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Potential Utilization of Phenolic Acid Compounds as Anti-Inflammatory Agents through TNF-α Convertase Inhibition Mechanisms: A Network Pharmacology, Docking, and Molecular Dynamics Approach

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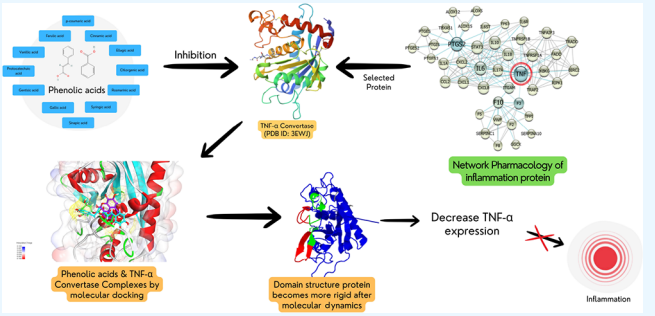
ABSTRACT: Inflammation is a dysregulated immune response characterized by an excessive release of proinflammatory mediators, such as cytokines and prostanoids, leading to tissue damage and various pathological conditions. Natural compounds, notably phenolic acid phytocompounds from plants, have recently garnered substantial interest as potential therapeutic agents to bolster well-being and combat inflammation recently. Based on previous research, the precise molecular mechanism underlying the anti-inflammatory activity of phenolic acids remains elusive. Therefore, this study aimed to predict the molecular mechanisms underpinning the anti-inflammatory properties of selected phenolic acid phytocompounds through comprehensive network pharmacology, molecular docking, and dynamic simulations. Network pharmacology analysis successfully identified TNF-α convertase as a potential target for anti-inflammatory purposes. Among tested compounds, chlorogenic acid (−6.90 kcal/mol), rosmarinic acid (−6.82 kcal/mol), and ellagic acid (−5.46 kcal/mol) exhibited the strongest binding affinity toward TNF-α convertase. Furthermore, phenolic acid compounds demonstrated molecular binding poses similar to those of the native ligand, indicating their potential as inhibitors of TNF-α convertase. This study provides valuable insights into the molecular mechanisms that drive the anti-inflammatory effects of phenolic compounds, particularly through the suppression of TNF-α production via TNF-α convertase inhibition, thus reinforcing their anti-inflammatory attributes.



INTRODUCTION

Inflammation is a fundamental aspect of the body's innate defense mechanism, acting as a protective response to a range of infectious and noninfectious causes. Understanding different types of inflammations, namely, acute, chronic, and subacute. Acute inflammation promptly occurs after an injury and usually resolves within a few days. It is characterized by a rapid onset involving immune cells, vasodilation, and increased vascular permeability in the affected area. The primary objective of acute inflammation is to eliminate harmful agents and initiate tissue repair. This process is initiated by releasing soluble mediators such as cytokines, acute phase proteins, and chemokines, which facilitate the migration of neutrophils and macrophages to the inflamed area. These cells actively participate in the inflammatory process.[1] If inflammation persists beyond 6 weeks, it transitions from a subacute state to a chronic form. Chronic inflammation involves the migration of T lymphocytes and plasma cells to the site of inflammation. Failure to resolve chronic inflammation can lead to tissue damage and the development of fibrosis.[1,2]

Subacute inflammation is an intermediate stage between acute and chronic inflammation, occurring within a transitional period of 2−6 weeks. During this phase, the inflammatory response exhibits the characteristics of both acute and chronic inflammation as the body's attempt to resolve the injury progresses toward a long-term repair process.[3]

While inflammation is a natural immune response crucial for protecting the body against harmful stimuli, excessive or chronic inflammation, known as hyperinflammation, can lead

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to various pathological conditions including autoimmune diseases, chronic inflammatory disorders, and tissue damage. Hyperinflammation is often characterized by the overproduction of proinflammatory cytokines, particularly tumor necrosis factor-α (TNF-α).[4] This cytokine is predominantly synthesized intracellularly by activated macrophages. Precursor TNF undergoes proteolysis by the TNF-converting enzyme to produce soluble TNF, which mediates inflammation and apoptosis in various diseases. Several tumor necrosis factor (TNF)-α inhibitors, including etanercept (E), infliximab (I), adalimumab (A), certolizumab pegol (C), and golimumab (G), belong to a class of biologic agents that have gained FDA approval. These agents' function by eliciting apoptosis in inflammatory cells within inflamed mucosal tissues, modulating intracellular signaling pathways and curtailing the production of proinflammatory cytokines and chemokines.[5,6] Significantly, each of these drugs can neutralize both forms of TNF-α. This is achieved either through binding to the trans-membrane variant (m-TNF-α) or by obstructing the soluble form (s-TNF-α).[7] However, the utilization of these medications is not without the potential for various side effects. These may encompass headaches, local reactions at injection sites during subcutaneous administration, and infusion-related responses with intravenous delivery. Additional potential side effects encompass rashes, mild anemia, elevated liver enzymes (transaminitis), upper respiratory tract infections, sinusitis, cough, pharyngitis, diarrhea, nausea, and abdominal pain. Thus, alternative approaches are warranted in inhibiting TNF-α, which involves suppressing the activity of TNF-α convertase (also known as ADAM17).[8] This enzyme is responsible for cleaving pro-TNF-α from the cell membrane and releasing it in a soluble form. TNF-α convertase is elevated in certain conditions such as osteoarthritis and COVID-19.[9] The development of drugs targeting TNF-α convertase could potentially reduce the levels of TNF-α and its harmful side effects.

Another cytokine implicated in inflammation is IL-6, which is involved in immune responses and inflammation, bone metabolism, and embryonic development. IL-6 plays roles in chronic inflammation and the cytokine storm of coronavirus disease 2019 (COVID-19).[10−12] Proinflammatory cytokines and other mediators can trigger the coagulation system and significantly decrease the physiologic anticoagulant pathways.[13] Recent studies have highlighted the involvement of Factor X and Factor XA, proteins associated with coagulation and deep inflammation, in various diseases.[14,15] Additionally, the activity of COX-1/2 enzymes influences inflammation alongside cytokines. Understanding the protein−protein interactions involved in the inflammatory process is crucial to identifying potential drug targets. Nonsteroidal anti-inflammatory drugs (NSAIDs), including COX-1 and COX-2 inhibitors, are commonly used to alleviate pain and inflammation. However, these drugs can have adverse effects, particularly in the gastrointestinal tract.[14]

In recent years, interest in natural products and their bioactive compounds as potential alternative therapies for inflammatory diseases has grown rapidly. Among these natural compounds, phenolic acid phytocompounds have garnered considerable attention due to their anti-inflammatory properties. These compounds, widely found in various plant sources, hold promise as potential candidates for modulating inflammation. Medicinal plants are a valuable source of active compounds, including phenolic acids, which act as secondary metabolites with potential uses for promoting good health and well-being. Several notable phenolic acids, such as rosmarinic acid, cinnamic acid, ferulic acid, chlorogenic acid, and ellagic acid, have shown potential anti-inflammatory activity.[16,17]

However, the exact mechanism by which these phenolic acid compounds inhibit target proteins involved in the inflammatory process remains unclear. Consequently, more research is needed to investigate the natural products that target these inflammatory proteins. The primary objective of this study is to explore the roles of specific phenolic acid phytocompounds in healing hyperinflammation through their potential interactions with target proteins. To achieve a comprehensive understanding of their therapeutic mechanisms, a multifaceted approach was employed, integrating network pharmacology, docking studies, and molecular dynamics simulations.[18−20]

Network pharmacology enables the exploration of potential targets and pathways affected by phenolic acid phytocompounds, which offers a comprehensive perspective on the molecular interactions within the inflammatory network. This approach reveals the intricate interplay between these compounds and the biological system. Subsequently, through the identification of potential targets, docking studies have been conducted to investigate the binding interactions between phenolic phytocompounds and target proteins. Docking studies unveil the specific molecular mechanisms by which these compounds interact with the target proteins, potentially influencing their activity and subsequent inflammatory response. Additionally, molecular dynamics simulations have been employed to assess the dynamic behavior and stability of the complexes formed between phenolic acid phytocompounds and targeted proteins over time. By examination of binding affinity, conformational changes, and overall stability, molecular dynamic simulations provide further insight into the potential therapeutic efficacy of phenolic acid phytocompounds.

By synergistically integrating these approaches, this study seeks to enhance our understanding of the therapeutic capabilities of carefully selected phenolic phytocompounds in ameliorating hyperinflammation. The insight gleaned from this study has the potential to drive the advancement of novel anti-inflammatory agents and establish solid groundwork for future exploration in the area of drug discovery for inflammatory diseases, particularly focusing on natural products. The culmination of these efforts holds promise for significant contributions to the field, leading to improved treatment options and better management of inflammatory conditions.

MATERIALS AND METHODS

All computational work was performed on a Dell Workstation Linux Ubuntu 20.04.3 LTS OS, Intel Xeon W-2223 CPU @ 3.60 GHz octacore, 16 GB RAM, and NVIDIA Quadro P2200 GPU.

Construction of Protein−Protein Interaction (PPI) Network. The PPI network of receptors involved in inflammation was created using the STRING database (http://string-db.org) and Cytoscape v3.9.1 software (https://cytoscape.org/). The STRING database was used to expand and construct human protein interactions of targeted receptors or proteins such as IL6, TNF-α, COX2 (PTGS2), and Factor XA (F10). The results were restricted to "*Homo sapiens*" with the parameter set to default (i.e., full STRING network for network type, high confidence of 0.700 for confidence score, and medium 5% for FDR stringency).

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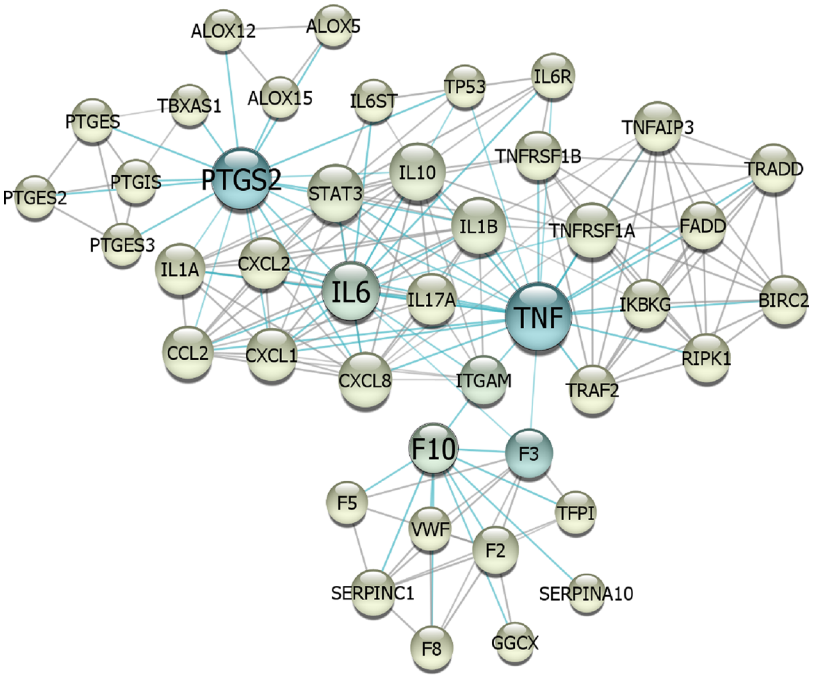

Figure 1. Topology of the protein−protein interaction network, the color intensity of nodes corresponding to their betweenness centrality values, the size of nodes corresponding to their degree values, and the blue color of edges corresponding to proteins that interact with target proteins.

