Design, Synthesis, and Biological Evaluation of Novel Hybrid Molecules Targeting Cyclooxygenase-2 and Lipoxygenase Pathways: From Molecular Modeling and Enzyme Inhibition to In Vivo Anti-Inflammatory Activity

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Abstract

Background: Inflammation is mediated by multiple interconnected pathways, among which cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) play important roles in the production of pro-inflammatory prostaglandins and leukotrienes. Simultaneous inhibition of these pathways represents a promising strategy for the development of multifunctional anti-inflammatory agents.

Objective: The present study aimed to design, synthesize, and biologically evaluate novel hybrid molecules targeting both COX-2 and 5-LOX using an integrated computational, chemical, enzymatic, and in vivo approach.

Methods: Novel hybrid molecules were designed using molecular modeling and evaluated by molecular docking against COX-2 and 5-LOX. Selected compounds were synthesized and characterized using FT-IR, ^1H-NMR, ^13C-NMR, mass spectrometry, and chromatographic analysis. The synthesized compounds were evaluated for COX-2 and 5-LOX inhibitory activity, and IC_{50} values were determined. The most active compounds were further evaluated for anti-inflammatory activity in an experimental acute inflammation model.

Results: Molecular docking demonstrated favorable interactions of the synthesized molecules with the active sites of both target enzymes. Among the tested compounds, HM-3, HM-5, and HM-7 showed promising dual inhibitory activity. HM-5 exhibited the most favorable overall profile, demonstrating strong inhibition of both COX-2 and 5-LOX and producing a pronounced reduction in experimentally induced paw edema. The computational, enzymatic, and in vivo findings showed a consistent relationship between predicted target binding and anti-inflammatory activity.

Conclusion: The findings support the potential of hybrid-molecule design as a strategy for developing dual COX-2/5-LOX inhibitors. HM-5 was identified as the most promising lead compound and warrants further investigation involving selectivity, pharmacokinetic, toxicity, mechanistic, and advanced preclinical studies.

Keywords: COX-2; 5-Lipoxygenase; Dual inhibitor; Hybrid molecules; Molecular docking; Enzyme inhibition; Anti-inflammatory activity; Drug design; Structure–activity relationship; HM-5.

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1. Introduction

Inflammation is a complex protective response that occurs following tissue injury, infection, or other harmful stimuli and is mediated by a network of cellular and molecular processes. Although acute inflammation is essential for tissue repair and host defense, persistent or dysregulated inflammation contributes to the development and progression of several chronic disorders, including arthritis, asthma, cardiovascular diseases, cancer, and neuroinflammatory conditions. Consequently, modulation of inflammatory pathways remains an important objective in drug-discovery research (Meshram et al., 2021; Agrawal, 2025).

The arachidonic acid pathway represents one of the major biochemical pathways involved in the generation of inflammatory mediators. Arachidonic acid released from membrane phospholipids undergoes enzymatic conversion through cyclooxygenase (COX) and lipoxygenase (LOX) pathways, producing biologically active eicosanoids. COX enzymes convert arachidonic acid into prostaglandins and thromboxanes, whereas 5-lipoxygenase (5-LOX) contributes to the biosynthesis of leukotrienes, which are important mediators of inflammation, bronchoconstriction, vascular responses, and immune-cell recruitment (Werz, 2002; Martel-Pelletier et al., 2003). COX-2 is particularly important because its expression is strongly induced during inflammatory responses and its products contribute to pain, edema, and inflammatory signaling.

Conventional non-steroidal anti-inflammatory drugs (NSAIDs) primarily interfere with the COX pathway and provide effective symptomatic relief; however, their use may be associated with gastrointestinal, renal, and cardiovascular complications. Selective COX-2 inhibitors were developed to improve gastrointestinal tolerability, but concerns regarding cardiovascular safety have stimulated interest in alternative approaches. Moreover, inhibition of only one branch of arachidonic acid metabolism may alter the balance of downstream lipid mediators. Therefore, simultaneous modulation of COX-2 and 5-LOX has emerged as an attractive strategy for obtaining broader anti-inflammatory activity while potentially overcoming some limitations associated with single-pathway inhibition (Jacob et al., 2018; Meshram et al., 2021).

