an AMES toxicity test of an investigational lead is one of the major obstacles to progressing to its clinical trials.[28] Inhibition of the cardiac ion channel encoded by the human ether-a-go-go-Related Gene (hERG) may lead to cardiac arrhythmia, which has become a major concern in drug discovery and development.[29] The importance of the carcinogenicity study is to identify the tumorigenic potential of a chemical in animals and to assess the relevant risk in humans.[30] All the investigational compounds here are non-AMES toxic (NAT), non-carcinogenic (NC) and weak hERG inhibitor. A chemical that enters the body through the oral route at a certain time and has any adverse effect with a short delay is orally and acutely toxic. An investigational drug's acute oral toxicity (AcOrlT) is studied based on its median lethal dose (LD_{50}) against the body weight. Based on it there are four categories of

Table 2: Physico-chemical properties of hit compounds

SL.No.	*Pub- Chem CID*	*Properties of the compound*								
		MLogP ≤5)	*TPSA ≤140*	*Atms 10–40*	*MW < 500*	*HA < 10*	*HD < 5*	*Vlns ≤ 1*	*Rotb ≤ 10*	*Volume 100–400 Å*
1	538521	8.56	17.07	31	428.75	1	0	1	5	472.54
2	11595333	5.05	52.61	25	342.44	4	0	1	2	324.34
3	11645561	5.45	39.45	24	326.44	3	0	1	2	315.98
4	5280794	7.87	20.23	30	412.70	1	1	1	5	450.33
5*	10383930	4.71	59.67	26	360.49	4	1	0	2	352.71
6*	11419457	1.76	112.28	32	446.50	8	1	0	4	397.85
7*	11580921	4.73	56.52	25	340.42	4	0	0	3	318.40
8*	581589	2.06	65.11	21	300.34	5	0	0	4	251.68
9*	11165955	4.11	85.98	30	416.51	6	1	0	4	391.61
10*	5322052	2.59	77.75	19	256.26	4	3	0	3	225.91
11	14985	9.04	29.46	31	430.72	2	1	1	12	474.50
12*	44260092	2.90	96.61	27	370.31	8	1	0	2	297.98
13*	5280343	1.68	131.35	22	302.24	7	5	0	1	240.08
14*	11530252	4.29	59.67	25	342.44	4	1	0	2	324.2
15*	11616598	4.84	50.44	23	312.41	**3**	1	0	1	298.45
16*	5711223	3.31	66.76	20	270.28	4	2	0	4	243.43
17	11594457	5.32	20.23	21	288.48	1	1	1	2	310.86
18	173183	8.30	20.23	29	400.69	1	1	1	5	439.71
19*	15381600	4.09	59.67	25	344.45	4	1	0	2	330.46
20*	14079439	3.17	55.77	21	282.30	4	1	0	2	250.36
21*	5326346	4.26	51.21	22	292.33	3	0	0	3	268.29
22*	11235450	2.06	103.05	30	418.49	**7**	1	0	4	382.86
23*	73299135	3.19	65.00	23	312.32	5	1	0	3	275.91
24*	11186554	3.72	67.51	24	330.42	4	1	0	1	313.08
25	73145	8.02	20.23	31	426.73	1	1	1	0	460.70
26*	10893432	3.68	83.83	25	348.44	5	2	0	1	326.99
27*	11703693	4.99	65.75	28	384.47	**5**	0	0	4	360.53
28	222284	8.62	20.23	30	414.72	1	1	1	6	456.52
29	5280459	0.64	190.28	32	448.38	11	7	2	3	363.95
30	92157	8.71	26.30	34	468.77	2	0	1	3	498.12
31	259846	8.29	20.23	31	426.73	1	1	1	1	461.60
32	3003908	5.23	34.14	27	416.63	2	0	1	0	362.07
33	73170	8.08	20.23	31	426.73	1	1	1	0	461.05
X1	439155	-2.77	182.65	26	384.42	11	7	2	7	317.36
X2	100252	-3.44	190.26	21	324.19	12	6	2	5	243.03

Legend: * Compound selected for further analysis, X1&X2 - native ligands, TPSA-topological polar surface area; HA-hydrogen acceptors; HD- hydrogen donors; Vlns.-violations; Rotb.-rotatable bonds.

