Dual-Target Inhibition Strategy Against Dengue Virus: Molecular Docking and Pharmacokinetic Evaluation of *Clitoria ternatea* Metabolites

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Abstract

Background

Dengue virus (DENV) continues to be a significant health issue on the planet, and there is minimal specific antiviral therapy. The multi-target approach to the inhibition of viral replication and RNA capping involves the targeting of viral enzymes like the NS2B/NS3 protease and NS5 methyltransferase. Phytochemicals of plant origin, especially *Clitoria ternatea* have an antiviral potential that is broad-spectrum and should be investigated systematically by computational methods.

Purpose

The purpose of the study was to discover dual-target anti-dengue lead compounds of selected *Clitoria ternatea* phytochemicals through an integrated in silico drug discovery model comprising structural validation, molecular docking, network pharmacology and ADMET profiling.

Methods

Eight phytochemicals were structurally optimized and docked into DENV NS2B/NS3 protease (2FOM) and NS5 methyltransferase (3L6P). Cavity-based blind docking was used to identify the active-size pockets (CB-Dock2). AutoDock Vina was used in molecular docking through PyRx with AutoDock Vina, and protein stereochemical quality was measured in the form of Ramachandran plot and G-factor analysis. BIOVIA Discovery Studio was used to analyse post-docking interactions. Network pharmacological potential was determined by Network pharmacology (STITCH) whereas the physicochemical, pharmacokinetic and toxicity properties were evaluated by SwissADME, pkCSM, and ADMETlab.

Findings:

The highest binding affinities of Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine and Taraxerone to 2FOM and 3L6P, respectively, were the highest. Interaction profiling showed that there were stable hydrogen bonding and hydrophobic stabilization of catalytic domains. Analysis of ADMET showed that glycosylated flavonoids and Taraxerone had good systemic persistence and high membrane permeability, respectively, but were rapidly cleared. Compounds were all lowly estimated cardiotoxic.

Conclusion

The combined workflow of computation found Delphinidin derivative and Taraxerone as promising dual-target antiviral leads. These results will give a strong preclinical basis to further molecular dynamics modeling and experimental validation on the development of multi-target anti-dengue drugs.

Introduction

Dengue is a fast-spreading mosquito-borne viral pathogen that is caused by the Dengue virus (DENV) belonging to the Flaviviridae family and is one of the most rapidly spreading arbovirus pathogens across the world (World Health Organization [WHO], 2024). Aedes aegypti and Aedes albopictus are the most common vectors that transmit the virus, which can be found in tropical and subtropical areas (Guzman & Harris, 2015). WHO (2024) reports that almost half of the world population is now at risk of getting dengue infection and over the last 20 years the number of incidences has gone up exponentially and in the years 2024, there have been record cases of infection. Other conditions include the rapid urbanization, the growth in global travel and climate change which have greatly increased the rate of transmission of the viruses (Guzman and Harris, 2015). Dengue clinically manifests itself with high fever, myalgia, thrombocytopenia, and severe cases with dengue hemorrhagic fever or dengue shock syndrome (Simmons et al., 2012). Though it is increasingly becoming a heavy burden, no universally effective antiviral treatments can be used, and treatment is largely supportive (Diamond & Pierson, 2015). This growing international medical issue highlights why new and efficient treatment methods are immediately needed.

DENV can be four antigenically different serotypes (DENV-1 through DENV-4), with each of them being able to induce an infection and severe disease (Halstead, 2014). Another significant dengue pathogenesis complication is antibody-dependent enhancement (ADE) whereby previous infection with a specific serotype can boost the further infections by enabling the virus to enter the host cells via the Fc-receptor-based mechanism (Halstead, 2014). After being internalized into the host cytoplasm, the virus quickly replicates and produces structural as well as non-structural proteins needed in the development of the virus. Among them, the NS2B-NS3 protease, and NS5 RNA-dependent RNA polymerase/methyltransferase are essential in processing poly proteins and capping RNAs, respectively, and therefore are the most ideal antiviral targets (Saleem et al., 2019). Nevertheless, their structural preservation, flexibility and the rapid mutation rate of the RNA viruses complicate drug development and instead they induce multi-target treatment with therapeutic approaches as opposed to a single enzyme inhibitor approach. At present, there is no particular antiviral medication that can be used to treat dengue, and the treatment of patients is based on symptomatic management, including antipyretics and intravenous fluids (WHO, 2024). The live attenuated vaccine Dengvaxia is partially protective, but increases the risk of safety in seronegative patients (Sridhar et al., 2018). In vitro activity of experimental protease and polymerase inhibitors has been shown, but they are facing the limitations of hepatotoxicity and off-target effects (Saleem et al., 2019). These restrictions make it clear that there is a need to move to safer multi-mechanistic antiviral solutions.

The use of plant-based medicines in the traditional system dates back to the use of the use of febrile and infectious diseases. Many medicinal plants have already shown antiviral activity against flaviviruses which can be greatly explained by the abundance of phytochemical composition (Jassim & Naji, 2003). Plant extracts (*Andrographis paniculata*, *Carica papaya*, and *Azadirachta indica*) have been explored in the treatment of dengue symptoms (Ahmad et al., 2011; Subenthiran et al., 2013). Among the known bioactive phytochemicals are flavonoids, terpenoids, alkaloids and phenolic compounds which inhibit viral polymerases, disrupt protease activities and control host immune responses (Jassim & Naji, 2003). Plant metabolites that have multi-target biochemical activity can provide a benefit over rapidly mutating RNA viruses, as compared to synthetic drugs. **(Shown in figure-1)** However, inconsistency in extracted contents as well as lack of mechanistic endorsement has impeded their clinical translation. Recent breakthroughs in computational docking and virtual screening have made it possible to screen phytochemicals in a systematic fashion with respect to their use against viral targets (Saleem et al., 2019). Regardless of these advances, a number of strong botanical-based antiviral agents against dengue have not been explored, thus highlighting the necessity of systematic *in-silico* screening and target-based confirmation of medicinal plant extracts.

Butterfly pea is a well-known phytochemical with a traditional medical history and is likely to be utilized as medicine in the future due to its high phytochemical content (*Clitoria ternatea* L., Family: Fabaceae). The plant has a variety of bioactive metabolites such as flavonoid, anthocyanin, phenolic acid and triterpenoid with high concentrations reported to have antiviral activity. With the help of the IMPPAT database, 54 phytoconstituents of *C. ternatea* were first discovered. Following a systematic screening of documented or predicted antiviral relevance and elimination of compounds which are mainly related to unrelated pharmacological activities, a shortlist of eight key candidates was selected and further explored. They were kaempferol (CID: 5280863), quercetin (CID: 5280343), delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine (CID: 25201902), flavonol-3-O-D-glucoside (CID: 11953828), clitorin (CID: 11592917; Kaempferol and quercetin are also known to have a broad-spectrum antiviral and enzyme-inhibitory effect, especially in viral polymerases and proteases (Jassim and Naji, 2003).

Given the structure of the positively charged flavylium backbone (polyacylated ternatins) of anthocyanin derivatives, structural rigidity and increased potential of electrostatic interaction are allowed, and triterpenoids such as taraxerone combine hydrophobic moieties that can stabilize ligand binding within catalytic pockets. Hydrogen-bonding as well as radical-scavenging is also made by phenolic acids like cinnamic acid. In spite of this structural heterogeneity and theoretical antiviral activity, a thorough comparison of these metabolites with dengue-specific molecular targets has not been done to date, thus necessitating systematic in silico docking studies. Dengue viral NS2B-NS3 protease (PDB ID: 2FOM) is an important viral polyprotein processing and maturation protein (Erbel et al., 2006). It has a catalytically active closed form that depends on accurate hydrogen bonding between the catalytic triad thereby making it a prospective site of flavonoid interaction. Quercetin and kaempferol are polyhydroxylated and structurally positioned to develop hydrogen bonds and π–π stacking interactions in the protease active site. Sugar-mediated polar contacts may also be improved by glycosylated flavonoid clitorin and flavonol-3-O-D-glucoside. **(Shown in figure-2)**

Equally, the methyltransferase of the viral RNA (PDB ID: 3L6P) controls the viral capping of the viral RNA through the use of a recognized S-adenosyl-L-methionine (SAM)-binding pocket which is essential in evading the immune system (Geiss et al., 2009). The anthocyanins which have a positively charged flavylium core can have positive electrostatic complementarity in this binding groove. NS2B-NS3 protease and NS5 methyltransferase dual inhibition would thus be able to inhibit viral replication at two mechanistically different parts. Nonetheless, the comparative analysis of docking studies conducted on 2FOM, 3L6P and *C. ternatea* phytochemicals have not been thoroughly conducted and this highlights the untapped potential of the plants in therapeutic use.

In the current study, a multi-layered and systematic computational workflow was used to examine the anti-dengue properties of the choosable *Clitoria ternatea* phytoconstituents towards two vital viral enzymes, namely, NS2B-NS3 protease (PDB ID: 2FOM) and NS5 methyltransferase (PDB ID: 3L6P). The three-dimensional structure of eight major metabolites was retrieved and structurally optimized before docking analysis. The corresponding protein structures were retrieved in the Protein Data Bank and confirmed with the aid of Ramachandran plot and G- factor analysis by using PROCHECK to guarantee the reliability of the stereochemical system and the integrity of the structure (Laskowski et al., 1993). CB-Dock2 was used to identify the active-site and cavity-based blind docking and then molecular docking simulations were performed using AutoDock Vina that is built into PyRx to approximate binding affinities and conformational stability (Trott and Olson, 2010). Visualization of hydrogen bonding, hydrophobic contacts and catalytic residue interactions was performed after docking with analysis in UCSF Chimera and BIOVIA Discovery Studio, which allowed the interpretation of the structural interactions between ligands and proteins and their complexes in detail.

