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EVALUATION OF THE MOLECULAR DOCKING-BASED INTERACTION OF AMINO ACIDS IN THE "AS-CAPSIPOL" FOOD SUPPLEMENT WITH CYCLOOXYGENASE-2 (COX-2)

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Abstract*:This article investigates the molecular docking-based interaction of selected amino acids contained in the "As-Capsipol" food supplement with the cyclooxygenase-2 (COX-2) enzyme. Arginine, phenylalanine, glutamic acid, tyrosine, and tryptophan were selected as ligands, and their binding affinity, inhibition constant, and interaction profiles with amino acid residues in the active site of the enzyme were evaluated. According to the results, tryptophan and tyrosine exhibited relatively high binding affinity toward COX-2, which may be important for explaining the potential molecular mechanism underlying the anti-inflammatory activity of the "As-Capsipol" complex. The obtained findings provide a scientific basis for evaluating the biological activity of this food supplement using an* in silico *approach.*

Keywords: *As-Capsipol, COX-2, molecular docking, amino acids, tryptophan, tyrosine, arginine, binding affinity, inflammation,* in silico.

ОЦЕНКА ВЗАИМОДЕЙСТВИЯ АМИНОКИСЛОТ, СОДЕРЖАЩИХСЯ В ПИЩЕВОЙ ДОБАВКЕ "AS-CAPSIPOL", С ФЕРМЕНТОМ ЦИКЛООКСИГЕНАЗОЙ-2 (COX-2) НА ОСНОВЕ МОЛЕКУЛЯРНОГО ДОКИНГА

Аннотация*:В данной статье изучено взаимодействие отдельных аминокислот, входящих в состав пищевой добавки «As-Capsipol», с ферментом циклооксигеназой-2 (COX-2) на основе молекулярного докинга. В качестве лигандов были выбраны аргинин, фенилаланин, глутаминовая кислота, тирозин и триптофан; оценены их аффинность связывания, константа ингибирования, а также профили взаимодействия с аминокислотными остатками активного центра фермента. Согласно полученным результатам, триптофан и тирозин проявили относительно высокую аффинность связывания с COX-2, что может иметь важное значение для объяснения предполагаемого молекулярного механизма противовоспалительного действия комплекса «As-Capsipol». Полученные данные служат научной основой для оценки биологической активности данной пищевой добавки с использованием подхода* in silico.

Ключевые слова: *As-Capsipol, COX-2, молекулярный докинг, аминокислоты, триптофан, тирозин, аргинин, аффинность связывания, воспаление,* in silico.

"AS-CAPSIPOL" OZIQ-OVQAT QO' SHILMASI TARKIBIDAGI AMINOKISLOTALARNING COX-2 FERMENTI BILAN MOLEKULYAR DOKING ASOSIDAGI O' ZARO TA'SIRINI BAHOLASH

Annotatsiya; *Ushbu maqolada "As-Capsipol" oziq-ovqat qo' shilmasi tarkibidagi ayrim aminokislotalarning siklooksigenaza-2 (COX-2) fermenti bilan molekulyar doking asosidagi o' zaro ta'siri o' rganildi. Tadqiqotda arginin, fenilalanin, glutamin kislota, tirozin va triptofan ligand sifatida tanlanib, ularning bog' lanish affiniteti, ingibitsiya konstantasi hamda faol markazidagi aminokislota qoldiqlari bilan o' zaro ta'sir profillari baholandi. Natijalarga ko' ra, triptofan va tirozin COX-2 bilan nisbatan yuqori bog' lanish affinitetini namoyon qilib, "As-Capsipol" majmuasining yallig' lanishga qarshi ehtimoliy molekulyar mexanizmini izohlashda muhim ahamiyat kasb etishi mumkin. Olingan natijalar mazkur oziq-ovqat qo' shilmasining biologik faolligini in silico yondashuv asosida ilmiy baholashga xizmat qiladi.*

Kalit so‘ zlar: *As-Capsipol, COX-2, molekulyar doking, aminokislotalar, triptofan, tirozin, arginin, bog‘ lanish affiniteti, yallig‘ lanish, in silico.*

INTRODUCTION

In recent years, scientific interest in the study of bioactive dietary supplements, phytocomplexes of natural origin, and their molecular mechanisms of action has been steadily increasing. In particular, the evaluation of the pharmacobiological potential of supplements developed on the basis of natural components is of significant scientific and practical importance in pathological conditions associated with inflammation, oxidative stress, and liver injury. This approach is closely related to the search for safer, biologically compatible agents with multicomponent mechanisms of action compared with synthetic drugs.