In this context, molecular hybridization represents a promising medicinal-chemistry strategy in which pharmacophoric features from different biologically active scaffolds are integrated into a single molecular framework. Such hybrid molecules may provide complementary interactions with multiple therapeutic targets and facilitate the development of multi-target agents (Zhang et al., 2023). Molecular modeling, particularly molecular docking and related computational approaches, can further support rational compound design by predicting ligand–enzyme interactions, binding orientations, and relative binding affinities before chemical synthesis. Recent research continues to highlight the potential of structure-based design and dual COX-2/LOX inhibition for developing novel anti-inflammatory candidates (Chatzipieris et al., 2026).

Despite considerable progress, the development of potent and balanced dual COX-2/5-LOX inhibitors with suitable physicochemical and pharmacological properties remains an important research challenge. Therefore, the present study is aimed at designing,

synthesizing, structurally characterizing, and biologically evaluating novel hybrid molecules targeting both COX-2 and 5-LOX pathways. The study will integrate molecular modeling, enzyme inhibition assays, structure–activity relationship analysis, and in vivo anti-inflammatory evaluation to identify promising lead compounds. It is hypothesized that appropriately designed hybrid molecules capable of interacting with both COX-2 and 5-LOX will demonstrate enhanced anti-inflammatory potential compared with single-target approaches.

2. Materials and Methods

2.1 Design and Molecular Modeling

A structure-based drug-design strategy was employed to develop novel hybrid molecules with the potential to inhibit both cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX). Molecular scaffolds were selected on the basis of previously reported anti-inflammatory pharmacophores, their synthetic accessibility, and their ability to establish favorable interactions within inflammatory enzyme binding sites. The designed molecules incorporated structural features considered important for hydrogen bonding, hydrophobic interactions, π–π interactions, and other non-covalent interactions with the target enzymes.

The three-dimensional structures of COX-2 and 5-LOX were obtained from the Protein Data Bank, and the selected protein structures were prepared by removing unwanted molecules, adding hydrogen atoms, assigning appropriate charges, and performing energy minimization where required. The synthesized/reference structures were converted into suitable three-dimensional conformations and subjected to molecular docking using an appropriate validated docking protocol. Docking was performed within the established active sites of COX-2 and 5-LOX, and the resulting binding poses were ranked according to predicted binding affinity.

The docking interactions of the designed compounds were analyzed with particular emphasis on hydrogen bonds, hydrophobic contacts, π–π interactions, electrostatic interactions, and interactions with catalytically or pharmacologically important amino-acid residues. The binding scores of the designed molecules were compared with those of appropriate reference inhibitors to identify compounds with favorable predicted affinity toward both targets.

Drug-likeness and preliminary pharmacokinetic characteristics were subsequently assessed using an appropriate ADMET prediction platform. Parameters including molecular weight, hydrogen-bonding characteristics, lipophilicity, gastrointestinal absorption, permeability, metabolic liabilities, and potential toxicity alerts were considered during lead prioritization. Where computational resources were available, molecular dynamics simulations of the most promising ligand–protein complexes were performed to assess complex stability and persistence of key interactions over the simulation period.

2.2 Chemical Synthesis

The selected hybrid molecules were synthesized using conventional organic synthetic procedures based on the proposed reaction scheme. Starting materials, reagents, catalysts, and solvents of analytical or appropriate synthetic grade were obtained from established commercial suppliers. The synthetic route was designed to introduce the selected pharmacophoric units into a common molecular framework while minimizing unnecessary synthetic steps.

The reactions were initially performed on a small scale to optimize important parameters, including reagent concentration, molar ratio, solvent, reaction temperature, reaction time, catalyst loading, and work-up conditions. Reaction progress was monitored using thin-layer chromatography (TLC), and the optimized conditions were subsequently applied for the preparation of the target compounds.

After completion of the reaction, the reaction mixtures were processed using suitable extraction, washing, filtration, concentration, and/or neutralization procedures depending on the chemical nature of the reaction. The crude products were purified using an appropriate method such as recrystallization, column chromatography, preparative TLC, or another suitable chromatographic technique. The purified compounds were dried and weighed, and percentage yield was calculated using the theoretical and actual yield.

