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TNF-α inhibitory potential of bioactive compounds from *Caesalpinia sappan* L.: A virtual screening study

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Abstract

Background: Tumor necrosis factor alpha (TNF-α) is a multifunctional proinflammatory cytokine that plays a crucial role in many pathological conditions such as inflammation, autoimmune diseases, and cancer. There is a large body of evidence that *Caesalpinia sappan* L. extract inhibits the TNF-α signaling pathway. Nevertheless, no studies evaluating the TNF-α inhibitory capacity of bioactive metabolites in *C. sappan* have been reported to date, and the mechanism of this process remains unclear. This study assessed the *in silico* binding ability of bioactive compounds in *C. sappan* with TNF-α through molecular docking simulation.

Methods: The secondary metabolite structures reported from *C. sappan* were retrieved from the PubChem database. A model of molecular docking simulation was constructed based on the crystal structure of TNF-α and the small molecule SPD304 with the PDB ID 2AZ5 (rcsb.org/structure/2AZ5). A high throughput docking campaign was conducted by using Autodock Vina with a simple bash script. The optimal docking pose of each compound was visually analyzed using BIOVIA Discovery Studio Visualizer.

Results: Most of the compounds in *C. sappan* showed good *in silico* binding ability to TNF-α. The fifteen metabolites were recognized as potential TNF-α inhibitors with better binding energy than the reference ligand SPD304. Neoprotosappanin was discovered as the best potential structure with a binding energy of -10.06 kcal/mol and meaningful *in silico* interactions with TNF-α.

Conclusions: Bioactive compounds from sappanwood may have the potential to inhibit TNF-α. The most promising compounds should be further evaluated by molecular dynamics simulations and predicted pharmacokinetic and toxicological properties before performing *in vitro* assays.

Keywords: *in silico*, *Caesalpinia sappan* L., sappanwood, inhibitor, TNF-α

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1. INTRODUCTION

Tumor necrosis factor alpha (TNF-α) is a pleiotropic proinflammatory cytokine involved in regulating immune responses, inflammation, cell proliferation, and apoptosis [1]. TNF-α exerts its biological functions through two membrane receptors, TNFR1 (p55) and TNFR2 (p75), which activate several downstream signaling pathways, including JNK, MAPK/ERK, and NF-κB, thereby modulating inflammatory responses and cell survival [2,3]. Under physiological conditions, TNF-α plays an essential role in host defense and immune homeostasis. However, dysregulated or persistent TNF-α expression has been strongly implicated in the pathogenesis of numerous inflammatory, autoimmune, and malignant diseases [4]. Excessive TNF-α signaling contributes to chronic inflammation and tissue damage in autoimmune disorders, such as rheumatoid arthritis and lupus [5].

In cancer, it may promote tumor progression by inducing angiogenic and tumor-promoting factors, including VEGF, bFGF, IL-8, and thymidine phosphorylase [6,7]. Targeting TNF-α has therefore become an important therapeutic strategy.

Several biologic TNF-α inhibitors, such as adalimumab, certolizumab, etanercept, golimumab, and infliximab, have been widely used in clinical practice. Nevertheless, these biologics present significant limitations, including high production cost, parenteral administration, and the risk of serious adverse effects [8]. Consequently, increasing attention has been directed toward the discovery of small-molecule TNF-α inhibitors. For example, the small molecule SPD304 has been reported to disrupt the TNF-α trimer by binding to the dimer interface, thereby inhibiting its biological activity [9]. However, despite these advances, clinically approved orally active small-molecule TNF-α inhibitors remain unavailable, highlighting the need for the identification of new candidate compounds.

Natural products are valuable resources for drug discovery. *Caesalpinia sappan* L. (*Biancaea sappan* L.), from the Fabaceae family, has been traditionally recognized for its anti-inflammatory, immunomodulatory, and anticancer activities [10]. Several of its metabolites, such as sappanchalcone and brazilin, as well as episappanol, protosappanin C, and sappanol, have shown inhibitory effects on TNF-α signaling in previous studies [11-13]. Extracts of *C. sappan* have also been found to suppress TNF-α-related inflammatory pathways [14].

