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Exploration of 5-Hydroxybowdichione Flavonoids As Inhibitors of Dengue Virus Ns5 RNA-Dependent RNA Polymerase Using Molecular Docking Approach

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Abstract

Objectives: *Human lives are now seriously threatened by dengue fever, which is brought on by the dengue virus (DENV). Aedes aegypti mosquitoes, which reproduce in still water, are the primary vectors of the arboviral virus known as dengue. It is well-recognized that phytochemicals have a high potential to eliminate bacterial, viral, and fungal infections in people. Thus, it demonstrates its inhibitory effects on the dengue virus. This study identifies some of the 5-hydroxybowdichione flavonoids' natural dengue virus inhibitors.* ***Methods:*** *5-hydroxybowdichione flavonoids were employed in a computational technique to evaluate the effective inhibitor against dengue virus NS5 RNA-dependent RNA polymerase. 15 flavonoids in total, including 5-hydroxybowdichione and its derivatives, were chosen. The findings analysis was carried out using a variety of methods and instruments. To identify the phytocompounds, PubChem was employed. Protein was retrieved from the Protein Data Bank and confirmed using a variety of tools, including the BIOVIA discovery studio software, PDBsum generates, and the Swiss model. The PyRx software was used to carry out the molecular docking. For the pharmacological research, ADMET studies on these flavonoids were conducted.* ***Results:*** *The ligands Cudraflavone-C, Paratocarpin-B, Paratocarpin-C, and Shancio-H have the lowest binding affinities and may have an inhibitory impact on DENV, according to the ADMET analysis and docking data. These ligands have the best ADMET characteristics and pose the least amount of hazardous risk.* ***Conclusion:*** *The best binding affinity for protein is -9.7, and it belongs to the ligand Shanciol-H. In vitro studies can be used to learn more about these substances.*

Keywords: ADMET, molecular docking, phytocompounds, RNA-dependent RNA polymerase, Dengue virus, 5-hydroxybowdichione.

INTRODUCTION

Dengue fever is a viral disease caused by the dengue virus (DENV), which belongs to the Flavivirus genus and family [1,2]. It is prevalent in many parts of the world, where half of the global population is at risk of contracting the disease [3]. Dengue is transmitted by mosquitoes, mainly Aedes aegypti, and to a lesser extent, Aedes albopictus, which carry the virus and spread it to humans through their bites [4].

Patients who have a primary or initial secondary dengue infection will experience mild-acute febrile sickness that is not otherwise distinguished from classical dengue fever (DF) [4].

A viral ailment called dengue is spread by mosquitoes [5]. The Flaviviridae family is a group of viruses that includes many important human pathogens such as the dengue virus (DENV) and the

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Zika virus. Specifically, the family Flaviviridae includes the four distinct serotypes of the dengue virus: DENV-1, DENV-2, DENV-3, and DENV-4. These viruses are transmitted to humans by infected mosquitoes and can cause a range of symptoms from mild flu-like illness to severe and potentially fatal diseases such as dengue hemorrhagic fever and dengue shock syndrome. Dengue viruses are a significant public health concern in many parts of the world, especially in tropical and subtropical regions [6]. The majority of flaviviruses are found in tropical and temperate regions of the world, where they are known to cause a variety of diseases in people, including dengue fever, tick-borne encephalitis, west Nile fever, and yellow fever [7]. Yes, that's correct. Aedes aegypti and Aedes albopictus are the two species of mosquitoes that are primarily responsible for transmitting dengue virus (DENV) infections to humans. Aedes aegypti is considered the main vector of DENV transmission and is found in many tropical and subtropical regions around the world. Aedes albopictus, also known as the Asian tiger mosquito, is another important vector of DENV and has a wider geographical range, including many temperate regions. Both species of mosquitoes typically breed in standing water, such as in containers, discarded tires, and other objects that can hold water. Effective mosquito control measures such as removing standing water, using insect repellents, and using mosquito nets can help reduce the risk of dengue virus transmission [8]. The 11 KB DENV genome codes for a polyprotein that has 10 structural and non-structural proteins [9]. Three proteins make up structural proteins: an envelope protein, a membrane-associated protein, and a core/capsid protein.

The seven proteins that make up non-structural proteins are NS, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [8]. Yes, it is believed that around 75 percent of people on the planet use medicinal plants as a source of health care. Traditional medicinal systems, such as Ayurveda, Traditional Chinese Medicine, and Indigenous medicine, have been using plants as a source of medicine for thousands of years. Even today, many modern medicines have been developed based on natural compounds found in plants.

Plants contain a variety of chemical compounds that can have medicinal properties, such as alkaloids, flavonoids, and terpenes. These compounds can have a range of effects on the human body, including anti-inflammatory, antimicrobial, and analgesic properties.

Despite the widespread use of medicinal plants, it's important to note that not all plants are safe for consumption, and some can have harmful side effects. Additionally, the effectiveness of medicinal plants can vary widely depending on factors such as the plant species, the preparation method, and the dosage. It's important to consult with a qualified healthcare professional before using any medicinal plant or herbal supplement for the treatment of health conditions [8]. Alkaloids, limonoids, flavonoids, furyl compounds, polyenes, thiophenes, organosulfur compounds, peptides, coumarins, terpenoids, polyphenolics, and saponins are only a few of the many phytochemicals that can be found in medicinal plants [8]. The flavivirus genome encodes the NS5 protein, which is the biggest [10] and most highly conserved viral protein [1, 11]. Particularly, this protein reveals that the four dengue serotypes share 67-82% of their amino acid sequence.

Yes, that's correct. NS5 is a non-structural protein found in viruses belonging to the Flaviviridae family, such as the dengue virus and the Zika virus. NS5 is a large protein that consists of two functional domains: a methyltransferase (MTase) domain and an RNA-dependent RNA polymerase (RdRp) domain.

The MTase domain is located at the N-terminal region of NS5 and is responsible for adding a methyl group to the 5' cap of viral RNA, which helps to protect the viral RNA from degradation by host cell enzymes. The RdRp domain is located at the C-terminal region of NS5 and is responsible for catalyzing the synthesis of viral RNA from a RNA template.

Both the MTase and RdRp domains are critical for the replication of the viral genome and are considered attractive targets for the development of antiviral drugs. Inhibition of the MTase domain can

prevent viral RNA capping and destabilize the viral genome, while inhibition of the RdRp domain can prevent viral RNA replication and ultimately lead to the suppression of viral replication. (Klema et al., 2016). A brief sequence of poorly conserved amino acids connects these domains [12]. Both the MTase and RdRp domains are necessary for the viral replication cycle and exhibit enzymatic activity. An RNA methyltransferase (MTase) domain is located in the N-terminal region of the protein, while an RNA-dependent RNA polymerase domain is located in the C-terminal two-thirds [13, 14].

Through the replication of viral RNA, DENV NS5 RdRp is essential to the viral life cycle [12]. To ascertain the interaction mechanism between DENV RdRp and its inhibitor, in silico research was used [15]. Because Rdrp plays a significant part in the viral replication process, it was selected as a target [16].

The drug research and development process can be made more effective with the help of computer-aided drug design (CADD) [17]. Computer-aided drug design (CADD) involves the use of various computational tools and technologies to aid in the design and optimization of new drugs. Some of the most commonly used CADD technologies include:

Quantitative structure-activity relationship (QSAR): This involves the use of mathematical models to predict the activity of a drug based on its chemical structure and physicochemical properties.

