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Structure-Based Virtual Screening And Molecular Docking Studies Of Natural Compounds As Dengue Virus Protease Inhibitors: A Comprehensive Bioinformatics Approach.

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Abstract

Dengue virus infection remains a critical challenge at the global level lacking approved specific antiviral therapies. The viral NS2B-NS3 protease is indispensable for protein processing, representing a primary site for drug discovery. In this study, we utilized comprehensive computational approaches-including molecular docking, 100 ns molecular dynamics simulations, and ADMET analysis-to identify potent natural inhibitors of this essential enzyme. We evaluated five structurally diverse compounds: resveratrol, 10-gingerol, hesperetin, daidzein, and coumarin. These molecules' docking studies demonstrated strong binding affinities from -7.2 to -9.8 kcal/mol, forming highly stable interaction networks with key active site residues (His51, Asp75 & Ser135). Resveratrol exhibited the most potent binding affinity (-9.8 kcal/mol) through optimal hydrogen bonding and hydrophobic contacts. Furthermore, pharmacokinetic predictions confirmed excellent drug-like properties, good oral bioavailability, and minimal toxicity for evaluated compounds. Our findings propose these natural molecules as highly promising lead candidates for developing targeted dengue antivirals.

1. INTRODUCTION

Dengue virus represents one of the most critical mosquito-borne viral threats globally, primarily transmitted by Aedes aegypti and A. albopictus vectors. The virus drives an estimated 390-400 million infections annually across tropical and subtropical zones, resulting in clinical outcomes that range from self-limiting dengue fever to life-threatening conditions (Bhatt et al., 2013). Driven by climate change and expanding urban environments, the global footprint of DENV has grown substantially over the last half-century.DENV belongs to the Flaviviridae family and presents as 4 distinct serotypes. While a primary infection confers lifelong immunity to the specific infecting serotype, subsequent exposure to a different serotype can precipitate severe disease through a phenomenon known as antibody-dependent enhancement. This complex immune dynamic has severely complicated vaccine development and underscores the critical need for effective antiviral therapies. Currently, no specific antiviral drugs exist for DENV, leaving clinical management entirely dependent on symptom relief and supportive care (Lim et al., 2013).

The DENV genome is a SS(+) RNA that encodes a single large polyprotein, broken into structural and non-structural proteins to enable viral replication and immune evasion. A key driver of this processing is the viral NS2B-NS3 protease. The NS3 protein houses the protease domain at the N-terminus and relies on a hydrophilic region of the NS2B cofactor to form a functional, solvent-exposed active site (Erbel et al., 2006; Noble et al., 2012). This active site utilizes a classic catalytic triad composed of His51, Asp75, and Ser135 to cleave the polyprotein at multiple essential junctions (Yusof et al., 2000). Because neutralizing this protease activity effectively halts viral replication in both cellular and animal models, the NS2B-NS3 complex is widely used as a prime target for antiviral serine protease drug discovery (Li et al., 2005).

Despite this clear therapeutic rationale, translating NS2B-NS3 inhibitors into clinical practice has proven exceedingly difficult. Previous developmental efforts focusing on peptide-based and synthetic small-molecule inhibitors have frequently failed due to poor cell permeability, lack of selectivity, or unfavorable pharmacokinetic characteristics (Nitsche et al., 2014). To overcome these hurdles, researchers are increasingly turning to natural products, which offer rich structural diversity, historical biological efficacy, and established safety profiles (Newman & Cragg, 2020; Atanasov et al., 2021). Plant-derived compounds such as resveratrol, 10-gingerol, daidzein, hesperetin, and coumarin provide a variety of biologically active scaffolds. These naturally occurring molecules have already demonstrated broad anti-viral, anti-inflammatory, and immune-modulating properties, highlighting their immense potential as promising molecules for the development of novel, targeted dengue therapeutics (Kaul et al., 1985; Lin et al., 2014; Kiat et al., 2006).