To narrow down and prioritize further on the possible leads, an integrated pharmacokinetic and toxicity analysis was performed. The use of network pharmacology methods was used to evaluate potential polypharmacological interactions, whereas extensive ADMET profiling, including physicochemical descriptors, gastrointestinal absorption, blood-brain barrier permeability, metabolism, clearance, and toxicity risks, was done using SwissADME, pkCSM, and ADMETlab platforms (Daina et al., 2017; Pires et al., 2015). Dual-target phytochemical candidates with the ability to inhibit the viral protease activity and the RNA capping activity at the same time were developed through the systemic correlation of docking-derived binding energetics with pharmacokinetic feasibility and toxicity predictions. This *in-silico* approach of integrating compounds offers an acceptable, reproducible, and affordable framework on the evolution of *C. ternatea*-derived compounds into experimental validation and multi-target antiviral drug development.

Materials and Methods

2.1 Phytochemical Retrieval from IMPPAT Database

Screenings of phytochemical databases were performed using the IMPPAT database to retrieve and select phytochemicals. A systematized phytochemical mining and database search approach was used to discover the therapeutic potential of *Clitoria ternatea* (Butterfly Pea). Discovery of bioactive secondary metabolites is a key critical step in computational drug discovery processes. The Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database was used in this research to venture into the phytochemical space of *C. ternatea*. The IMPPAT platform is an integrated, digital atlas between

conventional Indian medicine and contemporary phytochemical research, allowing the virtual screening of a large number of natural products (Mohanraj et al., 2018).

An exhaustive list of metabolites extracted in the different parts of the plant (roots, seeds and flowers) was created using the botanical nomenclature as the main search query. A strong filtering criterion was used to favor those that had known ethnopharmacological and initial antiviral or anti-inflammatory bioactivity. This identified eight main phytochemical ligands which were subject to further computation: are kaempferol (CID: 5280863), quercetin (CID: 5280343), delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine (CID: 25201902), flavonol-3-O-D-glucoside (CID: 11953828), clitorin (11592917) (kaempferol-3-O-rutinoside), Cinnamic acid (CID: 444539), Taraxerone (CID: 92785), and Flavylium (CID: 145858). To confirm the integrity of the structures in terms of molecular docking, the chemical structures and properties were checked with PubChem database. In using the curated datasets in IMPPAT, the researchers will be able to ensure that the chosen ligands have a history of medicinal safety in use and thereby serves as a reliable starting point to conduct further pharmacokinetic profiling and molecular interaction research on Dengue virus proteins (Vivek-Ananth et al., 2023).

2.2 Preparation of Structural Acquisition and Ligand

They were prepared in a systematic way to ascertain correctness and reliability in the analysis of molecular docking of the selected phytochemical ligands. The compounds were obtained as three-dimensional structures of the PubChem database of the National Center of Biotechnology Information (NCBI) with the help of their respective Compound Identifiers (CIDs) (Kim et al., 2023). The structural refinement of the structures was done before docking and the structures were downloaded in Spatial Data File (SDF). The conversion of SDF to open Babel, an open-source chemical toolbox, was used to convert SDF to PDFQT, the format needed to run simulations with AutoDock (O'Boyle et al., 2011). Molecular mechanics force fields were used to minimize the energy of each ligand in order to eliminate steric clashes and optimize bond lengths, bond angles and torsional parameters. The Gasteiger method of assigning partial charges to atoms was used so that the electrostatic interactions in the docking process could be correctly modeled (Morris et al., 2009). Rotatable bonds were designed in such a way that they were able to provide conformational flexibility which increased the prediction of realistic binding poses. This single-step ligand preparation strategy provided structural stability, acceptable charge distribution, and optimal geometry, which would make it reliable in the docking scores and binding affinity prediction in the future in virtual screening studies (Forli et al., 2016).

2.3 STITCH Database Interaction Analysis.

Network pharmacology analysis was conducted on the STITCH Database (Search Tool for Interacting Chemicals) to determine any possible protein-ligand interactions of phytochemicals of *Clitoria ternatea.* STITCH is an experimental data and curated pathway information alongside text-mined evidence predictor of chemical-protein association (Kuhn et al., 2008). The individual phytochemicals of *Clitoria ternatea* were inputted into PubChem Compound Identifiers (CIDs) and interactive networks were generated. The study allowed the determination of direct binding interactions and indirect functional associations of the bioactive compounds and possible protein targets. The confidence scores of interaction given by the STITCH platform were assessed to test the strength and reliability of the predicted associations. This calculus assay made it possible to identify the targets *in-silico* and rationalized the pharmacological relevance of *Clitoria ternatea* phytochemicals in viral and host-related protein regulation, thus closing the gap between traditional and modern molecular understanding of the human body (Szklarczyk et al., 2021).

2.4 CB-Docking-Based Active Site Identification and Grid Box Determination

Active site prediction and grid box determination were performed using the CB-Dock2 web server, a cavity-detection–guided blind docking platform designed to identify potential ligand-binding pockets without prior specification of active residues. CB-Dock2 integrates the CurPocket algorithm to systematically analyze protein surface topology, curvature, and solvent accessibility in order to detect probable catalytic and allosteric binding sites (Liu et al., 2022). The high-resolution crystal structures of Dengue virus NS2B/NS3 proteases (PDB IDs: 2FOM and 3L6P) were retrieved from the Protein Data Bank and uploaded to the CB-Dock2 server along with optimized phytochemical ligands derived from *Clitoria ternatea.*

For each ligand–protein complex, the server automatically identified the top-ranked binding cavities and generated corresponding grid box parameters, including center coordinates (X, Y, Z axes) and spatial dimensions. These calculated grid parameters were recorded and used to define focused docking regions, ensuring that subsequent molecular docking simulations were restricted to biologically relevant and sterically favorable binding pockets. This automated cavity-guided strategy minimized search space bias, enhanced computational efficiency, and improved the reliability of predicted binding interactions by targeting the most probable active and allosteric sites.

2.5 Preparation and Docking of Proteins Protocol.

Crystallographic structures of Dengue virus NS2B/NS3 proteases (PDB IDs: 2FOM and 3L6P) were obtained in the RCSB Protein Data Bank and subsequently, structural refinement was performed before docking analysis. UCSF Chimera was used to perform protein preprocessing, which involves deletion of the co-crystallized ligands, water molecules, and non-standard residues, polar hydrogen atoms, and Kollman charges to provide proper electrostatic representation (Pettersen et al., 2004; Goddard et al., 2018). The macromolecules were then optimized and transformed into PDBQT to be able to dock them. The grid box parameters (center coordinates and size) were determined using previously known active site cavities to limit the search space by the docking to the regions of biological interest.

PyRx in conjunction with the AutoDock Vina scoring algorithm was used to perform docking simulations (Dallakyan and Olson, 2015; Trott and Olson, 2010). The phytochemicals of *Clitoria ternatea* were docked to the viral protease targets in the rigid receptor conditions by preparing the phytochemicals. Several ligand poses were produced and ranked based on the estimated binding energies (kcal/mol). The most stable complex (obtained with the lowest binding energy, the most negative value) was chosen to be further interacted and structurally analyzed.

2.6 Post-Docking Interaction Analysis and Visualization by BIOVIA Discovery Studio

After the molecular docking simulations, BIOVIA Discovery Studio Visualizer was used to seek the details of the interactions between the ligand-protein complexes. The imports into the structural study were the most thermodynamically stable conformations of the selected phytochemicals of *Clitoria ternatea* docked against Dengue virus NS2B/NS3 proteins (PDB IDs: 2FOM and 3L6P). The software allowed high-resolution two-dimensional maps of interaction and three-dimensional models of the binding interface to be generated in order to accurately model the orientation of the ligand in the catalytic pocket. Timely analysis has been done to determine the main amino acidic residues in the process of ligand stabilization. The traditional and non-standard hydrogen bonds were assessed based on the determination of donor-acceptor reactions between functional groups of flavonoids (e.g., hydroxyl and glycosidic groups of flavonoids) and active-site residues. Also, hydrophobic contacts π–π stacking, π-alkyl contact, and van der Waals interactions were carefully characterized to find out the stabilization in hydrophobic cavities of the proteases. This structural interaction profiling offered mechanistic understanding of the binding modes of the ligands, elucidated the structure-activity relationships and validated the predicted antiviral significance of the studied phytochemicals using the refined spatial confirmation of the catalytic areas.

2.7 Validation of Target proteins by using PROCHECK.

Stereochemical validation of the Dengue virus NS2B/NS3 protease crystal structures (PDB IDs: 2FOM and 3L6P) was done to ensure structural reliability before docking analysis by the use of PROCHECK. Structural validation was done using Ramachandran plot analysis to assess the distribution of backbone dihedral angles (φ and ψ) of amino acid residues to assess and verify structural integrity. A widely used technique to evaluate protein stereochemistry is the Ramachandran plot, which classifies the residues in favored, additionally allowed, generously allowed, and disallowed regions depending on the statistically determined conformational parameters (Laskowski et al., 1993).