Meanwhile, Cytoscape was used to analyze the PPI and create the network. The topological network was analyzed based on the degree, betweenness centrality, and closeness centrality values.

Gene Ontology (GO) and KEGG Pathway Enrichment. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (60) analysis and gene ontology (GO) enrichment analysis (consisting of GO Biological Processes, GO Cellular Components, and GO Molecular Functions) were performed to explore the functions of target proteins using the Metascape database (https://metascape.org).[21,22] Subsequently, the results of pathway enrichment analysis were visualized using Cytoscape v3.9.1 (https://cytoscape.org/).[23]

Molecular Docking. AutoDock Tools 1.5.6 was used to set the partial charges of protein and ligand, add the missing hydrogens, and determine the gridbox parameters. Initially, the structure of TNF-α convertase (PDB ID: 3EWJ) was obtained in a PDB format from the Protein Data Bank (https://www.rcsb.org/), based on recommendations from previous studies on network pharmacology. Subsequently, the structure underwent meticulous examination to identify and correct errors, such as missing parts, incomplete loops, and incorrect side-chain orientations. Water molecules were eliminated from the structure, and the protein structure file was assigned Kollman charges to represent electrostatic interactions between the protein and the ligand.

Two-dimensional structures of phenolic acid derivatives, namely, vanillic acid, protocatechuic acid, gentisic acid, gallic acid, syringic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, sinapic acid, rosmarinic acid, chlorogenic acid, and ellagic acid, were created using ChemDraw. The structures were optimized and minimized by using the MMFF94 force field in Chem3D to identify the most stable conformations.

AutoDock4 was used to perform docking simulations to predict the binding affinity of the test compounds against TNF-α convertase. These simulations also facilitated the observation of molecular interactions. Validation was carried out by redocking the native ligand onto its respective receptor. The accuracy of the docking was assessed by calculating the root-mean-square deviation (RMSD) value with successful docking defined as achieving an RMSD value of less than 2.0 Å. Additionally, the reliability of the docking poses was confirmed through visual monitoring using the BIOVIA Discovery Studio 2020.

Molecular Dynamics. Molecular dynamics (MD) simulations were carried out using the Desmond module in the Maestro Schrödinger 2020-1 software.[24,25] The MD system was generated by immersing the ligand−protein complexes in the SPC (simple point charge) water box with a distance of 10 Å between the outer surface of the protein and the walls of the water box. To simulate physiological conditions, the system charges were subsequently neutralized by adding counterions and salts consisting of sodium and chloride set to 0.15 M. The MD was carried out using the OPLS_2005 force field and under NPT conditions (temperature at 310 K and pressure at 1.63 bar) for 100 ns with recording intervals set to 1.2 ps for energy and 20 ps for trajectory. The protein domain movement was analyzed using the DynDom program (http://dyndom.cmp.uea.ac.uk/dyndom/runDyndom.jsp). Molecular mechanics/generalized born surface area (MM-GBSA) calculations were employed to determine the binding free energy of ligand-protein complexes using thermal_mmgbsa.py. This estimation plays a crucial role in evaluating the strength of interactions between potential drug candidates, as derived from the following equations.[26]

$$\Delta G_{\text{bind}} = E_{\text{complex(minimized)}} - (E_{\text{protein(unbound,minimized)}} + E_{\text{lig(unbound,minimized)}}) \quad (1)$$

The ΔG_{bind} represents the computed binding energy, $E_{\text{complex(minimized)}}$ indicates the minimized molecular mechanics with generalized born surface area (MM-GBSA) energy of the

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optimized complex, $E_{protein(unbound,minimized)}$ denotes the MM-GBSA energy of the protein that has been minimized and separated from its bound ligand, and $E_{lig(unbound,minimized)}$ refers to the MM-GBSA energy of the ligand that has been relaxed and separated from the crystal complex.

RESULTS

Protein−Protein Interaction (PPI) Network. The target proteins were extended, and protein interactions were constructed using the STRING database. The network obtained from STRING was subsequently analyzed by using Cytoscape. The analysis showed that the extended network had 43 nodes with 186 edges (Figure 1). The color intensity in Figure 1 indicates that TNF protein exhibits the highest intensity, followed closely by PTGS2, which shares a similar color intensity with TNF. While PTGS2 initially appears to have a similar node size and color intensity, our selection of TNF as the primary target is based on an in-depth network pharmacology analysis. This analysis considers various network criteria, including degree, betweenness centrality, and closeness centrality, where TNF consistently demonstrates higher values compared to PTGS2 (see Supporting Information Table S1). Specifically, TNF boasts centrality values of 0.337, 24, and 0.677 for betweenness centrality, degree centrality, and closeness centrality, respectively. PTGS2 follows with centrality values of 0.336, 15, and 0.552, respectively. In our experimental study, "degree" represents the number of direct neighbors of a node, where a greater number of direct connections signifies a more influential role within the network.[27] "Betweenness centrality" quantifies the shortest paths between all node pairs, with an emphasis on specific nodes that facilitate communication within the network.[28,29] "Closeness centrality" is defined as the reciprocal of the average shortest path distance between a node and all other nodes in the network, with higher values indicating greater centrality and faster signal transmission to other nodes in the network.[30]

In addition, TNF is on a network that is a backbone of the PPI network. On the other hand, PTGS2 is one central protein/gene of the subnetwork. Therefore, we selected TNF, the gene or protein with the highest centrality value, and the central protein/gene of the backbond network based on centrality analysis and network topology. TNF plays an important and central role in the entire network and as a bridge between subnetworks. In addition, TNF has the highest degree value, indicating that TNF is the regulatory protein of the entire PPI network (Supporting Information Table S1).

GO and KEGG Pathway Enrichment Analysis. Enrichment analysis was conducted to identify classes of selected target proteins that could be associated with diseases. Metascape was used to analyze the association between selected target proteins and gene ontology (i.e., biological process, cellular component, and molecular function) and KEGG pathway enrichment with a p-value of 0.01 as a cutoff. The results showed that IL6, TNF, F10, and PTGS2 proteins had 57 KEGG pathways, 670 GO biological processes, 11 cellular components, and 13 molecular functions (see Supporting Information Figure S1).

KEGG pathway contains information about molecular interactions, reactions, and relation networks. The top three KEGG pathways with Log 10(P) values of −6.93, −6.8, and −6.7 were related to the IL-17 signaling pathway, C-type lectin receptor signaling pathway, and TNF signaling pathway, respectively. Meanwhile, gene ontology (GO) contains information about molecular functions, biological roles, and cellular locations of the gene products. The top three biological processes of target proteins with Log 10(P) values of −8.54, −8.03, and −7.82 were positive regulations of acute inflammatory response, neuroinflammatory response, and acute inflammatory response, respectively. Moreover, cellular components indicate the location of the proteins performing a certain function. The top three cellular components of target proteins with Log 10(P) values of −5.36, −3.4, and −3.17 were the endoplasmic reticulum lumen, interleukin-6 receptor complex, and membrane raft, respectively. Finally, molecular functions indicate the activity of the protein at the molecular level. The top three molecular functions of target proteins with Log 10(P) values of −3.45, −3.33, and −2.8 were cytokine activity, cytokine receptor binding, and receptor−ligand activity, respectively. Based on the enrichment data, target proteins are related to inflammatory regulation and cytokine activity.

Molecular Docking. Based on the results of the network pharmacology analysis, TNF-α acted as the most prominent candidate with the highest degree score, as well as a crucial inflammatory mediator. To counteract the effects of TNF-α, one viable strategy is to inhibit its production. This can be accomplished by targeting TNF-α convertase, an enzyme responsible for cleaving the precursor form of TNF-α into its active form, which performs various functions in the body. Commonly termed TACE (tumor necrosis factor-α converting enzyme), TNF-α convertase facilitates the release of active TNF-α from the cell membrane. By inhibition of the activity of TNF-α convertase, a promising anti-inflammatory approach can be pursued. Inhibiting the TNF-α convertase activity has potential benefits. Impeding the release of active TNF-α allows the levels of circulating TNF-α in the body to reduce. In turn, inflammation can be diminished, and symptoms associated with inflammatory diseases can be alleviated. It is also important to note that targeting TNF-α convertase as an additional therapeutic target has the potential advantage of reducing the side effects typically associated with direct TNF-α inhibition. By selective inhibition of TNF-α conversion, it becomes feasible to regulate inflammation more precisely and minimize the risk of undesired systemic effects.

However, inhibiting TNF-α convertase as an additional target in the treatment of inflammation has the potential to reduce the side effects associated with direct TNF-α inhibition. By selectively inhibiting TNF-α conversion, we might be able to control inflammation more specifically. In this study, TNF-α convertase became a target that acted as an anti-inflammatory agent using an in silico technique with molecular docking and molecular dynamics to evaluate the binding affinity and mechanism of interactions of phenolic acid compounds against TNF-α convertase. The active site coordinates of TNF-α were used (X = −6.463, Y = −12.801, and Z = 2.255). In addition, a grid box with dimensions of 40 × 40 × 40 was employed.

Our docking protocol underwent thorough internal validation by redocking the native ligand, (1*S*,3*R*,6*S*) 4-oxo-6-{4-[(2-phenylquinolin-4-yl)methoxy]phenyl}-5-azaspiro[2.4]heptane-1-carboxylic acid, back into its original position. This meticulous validation process yielded a commendable RMSD value of 0.9010 Å, strongly indicating the robustness and suitability of our docking protocol for predicting the binding energy of phenolic compounds against TNF-α convertase, as illustrated in Figure 2.

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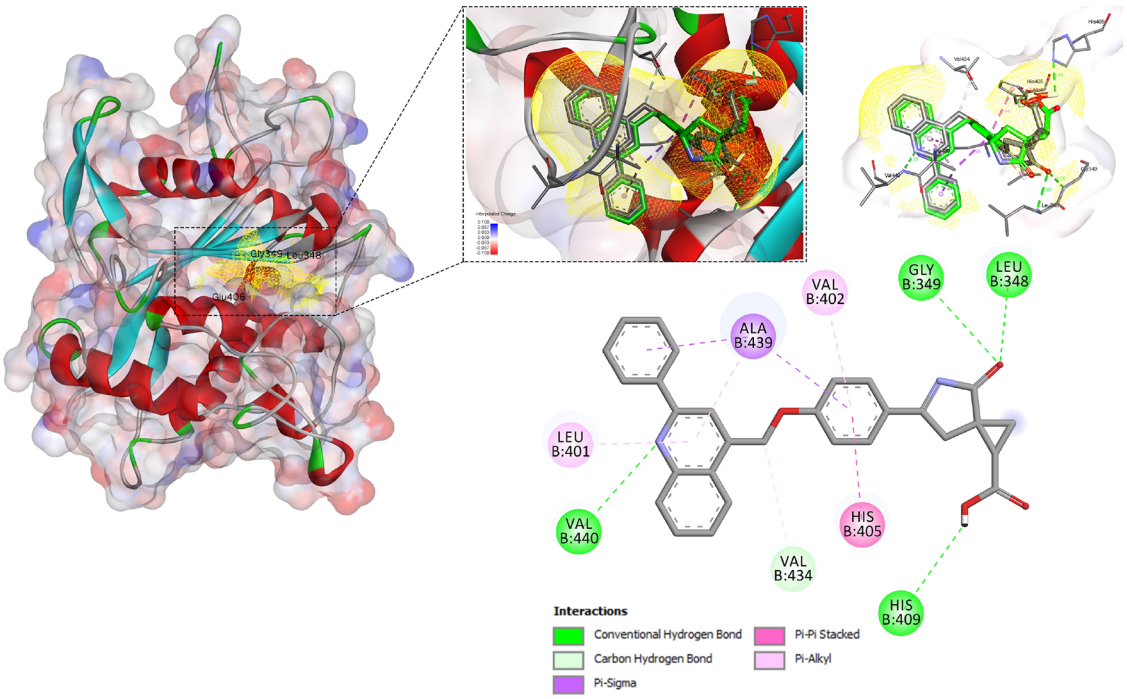

Figure 2. Redocked native ligand ((1*S*,3*R*,6*S*) 4-oxo-6-{4-[(2-phenylqui-nolin-4-yl)methoxy] phenyl}-5-azaspiro [2.4]heptane-1-carboxylic acid) is shown in gray and compared to its original position (green), with an RMSD value of 0.9010 Å. A plot of two-dimensional molecular interactions showed different types of interactions between the native ligand and the amino acid residues present in the receptor binding site.

