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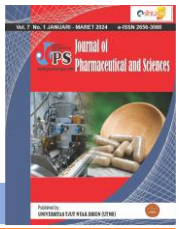

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Prediction of the Potential of Active Compounds in Kratom Leaves (Mitragyna speciosa) as Anti-Inflammatory Candidates through a Molecular Docking Approach to COX-1, COX-2, TNF-α, and IL-1β

Prediksi Potensi Senyawa Aktif Daun Kratom (Mitragyna speciosa) sebagai Kandidat Antiinflamasi melalui Pendekatan Molecular Docking terhadap COX-1, COX-2, TNF-α, dan IL-1β

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Abstract

Inflammation is a central component of numerous pathological conditions, and the limitations of currently available anti-inflammatory therapies continue to motivate the search for structurally diverse therapeutic candidates. Mitragyna speciosa Korth. contains bioactive indole alkaloids, particularly mitragynine and 7-hydroxymitragynine, that have been associated with anti-inflammatory effects. This study evaluated the predicted binding affinity and ligand-protein interaction profiles of mitragynine and 7-hydroxymitragynine against four inflammation-related targets—cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α)—using molecular docking. Protein structures were obtained from the Protein Data Bank (PDB IDs 1EQG, 3LN1, 5R87, and 3EWJ, respectively). Protein and ligand preparation and interaction visualization were performed using Discovery Studio Visualizer, docking was conducted using PLANTS, and protocol validation was assessed by redocking and root mean square deviation (RMSD) analysis in Molegro Molecular Viewer. All protocols met the prespecified RMSD acceptance criterion of ≤2.0 Å, with values of 1.57 Å for COX-1, 0.77 Å for COX-2, 1.36 Å for IL-1β, and 0.55 Å for TNF-α. Within each protein target, 7-hydroxymitragynine showed the most favorable predicted score among the tested alkaloids for COX-1 and COX-2 (−17.39 and −19.56 kcal/mol, respectively), whereas mitragynine showed the more favorable predicted score for IL-1β and TNF-α (−16.72 and −20.42 kcal/mol, respectively). These results indicate target-dependent binding behavior and provide a computational basis for further structural, pharmacokinetic, and experimental evaluation of both alkaloids as potential anti-inflammatory candidates.

Keywords: Mitragyna speciosa, mitragynine, 7-hydroxymitragynine, molecular docking, anti-inflammatory.

Abstrak

Inflamasi merupakan komponen penting dalam berbagai kondisi patologis, sedangkan keterbatasan terapi antiinflamasi yang tersedia saat ini mendorong pencarian kandidat terapi dengan keragaman struktur yang lebih luas. Mitragyna speciosa Korth. mengandung alkaloid indol bioaktif, terutama mitraginin dan 7-hidroksimitraginin, yang telah dikaitkan dengan efek antiinflamasi. Penelitian ini mengevaluasi prediksi afinitas pengikatan dan profil interaksi ligan-protein mitraginin serta 7-hidroksimitraginin terhadap empat target yang berperan dalam inflamasi, yaitu cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), interleukin-1 beta (IL-1β), dan tumor necrosis factor-alpha (TNF-α), menggunakan molecular docking. Struktur protein diperoleh dari Protein Data Bank dengan PDB ID 1EQG, 3LN1, 5R87, dan 3EWJ. Preparasi protein dan ligan serta visualisasi interaksi dilakukan menggunakan Discovery Studio Visualizer, docking dilakukan menggunakan PLANTS, sedangkan validasi protokol dilakukan melalui redocking dan analisis root mean square deviation (RMSD) menggunakan Molegro Molecular Viewer. Seluruh protokol memenuhi kriteria penerimaan RMSD yang telah ditetapkan, yaitu ≤2,0 Å, dengan nilai 1,57 Å untuk COX-1, 0,77 Å untuk COX-2, 1,36 Å untuk IL-1β, dan 0,55 Å untuk TNF-α. Pada masing-masing target protein, 7-hidroksimitraginin menunjukkan skor prediksi yang paling menguntungkan di antara alkaloid uji terhadap COX-1 dan COX-2 (masing-masing −17,39 dan −19,56 kkal/mol), sedangkan mitraginin menunjukkan skor prediksi yang lebih menguntungkan terhadap IL-1β dan TNF-α (masing-masing −16,72 dan −20,42 kkal/mol). Hasil ini menunjukkan pola pengikatan yang bergantung pada target dan memberikan dasar komputasional untuk evaluasi struktural, farmakokinetik, serta eksperimental lebih lanjut terhadap kedua alkaloid sebagai kandidat antiinflamasi potensial.

Kata Kunci: Mitragyna speciosa, mitragynine, 7-hydroxymitragynine, molecular docking, anti-inflamasi.

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Introduction

Inflammation is a coordinated host response to infection, tissue injury, and other harmful stimuli, mediated by interconnected cellular pathways that include cyclooxygenase enzymes and pro-inflammatory cytokines [1–4]. Although acute inflammation contributes to host defense and tissue repair, persistent or dysregulated signaling is implicated in chronic disorders, including atherosclerotic, autoimmune, metabolic, and neoplastic diseases [1,3,4]. Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), together with tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), are therefore relevant molecular targets in inflammatory disease [5,6]. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce prostaglandin synthesis through COX inhibition, but their use may be limited by gastrointestinal, renal, and cardiovascular adverse effects depending on the drug, dose, duration, and patient risk profile [7–10]. Biologic agents directed against cytokine pathways can be highly effective in selected diseases, yet cost, parenteral administration, and susceptibility to infection remain clinically important considerations [6,10]. These limitations continue to support the search for mechanistically distinct anti-inflammatory candidates [5,11].