Despite these encouraging findings, previous studies have mainly focused on the pharmacological activity of individual compounds or plant extracts, and the molecular interactions between the secondary metabolites of *C. sappan* and TNF-α have not been systematically investigated. In particular, a comprehensive structure-based evaluation of these compounds as potential TNF-α inhibitors remains lacking. Therefore, the present study specifically aims to systematically investigate the potential inhibitory activity of secondary metabolites from *C. sappan* against TNF-α using molecular docking analysis. The objectives are to elucidate the molecular binding interactions of these compounds with TNF-α and to identify promising natural compounds with the potential for development as novel TNF-α inhibitors.

2. MATERIALS AND METHODS

2.1. Materials

The study materials comprised secondary metabolites (small organic compounds produced by plants) of *C. sappan* reported in the PubChem database and the co-crystallized structure of the tumor necrosis factor-alpha (TNF-α, a signaling protein involved in inflammation) cytokine with the small-molecule inhibitor SPD304, which was retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB, an online database of protein structures) under the accession code 2AZ5. The protein structure 2AZ5 was determined by X-ray diffraction (a technique for determining the 3D atomic structure of molecules) at a resolution of 2.10 Å (angstroms, a unit equal to 0.1 nanometers).

Virtual screening (computer-aided testing of chemical compounds for potential biological activity) was performed on a personal computer equipped with an Intel Core i7-14700K processor (20 cores, 28 threads), 64 GB RAM, and 1.5 TB SSD storage, running Windows 11 Pro version 24H2.

2.2. Methods

2.2.1. Protein structure preparation

The crystal structure of the target protein (PDB ID: 2AZ5) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/structure/2AZ5). Protein preparation and standardization were performed using UCSF ChimeraX version 1.9 [15]. All non-protein components, including crystallographic water molecules, ligands, and extraneous peptide chains, were removed. Only chains A and B were retained for further analysis. Missing amino acid residues were reconstructed by homology modeling using Modeller version 10.6 [16] through the UCSF ChimeraX interface. Five homology models were generated, and their quality was evaluated using the GA341 score and the z-DOPE score. The model with the best evaluation scores was selected for subsequent analysis and saved in PDB format. The selected structure was further prepared for docking by adding polar hydrogens and assigning Kollman charges. Finally, the processed protein structure was converted to PDBQT format using AutoDock Tools version 1.5.7 for molecular docking.

2.2.2. Ligand preparation

Ligands were secondary metabolites of Caesalpinia sappan retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/taxonomy/483143), which lists 165 compounds associated with this species. The available three-dimensional (3D) structures were downloaded in SDF format from PubChem. The structures were subsequently cleaned and duplicate entries were removed using MOE version 2022.02, resulting in a curated library of 119 unique compounds. These structures were then subjected to energy minimization using the MMFF94 force field [17]. Finally, the minimized structures were converted to PDBQT format using Open Babel version 3.1.1 [18] for subsequent molecular docking analysis.

2.2.3. Molecular docking

Molecular docking simulations were performed using AutoDock Vina version 1.2.5. Docking workflow automation was achieved with a custom Perl script. Parameters were specified in a text configuration file. The search space was a rectangular grid box centered at (-19.52 Å, 74.85 Å, 33.90 Å) with dimensions of 24.3 × 24.3 × 24.3 Å. The Vina scoring function ranked the ten best binding poses for each ligand in an energy range of 4 kcal/mol. The exhaustiveness parameter was set to 32 to fully utilize the system's computational capacity.

The docking protocol was validated by redocking the co-crystallized ligand SPD304 into the TNF-α binding site using the same docking parameters. The predicted pose was compared with the crystallographic conformation, and the root-mean-square deviation (RMSD) was calculated. An RMSD value below 2.0 Å was considered acceptable for validating the reliability of the docking protocol.

SPD304 was used as a reference inhibitor. Compounds showing more favorable binding affinity than SPD304 were considered potential candidates for further analysis. Among these candidates, the five compounds with the most favorable binding affinities were selected for detailed ligand–protein interaction analysis.

2.3. Data analysis

Docking results were analyzed for protein-ligand interactions using BIOVIA Discovery Studio Visualizer version 24.1.0.23298 [19]. Docking scores were automatically compiled using a custom Python script and exported as CSV files to identify the binding poses with the most favorable binding energies.