Molecular docking requires high-quality protein models to ensure the correct prediction of binding since geometric distortions in the backbone geometry or steric clash in the active sites can produce erroneous prediction of binding. Thus, most of the non-glycine and non-proline residues were confirmed to be in the most preferred parts, which meant that they had stable and energetic allowed conformations. This step of validation reduced the possibility of structural artifact contributions to the ligand-binding results and verified that further docking results were obtained based on geometrically sound and physically realistic receptor structures. The ability of such stereochemical assessment is to enhance the validity of the predicted binding affinities and interaction patterns (Morris et al., 2009; Chen et al., 2010).

2.8 *In-Silico* ADMET and Drug-Likeness Assessment.

Computational workflow would be completed within silico pharmacokinetic and toxicity profiling of the selected phytochemicals using SwissADME and pkCSM. PubChem retrieved SMILES representations of the eight bioactive compounds, which underwent predictive analysis of the eight ADMET properties to these web servers, off the canonical form of their SMILES. The evaluation of drug-likeness was mostly done using Lipinski Rule of five which analyzed the molecular weight, lipophilicity (LogP), the number of hydrogen bond donors, and the number of hydrogen bond acceptors in an attempt to predict oral bioavailability (Lipinski et al., 2001). Topological polar surface area (TPSA), water solubility, and bioavailability score were also analysed to find the systemic suitability.

To analyze the absorption and distribution behavior, pharmacokinetic parameters such as human gastrointestinal absorption, blood-brain barrier permeability and P-glycoproteinsubstrate specificity were projected. The cytochrome P450 (CYP450) isoform inhibition profiling studied was employed to assess the metabolic stability and indicate possible risks of drug-drug interactions (Daina et al., 2017). Endpoints of toxicology estimated using pkCSM were AMES mutagenicity, hepatotoxicity, and inhibition of the human ether-a-go-go-related gene (hERG) channels (cardiotoxicity indicator), and the potential to cause skin sensitization (Pires et al., 2015). This combined ADMET test helped to assure that compounds with good binding affinity to the Dengue virus NS2B/NS3 proteases (2FOM and 3L6P) have good pharmacokinetic and acceptable safety characteristics, which further boosted their candidacy to undergo further experimental validation.

Results and Discussion

3.1 Phytochemical Retrieval and Ligand Library Characterization

The IMPPAT database was systematically mined, which allowed identifying eight major phytoconstituents reported to exist in *Clitoria ternatea*, namely Kaempferol (CID: 5280863), Quercetin (CID: 5280343), Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine (CID: 25201902), Flavonol-3-O-D-glucoside The selection was based on the database that excluded compounds that lacked reported phytochemical occurrence and pharmacological relevance. IMPPAT is a unified database containing phytochemistry, traditional medicine, and molecular annotations, thus facilitating rational compound prioritization during computational drug discovery processes (Mohanraj et al., 2018). The chosen metabolites were structurally different chemical scaffolds, such as flavonoids, anthocyanins, phenolic acids, and triterpenoids. This structural diversity is very beneficial in the discovery of antiviral drugs, specifically in viral proteases where ligand binding is promoted by a balance of hydrogen bonding, hydrophobic interactions and π–π stacking in catalytic domains.

Natural flavonoids like kaempferol and quercetin have been reported in large numbers to have antiviral potential because they inhibit proteases and alter viral replication pathways (Zakaryan et al., 2017). On the same note, anthocyanin analogs and phenolic acid have been shown to display bioactivity against RNA virus via antioxidant and enzyme-inhibitory activity (Wozniak et al., 2021). The curated phytochemical library thus put forward a chemically diverse and biologically significant dataset to be used in subsequent molecular docking, interaction profiling and ADMET analysis. A combination of ethnobotanical information and structure-based computational screening represents one of the modern trends in discovering antiviral drugs guided by natural products (Newman and Cragg, 2020). (Shown in figure-3)

The figure shows the search box to query Clitoria ternatea and the database home page to show phytochemical related associations and therapeutic data.

3.2 Structural Acquisition and Ligand Preparation

The three-dimensional structures of the selected *Clitoria ternatea* phytochemicals were successfully retrieved from the PubChem database and converted into the highly optimized PDBQT format utilizing the Open Babel computational suite. This acquisition and preparation phase comprehensively processed Kaempferol (CID: 5280863), Quercetin (CID: 5280343), Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine (CID: 25201902), Flavonol-3 O-D-glucoside (CID: 11953828), Clitorin (CID: 11592917), Cinnamic acid (CID: 444539), Taraxerone (CID: 92785), and Flavylium (CID: 145858). **(Shown in figure-4 & Table-1)** Subsequent energy minimization protocols effectively resolved localized steric strain and thoroughly optimized the torsional geometry of each compound, establishing the most thermodynamically stable, lowest-energy conformations. Furthermore, the precise assignment of Gasteiger partial charges ensured highly accurate electrostatic modeling, which is a critical prerequisite for evaluating intermolecular non-covalent forces. These rigorous preparatory steps provided the necessary conformational flexibility for the complex glycosides while perfectly maintaining the planar structural rigidity inherent to the flavonoid backbones. Notably, the optimized lowest-energy conformations of the more structurally rigid flavonoids, specifically Kaempferol (CID: 5280863) and Quercetin (CID: 5280343), are characterized by minimal rotatable bonds. This inherent structural stability directly contributed to their highly favorable and stable docking orientations deeply within the active site clefts of both the 2FOM and 3L6P Dengue virus targets, thereby fundamentally maximizing the computational reliability of the subsequent AutoDock Vina binding predictions.

Table 1
Phytochemicals of *Clitoria ternatea* retrieved from the IMPPAT database, including compound name, IMPPAT ID, molecular formula, molecular weight (Da), and canonical SMILES. These compounds were selected for molecular docking and ADMET analysis.

Sl.no	Compound name	IMPPAT CODE	Molecular formula	Molecular weight (Daltons)	Smiles
1	Kaempferol	IMPHY004388	$C_{15}H_{10}O_6$	286.24 Da	Oc1ccc(cc1)c1oc2cc(O)cc(c2c(= O)c1O)O
2	Quercetin	IMPHY004619	$C_{15}H_{10}O_7$	302.24 Da	Oc1cc(O)c2c(c1)oc(c(c2 = O)O)c1ccc(c(c1)O)O
3	Delphinidin 3-O-beta-D-glucoside-5-O-beta-D-glucoside betaine	IMPHY002910	$C_{27}H_{30}O_{17}$	626.52 Da	OC[C@H]1O[C@@H](Oc2cc(= O)cc3-c2cc(O[C@@H]2O[C@H](CO)[C@H]([C@@H]([C@H]2O)O)O)c(o3)c2cc(O)c(c(c2)O)O)[C@@H]([C@H]([C@@H]1O)O)O
4	Flavonol 3-O-D-glucoside	IMPHY010909	$C_{21}H_{20}O_8$	400.38 Da	OC[C@H]1O[C@@H](Oc2c(oc3c(c2 = O)cccc3)c2ccccc2)[C@@H]([C@H]([C@@H]1O)O)O
5	Clitorin	IMPHY001873	$C_{33}H_{40}O_{19}$	740.66 Da	Oc1ccc(cc1)c1oc2cc(O)cc(c2c(= O)c1O[C@@H]1O[C@H](CO[C@@H]2O[C@@H](C)[C@@H]([C@H]([C@H]2O)O)O)[C@H]([C@@H]([C@H]1O[C@@H]1O[C@@H](C)[C@@H]([C@H]([C@H]1O)O)O)O)O)O)O
6	Cinnamic acid	IMPHY011960	$C_9H_8O_2$	148.16 Da	OC(= O)/C = C/c1ccccc1
7	Taraxerone	IMPHY007426	$C_{30}H_{48}O$	424.71 Da	O=C1CC[C@]2([C@H](C1(C)C)CC[C@@]1([C@@H]2CC[C@@]2(C1 = CC[C@@]1([C@H]2CC(C)(C)CC1)C)C)C)C
8.	Flavylium	IMPHY002588	$C_{15}H_{11}O^+$	207.25 Da	c1ccc(cc1)c1ccc2c([o+]1)cccc2

3.3 Protein-Ligand Interaction Network Analysis via STITCH

The potential pharmacological and polypharmacological activity of the specific *Clitoria ternatea* phytochemicals was assessed by performing a comprehensive network pharmacology analysis with the STITCH database. This type of target-fishing produced a comprehensive interactome between eight phytochemicals, such as Quercetin (CID: 5280343), Kaempferol (CID: 5280863), Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine (CID: 25201902), and Taraxerone (CID: 92785) and various host and viral-associated The subsequent network of interaction showed a high predictive extent of proteins that participated in inflammatory signaling, oxidative stress management, and signal transduction conducted by kinases and enzyme-mediated modulation. Importantly, one can observe that Quercetin and Kaempferol scored highly on the interaction scores and had a high level of connectivity within central network nodes, which suggest their established multi-target pharmacological attributes (Szklarczyk et al., 2021). These hub-centric interactions imply the ability of such flavonoids to regulate important host pathways which affect the viral replication and immune responses.