Molecular docking: This involves the computational analysis of the interactions between a small molecule (the drug) and a target protein, in order to predict the binding affinity of the drug for the target.

Molecular dynamics simulation (MD): This involves the simulation of the dynamic behavior of molecules over time, in order to predict their interactions and behavior in different conditions.

Pharmacophore modelling (PM): This involves the identification of common structural features or motifs (known as pharmacophores) that are important for the activity of a drug, in order to aid in the design of new compounds.

These technologies can help to accelerate the drug discovery and development process by providing insights into the activity, toxicity, and other properties of potential drug candidates, and helping to optimize their chemical structures and properties for better efficacy and safety [4]. While molecular dynamics is used to monitor the stability of the bonds in a dynamic picture, as opposed to docking, which is a static picture, it is used to analyze the model and affinity of the interaction of enzymes/proteins with ligands/inhibitors [18–20].

MATERIALS & METHODS

Receptors and Ligand Selection

Natural compounds discovered in *Dalbergia candenatensis* and *Dalbergia parviflora* include 5-hydroxybowdichione [21]. For this study, a total of fifteen 5-hydroxybowdichione flavonoids with inhibitory activity were chosen [8] listed in Table 1. These ligands were obtained in SDF format along with the PubChem CID, canonical SMILES, molecular formula, and molecular weight from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [22]. Using the OpenBabel programme, these ligands were translated to the.pdb format [23].

Protein Retrieval and Purification

The dengue virus NS5 RNA-dependent RNA polymerase protein structure with PDB id-2J7U [24] was obtained from the RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank) (https://www.rcsb.org/) in the .pdb format [25]. The 1.85 resolution of the X-RAY diffraction method was used to resolve the protein. The protein structure of the NS5 RNA-dependent RNA polymerase from the dengue virus has been the subject of many studies in recent years. However, one

of the challenges in studying this protein is that it has several missing residues, which can make it difficult to accurately predict its structure and function.

To overcome this challenge, researchers have used a variety of computational and experimental techniques to obtain a better understanding of the NS5 RNA-dependent RNA polymerase structure and function. These techniques include homology modeling, molecular dynamics simulations, X-ray crystallography, and cryo-electron microscopy.

By using these techniques, researchers have been able to build accurate models of the NS5 RNA-dependent RNA polymerase structure, and to gain insights into its interactions with other viral and host proteins, its role in viral replication, and its potential as a target for antiviral drugs. This research is important for the development of new treatments for dengue virus infection, which remains a major public health concern in many parts of the world. Due to this, homology modelling was carried out utilising the SWISS-MODEL web server (https://swissmodel.expasy.org/) to create the model [26]. Using the BIOVIA discovery Studio programme, the protein structure was cleaned by eliminating heteroatoms, ligand groups, and water molecules from the protein crystal structure. Once the structure has been cleaned up, it is saved in files with the. pdb extension.

Validation of the Protein Structure

In terms of torsion angles (phi), the Ramachandran plot offers a straightforward two-dimensional graphic representation of all potential protein structures (psi). While the torsion angle surrounding the link between the -carbon and the carbonyl carbon atoms is known as that of the bond between the amino nitrogen and the -carbon atoms is known as [27]. The backbone conformation-representing alphaL-region, along with the two bigger "allowed" regions known as the alpha region and the beta region, comprise the three main regions [28]. The favourable and unfavourable regions are represented by the Ramachandran plot. For the creation of the Ramachandran plot, pure protein was employed and submitted to the PDB sum-generaTE process.

Molecular Docking

Molecular docking is a widely used computational approach in the field of drug discovery and design, which involves the prediction of the binding mode and affinity of a small molecule (such as a drug candidate) to a target protein (such as an enzyme or receptor).

By using molecular docking, we can gain insights into how small molecules interact with proteins at the atomic level, and how these interactions influence the activity, selectivity, and other properties of the small molecules. This information can help us to design and optimize new drugs that are more potent, selective, and less toxic than existing drugs.

In molecular docking, the small molecule and the protein are represented by 3D structures, and their interactions are evaluated using computational algorithms that calculate the energy of the complex. The goal is to identify the most favorable binding mode and energy for the small molecule, which is expected to correspond to the most potent and selective drug candidate.

Molecular docking can also be used to explore the flexibility and dynamics of the small molecule and protein, and to predict how these factors may affect the binding affinity and selectivity. This information can be used to guide the design of more effective drug candidates and to optimize the drug discovery process [29]. Python is a programming language that may be used to create PyRx, which is compatible with almost all current computers, including PCs and supercomputers. PyRx is a freely available software program that can be used for virtual screening in computational drug discovery. Virtual screening is a computational technique used to identify potential drug candidates from large libraries of compounds, by predicting their binding affinity and specificity for a specific protein target.

PyRx can be used to perform both ligand-based and structure-based virtual screening, using a variety of computational algorithms and methods. Ligand-based virtual screening involves the use of known ligands (small molecules that bind to the target protein) to identify new compounds with similar chemical structures and properties. Structure-based virtual screening, on the other hand, involves the use of the 3D structure of the target protein to predict the binding affinity and specificity of potential drug candidates.

PyRx allows researchers to easily prepare their input data (such as the protein structure, ligands, and compound libraries) and to perform virtual screening using a user-friendly graphical interface. It also provides a range of visualization and analysis tools to help researchers interpret and prioritize their results, and to guide the selection of promising drug candidates for further experimental validation.

PyRx is widely used in both academic and industrial research, and has contributed to the discovery of several new drug candidates in various therapeutic areas [30]. The purified protein was included in PyRx as a macromolecule and flavonoids were incorporated as a ligand. Energy minimization was carried out once the ligands had been loaded, and the ligands were then transformed into.pdbqt format. The following grid was produced, with dimensions (Angstrom) X:70.7232 Y:71.3954 Z:66.9513 with a central coordinate of X:24.3516 Y:60.8939 Z:16.3879. Nine distinct docking conformations were tested in PyRx to find the one with the best binding score based on binding affinity. As they exhibit the lowest binding scores among all the conformations, the binding affinity corresponding to zero RMSD (Root Mean Square Deviation) values was evaluated as the optimal docking conformation. As the ideal binding complex for each target protein, the top four conformations with the lowest binding affinity were chosen. The docked ligand structures were extracted as.pdb files, and BIOVIA Discovery Studio was used to display the interaction.

Visualisation

The PyRx's best binding conformations were downloaded in.pdb format and the BIOVIA Discovery Studio Visualizer was used to display them. The models of two-dimensional, three-dimensional, and non-bond interactions were examined.

Physiochemical Studies

The two main factors that contribute to drug failure are lack of efficacy and safety, which implies that chemical properties like absorption, distribution, metabolism, excretion and toxicity (ADMET) are crucial at every stage of drug research and development [31]. As a thorough resource and open-source tool for the forecasting of chemical ADMET characteristics, admetSAR was created. AdmetSAR 2.0 will serve as a platform for lead optimization and ADMET prediction in drug development [32]. The ligands' canonical SMILES were obtained from PubChem and evaluated using ADMET in the ADMETlab 2.0 website (http://lmmd.ecust.edu.cn/admetsar2/).