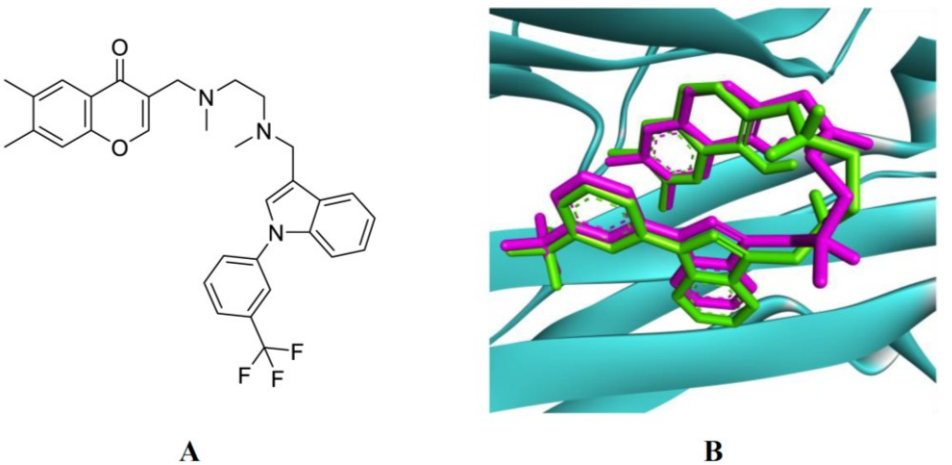
3. RESULTS

3.1. Protein structure preparation results

Table 1: Results of homology modeling for TNF-α chains A and B.

Homology model	GA341 score	z-DOPE score
I	1	-1.26938
II	1	-1.36324
III	1	-1.33445
IV	1	-1.30509
V	1	-1.29348

Five homology models of TNF-α (chains A and B) were generated using Modeller and evaluated based on GA341 and z-DOPE scores (**Table 1**). All models achieved a GA341 score of 1, indicating high reliability. The z-DOPE values ranged from -1.26 to -1.36, suggesting acceptable structural quality. Model II, which exhibited the lowest z-DOPE value, was selected for subsequent analyses.



A **B**

Figure 1. (A) Chemical structure of SPD304. (B) Validation showing how SPD304 fits back into the TNF-α binding site, comparing the original ligand position (green) with the model's result after fitting (magenta).

Figure 1 illustrates the chemical structure of the ligand SPD304 and the superposition of this ligand before and after re-docking into the selected homology model (Model II). The degree of overlap between the two ligand conformations was quantified using the root-mean-square deviation (RMSD), which was 1.09 Å.

3.2. Molecular docking results

The results from docking 119 compounds from PubChem are summarized and ranked by predicted binding strength in Table 2. Each compound ID matches its PubChem number. This docking analysis provides a comparative basis for evaluating compound affinities.

Table 2. Molecular docking results of 119 reported compounds from C. sappan and the reference ligand SPD304.

CID	$\Delta G_{binding}$ (kcal/mol)	CID	$\Delta G_{binding}$ (kcal/mol)	CID	$\Delta G_{binding}$ (kcal/mol)	CID	$\Delta G_{binding}$ (kcal/mol)
SPD304	-8.746	13846696	-7.232	14079439	-7.785	163094896	-8.136
7311726	-3.593	5487855	-7.356	5319633	-7.804	13846649	-8.136
12329	-4.306	5280417	-7.365	57396358	-7.813	71450773	-8.152
5354833	-4.332	124355840	-7.399	24854301	-7.829	13888976	-8.175
52914864	-4.763	13846659	-7.418	13888975	-7.83	162881922	-8.209
985	-5.033	71452537	-7.423	5281691	-7.832	24854208	-8.266
439263	-5.287	5320287	-7.451	71450718	-7.857	24854300	-8.283
370	-5.58	71457914	-7.455	24854205	-7.869	24854298	-8.287
53493060	-5.593	162959860	-7.505	13846647	-7.87	15703605	-8.294
11301173	-5.652	5319632	-7.508	13846645	-7.872	24854297	-8.315
57401583	-6.062	13888973	-7.533	13846641	-7.878	24854207	-8.326
3806	-6.173	13846680	-7.539	6918855	-7.88	222284	-8.466
11711453	-6.61	57391100	-7.57	91825567	-7.883	13846642	-8.503
163020472	-6.621	13846692	-7.572	73353608	-7.888	162961319	-8.641
11742971	-6.647	71457913	-7.602	24854206	-7.892	163065794	-8.719
162883538	-6.826	14522836	-7.619	24854209	-7.903	5742590	-8.755
5321124	-6.936	163076843	-7.65	162870707	-7.918	6710643	-8.839
44446908	-7.001	36689482	-7.663	85044918	-7.922	173183	-8.846
163068081	-7.01	71452598	-7.672	73299135	-7.933	5280794	-8.922
162855320	-7.05	13846648	-7.679	24854299	-7.936	162869498	-9.142
154832043	-7.061	5319517	-7.688	44443280	-7.953	162867721	-9.247
25201046	-7.114	13846690	-7.717	15299271	-7.957	10024975	-9.259
5319773	-7.117	13846660	-7.718	44135406	-7.968	11876269	-9.382
23259347	-7.155	71307370	-7.729	128001	-7.995	154497287	-9.437
162922147	-7.205	24824767	-7.742	162970399	-7.997	162869500	-9.587
13846639	-7.206	5280343	-7.743	13846643	-8.032	14608470	-9.628
71463283	-7.207	162848074	-7.747	13846678	-8.056	14608471	-9.759
13888974	-7.213	11347693	-7.75	163106093	-8.063	5319635	-9.79
71454364	-7.221	163047545	-7.751	162969168	-8.066	162869499	-9.835
146817	-7.225	162903062	-7.771	13846640	-8.091	11353625	-10.06