Further, multi-target interaction patterns (polypharmacological behavior) were observed with the structurally complex metabolites like Delphinidin glycosides and Taraxerone. The biologically relevant and promiscuous binding profile indicates that this family of compounds could potentially have dual modulatory effects which could be direct antiviral inhibition and also regulating the host-cell machinery that is needed to sustain viral survival and replication. Polypharmacology is also being considered as a strategic benefit in the development of antiviral agents, especially against rapidly evolving RNA viruses that can be better targeted through the use of simultaneous viral and host inflammatory pathway attack with antiviral agents (Hopkins, 2008; Zandi et al., 2021). **(Shown in figure-5)** It is inferred that the identified network topology offers a mechanistic rationale to proceed with the development of these *Clitoria ternatea* metabolites to focus on molecular docking studies on the catalytic domains of the Dengue virus NS2B/NS3 proteins. All these contribute to the overall translational justification of these phytochemicals as multi-pathway antiviral compounds.

3.4 Active Site Prediction and Grid Box Determination by (CB-Docking)

Active-site identification was performed using the CB-Dock2 web server, which applies the CurPocket algorithm to automatically detect potential ligand-binding cavities. The experiment of blind docking was performed on the Dengue virus NS2B/NS3 protease structures 2FOM and 3L6P to ensure the removal of structural bias during active-site selection. In the case of 2FOM, the binding cleft found to be dominant was C1 (1143 Å³; centered at X = − 13, Y = − 11, Z = 7). Docking scores: Quercetin and Kaempferol scored the highest affinity (-7.8 kcal/mol) next came Clitorin –(-7.6 kcal/mol; C3), Taraxerone (-7.4 kcal/mol;

C2) and Flavylium (-7.2 kcal/mol:C1). Flavonol-3-O-D-glucoside (-6.8 kcal/mol C1) and Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine(-6.7 kcal/mol C3) showed intermediate affinity, and Cinnamic acid had the lowest affinity (-5.6 kcal/mol C1).

In the case of 3L6P, cavity C1 (539 Å³; centered at X = − 32, Y = − 13, Z = 26) became the 3L6P main pocket. Taraxerone presented the most powerful interaction (-8.7 kcal/mol; C1 and C3), Clitorin (-7.9 kcal/mol; C3) and Delphinidin glycoside (-7.6 kcal/mol; C1). The affinities of Quercetin (-7.4 kcal/mol; C1) and Flavonol-3-O-D-glucoside (-7.3 kcal/mol; C1) were also favorable with a comparatively lower score being Kaempferol (-6.9 kcal/mol; C1) flavylium (-6.8 kcal/mol: C1) and Cinnamic acid (-5.5 kcal/mol; C4). The size of the grid boxes was dynamically changed (18–25 A3) based on the size and structure complexity of the ligand, and allows sufficient sampling of conformations and enhances docking accuracy, which is consistent with standard-of-art computational docking guidelines (Morris et al., 2009; Liu et al., 2022). Comparative study shows that flavonols like Quercetin and Kaempferol are highly affined to 2FOM which is probably because of their planar aromatic frameworks which enable π–π stacking and the development of hydrogen bonds in the catalytic triad region. (Shown in figure-6)

Conversely, Taraxerone was shown to bind 3L6P in a superior way, which may be a result of increased hydrophobic complementarity in the smaller cavity architecture of this target. Multi-cavity accommodation was consistent with glycosylated derivatives like Clitorin, Flavonol-3-O-D-glucoside and Delphinidin glycoside, and indicated that the derivatives were adaptively flexible in terms of binding. These data are consistent with structural research on the vulnerability of NS2B/NS3 proteins to the effects of polyphenolic and triterpenoid scaffolds that have the capacity to interact with both catalytic and allosteric subsites (Nitsche et al., 2014; Zou et al., 2015). The blind docking approach using cavity guidance reduced investigator bias and had biologically relevant grid placements, which further enhanced the use of the binding predictions. On balance, Taraxerone, Clitorin, Flavonol-3-O-D-glucoside and Delphinidin glycoside have been discovered as the most promising ones among the two Dengue protease targets, helping to develop it further into molecular dynamics simulation and enzymatic inhibition validation

3.5 Molecular Docking Analysis with UCSF Chimera and PyRx

Specific binding preferences of the chosen phytochemicals toward the Dengue virus NS2B/NS3 protease structures 2FOM and 3L6P were revealed by the comparative docking analysis. With a substantial binding affinity of − 8.7 kcal/mol against 2FOM and − 7.6 kcal/mol against 3L6P, delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine demonstrated persistent accommodation within both protease cavities, with somewhat higher thermodynamic favorability toward 2FOM. Taraxerone had the highest affinity of any studied drug against 3L6P (− 9.4 kcal/mol), although its binding score for 2FOM was − 7.9 kcal/mol.

Strong hydrophobic stabilization and ideal steric complementarity inside the catalytic groove are reflected in this better interaction with 3L6P, highlighting the significance of large, rigid triterpenoid scaffolds in occupying hydrophobic subsites of flaviviral proteases. While Flavonol-3-O-D-glucoside consistently maintained high binding energies against both 2FOM (− 8.1 kcal/mol) and 3L6P (− 8.0 kcal/mol), Clitorin showed strong binding toward 2FOM (− 8.2 kcal/mol) and moderate affinity toward 3L6P (− 7.6 kcal/mol), suggesting balanced interaction capability across protease variants. **(Shown in figure-7 & Table-2)**

Taraxerone was the most effective ligand among the 3L6P protease complexes, demonstrating the importance of large, conformationally restricted triterpenoid frameworks in maintaining hydrophobic and van der Waals interactions within enzyme catalytic grooves. Concurrently, glycosylated flavonoids like Clitorin and Delphinidin glycoside maintained a significant binding capacity, demonstrating their propensity to interact with polar and nonpolar residues concurrently through aromatic stacking and hydrogen bonding. The idea that longer conjugation systems and more molecule surface area improve cumulative non-covalent interactions and overall binding stability is further supported by the generally lower docking efficiencies seen for smaller phenolic scaffolds.

These results are in line with recent computational studies that show flavonoids and triterpenoids can inhibit Dengue NS2B/NS3 proteases via allosteric or competitive mechanisms backed by stable interaction profiles and favorable docking energies (Qamar et al., 2014; Mirza et al., 2016). Together, these two scaffolds show promise as lead candidates for further molecular dynamics simulations and experimental protease inhibition studies due to the strong thermodynamic stability of Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine toward 2FOM and the superior binding of Taraxerone toward 3L6P.

Table 2
AutoDock Vina–Predicted Binding Affinities (kcal/mol) of *Clitoria ternatea* Phytochemicals Against Dengue Virus NS2B/NS3 Proteases (PDB IDs: 2FOM and 3L6P).

Phytochemical Ligand	PubChem CID	Binding Affinity against 2FOM (kcal/mol)	Binding Affinity against 3L6P (kcal/mol)
Kaempferol	5280863	-7.5	-8.0
Quercetin	5280343	-7.8	-8.1
Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine	25201902	-8.7	-7.6
Flavonol-3-O-D-glucoside	11953828	-8.1	-8.0
Clitorin	11592917	-8.2	-7.6
Cinnamic acid	444539	-5.6	-4.9
Taraxerone	92785	-7.9	-9.4
Flavylium	145858	-6.7	-6.6

3.6 Post-Docking Interaction Profiling using BIOVIA Discovery Studio Visualizer

Two-dimensional (2D) interaction diagrams of the stabilized ligand–protease complexes were produced using post-docking interaction analysis using BIOVIA Discovery Studio Visualizer. Van der Waals forces, π–π stacking, π–alkyl interactions, hydrogen bonding, and hydrophobic contacts within the catalytic domains of 2FOM and 3L6P were all thoroughly explained by the 2D structure maps. In the catalytic triad areas of both proteases, delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine formed a vast hydrogen-bonding network, according to the interaction profiling. The hydroxyl groups of the glycoside moieties and important active-site residues created several conventional hydrogen bonds, which greatly improved electrostatic stability and anchored the ligand in the binding pocket. Improved binding selectivity and stability in flaviviral protease inhibition investigations are often linked to such extensive hydrogen bond interactions. (Qamar et al., 2014). **(Shown in figure-8)**

Taraxerone, on the other hand, mostly interacted through π-alkyl and hydrophobic contacts, deeply occupying nonpolar subpockets to match its greater docking affinity against this target, especially within 3L6P. Significant van der Waals connections that supported hydrophobic stabilization and steric complementarity were depicted in the 2D figures. Quercetin and Kaempferol are examples of structurally rigid flavonoids that sustain their moderate-to-strong binding affinities by forming π–π interactions and classical hydrogen bonds with catalytic and surrounding residues. According to recognized structure–activity connections, triterpenoid frameworks increase the hydrophobic occupancy of protease subsites, whereas polyphenolic scaffolds promote hydrogen bonding and aromatic stacking (Mirza et al., 2016). The antiviral potential of complex polyphenolic and triterpenoid scaffolds against Dengue NS2B/NS3 proteases is further supported by the structural validation of the docking-derived binding affinities and the confirmation of correct ligand orientation within the catalytic domains provided by the 2D interaction profiling.