Natural products remain a major source of chemically diverse scaffolds for drug discovery [11,12]. Mitragyna speciosa Korth. (kratom), a Southeast Asian medicinal plant, has attracted pharmacological interest because its indole alkaloids—particularly mitragynine and 7-hydroxymitragynine—exhibit diverse biological activities [13–17,26]. Recent experimental evidence further supports an anti-inflammatory signal associated with kratom preparations and alkaloid-rich fractions. An ethanolic M. speciosa extract reduced inflammatory mediator and cytokine expression in vitro [27], while an alkaloid extract containing approximately 46% mitragynine suppressed COX-2/5-LOX-related inflammatory responses and reduced TNF-α in LPS-stimulated RAW 264.7 macrophages [28]. In vivo work on a traditional Thai herbal kratom preparation also demonstrated anti-inflammatory activity in carrageenan-induced paw edema and ear inflammation models, although the multicomponent formulation prevents attribution of the effect to a single alkaloid [29]. Collectively, these findings support mechanistic investigation of kratom alkaloids at defined inflammation-related molecular targets.

Despite increasing pharmacological evidence, direct structural information on the recognition of major kratom alkaloids by canonical inflammatory targets remains incomplete [13,21,22,26]. Previous computational work has addressed other targets or broader network-pharmacology frameworks, but a within-study comparison of mitragynine and 7-hydroxymitragynine across COX-1, COX-2, IL-1β, and TNF-α using a single validated docking workflow remains limited. Molecular docking is appropriate for generating structural hypotheses regarding plausible ligand orientations and residue-level contacts within defined binding regions, but its scores are model-dependent and should be interpreted as predictions rather than measurements of biological potency [22,23]. The therapeutic interpretation of kratom also requires caution because its pharmacological effects are accompanied by safety concerns related to dose, product composition, co-exposure, dependence liability, and reported organ toxicity [13,18,24–26]. Accordingly, structural docking findings should not be extrapolated directly to clinical efficacy or safety. Instead, they are most informative when used to prioritize mechanistic hypotheses for subsequent molecular-dynamics, pharmacokinetic, toxicological, and experimental validation.

Accordingly, this study investigated the predicted molecular interactions of mitragynine and 7-hydroxymitragynine with COX-1, COX-2, IL-1β, and TNF-α using a common, validated molecular-docking protocol. The novelty of the analysis lies in the comparative evaluation of the two principal kratom alkaloids against four canonical inflammation-related targets within the same computational framework, with protocol validity assessed by redocking. Docking scores and ligand-protein interaction patterns were interpreted

within each target and compared with the corresponding crystallographic reference ligand to identify target-dependent differences in predicted binding behavior.

Methods

Materials and Apparatus

The molecular docking study was conducted to evaluate the potential anti-inflammatory activity of the major bioactive compounds of Mitragyna speciosa against four inflammatory protein targets, namely cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α). The three-dimensional structures of the target proteins were obtained from the Protein Data Bank (PDB) with PDB IDs 1EQG, 3LN1, 5R87, and 3EWJ, respectively.

Protein preparation and visualization of protein-ligand interactions were performed using Discovery Studio Visualizer. Molecular docking calculations were carried out using PLANTS (Protein-Ligand ANT System), and docking-protocol validation was performed by redocking the crystallographic ligands and calculating root mean square deviation (RMSD) values using Molegro Molecular Viewer.

The test compounds were mitragynine and 7-hydroxymitragynine, selected because they are pharmacologically important alkaloids of M. speciosa. Three-dimensional ligand structures were obtained from PubChem and prepared before docking. The corresponding co-crystallized ligands from each PDB structure were used as reference ligands for protocol validation and intra-target comparison.

Target Protein Preparation

The three-dimensional structures of COX-1, COX-2, IL-1β, and TNF-α were downloaded from the Protein Data Bank using PDB IDs 1EQG, 3LN1, 5R87, and 3EWJ, respectively. The selected protein structures were prepared using Discovery Studio Visualizer.

Protein preparation included removal of crystallographic water molecules and non-essential heteroatoms. The co-crystallized ligand was retained only for identification of the experimental binding site and for redocking validation; it was removed from the receptor structure before docking mitragynine and 7-hydroxymitragynine. Hydrogen atoms were added, and the prepared receptor structures were saved in a format compatible with PLANTS. For each target, the docking region for the test compounds was defined from the position of the corresponding co-crystallized ligand. The same binding-site definition used for redocking the native ligand was then applied to mitragynine and 7-hydroxymitragynine, thereby maintaining an identical search region for intra-target comparison.

Docking Method Validation

The molecular docking protocols for COX-1, COX-2, IL-1β, and TNF-α were validated using a redocking procedure. The respective co-crystallized ligands were separated from their protein structures and subsequently redocked into the corresponding binding sites using PLANTS under the same docking conditions applied to the test compounds.

The reliability of each docking protocol was evaluated by comparing the experimentally determined crystallographic ligand conformation with the predicted conformation obtained from redocking. The comparison was performed using Molegro Molecular Viewer by calculating the root mean square deviation (RMSD) between the crystallographic and redocked ligand poses.

An RMSD value of ≤2.0 Å was used as the acceptance criterion for the docking protocol, indicating that the docking procedure was able to reproduce the experimentally observed ligand-binding orientation with acceptable accuracy. The RMSD values obtained for COX-1 (1EQG), COX-2 (3LN1), IL-1β (5R87), and TNF-α (3EWJ) were recorded and used to determine the validity of each docking protocol before proceeding with the test-compound docking experiments.

Test Compound Preparation

Mitragynine and 7-hydroxymitragynine were prepared from three-dimensional structures obtained from PubChem. Hydrogen atoms were added and the ligand geometries were converted to a format compatible with PLANTS. Reference ligands corresponding to the co-crystallized ligand of each target were processed using the same general preparation workflow to support redocking validation and within-target comparison.

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Reference ligands corresponding to the native ligands of each protein target were also prepared using the same general procedure. These reference compounds were used for comparison of the predicted binding affinity and interaction profiles of the test compounds.