Based on the results of molecular docking (Table 1), several phenolic acid compounds with negative binding energy values, which correspond to favorable interactions with the receptor, were identified. Among the tested compounds, chlorogenic acid (−6.90 kcal/mol), rosmarinic acid (−6.82 kcal/mol), and ellagic acid (−5.46 kcal/mol) exhibited the highest binding affinities based on the lowest binding energy values among the phenolic acid compounds tested. This suggested their potential as inhibitors of TNF-α convertase receptors (Table 1). The binding energy reflects the strength of the interaction between the ligand and its target receptor, with a negative value indicating a stronger interaction. Compounds with stronger binding energies hold promise as potential candidates for further studies in the development of TNF-α convertase inhibitors, as they may exhibit similar activities to the native ligand of the receptor.

Table 1. Binding Energy of Phenolic Acid Derivatives against TNF-α Convertase

no	ligands	binding energy (kcal/mol)
1	(1*S*,3*R*,6*S*) 4-oxo-6-{4-[(2-phenylquinolin-4-yl) methoxy]phenyl}-5-azaspiro[2.4]heptane-1-carboxylic acid	−14.97
2	vanillic acid	−4.27
3	protocatechuic acid	−4.41
4	gentisic acid	−4.01
5	gallic acid	−3.88
6	syringic acid	−3.97
7	cinnamic acid	−4.99
8	*p*-coumaric acid	−5.20
9	ferulic acid	−5.09
10	sinapic acid	−4.84
11	rosmarinic acid	−6.82
12	chlorogenic acid	−6.90
13	ellagic acid	−5.46

Figure 2 illustrates the postdocking visualization, revealing the molecular interactions of the top three phenolic compounds with the best binding affinities against the TNF-α convertase receptor. Chlorogenic acid (Figure 3A) interacted with several amino acids on the receptor's binding site, indicating its potential as a potent ligand. The quinic acid moiety of chlorogenic acid formed hydrogen bonding interactions with Glu406, Gly346, and Ala439 in TNF-α convertase. Hydrogen bonding was also observed between the hydroxyl group on the aromatic ring of chlorogenic acid and Ile438. Additionally, hydrophobic interactions, such as π-sigma interactions between the imidazole group of His405 and the σ bond as well as interactions between the aromatic group of chlorogenic acid and the alkyl group of Leu401, were observed. Similarly, rosmarinic acid (Figure 3B) interacted with several amino acids in the active site of the TNF-α convertase receptor. The hydroxyl group attached to the carboxylic acid of rosmarinic acid interacted with His405, while the hydroxyl group attached to the aromatic ring interacted with Tyr433, Pro437, and Asn447. On the other hand, ellagic acid (Figure 3C) exhibited limited interactions. It formed hydrogen bonds with Glu406 and showed hydrophobic interactions, such as π single pairs with Pro437, $\pi-\pi$ T-shape with His405, and π-alkyl with Ala439.

The postdocking visualization of these phenolic compounds highlights multiple molecular interactions. Hydrogen bonding interactions are crucial for forming stable complexes with the receptor, enhancing the effectiveness of the compounds as inhibitors of TNF-α convertase. Chlorogenic acid formed hydrogen bonds with Glu406, Gly346, Ala439, and Ile438, while rosmarinic acid formed hydrogen bonds with His405, Tyr433, Pro437, and Asn447. However, ellagic acid formed

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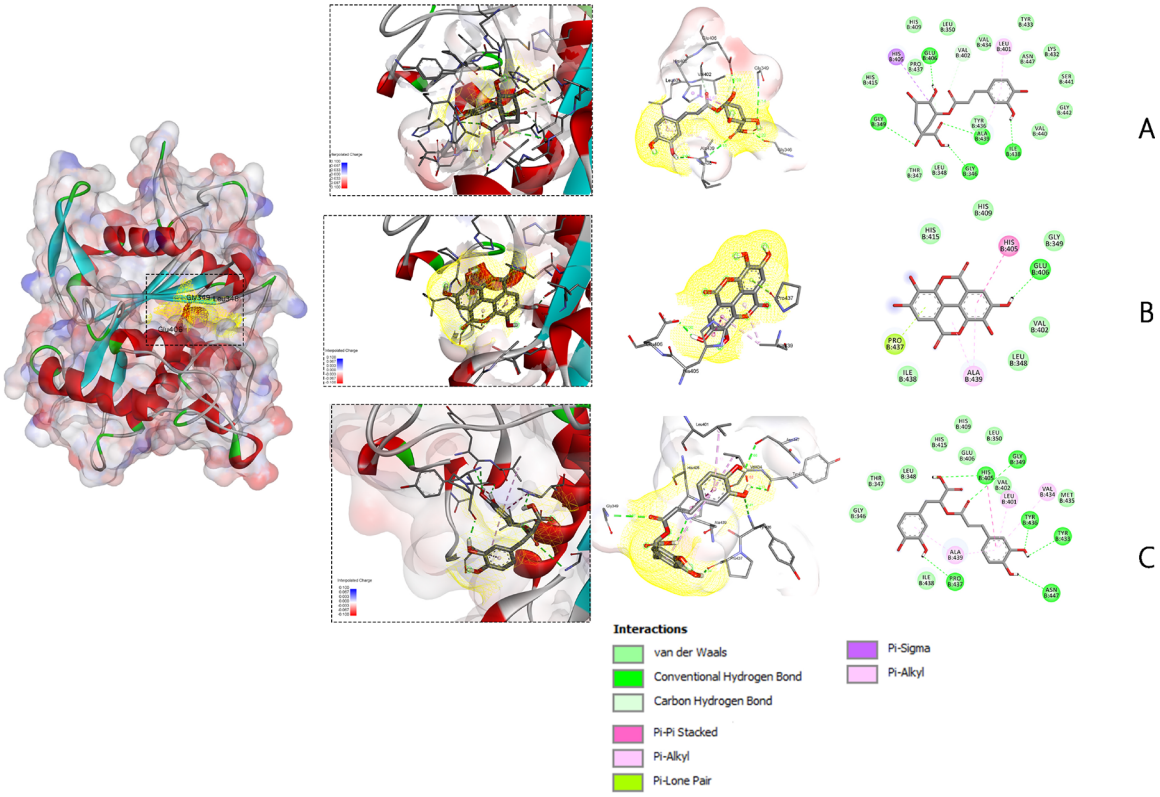

Figure 3. Molecular interactions of the top three phenolic acid compounds (A = chlorogenic acid, B = ellagic acid, and C = rosmarinic acid) with the lowest binding affinity against TNF-α convertase.

fewer hydrogen bonds, which could be attributed to its lower affinity compared with chlorogenic and rosmarinic acids. In addition to hydrogen bonding, hydrophobic interactions played an important role in forming stable protein−ligand complexes and contributed to the binding affinity of ligands. These interactions occurred between nonpolar amino acids and nonpolar functional groups on the ligand. For example, the π-sigma interaction between the cyclic group of His405 and the σ bond as well as the π interaction between the aromatic group and the alkyl bond of Leu401, enhanced the binding affinity of chlorogenic acid. Similarly, in rosmarinic acid, the π interactions between the aromatic system and the alkyl groups of Leu401, Val434, and Ala439 contributed to its binding affinity.

Overall, these observations provide valuable insight into the potential use of phenolic compounds as anti-inflammatory agents by inhibiting TNF-α convertase activity, as suggested by the docking studies.

Molecular Dynamics. Molecular dynamics simulations were carried out to investigate the stability of the ligand−receptor complexes and the effects of the ligand on protein dynamics under physiological conditions. The simulations were performed for 100 ns at a temperature of 310 K. The RMSD (root-mean-square deviation) values were analyzed to assess the conformational stability of the protein in complexes with each ligand, as shown in Figure 4. A lower RMSD value, typically below 3 Å, suggested a relatively stable protein conformation with minor fluctuations from the initial conformation.

In the unbound of ligand condition of the TNF-α convertase structure (C-α) exhibits a strikingly consistent and notably low root-mean-square deviation (RMSD) value (<3 Å) throughout

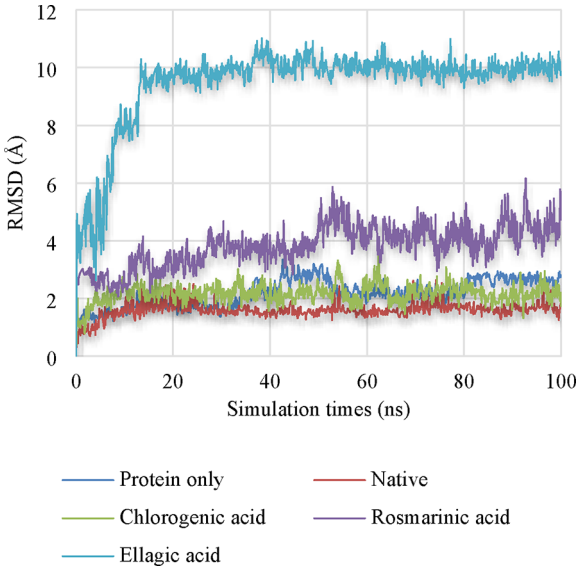

Figure 4. Root-mean-square deviation (RMSD) plot after 100 ns of simulation times. The plot illustrates the overall structural deviation of the system from its initial conformation.

the duration of simulations (Figure 4). This observed minimal RMSD underscores the structural stability inherent in its conformation. The fundamental objective behind probing the ligand-free state of TNF-α convertase (C-α) is to discern the potential impact of ligand presence, or lack thereof, on the overall stability of the TNF-α convertase structure. However, when a native ligand bound to the TNF-α convertase, the structure of the protein−ligand complexes appeared to be

