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MOLECULAR DOCKING ANALYSIS OF CHLOROGENIC ACID AND 3,5-DI-O-CAFFEOYLQUINIC ACID REVEALS STRONG INHIBITORY INTERACTIONS WITH COX-2 AND MAPK SIGNALING PROTEINS

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Abstract: Inflammation is a key factor in many chronic diseases, and natural polyphenols are emerging as good regulators of inflammatory signaling. An in silico molecular docking analysis was done to assess the potential inhibitory effects of chlorogenic acid and 3,5-di-O-caffeoylquinic acid at two relevant pro-inflammatory targets Cyclooxygenase-2 (COX-2) and Mitogen-activated Protein Kinases (MAPKs). The docking resulted demonstrated both ligands were able to form stable and favorable energy-complex binding to the active sites of COX-2 and MAPKs. Chlorogenic acid was found to have a −7.4 kcal/mol binding energy to MAPKs and formed hydrogen bonding and hydrophobic interactions with ALA93, ARG5, PHE348, and ASP88. For 3,5-di-O-caffeoylquinic acid, a larger −9.95 kcal/mol binding energy was calculated and multiple stabilizing interactions at TYR311, TYR132, ARG136, ASN82, and the remaining residues of the catalytic-region were observed.Concerning COX-2, chlorogenic acid engaged through hydrogen bonds with CYS36, HIS39, ASN43, and ARG44, and 3,5-di-O-caffeoylquinic acid exhibited remarkably favorable predicted ΔGtab and Ki value of −10.9 kcal/mol and kang of around 10 nM, respectively, primarily due to strong hydrogen bonding interactions and π–π interactions with the COX-2 binding pocket. Similar to chlorogenic acid, we conclude that both polyphenols possess robust dual-target inhibitory potential worth considering, especially in the case of 3,5-di-O-caffeoylquinic acid, which may be a potent modulator of inflammation through inhibiting COX-2 and MAPK-mediated signaling. The findings provide important rationales in developing further experimental studies and elucidate the clinical translation of natural compound-therapeutics for anti-inflammatory pathways.

Introduction

Inflammation is a multi-faceted biological response of the immune system that serves to protect the host from damaging stimuli including pathogens, dead cells, and irritants. However, inappropriate or prolonged inflammation can result in the etiology of several diseases, including cancer, diabetes, cardiovascular, and neurodegenerative diseases. Cyclooxygenase-2 (COX-2) and mitogen-activated protein kinases (MAPKs are all important molecular regulators of inflammation that participate in regulating inflammation-associated pathways. Currently available anti-inflammatory drugs (e.g., NSAIDs and corticosteroids) provide relief from symptoms; however, these drugs are oftentimes related to adverse events such as gastrointestinal injury, renal toxicity, and cardiovascular risks. Because of this, there has been a substantial increased interest into developing safer and more effective natural inhibitors with fewer adverse events.

Chlorogenic acid (CGA) and its isomer 3,5-Di-O-Caffeoylquinic acid (3,5-diCQA) are naturally occurring polyphenolic compounds widely found in coffee, fruits, and medicinal plants. Both compounds possess remarkable antioxidant, anti-inflammatory, and hepatoprotective

properties and have been reported to modulate several molecular targets involved in oxidative stress and inflammation. Recent studies have demonstrated that these compounds can suppress inflammatory mediators through inhibition of the NF-κB and MAPK signaling pathways, thereby reducing the production of COX-2.

Despite increasing evidence of their pharmacological potential, the molecular interactions between these bioactive compounds and major pro-inflammatory proteins remain incompletely understood. Molecular docking studies provide valuable insights into the binding affinity and interaction mechanisms between small molecules and their target proteins, allowing the prediction of possible inhibitory activities at the molecular level.

Therefore, the present study aims to evaluate the binding interactions and affinities of 3,5-Di-O-Caffeoylquinic acid and Chlorogenic acid with key inflammatory mediators — COX-2 and MAPK— using molecular docking analysis. This in silico approach may help to elucidate the potential of these natural compounds as promising anti-inflammatory agents, offering a theoretical foundation for further experimental and clinical validation.

Materials and Methods

Datasets and softwareThis study utilized publicly available structural and computational resources. The three-dimensional crystallographic structures of the target inflammatory proteins Cyclooxygenase-2 (COX-2, PDB ID: 5IKQ) and Mitogen-activated protein kinase (MAPK, PDB ID: 6GES), were obtained from the Protein Data Bank (PDB). The ligand structures of Chlorogenic acid (CGA) and 3,5-Di-O-Caffeoylquinic acid (3,5-diCQA) were retrieved from the PubChem database in SDF format and converted into PDB format using Open Babel software. PyMOL (version 2.5) was used for molecular visualization and analysis, while AutoDock 4.2 and AutoDock Tools (ADT) were used for docking simulations.