All compounds were fitted into the space between TNF-α chains A and B. The predicted binding strengths ranged from -10.06 to -3.593 kcal/mol, showing a broad range of attraction to the protein. Notably, fifteen compounds exhibited more favorable predicted binding energies than the reference ligand SPD304, among which neoprotosappanin (CID: 11353625) showed the strongest binding affinity.

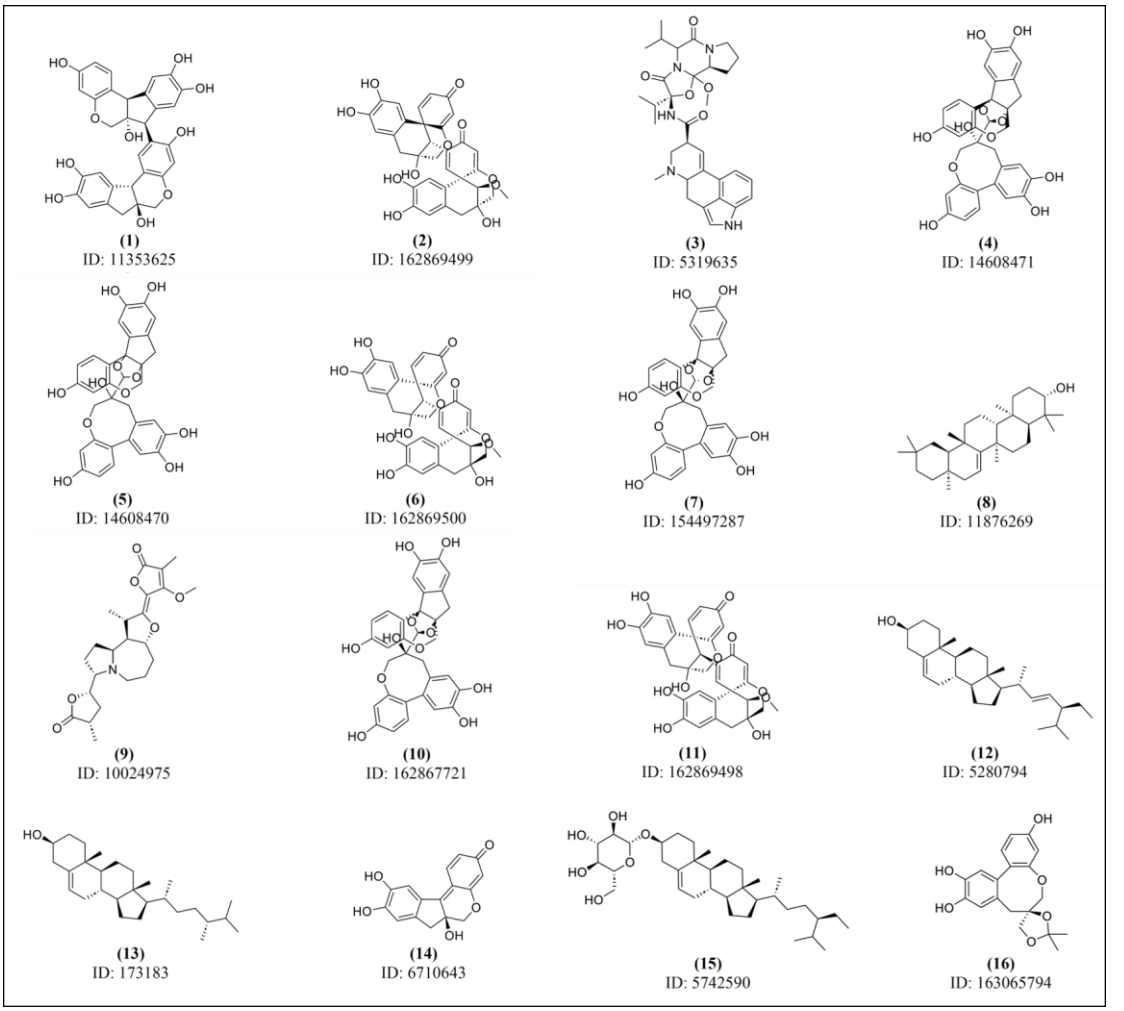


Figure 2. Chemical structures of the 16 compounds from C. sappan exhibiting the highest binding affinities toward