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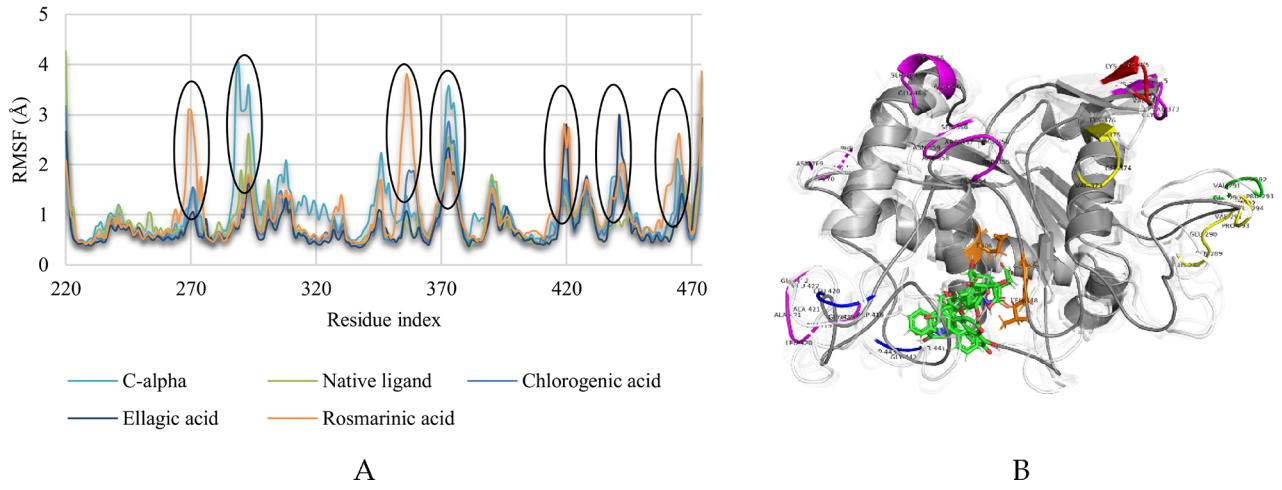

Figure 5. (A) Root-mean-square fluctuation (RMSF) plot after 100 ns simulation times and (B) region fluctuations of amino acid domains.

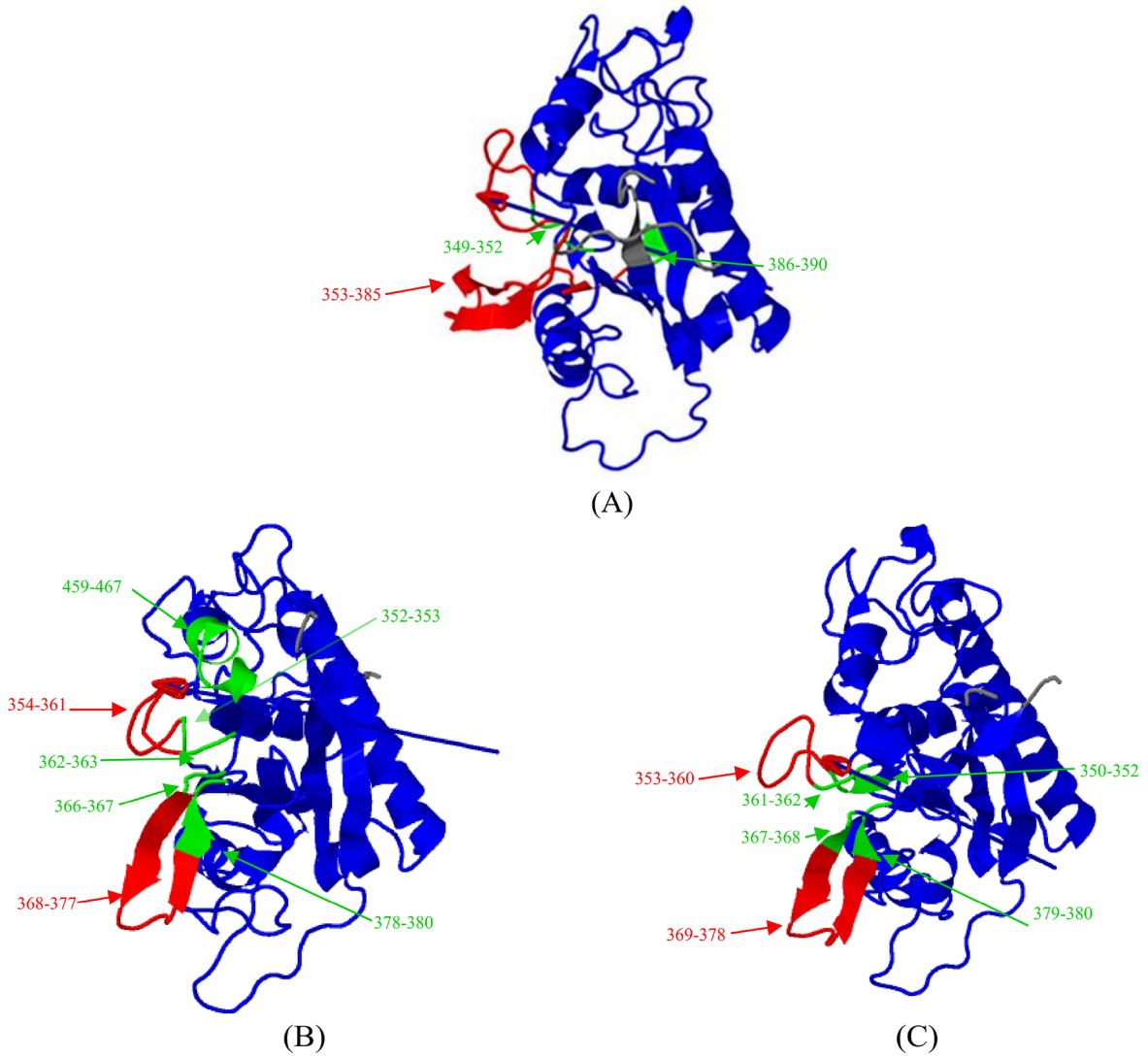

Figure 6. DynDom domain movement analysis of TNF-α convertase in the absence of (A) ligand and in complex with (B) ellagic acid and (c) rosmarinic acid after 100 ns simulation times. The fixed domains are depicted in blue, the moving domains in red, and the hinge domains in green. The locations of the moving domains are labeled for each complex, and the arrow indicates the hinge axis. This figure was generated from the DynDom output.

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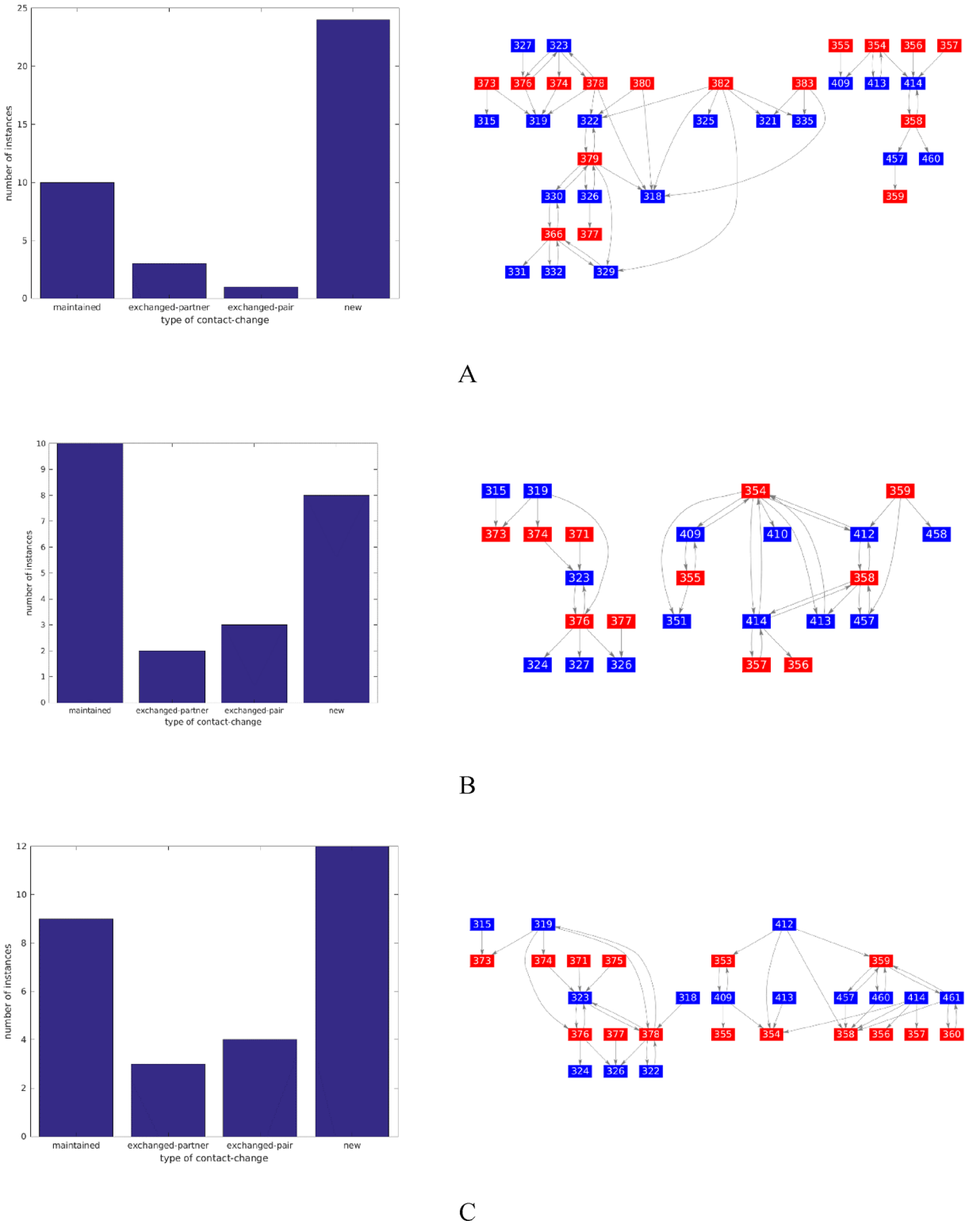

Figure 7. Dynamic contact analysis of TNF-α convertase in the absence of (A) ligand and complexes with (B) ellagic acid and (C) rosmarinic acid. The left graph shows the types of domain movement and their number of instances, while the right graph presents the dynamic contact graph (DCG) between residues in the fixed domain (blue) and moving domain (red) of TNF-α convertase.[31]

more rigid, as indicated by lower RMSD values than those of the protein alone. This suggested that ligand binding influences the conformational stability of the protein structure. A similar observation was made with chlorogenic acid, which is also bound to the TNF-α convertase receptor and showed a lower RMSD value, particularly at 40−50 ns throughout the simulations. Minor fluctuations were observed at 53−55 and 59−65 ns, indicating localized movements within specific areas of the protein structure. These findings suggested that chlorogenic acid exhibits binding characteristics similar to those of the native ligand, albeit potentially less optimal.

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In contrast, rosmarinic acid and ellagic acid, which are also bound to the TNF-α convertase receptor, exhibited significantly higher RMSD values of >3 Å throughout the 100 ns simulations. This suggested a substantial change in the protein conformation upon binding of these phenolic acid compounds, which suggested a greater impact on the protein structure.

Figure 5A presents the root-mean-square fluctuation (RMSF) plot, which provides information about the displacement of different regions within the protein from its initial state as a reference over time. Higher RMSF values suggested greater fluctuations of the corresponding residues.