AcOrlT. Grade-I is very toxic (<5 mg/kg), Grade-II is toxic (>5 <50 mg/kg), Grade-III is harmful (>50 <500 mg/kg) and Grade-IV (>500 <2000 mg/kg) is safe.[31] Except Neocaesalpin H (Gr.I), quercetin and Caesaldekarins F (Gr. II) all others are in the safe category, gradeIII acute oral toxicity (Table 4).

Based on the physicochemical parameters, pharmacokinetic properties and toxicity analysis, caesaldekarin A, taepeenin C, taepeenin B, caesalpinin F and taepeenin D were found to be more appropriate for further evaluation. Compounds with high molecular weight and lipophilic potential have a high risk of degeneration at each stage

Table 3: Bioactivity score based on molinspiration and drug-likeness score in MOLSOFT

Sl. No.	PubChem CID	Bioactivity (>0.00: active; -0.50 - 0.00: moderate; <-0.50: inactive)						MolSoft
		GPCRL	ICM	KI	NRL	PI	EI	DL (0-1.5)
1*	10383930	0.06	0.22	-0.36	0.56	0.12	0.40	0.29
2	11419457	0.22	0.31	-0.28	0.46	0.08	0.45	-0.48
3	11580921	0.31	0.12	-0.23	0.53	0.01	0.38	-0.20
4	581589	-0.82	-1.31	-0.65	-1.07	-0.64	-0.18	-0.45
5	11165955	0.22	0.43	-0.32	0.59	0.17	0.44	-0.16
6	5322052	-0.18	-0.13	-0.33	-0.05	-0.35	0.03	-0.05
7	44260092	-0.10	-0.38	0.03	-0.06	-0.34	0.10	-0.43
8*	5280343	-0.06	-0.19	0.28	0.36	-0.25	0.28	0.52
9*	11530252	0.51	0.17	-0.22	0.70	0.01	0.44	0.67
10*	11616598	0.55	0.14	-0.29	0.81	-0.07	0.54	0.18
11	5711223	-0.13	-0.18	-0.30	0.01	-0.32	0.04	0.51
12*	15381600	0.16	0.34	-0.44	0.53	0.02	0.22	0.47
13	14079439	-0.22	-0.28	-0.34	0.06	-0.45	-0.01	0.24
14	5326346	-0.18	-0.10	-0.43	-0.17	-0.21	0.20	-0.14
15*	11235450	-0.05	0.08	-0.52	0.44	-0.01	0.38	0.33
16	73299135	-0.20	-0.27	-0.32	-0.07	-0.39	-0.03	-0.06
17	11186554	-0.04	-0.24	-0.47	0.69	0.30	0.68	-0.38
18*	10893432	0.18	0.12	-0.35	0.53	0.04	0.70	0.30
19*	11703693	0.35	0.08	-0.29	0.43	-0.05	0.34	0.78
X1	439155	1.04	0.44	0.47	-1.18	0.51	1.23	0.29
X2	100252	1.04	0.85	0.53	-0.71	0.53	1.38	0.95

Legend: * Compound selected for further analysis, GPCRL – G-protein coupled receptor ligands, ICM- Ion channel modulator, KI-kinase inhibitor, NRL-nuclear receptor ligands, PI-protein inhibitor, EI-enzyme inhibitor, DL-drug-likeness score, X1, X2 - native ligand.

Table 4: Adme/Toxicity validation of investigational compounds

Sl. No.	*Pubchem CID*	*HIA*	*BBB*	*CYP-IPro*	*AcOrlT level*	*PGPS/I*	*AMEST*	*Carcinogens*	*HERGI*
1	10383930	+	+	Low	III	S/I	NAT	NC	weak
2	5280343	+	-	High	II	S/NI	NAT	NC	weak
3	11530252	+	+	Low	III	S/NI/I	NAT	NC	weak
4	11616598	+	+	Low	III	S/NI/I	NAT	NC	weak
5	15381600	+	+	Low	II	S/I	NAT	NC	weak
6	11235450	+	+	Low	III	S/I	NAT	NC	weak
7	10893432	+	+	Low	I	S/NI	NAT	NC	weak
8	11703693	+	+	Low	III	S/I	NAT	NC	weak
X1	439155	+	-	Low	III	S/NI	NAT	NC	Weak
X2	100252	+	+	Low	III	NS/NI	NAT	NC	Weak