3.7 Stereochemical Validation of Target Receptors by PROCHECK

The proteins structure of the Dengue virus NS2B/NS3 proteases 2FOM and 3L6P were obtained in the Protein Data Bank and then were validated with stereochemistry before molecular docking. Structural quality measurement was conducted with the help of PROCHECK by investigating the backbone structure with the help of the Ramachandran plot that evaluates backbone dihedral angles (φ and ψ) to verify the conformational integrity (Laskowski et al., 1993). In the case of 2FOM, 145 residues (91.8%) were found in the most favored regions and 13 residues (8.2%) found in additional allowed regions, and none in generously allowed and disallowed regions. Thus 3L6P showed 139 residues (89.1% in the most preferred regions and 17 residues (10.9% in the other permitted regions) and zero residues in prohibited regions. The fact that residues are not found in disallowed conformational space is a confirmation of good backbone geometry and low levels of steric strain, accepted structural validation criteria of high-quality protein models in structure-based drug design (Morris et al., 2009; Laskowski et al., 1993).

There was strong conformational reliability as evidenced by further stereochemical analysis using G-factor. The 2FOM structure had a phi-psi G-factor of -0.37 with the total average G-factor of -0.04, whereas 3L6P had a phi-psi distribution of -0.47 and an average G-factor of -0.20. Because the values of the G-factor less than − 0.5 are regarded as very unnatural, whereas the values of the G-factor less than − 1.0 are regarded as very, very unnatural, the near values of both proteases indicate that they have normal covalent geometry and that they do not have any abnormal torsional strain. This kind of validation is important since incorrect protein backbone conformation may severely affect the docking predictions and binding energy calculations (Kumar & Zhang, 2018). Thus, the stereochemical integrity of the two proteases has been confirmed, which means that the positive docking relationships with the lead phytochemicals based on Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine and Taraxerone are due to physically realistic and thermodynamically plausible ligand-enzyme complexes and not on artifacts due to the impaired geometry of receptors. Values indicate backbone stereochemical quality, where > 90% residues in favored regions and G-factors confirm structural reliability for docking studies.**(Shown in Table-3 & figure-9)**

Table 3: Ramachandran plot distributions and G-factor values were calculated using PROCHECK for 2FOM and 3L6P structures from the Protein Data Bank.

Validation Parameter	NS2B/NS3 Protease (2FOM)	NS2B/NS3 Protease (3L6P)
Most Favored Regions	145 (91.8%)	139 (89.1%)
Additionally Allowed Regions	13 (8.2%)	17 (10.9%)
Generously Allowed Regions	0 (0.0%)	0 (0.0%)
Disallowed Regions	0 (0.0%)	0 (0.0%)
Total Number of Residues	196	196
Phi–psi Distribution Score	-0.37	-0.47
Overall Average G-Factor	-0.04	-0.20

3.8 *In-Silico* Pharmacokinetic and Toxicity (ADMET) Profiling

The combined ADMET evaluation indicated that there is a strong variability of pharmacokinetic properties between phytochemicals under investigation that were predominantly controlled by the physicochemical characteristics of the compounds. Smaller phenols like Cinnamic acid and triterpenoid Taraxerone

also showed relatively high Caco-2 permeability and high predicted intestinal absorption to favour effective epithelial uptake. Glycosylated flavonoids, in contrast, such as Delphinidin derivative, Clitorin, and Flavonol-3-O-D-glucoside, had lower permeability, which explained their high molecular weights, topological polar surface areas (TPSA) and high hydrogen-bonding capacity. These characteristics surpass classical drug-likeness criteria especially the ones put forth by Lipinski rule of five that correlates greater polarity and hydrogen bonding with reduced membrane diffusion (Lipinski et al., 2001). Distribution profiling also revealed that Kaempferol, Quercetin, Flavonol glucoside, Taraxerone and Flavylium had very high plasma protein binding (> 95) and could decrease the free pharmacologically active fraction. It is interesting to note that Taraxerone and Flavylium had a high blood-brain barrier penetration potential indicating potential CNS exposure, but most flavonoids were mostly barred CNS entry owing to their polar nature. The analysis of metabolic interaction indicated a pattern of cytochrome P450 differences of modulation. Strong chances of CYP3A4 inhibition were observed with Kaempferol, Quercetin, and Taraxerone, and it suggests that these drugs may cause drug-drug interaction during combination therapy.

On the other hand, Delphinidin derivative, Clitorin, and Cinnamic acid showed acceptable non-inhibitory CYP and a relative better predicted metabolic stability. Prediction of clearance and half-life also differentiated the candidates: Delphinidin derivative (1.619 ml/min/kg; t1/2 6.227 h) and Clitorin (0.981 ml/min/kg; t1/2 4.962 h) had low systemic clearance with long half-lives, meaning that they were in circulation. Taraxerone on the other hand exhibited a high plasma clearance (10.123 ml/min/kg) and ultra-short half-life (0.184 h) indicating a high rate of hepatic clearance and low systemic persistence. These pharmacokinetic differences would highlight the need to adjust between metabolic and permeability concerns during lead optimization (Pires et al., 2015; Xiong et al., 2021). Potential mutagenicity and hepatotoxicity liabilities of various flavonoid derivatives were predicted, especially Delphinidin and Flavylium, but Taraxerone and Cinnamic acid had lower predicted toxicity liabilities. Notably, every compound had an insignificant hERG inhibition probability, which implies low cardiotoxicity. Taken together, Taraxerone proves to have the best lipophilicity and good docking compatibility, but its high metabolic rate can be a reason to chemically modify it or employ sophisticated formulation to improve systemic stability. Alternatively, Delphinidin derivative and Clitorin are better in terms of pharmacokinetic persistence but suffer poor membrane permeability. These results highlight the concept that the rational prioritization of antiviral hits in modern computational drug discovery platforms needs to be done through a combination of physicochemical profiling, ADMET modeling, and molecular docking (Lipinski et al., 2001; Pires et al., 2015; Xiong et al., 2021). **(Shown in figure-10 & Table-4 and 5)**

Table 4

The table presents physicochemical descriptors and predicted ADMET parameters including absorption (Caco-2, HIA), distribution (BBB, PPB), metabolism life, and toxicity (AMES, DILI, hERG) for selected Clitoria ternatea phytochemicals.

Compound	Caco-2 (Log Papp)	HIA+ Prob.	P-gp Substrate	VDss (Log L/kg)	PPB (%)	BBB+ Prob.	CYP3A4	CYP2D6	CYP2C9	HLM Instability	Clearance (ml/min/kg)	t½ (h)	AMES
Kaempferol	-5.969 (Low)	0.716	No (0.163)	-0.812	97.88	0.001 (No)	Inhib. (0.975)	Sub. (0.995)	Inhib. (0.799)	0.988 (Unstable)	5.694	1.39	Risk
Quercetin	-6.177 (Low)	0.991	No (0.031)	-0.879	98.66	0.000 (No)	Inhib. (0.937)	Sub. (0.978)	Sub. (0.030)	0.992 (Unstable)	8.289	1.59	Risk
Delphinidin Deriv.	-6.726 (Low)	1.000	No (0.038)	-0.189	74.00	0.000 (No)	Non-Inhib.	Non-Inhib.	Non-Inhib.	0.000 (Stable)	1.619	6.23	Risk
Flavonol-3-O-D-gluc.	-5.746 (Low)	0.112	No (0.025)	-0.477	95.28	0.287 (No)	Non-Inhib.	Non-Inhib.	Non-Inhib.	0.410 (Moderate)	2.568	2.89	Risk
Clitorin	-6.655 (Low)	0.985	Yes (0.995)	-0.079	81.99	0.000 (No)	Non-Inhib.	Non-Inhib.	Non-Inhib.	0.056 (Stable)	0.981	4.96	Low
Taraxerone	-4.684 (High)	0.982	No (0.000)	+ 0.556	95.73	0.756 (Yes)	Inhib. (0.999)	Non-Inhib.	Non-Inhib.	0.975 (Unstable)	10.123	0.18	Low
Cinnamic acid	-4.441 (High)	0.890	No (0.019)	-0.838	80.31	0.025 (No)	Non-Inhib.	Non-Inhib.	Non-Inhib.	0.003 (Stable)	6.162	1.88	Low
Flavylium	-4.527 (High)	0.533	Yes (0.523)	+ 0.120	97.83	0.873 (Yes)	Sub. (0.580)	Sub. (0.991)	Sub. (0.987)	0.876 (Unstable)	8.213	0.96	Risk

Table 5: The table presents physicochemical properties and predicted ADMET parameters (absorption, distribution, metabolism, clearance, half-life, and toxicity risks) of selected *Clitoria ternatea* phytochemicals.Data were generated using validated in silico tools (pkCSM and ADMETlab) to assess drug-likeness, pharmacokinetic behavior, and safety for antiviral lead optimization.