By analyzing the RMSF plot, regions that underwent significant fluctuations throughout the simulations were identified. This study focuses on the RMSF values of amino acids at the catalytic sites (Leu348, Gly349, and Glu406), which are crucial for protein activity. The results suggested that chlorogenic, rosmarinic, and ellagic acids maintained their interactions with TNF-α convertase catalytic residues throughout the simulations. In particular, chlorogenic acid exhibited interactions with Leu348 (0.508 Å), Gly349 (0.496 Å), and Glu406 (0.519 Å), which were comparable to the interactions of the native ligand with Leu348 (0.506 Å), Gly349 (0.625 Å), and Glu406 (0.481 Å). Similarly, rosmarinic acid exhibited low RMSF values of 0.604 Å for Leu348, 0.592 Å for Gly349, and 0.671 Å for Glu406 at the catalytic site. Ellagic acid exhibited an RMSF value of 0.714 Å for Leu348, 0.557 Å for Gly349, and 0.528 Å for Glu406. These low RMSF values suggested that these compounds maintained stable interactions with the catalytic sites of TNF-α convertase, making them less fluctuated.

On the other hand, in the absence of ligand, the beta-strand region underwent major conformational changes, with residues Val373-Lys376 and Pro288-Gly294 exhibiting RMSF values of more than 3 Å, indicating significant fluctuations over 100 ns simulation times. However, when these residues bound to certain ligands, they exhibited lower RMSF values. This suggested that ligand binding causes a higher rigidity in several regions of the protein. It is also interesting to note that the molecular dynamics simulations showed that rosmarinic acid and ellagic acid induced an increase in the RMSF values of several amino acid residues in the TNF-α convertase. These residues included Asn269-Lys373, Gly354-Ser360, Val373-Lys376, Asp418-Glu422, and Glu463-Ala466. In particular, these residues were located far from the active sites of the receptor, specifically in the loop region of the protein. Specifically, both rosmarinic acid and ellagic acid increased the RMSF values in the regions of Asp418-Glu422 and Ser441-Asp443 (Figure 5B).

To analyze the protein domain movement, dynamic domain analysis using DynDom was employed to identify the rotation and translation of the hinge domains relative to the fixed and moving domains as well as calculate the root-mean-square deviation (RMSD) of the domain movement. The results provide valuable insight into the conformational changes and interactions between protein domains during enzymatic activity. It is interesting to note that no rigid body movement was observed when the native ligand and chlorogenic acid were bound to the protein. This suggested that chlorogenic acid can inhibit protein movement in a way similar to that of the native ligand.

Additionally, it was found that when TNF-α convertase was unbound to any ligand, a specific segment (353−385) of the protein exhibited rigid body movement. This movement was facilitated by two hinges (349−352 and 386−390). Within this moving segment, four residues (Val373, Gly374, Lys375, and Lys376) exhibited higher RMSF values compared to when they were bound to the ligand, as shown in Figure 6. When ellagic acid bound to the TNF-α convertase, it induced two rigid body movements (354−361 and 368−377) and involved five hinges (352−353, 362−363, 366−367, 378−380, and 459−467), as illustrated in Figure 6B and Supporting Information Table S2.

The RMSF plot in Figure 5 demonstrated that amino acid residues with high RMSF values when bound to ellagic acid included Lys367, Ala368, Tyr369, Tyr370, Ser371, Pro372, Val373, Gly374, Lys375, Lys376, and Asn377. Similarly, rosmarinic acid also caused two rigid body movements in residues 353−360 and 369−378 and involved four hinges (350−352, 361−362, 367−368, and 379−380), as shown in Figure 6C and Supporting Information Table S2. The RMSF plot in Figure 5 indicated that amino acid residues with the highest RMSF values during body movements when bound to rosmarinic acid included Gly353, Gly354, Ser355, Pro356, Arg357, Ala358, Asn359, and Ser360.

The TNF-α convertase structure exhibited four types of domain movements, namely, “maintained”, “exchanged-partner”, “exchanged-pair”, and “new”, as observed in the graph of dynamic contact analysis resulting from DynDom (Figure 7). When TNF-α convertase was unbound to a ligand (Figure 7A), 24 “new” (open-closed) domain movements were observed, involving residues such as Pro305 → His409, Pro305 → Lys460, Gly354 → Glu414, Gly354 → His409, Pro356 → Glu414, Arg357 → Glu414, Val373 → Lys315, Val373 → Glu319, Ile378 → Glu319, Ile378 → Ser322, Ile378 → Leu318, Tyr379 → Leu318, Tyr379 → Ala329, Leu380 → Leu318, Leu380 → Ser322, Ser382 → Ala335, Ser382 → Phe321, Ser382 → Ala329, Ser382 → Ile325, Ser382 → Leu318, Ser382 → Ser322, Gly383 → Leu318, Gly383 → Ala335, and Gly383 → Phe321. Moreover, “maintained” (anchored) domain movements were observed between residues Phe323 → Lys376, Phe323 → Ile378, Gly354 → Ala413, Ala358 → Glu414, Tyr379 → Ser322, Tyr379 → Ser330, Tyr379 → Ala326, Pro366 → Ser330, Pro366 → Val332, and Pro366 → Ala329. Two “exchanged-partner” (sliding-twist) domain movements occurred between residues Glu327 → Lys376 → Glu319 and Glu327 → Gly374 → Val314 and one “exchanged-pair” (seesaw) domain movement occurred between residues Gly354 → His409 and Ala413 → Gly354.

In contrast, the binding of ellagic acid to TNF-α convertase (Figure 7B) resulted in eight “new” (open-closed) domain movements involving residues Lys315 → Val373, Gly354 → Asn410, Ser355 → Ala351, Asn359 → Gly412, Asn359 → Ile458, Asn359 → Ser457, Ser371 → Phe323, and Asn377 → Ala326. Additionally, 10 “maintained” (anchored) domain movements were observed between residues Gly354 → His409, Gly354 → Glu414, Gly354 → Ala413, Gly354 → Gly412, Ser355 → His409, Arg357 → Glu414, Ala358 → Glu414, Ala358 → Ser457, Ala358 → Gly412, and Lys376 → Phe323. Two “exchanged-partner” (sliding-twist) domain movements occurred between residues Glu319 → Gly374 → Phe323 and Glu319 → Lys376 → Glu327, while three “exchanged-pair” (seesaw) domain movements occurred between residues Glu414 → Pro356 and Arg357 → Glu414, Ser457 → Ala358 and Asn359 → Ser457, and Ala413 → Gly354 and Ala358 → Ala413.

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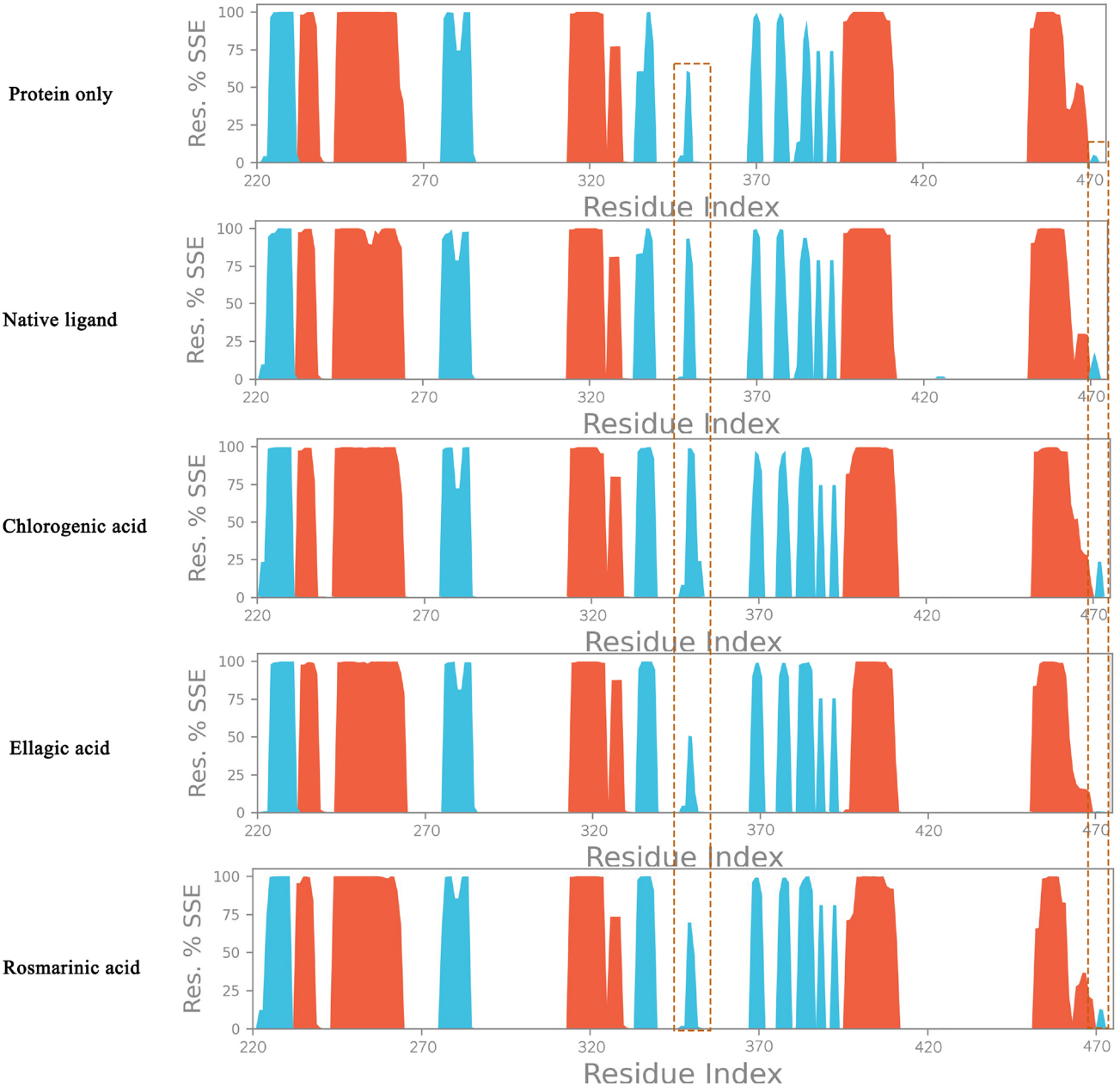

Figure 8. Protein secondary structure elements (SSE) (α-helices (orange) and β-strands (blue)) were monitored after 100 ns simulation times.

In the case of the complexes of rosmarinic acid and TNF-α convertase (Figure 7C), 12 "new" (open-closed) domain movements were observed involving residues Lys315 → Val373, Leu318 → Ile378, Glu319 → Val373, Glu319 → Lys376, Ser371 → Phe323, Asn377 → Ala326, Gly412 → Gly354, Ala413 → Gly354, Glu414 → Gly354, Glu414 → Pro356, Glu414 → Arg357, and Glu414 → Ala358. Additionally, nine "maintained" (anchored) domain movements were observed between residues Gly353 → His409, Asn359 → Ser457, Asn359 → Lys460, Asn359 → Thr461, Ser360 → Thr461, Lys376 → Phe323, Ile378 → Ser322, Ile378 → Phe323, and Ile378 → Glu319. Three "exchanged-partner" (sliding-twist) domain movements occurred between residues Leu318 → Ile378 → Ala326, Glu319 → Gly374 → Phe323, and Glu319 → Lys376 → Asp324, while four "exchanged-pair" (seesaw) domain movements occurred between residues Phe323 → Lys376 and Ile378 → Phe323, Asn359 → Ser457 and Lys460 → Asn359, Ile378 → Ala326 and Ser322 → Ile378, and Thr461 → Ala358 and Ser360 → Thr461.