Legend: HIA – human intestinal absorption, BBB-blood brain barrier, CYPIpro.-Cytochrom P450 promiscuity, AcOrlT-Acute oral toxicity, PGPS/I - P-glycoprotein substrate/inhibitor, AMEST- ames toxicity, HERGI- Human Ether-a-go-go-related gene inhibitor, X1, X2 – native ligands.

of clinical development. Molecular weight (Mw) can be up to 500 Da, but less than 350 Da is more suitable.[32] Here the Mw of all the five experimental compounds are below 400 g/mol except caesalpinin F. Lipid solubility affects the volume of distribution (Vd) of a drug which represent the amount of drug in the body in comparison with the concentration of drug in the plasma. Highly lipid soluble drugs have high Vd.[33] Lipid solubility of the selected compounds ranging from 2.06 to 4.99 that is in the preferred range. If the number of hydrogen bond acceptors such as nitrogen and oxygen increases, 'ClogP' will decrease and there will be a significant increase in topological polar surface area (TPSA),[21] thereby decreasing the absorption of compounds. The hydrogen

bond receptors of all the above compounds are between 3 to 7, which is <10 at the allowable level. Hydrogen bond donors of those compounds are one except taepeenin D which has no donor elements and is in the limit of 5. The volume of the compounds is in the permissible range, between 298 to 383 (standard range lies from 100–400 Å) (Table 2). In terms of bioactivity, all the said compounds were moderately active and showed good drug likeness (Table 3). Veber and colleagues[34] found that the number of rotating bonds (NROT) was an important parameter, with a maximum of seven seeming to be the most suitable for oral bioavailability. Oral bioavailability is thought to be low for passively absorbed molecules with polar surface area (PSA) 110 to 140 Å. TPSA of the said compounds are between 59.67 and 103.05 and NROT two to four (Table 2). The number of atoms in each compound is also a factor influencing absorption. Compounds with less than 40 atoms are considered more drug-like. Here all the said three compounds comprise ≤ 30 atoms (Table 2). Brenks' lead formulation criteria[35] are also important for the optimization and selection of new chemical entities for drug development. As suggested by Brenk, the number of hydrogen-bond donors and acceptors of the selected compounds is less than four and seven, respectively and the number of rotating bonds is less than seven and the number of ring systems is less than five. The molecular interaction of these three compounds truly emphasizes their binding affinity with the targets, depicted in Tables 5 and 6.

Caesaldekarin-A (CA) is strongly binding to the Mtase domain of NS5 (ΔG_{bind} -9 Kcal/mol) and the active site of IMPDH-II (ΔG_{bind} -8.78 Kcal/mol). It established six conventional H-bonds and pi-donor interactions within the threshold range of bond distance with IMPDH-II. Active site residues, including Asp364, Ser329 and Tyr411 are part of these interactions. Cys331, which forms highly stereo-specific mobile regions of the site, is also among the hydrophobic residues involved. CA made three conventional H-bonds and hydrophobic interactions with NS5 where active site residues Lys105 and His110 are part of hydrophobic contacts. Caesalpinin-F (CF) is firmly binding the targets NS5 (ΔG_{bind} -9 Kcal/mol) and IMPDH-II (ΔG_{bind} -8.01 Kcal/mol). Interaction of CF with IMPDH-II made 3 conventional H-bonds, a carbon H-bond and a pi-sulfur bond in addition to hydrophobic interactions of Met70 and Met414. One of the H-bonds of it is established with the active residue, Ser329. Interaction of CF with the target NS5 shows 4 conventional H-bonds, among which one is with the active residue Val132 and among two carbon H-bonds one is with the active residue Asp131. Distance of H-bonds with CF is also within the allowable range. Taepeenin-D (TD) is bonded firmly with NS5 (ΔGbind -8.91 Kcal/mol) and formed relatively weak bonding with IMPDH-II (ΔG_{bind} -7.07 Kcal/mol). With NS5, it formed 3 conventional H-bonds, carbon H-bonds, and a pi-donor H-bond. One of the H-bonds is with the active residue Lys105, and the hydrophobic interactions also involved active site residue His110. Interaction of TD with IMPDH-II established two H-bonds, a carbon H-bond and a pi-bond. 3D and 2D graphical depictions of the lead molecules observed using discovery studio visualizer are represented in Figs. 2 and 3.