Inflammation is a complex protective response of the organism to various endogenous and exogenous factors. However, when this process becomes prolonged or uncontrolled, it may lead to tissue damage, metabolic disturbances, and the development of chronic diseases. Prostaglandins, cytokines, reactive oxygen species, and other mediators play an important role in this process. In particular, cyclooxygenase enzymes are involved in the biosynthesis of prostaglandins through arachidonic acid metabolism and are considered among the key factors determining the molecular basis of inflammatory responses.

Cyclooxygenase-2 (COX-2) is an inducible enzyme that is expressed at low levels under normal physiological conditions; however, its activity markedly increases during inflammation, oxidative stress, infectious processes, and tissue injury. Activation of COX-2 enhances the formation of prostaglandin E2 and other pro-inflammatory mediators, thereby contributing to pain, edema, increased vascular permeability, and modulation of the immune response. Therefore, COX-2 is regarded as a promising molecular target in many inflammatory diseases, including hepatitis and inflammatory lesions of liver tissue.

Molecular docking is an effective in silico approach that enables the preliminary assessment of the binding energy, spatial orientation, types of interactions with amino acid residues, and potential inhibitory activity of biologically active compounds against protein targets. This method represents an important complementary stage to laboratory experiments and is widely used to elucidate the molecular mechanisms of natural compounds, select promising ligands, and provide a scientific basis for subsequent experimental studies.

The aim of the present study was to evaluate the molecular docking-based binding properties of selected amino acids contained in the “As-Capsipol” dietary supplement with the COX-2 protein, as well as to determine their binding affinity, inhibition constant, and ligand–protein interaction profiles. The results of this study may contribute to explaining the possible molecular mechanisms underlying the anti-inflammatory activity of the “As-Capsipol” complex and to its scientific substantiation as a bioactive dietary supplement.

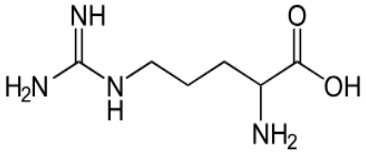
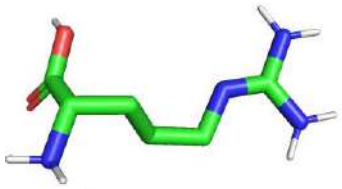
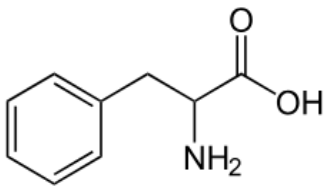
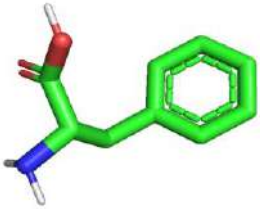
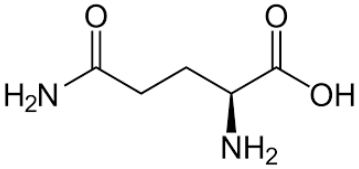
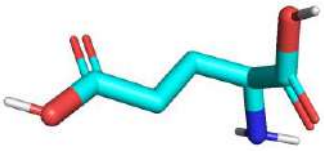
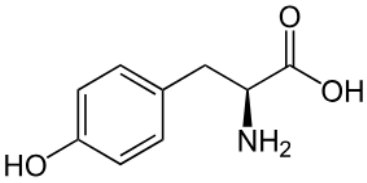
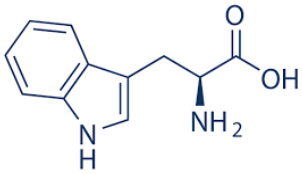
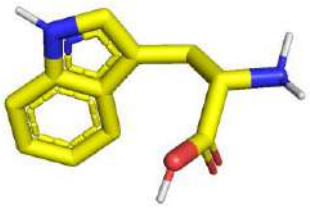
MATERIALS AND METHODS

All computational analyses were performed using freely available academic software tools. The three-dimensional structures of the selected target proteins, including cyclooxygenase-2 (COX-2, PDB ID: 5IKR), were retrieved from the Protein Data Bank (PDB) database. The structures of the ligands representing the amino acids contained in the “As-Capsipol” dietary supplement were obtained from the PubChem database. Their three-dimensional conformations were downloaded in appropriate formats, and their geometries were optimized prior to the docking procedure (Table 1). Protein preparation and structural analysis were carried out using PyMOL software, version 1.2 [1].