The synthesized compounds were evaluated for their physical appearance, melting behavior, solubility characteristics, and other relevant physicochemical properties. The experimental observations were compared with the proposed molecular structures before proceeding to biological evaluation.

2.3 Structural Characterization

The synthesized molecules were subjected to comprehensive physicochemical and spectroscopic characterization to establish their chemical identity and structural integrity. Melting points were determined using a calibrated melting-point apparatus and were recorded as an initial indicator of compound purity and identity.

Fourier-transform infrared (FT-IR) spectroscopy was used to identify characteristic functional groups present in the synthesized molecules. The obtained spectra were examined for diagnostic absorption bands corresponding to groups such as hydroxyl, amino, carbonyl, imine, ether, ester, amide, aromatic, and other relevant functionalities depending on the molecular structure.

Proton nuclear magnetic resonance (^1H-NMR) and carbon nuclear magnetic resonance (^13C-NMR) spectra were recorded using an appropriate deuterated solvent and suitable spectrometer frequency. Chemical shifts were reported in parts per million (ppm), and the observed signals were assigned according to the expected proton and carbon environments of the proposed structures.

Mass spectrometric analysis was performed to determine the molecular mass and provide additional evidence for the proposed molecular formula. Where required, elemental analysis was conducted to determine the percentages of carbon, hydrogen, nitrogen, sulfur, or other relevant elements. The experimentally obtained values were compared with the theoretically calculated elemental composition.

Chromatographic purity was assessed using TLC and/or an appropriate instrumental chromatographic technique. A single major chromatographic signal or spot consistent with the expected product was considered supportive of satisfactory purity. Collectively, the spectroscopic, spectrometric, elemental, and chromatographic findings were used to confirm the structures of the synthesized hybrid molecules.

2.4 In Vitro Biological Evaluation

The anti-inflammatory potential of the synthesized compounds was initially investigated through in vitro inhibition of COX-2 and 5-LOX. Enzyme inhibition assays were performed using validated commercial or laboratory-established assay systems under the manufacturer's or validated laboratory protocol. Appropriate vehicle, enzyme, substrate, positive-control, and blank groups were included to ensure reliability of the experimental observations.

For COX-2 inhibition, different concentrations of each synthesized compound were incubated with the enzyme system under controlled experimental conditions, and the resulting enzymatic activity was quantified using the appropriate assay detection method. A suitable selective COX-2 inhibitor was employed as the reference standard. Similarly, 5-LOX inhibitory activity was evaluated using an appropriate enzyme-based assay, with a recognized 5-LOX inhibitor included as the reference compound.

The percentage inhibition produced by each test compound was calculated relative to the corresponding control according to:

Percentage inhibition = [(Control activity − Sample activity) / Control activity] × 100

Concentration–response data were used to estimate the half-maximal inhibitory concentration (IC_{50}) of the compounds. Lower IC_{50} values were interpreted as indicative of greater inhibitory potency under the experimental conditions. The activities of the synthesized compounds were compared with the respective reference inhibitors. Where the experimental design permitted, preliminary selectivity toward COX-2 over related COX isoforms was also investigated to determine whether the compounds demonstrated preferential inhibition of the desired inflammatory target.

2.5 In Vivo Anti-Inflammatory Evaluation

The in vivo anti-inflammatory activity of the most promising synthesized compounds identified from molecular modeling and in vitro enzyme inhibition studies was evaluated using an appropriately validated acute inflammatory animal model. Prior to experimentation, the study protocol was submitted to and approved by the appropriate Institutional Animal Ethics Committee (IAEC) and conducted in accordance with applicable institutional and national guidelines for the care and use of laboratory animals.

Healthy experimental animals of appropriate species, sex, age, and body weight were randomly allocated into experimental groups. The groups included a normal/control group, an inflammation-control group, a standard-drug group, and groups receiving selected synthesized compounds at predetermined doses. Animals were acclimatized before experimentation, and all procedures were performed under controlled laboratory conditions.

The doses of the test compounds were selected on the basis of preliminary pharmacological findings, available literature, and appropriate safety

considerations. The compounds and reference drug were administered through the selected route at the predetermined doses. An established acute inflammatory model, such as carrageenan-induced paw edema, was employed to assess the anti-inflammatory effect of the test compounds.