Property	Kaempferol	Quercetin	Delphinidin	Flavono 3-O-D-Glucoside	Taraxerone	Clitorin	Cinnamic acid	Flavylium
Molecular Weight	286.05	302.04	626.15	400.12	424.37	740.22	148.05	207.08
LogP	1.965	1.448	-2.088	0.76	5.735	1.002	2.619	1.412
TPSA (Å²)	111.13	131.36	289.66	129.59	17.07	308.12	37.3	11.3
H-Bond Donors	4	5	11	4	0	11	1	0.0
H-Bond Acceptors	6	7	17	8	1	19	2	1.0
Rotatable Bonds	1	1	7	4	0	8	2	1.0
Caco-2 Permeability	-5.969	-6.177	-6.726	-5.746	-4.684	-6.655	-4.441	-4.527
HIA (Probability)	0.015	0.134	0.955	0.027	0.0	0.136	0.095	0.002
BBB Permeability	0.001	0.0	0.0	0.287	0.756	0.0	0.025	0.873
Plasma Protein Binding	97.881%	98.66%	74.007%	95.281%	95.735%	81.997%	80.311%	97.837
Clearance (ml/min/kg)	5.694	8.289	1.619	2.568	10.123	0.981	6.162	8.213
Biological Half-Life (hr)	1.388	1.586	6.227	2.891	0.184	4.962	1.882	0.96
AMES Mutagenicity	0.546	0.586	0.984	0.804	0.236	0.738	0.214	1.0
Hepatotoxicity (DILI)	0.703	0.783	0.99	0.95	0.439	0.877	0.673	1.0
hERG Cardiotoxicity	0.069	0.053	0.002	0.033	0.209	0.01	0.059	0.011

Conclusion

The Present research paper has systematically investigated the antiviral properties of *Clitoria ternatea* phytochemicals against Dengue virus NS2B/NS3 protease (2FOM) and NS5 methyltransferase (3L6P) using an integrated in silico pipeline using network pharmacology, cavity-guided molecular docking, stereochemical validation, and ADMET profiling. Docking study showed that Delphinidin-3-O-βD-glucoside-5-O-β-D-glucoside betaine and Taraxerone had the highest binding affinities, and for which the strong stabilization in catalytic domains was facilitated by hydrogen bonding, hydrophobic interactions, and π–π stacking. Taraxerone was more affinitive to 3L6P, which represents an example of hydrophobic complementarity in the SAM-binding pocket, and the Delphinidin derivative had been demonstrated to be more stabilized in 2FOM by extensive polar interactions. High stereochemical integrity of both receptor structures was verified by Ramachandran plot and G-factor validation, which guaranteed that the interaction between a ligand and protein would be geometrically and thermodynamically sound as predicted.

The polypharmacological nature of the major flavonoid was also supported by network pharmacology analysis and confirmed their ability to regulate the viral and host-associated pathways. The importance of translational considerations was identified by ADMET profiling. Although Taraxerone showed a good level of membrane penetration, high docking activity, its rapid clearance and rapid metabolite nature indicate that structural optimization or formulation of the drug may be required. On the other hand, Delphinidin derivative and Clitorin had better systemic persistence and metabolic stability but were restricted by low permeability because of high polarity and molecular weight. The majority of the compounds exhibited insignificant projected hERG inhibition, a feature of low cardiotoxic liability, even though there were flavonoid compounds with moderate mutagenicity and hepatotoxicity potential, which require experimental confirmation. Together, structural docking, pharmacokinetic modeling, and toxicity testing give a solid computation base of prioritizing the use of Delphinidin derivatives and Taraxerone as dual-target antiviral leads. Such results are helpful in further confirming them by molecular dynamics simulations, enzymatic inhibition tests, and in vitro antiviral experiments to take the next step in the development of multi-target, plant-derived therapeutics against dengue virus infection.

Declarations

Conflicts of Interest:

“The authors declare no conflict of interest.”

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

Clinical Trial Number

Not applicable

Consent Statement

I have read and understood the study details, including its purpose, procedures, and potential risks or benefits. I understand that participation is voluntary, involves tasting the product, and I may withdraw at any time without penalty. I consent to participate and allow my data to be used for research purposes. We confirm that all research was performed in accordance with relevant guidelines/regulations, and include in their manuscript a statement confirming that informed consent was obtained from all participants and/or their legal guardians.

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Author Contributions

Dr.K.R.Padma contributed to writing, and drawing figures and tables in this research article. KRP and Battula Tanmai Subhashini solely drafted this research article. Research work performed by Battula Tanmai Subhashini Under Guidance of Dr.K.R.Padma and M.Sankari.

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Availability of data and materials

"The datasets used and/or analysed during the current study available from the corresponding author on reasonable request."

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Figures

Figure 1

An overview of the significant pharmacological uses of *Clitoria ternatea*, antiviral, antidiabetic, antioxidant, cognitive-enhancing, immunomodulatory, and cosmetic uses. These are mainly due to its high phytochemical content, especially flavonoid, anthocyanins (ternatins), phenolic compounds and bioactive peptides.

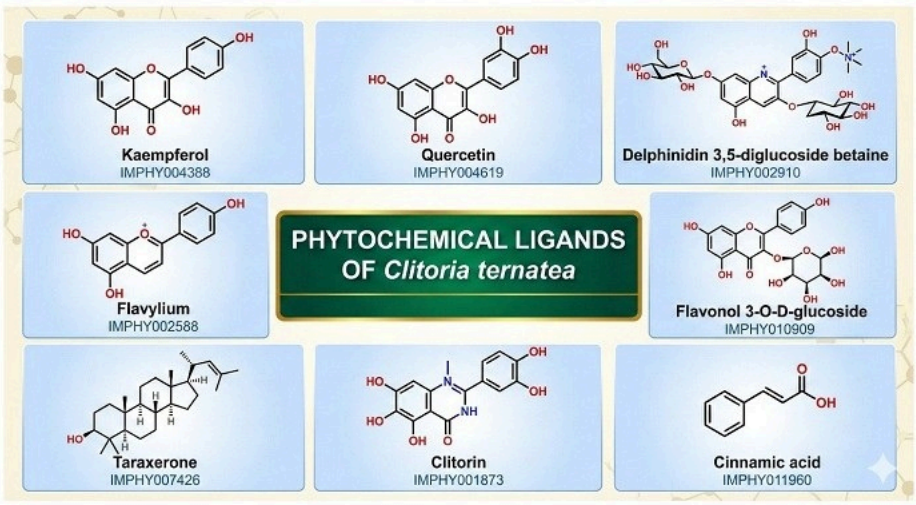

Figure 2

Phytochemical ligands found in *Clitoria ternatea* and their chemical structures and IMPPAT IDs. These compounds are flavonoids, anthocyanins, phenolic acids and triterpenoids which have the potential to be antiviral.

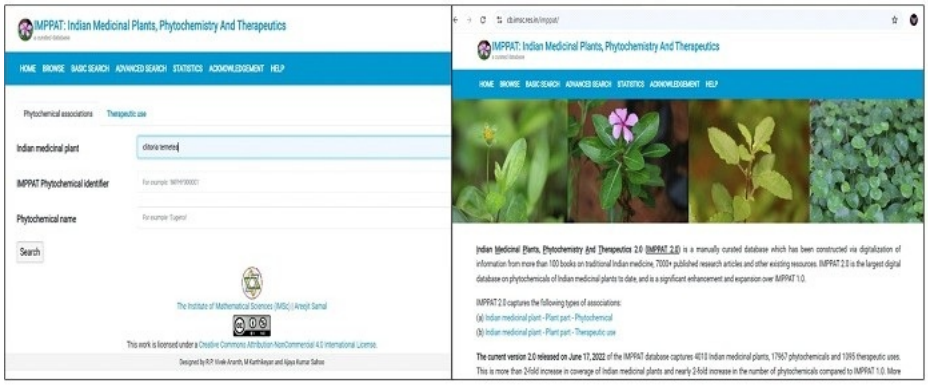

Figure 3

Interface of the IMPPAT used for phytochemical retrieval.

The figure shows the search box to query *Clitoria ternatea* and the database home page to show phytochemical related associations and therapeutic data.

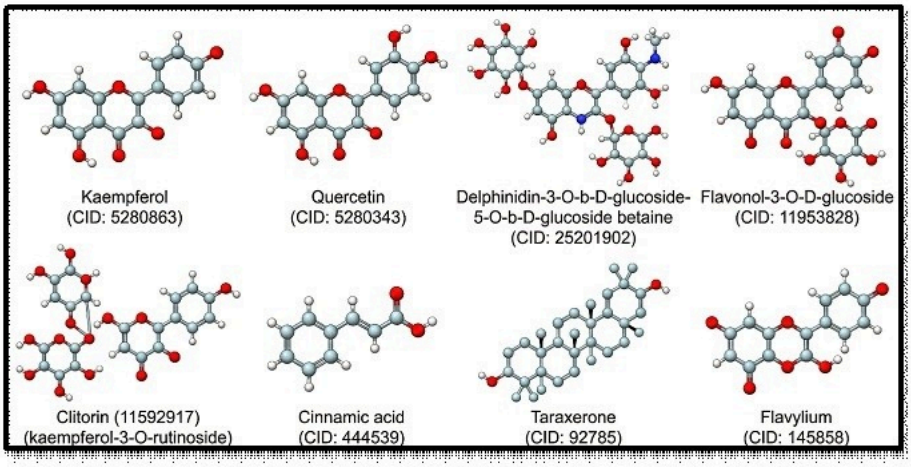

Figure 4

3D Molecular Structures - Ball-and-stick molecular models of key bioactive phytochemicals derived from *Clitoria ternatea*. The panel includes Kaempferol, Quercetin, Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine, Flavonol-3-O-D-glucoside, Clitorin (kaempferol-3-O-rutinoside), Cinnamic acid, Taraxerone, and Flavylium, with carbon, oxygen, nitrogen, and hydrogen atoms color-coded for structural clarity.