The active binding sites of the proteins were identified using Discovery Studio software, which allowed accurate localization of the docking regions. Visualization and analysis of ligand–protein interactions were also performed using PyMOL.

Molecular docking simulations were conducted using AutoDock 4.2 software. AutoDock Tools (ADT) was used for the preparation of proteins and ligands for docking. During this process, polar hydrogen atoms were added, partial charges were assigned, and rotatable bonds in the ligands were defined. The grid box parameters were set based on the previously predicted active sites [2]. This methodology enabled reliable prediction of the binding modes of the ligands with the target proteins, their spatial orientations, and their interaction profiles.

Table 1. Structural representation of the selected amino acid ligands used in molecular docking analysis in 2D and 3D formats

Ligands	2D	3D
Arginine		
Phenylalanine		
Glutamic acid		
Tyrosine		
Tryptophan		

Preparation of Ligand and Protein Files:

Prior to molecular docking, all PDB structures retrieved from the Protein Data Bank were subjected to a comprehensive preprocessing procedure, since the original files obtained from the PDB database are not fully suitable for direct docking analysis [3]. Such structures often contain crystallographic water molecules, co-crystallized ligands, ions, and other heteroatoms, which may adversely affect the accuracy of docking calculations.

Each protein structure was imported into AutoDock Tools (ADT) for preparation. Crystallographic water molecules and all non-essential heteroatoms were removed to eliminate undesirable interactions. Co-crystallized ligands were deleted prior to docking simulations; however, when necessary, they were preserved separately for validation purposes [4]. In addition, all non-protein components, including metal ions such as calcium, were removed to prevent artificial electrostatic effects under the rigid receptor docking model and to ensure a uniform preparation procedure for all target proteins.

The structural integrity of the selected protein models was visually inspected using PyMOL. Particular attention was paid to the presence or absence of missing amino acid residues in functionally important regions. No missing residues of critical importance were detected in the selected models [5]. In the next step, polar hydrogen atoms were added to better represent electrostatic properties, and Kollman united atom charges were assigned. In accordance with standard ADT protocols, the protonation states were adjusted to approximate physiological conditions (pH 7.4). The prepared receptor structures were subsequently saved in ".pdbqt" format, which contains information on atom types and partial charges required for docking calculations.

Ligand preparation was performed using a similar procedure. The selected amino acid ligands were imported into ADT, hydrogen atoms were added, and Gasteiger partial charges were assigned. In accordance with the standard AutoDock protocol, nonpolar hydrogens were merged [6]. To ensure conformational flexibility during the docking process, rotatable bonds and root atoms were automatically detected by the software. In the final step, the ligands were saved in ".pdbqt" format, containing all the structural and electrostatic information required for subsequent docking simulations [7].

Calculation of the Inhibition Constant (Ki) Based on Binding Energy:

In molecular docking studies, the affinity between a ligand and its target protein is usually expressed in terms of the free binding energy (ΔG), which is measured in kcal/mol. A lower, i.e., more negative, ΔG value indicates a stronger and more energetically favorable interaction between the ligand and the protein. The inhibition constant (Ki), which reflects the binding strength of the ligand, can be estimated from the calculated binding energy using the standard thermodynamic relationship [8]:

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

Where:

Ki is the inhibition constant, expressed in mol/L;
ΔG is the free binding energy, expressed in kcal/mol;
R is the universal gas constant, equal to 1.987 cal/(mol·K);
T is the temperature on the Kelvin scale, usually taken as 298.15 K;
1000 is the conversion factor used to convert kcal to cal.

Computational Tools and Databases Used in Molecular Docking Analysis:

An *in silico* molecular docking study was conducted to identify the possible molecular mechanisms underlying the biological activity of the "As-Capsipol" complex. Although the "As-Capsipol" complex contains a range of amino acids, several representative compounds, namely arginine, phenylalanine, glutamic acid, tyrosine, and tryptophan, were selected for the targeted docking analysis. Their selection was based primarily on their binding affinity profiles and biological significance.

These amino acids were selected because they demonstrated favorable binding energies toward the target proteins investigated in the preliminary docking results. Among them, tryptophan and tyrosine showed the most pronounced binding affinity toward several target proteins, indicating their strong interaction potential [9]. Phenylalanine also exhibited consistently favorable binding energies, suggesting its possible role in stabilizing ligand–protein complexes through hydrophobic interactions and π–π stacking interactions between aromatic rings.

Arginine was included in the analysis because of its ability to form strong electrostatic interactions and hydrogen bonds through its positively charged guanidinium group. This property facilitates its interaction with negatively charged amino acid residues located in the active sites of proteins [10]. Glutamic acid, as a negatively charged amino acid, was selected based on its ability to participate in ionic interactions and its involvement in the regulation of inflammatory signaling pathways.