Molecular Docking of Test Ligands to the Proteins

Mitragynine and 7-hydroxymitragynine were independently docked to COX-1, COX-2, IL-1β, and TNF-α using PLANTS. For each receptor, docking was performed within the binding region defined by its co-crystallized ligand, and the same validated search-space definition was applied to the reference and test ligands. The docking settings used in the original calculation were maintained consistently for all ligands evaluated within a given target.

The generated poses were ranked according to the PLANTS docking score. For each ligand-target pair, the pose reported for structural interpretation was the pose with the most favorable score that remained appropriately located within the predefined binding region. This selection criterion was applied consistently to the reference ligands and the two test alkaloids.

The docking scores obtained for mitragynine and 7-hydroxymitragynine were compared with those of the respective reference ligands. More favorable docking scores were interpreted as indicating stronger predicted ligand-protein interactions within the same docking protocol. However, docking scores were interpreted as computational predictions and were not considered direct evidence of biological activity.

Analysis and Visualization of Molecular Docking Results

The molecular docking results were analyzed based on docking scores, ligand binding poses, and protein-ligand interaction patterns. The best-ranked poses of mitragynine and 7-hydroxymitragynine for each target protein were subjected to further structural analysis.

Protein-ligand interactions were visualized using Discovery Studio Visualizer. The interactions analyzed included hydrogen bonds, hydrophobic interactions, π-π interactions, π-alkyl interactions, electrostatic interactions, and other relevant non-covalent interactions between the ligand and amino acid residues within the binding pocket.

The amino acid residues involved in the interactions were identified and compared between the test compounds and their corresponding reference ligands. Particular attention was given to residues located within the established binding sites of COX-1, COX-2, IL-1β, and TNF-α.

The docking scores and interaction profiles were subsequently compared among the two test compounds and the reference ligands. For COX-1 and COX-2, the relative docking scores and interaction patterns were further evaluated to determine the predicted selectivity profile of the compounds toward COX-2. The combined docking results were used to identify mitragynine or 7-hydroxymitragynine as potential candidate compounds for further investigation as anti-inflammatory agents.

Results and Discussion

Validation of the Docking Protocol

Validation of the molecular docking protocol was performed to ensure that the applied method could consistently reproduce the ligand orientation within the protein binding site. Validation was conducted by redocking the reference ligand into each target protein, followed by evaluation using the root mean square deviation (RMSD) value. RMSD represents the degree of similarity between the ligand pose obtained from redocking and the experimentally determined pose in the crystal structure. In general, an RMSD value below 2 Å indicates that the docking protocol is capable of reproducing the reference ligand pose with acceptable accuracy.

In this study, validation was performed for four target proteins: COX-1 (PDB ID: 1EQG), COX-2 (PDB ID: 3LN1), IL-1β (PDB ID: 5R87), and TNF-α (PDB ID: 3EWJ). All target proteins exhibited RMSD values below 2 Å. COX-1 (1EQG) produced an RMSD value of 1.57 Å, COX-2 (3LN1) produced 0.77 Å, IL-1β (5R87) produced 1.36 Å, and TNF-α (3EWJ) produced 0.55 Å.

The lowest RMSD value was obtained for TNF-α (3EWJ; 0.55 Å), whereas COX-1 (1EQG) showed the highest value (1.57 Å). All values nevertheless remained below the prespecified 2.0 Å threshold. Differences in RMSD among targets can arise from target-specific binding-site geometry, ligand conformational flexibility, and the extent to which the docking search reproduces the crystallographic pose. Because these factors were

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not separately quantified in the present study, the higher COX-1 RMSD should be interpreted as a target-specific validation result rather than attributed to a single structural cause.

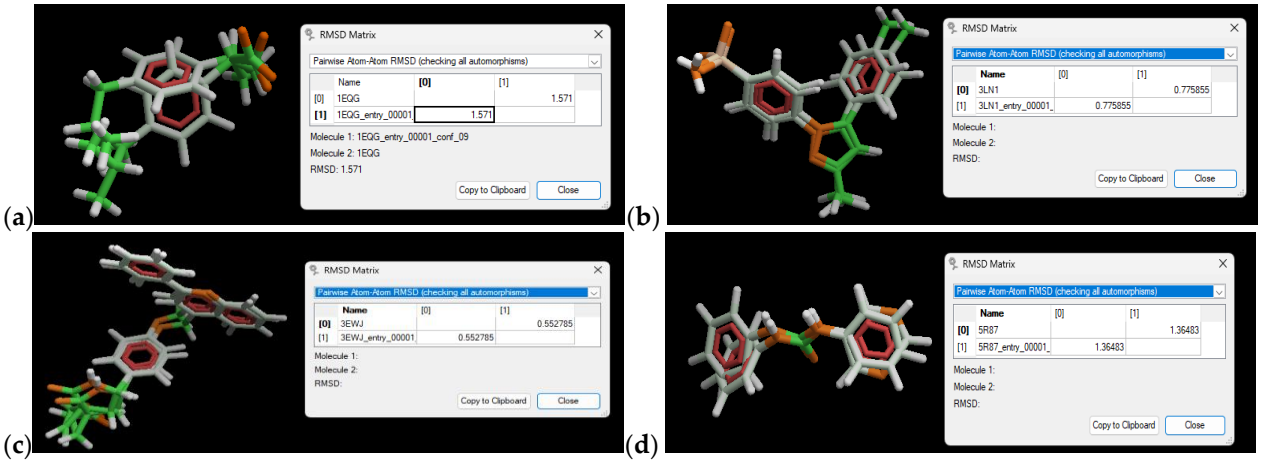

Figure 1. RMSD of the test protein (**a**) COX-1 (1EQG), (**b**) COX-2 (3LN1) , (**c**) TNF-α (3EWJ), (**d**) IL-1β (5R87)**.**

Molecular docking of mitragynine and 7-hydroxymitragynine was subsequently performed using PLANTS. Protein preparation and protein–ligand interaction visualization were conducted using Discovery Studio, while RMSD validation was performed using Molegro. Based on these validation results, the docking protocol was considered valid and suitable for evaluating the interactions of mitragynine and 7-hydroxymitragynine with COX-1, COX-2, IL-1β, and TNF-α.