2.2. Ligand and receptor preparation

The receptor proteins were prepared by removing all water molecules, co-crystallized ligands, and heteroatoms from the PDB structures. Polar hydrogen atoms were added, and Kollman partial charges were assigned using AutoDock Tools. Ligand structures were energy-minimized to achieve the most stable conformations and then saved in the .pdbqt format compatible with AutoDock.

2.3. Grid parameter setup

A three-dimensional grid box was generated around the active site of each protein to define the docking search area. The grid box dimensions were adjusted to completely cover the binding pocket and allow ligand flexibility. All parameters were stored in a .gpf (Grid Parameter File).

2.4. AutoGrid operation

The AutoGrid4 module was used to create atomic affinity maps for each ligand atom type. These maps represent the potential energy landscape around the protein and were later used by AutoDock to estimate interaction energies between the ligand and receptor.

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2.5. Docking protocol and binding energy calculation

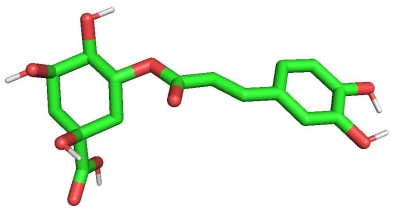
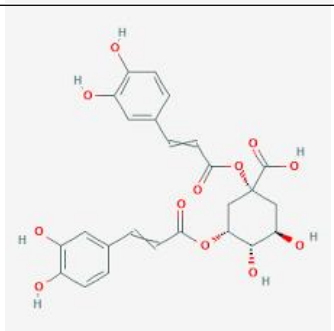
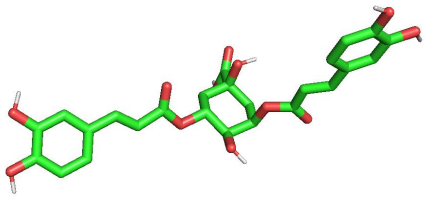
Docking simulations were carried out using AutoDock 4.2 with the Lamarckian Genetic Algorithm (LGA) as the search method. Default parameters were used for population size, energy evaluations, and mutation rates to ensure reproducibility. Each ligand was docked with all five target proteins to predict the most favorable binding conformation.

Binding free energy (ΔG, kcal/mol) values were automatically calculated by AutoDock based on the scoring function. The inhibition constant (Ki, μM) was determined from the binding energy using the following equation:

$$Ki = e^{\wedge} \frac{\Delta G x 1000}{RxT}$$

where ΔG is the binding energy in kcal/mol, R is the universal gas constant (1.987 cal·mol^{-1}·K^{-1}), and T is the absolute temperature (298.15 K).

Table 1. 2D and 3D Structures of Ligands Selected for Molecular Docking Analysis.

Ligands	2D	3D
Chlorogenic acid		
3,5-Di-O-Caffeoylquinic acid		

2.6. Visualization and interaction analysis

The docked complexes were analyzed using PyMOL and Discovery Studio Visualizer to pinpoint hydrogen bonding, hydrophobic and π–π interactions between ligands and key receptor residues. 2D and 3D interaction diagrams were created to illustrate the amino acids involved in ligand recognition to help provide information about possible inhibitory mechanisms of 3,5-

diCQA and CGA on pro-inflammatory proteins.In addition, Discovery Studio allowed for autorigation in the identification and characterization the ligand binding pockets that included mapping the hydrophobic area, clusters of aromatic residues, and the exact coordinates for the active site. This facilitated accurate identification of the functional binding cavity where ligand-protein interaction may occur.

Results

COX-2

Cyclooxygenase-2 (COX-2) is an isoform of the cyclooxygenase enzyme (COX) that can be induced and is central to the formation of prostaglandins from arachidonic acid. This enzyme plays a critical role in inflammation, pain, and fever. COX-1 is different than COX-2 due to its constitutive expression in many different tissues related to activities such as maintaining physiologic processes (e.g., gastric mucosal defense and platelet aggregation); COX-1 is always present in the tissues. COX-2 is expressed at low levels under normal physiologic conditions and is significantly upregulated with inflammation, cytokines, and growth factors.