TNF-α, ranked in descending order of predicted binding strength from (1) to (16).

As shown in **Figure 2**, fifteen compounds displayed superior in silico binding affinities compared with the reference ligand SPD304, while one compound exhibited a binding affinity comparable to that of SPD304.

3.3. Analysis of molecular docking results

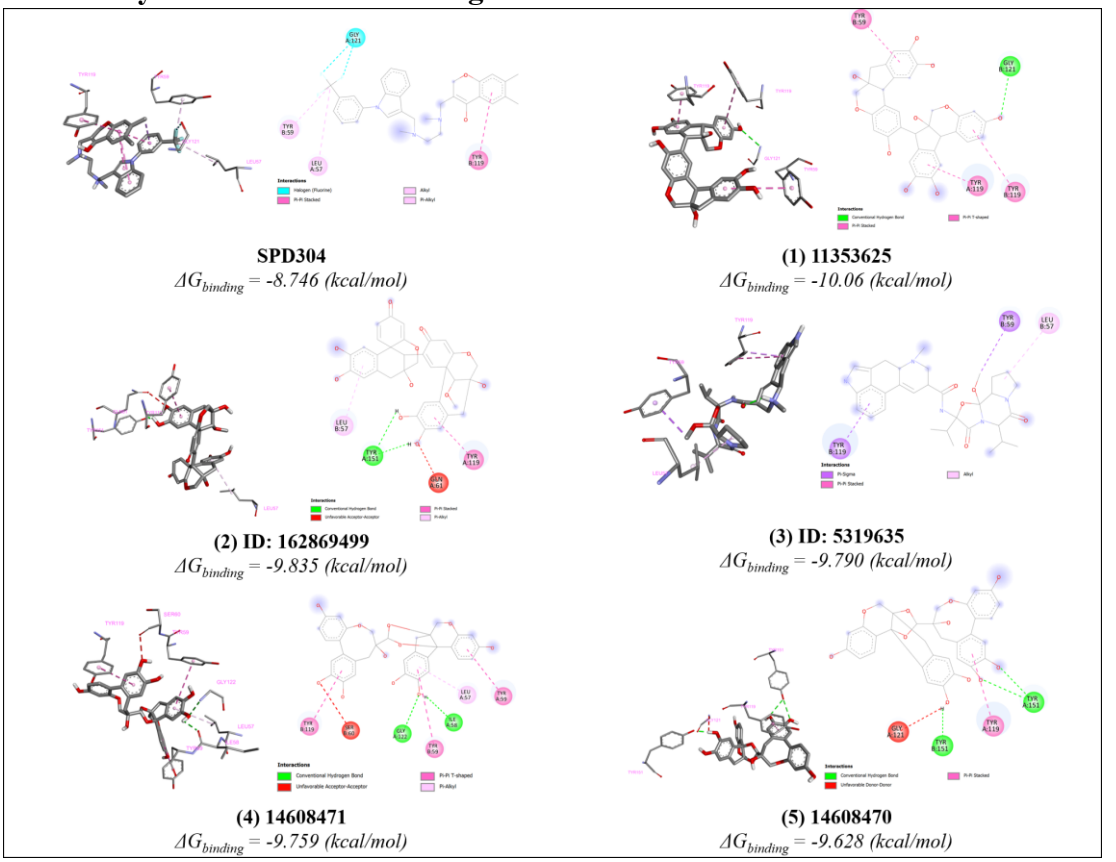


Figure 3. Three-dimensional (left) and two-dimensional (right) interaction profiles of the five top-ranking bioactive compounds from C. sappan binding to TNF-α.