RESULT

Selection of Phytocompounds

Based on the docking results obtained from the PyRx mentioned in Table 1, the top four ligands that exhibit the best binding affinity were chosen. Figure 1 depicts the ligands' 2D structural layout. These diagrams were created using the free sketching programme Marvin-Chemaxon.

Table 1. Ligand with their PubChem CID and canonical smiles.

Phytochemical	PubChem CID	Canonical Smile
Shanciol H	102097660	CC(=O)OCC1C(OC2=C1C=C3C(=C2)CCC4=C3C(=CC(=C4)O)OC)C5=CC(=C(C=C5)O)OC
Cudraflavone C	5319924	CC(=CCC1=C(C2=C(C=C1O)OC(=C(C2=O)CC=C(C)C)C3=C(C=C(C=C3)O)O)O)C
Paratocarpin B	42607541	CC(=CCC1=C(C=CC(=C1)C=CC(=O)C2=C(C3=C(C=C2)OC(C=C3)(C)C)O)O)C
Paratocarpin C	42607538	CC(=CCC1=C(C=CC(=C1O)C(=O)C=CC2=CC3=C(C=C2)OC(C=C3)(C)C)O)C

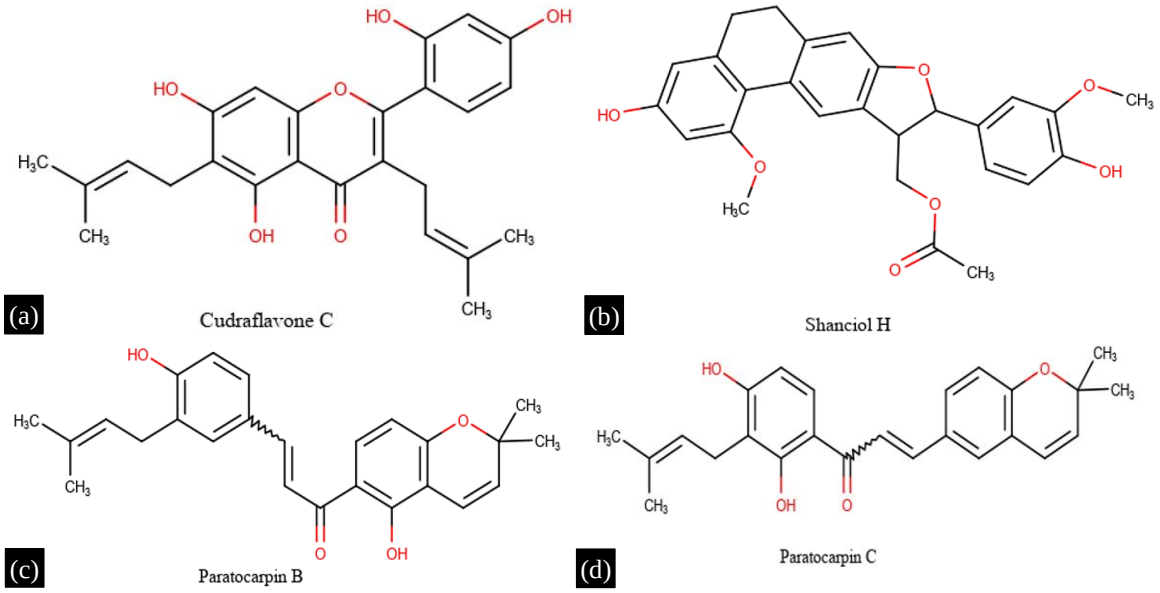

Figure 1. (a–d) 2D structure of the ligands retrieved from *Dalbergia candenatensis*.

Protein Modelling

Using the Swiss model, we have remodel led the protein. From the sequence, 30 templates and 1 model were produced. Model 1 has been chosen, with a QMEANDisco value of 0.88 ± 0.05 and a 99.84% sequence similarity. Figure 2 depicts the remodelled structure of the protein (Dengue virus NS5 RNA dependent RNA polymerase domain) with the pdb id- 2J7U.

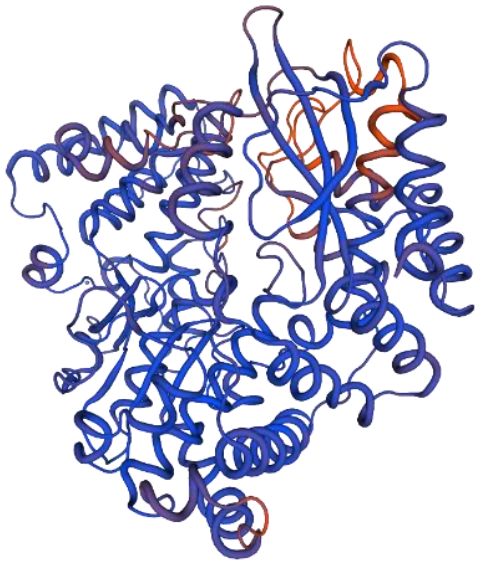

Figure 2. Structure of the remodelled protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

Protein Structure Analysis

For the creation of the protein's Ramachandran plot, the PDBsum generator was used. The Ramachandran plot statistics show that 90.6% of the residues in the purified 3D structure of the protein are located in the most favoured parts of the plot, 8.5% in additional allowed regions, 0.6% in generously allowed regions, and 0.5% in banned regions. These statistical findings confirm that the 3D structures that were modelled are high-quality models. Figure 3 depicts the Ramachandran plot for the protein (Dengue virus NS5 RNA dependent RNA polymerase domain) with the pdb id- 2J7U.

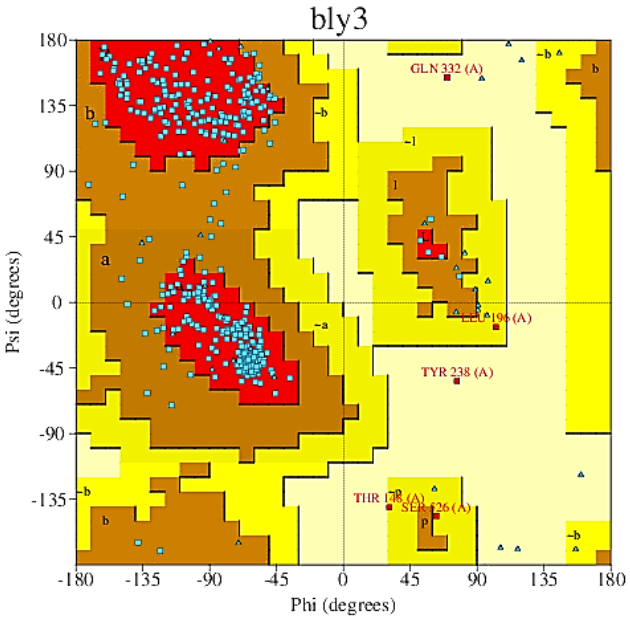

Figure 3. Ramachandran plot of protein (Dengue virus NS5 RNA dependent RNA polymerase domain)

Secondary Structure

Protein's secondary structure was studied using PDBsum. 4 sheets, 1 beta alpha beta, 3 beta hairpins, 2 beta bulges, 10 strands, 31 helices, 32 helix-helix interacs, 36 beta turns, and 8 gamma turns are among the PDBsum findings for protein secondary structure prediction. There are 612 total residues in the structure. Figure 4 depicts the secondary structure for the protein (Dengue virus NS5 RNA dependent RNA polymerase domain) with the pdb id- 2J7U.