It has been concluded that the diterpenes caesaldekarin-A isolated from the roots,[36] caesalpinin-F from seeds and a meroterpenoid Taepeenin-D derived from stem, root, and seeds of *C. bonduc*[15] are potential compounds capable of inhibiting the natural functions of the experimental targets, Human IMPDH-II and Dengue viral NS5 Mtase. Most of the mero terpenoids and diterpens exhibit anti-

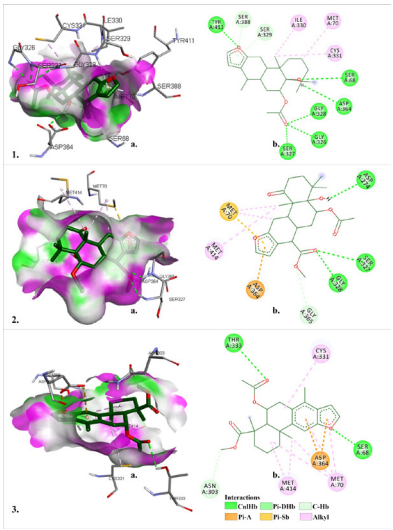

Fig. 2: Interaction of lead molecules with the target IMPDH-II IN Discovery Studio visualizer. 1. Caesaldekarin-A, 2. Caesalpinin-F and 3. Taepeenin-D; a. 3D and b. 2D view; CnlHb – Conventional H-bond, Pi-DHb – Pi-donor H-bond, C-Hb – Carbon H-bond, Pi-A - Pi bond Acceptor, Pi-Sb – Pi-Sulfur bond.

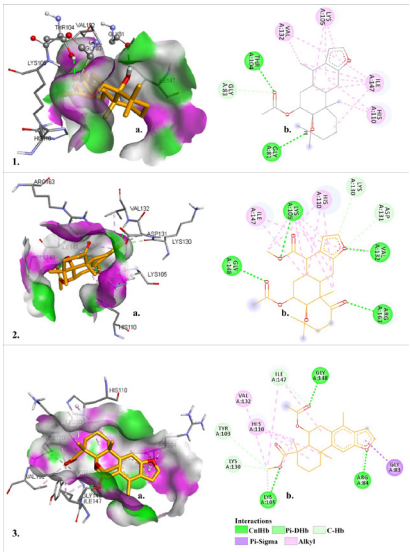

Fig. 3: Interaction of lead molecules with the target NS5-Mtase IN Discovery Studio visualizer. 1. Caesaldekarin-A, 2. Caesalpinin-F and 3. Taepeenin-D; a. 3D and b. 2D view; CnlHb – Conventional H-bond, Pi-DHb – Pi-donour H-bond, C-Hb – Carbon H-bond.