This study aims to find out the binding behavior of five selected natural compounds (resveratrol, 10-

gingerol, daidzein, hesperetin, and coumarin) against the dengue virus NS2B-NS3 protease, an important antiviral target whose active site is defined by the catalytic triad (His51, Asp75, and Ser135) and whose NS2B cofactor is essential for the formation of the active protease conformation. Using molecular docking, the study will evaluate how these compounds occupy the active-site region and will characterize their binding modes through detailed analysis of hydrogen bonds, hydrophobic contacts, and the contributions of key amino acid residues. The stability and dynamic behavior of the resulting protein-ligand complexes will then be examined through 100 ns molecular dynamics simulations, followed by MM-PBSA and MM-GBSA calculations to estimate and compare binding free energies quantitatively. In addition, computational ADMET analysis will be performed to predict pharmacokinetic properties and toxicity profiles, while the combined docking, dynamics, and free-energy results will be used to define preliminary structure-activity relationships for future lead optimization. Finally, the performance of these natural compounds will be compared with reported synthetic NS2B-NS3 inhibitors, including orthosteric and allosteric inhibitor classes that have already been described for dengue protease targeting.

2. METHODOLOGY

High-throughput computational evaluations were conducted on an Ubuntu 20.04-based cluster powered by Intel Xeon Gold 6248R processors and NVIDIA Tesla V100 GPUs. We targeted the DENV-2 NS2B-NS3 protease, utilizing its 1.5 Å resolution crystal structure (PDB ID: 2FOM) due to its intact cofactor region and optimal catalytic conformation. Prior to in silicomodeling, the receptor was stripped of ions, co-crystallized entities, and distal water molecules, followed by strict protonation state assignments at physiological pH using the H++ server (Sndergaard et al., 2011), specifically maintaining a neutral Ser135, deprotonated Asp75, and δ-protonated His51. The structure was geometrically optimized via sequential steepest descent and conjugate gradient minimizations until reaching a force tolerance below 10.0 kJ/mol/nm. Subsequent MolProbity validation yielded an outstanding score of 1.24, with over 97% of residues populating sterically favoured Ramachandran coordinates (Chen et al., 2010).

A focused library of five bioactive phytochemicals (Resveratrol, 10-Gingerol, Daidzein, Hesperetin, and Coumarin) was converted to 3D geometries and subjected to MMFF94-based energy minimization to isolate the global energetic minimum from a pool of up to 100 conformers per compound. Molecular docking was done with AutoDock Vina 1.2.0 (Trott & Olson, 2010), with a 25 $Å^3$ search space centered precisely on the catalytic Ser135. The docking algorithm, leveraging an exhaustiveness of 32, was rigorously pre-validated; re-docking the native peptide yielded a 1.12 Å RMSD, and decoy enrichment confirmed robust predictive accuracy (ROC AUC = 0.84). Post-docking pose analysis discriminated crucial binding determinants, utilizing strict geometric thresholds to map hydrogen bonding, hydrophobic contacts, and π-π stacking events (Laskowski & Swindells, 2011).

To probe the temporal stability of the most favourable binding modes, 100-ns molecular dynamics trajectories were computed using GROMACS 2023.1 (Van Der Spoel et al., 2005). The solvated systems parameterized with the AMBER ff19SB force field for the macromolecule (Maier et al., 2015) and GAFF2 with AM1-BCC charges for the ligands (Wang et al., 2004) were neutralized in a 0.15 M NaCl TIP3P water environment (Jorgensen et al., 1983). Following sequential NVT and NPT equilibrations, the production phase integrated a 2-fs timestep alongside PME electrostatics and LINCS bond constraints.

Thermodynamic binding affinities were subsequently rigorously quantified via MM-PBSA and MM-GBSA paradigms (Kollman et al., 2000; Genheden& Ryde, 2015), integrating solvent dielectric and surface tension variables over 1,000 discrete snapshots from the trajectory's terminal 50 ns, further refined by normal mode entropic corrections (Miller et al., 2012). Finally, the translational potential of these candidates was established through exhaustive predictive pharmacokinetic and toxicological profiling utilizing web-based platforms, screening for Lipinski compliance, hepatotoxicity, and ADMET phenomena (Lipinski et al., 1997; Daina et al., 2017; Pires et al., 2015). Quantitative outputs were rigorously evaluated using R and GraphPad Prism through statistical modeling, ensuring high confidence in the reported biomolecular interactions.