In addition to DynDom, protein-domain flexibility analysis can also be carried out by calculating the secondary structure elements (SSE) of protein. Peptide bonds, which link amino acids, are not rigid, thus resulting in the formation of three-dimensional structures. This folding process can give rise to characteristic structures through hydrogen bonding between nonconsecutive amino acids. These structures are referred to as secondary structures, with the α-helix and β-sheet being the most common ones. The α-helix is formed when a section of the protein backbone adopts a helical shape, while the β-sheet occurs when distant segments of the backbone are connected in a parallel manner.[32]

The analysis revealed that TNF-α convertase maintained its secondary structures throughout the simulations. However, changes were observed in the β-strand structure, specifically in

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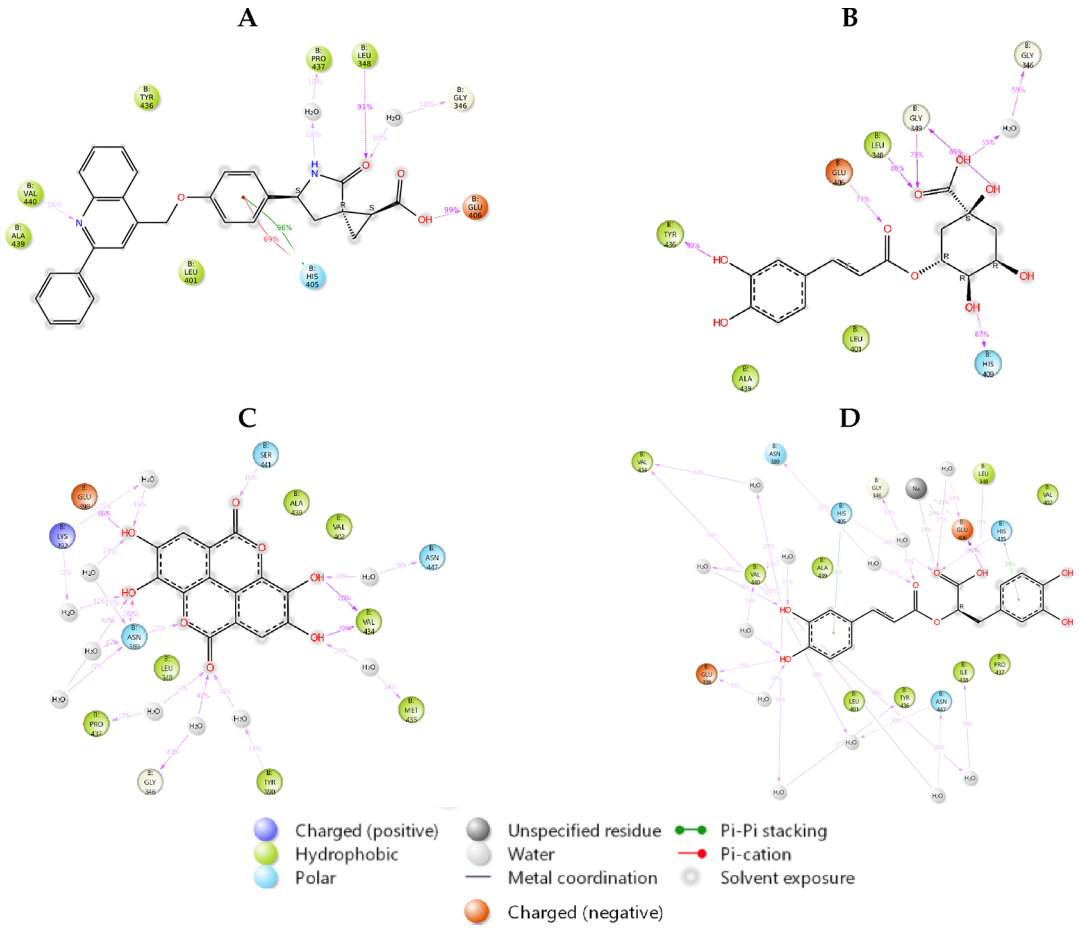

Figure 9. Molecular interaction and bonding type of ligands after 100 ns MD simulation (A = native ligand-TNF-α convertase, B = chlorogenic acid-TNF-α convertase, C = ellagic acid-TNF-α convertase, and D = rosmarinic acid- TNF-α convertase).

two regions, namely, the 350−360 and 470−472 residues. These changes were evident from the decrease in the β-strand structure to below 75% in residues 350−360, along with a similar decrease observed in residues 470−472, which dropped to less than 25% (Figure 8). Rosmarinic acid caused the highest decrease in the β-strand structure in both regions, where the percentage of SSE in residues 350−360 dropped below 50% and was nearly nonexistent in residues 470−472. Such a reduction in the number of secondary structures may have implications for the inhibitory activity of the protein.[33]

The decrease in the β-strand secondary structure of a protein may significantly impact its stability and function. The β-strands are protein segments formed by polypeptide chains, creating parallel or antiparallel sheets connected by hydrogen bonds. Changes in the β-strand structure can occur during molecular dynamics, due to either fluctuations in temperature and pressure or interactions with the ligand.

One of the primary consequences of the decrease in the β-strand secondary structure is the loss of protein stability. A disrupted secondary structure can disrupt the energetic balance of the protein and lead to an irregular folding. This can result in a loss of structural stability or even folding into nonfunctional conformations. Furthermore, these changes in the secondary structure can have implications for binding models. The decrease in the β-strand secondary structure can affect the intra- or intermolecular bonds within the protein. Disruption of the hydrogen bonds that maintain the β-strand can alter the interactions of the protein with the ligand or other target molecules. Additionally, changes in secondary structure can impact the active sites of the protein, modifying the availability and affinity for binding substrates or inhibitors.[33]

It is important to investigate the molecular interactions occurring between ligands and proteins during molecular dynamics simulations, because these interactions provide insight into the interaction patterns of the protein and its relationships with other molecules. Figure 9 visualizes the intermolecular contact analysis, highlighting the molecular interactions between various ligands and the TNF-α convertase. Among the ligands studied, chlorogenic acid primarily interacted with Gly349 through hydrogen bonding. The carboxylic group of chlorogenic acid served as a hydrogen bond acceptor, while the OH group attached to the cyclohexyl phenolic acid acted as a donor. Additionally, Leu348 acted as a hydrogen bond donor and interacted with the carboxylic group of the chlorogenic acid. The alkyl groups in Leu348 contributed to the hydrophobic bonding with the ligand. Glu406 also formed interactions with the carbonyl group of chlorogenic acid through hydrogen bonding (Figure 9B). However, these interactions were relatively weaker compared to the native ligand, which exhibited stronger interactions (>90%) with Glu406 and Leu348. Furthermore, His405 supported the binding mode of the native ligand through $\pi-\pi$-stacking and π-cation interactions (Figure 9A).

On the other hand, ellagic acid bound to the TNF-α convertase primarily through hydrophobic interactions, particularly with Val434 (>50%) in each phenolic group. It also

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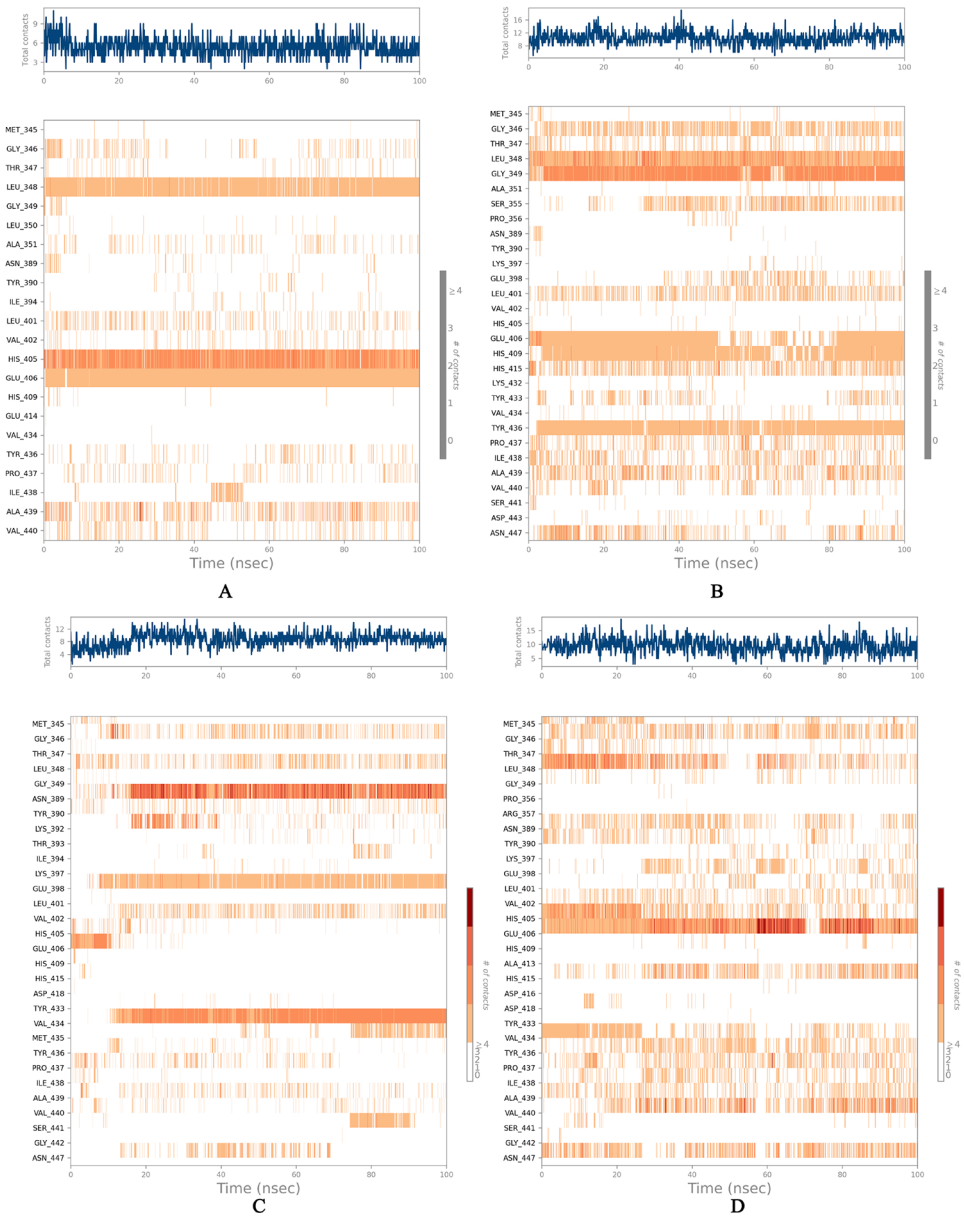

Figure 10. Timeline representation of the interactions and contacts (i.e., H-bonds, hydrophobic, ionic, and water bridges) (A = native ligand, B = chlorogenic acid, C = ellagic acid, and D = rosmarinic acid). The top panel shows the total number of specific contacts the protein made with the ligand throughout the trajectory. The bottom panel shows which residues interacted with the ligand in each trajectory frame. Some residues made more than one specific contact with the ligand, which is represented by a darker shade of orange, according to the scale to the right of the plot.

formed a negative charge interaction with Glu398 (86%). A weaker hydrogen interaction was observed in Ser441 (15%) (Figure 9C). Rosmarinic acid interacted with the TNF-α convertase through hydrophobic interactions with His405 (85%) and a negative charge interaction with Glu406 (92%) (Figure 9D).

