

In Silico Design, Network Pharmacology and Evaluation of Novel Curcumin Derivatives as Potential NS2B–NS3 Protease Enzyme Inhibitors for Dengue Fever

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Abstract: Dengue is a mosquito-borne viral disease for which no specific antiviral drug is in routine clinical use, and the viral NS2B–NS3 serine protease, which processes the viral polyprotein, is an attractive target for direct-acting antivirals. Five curcumin-based compounds were evaluated with an integrated in silico workflow: curcumin (C1), desmethoxycurcumin (C2), bisdemethoxycurcumin (C3), a piperidone–sulfonyl curcumin analogue (C4) and a benzothiazole-linked curcumin hybrid (C5). Drug-likeness was assessed with Molinspiration and SwissADME, toxicity with ProTox-3.0, and the multi-target context of curcumin in dengue by network pharmacology. Binding to the NS2B–NS3 protease (PDB ID: 3L6P) was predicted by docking with PyRx/Auto Dock, and the C5 complex was examined in a 100 ns molecular dynamics simulation in GROMACS. Network analysis identified 37 targets shared between curcumin and dengue and ten host hub genes (IL1B, IFNG, CCL2, CXCL10, IL6, TLR4, IL10, IL17A, TNF and CSF2) enriched in cytokine and inflammatory signaling. Docking scores were –6.3, –7.2, –6.8, –9.2 and –9.8 kcal/mol for C1–C5, respectively. The two designed analogues scored best, and their predicted poses involved Asp75, Ser135 and His51 of the catalytic triad. In the 100 ns simulation the protease backbone remained stable (RMSD about 0.15–0.20 nm) and compact, but protein–ligand hydrogen bonds were transient. All results are computational predictions; C5 is regarded as a candidate for synthesis, enzymatic inhibition assays and further optimization rather than a confirmed inhibitor.

Keywords: dengue virus; NS2B–NS3 protease; curcumin; network pharmacology; molecular docking; molecular dynamics; ADMET; PyRx; in silico drug design.

INTRODUCTION

Dengue is caused by the dengue virus (DENV), a mosquito-borne flavivirus with four serotypes (DENV-1 to DENV-4), and is transmitted mainly by *Aedes aegypti* [1]. About half of the world's population is at risk, and modelling studies estimate hundreds of millions of infections each year [1–3]. Clinical illness ranges from a self-limiting febrile illness to severe dengue with plasma leakage and shock. Management remains

largely supportive, and no specific antiviral drug is in routine clinical use [1,3]. The DENV genome encodes a single polyprotein that is cleaved into structural and non-structural proteins. Cleavage at several of these sites is carried out by the NS2B–NS3 serine protease, in which the NS3 protease domain carries the catalytic triad His51, Asp75 and Ser135 and the NS2B cofactor is required for the active conformation [4,5]. Because the protease is essential for replication, structurally

characterized and conserved among flaviviruses, it is a widely pursued target for direct-acting antivirals [4,6]. Curcumin, a diarylheptanoid from *Curcuma longa*, has many reported biological activities and is a frequent starting point for medicinal chemistry [7,8]. Its clinical use is limited by poor aqueous solubility, low bioavailability, rapid metabolism and chemical instability [8,9], and curcuminoids have been reported to inhibit dengue virus in cell-based studies [10]. Modification of the central linker and introduction of heterocyclic groups are common strategies for improving such scaffolds. Here, curcumin, its two natural analogues desmethoxycurcumin and bisdemethoxycurcumin, and two designed analogues carrying a piperidone–sulfonyl or a benzothiazole group were evaluated by drug-likeness and toxicity prediction, network pharmacology, molecular docking and molecular dynamics, with the aim of prioritizing candidates for experimental testing against the NS2B–NS3 protease.

2. Materials and Methods

2.1 Ligand Design

Five compounds were studied and coded C1–C5: curcumin (C1), desmethoxycurcumin (C2), bisdemethoxycurcumin (C3), a piperidone–sulfonyl curcumin analogue (C4) and a benzothiazole-linked curcumin hybrid (C5). C4 and C5 were designed by introducing a piperidone/sulfonyl or a benzothiazole pharmacophore into the curcumin-derived scaffold. Structures were drawn and their formulas and masses calculated with ACD/ ChemSketch.