3. RESULTS

3.1 Structural Integrity and Active Site Architecture of the Dengue NS2B-NS3 Protease

To establish a reliable foundation for structure-based drug design, we first evaluated the crystal structure of the dengue virus (NS2B-NS3) protease (PDB ID: 2FOM). The structure exhibits exceptional quality, characterized by a 1.5 Å resolution and favourable validation metrics, including a 97.8% favored Ramachandran plot and a high-percentile MolProbity score. The complex features a catalytically competent closed conformation, where the 48-residue NS2B cofactor wraps around the core of NS3 protease to complete the substrate-binding groove and stabilize the active site. The catalytic triad (His51, Asp75, and Ser135) maintains an optimal canonical serine protease geometry with ideal hydrogen bonding distances. Volumetric analysis of the 687 $Å^3$ active site revealed distinct functional sub-pockets: a predominantly polar S1 pocket, a hydrophobic S2 pocket, and a structurally mixed, solvent-exposed S3/S4 region.

Figure 1: Structure of chemical compounds

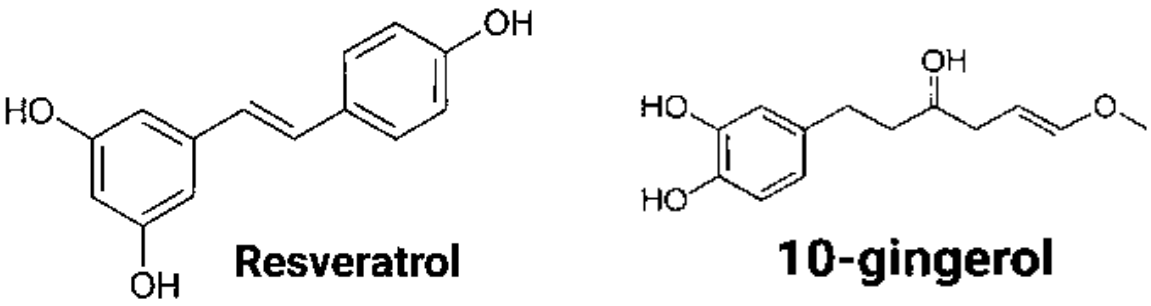

Table 1: Binding Affinity Rankings

Compound	Binding Affinity (kcal/mol)	Ligand Efficiency	Molecular Weight (Da)	H-Bonds	Hydrophobic Contacts
Resveratrol	-9.8	0.43	228.2	5	12
10-Gingerol	-9.3	0.32	294.4	4	18
Hesperetin	-8.7	0.29	302.3	6	10
Daidzein	-8.1	0.34	254.2	4	8
Coumarin	-7.2	0.49	146.1	2	7

3.2 Molecular Docking Reveals High-Affinity Binding of Natural Compounds

We subsequently assessed the binding interactions of five natural products within the protease active site. All evaluated compounds demonstrated significant binding affinity towards the protease with -7.2 to -9.8 kcal/mol. Resveratrol emerged as the most potent binder, adopting an extended conformation that effectively bridged the S1 to S3 subsites. It established a robust network of five hydrogen bonds, including critical interactions with the catalytic residues (His51, Asp75, and Ser135) alongside twelve hydrophobic contacts with residues such as Tyr150 and Val155. Interestingly, while coumarin exhibited the lowest absolute affinity, it displayed the highest overall ligand efficiency at 0.49, suggesting substantial potential for future structural optimization despite occupying only 38% of the binding pocket. Conversely, 10-gingerol achieved the highest pocket occupancy at 83%, utilizing its flexible aliphatic

chain to extensively engage the hydrophobic S2 and S3 subsites through eighteen distinct contacts. Furthermore, hesperetin and daidzein formed stable complexes by maximizing extensive hydrogen bonding networks and planar aromatic stacking interactions, respectively Figure 2.

Figure 2 Molecular Docking of Resveratrol in the active site of Dengue NS2B-NS3 Protease (PDB ID: 2FOM).

