Contents lists available at NCBI

The American Journal of Science and Medical Research

Journal homepage: http://ajsmrjournal.com/

Research Article

Evaluating the Anti-Cancer Potential of Natural Flavonoids COX-2 Inhibition through Molecular Docking

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https://dx.doi.org/10.5281/zenodo.15543983

Received: 18 April 2025;
Accepted: 25 May 2025;
Published: 29 May, 2025

ISSN: 2377-6196© 2025 The Authors.
Published by AIRA

Keywords: *Cyclooxygenase-2, Anti-Inflammatory, Molecular Docking, Flavonoid compounds.*

ABSTRACT

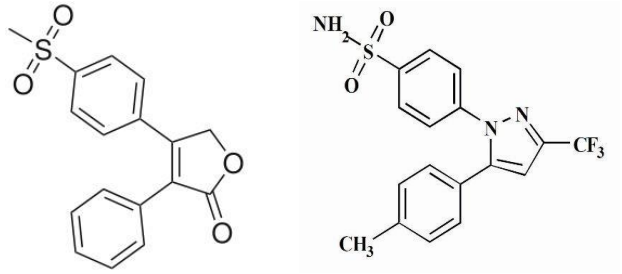
The cyclooxygenase-2 (COX-2) receptor is a therapeutic target for the development of potential drugs with anti-inflammatory activity. Rofecoxib, one of the first selective COX-2 inhibitors (coxibs), was approved for use in humans. The primary aim of this study is to inhibit the overactivity of COX-2, a key enzyme implicated in colorectal cancer. This research investigates the natural inhibitory potential of 20 flavonoid compounds against the COX-2 enzyme (PDB ID: 5KIR). Molecular docking was performed to evaluate binding affinities and interaction patterns. Rofecoxib exhibited a binding affinity of ΔG = −7.6 kcal/mol against COX-2. Notably, several flavonoid-based natural compounds demonstrated even higher binding affinities: 4-Hydroxywogonin (ΔG = −9.9 kcal/mol), 2′,3′,5,7-Tetrahydroxyflavone (ΔG = −9.5 kcal/mol), and 2′R,4′-Hydroxyemoroidocarpan (ΔG = −9.4 kcal/mol). These findings exhibit significant binding to COX-2, indicating their potential as anti-inflammatory agents. This research lays the groundwork for novel flavonoid-based therapeutics targeting COX-2-related pathways.

1. Introduction

Cyclooxygenase (COX) enzymes are members of the myeloperoxidase family, which regulates the prostaglandin (PG) biosynthesis. COX isozymes metabolize arachidonic acid to prostaglandins and thromboxane (Chandrasekharan & Simmons, 2004). COX-2 is also known as prostaglandin-endoperoxide synthase 2 and is encoded by the PTGS2 gene in humans. It is a homodimer of 581 amino acids with a molecular weight of 70 kDa. The functional role of COX-2 is the conversion of arachidonic acid to prostaglandins, which is further converted into prostaglandin H2 (PGH2), that finally metabolized by tissue-specific synthases into 5 major prostaglandins (PGD2, PGE2, PGF2α), prostacyclin (PGI2), and thromboxane A2 (TXA2) (3). Prostaglandins regulate various physiological pathways such as tumor development, migration, differentiation, inflammation, and cell-to-cell adhesion, while thromboxane triggers platelet aggregation, proliferation, and vasoconstriction (Wang & DuBois, 2006; Mamidala et al., 2013). In India, cancer incidence is rising, with around 1.46 million new cases reported in 2022, primarily affecting the breast, lung, cervix, and colorectal regions (Sathishkumar et al. 2022). In normal conditions, COX-2 is unexpressed in cells, while in response to stimuli such as inflammation; COX-2 is highly expressed (Zarghi & Arfaei, 2011; Ai-Masri et al., 2024).