The binding interfaces of the five most promising ligands, selected based on their lowest predicted binding free energies from molecular docking simulations, were analyzed using BIOVIA Discovery Studio Visualizer and are illustrated in **Figure 3**.

Analysis of the docking poses revealed that all five ligands interacted with the key residue Tyr119 of TNF-α (Figure 3). In addition, several other residues located in the binding interface were involved in ligand interactions, including LeuA57, TyrA59, GlnA61, TyrA119, GlyA121, GlyA122, TyrA151, LeuB57, TyrB59, SerB60, TyrB119, GlyB121, and TyrB151.

Among the five compounds, structure (4) (CID: 14608471) exhibited the the greatest number of interactions, engaging seven amino acid residues: TyrB119, SerB60, GlyA122, TyrB59, IleA58, LeuA57, and TyrA59. In contrast, structure (3) showed the fewest interactions, involving only three residues (TyrB59, LeuB57, and TyrB119). Structures (1), (2), and (5) displayed comparable interaction profiles, each interacting with four TNF-α residues.

The dominant interaction type observed among these ligands was hydrophobic interaction, while hydrogen bonding was also detected in most complexes, except in structure (4).

4. DISCUSSION

Sappanwood is a medicinal plant with diverse anti-inflammatory and anticancer activities. Substantial evidence supports the inhibitory effect of *C. sappan* extracts on the TNF-α signaling pathway. Several phytochemical studies have shown that this plant contains numerous phenolic and

brazilin-derived compounds that exhibit anti-inflammatory effects through modulation of cytokine-mediated signaling pathways, including TNF-α-related pathways. These findings suggest that secondary metabolites from *C. sappan* may represent potential small-molecule modulators of TNF-α activity.

In the present study, a systematic *in silico* screening strategy was employed to identify potential TNF-α-inhibitory metabolites from this medicinal plant. The workflow consisted of: (i) compiling a database of reported secondary metabolites from the PubChem database; (ii) preparing the TNF-α protein structure through homology modeling based on the crystal structure of TNF-α complexed with SPD304 (PDB ID: 2AZ5); (iii) performing molecular docking simulations; and (iv) analyzing the interaction interfaces of compounds exhibiting the lowest binding free energies to identify candidates with high potential for experimental TNF-α inhibition. This computational strategy is commonly applied in early-stage drug discovery to prioritize natural compounds for experimental validation and to explore possible binding mechanisms at the molecular level.

The homology modeling results for TNF-α chains A and B demonstrated that all generated models achieved a GA341 score of 1.0, indicating high reliability and compliance with fundamental quality criteria. Based on the z-DOPE assessment, Model II was identified as the optimal structure, exhibiting the lowest z-DOPE value (−1.36). In contrast, Model I showed the highest z-DOPE score (−1.27), suggesting comparatively lower structural quality. Nevertheless, the differences among z-DOPE values were minor, reflecting the overall stability and consistency of the generated models. Accordingly, Model II was selected as the most suitable structure for subsequent molecular docking simulations. The relatively small variation among z-DOPE scores further indicates that the structural features of the modeled TNF-α chains are stable and suitable for docking analysis.

The docking protocol was further validated by re-docking the reference ligand SPD304 into the predefined binding pocket. The re-docking results revealed a good overlap between the co-crystallized ligand and the top-ranked docked pose (Figure 1B), with a root-mean-square deviation (RMSD) of 1.09 Å, which is below the commonly accepted threshold of 2.0 Å. These findings confirm the reliability of the molecular docking model. Such RMSD values indicate that the docking protocol can accurately reproduce the experimentally observed binding mode of SPD304 and therefore provides a reliable framework for evaluating potential TNF-α inhibitors.

Molecular docking simulations yielded promising results, as numerous secondary metabolites from *C. sappan* exhibited strong binding affinities toward TNF-α. Notably, 15 compounds exhibited binding energies that were significantly more favorable than those of the reference ligand SPD304. SPD304 has been reported to induce dissociation of the TNF-α trimer by binding to aromatic residues, with six interacting residues identified as tyrosines. Among these, Tyr119 has been described as the most critical residue, as it is located at the interface between TNF-α dimers and monomers and plays a key role in ligand-induced conformational disruption (**Figure 4**).