The docking analysis was performed against a key protein target involved in the development of inflammatory processes and liver injury. Cyclooxygenase-2 (COX-2, PDB ID: 5IKR) was selected as the target protein. This protein target was chosen because it plays a central role in inflammatory signaling pathways and in the pathogenesis of liver diseases [11].

Quantitative determination of the amino acid content in the sample:

The precipitation of proteins and peptides from the aqueous extract of the As-Capsipol food supplement was carried out in centrifuge tubes. For this purpose, 1 mL of the investigated sample was mixed with an equal volume, namely 1 mL, of 20% trichloroacetic acid (TCA). After 10 minutes, the precipitated proteins were separated by centrifugation at 8000 rpm for 15 minutes. Then, 0.1 mL of the obtained supernatant was collected and subjected to lyophilization.

The dried residue was subsequently evaporated, dissolved in a triethylamine–acetonitrile–water mixture at a ratio of 1:7:1, and dried again. This procedure was repeated twice in order to neutralize residual acidity. Free amino acids were reacted with phenyl isothiocyanate (PITC) and converted into phenylthiocarbamyl (PTC) amino acid derivatives according to the method described by Steven A. and Cohen Daviel [12]. The results obtained are summarized in Table 2.

The identification of amino acid derivatives was performed using high-performance liquid chromatography (HPLC). The analytical conditions were as follows: an Agilent Technologies 1200 chromatographic system equipped with a DAD detector and a Discovery HS C18 column (75×4.6 mm) was used. Mobile phase A consisted of 0.14 M sodium acetate (CH_3COONa) containing 0.05% triethylamine (TEA), adjusted to pH 6.4, while acetonitrile (CH_3CN) was used as mobile phase B. The flow rate was set at 1.2 mL/min, and detection was performed at a wavelength of 269 nm. The gradient program for solvent B was as follows: 1-6% from 0 to 2.5 min; 6-30% from 2.51 to 40 min; 30-60% from 40.1 to 45 min; 60-60% from 45.1 to 50 min; and 60-0% from 50.1 to 55 min.

Table 2. Amino acid composition and quantitative content of the As-Capsipol food supplement

Amino acids	**As-Capsipol**
	Concentration (mg/g)
Glutamic acid	1.237

Arginine	0.458
Tyrosine	1.115
Tryptophan	7.147
Phenylalanine	2.037

Rationale for the selection of representative amino acid ligands:

The selection of arginine, phenylalanine, glutamic acid, tyrosine, and tryptophan as representative ligands was based on a combination of analytical, structural, and biological criteria. First, these amino acids were experimentally detected in the As-Capsipol food supplement by HPLC analysis, confirming their real presence in the investigated sample. Second, they represent different chemical classes of amino acids, including acidic amino acids (glutamic acid), basic amino acids (arginine), and aromatic amino acids (phenylalanine, tyrosine, and tryptophan). This chemical diversity is important because it allows the evaluation of different types of ligand-protein interactions, such as hydrogen bonding, electrostatic interactions, hydrophobic contacts, and π-mediated interactions within the COX-2 binding site.

In addition, these amino acids were selected due to their potential biological relevance to inflammatory and metabolic processes. Arginine may participate in immune regulation and nitric oxide-related pathways, glutamic acid is involved in nitrogen metabolism and cellular signaling, whereas aromatic amino acids such as phenylalanine, tyrosine, and tryptophan may contribute to hydrophobic and π-related interactions with protein targets. Among them, tyrosine and tryptophan are of particular interest because of their relatively high binding affinity toward COX-2 in the preliminary docking results. Therefore, the selected amino acids can be considered chemically diverse and biologically informative representative constituents of the As-Capsipol complex for targeted molecular docking analysis.

Table 3. Criteria for the selection of representative amino acid ligands from the As-Capsipol complex

Amino acid	Selection criterion	Rationale for representativeness
Glutamic acid	Acidic amino acid	Selected to evaluate the potential of acidic amino acids to interact with COX-2 through electrostatic interactions and hydrogen bonding.
Arginine	Basic amino acid	Represents positively charged amino acids; its guanidinium group can form strong hydrogen bonds and electrostatic interactions with residues in the COX-2 binding site.
Phenylalanine	Hydrophobic aromatic amino acid	Selected to assess hydrophobic contacts and π-mediated interactions associated with aromatic amino acid residues.
Tyrosine	Polar aromatic amino acid	Represents aromatic amino acids capable of forming both hydrogen bonds and π-alkyl interactions within the COX-2 active region.
Tryptophan	Indole-containing aromatic amino acid	Detected at a relatively high level in the sample and showed the strongest binding affinity toward COX-2, supporting its relevance as an informative representative ligand.