Flavonoids represent a structurally diverse class of polyphenolic compounds, unified by a common backbone consisting of two aromatic rings (designated A and B) connected via a three-carbon bridge that forms a heterocyclic C ring (Kashyap et al., 2022; Raghav Mishra & Kaushal, 2024). This core structure permits a variety of substitutions, giving rise to several subclasses, including flavones, flavonols, and flavanones. Numerous studies have established the broad spectrum of biological activities associated with flavonoids. These include antioxidant, anti-inflammatory, and anticancer properties (Kandeel et al., 2023; Ameen et al., 2021). Notably, Jain et al. (2021) emphasized the role of flavonoids in modulating enzymatic activity and inhibiting cellular proliferation. Additionally, flavonoids contribute to disease prevention by influencing multiple cellular signaling pathways, thereby helping to mitigate the risk of chronic diseases such as cardiovascular conditions and certain forms of cancer (Raghav Mishra & Kaushal, 2024). Rofecoxib (Vioxx) was approved by the Food and Drug Administration (FDA) for human use in

May 1999 and withdrawn from the market in September 2004 (Sibbald, 2004; Babu et al., 2024). The role of COX-2 in inflammation and cancer progression, the development of selective COX-2 inhibitors remains challenging due to adverse side effects associated with existing drugs such as Rofecoxib and Celecoxib (Janakiramulu and Estari, 2025; Daipule et al., 2020). Chemical structures of the FDA-approved COX-2 inhibitors are shown in Fig 1.



A) Rofecoxib B) Celecoxib

Fig 1. Chemical structures of the FDA-approved COX-2 inhibitors (a) Rofecoxib, (b) Celecoxib

The virtual screening strategy was chosen once it was widely applied in the early phase of drug discovery, accelerating hit discovery and reducing drug development costs (Leão et al., 2020). Molecular docking has emerged as a powerful computational tool in drug discovery, allowing researchers to predict the binding affinity and orientation of small molecules, such as flavonoids, within the active site of COX-2 (Morris 2009; Davella et al., 2021). This technique provides vital insights into the ligand-receptor interactions at a molecular level, revealing the key residues involved in binding (Trott and Olson 2010). We seek to provide valuable insights into the molecular mechanisms underlying the anti-inflammatory and anti-cancer activities of these natural compounds

2. Materials and Methods

2.1. Target selection

The target of this study is the COX-2 enzyme, which is a crucial target for anti-inflammation by using different standard synthetic COX-2 inhibitors. The target is selected from the Protein Data Bank (www.rcsb.org). This structure was downloaded and visualized using Discovery Studio Visualizer (Sanober & Mamidala, 2024; Gidhamaari et al., 2012).

2.2. Ligand preparation

The three-dimensional structures of the selected flavonoid compounds were obtained in MDL Molfile format from the NPACT database (Figure-1). Using Open Babel, the Molfiles were converted into PDBQT format, as AutoDock Vina supports only PDBQT files for molecular docking. This conversion ensures that the ligand structures are in a compatible format for subsequent molecular docking simulation (Kumar & Mamidala, 2025; Gujjeti et al., 2013). To ensure optimal molecular geometry and eliminate any steric hindrance, the compounds underwent energy minimization. Following optimization, the ligands were prepared in PDBQT format for compatibility with AutoDock Vina docking simulations.

2.3. Protein Preparation

The three-dimensional structure of the enzyme COX-2 (PDB ID: 5KIR, resolution 2.70 Å) was retrieved from the Protein Data Bank (www.rcsb.org) (Fig 2). The protein was prepared by removing all water molecules, heteroatoms, and co-crystallized ligands that could interfere with the docking process (Kumar & Mamidala, 2025). Polar hydrogen atoms were added, and Kollman charges were assigned to ensure proper interaction modelling (Sanober & Mamidala, 2024). The cleaned and optimized protein structure was then saved in PDBQT format using AutoDock Tools, making it suitable for use in AutoDock Vina docking simulations.

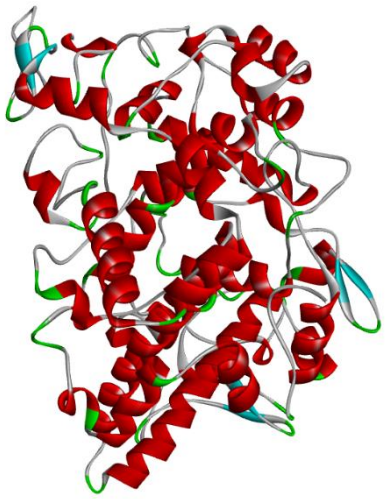


Fig 2. 3D Structure of the COX-2 PDB ID (5KIR)