Molecular Docking with COX-1

COX-1 was represented by the PDB structure 1EQG, with ibuprofen used as the reference ligand. The docking results showed that ibuprofen produced a best docking score of −69.62 kJ/mol or −16.63 kcal/mol at conformer 10. Mitragynine produced a best docking score of −54.68 kJ/mol or −13.06 kcal/mol at conformer 9, whereas 7-hydroxymitragynine produced the most negative docking score, −72.79 kJ/mol or −17.39 kcal/mol, at conformer 10.

Within COX-1, 7-hydroxymitragynine produced the most negative docking score among the evaluated ligands (−72.79 kJ/mol; −17.39 kcal/mol), compared with ibuprofen (−69.62 kJ/mol; −16.63 kcal/mol) and mitragynine (−54.68 kJ/mol; −13.06 kcal/mol). Under the applied docking conditions, this indicates the most favorable predicted binding score for 7-hydroxymitragynine within this target. The result does not, however, establish greater COX-1 inhibitory activity because docking scores are computational estimates and do not incorporate enzyme kinetics, protein dynamics, solvation, pharmacokinetics, or experimental efficacy.

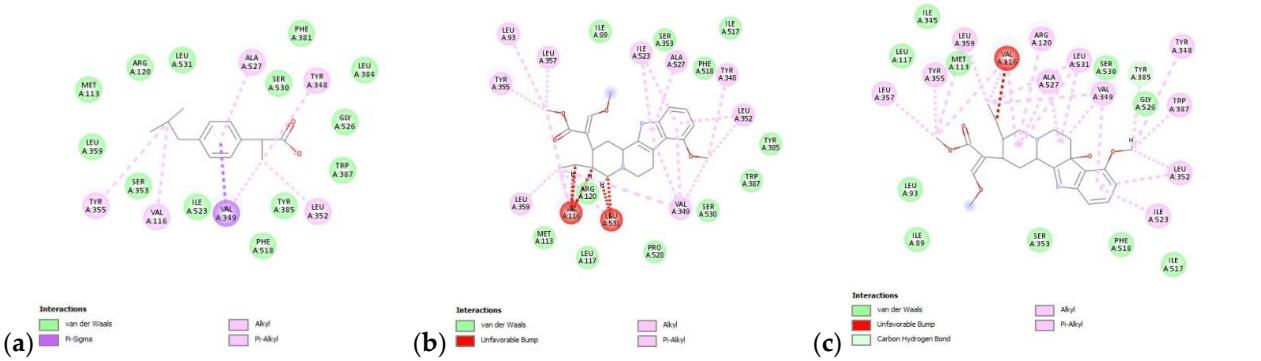

Figure 2. Visualization of docking best score Protein 1EQG (**a**) Native-Ibuprofen, (**b**) mitragynine (**c**) 7-hydroxymitragynine

The difference in affinity between mitragynine and 7-hydroxymitragynine may be associated with their structural differences. The hydroxyl group at position 7 in 7-hydroxymitragynine may enhance the ability of the molecule to establish polar interactions or hydrogen bonds with residues within the binding site. In addition, the indole scaffold and substituents of 7-hydroxymitragynine may contribute to hydrophobic interactions that help maintain the ligand orientation within the binding pocket.

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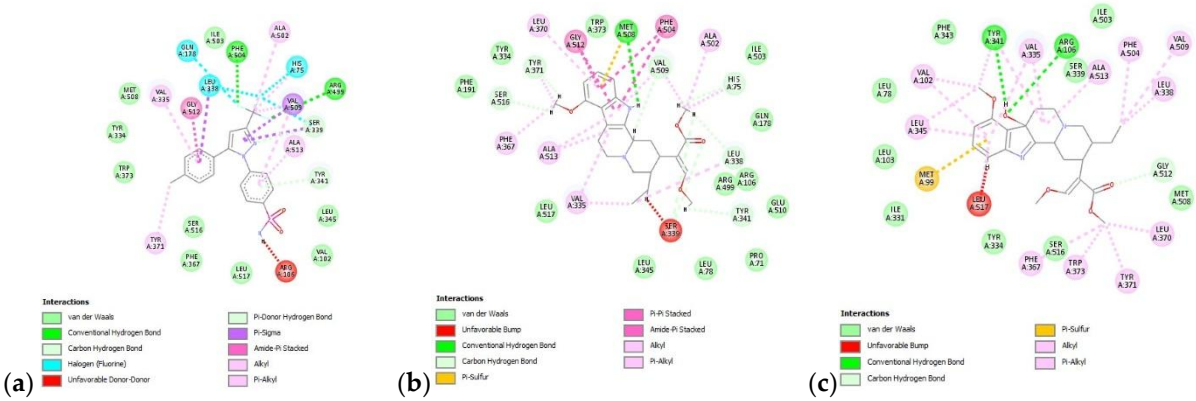
Several residues were identified around the docked pose of 7-hydroxymitragynine, including Arg120, Ala527, and Leu531 in one conformer, as well as Val349, Tyr348, and Trp387 in another conformer. These residues indicate the presence of both hydrophobic and polar environments surrounding the ligand. However, the presence of a residue in a docking pose does not by itself confirm a specific type of interaction. Therefore, identification of hydrogen bonds, hydrophobic interactions, or other interaction types should be supported by interatomic distance analysis and three-dimensional visualization.