The selected amino acids were considered chemically diverse and biologically informative representative constituents of the As-Capsipol complex for targeted molecular docking analysis.

RESULTS AND DISCUSSION

Cyclooxygenase-2 (COX-2) is an inducible enzyme that plays an important role in the conversion of arachidonic acid into prostaglandins, particularly during inflammatory responses. Unlike COX-1, which is constitutively expressed in most tissues and participates in the maintenance of physiological homeostasis, COX-2 expression is generally low under normal conditions. However, its expression markedly increases under the influence of inflammatory stimuli such as cytokines, oxidative stress, and viral infections. Under hepatitis conditions, COX-2 has been shown to play an important role in the pathogenesis and progression of hepatic inflammation [10].

Various forms of hepatitis, including viral hepatitis such as hepatitis B and C, are associated with increased COX-2 expression in hepatocytes and non-parenchymal liver cells. This activation leads to enhanced production of prostaglandin E2 (PGE2). PGE2 contributes to hepatic inflammation by promoting vasodilation, increasing vascular permeability, and modulating immune responses. In addition, PGE2 may influence the balance between pro-inflammatory and anti-inflammatory cytokines, thereby affecting the severity and duration of liver injury [11].

Chronic activation of COX-2 in hepatitis is also associated with the progression of liver diseases toward fibrosis, cirrhosis, and hepatocellular carcinoma. There is evidence that COX-2-derived mediators may stimulate the activation of hepatic stellate cells, leading to excessive accumulation of extracellular matrix components and the development of fibrosis. Furthermore, COX-2 may contribute to carcinogenesis by enhancing cell proliferation, suppressing apoptosis, and

promoting angiogenesis within the hepatic microenvironment. Due to its important involvement in hepatic inflammation and disease progression, COX-2 is considered a promising therapeutic target in hepatitis-related conditions [2]. Selective inhibition of COX-2 has been proposed as a strategy to reduce hepatic inflammation and limit disease progression. However, the therapeutic use of COX-2 inhibitors should be carefully evaluated, particularly because of the potential systemic adverse effects associated with long-term administration.

Arginine exhibited a binding affinity of −6.1 kcal/mol toward COX-2. This corresponds to a Ki value of approximately 3.4×10^{-5} M, or about 34 μM, indicating moderate inhibitory potential and stable interaction within the active region of the enzyme. The docking simulation was performed using a grid box centered at the coordinates center_x = 36.323000, center_y = 26.465000, and center_z = 23.686000. This allowed the ligand to be accurately positioned within the functional binding pocket of COX-2 (Figure 1).

According to the docking analysis, arginine formed conventional hydrogen bonds with Gln192, Ser353, Leu352, and Met522 (Figure 2). At the same time, an unfavorable positive–positive interaction was observed with Arg513. The affinity toward these amino acid residues can be explained by the structural characteristics of arginine, particularly its polar guanidinium group. This group readily forms hydrogen bonds with polar amino acid residues.

Gln192 and Ser353 play an important role in stabilizing ligand binding through hydrogen bonding and contribute to the firm positioning of the ligand within the active site. Although Leu352 is predominantly hydrophobic, it contributes to the spatial orientation of the ligand through close-contact interactions. Met522, located within the hydrophobic channel of COX-2, is known to participate in substrate positioning. This suggests that arginine may occupy a catalytically significant region of the enzyme.

The unfavorable interaction observed with Arg513 is likely associated with electrostatic repulsion between positively charged groups present in both the ligand and the amino acid residue. However, the presence of several stabilizing hydrogen bonds may compensate for this unfavorable effect and contribute to an overall favorable binding energy.

From a functional perspective, the localization of arginine within the active site may partially block substrate access or disrupt the proper spatial orientation required for enzymatic catalysis. Considering that COX-2 is an enzyme responsible for the conversion of arachidonic acid into pro-inflammatory prostaglandins, such interactions may reduce enzymatic activity and contribute to the attenuation of inflammatory processes. This may be particularly important under hepatitis conditions, where COX-2 expression is elevated.