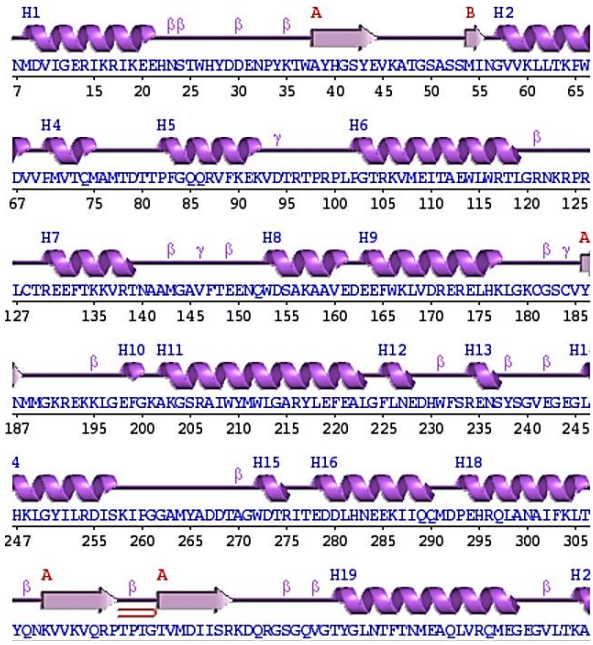

Figure 4. Secondary structure of the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

Molecular Docking and Visualization

In this work, the protein was docked with fifteen ligands. Table 2 lists the ligands' protein binding affinities. After docking, ligands with the lowest root mean square deviation and binding affinities were

chosen. Our ligands were docked with the desired proteins, and measurements of the binding affinity, RMSD/ub, and r/lb were taken. A total of 4 ligands were chosen for additional study. These ligands are as follows *Cudraflavone-C, Paratocarpin-B, Paratocarpin-C, and Shanciol-H* based on their lowest binding affinity.

Table 2. Binding affinity of the ligands with the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

Ligand	PubChem CID	Binding Affinity	rmsd/ub	rmsd/lb
2'57-_Trihydroxyflavone	21611827	-7.9	0	0
3'-Chloro-2'5-dihydroxy-3,7,8-trimethoxyflavone	5281606	-7.7	0	0
4-methoxyflavan	12243061	-7.2	0	0
5-hydroxybowdichione	5491730	-8.2	0	0
Cudraflavone_C	5319924	-9.1	0	0
Paratocarpin_B	42607541	-9.1	0	0
Tephrosol	14704585	-8.7	0	0
Unanisoflavan	15838234	-7.6	0	0
Paratocarpin_c	42607538	-9	0	0
Paratocarpin_d	42607533	-8.3	0	0
Shanciol_D	102121476	-8.8	0	0
Shanciol_H	102097660	-9.7	0	0
Tambulin	5281700	-7.6	0	0
Tenaxin__I	159029	-8.7	0	0
Villosin_A	14543624	-7.5	0	0

Using Dassault Systems BIOVIA Discovery Studio Visualizer, the chosen ligands were visualised, and two-dimensional and three-dimensional models were collected. Additionally, data on the type and category of interactions, as well as the bonding distance for the relevant amino acid residues in the ligand, were also gathered. Figures 5,6 shows 2D& 3D interactions of top ligands interacting with protein (Dengue virus NS5 RNA dependent RNA polymerase domain)**.**

ADMET Analysis

ADMET was tested for in Cudraflavone-C, Paratocarpin-B, Paratocarpin-C, and Shanciol-H. Tables 3, 4, 5, 6, 7, and 8 show how their physiochemical characteristics, medicinal chemistry, absorption, distribution, metabolism, excretion, and toxicity were determined using a web programme called ADMETlab 2.0.

Table 3. Physicochemical properties of the top ligands with the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

PubChem CID	MW	Vol	nHA	nHD	nRot	nRing	nHet	fChar	Flex	TPSA	LogS	PAINS	Lipinski
5319924	422.17	441.663	6	4	5	3	6	0	0.25	111.13	-2.763	0	Accepted
42607541	390.18	424.083	4	2	5	3	4	0	0.25	66.76	-3.889	0	Accepted
42607538	390.18	424.083	4	2	5	3	4	0	0.25	66.76	-3.811	0	Accepted
102097660	462.17	467.933	7	2	6	5	7	0	0.222	94.45	-4.066	0	Accepted

MW: Molecular weight; **nHA:** Number of hydrogen bond acceptors; **nHD:** Number of hydrogen bond donors; **nRot:** Number of rotatable bonds; **nRing**: Number of rings; **MaxRing:** Number of atoms in the biggest ring; **nHet:** Number of heteroatoms; **nRig:** Number of rigid bonds: **Flex:** Flexibility; **TPSA:** Topological polar surface area; **logS:** The logarithm of aqueous solubility value; **PAINS:** Pan Assay Interference Compounds; ; **Lipinski Rule of 5:** Molecular weight less than 500 daltons, nHD<5, nHA<10, lipohilicity<4.15 and TPSA: 40-130 $Å^2$.