Although 7-hydroxymitragynine produced a more negative docking score than ibuprofen, this result cannot directly be interpreted as evidence that 7-hydroxymitragynine has greater COX-1 inhibitory activity than ibuprofen. Docking scores represent computational predictions and do not account for all factors determining the actual biological activity of a compound.

Molecular Docking with COX-2

COX-2 was represented by the PDB structure 3LN1, with celecoxib used as the reference ligand. Celecoxib produced a best docking score of −107.43 kJ/mol or −25.66 kcal/mol at conformer 3. Mitragynine produced a best docking score of −65.91 kJ/mol or −15.74 kcal/mol at conformer 9, whereas 7-hydroxymitragynine produced a docking score of −81.89 kJ/mol or −19.56 kcal/mol at conformer 3.

Within COX-2, 7-hydroxymitragynine produced a more negative docking score than mitragynine, suggesting a more favorable predicted interaction under the applied protocol. Its score remained less negative than that of celecoxib. These results therefore support a target-specific structural interaction of 7-hydroxymitragynine with the COX-2 binding region, but they should not be interpreted as evidence of celecoxib-like potency or clinical anti-inflammatory efficacy.



(**a**) (**b**) (**c**)

Figure 3. Visualization of docking best score Protein 3LN1 (**a**) Native- celecoxib, (**b**) mitragynine (**c**) 7-hydroxymitragynine

Several residues were identified around the 7-hydroxymitragynine docking pose, including Leu345, Val102, and Val335. Other residues identified in several relatively favorable poses included Ala513, Phe504, Val509, Leu338, Leu370, and Tyr371. The presence of several hydrophobic residues within the interaction environment may indicate a contribution of hydrophobic interactions in maintaining the ligand within the binding site.

The more favorable COX-2 score of 7-hydroxymitragynine relative to mitragynine may be related to changes in polarity and local complementarity introduced by the 7-hydroxyl substituent. A hydroxyl group can create additional opportunities for polar contacts, but any energetic benefit depends on ligand orientation, donor-acceptor geometry, desolvation, and the local binding environment. Because hydrogen-bond distances and per-interaction energies were not available in the supplied docking output, the contribution of the 7-hydroxyl group cannot be quantified from the present dataset and should be regarded as a structure-based hypothesis rather than a demonstrated mechanism.

Molecular Docking with IL-1β

IL-1β was represented by the PDB structure 5R87, with the native ligand K0G, N-phenyl-N′-pyridin-3-ylurea, used as the reference ligand. The native ligand produced a best docking score of −68.99 kJ/mol or −16.48 kcal/mol. Mitragynine produced a best docking score of −69.99 kJ/mol or −16.72 kcal/mol at conformer 8, whereas 7-hydroxymitragynine produced a docking score of −67.33 kJ/mol or −16.08 kcal/mol at conformer 3.

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In contrast to the results obtained for COX-1 and COX-2, mitragynine exhibited the most negative docking score against IL-1β. This value was slightly more negative than that of the native ligand K0G. These results indicate that, under the applied docking conditions, mitragynine has a favorable predicted interaction with the IL-1β binding site.

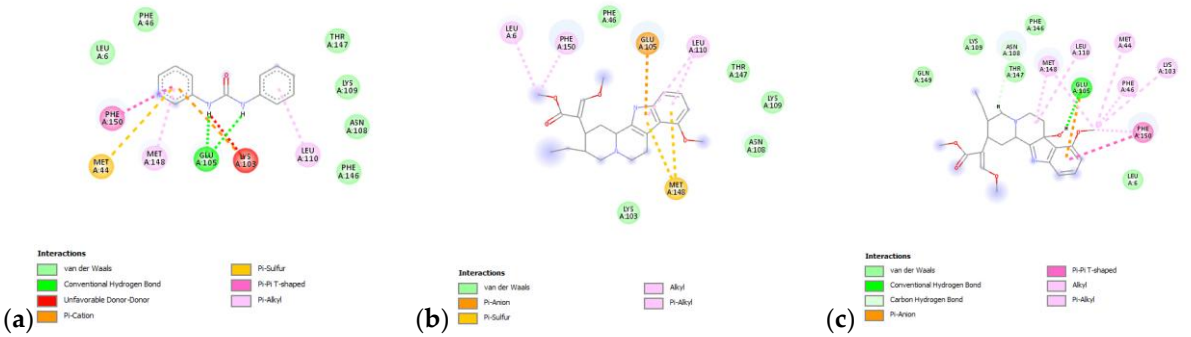

Figure 4. Visualization of docking best score Protein 5R87 (**a**) Native- (K0G) N-phenyl-N'-pyridin-3-ylurea, (**b**) mitragynine (**c**) 7-hydroxymitragynine

Mitragynine interacted with several residues, including Leu6, Phe150, Leu110, Glu150, and Met148. Several of these residues, particularly Phe150, Leu110, and Met148, were located within the ligand-binding environment. Similarity or proximity of interacting residues may provide an initial indication that mitragynine occupies a binding region comparable to that of the reference ligand. However, this interpretation should be further supported by pose-overlay analysis and three-dimensional structural visualization.

For 7-hydroxymitragynine, the identified residues included Glu105, Asn108, Met148, Leu110, Met44, Phe46, Lys103, and Phe150. Although several residues were identified around the ligand, its docking score remained slightly less negative than those of mitragynine and the native ligand.

These results demonstrate that the presence of a hydroxyl group in 7-hydroxymitragynine does not necessarily improve docking affinity. Although a hydroxyl group can increase the potential for polar interactions, it may also influence charge distribution, ligand orientation, flexibility, and shape complementarity with the binding site. Therefore, the effect of structural modification on docking affinity depends on the specific characteristics of the target protein.