The final SMILES for C4 and C5 are CS(=O)(=O)N1CC(=CC2=CC=C(O)C(=C2)OC)C(=O)C(=CC3=CC=C(O)C(=C3)OC)C1 and COC1=C(O)C=CC(=CC=C2C=C3N4C(=NSC5=CC=CC=C54)N=C2C=CC6=CC=C(O)C(=C6)OC)C1

2.2 Drug-Likeness, ADME and Toxicity Prediction

Molecular weight, miLogP, topological polar surface area (TPSA), hydrogen-bond acceptors and donors (nON, nOHNH) and rotatable bonds were calculated for C1–C5 with Molinspiration (property engine v2022.08) [11]. Drug-likeness was judged against Lipinski's rule of five [12] and the rotatable-bond and TPSA limits of Veber et al. [13]. SwissADME [14] was used for gastrointestinal absorption, blood–brain barrier permeation, solubility, drug-likeness filters

and medicinal-chemistry alerts (PAINS [15] and Brenk [16]) for C1–C3. Toxicity endpoints (hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity) were predicted with ProTox-3.0 [17] from the submitted structures.

2.3 Network Pharmacology

Targets of curcumin were predicted with Swiss Target Prediction [18] and cross-checked against compound–target databases. Dengue-associated genes were retrieved from Gene Cards [19] using “dengue virus infection” and related immunopathology terms. Overlapping targets were identified with Venny 2.0 [20]. A protein–protein interaction (PPI) network was built in STRING [21] (confidence score > 0.4), visualized in Cytoscape [22], and hub genes were ranked with the cytoHubba plugin [23]. Gene Ontology (biological process, molecular function, cellular component) and KEGG enrichment of the ten hub genes were performed with ShinyGO [24]. A viral–host protein interaction network of the ten DENV proteins with host proteins was also examined (ViralHostNet). The network pharmacology approach follows the multi-target concept described by Hopkins [25].

2.4 Molecular Docking

The crystal structure of the NS2B/NS3 protease (PDB ID: 3L6P; X-ray diffraction, 2.20 Å) [26] was obtained from the RCSB Protein Data Bank. Water molecules were removed and hydrogen atoms and charges were added. Ligands were energy-minimized and converted to the docking format in PyRx [27]. Docking was performed with PyRx using Auto Dock Vina [28,29], with the search space placed over the catalytic region containing His51, Asp75 and Ser135. The grid centered at X = -2.7, Y = -11.3, Z = -13.5 with grid-box dimensions of 16 Å (X), 24 Å (Y), 22 Å (Z). The top-scoring pose of each ligand was retained, and binding affinity (kcal/mol) was used as the ranking criterion, with more negative values indicating more favorable predicted binding [30]. Interactions were visualized and mapped in BIOVIA Discovery Studio.

2.5 Molecular Dynamics Simulation

The C5–3L6P complex was simulated for 100 ns with GROMACS [31]. The protein was described and the ligand with GAFF2 [33] parameters generated with ACPYPE [34].

The complex was solvated with TIP3P water [32] in a dodecahedral box with at least 1.0 nm between solute and box edge and neutralized with 0.15 M NaCl. After energy minimization and NVT and NPT equilibration, production dynamics used a 2-fs time step with LINCS constraints on hydrogen bonds, particle-mesh Ewald electrostatics with 1.0–1.2 nm cut-offs, the V-rescale thermostat at 310 K and the Parrinello–Rahman barostat at 1 bar. The trajectory was analyzed for backbone RMSD of the protein and ligand, per-residue RMSF, radius of gyration (Rg), solvent-accessible surface area (SASA), intermolecular hydrogen bonds and minimum protein–ligand distance. [[Add MM-PBSA/MM-GBSA method details (gmx_MMPBSA [35,36], frames, entropy treatment) once the calculation is finalized.]]

3. Results

3.1 Compounds Studied

The panel comprises the parent compound, its two natural analogues and two designed analogues (Figure 1, Table 1).

Table 1. Compounds C1–C5 and their structural modification relative to curcumin.