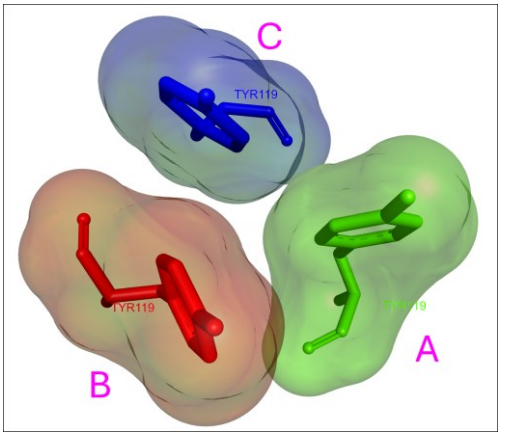


Figure 4. Illustration of hydrophobic interactions involving Tyr119 residues from chains A (green), B (red), and C (blue) in the TNF-α trimer complex.

The importance of Tyr119 has been highlighted in structural studies demonstrating that ligand binding at this residue can destabilize the trimeric assembly of TNF-α, thereby interfering with receptor recognition and downstream inflammatory signaling [9,20]. Small molecules targeting this interface pocket therefore represent an attractive strategy for modulating TNF-α activity.

Analysis of the binding interface between SPD304 and TNF-α revealed that the binding affinity is predominantly driven by hydrophobic interactions and halogen bonding (Figure 1). In addition, SPD304 was found to interact with Tyr119, a proposed key residue in the destabilization of the TNF-α trimer. Interaction with Tyr119 may induce an outward rotation of this residue, thereby facilitating conformational changes that promote dissociation of the TNF-α trimer into dimeric forms. These findings are consistent with the mechanism previously described by He et al. (2005) [9].

Hydrophobic interactions are known to play a crucial role in stabilizing ligand binding within the SPD304 pocket of TNF-α, which is largely composed of aromatic and nonpolar residues located at the trimer interface. Notably, structure (1), also known as neoprotosappanin, simultaneously interacted with Tyr119 residues from both chain A and chain B. This dual interaction is particularly significant, as it suggests a high likelihood of inducing conformational changes in the Tyr119 side chains, potentially promoting the dissociation of the TNF-α trimer, as proposed for SPD304. Consequently, neoprotosappanin appears to be the most promising candidate for inhibiting the formation of the active TNF-α complex. This interaction pattern may enhance ligand stability within the interface pocket and increase the probability of disrupting the TNF-α trimer architecture. Therefore, neoprotosappanin may represent a promising scaffold for the development of novel small-molecule TNF-α inhibitors derived from natural products.

Despite the encouraging results obtained from molecular docking, several limitations of this *in silico* study should be acknowledged. Docking simulations primarily evaluate static binding interactions and may not fully capture the dynamic behavior of protein–ligand complexes in physiological environments. Additionally, docking scores provide only approximate estimates of binding affinity and may be influenced by the scoring function used. Therefore, further studies such as molecular dynamics simulations and experimental validation, including

biochemical binding assays or cell-based inflammatory models, are required to confirm the inhibitory activity of the identified compounds against TNF-α.

5. CONCLUSION

Secondary metabolites from *C. sappan* represent promising candidates for inhibition of the TNF-α signaling pathway. Based on the molecular docking analysis performed in this study, this work provides preliminary molecular-level insights into the mechanism by which *C. sappan* extracts may interact with and potentially modulate the proinflammatory cytokine TNF-α. Among the screened compounds, neoprotosappanin was identified as the most promising structure based on its predicted binding affinity and interactions with key residues within the TNF-α binding interface, with the highest potential to inhibit TNF-α. In addition, fifteen other compounds from *C. sappan* exhibited in silico binding affinities comparable to or greater than that of the reference ligand SPD304.

These findings suggest that secondary metabolites from *C. sappan* may serve as potential starting points for further investigation of TNF-α modulators. Nevertheless, the present results are limited to virtual screening based on molecular docking simulations. Docking primarily evaluates static protein–ligand interactions and does not fully account for protein flexibility, solvent effects, or the dynamic behavior of biomolecular complexes under physiological conditions. Therefore, the current findings should be interpreted as preliminary computational predictions rather than evidence of therapeutic efficacy. Accordingly, the identified candidate compounds should be further evaluated through comprehensive in silico analyses, including pharmacokinetic and toxicity predictions as well as molecular dynamics simulations, to better elucidate their interactions with TNF-α prior to *in vitro* validation.

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