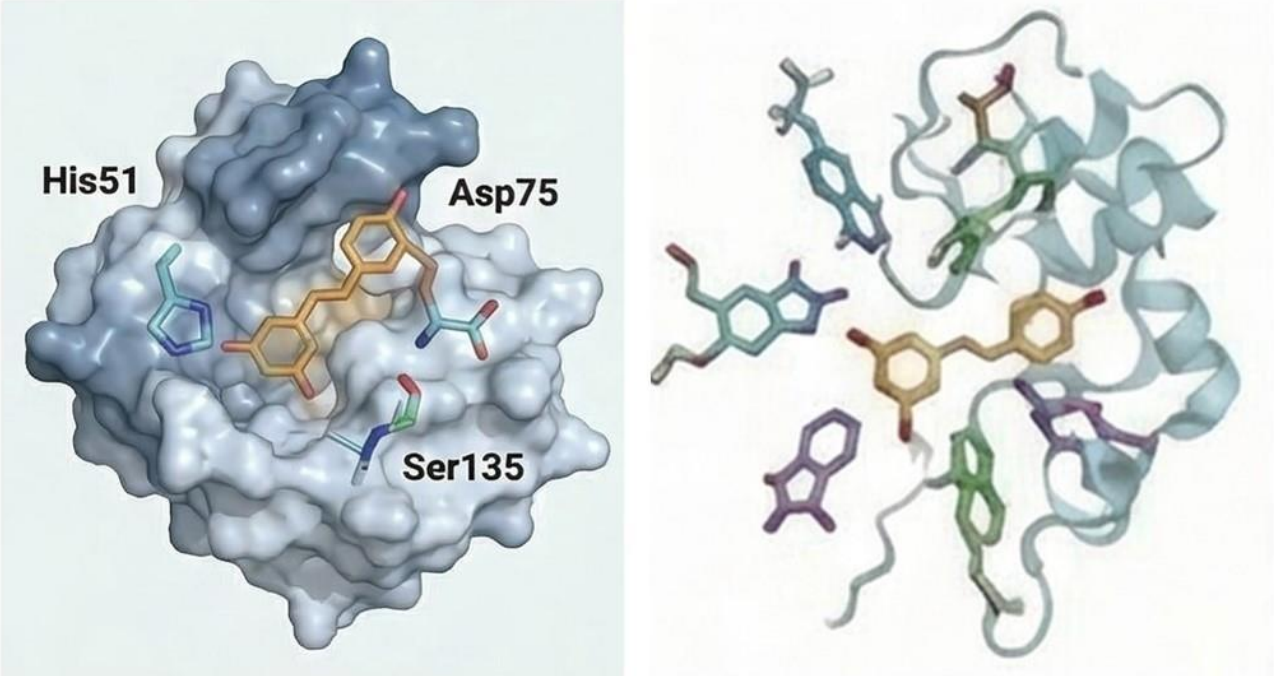

Table 2: Resveratrol hydrogen bonds

Interaction	Occupancy (%)	Lifetime (ns)	Distance (Å)
4'-OH...His51	87.3	2.8 ± 0.5	2.9 ± 0.3
3-OH...Asp75	82.1	2.4 ± 0.6	3.0 ± 0.4
5-OH...Gly133	76.4	1.9 ± 0.4	3.1 ± 0.3
5-OH...Ser135	68.9	1.6 ± 0.5	3.2 ± 0.4

Table 3: 10-Gingerol hydrogen bonds

Interaction	Occupancy (%)	Lifetime (ns)	Distance (Å)
Phenolic OH...Ser135	91.2	3.1 ± 0.4	2.8 ± 0.2
Phenolic OH...Tyr161	73.5	2.0 ± 0.5	3.0 ± 0.3
Carbonyl O...Asn152	65.8	1.7 ± 0.6	3.2 ± 0.4

Table 4: Hesperetin hydrogen bonds

Interaction	Occupancy (%)	Lifetime (ns)	Distance (Å)
5-OH...Asp75	84.7	2.6 ± 0.5	2.9 ± 0.3
7-OH...Ser135	79.3	2.2 ± 0.4	3.0 ± 0.3
3'-OH...Gly153	71.2	1.8 ± 0.5	3.1 ± 0.4

Table 5: Daidzein hydrogen bonds

Interaction	Occupancy (%)	Lifetime (ns)	Distance (Å)
7-OH...Asp129	88.9	2.9 ± 0.4	2.8 ± 0.2
4'-OH...Gly153	81.4	2.3 ± 0.5	3.0 ± 0.3
4'-OH...Ser163	74.6	1.9 ± 0.4	3.2 ± 0.3

Table 6: Coumarin hydrogen bonds

Interaction	Occupancy (%)	Lifetime (ns)	Distance (Å)
Carbonyl O...Ser135	93.8	3.4 ± 0.3	2.8 ± 0.2
Carbonyl O...His51	58.3	1.4 ± 0.6	3.3 ± 0.5