Code	Compound	Structural modification
C1	Curcumin	Parent diferuloylmethane scaffold with two methoxy groups
C2	Demethoxycurcumin (DMC)	One aromatic methoxy group removed
C3	Bisdemethoxycurcumin (BDMC)	Both aromatic methoxy groups removed
C4	Piperidone-sulfonyl curcumin analogue	Curcumin-derived aromatic system linked to a piperidone/sulfonyl pharmacophore
C5	Benzothiazole-linked curcumin hybrid	Benzothiazole heterocycle incorporated into the curcumin-derived scaffold

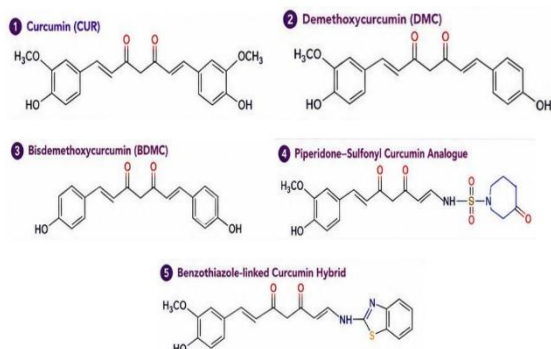


Figure 1. Structures of C1–C5 as drawn for the design.

3.2 Drug-Likeness and ADME

Molinspiration properties are given in Table 2. C1, C2, C3 and C5 satisfied Lipinski's criteria, with C5 close to the 500 Da limit (499.57). C4 has a molecular weight of 569.60 and

therefore breaks the weight criterion. All compounds had TPSA below 140 Å² and no more than ten rotatable bonds.

Table 2. Molinspiration physicochemical properties of C1–C5. MW, molecular weight; TPSA, topological polar surface area; nON, hydrogen-bond acceptors (N + O); nOHNH, hydrogen-bond donors; nroth, rotatable bonds.

Code	miLogP	TPSA (Å ²)	MW (g/mol)	nON	nOHNH	nroth	Lipinski violations
C1	3.36	93.06	368.38	6	2	6	0
C2	2.83	73.55	338.35	5	2	6	0
C3	2.31	54.05	308.31	4	2	6	0
C4	2.74	113.81	569.60	9	2	7	1 (MW > 500) ^a
C5	3.18	91.74	499.57	7	2	6	0

a Recounted from the listed values; the Molinspiration output reported no violation.

SwissADME predictions for C1–C3 are summarized in Table 3. All three were predicted to have high gastrointestinal absorption, to pass Lipinski, Ghose, Veber, Egan and Muegge filters without violation, to carry no PAINS alert, and to raise two Brenk alerts (a β -keto/anhydride-type and a Michael-acceptor alert) arising from the enone–diketone linker. Only C3 was predicted to cross the blood–brain barrier.

Table 3. SwissADME predictions for C1–C3.

Parameter	C1 (curcumin)	C2 (DMC)	C3 (BDMC)
Formula / MW (g/mol)	C21H20O6 / 368.38	C20H18O5 / 338.35	C19H16O4 / 308.33
TPSA (Å ²)	93.06	83.83	74.60
Consensus Log P	2.97	3.05	3.10
Water solubility (ESOL)	Soluble (–3.94)	Soluble (–3.92)	Soluble (–3.80)
GI absorption	High	High	High
BBB permeant	No	No	Yes
P-gp substrate	No	No	No
Lipinski violations	0	0	0
Bioavailability score	0.55	0.55	0.55
PAINS / Brenk alerts	0 / 2	0 / 2	0 / 2
Lead-likeness	No (2 violations)	Yes	Yes
Synthetic accessibility	2.97	2.82	2.59

3.3 Toxicity Prediction

ProTox-3.0 predictions are summarized in Table 4 (class and probability). All four evaluated compounds were predicted inactive for carcinogenicity and mutagenicity. Immunotoxicity was predicted active for C1, C2 and C5, and respiratory toxicity for C2 and C5. For C5, neurotoxicity and cytotoxicity were predicted active with low probabilities (0.59 and 0.54). These are probabilistic model outputs and require experimental confirmation.

Table 4. ProTox-3.0 predicted toxicity endpoints (prediction, probability).