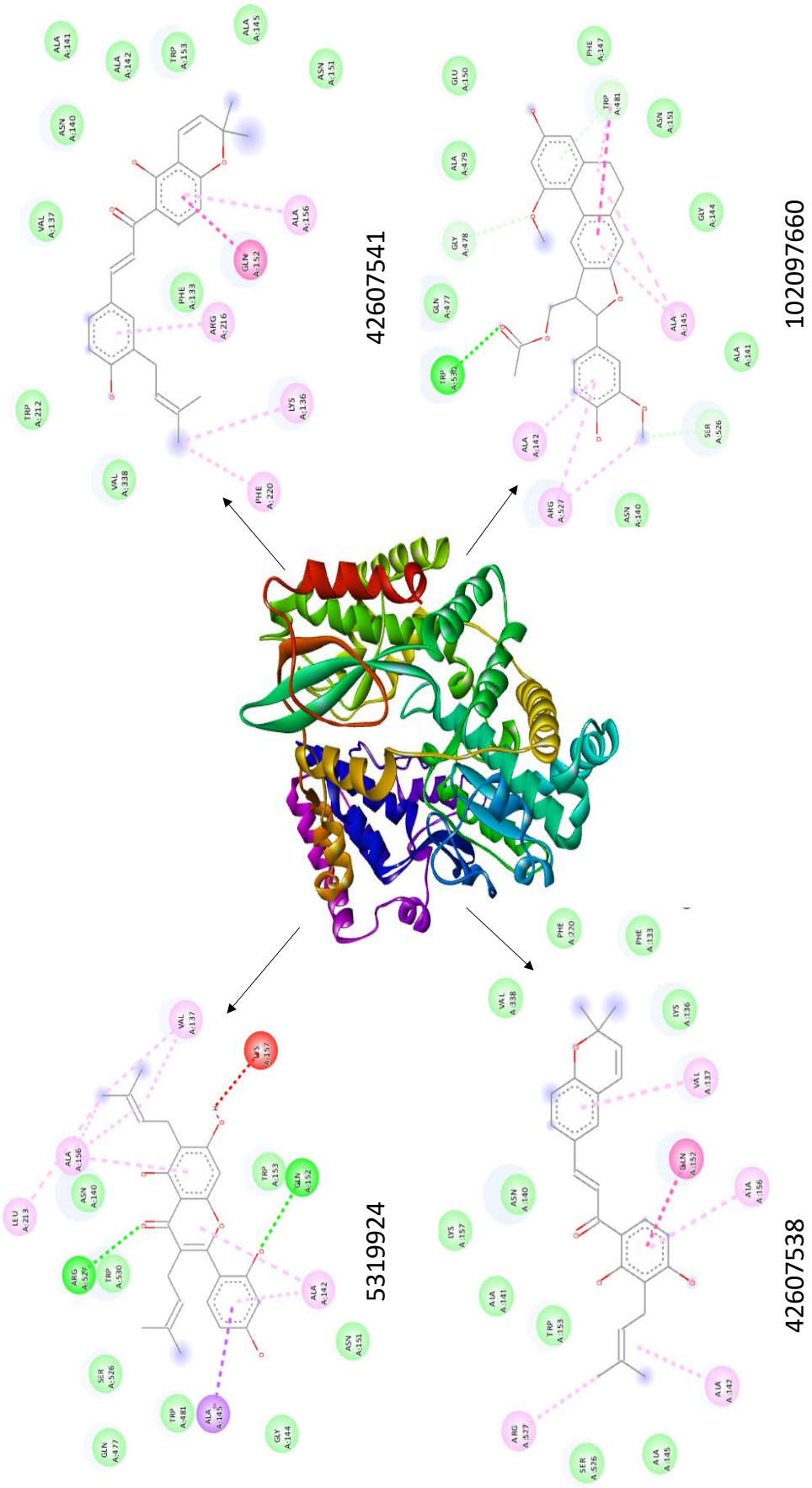

Figure 5. 2D interactions of top ligands interacting with protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

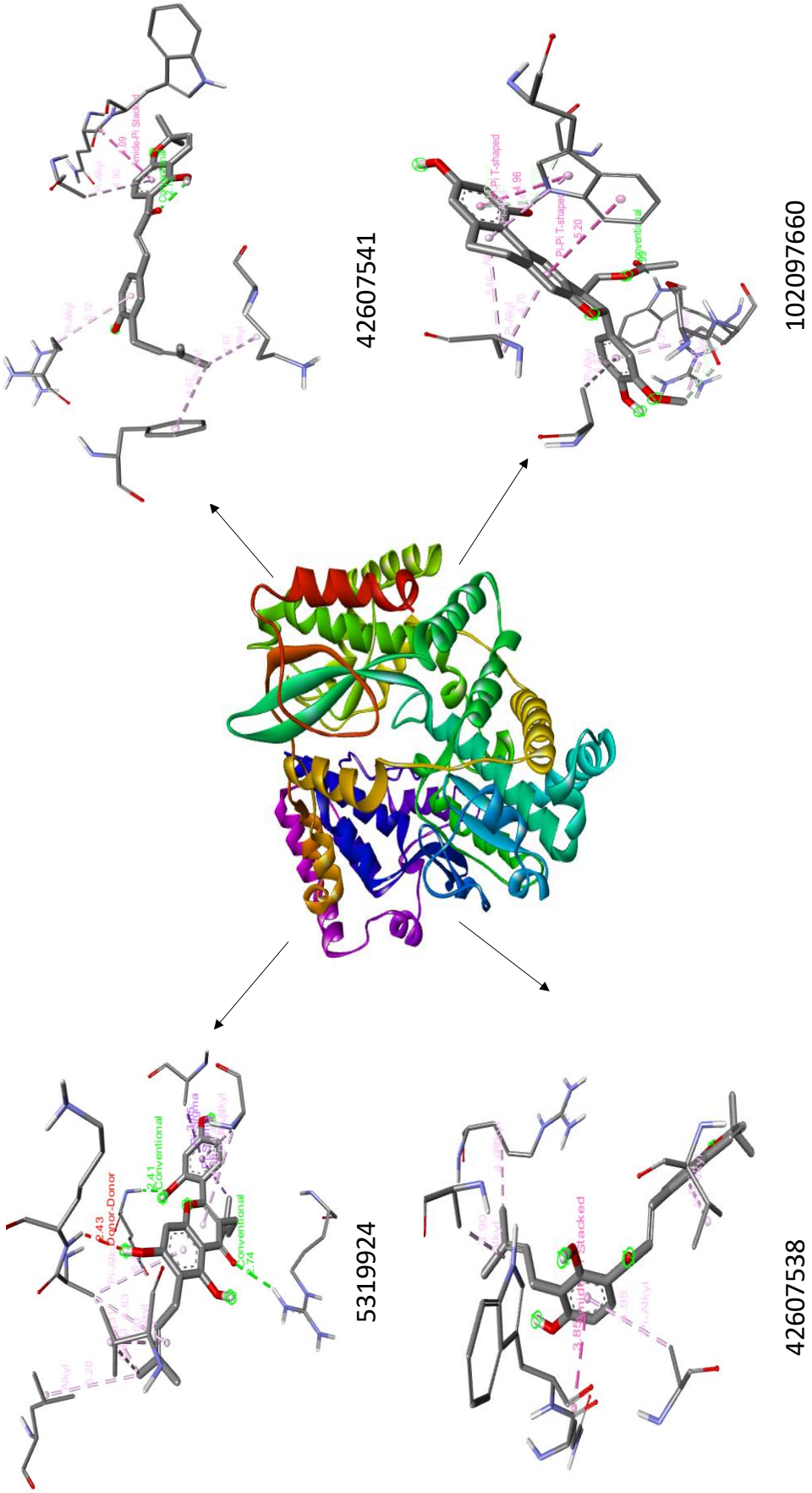

Figure 6. 3D interactions of top ligands interacting with protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

Table 4. Medicinal chemistry properties of the top ligands with the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

PubChem CID	QED	Synth	Fsp3
5319924	0.415	3.134	0.24
42607541	0.391	2.966	0.24
42607538	0.391	2.978	0.24
102097660	0.533	3.624	0.296

QED:A measure of drug-likeness based on the concept of desirability; **Fsp3:** the number of sp3 hybridized carbons/total carbon count; **Synth:** Synthetic accessibility score were accounted.

Table 5. Adsorption properties of the top ligands with the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

PubChem CID	Caco-2	MDCK	Pgp-inh	Pgp-sub	HIA	F(20%)
5319924	-4.969	1.28E-05	0.4	0.801	0.026	0.977
42607541	-4.885	2.17E-05	0.996	0.002	0.009	0.011
42607538	-4.935	2.26E-05	0.995	0.003	0.01	0.018
102097660	-5.259	2.03E-05	0.085	0.008	0.009	0.005

Caco-2: Caco-2 Permeability; **MDCK:** Madin−Darby Canine Kidney cells (MDCK) Permeability; **Pgp-inh/ Pgp-sub:** the inhibitor and substrate of P-glycoprotein; **HIA:** Human intestinal absorption; **F(30%):** the human oral bioavailability 30%

Table 6. Distribution properties of the top ligands with the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

PubChem CID	BBB	PPB	VDss	Fu
5319924	0.003	92.43%	0.84	8.19%
42607541	0.01	103.27%	0.755	0.90%
42607538	0.017	103.00%	0.908	1.18%
102097660	0.019	96.16%	0.687	2.31%

BBB: Blood–brain barrier; **PPB:** Plasma protein binding; **VDss:** Volume Distribution; **Fu:** fraction unbound in plasma.