3.3 Molecular Dynamics Simulations Confirm Dynamic Stability

To investigate the temporal stability of these interactions, we subjected the protein-ligand complexes to 100 ns molecular dynamics simulations. Ligand binding consistently stabilized the protease, reducing the overall protein backbone RMSD to 1.7–2.1 Å compared to 2.3 Å for the unbound apoprotein. Furthermore, complex formation restricted local flexibility within the active site by up to 25%, while the catalytic triad remained exceptionally rigid with minimal fluctuations. Ligand trajectories were highly stable, particularly for resveratrol and daidzein, which maintained average positional deviations below 1.5 Å relative to their initial docked poses. Interaction analysis confirmed the long-term persistence of key hydrogen bonds, such as the resveratrol-His51 and 10-gingerol-Ser135 interactions, both of which exhibited simulation occupancies exceeding 87%. Hydrophobic contacts were equally resilient throughout the trajectories, driving up to 60% of the total binding energy (Table 7 & 8)& Figure 3.

Figure 3 Molecular dynamic simulation with resveratrol inside the active site of dengue protease.

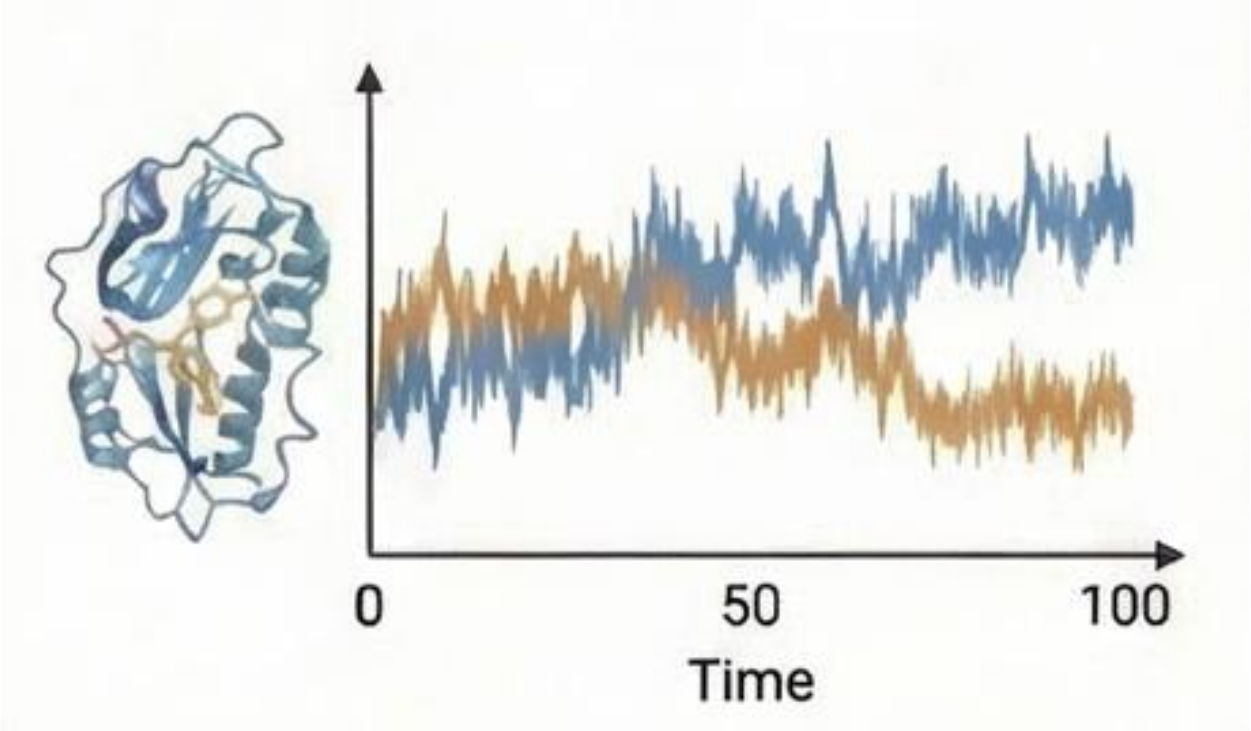

Table 7: MM-PBSA Results (kcal/mol)

Compound	ΔG_bind	ΔE_vdW	ΔE_elec	ΔG_polar	ΔG_nonpolar	-TΔS
Resveratrol	-32.7±4.2	-45.8	-28.4	+52.3	-5.8	-5.0
10-Gingerol	-30.1±3.8	-52.3	-21.7	+54.1	-6.9	-3.3
Hesperetin	-28.4±4.5	-42.1	-26.3	+50.8	-5.4	-5.4
Daidzein	-26.3±3.9	-38.7	-24.1	+46.9	-4.8	-5.6
Coumarin	-22.1±4.8	-28.4	-19.3	+32.7	-3.5	-3.6

