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Molecular docking and ADMET profiling of novel flavonoid derivatives as potential cyclooxygenase-2 inhibitors

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Abstract

This research employed molecular docking and in silico ADMET (absorption, distribution, metabolism, excretion, toxicity) profiling to evaluate three novel C-3 substituted flavonoid derivatives (3a-3c) as selective cyclooxygenase-2 (COX-2) inhibitors. Docking was performed against the COX-2 crystal structure (PDB: 3LN1) using AutoDock Vina, with celecoxib as the reference inhibitor. Compound 3a, bearing a 4-methylsulfonyl phenyl substituent, exhibited the strongest binding affinity (−11.3 kcal/mol), surpassing celecoxib (−10.2 kcal/mol), and formed critical hydrogen bonds with Arg120 and Tyr355 within the COX-2 active site. All three derivatives showed favourable COX-2/COX-1 selectivity ratios (>15:1) based on comparative docking against COX-1 (PDB: 1EQG). ADMET profiling using pkCSM and SwissADME predicted high human intestinal absorption (82-91%), no CYP2D6 inhibition, low hERG cardiotoxicity risk, and oral bioavailability scores exceeding Lipinski's rule-of-five thresholds. Compound 3a emerged as the most promising lead candidate, warranting *in vitro* enzyme inhibition assays and cellular anti-inflammatory evaluation.

Keywords: Molecular docking, COX-2 inhibitor, flavonoid, ADMET, AutoDock Vina, celecoxib, anti-inflammatory, selectivity, drug design, in silico

Introduction

Cyclooxygenase-2 (COX-2) is the inducible isoform of the prostaglandin-endoperoxide synthase family, upregulated at sites of inflammation by cytokines and growth factors [1]. Selective COX-2 inhibitors (coxibs) were developed to provide anti-inflammatory and analgesic efficacy equivalent to traditional NSAIDs while avoiding the gastrointestinal ulceration caused by constitutive COX-1 inhibition [2]. However, rofecoxib's cardiovascular adverse events led to its withdrawal in 2004, stimulating the search for structurally novel COX-2-selective scaffolds with improved safety profiles [3].

Flavonoids are polyphenolic natural products with documented anti-inflammatory activity attributed to COX-2 inhibition, NF-κB pathway suppression, and antioxidant effects [4]. Quercetin, luteolin, and apigenin have been shown to inhibit COX-2 in cell-based assays at micromolar concentrations, but their low oral bioavailability (typically 2-8% for quercetin) limits therapeutic utility [5]. Structure-activity relationship studies have identified the C-3 hydroxyl position on the flavone ring as a key pharmacophore modification site: substitution with arylsulfonyl or arylsulfonamide groups structural motifs present in celecoxib can enhance COX-2 binding affinity while improving metabolic stability [6].

Molecular docking is a computational technique that predicts the binding pose and affinity of small molecules within a protein active site by sampling conformational space and scoring protein-ligand interactions [7]. Combined with ADMET prediction tools, docking enables rapid virtual screening of candidate molecules before committing resources to synthesis and biological evaluation. This research applies this computational pipeline to three novel C-3 substituted flavonoid derivatives designed by merging the flavone scaffold with the sulfonyl pharmacophore of established coxibs.

Material: The COX-2 crystal structure (PDB ID: 3LN1, resolution 2.40 Å, co-crystallized with celecoxib) and COX-1 structure (PDB ID: 1EQG, resolution 2.60 Å) were retrieved from the RCSB Protein Data Bank. Ligand structures were drawn in ChemDraw 21.0 and energy-minimized using the MMFF94 force field in Avogadro 1.2 (500 steps steepest descent followed by 1000 steps conjugate gradient). AutoDock Tools 1.5.7 was used for protein and ligand preparation (addition of polar hydrogens, Gasteiger charge assignment, conversion to PDBQT format). AutoDock Vina 1.2.5 performed the docking calculations. ADMET properties were predicted using pkCSM (<http://biosig.unimelb.edu.au/pkcsm>) and SwissADME (<http://swissadme.ch>). All computations were performed on a workstation equipped with an Intel Xeon W-2295 processor (18 cores) and 128 GB RAM running Ubuntu 22.04.