The enzyme is primarily expressed in inflamed tissues, activated macrophages, and the endothelial cell line. COX-2 catalyzes the conversion to prostaglandin E2 (PGE2), a critical signaling molecule that causes vasodilation, sensitization of pain, and random infiltration of leukocytes. COX-2 overexpression has been linked to chronic diseases including rheumatoid arthritis, cardiovascular disorders, neurodegenerative diseases, and many cancers. In relation to COX-2 and its relation to drugs, COX-2 has been an important target in the drug discovery and development of nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors (coxibs). These drug classes primarily exert their therapeutic effect at the active site of the enzyme, with downstream inhibition of prostaglandin and inflammation. Nevertheless, there have been associated adverse cardiovascular effects due to long-term use of some synthetic COX-2 inhibitors, which is now leading to interest in the identification of natural compounds with selectivity for COX-2 inhibition, and lower adverse profile in terms of side effects. Molecularly, COX-2 is a homodimeric membrane-bound protein that has a catalytic domain comprised of a hydrophobic channel that opens to the active site. Substrate binding and catalysis involve significant amino acid residues, including Arg120, Tyr355, Tyr385, and Ser530. The COX-2 binding pocket is also slightly larger and more flexible than COX-1, allowing for the binding of more bulky inhibitors.

In an in silico study, we contributed to knowledge of the binding interaction between biologically active compounds present in Gnaphalium species - chlorogenic acid and 3,5-di-O-caffeoylquinic acid - and the target protein Cyclooxygenase-2 (COX-2). The binding interaction of these compounds and target proteins release some energy, which is expressed by following equation:

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{desolv} + \Delta G\_tors$$

- ΔG_vdw – van der Waals and hydrophobic interaction energy
- ΔG_hbond – hydrogen bond interaction energy
- ΔG_elec – electrostatic interaction energy
- ΔG_desolv – desolvation (removal from solvent) energy
- ΔG_tors – compensation energy for the ligand's rotational (torsional) freedom

When chlorogenic acid interacted with COX-2, the calculated binding energy was –8.5 kcal/mol.The thermodynamic laws state that the lower (more negative) the binding energy is, the interaction between the ligand and the protein is stronger and more stable. With this energy value, the inhibition constant (Ki) can be calculated as follows:

$$Ki = e^{\wedge}\frac{\Delta Gx1000}{RxT} = e^{\wedge}\frac{-8.5x1000}{1.987x298.15} \approx 5.83 \times 10^{-7}\ \text{M} = 0.583\ \mu\text{M}$$

Thus, this finding suggests that chlorogenic acid has strong inhibition potential against COX-2, indicating the potential of chlorogenic acid as a potent anti-inflammatory.

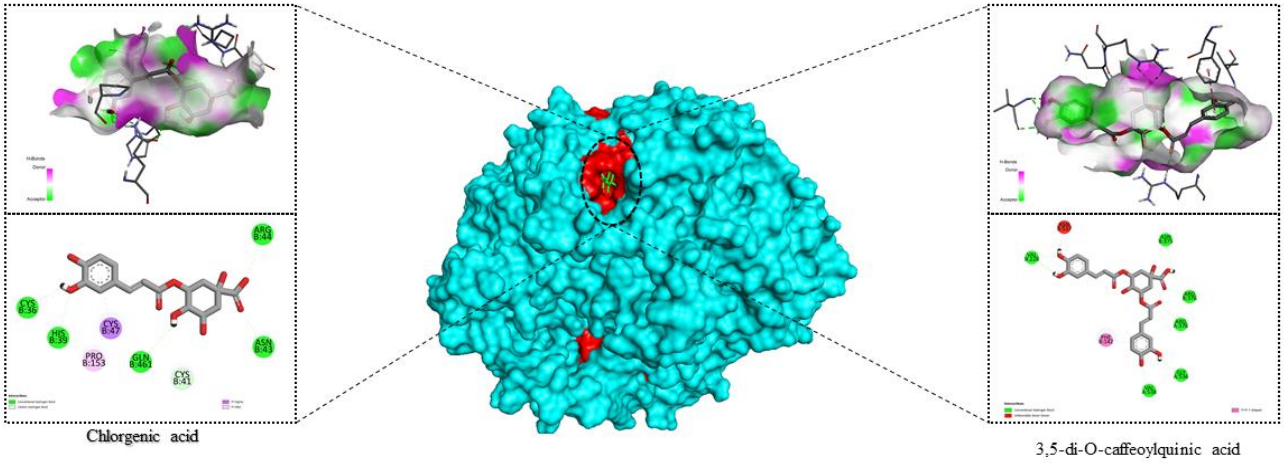


Figure 2. Binding Pocket Visualization of COX-2 Interactions with Chlorogenic Acid and 3,5-di-O-caffeoylquinic Acid