2.4. Molecular docking

The molecular docking simulations were carried out using AutoDock Vina to evaluate the binding affinities and interaction modes of the selected flavonoid compounds with the COX-2 protein (PDB ID: 5KIR). A grid box measuring 56 × 80 × 44 Å was defined to focus on the protein's active site, centered at the coordinates x = 22.126, y = 10.25, and z = 34.864, effectively encompassing the inhibitor to bind active site residues. AutoDock Vina calculates the binding free energy (ΔG) for each ligand-receptor complex, providing an estimate of the binding affinity in kcal/mol. A more negative ΔG value indicates stronger binding between the ligand and the protein. The docking results were ranked based on these binding energies, and the top-ranked poses were selected for further analysis (Swapna et al., 2024; Janakiramulu et al., 2025). The binding conformations and key interactions, such as hydrogen bonds, hydrophobic contacts, and π–π stacking, were visualized using molecular visualization tools like PyMOL and Discovery Studio Visualizer. These evaluations help to identify the most promising flavonoid compounds with potential COX-2 inhibitory activity.

3. Results and Discussion

3.1. Results of Molecular Docking

The analysis of the binding energies, hydrogen bond interactions, and hydrophobic interactions for various compounds reveals significant insights into their potential efficacy compared to the reference drug, Rofecoxib depicted in Table-1.

The binding energies of the compounds in relation to COX-2 show a significant range, with values from -10.8 to -6.6 kcal/mol. Notably, Agathisflavone demonstrates the strongest binding affinity at -10.8 kcal/mol. This suggests that it may effectively inhibit COX-2 activity more efficiently than Rofecoxib, which has a binding energy of -7.6 kcal/mol. The lower (more negative) binding energy of Agathisflavone indicates a stronger interaction with the enzyme, which is essential for its inhibitory function (Fig 3).

In terms of hydrogen bond interactions, the number of hydrogen bonds formed with COX-2 varies among the compounds. For instance, the 3,5,7,3',4'-Pentahydroxyflavonol-3-O-Beta-D (Fig 3). Glucopyranoside forms five hydrogen bonds with key amino acids such as Asn350 and His351. This extensive hydrogen bonding likely contributes to a more stable and effective interaction with COX-2, enhancing its inhibitory potential. In contrast, Agathisflavone only forms a single hydrogen bond with His39, which may suggest a less stable interaction compared to compounds with multiple hydrogen bonds. Rofecoxib's lack of hydrogen bonding interactions may limit its efficacy as an inhibitor of COX-2, highlighting the importance of these interactions in drug design.

Moreover, the hydrophobic interactions play a significant role in the binding of these compounds to COX-2. Agathisflavone engages in hydrophobic interactions with amino acids such as Pro153 and Cys47, while 3,5,7,3',4'-Pentahydroxyflavonol-3-O-Beta-D-Glucopyranoside also shows notable interactions with Tyr355. These hydrophobic contacts are critical for stabilizing the enzyme-ligand complex, thereby enhancing the overall binding affinity. Rofecoxib, while engaging in hydrophobic interactions with amino acids like Tyr115 and Ile112, may not achieve the same level of interaction as some of the other compounds, potentially affecting its inhibitory effectiveness.

The comparative analysis of binding energies, hydrogen bond interactions, and hydrophobic interactions with COX-2 illustrates that several compounds exhibit superior binding characteristics compared to Rofecoxib (Table-1). These findings underscore the potential of these compounds as more effective inhibitors of COX-2, which could lead to improved therapeutic outcomes in the treatment of inflammatory conditions. Further investigation into these interactions will be essential for the development of novel COX-2 inhibitors.