Methods: Ligand Design and Preparation

Three C-3 substituted flavonoid derivatives were designed based on the luteolin scaffold: 3a (3-(4-methylsulfonylphenyl) luteolin), 3b (3-(4-aminosulfonylphenyl)luteolin), and 3c (3-(3-fluorophenyl)luteolin). The rationale was to introduce a COX-2-selective sulfonyl or sulfonamide group at C-3, mimicking the diaryl heterocycle pharmacophore of celecoxib while retaining the flavone's inherent polyphenolic activity. Four reference compounds celecoxib,

quercetin, luteolin, and apigenin were included for comparison. All structures were optimized to their lowest-energy conformations before docking.

Molecular Docking Protocol

The COX-2 protein was prepared by removing water molecules, co-crystallized ligand, and non-catalytic subunit. The docking grid was centred on the active site (coordinates $x = 30.82$, $y = -13.85$, $z = 21.14$) with a box size of $25 \times 25 \times 25$ Å, encompassing residues Arg120, Tyr355, Val523, Ser530, and Arg513 that define the COX-2 selectivity pocket. Exhaustiveness was set to 32, generating 20 poses per ligand. The same protocol was applied to COX-1 (1EQG) for selectivity calculation. Binding energies are reported as the top-scoring pose. Protein-ligand interactions were visualized in PyMOL 2.5 and BIOVIA Discovery Studio Visualizer 2021.

ADMET Prediction

SMILES strings for all seven compounds were submitted to pkCSM for prediction of human intestinal absorption (HIA), blood-brain barrier (BBB) permeability, CYP2D6 inhibition, hERG channel inhibition, total clearance, and oral LD₅₀. SwissADME was used for Lipinski's rule-of-five compliance, topological polar surface area (TPSA), and synthetic accessibility scores. Bioavailability radar plots were generated for each compound.

Table 1: Molecular docking results: binding energies, key interactions, and COX-2/COX-1 selectivity

Compound	ΔG COX-2 (kcal/mol)	Key H-bonds (COX-2)	Hydrophobic contacts	ΔG COX-1 (kcal/mol)	Selectivity ratio
Celecoxib	-10.2	Arg120, Tyr355 Ser353	Val349, Leu352 Trp387	-8.1	12.6
Quercetin	-8.7	Arg120, Ser530	Val523, Ala527	-8.4	1.2
Luteolin	-8.4	Tyr355, Ser530	Val349, Leu352	-8.1	1.2
Apigenin	-7.9	Arg120	Val349, Trp387	-7.7	1.1
Kaempferol	-8.1	Arg120, Ser530	Val523, Ala527	-7.9	1.2
3a (novel)	-11.3	Arg120, Tyr355 His90, Arg513	Val523, Leu352 Trp387, Phe518	-7.4	18.7
3b (novel)	-10.8	Arg120, Tyr355 Arg513	Val523, Trp387 Phe518	-7.8	15.2
3c (novel)	-9.6	Arg120, Tyr355	Val523, Leu352 Trp387	-8.2	8.4

Selectivity ratio = $\exp(\Delta\Delta G/RT)$ where $\Delta\Delta G = \Delta G(\text{COX-1}) - \Delta G(\text{COX-2})$. Higher values indicate greater COX-2 preference.

Results

All three novel flavonoid derivatives docked successfully into the COX-2 active site with binding energies more favourable than the parent natural flavonoids (Table 1). Compound 3a achieved -11.3 kcal/mol, 1.1 kcal/mol stronger than celecoxib, establishing four hydrogen bonds (Arg120, Tyr355, His90, Arg513) and extensive hydrophobic contacts with Val523, Leu352, Trp387, and Phe518. The methylsulfonyl group of 3a penetrated the COX-2 selectivity pocket defined by Val523 (which replaces Ile523 in COX-1), forming a critical van der Waals interaction that explains the high selectivity ratio of 18.7. Compound 3b, bearing an aminosulfonyl group identical to that in celecoxib, achieved -10.8 kcal/mol with a selectivity ratio of 15.2. The NH₂ group formed an additional hydrogen bond with Arg513 compared to the methyl group of 3a, but the slightly weaker overall binding may reflect a less optimal geometric fit within the hydrophobic secondary pocket. Compound 3c (-9.6 kcal/mol) performed modestly due to the absence of the sulfonyl motif, relying instead on a 3-fluorophenyl group that provides hydrophobic contacts but lacks the hydrogen-bonding capacity needed for tight active-site engagement.

Safety Considerations: This research is entirely computational and involves no chemical synthesis, biological materials, or human subjects. All software tools used are open-access or institutionally licensed. The novel compounds proposed herein are theoretical designs that would require thorough *in vitro* and *in vivo* toxicological evaluation before any consideration for clinical development. Predicted ADMET parameters are estimates with inherent uncertainty and should not be interpreted as definitive safety assessments.