Table 7. Metabolism and excretion properties of the top ligands with the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

PubChem CID	CYP1A2-inh	CYP1A2-sub	CYP3A4-inh	CYP3A4-sub	CL
5319924	0.813	0.176	0.272	0.068	8.063
42607541	0.941	0.283	0.702	0.19	7.643
42607538	0.916	0.356	0.597	0.241	8.661
102097660	0.194	0.892	0.8	0.84	3.701

CYP1A2-inh, CYP1A2-sub, CYP3A4-inh and CYP3A4-sub: substrate therapeutic molecules; **Cl:** clearance of toxicity

Table 8. Toxicity properties of the top ligands with the protein (Dengue virus NS5 RNA dependent RNA polymerase domain).

PubChem CID	hERG	H-HT	DILI	Ames	Carcinogenicity	Respiratory	IGC50	LC50
5319924	0.015	0.911	0.953	0.506	0.061	0.146	5.128	6.403
42607541	0.244	0.893	0.526	0.268	0.824	0.862	5.361	6.686
42607538	0.367	0.9	0.218	0.204	0.809	0.882	5.306	6.528
102097660	0.102	0.239	0.647	0.517	0.097	0.165	5.405	7.217

hERG: The human ether-a-go-go related gene; **DILI:** Drug-induced liver injury; **AMES:** The Ames test for mutagenicity; **FDAMDD:** The maximum recommended daily dose, carcinogenicity, and 96-hour fathead minnow LC_{50} were examined.

DISCUSSION

The single positive-stranded RNA virus of the family Flaviviridae, genus Flavivirus, or dengue virus (DENV), is what causes dengue fever [33]. It is spread by mosquitoes. Four different viral serotypes have been identified, and a fifth has reportedly been discovered [34], all of which are capable of causing

the full range of diseases. The symptoms mentioned are often associated with severe forms of dengue virus infection, which is a mosquito-borne viral disease that affects millions of people worldwide each year. Figure 7 represents Life cycle of dengue virus vector A. aegypti.

The symptoms typically start with a sudden onset of high fever, accompanied by severe headache, muscle and joint pain, and general malaise. Patients may also experience a rash on the skin, often characterized by small red bumps or patches, and may develop hemorrhagic symptoms such as bleeding from the gums, nose, or skin.

In severe cases, dengue fever can progress to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which are characterized by circulatory failure, low blood pressure, and organ failure. DHF and DSS can be life-threatening if not treated promptly, and require close monitoring and supportive care in a hospital setting.

Prompt diagnosis and management of dengue virus infection are critical to preventing complications and reducing the risk of mortality. Treatment typically involves supportive care, such as hydration and pain management, and in severe cases, blood transfusions and intensive care support. There is currently no specific antiviral treatment for dengue virus infection, although several drug candidates are being developed and tested in clinical trials [35].

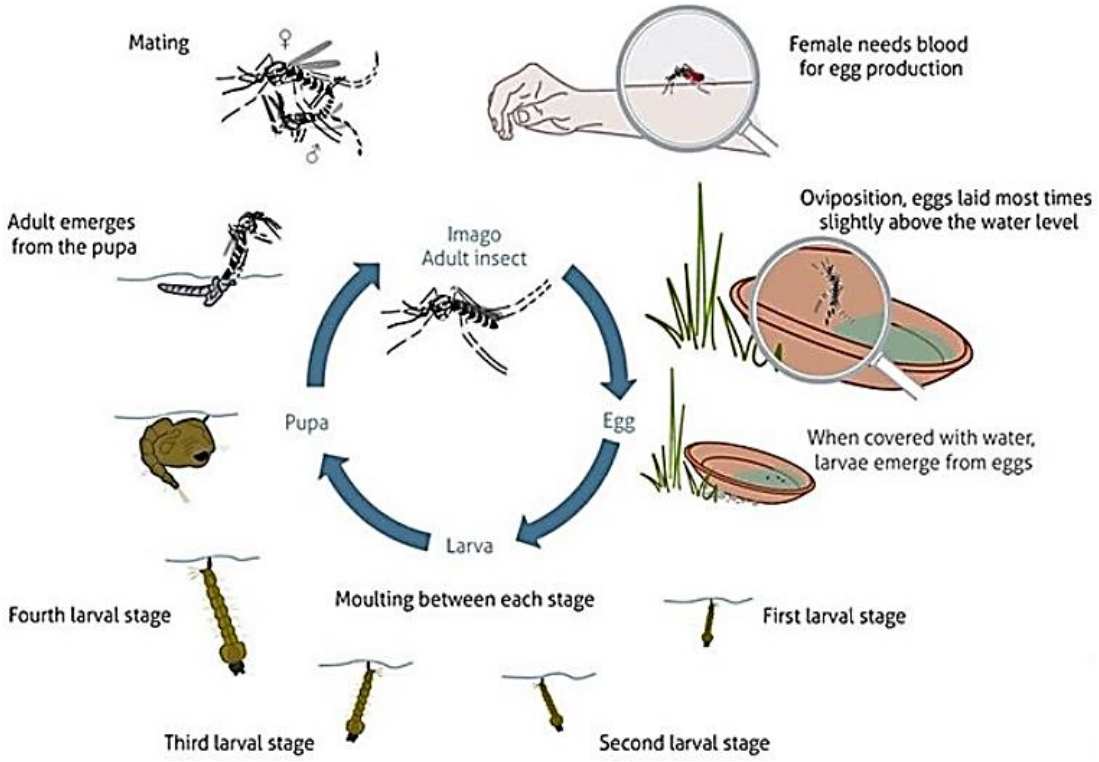

Figure 7. Life cycle of dengue virus vector A. aegypti.

The DENV genome is made up of a single positive-polarity RNA strand that is 10.8 kb long [36]. open reading frame that encodes a single polyprotein that is split into eight non-structural (NS) proteins, numbered NS1 through NS5 and the structural proteins capsid, membrane, and envelope. Dengue fever has become a major global public health concern over the past few decades, with a significant increase in the number of cases reported worldwide.

The factors contributing to the rise in dengue cases include rapid population growth, unplanned urbanization, inadequate mosquito control measures, and increased global travel and trade, which have

facilitated the spread of the virus to new areas. Climate change may also be playing a role, as rising temperatures and changing rainfall patterns can create favorable conditions for the mosquito vector that transmits the virus.

India has been particularly affected by the dengue virus, with frequent outbreaks reported in various parts of the country. The first virologically confirmed dengue fever pandemic in India occurred in Calcutta and the Eastern Coast of India in 1963-1964. However, the first documented case of dengue-like disease in India dates back to the 1780s in Madras (now Chennai).

Efforts to control the spread of dengue virus infection have focused on a combination of mosquito control measures, such as insecticide spraying and the elimination of mosquito breeding sites, and public education campaigns aimed at reducing mosquito bites and increasing awareness of the disease. Research efforts are also focused on developing effective vaccines and antiviral drugs to prevent and treat dengue virus infection [37].

Based on the conserved motif GDD present in various RNA-dependent RNA polymerases, it is believed that NS5, the virus' biggest protein, is involved in the creation of this replicative intermediate form. NS5 is the probable RNA-dependent RNA polymerase involved in viral RNA replication [38].

Yes, that's correct. In silico drug design, also known as computer-aided drug design (CADD), has become an increasingly popular approach in the field of drug discovery and development. This method involves using computational tools and techniques to evaluate the properties and interactions of potential drug candidates in a simulated environment, with the goal of identifying promising compounds that can be further tested and optimized in the laboratory.