Molecular Docking with TNF-α

TNF-α was represented by the PDB structure 3EWJ, with native ligand 642 used as the reference ligand. The native ligand produced a best docking score of −121.92 kJ/mol or −29.12 kcal/mol at conformer 5. Mitragynine produced a best docking score of −85.49 kJ/mol or −20.42 kcal/mol at conformer 6, whereas 7-hydroxymitragynine produced a docking score of −75.49 kJ/mol or −18.03 kcal/mol at conformer 10.

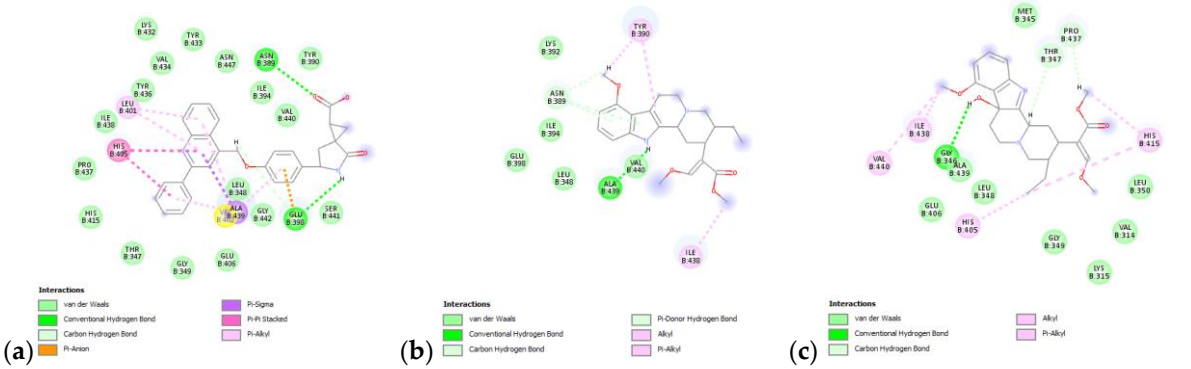

Figure 5. Visualization of docking best score Protein 3EWJ (a) Native ligand (642) (1S,3R,6S)-4-oxo-6-{4-[(2-phenylquinolin-4-yl)methoxy]phenyl}-5-azaspiro[2.4]heptane-1-carboxylic acid, (b) mitragynine (c) 7-hydroxymitragynine

Within TNF-α, mitragynine produced a more negative docking score than 7-hydroxymitragynine and therefore showed the more favorable predicted score of the two test alkaloids under the applied docking conditions. Both test compounds remained less negative than the native ligand 642. This comparison is

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restricted to the TNF-α target and should not be interpreted as a direct measure of cytokine inhibition or biological efficacy.

Mitragynine interacted with Tyr390, Ile438, Asn389, and Ala439. Tyr390 and Ile438 may contribute to hydrophobic interactions, whereas Asn389 has polar characteristics that may facilitate polar interactions with the ligand. The combination of these interactions may contribute to maintaining the position of mitragynine within the binding site.

Meanwhile, 7-hydroxymitragynine interacted with Thr347, Pro437, His405, His415, Ile438, Val440, and Gly346. Although a greater number of residues were identified, its docking score remained less negative than that of mitragynine. This finding demonstrates that the number of interacting residues is not the sole determinant of protein–ligand interaction strength. Ligand orientation, interatomic distances, interaction types, and geometric complementarity also contribute to the resulting docking score.

Docking Affinity Analysis

The overall docking results revealed distinct affinity patterns between mitragynine and 7-hydroxymitragynine across the four target proteins. A comparison of the best docking scores obtained for each compound and reference ligand is presented in Table 1.

Table 1. All scores docking

Target	Reference ligand	Mitragynine	7-Hydroxymitragynine
COX-1 (1EQG)	−69.62 kJ/mol or −16.63 kcal/mol	−54.68 kJ/mol or −13.06 kcal/mol	−72.79 kJ/mol or −17.39 kcal/mol
COX-2 (3LN1)	−107.43 kJ/mol or −25.66 kcal/mol	−65.91 kJ/mol or −15.74 kcal/mol	−81.89 kJ/mol or −19.56 kcal/mol
IL-1β (5R87)	−68.99 kJ/mol or −16.48 kcal/mol	−69.99 kJ/mol or −16.72 kcal/mol	−67.33 kJ/mol or −16.08 kcal/mol
TNF-α (3EWJ)	−121.92 kJ/mol or −29.12 kcal/mol	−85.49 kJ/mol or −20.42 kcal/mol	−75.49 kJ/mol or −18.03 kcal/mol