In the case of chlorogenic acid interacting with COX-2 protein, there are amino acid residues that are important for ligand binding as well as for regulation of the enzyme catalytic activity. The residues CYS B:36, HIS B:39, GLN B:461, ASN B:43, and ARG B:44 formed conventional hydrogen bonds with the ligand that contribute to stabilization of the complex due to electrostatic and polar interactions taking place within the active site. The residues fall within the binding domain of COX-2 which help to orient the ligand and determine affinity to the binding site. The residue PRO B:153 made a pi-alkyl interaction contributing to hydrophobic stabilization between the aromatic ring of chlorogenic acid and the non-polar pocket of the protein. In addition, CYS B:153 made a pi-sigma and carbon–hydrogen interaction suggesting its involvement in structural stability and establishment of weak van der Waals interactions to facilitate ligand binding specificity (Figure 1). For the 3,5-di-O-caffeoylquinic acid and COX-2 complex, the predicted ΔG is -10.9 kcal/mol, indicative of spontaneous association without the need for free energy from an external source. The corresponding Ki is nearly 10 nM (Ki ≈ 1.0 × 10^{-8} M at 298 K), which is reflective of a high-affinity, potent inhibition. At the contact level, standard hydrogen bonds are formed with VAL B:228, ASN B:375, ARG B:376, GLY A:536, and VAL A:538. forming favorable orienting and stabilizing interactions that hold the ligand in the binding cavity. PHE B:142 makes a π–π T-shaped contact with the aromatic system of the ligand, providing to support a hydrophobic and dispersion stabilization mode. A potential unfavorable donor–donor contact is predicted for ASN B:537, which suggests that a

local geometric or protonation mismatch does not contribute to a favorable binding interaction, and likely indicates that a strained pose is present at this position. All of the hydrogen bonds, in conjunction with the π–π contact, make for a strong binding and low Ki observed for this ligand–COX-2 complex overall (Figure 1).

MAPK

Mitogen-activated protein kinases (MAPKs) are an excellent target because they are central to many disease-related signalling pathways. Following cellular stress, inflammation, oxidative damage, or bioactive compound exposure, the MAPKs are among the first signalling pathways to become activated. This activation leads to the phosphorylation of transcription factors and, ultimately, the expression of the pro-inflammatory factors TNF-α, IL-1β, IL-6, COX-2, and numerous chemokines. These signal molecules are very important in the contexts of chronic inflammation, injury to tissues, metabolic dysfunction, and progression of cancer. Therefore, aberrant MAPK signalling directly contributes to disease processes. Based on these reasons, it is scientifically valid to evaluate both ligand binding and inhibitory potential against MAPK proteins. Exploration of MAPK inhibition would unveil compounds with potential anti-inflammatory, antioxidant, and/ or anti-cancer effects, which makes this pathway a crucial and clinically relevant targeted therapy in contemporary drug discovery. In our in silico experiments, we performed molecular docking with the ligands chlorogenic acid and 3,5-di-O-caffeoylquinic acid and the Mitogen-activated Protein Kinases. The binding energies were -7.4 kcal/mol (Ki ≈ 3.7 μM) and -9.9 kcal/mol (Ki ≈ 0.05 μM), indicating a strong intereaction and ingibiton