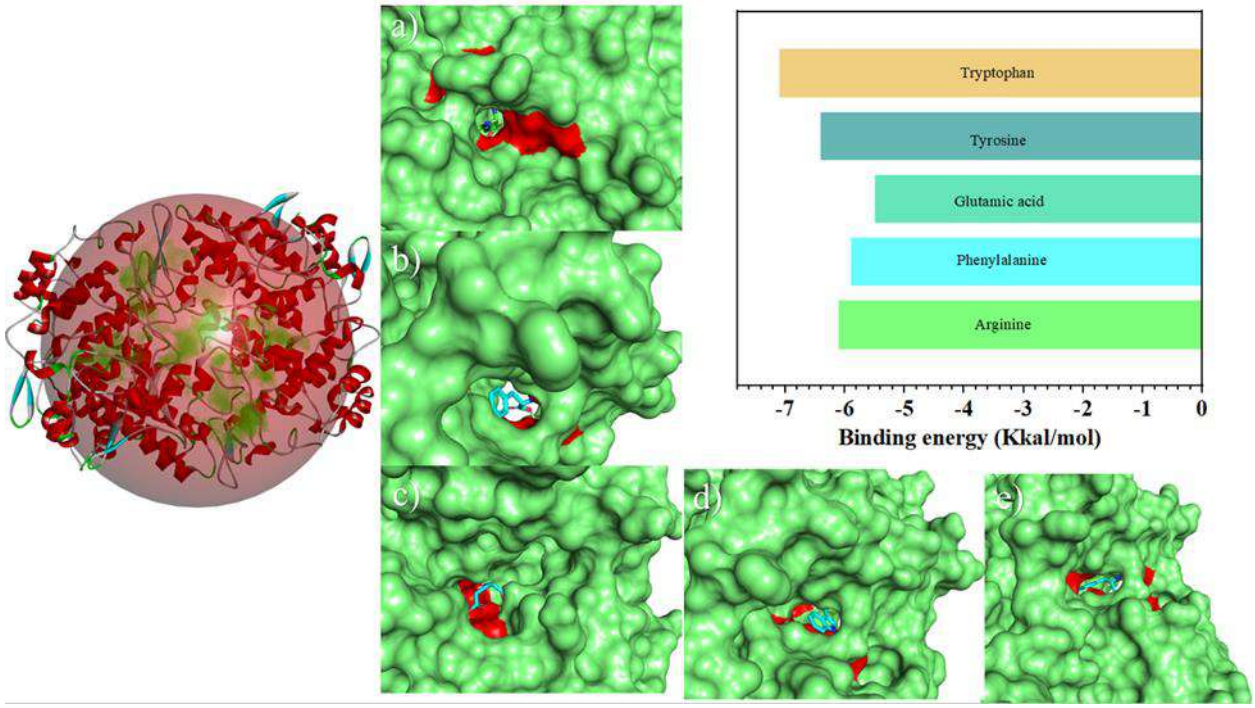


Figure 1. Integrated molecular docking analysis of selected amino acid ligands from the "As-Capsipol" complex with COX-2, illustrating their binding interactions and affinity profiles: (a) arginine, (b) phenylalanine, (c) glutamic acid, (d) tyrosine, and (e) tryptophan.

Phenylalanine exhibited a binding affinity of −5.9 kcal/mol toward COX-2 (Figure 1). This value corresponds to a Ki value of approximately 5.2×10^{-5} M, or about 52 μM. This result indicates that phenylalanine possesses a moderate inhibitory potential. Although this value is slightly weaker compared with that of arginine, it confirms that phenylalanine interacts significantly with the enzyme.

Interaction analysis showed that phenylalanine formed conventional hydrogen bonds with Cys36, Cys41, and Gln461. This indicates that the ligand is stably positioned within the binding pocket. These amino acid residues possess polar or semi-polar properties and are capable of stabilizing the ligand through hydrogen bonding. In particular, cysteine residues such as Cys36 and Cys41 may participate in hydrogen bonding through their thiol groups, thereby contributing to the spatial orientation and stabilization of the ligand. Gln461, as a polar amino acid residue, further enhances the specificity of binding through hydrogen bond formation.

In addition to hydrogen bonds, phenylalanine also exhibits π-related interactions due to its aromatic benzene ring. A π–sigma interaction was observed with Leu152, whereas a π–alkyl interaction was detected with Arg469. Such interactions are important because aromatic amino acids such as phenylalanine are characterized by their ability to form hydrophobic and π-mediated contacts within hydrophobic regions of proteins. This substantially increases the stability of the ligand within the protein binding pocket. These interactions help maintain the ligand in a favorable spatial orientation inside the binding pocket.