The in silico drug design process typically involves several steps, including pharmacophore screening, molecular docking, analysis of post-docking interactions, and molecular dynamics simulations (MDS), among others. By using these techniques, researchers can predict the binding affinity and selectivity of potential drug candidates against specific target proteins or disease pathways, as well as analyze the stability and conformational dynamics of drug-protein complexes.

In recent years, in silico drug design has been applied to a wide range of diseases, including cancer, infectious diseases, and neurological disorders, among others. This approach has the potential to accelerate the drug discovery process and reduce the cost and time required to bring new drugs to market. However, it is important to note that in silico predictions must be validated through experimental studies, and that the success of this approach depends on the availability and accuracy of relevant data and computational tools [39].

Molecular docking is a method for predicting how two or more molecules would interact when they have the lowest possible binding affinity and the highest compositional confirmation [40].

Based on their pharmacological action, fifteen 5-hydroxybowdichione flavonoids were chosen for this study. The targeted protein dengue virus NS5 RNA-dependent RNA polymerase has the lowest binding affinity to the ligands Cudraflavone-C, Paratocarpin-B, Paratocarpin-C, and Shanciol-H. These ligands were chosen for further visualisation as a result. These ligands had binding affinities of 9.1, 9.1, 9.0, and 9.7, respectively. The BIOVIA discovery studio was used to visualise its two-dimensional structure. The most prevalent amino acids found in the 2-D structure were found to be GLN:152, ALA:142, and ARG:527.

The top 4 compounds were further put through additional in-silico testing to examine their ADMET and drug-likeness characteristics. According to the physiochemical characteristics of these drungs, Shanciol-H exhibits the best characteristics, with nHA = 7 hydrogen bond acceptors, nHD = 2 hydrogen

bond donors, nRot = 6 rotatable bonds, nRing = 5 rings, nHet = 7 heteroatoms, and with logs = -4.066. Lipinski's selection standards: There are 10 H bond acceptors, 5 H bond donors, and a molecular weight of 150–500 g/mol. All of the phytochemicals in the table exhibit potential pharmacological properties, according to the Lipinski filter study. Shanciol-H can be synthesised in a dry lab as well because it has a synthetic accessibility of 3.624. In comparison to other ligands, it also has a sufficient quantity of sp3 hybridised carbons. Additionally, human intestinal absorption of an oral medicine is necessary for its apparent efficacy since the drug must cross the gastrointestinal membrane before reaching the systemic circulation. Caco-2 cell permeability is used as an indicator for an appropriate candidate drug molecule. It was anticipated that all four ligands would cross caco-2 and enter the human gut. Shanciol-H is more likely to be adsorbed in the gastrointestinal membrane, in light of this. Paratocarpin-B and Paratocarpin-C are predicted to block p-glycoprotein, while Cudraflavone-C may act as p-glycoprotein substrates in terms of predicting P-glycoprotein efflux from the cell. The effectiveness of chemical absorption into the body and the impact of the blood-brain barrier are measured using Papp values of MDCK cell lines, and all four chemicals show low permeability. Due to its strong ability to bind to plasma proteins, paratocarpin-B may be able to reach its target area in high to moderate levels. All of the substances may possibly enter the CNS because they are projected to cross the blood-brain barrier (BBB). The most frequent safety concern related to drug recalls over the past 50 years has been drug-induced liver injury (DILI). The results indicate that none of the chemicals are harmful to liver cells and that daily doses are safe for people. Shanciol-H is a mild carcinogen, while Paratocarpin-C is the ligand with the least capacity to cause mutations. The LC50 for the top four ligands falls short of the desired result.

CONCLUSION

The ligand shanciol-h has the best binding affinity for protein, with a value of -9.7, according to the study's findings. The best result in the ADMET characteristics is displayed by Shanciol-H. These substances can be studied further in in vitro experiments.

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Abbreviations

DENV	Dengue Virus
NS5	Non-Structural protein 5
RdRp	RNA-dependent RNA polmerase
BBB	Blood-Brain Barrier

REFERENCES

1. G. Zou *et al.*, “Functional analysis of two cavities in flavivirus NS5 polymerase.,” *J. Biol. Chem.*, vol. 286, no. 16, pp. 14362–14372, Apr. 2011, doi: 10.1074/jbc.M110.214189.
2. S. P. Lim *et al.*, “A crystal structure of the dengue virus non-structural protein 5 (NS5) polymerase delineates interdomain amino acid residues that enhance its thermostability and de novo initiation activities.,” *J. Biol. Chem.*, vol. 288, no. 43, pp. 31105–31114, Oct. 2013, doi: 10.1074/jbc.M113.508606.
3. N. Ochida *et al.*, “Modeling present and future climate risk of dengue outbreak, a case study in New Caledonia,” *Environ. Heal.*, vol. 21, no. 1, p. 20, 2022, doi: 10.1186/s12940-022-00829-z.
4. C. X. Yu, J. W. Tan, K. Rullah, S. Imran, and C. L. Tham, “Insight parameter drug design for human β-tryptase inhibition integrated molecular docking, QSAR, molecular dynamics simulation, and pharmacophore modelling studies of α-keto-[1,2,4]-oxadiazoles,” *bioRxiv*, p. 2022.07.17.500327, Jan. 2022, doi: 10.1101/2022.07.17.500327.
5. M. G. Guzmán and G. Kourí, “Dengue: an update.,” *Lancet. Infect. Dis.*, vol. 2, no. 1, pp. 33–42, Jan. 2002, doi: 10.1016/s1473-3099(01)00171-2.