Table 5: Molecular interaction of lead molecules with IMPDH-II

Lead name and CID	ΔG_{bind} (Kcal/mol)	H- bond/S-bond/Pi-Pi-bond interaction	Bond Types	BD	DHAA	HPh. Residues (Distance)
Caesaldekarin-A (CA) 10383930	-8.78	SER68:HG -- O:LIG	CnlHb	2.90	129.02	Met70 (4.22) Ile330 (4.97) Cys331 (3.63)
		GLY326:HN-- O:LIG	CnlHb	2.27	110.43	
		SER327:HN -- O:LIG	CnlHb	1.73	160.27	
		GLY328:HN -- O:LIG	CnlHb	2.59	148.79	
		TYR411:HH -- O:LIG	CnlHb	1.74	140.77	
		LIG1:H-- OD2:ASP364	CnlHb	2.50	157.33	
		SER329:HN -- LIG	Pi-DHb	2.42	-	
		SER388:HN -- LIG	Pi-DHb	2.65	-	
Caesalpinin-F (CF) 11235450	-8.01	SER327:HN-- O :LIG	CnlHb	2.92	137.141	Met70 (3.70, 4.72) Met414 (5.31)
		GLY328:HN-- O:LIG	CnlHb	2.06	133.63	
		LIG1:H-- OD2:ASP274	CnlHb	2.27	162.53	
		GLY365:CA-- O:LIG	C-Hb	3.05	-	
		MET70:SD -- LIG1	Pi-Sb	4.34	-	
Taepeenin-D (TD) 11703693	-7.07	SER68:HG -- O:LIG	CnlHb	2.08	143.296	MET70 (5.41, 4.27, 5.12, 4.97) CYS331(4.77,5.12)
		THR333:HG1-- O:LIG	CnlHb	2.93	97.327	
		LIG1:C-- OD1:ASN303	C-Hb	3.21	-	
		ASP364:OD1 -- LIG1	Pi-A	3.14	-	
		ASP364:OD2 -- LIG1	Pi-A	4.14	-	

Legend: CnlHb-Conventional H-bonds (Hb), C-Hb – Carbon Hb, Pi-DHb-pi donor Hb, Pi-S - pi Sulfur bond, Pi-A - pi bond acceptors, BD- bond distance, DHAA-donor hydrogen acceptor angle, HPh.-hydrophobic.

Table 6: Molecular interaction of lead molecules with NS5-Mtase

Lead name and CID	ΔG_{bind} (Kcal/mol	H- bond/S-bond/Pi-Pi-bond interaction	Bond Types	BD	DHAA	HPh. Residues (Distance)
CA 10383930	-9.00	THR104:HG1 -- O:LIG	CnlHb	2.33	127.187	LYS105 (5.34, 4.66, 4.29); ILE147 (4.43, 4.42, 4.10, 4.51, 5.03); HIS110 (5.41, 4.52); VAL132 (5.31)
		LIG:H -- O:GLY81	CnlHb	2.11	158.402	
		GLY83:CA -- O:LIG	CnlHb	2.86		
CF 11235450	-9.00	LYS105:HN -- O:LIG	CnlHb	2.26	156.391	LYS105 (4.86,3.60,4.11,4.60,4.39); ILE147 (4.50,5.28); HIS110 (4.70,3.98,4.14); VAL132(4.05)
		VAL132:HN -- O:LIG	CnlHb	2.04	153.855	
		GLY148:HN -- O:LIG	CnlHb	1.87	143.328	
		ARG163:HH11 -- O:LIG	CnlHb	2.24	168.514	
		ASP131:CA -- O:LIG	C-Hb	3.07	-	
		LIG:C -- O:LYS130	C-Hb	2.67	-	
TD 11703693	-8.91	ARG84:HN -- O:LIG	CnlHb	2.29	168.37	GLY83 (3.88); ILE47 (5.42,5.36); LYS105 (4.67,5.21); VAL132 (4.04); HIS110 (4.94, 5.15, 3.82); ARG84 (5.03)
		LYS105:HN -- O :LIG	CnlHb	2.15	139.774	
		GLY148:HN -- O:LIG	CnlHb	2.07	129.27	
		ILE147:CA -- O:LIG	C-Hb	2.73	-	
		LIG1:C -- O:TYR103	C-Hb	3.74	-	
		LIG1:C -- O:LYS130	C-Hb	3.58	-	
		ARG84:HN -- LIG	Pi-DHb	3.07	-	

Legend: Molecular interaction of lead molecules with targets NS5. CnlHb-Conventional H-bonds (Hb), C-Hb – Carbon Hb, Pi-DHb-pi donor Hb, Pi-S - pi Sulfur bond, Pi-A - pi bond acceptors, BD- bond distance, DHAA-donor hydrogen acceptor angle**,** HPh.-hydrophobic.

tumor, anti-inflammatory, antimicrobial, antiviral and antioxidant properties.[37-39] However, *in-vitro* evaluation and *in-vivo* clinical trials are required to establish the anti-dengue activity of the selected compounds for the development of antiviral drugs.

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