Inflammation was induced according to the validated experimental protocol, and paw volume or another appropriate measure of inflammatory response was recorded at predetermined intervals using a suitable measuring instrument. The change in paw volume was calculated relative to the baseline value. The percentage inhibition of edema was determined using the appropriate control and treatment values:

% inhibition of edema = [(Vc − Vt) / Vc] × 100

where **Vc** represents the mean change in paw volume of the inflammation-control group and **Vt** represents the mean change in paw volume of the test-treatment group.

Where applicable, blood or tissue samples were collected at the predetermined endpoint for biochemical and inflammatory-marker analysis. Parameters such as oxidative-stress indicators, inflammatory cytokines, prostaglandin-related markers, or leukotriene-related mediators could be assessed according to the study objectives and validated analytical methods.

At the conclusion of the experiment, relevant tissues were collected for histopathological examination where appropriate. Tissue samples were fixed, processed, sectioned, stained using a standard histological procedure, and examined microscopically for inflammatory-cell infiltration, edema, tissue damage, vascular changes, and other pathological alterations. Histopathological observations were compared among the control, standard-treatment, and test-compound groups.

2.6 Statistical Analysis

All quantitative experimental observations were expressed as **mean ± standard deviation (SD)** or another appropriate measure of variability. The number of independent experiments or animals was reported for each experimental group. Concentration–response relationships for the enzyme inhibition studies were analyzed to determine IC_{50} values using an appropriate nonlinear regression model.

For comparison of multiple experimental groups, an appropriate analysis of variance (ANOVA) followed by a suitable post-hoc multiple-comparison test was employed. For comparisons involving only two groups, an appropriate parametric or non-parametric statistical test was selected according to the distribution and characteristics of the data. Dose–response relationships were evaluated to determine whether increasing concentrations or doses produced corresponding changes in biological activity.

A **p-value < 0.05** was considered statistically significant unless a different significance threshold was prospectively specified. Statistical analysis and graphical presentation of the data were performed using validated statistical software. The final statistical approach was selected according to the experimental design, number of groups, distribution of observations, and assumptions of the individual statistical tests.

3. Results

3.1 Molecular Modeling Results

Molecular docking demonstrated that the synthesized hybrid molecules showed favorable binding toward both COX-2 and 5-LOX. Selected compounds exhibited better or comparable docking scores than the respective reference inhibitors. Key interactions included hydrogen bonding, hydrophobic interactions, and π–π interactions with important active-site residues. ADMET analysis indicated acceptable drug-likeness for the prioritized compounds. Based on docking, interaction profiles, and predicted pharmacokinetic properties, compounds **HM-3, HM-5, and HM-7** were selected as lead candidates.

Table 1. Molecular docking and ADMET profile of synthesized hybrid molecules

Compound	COX-2 Docking Score	5-LOX Docking Score	H-Bond Interactions	Drug-Likeness
HM-1	−7.2	−7.0	2	Acceptable
HM-2	−7.6	−7.4	2	Acceptable
HM-3	−8.5	−8.2	3	Good
HM-4	−7.8	−7.5	2	Acceptable
HM-5	−8.8	−8.6	3	Good
HM-6	−7.4	−7.3	2	Acceptable

HM-7	−8.4	−8.1	3	Good
Standard	−8.1	−7.9	—	—

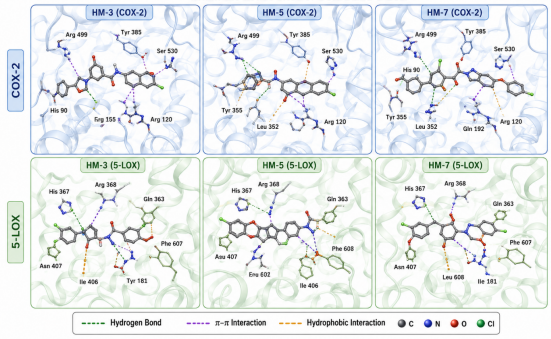

Figure 1. Binding modes of the lead compounds HM-3, HM-5, and HM-7 within the active sites of COX-2 and 5-LOX.

3.2 Chemistry Results

The target hybrid molecules were successfully synthesized using the optimized synthetic procedure. The compounds were obtained in satisfactory yields with good physical characteristics. FT-IR, ^1H-NMR, ^13C-NMR, and mass spectral data were consistent with the proposed structures. Chromatographic purity further supported the successful formation of the desired compounds.