The molecular docking analysis revealed that 36 flavonoid compounds exhibited varying binding affinities with the target protein, with binding energies ranging from −10.8 kcal/mol (for Agathisflavone) to −6.6 kcal/mol (for (3S)-3',7-Dihydroxy-2',4',5',8-Tetramethoxyisoflavan). Among them, Agathisflavone demonstrated the strongest binding affinity, suggesting its potential as an effective inhibitor. This compound formed a combination of hydrogen bonds and hydrophobic interactions with key amino acid residues such as His39, Pro153, Cys47, and Leu152, which likely contribute to its strong binding. In comparison, the standard inhibitor Rofecoxib exhibited a binding energy of −7.6 kcal/mol and formed only hydrophobic interactions, without any hydrogen bonding. Several flavonoids, particularly Agathisflavone, showed stronger binding affinities than Rofecoxib. However, some compounds, such as (3S)-7-Hydroxy-2',3',4',5',8-Pentamethoxyisoflavan and (3S)-3',7-Dihydroxy-2',4',5',8-Tetramethoxyisoflavan, displayed weaker interactions, indicating that not all flavonoids are equally effective as inhibitors of the target protein. These findings are consistent with the study by Ribeiro et al. (2015), which reported that flavonoids containing a catechol group in the B-ring can effectively inhibit COX-2 activity. In contrast, a study by Bai and Zhu (2008) and Swapna et al., (2024) and Namthabad et al., (2013) found that certain flavonoids, such as myricetin, quercetin, and fisetin, may stimulate COX-2 activity, leading to increased prostaglandin production. This discrepancy suggests that the inhibitory or stimulatory effect of

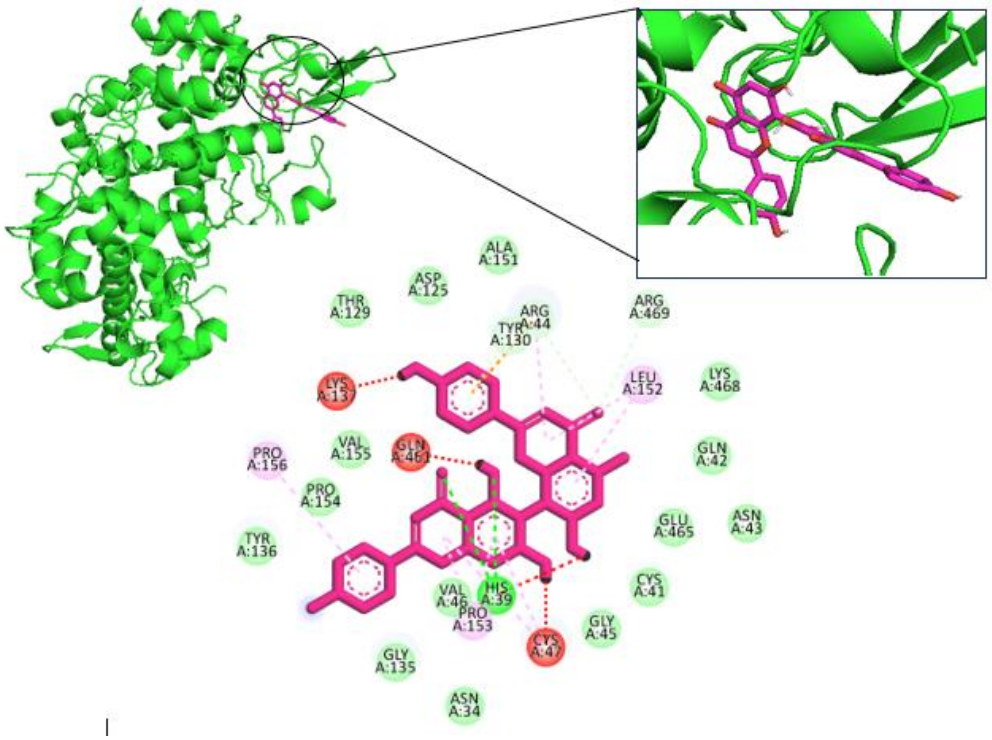


Fig 3. 3D and 2D View of the target Enzyme **COX-2** and **Agathisflavone** interactions

Table 1. Binding affinity energies, H-bond, and other hydrophobic interacting amino acid residues between selected flavonoids and targets