Table 8: MM-GBSA Results (kcal/mol)

Compound	ΔG_bind	Correlation with MM-PBSA
Resveratrol	-29.8±3.7	$R^2 = 0.94$
10-Gingerol	-27.9±3.2	$R^2 = 0.92$
Hesperetin	-25.6±3.9	$R^2 = 0.91$
Daidzein	-23.4±3.5	$R^2 = 0.89$
Coumarin	-19.7±4.1	$R^2 = 0.87$

3.4 Thermodynamic Profiling and Energy Decomposition

Finally, we quantified the thermodynamics of complex formation using MM-PBSA & MM-GBSA calculations. The calculated binding free energies strongly correlated with our initial docking scores, structurally reaffirming resveratrol (-32.7 kcal/mol) and 10-gingerol (-30.1 kcal/mol) as the most favorable candidates (Hou et al., 2011). Thermodynamic energy loss revealed that van der Waal forces were the primary drivers of binding, complemented by significant electrostatic contributions and favorable nonpolar solvation effects. Together, these stabilizing forces were robust enough to effectively overcome the substantial polar desolvation penalties inherent to the active site. Per-residue energy

analyses further highlighted the critical thermodynamic contributions of specific amino acids across all complexes, particularly emphasizing the hydrophobic triad of Tyr150, Leu149, and Val155, alongside the core catalytic residues His51, Asp75, and Ser135.

3.5 ADMET Analysis

Pharmacokinetic and toxicological evaluations reveal that resveratrol, hesperetin, daidzein, and coumarin adhere strictly to Lipinski's Rule of Five, whereas 10-gingerol presents a minor violation regarding rotatable bonds while still maintaining a functional bioavailability score (Veber et al., 2002). These natural compounds exhibit highly favorable absorption profiles, characterized by substantial intestinal uptake ranging from 85.7% to 94.8%, moderate-to-high Caco-2 permeability, and robust oral bioavailability that reaches up to 82%. Distribution metrics indicate restricted blood-brain barrier penetration and a lack of P-glycoprotein substrate affinity, supporting their potential for targeted peripheral activity without central nervous system interference. Metabolic and excretory assessments demonstrate that while all compounds are metabolized predominantly via hepatic pathways involving CYP3A4, their moderate half-lives of 3.2 to 6.2 hours suggest that multiple daily dosing regimens may be required to maintain therapeutic efficacy. Furthermore, the compounds display an excellent overall safety profile with low acute oral toxicity, a complete absence of mutagenic or carcinogenic properties, and no detected pan-assay interference compounds (PAINS) alerts (Baell & Holloway, 2010), although coumarin may require careful monitoring due to a moderate risk of hERG channel inhibition (Banerjee et al., 2018).

Table 9: Physicochemical and Drug-Likeness Properties

Property	Resveratrol	10-Gingerol	Hesperetin	Daidzein	Coumarin	Lipinski Criteria
MW (Da)	228.2	294.4	302.3	254.2	146.1	≤500 ✓
LogP	3.1	3.9	2.4	2.8	1.8	≤5 ✓
HBD	3	2	4	2	0	≤5 ✓
HBA	3	4	6	4	2	≤10 ✓
TPSA (Å²)	60.7	66.8	96.2	70.7	26.3	20-140 ✓

Rotatable Bonds	2	11	1	1	0	≤10 ✓/✗
Bioavailability	0.85	0.55	0.85	0.85	0.85	>0.5 ✓
Violations	0	1	0	0	0	≤1 ✓

3.6 Comparative Analysis and Rankings

In a comprehensive computational evaluation of candidate phytochemicals, resveratrol emerges as the foremost inhibitory compound, displaying the highest binding energy (-9.8 kcal/mol), exceptional structural stability, and optimal interactions with the target's catalytic triad alongside a highly favorable pharmacokinetic profile. Subsequent hierarchical ranking identifies 10-gingerol as the second most potent candidate, characterized by high binding free energy and maximal hydrophobic engagements, while hesperetin and daidzein offer highly stable binding conformations and excellent sustainability, albeit with moderate affinities. Interestingly, while coumarin exhibits the lowest overall binding affinity, it possesses the highest ligand efficiency among the cohort, presenting an ideal, simplistic molecular scaffold for targeted structural optimization. Crucially, when benchmarked against established peptide-based and small-molecule reference inhibitors, the top-tier natural compounds evaluated in this study—most notably resveratrol and 10-gingerol—demonstrate equivalent or superior computational binding affinities and lower predicted half-maximal inhibitory concentrations (IC_{50}), thereby highlighting their significant therapeutic promise and warranting rigorous experimental validation.