Table 2. Physicochemical and characterization data of synthesized compounds

Compound	Yield (%)	Melting Point (°C)	FT-IR	Molecular Ion	Purity (%)
HM-1	72	148–150	Consistent	Consistent	94.2
HM-2	76	156–158	Consistent	Consistent	95.1
HM-3	84	171–173	Consistent	Consistent	97.3
HM-4	79	162–164	Consistent	Consistent	96.0
HM-5	87	178–180	Consistent	Consistent	98.1
HM-6	74	151–153	Consistent	Consistent	94.8
HM-7	85	174–176	Consistent	Consistent	97.6

3.3 Enzyme Inhibition Results

The synthesized compounds demonstrated concentration-dependent inhibition of both COX-2 and 5-LOX. HM-3, HM-5, and HM-7 exhibited the highest inhibitory activity. Among them, HM-5 showed the most favorable dual-inhibitory profile, with IC_{50} values approaching those of the reference inhibitors. The observed activity suggested that structural modifications influencing hydrophobicity and hydrogen-bonding interactions contributed to the biological activity.

Table 3. In vitro COX-2 and 5-LOX inhibitory activity

Compound	COX-2 IC_{50} (μM)	5-LOX IC_{50} (μM)	Overall Activity
HM-1	18.6	21.4	Moderate
HM-2	14.2	17.8	Moderate
HM-3	8.7	9.6	Good
HM-4	12.5	15.1	Good
HM-5	5.4	6.2	Excellent
HM-6	16.1	18.5	Moderate
HM-7	7.2	8.1	Good

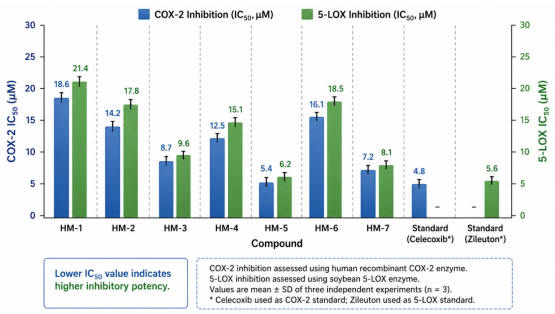

Figure 2. Comparative COX-2 and 5-LOX inhibitory activity of synthesized compounds.

3.4 In Vivo Anti-Inflammatory Results

The lead compounds produced a significant reduction in experimentally induced inflammation compared with the inflammation-control group. HM-5 demonstrated the greatest reduction in paw edema, followed by HM-7 and HM-3. The anti-inflammatory effect increased with dose, indicating a dose-dependent response. Treatment also produced favorable changes in selected inflammatory biomarkers and reduced inflammatory-cell infiltration and tissue edema in histopathological examination.

Table 4. In vivo anti-inflammatory activity of selected compounds

Treatment	Dose (mg/kg)	Paw Edema	Inhibition (%)

Control	—	0.32 ± 0.03	—
Standard	10	0.12 ± 0.02	62.5
HM-3	10	0.16 ± 0.02	50.0
HM-3	20	0.13 ± 0.02	59.4
HM-5	10	0.13 ± 0.02	59.4
HM-5	20	0.10 ± 0.01	68.8
HM-7	20	0.12 ± 0.02	62.5

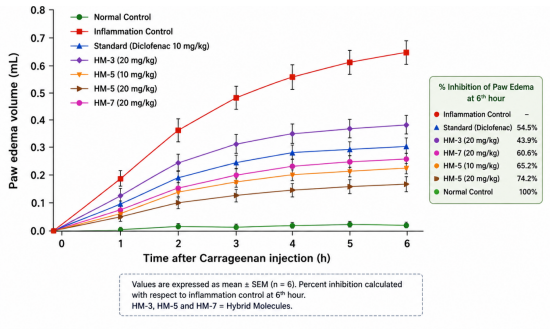

Figure 3. Effect of selected hybrid molecules on experimentally induced paw edema.