Endpoint	C1	C2	C3	C4	C5
Hepatotoxicity	Inactive (0.61)	Active (0.69)	Inactive (0.65)	Active (0.69)	Inactive (0.73)
Neurotoxicity	Inactive (0.81)	Active (0.87)	Inactive (0.88)	Active (0.87)	Active (0.59)
Nephrotoxicity	Active (0.59)	Inactive (0.90)	Active (0.56)	Inactive (0.9)	Inactive (0.55)
Respiratory toxicity	Inactive (0.77)	Active (0.98)	Inactive (0.63)	Active (0.98)	Active (0.90)
Cardiotoxicity	Active (0.55)	Inactive (0.77)	Inactive (0.62)	Inactive (0.77)	Inactive (0.69)
Carcinogenicity	Inactive (0.84)	Inactive (0.62)	Inactive (0.64)	Inactive (0.62)	Inactive (0.62)
Immunotoxicity	Active (0.92)	Active (0.96)	Inactive (0.90)	Active (0.92)	Active (0.88)
Mutagenicity	Inactive (0.88)	Inactive (0.97)	Inactive (0.82)	Inactive (0.93)	Inactive (0.70)
Cytotoxicity	Inactive (0.88)	Inactive (0.93)	Inactive (0.93)	Inactive (0.97)	Active (0.54)

3.4 Network Pharmacology

Dengue-associated genes (231) and predicted curcumin targets (116) shared 37 targets, 16.0% of the dengue set (Figure 2). PPI network analysis in STRING and Cytoscape identified ten hub genes: IL1B, IFNG, CCL2, CXCL10, IL6, TLR4, IL10, IL17A, TNF and CSF2 (Figure 3). Enrichment of these hub genes was dominated by inflammatory and immune processes (Table 5): inflammatory response and immune response each covered all ten genes, and cytokine-mediated signaling, cellular response to cytokine stimulus and regulation of inflammatory response covered eight each. At the molecular-function level the hub genes were associated with cytokine activity and cytokine-receptor binding, and KEGG enrichment highlighted viral protein interaction with cytokine and cytokine receptor. The viral–host interaction network linked the DENV proteins, particularly the non-structural proteins, with host immune and interferon-signaling genes such as STAT1, TLR7 and IFI27.

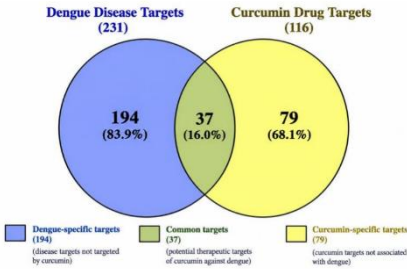


Figure 2. Venn diagram of dengue-associated targets (231) and curcumin targets (116); 37 targets are shared.

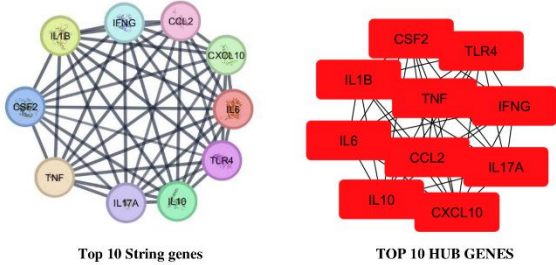


Figure 3. Top ten hub genes of the PPI network: STRING network (left) and cytoHubba ranking (right).

Table 5. Gene Ontology enrichment of the ten hub genes (number of hub genes annotated to each term).

Category	Term	Hub genes
Biological process	Inflammatory response	10
Biological process	Immune response	10
Biological process	Cytokine-mediated signalling pathway	8
Biological process	Regulation of inflammatory response	8
Biological process	Response to virus	6
Molecular function	Cytokine receptor binding	8
Molecular function	Cytokine activity	7
Molecular function	Chemokine activity	2
Cellular component	Extracellular space	8
Cellular component	Cell surface	4

3.5 Molecular Docking

Predicted binding affinities against the NS2B–NS3 protease (3L6P) was –6.3 (C1), –7.2 (C2), –6.8 (C3), –9.2 (C4) and –9.8 kcal/mol (C5) (Table 6). The two designed analogues therefore scored 2.0–3.5 kcal/mol more favorably than curcumin, with C5 the highest ranked. In the predicted poses, C5 formed hydrogen bonds with Asp75, Ser135 and Asn152, a π – π T-shaped contact with His51, and van der Waals contacts with Gly49, Thr134, Pro132 and Tyr161. C4 formed hydrogen bonds with Asp75 and Ser135, a π – σ contact with His51 and van der Waals contacts with Gly49, Thr134 and Tyr161 (Figure 4). Residues of the catalytic triad were thus engaged by both designed analogues.