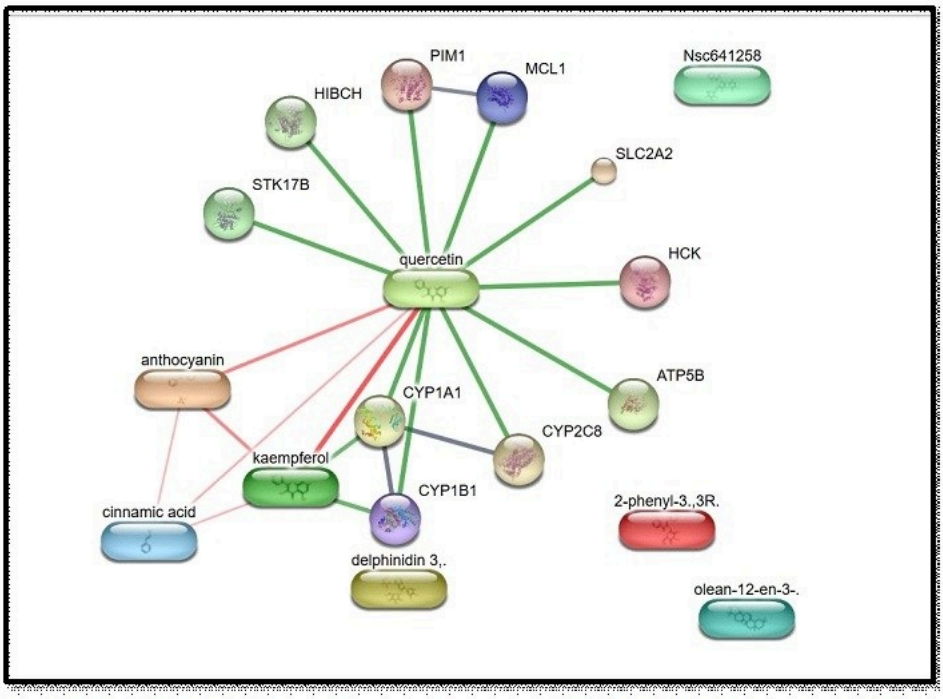

Figure 5

Protein-chemical interaction network generated via the STITCH database for the eight selected *Clitoria ternatea* phytochemicals. The network topology illustrates the polypharmacological potential of the compounds, mapping high-confidence interactions between the plant metabolites and various biological target proteins.

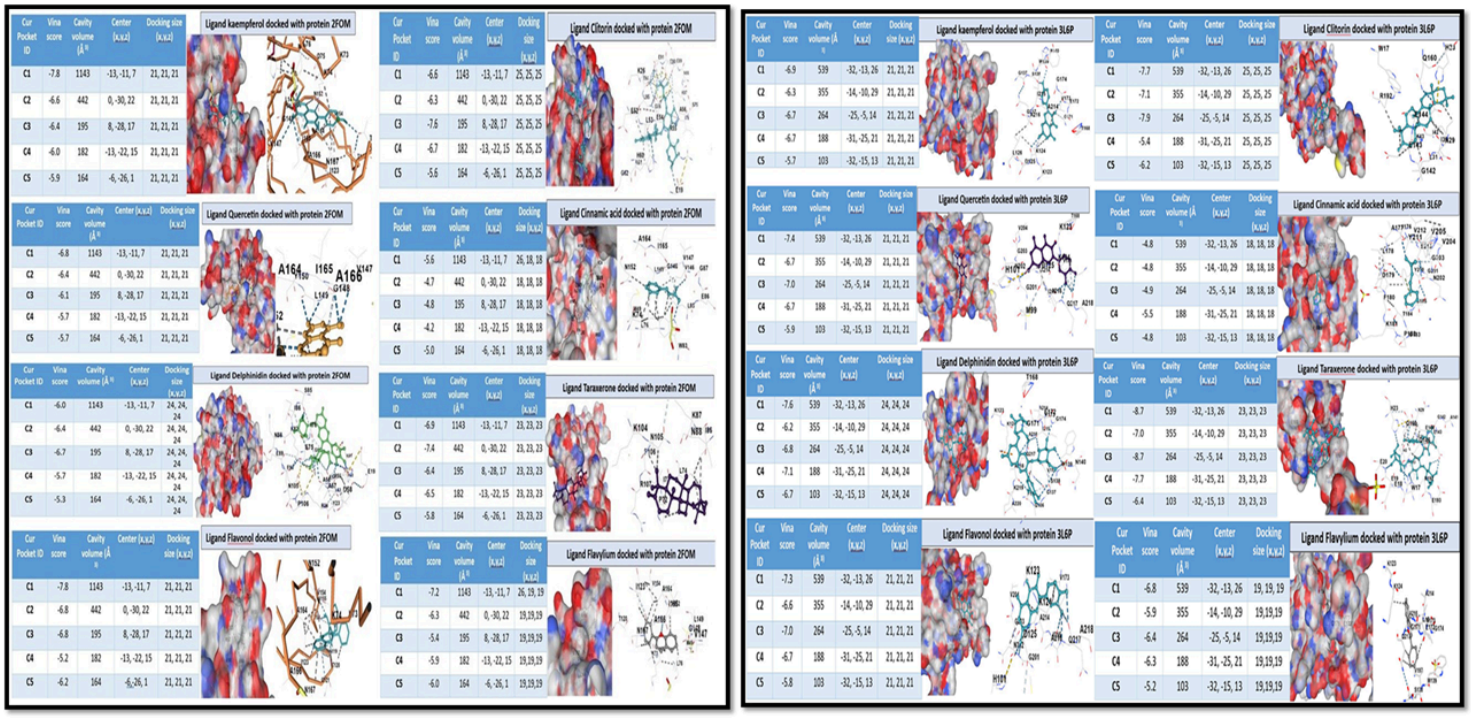

Figure 6

Cavity recognition and molecular docking of identified *Clitoria ternatea* phytochemicals of dengue viral proteins NS2B-NS3 protease (2FOM) and NS5 methyltransferase (3L6P). Panels depicting the predicted conformations of the binding of Kaempferol, quercetin, Delphinidin-3-O-β-D-glucoside-5-O-β-D-glucoside betaine, Flavonol-3-O-β-D-glucoside, Clitorin, Cinnamic acid, Taraxerone, and Flavylium.

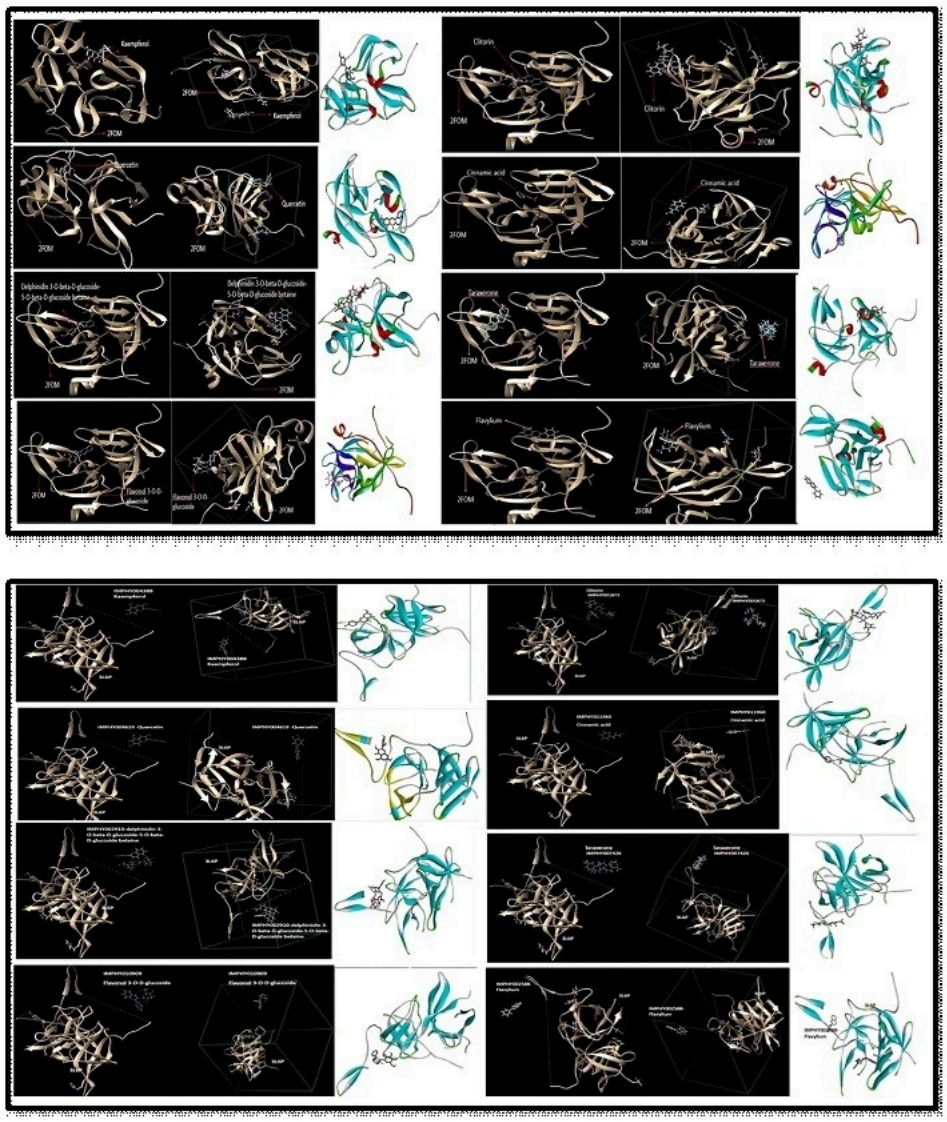

Figure 7

Molecular docking Visualization Three-dimensional validation of the presence of selected *Clitoria ternatea* phytochemicals in the interaction with dengue virus (NS2B-NS3 protease) (PDB ID: 2FOM) and (NS5 methyltransferase) (PDB ID: 3L6P). The figures show protein ribbon structure, docking grid box, spatial orientation of ligands in the active sites and binding affinities of the same.