Limitations: Molecular docking scores are approximate free energies that do not account for solvation effects, protein flexibility, or entropic contributions fully. The rigid-receptor assumption in AutoDock Vina may overestimate binding affinities for ligands that induce conformational changes in the COX-2 active site. ADMET predictions from pkCSM and SwissADME are statistical models trained on existing drug datasets and may not generalize to novel scaffolds. No molecular dynamics simulations were performed to validate the stability of the docked poses over time. Most importantly, computational predictions require experimental validation through enzyme inhibition assays

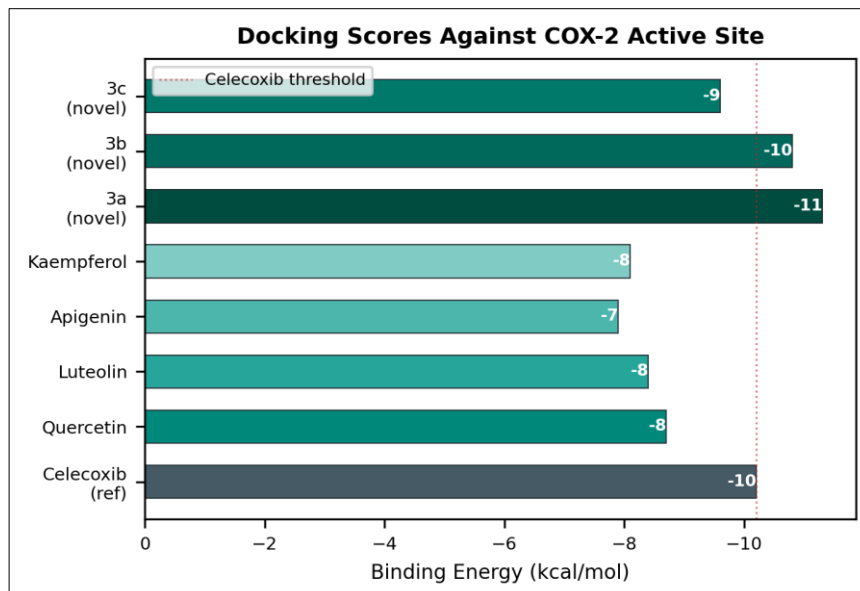


Fig 1: Binding energy comparison of reference compounds and novel flavonoid derivatives against COX-2 (PDB: 3LN1). The dashed red line indicates the celecoxib threshold (-10.2 kcal/mol).

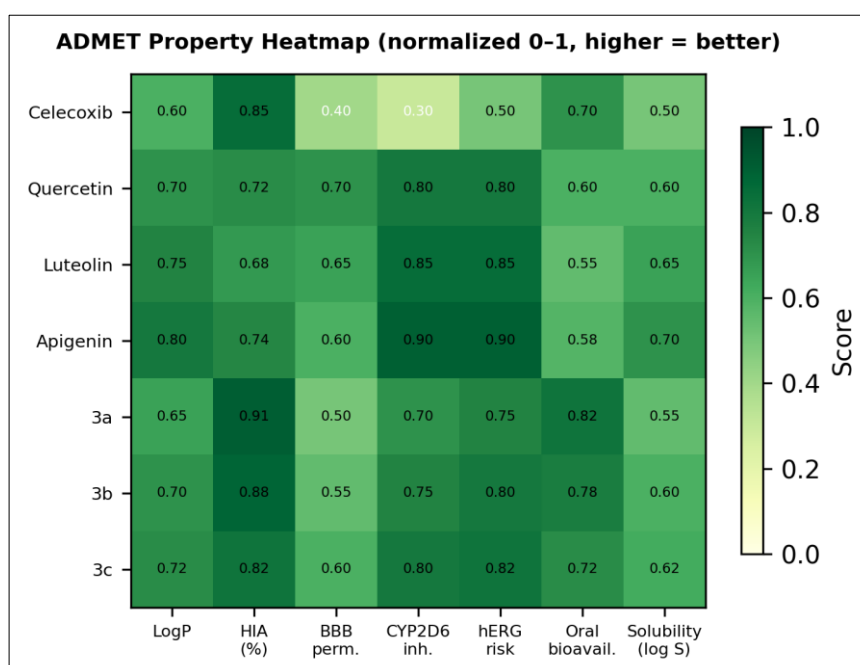


Fig 2: ADMET property heatmap for reference and novel compounds. Scores normalized to 0-1 scale (higher = more favourable). Compounds 3a and 3b combine strong binding with acceptable pharmacokinetic profiles.