Table 6. Docking scores of C1–C5 against the NS2B–NS3 protease (PDB ID: 3L6P) and key interacting residues of the two top-ranked compounds.

Code	Docking score (kcal/mol)	Key interactions
C1	−6.3	H bonds: Ala214, Trp 139 ; π - σ : Gln 217 ; van der Waals: Ser 138, Asp 125, Val 173
C2	−7.2	H-bonds: Ala 197 ; π - σ : Ala 198 , Tyr 62 , Leu 162 ; van der Waals: Asp 125, Val 173
C3	−6.8	H-bonds: His 201 , Asp 197 ; π - σ : Tyr 62, Leu 162 ; van der Waals: Trp 59, Asp 300, His101
C4	−9.2	H-bonds: Asp75, Ser135; π - σ : His51; van der Waals: Gly49, Thr134, Tyr161
C5	−9.8	H-bonds: Asp75, Ser135, Asn152; π - π T-shaped: His51; van der Waals: Gly49, Thr134, Pro132, Tyr161

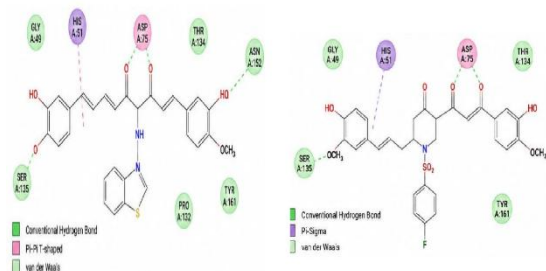


Figure 4. Two-dimensional interaction diagrams of the docked poses of C5 (left) and C4 (right) in the NS2B–NS3 protease. Green dashed lines, conventional hydrogen bonds; pink, π - π T-shaped; purple, π - σ ; light green circles, van der Waals contacts.

3.6 Molecular Dynamics of the C5–Protease Complex

Over 100 ns the protein backbone RMSD rose during the first nanoseconds and then fluctuated around 0.15–0.20 nm, while the ligand RMSD settled at about 0.2–0.3 nm, indicating that the complex equilibrated without large displacement of the ligand (Figure 5, Table 7). The radius of gyration stayed near 1.6–1.7 nm, so the protein remained compact. SASA drifted upward modestly, from about 90 to 93 nm². Per-residue RMSF showed peaks of up to about 0.3 nm near His51, Asp75 and Ser135, indicating that these catalytic residues are among the more mobile regions of the protein. Protein–ligand hydrogen bonds were intermittent, with zero or one bond at any time, and the minimum protein–ligand distance remained about 0.17–0.24 nm, consistent with the ligand staying in contact with the protein throughout.

Table 7. Summary of the 100 ns simulation of the C5–3L6P complex (values read from the trajectory plots).

Parameter	Observation
Simulation time	100 ns
Protein backbone RMSD	Equilibrated; about 0.15–0.20 nm
Ligand RMSD	About 0.2–0.3 nm after equilibration
Radius of gyration	About 1.6–1.7 nm, stable
SASA	About 89–94.5 nm ² , slight upward drift
RMSF	Up to about 0.3 nm at His51, Asp75 and Ser135; mostly 0.05–0.15 nm elsewhere
Intermolecular hydrogen bonds	Transient; 0–1 bond at any time
Minimum protein–ligand distance	About 0.17–0.24 nm
MM-PBSA $\Delta G(\text{bind})$	About −17 to 85 kcal/mol