The overall protein−ligand interactions are illustrated in Figure 10, which include hydrogen bonds, hydrophobic interactions, ionic interactions, and water bridges. The analysis focuses on the catalytic site of TNF-α convertase, with a particular emphasis on three crucial amino acid residues, namely Leu348, Gly349, and Glu406.[34] The native ligand exhibited an average of six interactions with TNF-α convertase. The analysis of the catalytic site interactions revealed that the ligand predominantly interacted with Leu348 and Glu406, while the interaction with Gly349 was observed only during the first 5 ns simulation times (Figure 10A). Meanwhile, chlorogenic acid showed a higher average of total interactions, namely, 12 interactions with TNF-α convertase. The interaction with the catalytic site suggested stable interactions with all three amino acids, namely, Leu348, Gly349, and Glu406. However, the interaction with Glu406 decreased between 50 and 80 ns before returning to normal levels after 83 ns until the end of the simulations (Figure 10B). Ellagic acid exhibited results similar to those of chlorogenic acid, with an average of a total of 10 interactions with TNF-α convertase. However, optimal binding to Leu348 was observed only transiently, and suboptimal interactions were observed at the other catalytic sites, namely, Gly349 and Glu406, during the first 10 ns (Figure 10C). Moreover, rosmarinic acid exhibited an optimal interaction with Glu406, as indicated by a prominent interaction spot. However, suboptimal interactions

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were observed in Leu348 and Gly349 throughout the simulations (Figure 10D).

MM-GBSA calculations were carried out to calculate the binding energy between the ligand and TNF-α convertase from the trajectory of molecular dynamics. MM-GBSA calculations are highly valuable in assessing the energetics and binding affinity of protein−ligand interactions. These calculations estimate the binding free energy of a protein−ligand complex by employing molecular mechanics force fields to represent the protein−ligand interactions, while a continuum solvent model approximates the solvation effect. The use of MM-GBSA calculations provides quantitative estimations of the binding free energy, offering insight into the energetics involved in ligand binding.

Table 2 shows that native ligands had the highest binding affinity, followed by chlorogenic acid, rosmarinic acid, and ellagic acid. This suggested that the native ligand has the strongest binding interaction with the TNF-α receptor. Meanwhile, chlorogenic acid had the strongest interaction with the protein among phenolic compounds in this study.

Table 2. Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) Calculations of Ligands after 100 ns of Simulation Times against TNF-α Convertase

no.	ligand	MMGBSA (kcal/mol)
1	native ligand	−74.8982
2	chlorogenic acid	−55.9266
3	rosmarinic acid	−34.4540
4	ellagic acid	−45.1447

DISCUSSION

Inflammation is a complex biological response involving the interplay of various proteins and molecules including coagulation factor X, interleukin-6 (IL-6), prostaglandin G/H synthase-2 (COX-2), and tumor necrosis factor (TNF). Network pharmacology studies offer valuable insight into the interactions among these components and their roles in inflammation. TNF, produced by immune cells such as macrophages and lymphocytes, plays an important role in regulating the inflammatory response. It has been consistently demonstrated that TNF contributes to immune cell activation, recruitment of inflammatory cells, and induction of proinflammatory mediators. Network pharmacology studies highlight the crucial role of TNF in inflammation, underscoring its potential as a therapeutic target. Notably, therapeutic interventions targeting TNF, such as infliximab and etanercept, have shown promising outcomes under various inflammatory conditions. These inhibitors specifically bind to TNF, preventing its interaction with cell surface receptors and reducing downstream inflammatory signaling.[18,35,36]

During immune cell activation, TNF-α convertase is responsible for cleaving the membrane-bound form of TNF-α, known as transmembrane TNF-α (tmTNF-α), to release the soluble form of TNF-α (sTNF-α) into the extracellular environment. The soluble form of TNF-α is biologically active and can bind to TNF receptors and initiate inflammatory signaling pathways. Inhibiting TNF-α convertase prevents the cleavage of tmTNF-α, which results in the reduction in sTNF-α release. Consequently, there is a decrease in the level of TNF receptor activation and downstream inflammatory signaling. This inhibition helps to modulate the inflammatory response and potentially alleviates symptoms associated with various inflammatory conditions.[18]

There are several strategies to inhibit TNF-α convertase. One approach involves the use of specific inhibitors that directly target enzyme activity. These inhibitors bind to TNF-α convertase, blocking its cleavage activity and preventing the release of sTNF-α. Another approach focuses on regulating the expression or activity of TNF-α convertase through various signaling pathways or molecules involved in its regulation.[35] The inhibition of TNF-α convertase and subsequent reduction in TNF-α production have been explored as a therapeutic strategy in the treatment of inflammatory diseases. Drugs targeting TNF-α convertase have been investigated for their potential to alleviate inflammation and improve patient outcomes. By preventing the cleavage of the TNF-α transmembrane, the inhibitors reduce the release of soluble TNF-α, thereby modulating the inflammatory response. They have shown promise in conditions such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease.[37]

Phenolic acid compounds have garnered significant attention for their potential anti-inflammatory properties. This study aims to investigate the anti-inflammatory activity of several well-known phenolic compounds, including vanillic acid, protocatechuic acid, gentisic acid, gallic acid, syringic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, sinapic acid, rosmarinic acid, chlorogenic acid, and ellagic acid.[38,39] In particular, the objective of this study was to assess their potential as anti-inflammatory agents by targeting TNF-α convertase, guided by a network pharmacology analysis.

Among the phenolic acid compounds analyzed, rosmarinic acid, chlorogenic acid, and ellagic acid exhibited the most favorable binding energies, indicating their strong affinities for TNF-α convertase. This suggested that these compounds have significant potential as TNF-α convertase inhibitors and have become promising candidates for anti-inflammatory intervention. Although their binding affinities may be lower than that of the native ligand, it is important to note that their potential as anti-inflammatory agents should not be disregarded. These compounds provide opportunities for further development as anti-inflammatory agents. Optimization of their molecular structures or exploration of their derivatives can improve their binding affinities and efficacy as TNF-α convertase inhibitors. The observed low binding energies of rosmarinic acid, chlorogenic acid, and ellagic acid suggested favorable interactions in the active site of TNF-α convertase and their capacity to modulate the enzymatic activity of TNF-α convertase and inhibit TNF-α production. This has the potential to alleviate the inflammatory response associated with various inflammatory conditions.

Chlorogenic acid is a depside acid formed by combinations of caffeic and quinic acids (1-hydroxyhexahydrogallic acid). This compound is also recognized as a phenylacrylate polyphenol that is synthesized through the shikimic acid pathway in plants during their aerobic respiration process. Naturally, chlorogenic acid can be discovered across a diverse array of sources, encompassing honeysuckle, potato, cork, eucommia leaves, chrysanthemum, strawberry, mango, blueberries, mulberry leaves, and green coffee. Chlorogenic acid showed promising pharmacological activities, including potent antioxidant capabilities, protection of liver and kidney functions, antimicrobial, antitumor, modulation of glucose and lipid metabolism, protection of the nervous and vascular system, and anti-inflammation.[40,41] In both in vivo and in vitro

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studies, chlorogenic acid showed remarkable anti-inflammatory potential. At a concentration of 20 μM, this compound has demonstrated ability to suppress the expression of key proinflammatory cytokines, including IL-1β and TNF-α, within LPS-activated RAW 264.7 cells over a 24 h period. Notably, chlorogenic acid has also exhibited a modulating effect on the mRNA levels of COX-2, IL-6, and CXCL1, all of which contribute to the inflammatory response induced by LPS. Moreover, this compound has effectively suppressed the production of nitric oxide, downregulated the expression of COX-2 and iNOS, and notably attenuated the levels of inflammatory cytokines, including IL-1β, TNF-α, and IL-6. Additionally, chlorogenic acid has been shown to reduce macrophage adhesion and significantly diminish the expression of ninjurin. Importantly, it has hindered the nuclear translocation of NF-κB, a pivotal regulator of inflammatory responses, which further supports its potent anti-inflammatory effects.[42] Another in vitro study has supported the anti-inflammatory efficacy of chlorogenic acid within macrophages, suggesting potential mediation through the inhibition of NF-κB signaling pathways using several proinflammatory cytokines, including TNF-α and IL-1β, that are widely recognized biomarkers indicative of inflammatory progression. Notably, the research unveiled that chlorogenic acid significantly decreases both the mRNA expression and the release of TNF-α and IL-1β in LPS-stimulated primary microglia and macrophages, showcasing its robust anti-inflammatory properties. Consequently, the attenuation of these proinflammatory cytokines, specifically TNF-α, can be considered as promising of anti-inflammatory action.[43] Some of these studies demonstrate the anti-inflammatory activity of chlorogenic acid involving various mechanisms.[40,42,43] Of particular interest is its remarkable ability to suppress, which plays a role in proinflammatory cytokines. Therefore, in this research, we predict the molecular mechanism of inhibiting TNF-α production through the inhibition of TNF-α convertase, through both docking and molecular dynamics. Our study successfully reveals that chlorogenic acid exhibits a binding pose similar to the native ligand (inhibitor) of TNF-α convertase, with commendable stability; nevertheless, it still possessing suboptimal binding affinity. However, when compared to other phenolic compounds, chlorogenic acid displays the most robust dynamic interaction stability in binding with TNF-α convertase.