S. No	Compound Name	Binding Energy (kcal/mol)	No. Of H-Bonds	H-Bond Interactions	Hydrophobic Interactions
1.	Agathisflavone	-10.8	1	His39	Pro153, Cys47, Leu152, Arg44, Lys137, Gln461, Pro156,
2.	2',3',5,7-Tetrahydroxy Flavone	-9.5	3	Cys36, Cys41, Arg44	Pro153, Leu152, Cys47
3.	2'R,4'-Hydroxyemoroidocarpan	-9.4	3	Asn34, His39, Arg44.	Pro156, Cys47, Cys36, Pro153, Leu152.
4.	3,5,7,3',4'-Pentahydroxyflavonol-3-O-Beta-D-Glucopyranoside	-9.2	5	Asn350, Gln192, His351, Phe580, Glu346.	Tyr355,
5.	2',5,6'7-Tetrahydroxy Flavone	-9.1	1	Gly45.	Leu152, Arg44, Pro153, Cys36.
6.	3,5,7,3',4',5'-Hexahydroxy Flavanone-3-O-Beta-D-Glucopyranoside	-9.1	7	His207, His388, Asn382, His386, Phe210, His214, Gln289.	Leu294, Val447, Val291.
7.	2',5-Dihydroxy-6,7,8-Trimethoxyflavone	-8.9	1	His388.	Ala202, Leu391, Val447, His386, His207, Phe210.
8.	3,5,7,3',4'pentahydroxyflavone-3-O-Beta-D-Glucopyranoside	-8.9	6	His207, His388, Asn382, His386, Phe210, Gln289.	Val291, Val447, Leu294.
9.	4'-Bromoflavone	-8.8	1	Arg44	Pro153, Cys47, Cys36, Leu152, Arg469,
10.	2',5,6',7-Tetrahydroxy Flavanone	-8.7	3	Cys47, Arg44, Cys41.	His39, Pro156, Cys36, Pro153, Leu152.
11.	3'-Formyl-2',4',6'-Trihydroxy-5'-Methyldihydrochalcone	-8.3	6	His386, Asn382, Thr212, His214, His207, His388.	Val447, Ala202
12.	(-)-Epicatechin	-8.0	2	Cys41, Gln461.	Tyr130, Pro153, Cys47, His39.
13.	3,5,7,3',4'-Pentahydroxyflavonol-3-O-Beta-D-Galactopyranoside	-8.0	4	Ser581, Ser579, Phe580, Gln192.	His356, His351,
14.	3'-O-Methyl-6-(1,1-Dimethylallyl)Eriodictyol	-8.0	4	Phe580, Ser581, His351, Asn350.	Phe577.
15.	Candenatenin A	-7.9	2	Arg44, Cys47.	Leu152, Arg469, Cys36, Pro156, Pro153, Glu465.
16.	2',4'-Dihydroxy-6'-Methoxy-3',5'-Dimethylchalcone	-7.3	3	Gly135, Pro154, Asn34.	Pro156, Pro153, Cys47, Cys36.
17.	3,3',4',5,6,7,8-Heptamethoxyflavone	-7.1	2	Gly135, His39.	Pro156, Val155, Pro154, Asn34, Cys47, Cys36, Pro153.
18.	(+)-Gallocatechin	-7.0	4	Lys473, Glu520, Glu480, Lys511.	-
19.	(3S)-7-Hydroxy-2',3',4',5',8-Pentamethoxyisoflavan	-7.0	3	Asn222, His214, Asn382.	Gln289, Lys211, Val291, His386.
20.	(3S)-3',7-Dihydroxy-2',4',5',8-Tetramethoxyisoflavan	-6.6	2	Asn34, Cys47.	Pro156, Gly135, Cys36, Pro153, Pro154, Asp157,
21.	Rofecoxib (Reference drug)	-7.6	-		Tyr115, Ile112, Leu93, Val89.

flavonoids on COX-2 may depend on their specific chemical structures and functional groups.

4. Conclusion

The molecular docking analysis demonstrated that several flavonoid compounds possess significant binding affinities toward the target protein, with Agathisflavone emerging as the most promising candidate due to its strong binding energy and favorable interactions with key amino acid residues. Its performance surpassed that of the standard inhibitor Rofecoxib, which lacked hydrogen bonding interactions. However, the variability in binding affinities among the flavonoids indicates that structural differences, particularly the presence or absence of functional groups such as the catechol moiety, play a critical role in their inhibitory potential. These findings support the notion that specific flavonoids could serve as effective COX-2 inhibitors, though further experimental validation is necessary. Additionally, the contrasting effects reported in previous studies highlight the importance of detailed structural evaluation when considering flavonoids for therapeutic applications.

Conflicting Interests

The authors have declared that no conflicting interests exist.

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