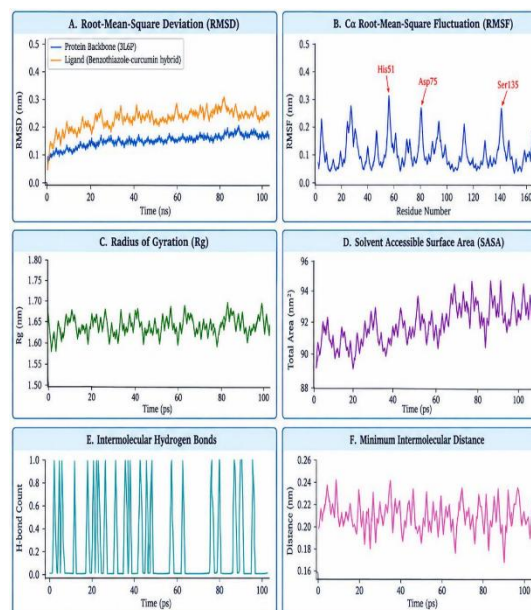


Figure 5. Trajectory analysis of the C5–3L6P complex: (A) backbone and ligand RMSD, (B) C α RMSF, (C) radius of gyration, (D) SASA, (E) intermolecular hydrogen bonds and (F) minimum protein–ligand distance.

4. Discussion

The ranking by docking placed the two designed analogues, C5 and C4, clearly ahead of curcumin and its natural analogues. The piperidone–sulfonyl and benzothiazole groups add hydrogen-bond acceptors and aromatic or hydrophobic surface that, in the predicted poses, allow contacts with Asp75, Ser135 and His51, which are the residues of the catalytic triad [4,5]. This is consistent with the design rationale, but a docking score is a ranking tool and not a measure of inhibition [30]. The 0.6 kcal/mol difference between C5 and C4 is smaller than the error usually attributed to docking scoring functions and should not be read as a firm preference. The 100 ns simulation of C5 supports a stable protein fold and continued ligand contact: the backbone RMSD and radius of gyration were steady and the minimum distance stayed short. The evidence for a strongly

anchored ligand is weaker, because hydrogen bonds were transient, the ligand RMSD was higher than that of the protein, and the catalytic residues showed the largest fluctuations. A single trajectory of this length, without replicates or a completed free-energy estimate [35,36], cannot establish binding stability. Drug-likeness predictions favour C1–C3 and C5 over C4, whose molecular weight (569.60) exceeds the Lipinski limit, while C5 lies at the limit (499.57). Curcumin and its analogues carry Michael-acceptor and related Brenk alerts, and curcumin is known as a frequent-hitter and unstable scaffold in assays [8], so activity in enzymatic assays should be interpreted with appropriate counter-screens. ProTox-3.0 raised immunotoxicity, respiratory and, for C5, low-probability neurotoxicity and cytotoxicity flags; these are predictions only. The network pharmacology results describe the host-response context rather than the protease mechanism. The hub genes, particularly TNF, IL6, IL10, IFNG and CXCL10, are central to the cytokine response in dengue [37], so modulation of these pathways by curcumin-type compounds is a hypothesis that is separate from, and complementary to, protease inhibition. The targets were predicted for curcumin and not for each analogue, and the enrichment counts are not accompanied by adjusted significance values. The main limitations are as follows. The study is entirely computational, with no enzymatic, cell-based or antiviral data. The protease structure 3L6P is a DENV-1 NS2B/NS3 construct [26], whereas dengue disease burden involves all four serotypes; although the catalytic triad is conserved, serotype-specific differences, particularly in the NS2B cofactor, may affect binding. Docking used a single run per ligand and no re-docking validation is reported. Interaction residues for C1–C3 are not included. Compounds were not docked against the human host proteins. Experimental synthesis, protease inhibition assays, cytotoxicity testing, and replicate and longer simulations are required to test the proposed mechanism.

5. Conclusions

An integrated in silico analysis of five curcumin-based compounds identified the piperidone–sulfonyl analogue (C4, –9.2 kcal/mol) and the benzothiazole-linked hybrid (C5, –9.8 kcal/mol) as the best-ranked binders of the dengue NS2B–NS3 protease, with predicted contacts to Asp75, Ser135 and His51.

A 100 ns simulation of the C5 complex showed a stable, compact protein but only transient hydrogen bonding. Network analysis placed curcumin-type compounds in a host cytokine and inflammatory context. These predictions are preliminary; C5 is a candidate for synthesis, protease-inhibition assays and further optimization.

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Conflicts of Interest: The authors declare no conflict of interest.

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