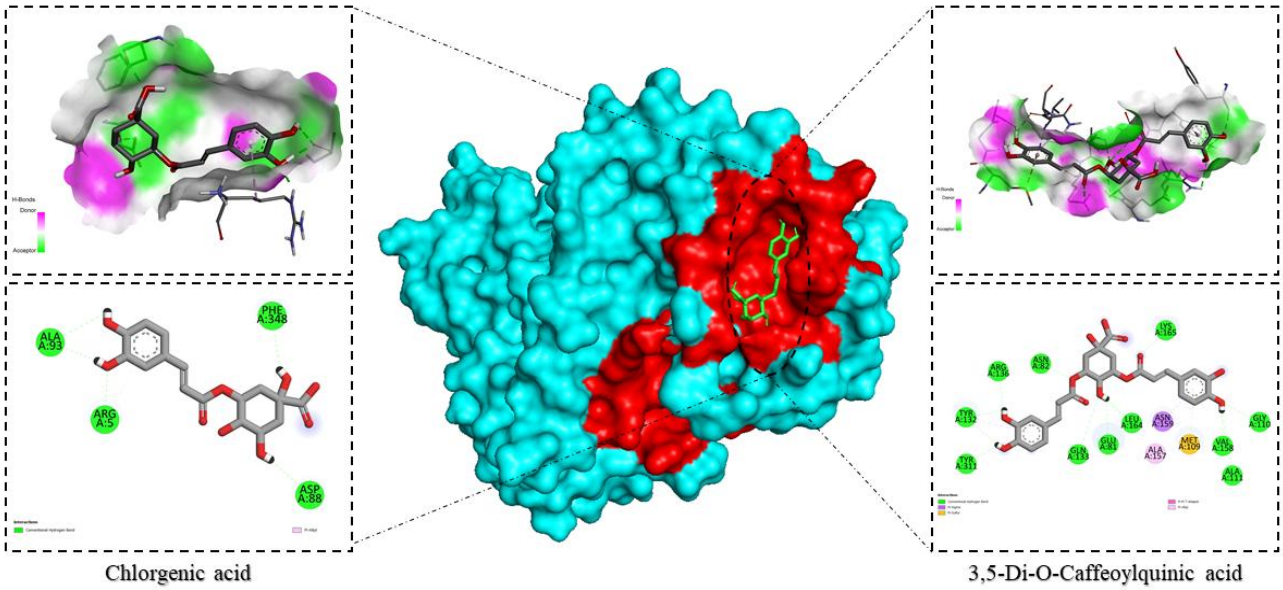


Figure 2. Binding Pocket Visualization of MAPK Interactions with Chlorogenic Acid and 3,5-di-O-caffeoylquinic Acid

Chlorogenic acid is bound to the MAPK residues ALA93, ARG5, PHE348, and ASP88 because they are present in the natural active pocket of the protein and provide a suitable chemical environment for stable interaction. ALA93 is small and hydrophobic, providing some ability to hold the ligand inside the pocket. ARG5 possesses a positive charge that will easily make strong hydrogen bonds with the negatively charged groups of chlorogenic acid. One of the residues in

this pocket, PHE348, is an aromatic residue that can assist in stabilizing the ligand by way of both hydrophobic and π–alkyl interactions with the aromatic ring. RES88, on the other hand, carries a negative charge and can provide a hydrogen bond to the hydroxyl groups of the ligand. Together, these residues create a relatively good binding pocket that enables chlorogenic acid to bind well and potentially inhibit the activity of MAPK.3,5-di-O-caffeoylquinic acid interacts with many important residues at the MAPK active site, such as TYR311, TYR132, ARG136, ASN82, LYS165, GLN133, GLU81, LEU164, VAL158, ALA111, and GLY110 with many traditional hydrogen bonds. These residues line the catalytic groove and present polar/hydrophobic surfaces accepting compatibility with the ligand's hydroxyl and aromatic groups. ASN159 interacts via a π–sigma interaction with the ligand's aromatic moiety while ALA157 interacts with π–alkyl interactions to stabilize the compound in the pocket. Additionally, TYR132 interacts via a π–π T-shaped interaction, enhancing aromatic stacking and contributing to binding affinity. Since these residues provide hydrogen-bond donors, acceptors, hydrophobic surface, and aromatic surface, the ligand is properly accommodated in this region and, also, has formed a stable complex indicating a strong inhibiting effect on MAPK activity (Figure 2).