At the same time, an unfavorable donor–donor interaction was observed with His39 (Figure 2). This can be explained by competition between hydrogen bond donor groups of phenylalanine and the histidine residue, resulting in a repulsive interaction. However, as in the case of arginine, this unfavorable contact does not dominate the overall binding process, since it is compensated for by several stabilizing interactions.

From structural and functional perspectives, the binding of phenylalanine is mainly supported by hydrophobic and aromatic interactions rather than strong electrostatic forces. This suggests that phenylalanine occupies a relatively hydrophobic region within the COX-2 binding site. As a result, it may interfere with substrate positioning or stabilize an inactive conformation of the enzyme. Considering that COX-2 activity depends on the proper positioning of arachidonic acid within the hydrophobic channel, the localization of phenylalanine in this region may disrupt substrate binding and reduce enzymatic activity.

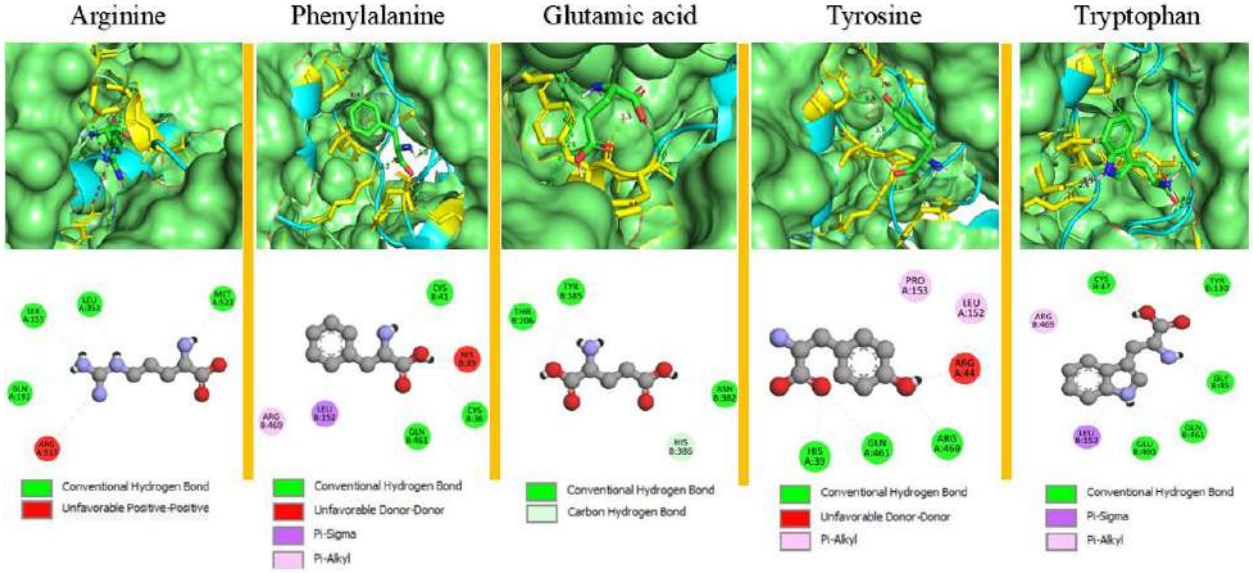


Figure 2. Molecular docking visualization of selected amino acid ligands from the "As-Capsipol" complex within the COX-2 active site, showing the binding modes, interaction networks, and structural conformations of arginine, phenylalanine, glutamic acid, tyrosine, and tryptophan.

Glutamic acid exhibited a binding affinity of −5.5 kcal/mol toward COX-2 (Figure 1). This value corresponds to an estimated Ki value of approximately 9.3×10^{-5} M, or about 93 μM, indicating the presence of moderate inhibitory activity. The docking simulation was performed using a grid box centered at the coordinates center_x = 36.323000, center_y = 26.465000, and center_z = 23.686000. This ensured the proper localization of the ligand within the active site region of the enzyme.

Interaction analysis showed that glutamic acid was stabilized within the binding pocket through several hydrogen bonds involving Thr206, Tyr385, and Asn382, as well as an additional carbon–hydrogen interaction with His386. These amino acid residues are located in the functional region of COX-2 and participate in processes associated with proper ligand positioning and enzymatic activity. In particular, Tyr385 plays an important role in the catalytic mechanism of COX-2 (Figure 2). Its involvement in interaction with the ligand suggests that glutamic acid may influence the functional activity of the enzyme.