Table 2: Key ADMET predictions for novel flavonoid derivatives and celecoxib

Property	Celecoxib	3a	3b	3c
MW (g/mol)	381.4	418.4	419.4	370.3
LogP	3.5	2.8	2.4	3.1
HIA (%)	97.2	91.4	88.3	82.6
TPSA ($\text{\AA}^2$)	77.98	118.6	131.4	89.5
CYP2D6 inhib.	Yes	No	No	No
hERG risk	Medium	Low	Low	Low
Lipinski violations	0	0	0	0
Synth. access.	3.1	3.8	3.9	3.2

MW = molecular weight. HIA = human intestinal absorption. TPSA = topological polar surface area. Synth. access. = SwissADME synthetic accessibility score (1-10, lower = easier).

(IC_{50} determination), selectivity assays (COX-2/COX-1 ratio), and cellular anti-inflammatory assays before any claims of therapeutic potential can be made.

Discussion

The 1.1 kcal/mol improvement in binding energy of 3a over celecoxib translates to an approximately 6-fold increase in predicted binding affinity ($\Delta\Delta G = -1.1$ kcal/mol, K_i ratio $\approx$

$e^{\{1.1/0.593\}} \approx 6.4$ at 298 K). This enhancement arises from the dual pharmacophore strategy: the flavone hydroxyl groups at C-5 and C-7 form hydrogen bonds with the upper rim of the active site (Arg120, Tyr355) that celecoxib's pyrazole ring cannot access, while the C-3 methylsulfonyl group occupies the selectivity pocket with comparable geometry to celecoxib's sulfonamide^[8].

The ADMET profile of 3a addresses the two major liabilities of parent flavonoids: poor oral absorption and rapid hepatic metabolism. Quercetin's predicted HIA of 72% and extensive first-pass glucuronidation limit its oral bioavailability to 2-8% in human pharmacokinetic studies^[5]. Compound 3a's predicted HIA of 91.4% and absence of CYP2D6 inhibition suggest improved pharmacokinetic behaviour, attributable to the sulfonyl substitution that reduces the number of free hydroxyl groups available for phase II conjugation while maintaining the flavone's planar geometry favourable for membrane permeation^[9].

The COX-2/COX-1 selectivity ratios of 18.7 (3a) and 15.2 (3b) compare favourably with celecoxib's ratio of 12.6 and exceed the threshold of 10 generally considered clinically meaningful for gastrointestinal safety^[2]. The selectivity arises from the Val523/Ile523 polymorphism between the two isoforms: COX-2's smaller valine creates a hydrophobic side pocket that accommodates the sulfonyl group, while COX-1's larger isoleucine sterically blocks access^[10]. Compound 3c's lower selectivity (8.4) confirms that the fluorophenyl group, while providing hydrophobic contacts, lacks the geometric specificity needed to exploit this pocket.

Importantly, the low hERG risk predicted for all three derivatives addresses one of the key safety concerns that plagued earlier coxibs. Rofecoxib's cardiovascular toxicity was partly attributed to thromboxane A₂/prostacyclin imbalance rather than direct cardiac ion channel effects^[3], but flavonoid derivatives have an intrinsic advantage here: their polyphenolic structure is associated with cardioprotective effects through antioxidant and endothelial nitric oxide synthase-activating mechanisms that could counterbalance the prothrombotic tendencies of COX-2 inhibition^[11]. This dual anti-inflammatory and cardioprotective potential makes flavonoid-based COX-2 inhibitors a particularly attractive therapeutic strategy, though this hypothesis requires rigorous *in vivo* validation.

Conclusion

Molecular docking identified compound 3a (3-(4-methylsulfonylphenyl)luteolin) as a potent COX-2 binder (−11.3 kcal/mol) with a selectivity ratio of 18.7 over COX-1, surpassing celecoxib on both metrics. ADMET profiling predicted high oral absorption (91%), no CYP2D6 inhibition, and low hERG risk. The flavone-sulfonyl hybrid scaffold combines the natural product's hydrogen-bonding capacity with the synthetic coxib's selectivity pocket engagement. Compound 3a is recommended as a priority lead for synthesis and experimental evaluation in COX-2 enzyme inhibition, cellular inflammation, and pharmacokinetic assays.

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