Discussion

In the current investigation, in silico molecular docking was utilized to assess the inhibitory capacity of chlorogenic acid (CGA) and 3,5-di-O-caffeoylquinic acid (3,5-diCQA) on two essential pro-inflammatory targets, COX-2 and Mitogen-activated Protein Kinases (MAPKs). The binding energies and interaction histograms demonstrate that both ligands can be accommodated by COX-2 and MAPK, and are likely to stabilize themselves by interacting with key residues involved in catalytic and regulatory functions. For COX-2, chlorogenic acid exhibited favorable binding and formed multiple conventional hydrogen bonds with residues CYS36, HIS39, GLN461, ASN43 and ARG44, which reside within the ligand-binding region of the enzyme and help stabilize and orient the ligand. Hydrophobic contacts, which include a π-alkyl interaction with PRO153 and additional π-sigma and carbon–hydrogen contacts interacted with CYS153, also helped to stabilize CGA in the hydrophobic regions of the COX-2 binding pocket. These interactions together suggest that CGA may inhibit the catalytic function of COX-2 by blocking access to residues involved in substrate turnover.The docking of 3,5-diCQA to COX-2 displayed a much more favorable binding energy (ΔG = -10.9 kcal/mol), which can be translated to a Ki value of approximately 10 nM suggesting rather high inhibitory potential classed as very strong affinity. This strong affinity is supported by several stabilizing hydrogen bonds with VAL228, ASN375, ARG376, GLY536, and VAL538, in addition to a strong π–π T-shaped bond of some kind with PHE142. While it is possible to notice the presence of an unfavorable donor–donor contact at ASN537, this isolated geometric mismatch does not outweigh the overall pattern of both strong stabilizing interactions and clearly demonstrates the case of high-affinity binding. In summary, the overall pattern of combined interactions conveys that 3,5-diCQA strongly occupies the COX-2 binding site and acts as a possible strong antagonist to the COX-2 active site.Docking against MAPKs as a whole reveal that both ligands can target the active pocket involved in the kinase domain. Chlorogenic Acid exhibited a ΔG of -7.4 kcal/mol (Ki ≈ 3.7 μM) and established hydrogen-bond and hydrophobic interactions with ALA93, ARG5, ASP88, and PHE348, all residues that lie within the ATP-binding cleft and are consequential to substrate positioning and catalytic activation. ARG5 provides very strong electrostatic attraction for the acidic groups of CGA while PHE348 and ALA93 provides the hydrophobic anchoring. ASP88, being negatively charged, forms additional polar stabilization with the hydroxyl groups of the ligand. These interactions collectively imply that CGA may

interfere with phosphorylation events by occupying the catalytic region responsible for ATP docking.In comparison, 3,5-diCQA demonstrated a substantially stronger interaction with MAPKs, reflected by a binding energy of –9.9 kcal/mol (Ki ≈ 0.05 μM). The ligand formed numerous hydrogen bonds with residues TYR311, TYR132, ARG136, ASN82, LYS165, GLN133, GLU81, LEU164, VAL158, ALA111, and GLY110. These residues collectively define the extended binding groove of MAPKs and facilitate multi-point stabilization. Additional π–sigma interaction with ASN159, π-alkyl contact with ALA157, and a π–π T-shaped interaction with TYR132 further strengthen the ligand's fit within the aromatic and hydrophobic sub-pockets of the kinase domain. This extensive interaction network reflects a highly optimized complementarity between the ligand's polyphenolic structure and the chemical architecture of the MAPK catalytic site.The negatively charged ASP88 provides additional polar stabilization with the hydroxyl groups of the ligand. Together, these interactions suggest that CGA could act to block phosphorylation events by taking up space within the ATP-docking catalytic region. In contrast, 3,5-diCQA had a significantly greater interaction with MAPKs (-9.9 kcal/mol, Ki ≈ 0.05 μM). The ligand made many hydrogen bonding interactions with residues TYR311, TYR132, ARG136, ASN82, LYS165, GLN133, GLU81, LEU164, VAL158, ALA111, and GLY110, which all define the extended binding groove of MAPKs and provide multi-point stabilization. Additionally, there was a π–sigma interaction with ASN159, π-alkyl interaction with ALA157, and a π–π T-shaped interaction with TYR132 to provide extra stabilization of the ligand in the aromatic and hydrophobic sub-pockets of the ATP-binding site, where these interactions provide additional compatibility of the polyphenolic structure of the ligand with the chemical architecture of the MAPK catalytic site.In summary, the docking results indicate that both natural products have strong potential to interact with COX-2 and MAPKs, two key regulators of inflammatory signaling. When looking at binding affinities, 3,5-diCQA showed a stronger association for both protein targets likely due in part to its larger aromatic system and a higher number of hydrogen-bond donors and acceptors, which would then facilitate a more dense and stable intricate web of affinities. In summary, the data suggest 3,5-diCQA is a more promising anti-inflammatory than chlorogenic acid. The observed binding interactions with the most relevant catalytic and regulatory amino acid residues of COX-2 and MAPK suggests that the mono- and di-CQA compounds can modulate inflammatory related pathways by blocking substrate access to the active site, inhibiting ATP binding, or stabilizing a protein in its inactive rather than active conformation as needed for the downstream pathways. Future experimental studies that include evaluation of enzyme inhibition, kinase activity, and cell-based anti-inflammatory models would help validate the computational assessments, which would enhance our knowledge of the biological significance of the work presented here.

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