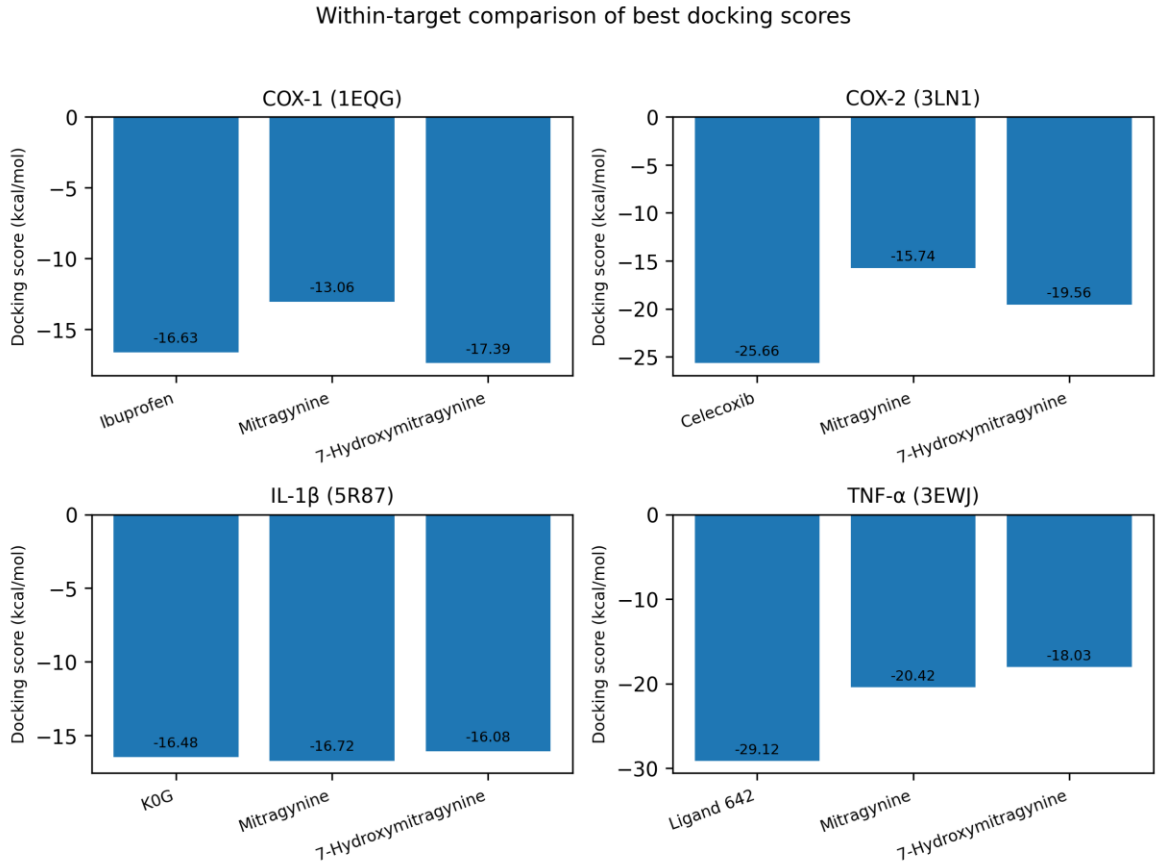

Figure 6. Within-target comparison of the best docking scores for the reference ligand, mitragynine, and 7-hydroxymitragynine. Numerical values should be interpreted only within each protein target and not as an absolute cross-target ranking.

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For COX-1, 7-hydroxymitragynine produced the most negative docking score, −72.79 kJ/mol (−17.39 kcal/mol), compared with ibuprofen at −69.62 kJ/mol (−16.63 kcal/mol) and mitragynine at −54.68 kJ/mol (−13.06 kcal/mol). A similar pattern was observed for COX-2, where 7-hydroxymitragynine produced a docking score of −81.89 kJ/mol (−19.56 kcal/mol), which was more negative than mitragynine at −65.91 kJ/mol (−15.74 kcal/mol) but less negative than celecoxib at −107.43 kJ/mol (−25.66 kcal/mol).

In contrast, mitragynine produced the most negative docking score against IL-1β, at −69.99 kJ/mol (−16.72 kcal/mol), slightly more negative than the native ligand K0G at −68.99 kJ/mol (−16.48 kcal/mol) and 7-hydroxymitragynine at −67.33 kJ/mol (−16.08 kcal/mol). Against TNF-α, mitragynine also showed a more negative docking score than 7-hydroxymitragynine, at −85.49 kJ/mol (−20.42 kcal/mol) and −75.49 kJ/mol (−18.03 kcal/mol), respectively. However, both compounds remained less negative than the native ligand 642, which produced a score of −121.92 kJ/mol (−29.12 kcal/mol).

Across the four targets, the two alkaloids displayed distinct within-target docking patterns: 7-hydroxymitragynine had the more favorable predicted score of the two alkaloids for COX-1 and COX-2, whereas mitragynine had the more favorable predicted score for IL-1β and TNF-α. This pattern is consistent with a target-dependent effect of the 7-hydroxyl substitution on ligand recognition. Importantly, docking scores from different proteins were not used to rank absolute potency because each receptor has a distinct binding-site geometry, composition, and scoring landscape.

Docking scores were therefore interpreted only within the same protein target. Cross-protein numerical differences were not treated as evidence that a compound binds one target more strongly than another, because PLANTS scores are influenced by receptor-specific binding-site characteristics and by differences in ligand and pocket physicochemical properties.

Comparative Interaction Analysis

Comparison of the interaction patterns revealed distinct binding characteristics between mitragynine and 7-hydroxymitragynine across the target proteins. For COX-1 and COX-2, 7-hydroxymitragynine produced more negative docking scores than mitragynine. In contrast, mitragynine produced more negative docking scores than 7-hydroxymitragynine against IL-1β and TNF-α.

Table 2. Comparison of Main Residues

Target	Ligand	Residues
COX-1 (1EQG)	Ibuprofen	Val349, Tyr355, Val116, Leu352, Tyr348, Ala527
	Mitragynine	Val116, Leu531, Leu93, Tyr355, Leu357, Ile523, Ala527, Tyr348, Leu352, Val349, Leu359
	7-Hydroxymitragynine	Val116, Leu357, Tyr355, Leu359, Arg120, Ala527, Leu531, Val349, Tyr348, Trp387, Leu352, Ile523, Tyr385
COX-2 (3LN1)	Celecoxib	Arg106, Gln178, Leu338, His75, Gly512, Val509, Val355, Ala502, Ala513, Tyr371, Phe504, Arg495
	Mitragynine	Ser339, Met508, Gly512, Phe504, Leu370, Ala503, Val335, Phe376, Ser516, Tyr371, Val509, His75, Leu388
	7-Hydroxymitragynine	Tyr341, Arg106, Ser516, Leu345, Val102, Val335, Ala513, Phe504, Val509, Leu338, Leu370, Tyr371, Trp373, Phe367, Leu517, Met99
IL-1β (5R87)	K0G	Lys103, Glu105, Phe150, Met148, Leu110, Met44
	Mitragynine	Leu6, Phe150, Leu110, Glu150, Met148
	7-Hydroxymitragynine	Glu105, Asn108, Met148, Leu110, Met44, Phe46, Lys103, Phe150
TNF-α (3EWJ)	Ligand 642	Asn389, Glu398, Ala439, His405, Leu401
	Mitragynine	Tyr390, Ile438, Asn389, Ala439
	7-Hydroxymitragynine	Thr347, Pro437, His405, His415, Ile438, Val440, Gly346

For COX-1, the 7-hydroxymitragynine docking poses involved Arg120, Ala527, Leu531, Val349, Tyr348, and Trp387. For COX-2, the identified residues included Leu345, Val102, Val335, Ala513, Phe504, Val509, Leu338, Leu370, and Tyr371. The presence of several hydrophobic residues in the interaction environment of both targets may contribute to the stabilization of the ligand through hydrophobic interactions.