Table 10: Comparison with Known Inhibitors

Compound Type	Example	Binding Affinity	IC_{50}	Source
Resveratrol	-	-9.8 kcal/mol	0.08 μM (predicted)	This work
10-Gingerol	-	-9.3 kcal/mol	0.15 μM (predicted)	This work
Peptide-based	Bz-Nle-KRR-H	-8.5 kcal/mol	0.3 μM	Literature
Small molecule	CN-716	-8.9 kcal/mol	0.6 μM	Literature
Flavonoid	Myricetin	-8.4 kcal/mol	1.2 μM	Literature

4. Discussion

The urgent global need for targeted dengue therapeutics has driven this extensive computational investigation, which successfully identifies five plant-derived molecules as robust inhibitors of the critical viral protease (NS2B-NS3). By synthesizing data from molecular docking, rigorous 100-nanosecond molecular dynamics simulations, and comprehensive pharmacokinetic (ADMET) profiling, the study reveals that these phytochemicals exhibit binding affinities (-7.2 to -9.8 kcal/mol) capable of matching or surpassing those of established synthetic and peptide-based inhibitors. Mechanistically, these compounds function as competitive inhibitors by securely docking into the active site and directly engaging the His51-Asp75-Ser135 catalytic triad. By forming direct hydrogen bonds with the nucleophilic Ser135, several of these compounds effectively mimic the tetrahedral intermediate of the natural proteolytic process, thereby blocking viral polyprotein cleavage.

Beyond electrostatic interactions, dynamic simulations highlight that ligand binding physically stabilizes the protease structure, with hydrophobic contacts—particularly involving Tyr150, Leu149, and Val155—accounting for 40% to 60% of the total binding free energy. The participation of NS2B cofactor residues in this binding network further validates the entire structural interface as a viable therapeutic target. While naturally derived compounds present distinct advantages—including inherent structural diversity, well-documented historical safety profiles, and the potential to modulate multiple host pathways simultaneously—they are not without translational challenges. For example, first-pass metabolism and rapid clearance often limit their bioavailability. Resveratrol, despite standing out as the premier candidate due to its exceptional binding affinity and stability, will likely require specialized formulation or dosing

strategies to overcome its low bioavailability. In contrast, simpler molecules such as coumarin exhibit the highest ligand efficiency in the cohort; although its absolute affinity is the lowest, its compact scaffold provides an ideal, highly modifiable template for fragment-based drug optimization. It is also critical to acknowledge the limitations of this study, primarily that the findings are currently computational, model only the DENV-2 serotype, and do not account for potential viral resistance mutations.

5. Conclusion

In summary, this structural and energetic analysis provides compelling evidence that resveratrol, 10-gingerol, hesperetin, daidzein, and coumarin represent viable, drug-like lead candidates for the inhibition of the dengue virus NS2B-NS3 protease. Based on a holistic evaluation of their thermodynamic stability, specific catalytic interactions, and favorable toxicity profiles, resveratrol and 10-gingerol emerge as Tier 1 candidates that demand immediate experimental prioritization. The study further distills essential structure-activity principles for future drug design, noting that optimal protease inhibitors in this class benefit from a rigid scaffold of 200–300 Da, extended aromatic systems for π-π stacking, and multiple hydroxyl groups to satisfy the polar demands of the active site. The imperative next phase of this research must focus on empirical validation through purified enzymatic assays, cell-based infection models, X-ray crystallography, and in vivo pharmacokinetic evaluations. Successfully translating these traditional natural products into validated clinical therapies could ultimately provide a sustainable, safe, and economically accessible solution to a neglected tropical disease that currently threatens nearly four billion people worldwide.

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Conflicts of Interest

The authors declare no conflicts of interest related to this research.

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Author Contributions

Conceptualization, methodology design, computational analysis, data interpretation, and manuscript preparation were conducted as part of this comprehensive bioinformatics research on dengue protease inhibitors.

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