Rosmarinic acid is a polyphenolic compound primarily found in *Rosmarinus officinalis* and has been extensively studied for its renowned antioxidant and anti-inflammatory properties. Numerous studies have demonstrated its efficacy in various models of inflammation, including reducing paw edema induced by carrageenin in rats. Moreover, in rat models of liver ischemia-reperfusion and thermal injury, rosmarinic acid exhibited a protective effect by lowering serum levels of transaminases and markers of multiorgan dysfunction. These findings underscore the substantial anti-inflammatory potential of rosmarinic acid and its potential utility in managing inflammation-associated injuries.[44] In another study investigating the effects of rosmarinic acid on lipopolysaccharide (LPS)-induced mastitis in mice, treatment with rosmarinic acid resulted in an improvement in mammary structural damage and a decrease in the expression of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6. The study also revealed that rosmarinic acid inhibited the TLR4/MyD88/NF-κB signaling pathway, indicating its potential as an effective treatment for LPS-induced mastitis.[45]

Ellagic acid, a natural compound found in various fruits, vegetables, and nuts, has gained recognition for its powerful antioxidant properties. Recent studies have shed light on its potential as an anti-inflammatory agent. While the exact mechanism is not yet fully understood, extensive research has identified multiple targets and pathways involved in the anti-inflammatory activity of ellagic acid. Network pharmacology analysis revealed that ellagic acid interacts with 52 targets associated with inflammation. Gene ontology and pathway analysis have further unveiled relevant signaling pathways and diseases linked to inflammatory processes. Notably, AKT1, VEGFA, TNF, MAPK3, ALB, SELP, MMP9, MMP2, PTGS2, and ICAM1 have emerged as particularly significant targets. Molecular docking and simulations have supported AKT1, PTGS2, VEGFA, and MAPK3 as the most likely targets of EA. These findings support previous evidence of the anti-inflammatory effects of ellagic acid and provide valuable insight into its underlying mechanisms of action.[46] Moreover, ellagic acid (at concentrations of 1, 5, 10, and 25 μg/mL) inhibits IL-1β and TNF-α-induced activation of AP-1 and MAPK signaling pathways, including ERK1/2, JNK, and p38.[47] However, it is noteworthy that this inhibition did not extend to NF-κB activation. Similarly, the ellagic acid at 0.1−10 μM suppress the TNF-α-triggered endothelial activation, ultimately leading to a reduction in the expression levels of both VCAM-1 and ICAM-1 molecules.[48]

In a study conducted on adjuvant-induced arthritis (AIA) in mice, ellagic acid treatment showed promising results. The primary objective of the study was to evaluate the preventive and therapeutic effects of ellagic acid on AIA. The mice were administered ellagic acid starting from 1 week before the induction of AIA, and treatment was continued for 3 weeks. The results demonstrated that ellagic acid significantly inhibited foot paw swelling and mitigated the pathological changes associated with AIA. Additionally, the ellagic acid treatment led to a decrease in the levels of proinflammatory cytokines, including interleukin 1L (IL-1ß), tumor necrosis factor-alpha (TNF-α), and interleukin 17 (IL-17). Conversely, it significantly increased the levels of the anti-inflammatory cytokines IL-10 and interferon γ (IFN-γ). However, ellagic acid treatment did not induce significant changes in the levels of transforming growth factor ß (TGF-ß).[49]

Despite the anti-inflammatory properties of phenolic compounds, particularly their capacity to inhibit the production of TNF-α have been well-documented, the precise mechanisms responsible for this inhibition remain not yet fully understood. It is widely believed that these anti-inflammatory activities are mediated through the modulation of various signaling pathways implicated in inflammation. One promising strategy for suppressing TNF-α production involves targeting TNF-α convertase. The present study employs computational methods with predictive outcomes, and the docking study has successfully identified several phenolic acid compounds, including chlorogenic acid, rosmarinic acid, and ellagic acid, which exhibit strong binding affinities for the TNF-α convertase protein. These interactions between the identified phenolic acid compounds and the protein offer valuable insights into the molecular mechanisms underlying TNF-α convertase inhibition. As a result, we propose that this approach could be useful in predicting the TNF-α inhibitory capabilities of phenolic compounds through the TNF-α

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convertase pathway. These findings further corroborate previous research, which indicated the anti-inflammatory potential of chlorogenic acid, rosmarinic acid, and ellagic acid in suppressing TNF-α production, shedding light on the still-unclear molecular mechanisms behind their anti-inflammatory action.[43,45,47,48] Our study contributes to a better understanding of how these compounds function as TNF-α inhibitors via TNF-α convertase inhibition.

Notably, successful docking programs are known to produce poses with RMSD values below 2 Å, and we are delighted to report that our computed RMSD values fall well within this established threshold. This unequivocally establishes the validity of our docking protocol.[50,51] Moreover, it is imperative to emphasize the significance of our redocking calculations, which displayed outstanding performance with RMSD values below 1.0 Å.[52] In our study, the RMSD resulting from the redocking process measured at a highly reassuring value of 0.9010 Å, further solidifying the validity of our docking methodology.

In addition to its role as a reference during the validation of our docking protocols, the native ligand assumes a pivotal role as the standard compound or reference control in our research, as documented in Table 1 and described well in previous research.[34] In this study, the native ligand, identified as (1S,3R,6S)-4-oxo-6-{4-[(2-phenylquinolin-4-yl)methoxy]-phenyl}-5-azaspiro[2.4]heptane-1-carboxylic acid, exhibits a remarkable TNF-α convertase inhibition activity with a K_i value of 143 nM.[34] Furthermore, this research furnishes valuable insights into the binding model of the native ligand with the TNF-α convertase receptor, revealing a reasonably effective binding structure. This inhibitory activity of the native ligand establishes our benchmark and enables us to employ it as a control in assessing the inhibitory potential of the selected phenolic test compounds. Our objective is to attain a lower binding affinity for these test phenolic compounds to evaluate their binding energy, which is signifying correlation to the stability of the ligand−receptor complex.

The inhibition mechanism of this enzyme involves various complex processes. One important aspect of these interactions is the involvement of catalytic residues in the protein, particularly, Leu348, Gly349, and Glu406. The interactions formed between the phenolic compounds and these residues contribute to the inhibition of the function of the TNF-α convertase enzyme. Leu348, Gly349, and Glu406 are key residues located at the active site of the enzyme. The binding of phenolic compounds to these residues can interfere with normal interactions between the enzyme and its substrates, thereby inhibiting enzyme activity. Furthermore, phenolic acid compounds can interact with important structural components of the enzyme, leading to alterations in its three-dimensional conformation and a disturbance of its catalytic function. Inhibition of reaction pathways involved in enzyme function is also a possible mechanism that can occur.[19,53]

To gain further insight into the stability of the molecular interactions, molecular dynamics simulations were employed. Molecular dynamics analysis provides valuable information about the stability and dynamics of protein−ligand complexes over time and space. By subjecting the protein−ligand complex to molecular dynamics simulations, the dynamic behavior and evolution of the interactions between the phenolic compound and TNF-α convertase could be investigated. This analysis allowed us to observe how the complexes responded to and evolved, providing a comprehensive understanding of the stability and strength of the interactions.

During the molecular dynamic simulations, various parameters such as bond lengths, angles, and torsion angles were monitored to assess the stability of the complexes. The simulations also provided insight into the flexibility of the protein and ligand as well as any conformational changes that occurred during the interactions. The molecular dynamics analysis unequivocally demonstrated the stability of the interactions between the phenolic acid compounds and the key residues in the active sites of TNF-α convertase. Throughout the simulations, the complexes exhibited minimal deviations and sustained consistent interactions, indicating a robust and enduring binding.[19]

The dynamic behavior of the protein−ligand complex was further investigated through protein rigidity analysis, which provided valuable insight into the flexibility and conformational changes occurring within the protein upon ligand binding. A decrease in protein rigidity indicated the inhibition of protein activity. In the case of binding between the phenolic compounds and TNF-α convertase, the protein exhibited reduced rigidity, indicating that the interactions of the compounds with active site residues impeded normal functional motions of the protein. This reduced flexibility and altered conformation of the protein strongly suggested inhibited enzymatic activity. It is important to note that these interactions occurred on residues located in the protein loop region, which are distant from the active sites. Both rosmarinic acid and ellagic acid increased the root-mean-square fluctuation (RMSF) values in the Asp418-Glu422 and Ser441-Asp443 regions. This suggested that ligand binding induced a "domino effect" on the protein, leading to changes in the positions of amino acid residues, even those situated far from the active sites. These findings provided evidence that rosmarinic acid and ellagic acid may inhibit the TNF-α convertase enzyme activity by triggering an allosteric inhibitory effect on the target receptor.[54,55]

The effects of phenolic acid compounds on protein rigidity provide a deeper understanding of the inhibitory mechanism at the structural level. Stabilization of the protein−ligand complex and subsequent decrease in protein rigidity provide compelling evidence for the disruption of the normal function of the enzyme by phenolic acid compounds.[56] TNF-α convertase inhibition has emerged as a potential strategy for anti-inflammation due to its crucial role in regulating inflammatory processes. TNF-α is proteolytically cleaved and released from its membrane-bound precursor form by TNF-α convertase, also known as TACE (TNF-α converting enzyme).[18] Inhibiting TNF-α convertase as a means of reducing TNF-α levels provides a promising approach for anti-inflammation. By attenuating TNF-α production and its downstream effects, TNF-α convertase inhibitors can help alleviate the inflammatory response, suppress immune cell activation, modulate inflammatory signaling pathways, and promote tissue repair.[57]

CONCLUSIONS

In summary, phenolic compounds with potential anti-inflammatory properties, namely, chlorogenic acid, rosmarinic acid, and ellagic acid, exhibit their anti-inflammatory activity through the inhibition of TNF-α convertase. These compounds demonstrate binding poses that are similar to the native ligand, even though they have lower binding affinities. This suggests a plausible mechanism of action for these three

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phenolic compounds as anti-inflammatories, particularly in the inhibition of TNF-α production through TNF-α convertase inhibition. These findings support previous research highlighting the anti-inflammatory activity of phenolic compounds based on suppressing TNF-α.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06450.

Table of the results of network analysis for proteins involved in the inflammatory process, the residue classification table obtained from DynDom analysis for domain movement analysis, and a figure depicting the network of GO and KEGG pathway interactions with targetted proteins (PDF)

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J.E. and W.A.K. were responsible for conceptualization, J.E. and M.I.S. were responsible for methodology, N.N., S.M., R.F., and N.S.R. were responsible for software, B.A.T., W.A.K., and M.S.Z. were responsible for validation, K.A.N. was responsible for formal analysis, T.Y. was responsible for data curation, J.E. was responsible for writing the original draft preparation, B.A.T., W.A.K., S.M., M.S.Z., and T.Y. were responsible for writing, review, and editing, N.N. and S.M. were responsible for visualization, and J.E. was responsible for supervision. All authors have read and agreed to the published version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

TNF-α tumor necrosis factor-alpha; COX2/PTGS2 cyclooxygenase-2/prostaglandin-endoperoxide synthase 2; FDR false discovery rate; MMFF94 molecular mechanic force field 94; RMSD root-mean-square deviation; MD molecular dynamics; SPC simple point charge; MM-GBSA molecular mechanics/generalized born surface area; IL-6 interleukin-6; NPT normal pressure and temperature; GO gene ontology; KEGG Kyoto Encyclopedia of Genes and Genomes; F10 Factor X; TACE tumor necrosis factor-alpha converting enzyme; ns nanoseconds; Å angstroms; RMSF root-mean-square fluctuation; DynDom dynamic domain motion; SSE secondary structure elements; tmTNF-α transmembrane tumor necrosis factor-alpha; sTNF-α soluble tumor necrosis factor-alpha; IL-1β interleukin-1 beta; LPS-activated RAW lipopolysaccharide-activated RAW; CXCL1 chemokine Ligand 1; iNOS inducible nitric oxide synthase; NF-κB nuclear factor-kappa B; TLR4 toll-like receptor 4; MyD88 myeloid differentiation primary response 88; AKT1 protein kinase B alpha 1; VEGFA vascular endothelial growth factor A; MAPK3 mitogen-activated protein kinase 3; ALB albumin; SELP selectin; MMP9 matrix metallopeptidase 9; MMP2 matrix metallopeptidase 2; ICAM1 intercellular adhesion molecule 1; ERK1/2 extracellular signal-regulated kinase 1/2; p38 p38 mitogen-activated protein kinase; JNK c-Jun N-terminal kinase; VCAM-1 vascular cell adhesion molecule 1; IL-17 interleukin-17; IFN-γ interferon-gamma; TGF-ß transforming growth factor-beta; AIA adjuvant-induced arthritis

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