6. S. C. Weaver and N. Vasilakis, "Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease.," *Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis.*, vol. 9, no. 4, pp. 523–540, Jul. 2009, doi: 10.1016/j.meegid.2009.02.003.
7. D. L. Akey *et al.*, "Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system.," *Science*, vol. 343, no. 6173, pp. 881–885, Feb. 2014, doi: 10.1126/science.1247749.
8. M. Tahir Ul Qamar *et al.*, "In-silico identification and evaluation of plant flavonoids as dengue NS2B/NS3 protease inhibitors using molecular docking and simulation approach," *Pak. J. Pharm. Sci.*, vol. 30, pp. 2119–2137, Nov. 2017.
9. J. L. Muñoz-Jordan, G. G. Sánchez-Burgos, M. Laurent-Rolle, and A. García-Sastre, "Inhibition of interferon signaling by dengue virus.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 100, no. 24, pp. 14333–14338, Nov. 2003, doi: 10.1073/pnas.2335168100.
10. B. Troost and J. M. Smit, "Recent advances in antiviral drug development towards dengue virus.," *Curr. Opin. Virol.*, vol. 43, pp. 9–21, Aug. 2020, doi: 10.1016/j.coviro.2020.07.009.
11. M. Bollati *et al.*, "Structure and functionality in flavivirus NS-proteins: perspectives for drug design.," *Antiviral Res.*, vol. 87, no. 2, pp. 125–148, Aug. 2010, doi: 10.1016/j.antiviral.2009.11.009.
12. S. P. Lim, C. G. Noble, and P.-Y. Shi, "The dengue virus NS5 protein as a target for drug discovery.," *Antiviral Res.*, vol. 119, pp. 57–67, Jul. 2015, doi: 10.1016/j.antiviral.2015.04.010.
13. E. V Koonin, "Computer-assisted identification of a putative methyltransferase domain in NS5 protein of flaviviruses and lambda 2 protein of reovirus.," *J. Gen. Virol.*, vol. 74 (Pt 4), pp. 733–740, Apr. 1993, doi: 10.1099/0022-1317-74-4-733.
14. E. V Koonin, "The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses.," *J. Gen. Virol.*, vol. 72 (Pt 9), pp. 2197–2206, Sep. 1991, doi: 10.1099/0022-1317-72-9-2197.
15. J. F. Fatriansyah, R. K. Rizqillah, and M. Y. Yandi, "Molecular Docking and Molecular Dynamics Simulation of Fisetin, Galangin, Hesperetin, Hesperidin, Myricetin, and Naringenin against Polymerase of Dengue Virus," *J. Trop. Med.*, vol. 2022, p. 7254990, 2022, doi: 10.1155/2022/7254990.
16. F. Picarazzi, I. Vicenti, F. Saladini, M. Zazzi, and M. Mori, "Targeting the RdRp of Emerging RNA Viruses: The Structure-Based Drug Design Challenge.," *Molecules*, vol. 25, no. 23, Dec. 2020, doi: 10.3390/molecules25235695.
17. X. Lin, X. Li, and X. Lin, "A Review on Applications of Computational Methods in Drug Screening and Design.," *Molecules*, vol. 25, no. 6, Mar. 2020, doi: 10.3390/molecules25061375.
18. M. P. Allen, "Introduction to molecular dynamics simulation," *Comput. soft matter from Synth. Polym. to proteins*, vol. 23, no. 1, pp. 1–28, 2004.
19. A. R. Leach, B. K. Shoichet, and C. E. Peishoff, "Prediction of protein− ligand interactions. Docking and scoring: successes and gaps," *J. Med. Chem.*, vol. 49, no. 20, pp. 5851–5855, 2006.
20. O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *J. Comput. Chem.*, vol. 31, no. 2, pp. 455–461, 2010.
21. A. Rutz *et al.*, "The LOTUS initiative for open knowledge management in natural products research," *Elife*, vol. 11, p. e70780, 2022, doi: 10.7554/eLife.70780.
22. S. Kim *et al.*, "PubChem in 2021: new data content and improved web interfaces," *Nucleic Acids Res.*, vol. 49, no. D1, pp. D1388–D1395, Jan. 2021, doi: 10.1093/nar/gkaa971.
23. N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, and G. R. Hutchison, "Open Babel: An open chemical toolbox," *J. Cheminform.*, vol. 3, no. 1, p. 33, 2011, doi: 10.1186/1758-2946-3-33.
24. Y. T. Leong *et al.*, "Crystal Structure of the Dengue Virus RNA-Dependent RNA Polymerase Catalytic Domain at 1.85-Angstrom Resolution," *J. Virol.*, vol. 81, no. 9, pp. 4753–4765, May 2007, doi: 10.1128/JVI.02283-06.

25. S. K. Burley *et al.*, "RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences," *Nucleic Acids Res.*, vol. 49, no. D1, pp. D437–D451, Jan. 2021, doi: 10.1093/nar/gkaa1038.
26. A. Waterhouse *et al.*, "SWISS-MODEL: homology modelling of protein structures and complexes," *Nucleic Acids Res.*, vol. 46, no. W1, pp. W296–W303, Jul. 2018, doi: 10.1093/nar/gky427.
27. P. Kumar and A. Arya, "Ramachandran Plot," Dec. 2018.
28. S. A. Hollingsworth and P. A. Karplus, "A fresh look at the Ramachandran plot and the occurrence of standard structures in proteins.," *Biomol. Concepts*, vol. 1, no. 3–4, pp. 271–283, Oct. 2010, doi: 10.1515/BMC.2010.022.
29. X.-Y. Meng, H.-X. Zhang, M. Mezei, and M. Cui, "Molecular docking: a powerful approach for structure-based drug discovery.," *Curr. Comput. Aided. Drug Des.*, vol. 7, no. 2, pp. 146–157, Jun. 2011, doi: 10.2174/157340911795677602.
30. S. Dallakyan and A. J. Olson, "Small-molecule library screening by docking with PyRx.," *Methods Mol. Biol.*, vol. 1263, pp. 243–250, 2015, doi: 10.1007/978-1-4939-2269-7_19.
31. L. Guan *et al.*, "ADMET-score - a comprehensive scoring function for evaluation of chemical drug-likeness.," *Medchemcomm*, vol. 10, no. 1, pp. 148–157, Jan. 2019, doi: 10.1039/c8md00472b.
32. H. Yang *et al.*, "admetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties," *Bioinformatics*, vol. 35, no. 6, pp. 1067–1069, Mar. 2019, doi: 10.1093/bioinformatics/bty707.
33. I. A. Rodenhuis-Zybert, J. Wilschut, and J. M. Smit, "Dengue virus life cycle: viral and host factors modulating infectivity," *Cell. Mol. Life Sci.*, vol. 67, no. 16, pp. 2773–2786, 2010, doi: 10.1007/s00018-010-0357-z.
34. V. D. Dwivedi, I. P. Tripathi, R. C. Tripathi, S. Bharadwaj, and S. K. Mishra, "Genomics, proteomics and evolution of dengue virus," *Brief. Funct. Genomics*, vol. 16, no. 4, pp. 217–227, Jul. 2017, doi: 10.1093/bfgp/elw040.
35. S. Hasan, S. F. Jamdar, M. Alalowi, and S. M. Al Ageel Al Beaiji, "Dengue virus: A global human threat: Review of literature.," *J. Int. Soc. Prev. Community Dent.*, vol. 6, no. 1, pp. 1–6, 2016, doi: 10.4103/2231-0762.175416.
36. T. S. Salles *et al.*, "History, epidemiology and diagnostics of dengue in the American and Brazilian contexts: a review," *Parasit. Vectors*, vol. 11, no. 1, p. 264, 2018, doi: 10.1186/s13071-018-2830-8.
37. N. Gupta, S. Srivastava, A. Jain, and U. C. Chaturvedi, "Dengue in India.," *Indian J. Med. Res.*, vol. 136, no. 3, pp. 373–390, Sep. 2012.
38. M. Kapoor, L. Zhang, M. Ramachandra, J. Kusukawa, K. E. Ebner, and R. Padmanabhan, "Association between NS3 and NS5 Proteins of Dengue Virus Type 2 in the Putative RNA Replicase Is Linked to Differential Phosphorylation of NS5 *," *J. Biol. Chem.*, vol. 270, no. 32, pp. 19100–19106, 1995, doi: https://doi.org/10.1074/jbc.270.32.19100.
39. P. Singh *et al.*, "The dual role of phytochemicals on SARS-CoV-2 inhibition by targeting host and viral proteins," *J. Tradit. Complement. Med.*, vol. 12, no. 1, pp. 90–99, 2022, doi: https://doi.org/10.1016/j.jtcme.2021.09.001.
40. P. Biswas *et al.*, "Evaluation of Melongosides as Potential Inhibitors of NS2B-NS3 Activator-Protease of Dengue Virus (Serotype 2) by Using Molecular Docking and Dynamics Simulation Approach," *J. Trop. Med.*, vol. 2022, p. 7111786, 2022, doi: 10.1155/2022/7111786.