For IL-1β, mitragynine interacted with Leu6, Phe150, Leu110, Glu150, and Met148, whereas 7-hydroxymitragynine interacted with Glu105, Asn108, Met148, Leu110, Met44, Phe46, Lys103, and Phe150. For TNF-α, mitragynine interacted with Tyr390, Ile438, Asn389, and Ala439, while 7-hydroxymitragynine involved Thr347, Pro437, His405, His415, Ile438, Val440, and Gly346.

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These differences indicate that relatively small structural changes between mitragynine and 7-hydroxymitragynine can influence ligand orientation and complementarity within different protein binding sites. The hydroxyl group in 7-hydroxymitragynine may provide an additional opportunity for polar interactions; however, this modification did not consistently result in improved docking affinity across all targets. In COX-1 and COX-2, the hydroxyl group may favor ligand complementarity with the binding sites, whereas in IL-1β and TNF-α, the structural modification may result in a less favorable orientation or molecular fit.

The number of identified interacting residues should also not be considered the sole indicator of interaction strength. A ligand involving more residues does not necessarily produce a more negative docking score. Ligand orientation, interaction type, interatomic distance, energy distribution, and geometric complementarity all contribute to the overall docking score.

Overall, the interaction patterns suggest that the two compounds may exhibit distinct multitarget interaction profiles. 7-hydroxymitragynine was more prominent against the COX targets, whereas mitragynine showed more favorable docking toward IL-1β and TNF-α. These findings provide an initial indication that the potential anti-inflammatory activity of the two compounds may involve multiple molecular pathways.

Biological Implications and Study Limitations

The findings indicate that mitragynine and 7-hydroxymitragynine exhibit predicted interactions with several proteins associated with inflammatory processes. 7-Hydroxymitragynine demonstrated the most favorable docking scores toward COX-1 and COX-2, whereas mitragynine showed the most favorable docking scores toward IL-1β and TNF-α. This pattern provides an initial indication of the potential involvement of both compounds in multiple inflammation-related molecular targets.

For COX-1, 7-hydroxymitragynine produced a more negative docking score than ibuprofen. For COX-2, 7-hydroxymitragynine exhibited a more negative score than mitragynine but remained less negative than celecoxib. For IL-1β, mitragynine produced a slightly more negative docking score than the native ligand K0G, whereas for TNF-α, both mitragynine and 7-hydroxymitragynine exhibited less negative scores than the native ligand 642.

These findings cannot directly establish that mitragynine or 7-hydroxymitragynine possesses greater pharmacological activity than the reference drugs or native ligands. Molecular docking provides a computational prediction of the potential interaction between a ligand and a protein under defined conditions. Actual biological activity is influenced by various additional factors, including target selectivity, protein dynamics, complex stability, pharmacokinetic properties, metabolism, bioavailability, and compound toxicity.

The present results should be interpreted within the inherent constraints of a docking-based study. The calculations represent ligand recognition in selected static protein conformations and do not fully capture receptor flexibility, explicit solvent effects, or the time-dependent stability of protein-ligand complexes. Molecular-dynamics simulations were not performed, ADMET properties were not evaluated, and the docking predictions were not validated experimentally. In addition, selection of a single best-scoring pose may introduce conformational-selection bias when multiple poses have similar scores. Consequently, the docking patterns reported here should be considered structural hypotheses requiring confirmation through complementary computational and experimental approaches.

Therefore, the findings of this study should be considered an initial computational assessment of the potential of mitragynine and 7-hydroxymitragynine as anti-inflammatory candidates. Further studies are required to strengthen these findings through detailed three-dimensional interaction analysis, molecular dynamics simulations, ADMET prediction, and biological evaluation using in vitro and in vivo approaches. Experimental validation is ultimately required to determine whether the predicted docking affinities correspond to the actual biological activity of these compounds.

Conclusions

Molecular docking revealed target-dependent interaction profiles for mitragynine and 7-hydroxymitragynine against COX-1, COX-2, IL-1β, and TNF-α, with all redocking protocols meeting the predefined RMSD acceptance criterion of ≤2.0 Å. Within the respective targets, 7-hydroxymitragynine showed the more favorable predicted score of the two alkaloids for COX-1 and COX-2, whereas mitragynine showed

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the more favorable predicted score for IL-1β and TNF-α. These differences suggest that hydroxyl substitution at position 7 alters ligand recognition in a protein-dependent manner rather than uniformly improving docking affinity. The findings are entirely computational and do not establish anti-inflammatory efficacy or clinical relevance. Molecular-dynamics simulation, ADMET assessment, biochemical target assays, and subsequent in vitro and in vivo validation are required to determine whether the predicted interactions translate into biologically meaningful activity.

Conflict of Interest

All authors declare that there are no conflicts of interest regarding this study. The research process, encompassing design, data collection, analysis or interpretation, and manuscript preparation, was conducted independently without external interference; furthermore, there are no personal, financial, or professional interests that could compromise the objectivity and integrity of the research findings.

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