Tyrosine showed a binding affinity of −6.4 kcal/mol toward COX-2. Its estimated Ki value was 2.0×10^{-5} M, or approximately 20 μM. This result indicates that tyrosine has a stronger inhibitory potential compared with the other amino

acids present in the "As-Capsipol" complex. The docking simulation was performed using a grid box centered at the coordinates center_x = 36.323000, center_y = 26.465000, and center_z = 23.686000. This allowed the ligand to be properly positioned within the active site of the enzyme (Figure 1).

Interaction analysis demonstrated that tyrosine formed conventional hydrogen bonds with His39, Gln461, and Arg469. These bonds contribute to the stable anchoring of the ligand within the binding pocket. In addition, hydrophobic π–alkyl interactions were observed with Pro153 and Leu152. This indicates that the aromatic ring of tyrosine is well positioned within the hydrophobic region of the active site. An unfavorable donor–donor interaction with Arg44 was also detected, which may be associated with repulsion between hydrogen bond donor groups. However, this unfavorable interaction does not substantially affect the overall stability of the complex.

The combined presence of hydrogen bonds and hydrophobic interactions indicates that tyrosine is effectively stabilized within the functional region of COX-2. While His39 and Gln461 play an important role in anchoring the ligand within the binding pocket, Arg469 provides an additional contribution to its spatial orientation (Figure 2). The involvement of Pro153 and Leu152 highlights the importance of hydrophobic contacts in maintaining the ligand in an appropriate orientation within the binding cavity.

From a functional perspective, the binding of tyrosine in this region may interfere with substrate entry into the active site or alter the spatial arrangement of catalytically important amino acid residues. Since COX-2 is responsible for converting arachidonic acid into pro-inflammatory prostaglandins, occupation of the active site by tyrosine may reduce enzymatic efficiency. Consequently, this interaction may contribute to the anti-inflammatory potential of the "As-Capsipol" complex, particularly under conditions such as hepatitis, where COX-2 expression is increased.

Tryptophan exhibited a binding affinity of −7.1 kcal/mol toward COX-2. Its estimated Ki value was 6.0×10^{-6} M, or approximately 6 μM. This result indicates that tryptophan has a stronger inhibitory potential than the other amino acids identified in the "As-Capsipol" complex. The docking analysis was performed using a grid box centered at the coordinates center_x = 36.323000, center_y = 26.465000, and center_z = 23.686000. This ensured precise positioning of the ligand within the active site of the enzyme (Figure 1).

The interaction profile showed that tryptophan was stabilized within the COX-2 binding pocket through several conventional hydrogen bonds involving Cys47, Tyr130, Gly45, Gln461, and Glu465 (Figure 1). These amino acid residues facilitate the anchoring of the ligand in the active site and support its proper spatial orientation. In addition to hydrogen bonds, π-related interactions, including π–sigma and π–alkyl contacts with Leu152 and Arg469, were also observed. This suggests that the aromatic indole ring of tryptophan plays an important role in stabilizing the ligand within the hydrophobic microenvironment.

The combined presence of polar and hydrophobic interactions indicates that tryptophan is well positioned within the functional region of COX-2. Gln461 and Glu465 contribute to stabilization through hydrogen bonding, whereas Tyr130 and Cys47 further strengthen ligand binding through polar contacts. The aromatic structure of tryptophan enables it to form strong interactions with hydrophobic amino acid residues. This may explain its relatively high binding affinity.

Functionally, the localization of tryptophan within the active site may significantly hinder substrate entry or disrupt the catalytic microenvironment required for prostaglandin synthesis. Since COX-2 is one of the key enzymes involved in the production of pro-inflammatory mediators, the strong binding of tryptophan may lead to reduced enzymatic activity. These findings suggest that tryptophan may play an important role in the anti-inflammatory effect of the "As-Capsipol" complex. This is particularly relevant for pathological conditions such as hepatitis, where COX-2 is highly expressed.

CONCLUSION

The molecular docking analyses demonstrated that the selected amino acids contained in the "As-Capsipol" dietary supplement are capable of forming stable interactions with the active site of the COX-2 enzyme. In particular, the relatively high binding affinities of tryptophan and tyrosine suggest that they may play an important role in modulating inflammatory processes at the molecular level. Arginine, phenylalanine, and glutamic acid were also found to interact with COX-2 through hydrogen bonds, hydrophobic interactions, and electrostatic interactions, thereby potentially contributing to the overall biological activity of the complex. The obtained results provide an in silico-based scientific rationale for the anti-inflammatory potential of the "As-Capsipol" complex and establish a theoretical basis for further experimental studies.

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