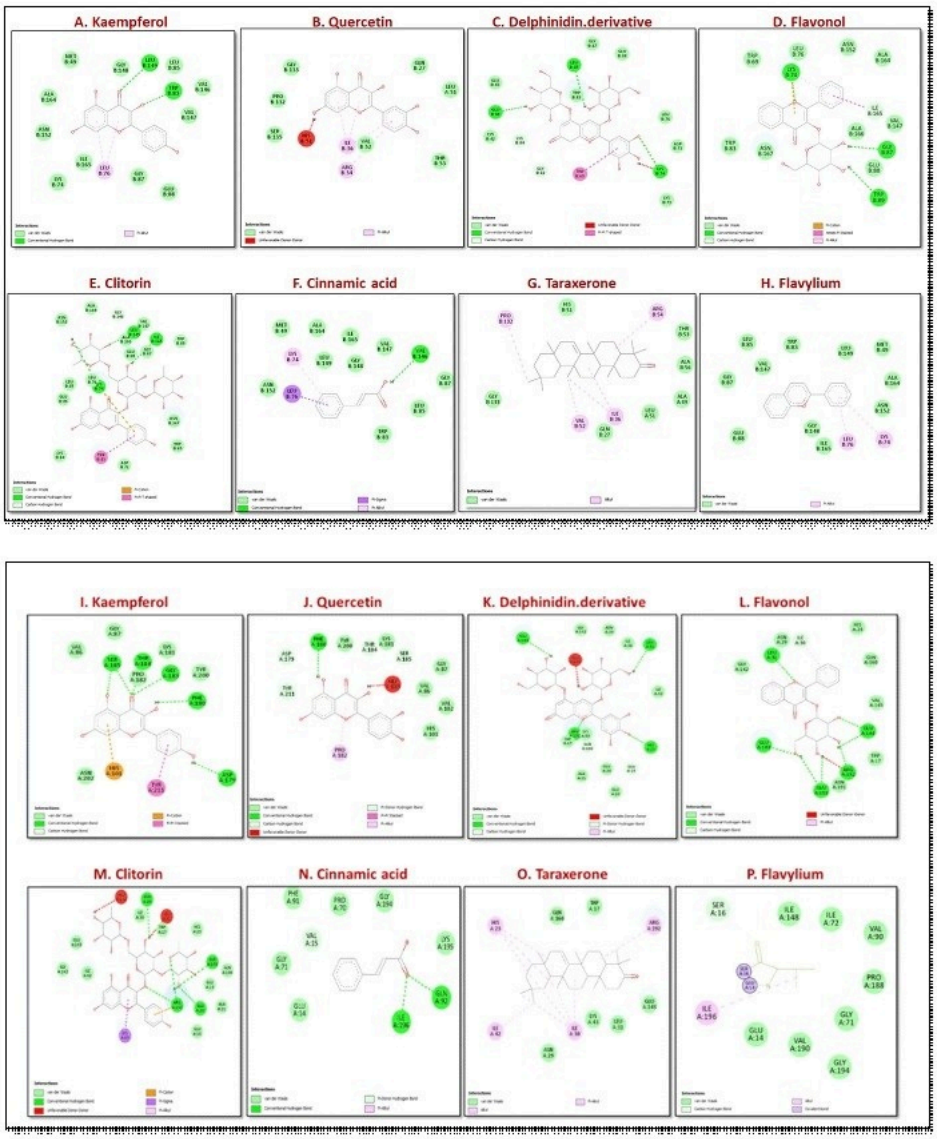

Figure 8

Two-dimensional protein-ligand interaction diagrams depicting binding interactions of *Clitoria ternatea*phytochemicals with dengue virus targets protease (2FOM) and methyltransferase (3L6P). Panels (A-P) indicate Kaempferol, Quercetin, Delphinidin, Flavonol-3-O-D-glucoside, Clitorin, Cinnamic acid, Taraxerone, and Flavylium, with major interactions among amino -acids shown as conventional hydrogen bonds (green), van der Waals forces (light green), π-interactions (pink/purple/orange) and unfavorable donor-donor interactions (red).

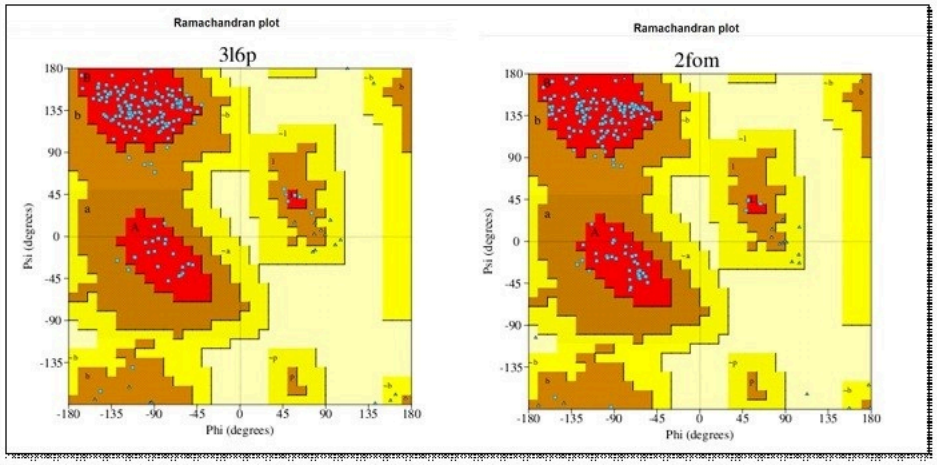

Figure 9

Ramachandran plots confirming the quality of dengue virus protein structure 2FOM and 3L6P as a stereochemical structure displaying the distribution of backbone dihedral angles (φ and ψ) of amino acids. The fact that the concentration of residues is concentrated in the red (most allowed) regions and less in the additionally allowed and generously allowed regions shows that they have stable and consistent protein conformations which can be used in molecular docking analysis.

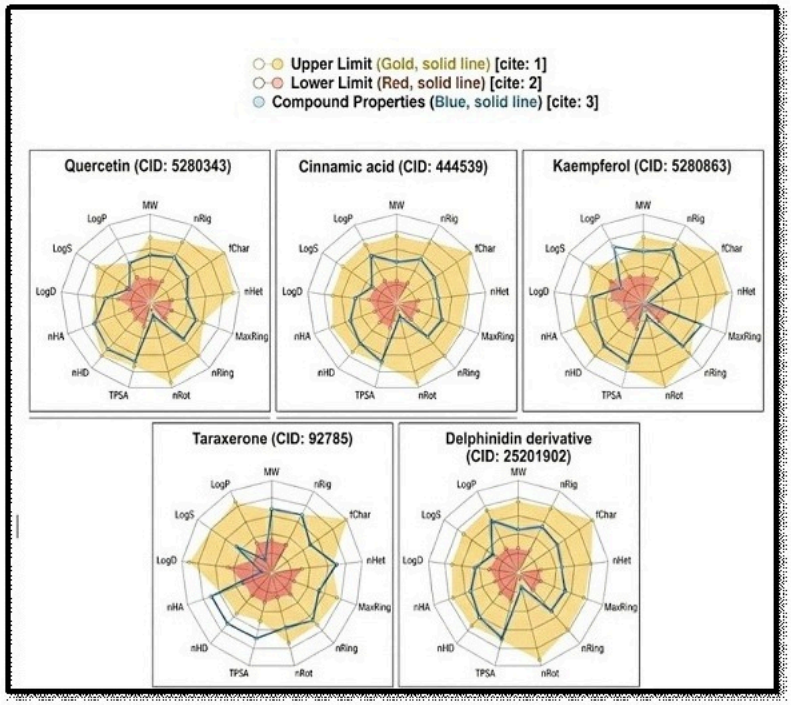

Figure 10

Bioavailability radar plots of physicochemical and drug-likeness characteristics of Clitoria ternatea selected phytochemicals: Quercetin (CID: 5280343), Cinnamic acid (CID: 444539), Kaempferol (CID: 5280863), Taraxerone (CID: 92785), and Delphinidin derivative (CID: 25201902). Key molecular descriptors are screened in the plots; they are MW, LogP, LogS, LogD, TPSA, hydrogen bond acceptors/donors, rotatable bonds, the number of rings, heteroatoms, charge, and rigidity. The gold region implies the best range (maximum), the red region implies the lowest possible tolerable limits, and the blue line implies the actual properties of the compound. The closer the blue polygon is to the optimal region, the closer is the predicted drug-likeness and pharmacokinetic suitability.