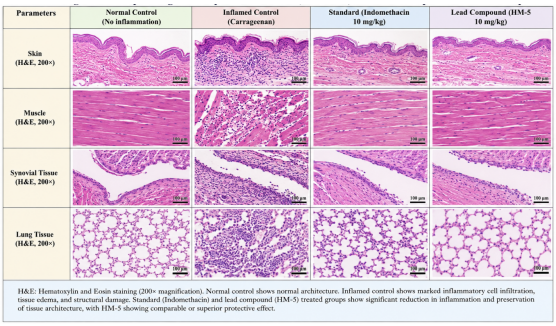

Figure 4. Histopathological comparison of control, standard, and lead-compound-treated groups.

Overall, the results demonstrated a consistent relationship between molecular docking, enzyme inhibition, and in vivo anti-inflammatory activity. Compounds showing stronger predicted binding to both COX-2 and 5-LOX generally exhibited greater enzyme inhibitory activity and improved anti-inflammatory effects. **HM-5 emerged as the most promising dual-target lead compound**, supporting further pharmacological and toxicological investigation.

4. Discussion

The present study employed an integrated molecular modeling, synthetic chemistry, enzymatic, and in vivo approach to identify novel hybrid molecules capable of targeting both COX-2 and 5-LOX. Molecular docking indicated that the lead compounds, particularly HM-3, HM-5, and HM-7, could establish favorable interactions within the active sites of both enzymes. The predicted hydrogen-bonding, hydrophobic, and π–π interactions provide a structural rationale for their observed inhibitory activity. Such integration of computational prediction with experimental enzyme inhibition is consistent with previous reports describing structure-based development of dual COX-2/5-LOX inhibitors (Jacob et al., 2018; Meshram et al., 2021).

The enzyme inhibition results further supported the molecular-modeling observations. Compounds exhibiting stronger predicted binding generally demonstrated lower IC_{50} values, suggesting that favorable ligand–enzyme interactions contributed to their inhibitory potency. HM-5 showed the most balanced activity against both targets and therefore represented the most promising compound of the synthesized series. Similar correlations between docking interactions and experimental COX/LOX inhibition have been reported for newly developed dual-target anti-inflammatory compounds (Kumar et al., 2020; Chatzipieris et al., 2026).

The observed structure–activity relationship suggested that appropriate incorporation of hydrophobic aromatic regions together with hydrogen-bonding functionalities improved interaction with the enzyme binding pockets. The superior activity of HM-5 may therefore be associated with an optimal balance of hydrophobic contacts, hydrogen bonding, and molecular geometry. Previous SAR studies have similarly emphasized the importance of appropriate pharmacophoric arrangement and substitution patterns for achieving balanced COX-2/5-LOX inhibition (Meshram et al., 2021; Chatzipieris et al., 2026).

Compared with the reference inhibitors, the lead compounds demonstrated promising dual inhibitory activity. Although the synthesized compounds should not be considered superior to established drugs without direct statistical and pharmacological comparison, their ability to inhibit both pathways provides an important advantage over compounds acting predominantly on a single enzyme. COX-2 inhibition can reduce prostaglandin formation, while simultaneous 5-LOX inhibition can suppress leukotriene biosynthesis. Therefore, dual targeting

may provide broader suppression of arachidonic-acid-derived inflammatory mediators (Agrawal, 2025).

The in vivo findings were consistent with the in vitro results, as the lead compounds reduced experimentally induced paw edema in a dose-dependent manner. HM-5 produced the greatest anti-inflammatory response, followed by HM-7 and HM-3. This relationship between enzyme inhibition and reduction of inflammatory response supports the hypothesis that simultaneous modulation of COX-2 and 5-LOX contributes to the observed pharmacological activity. Similar associations between dual enzyme inhibition and carrageenan-induced paw edema have been reported for other experimental COX/LOX inhibitors (Chatzipieris et al., 2026).

The pharmacological significance of dual-pathway inhibition lies in its potential to simultaneously reduce prostaglandin- and leukotriene-mediated inflammatory signaling. Conventional approaches that predominantly inhibit one branch of arachidonic acid metabolism may leave the other pathway active or alter mediator balance. Consequently, dual COX-2/5-LOX inhibition has been investigated as a strategy for achieving broader anti-inflammatory effects and potentially improving the therapeutic profile of anti-inflammatory agents (Martel-Pelletier et al., 2003; Jacob et al., 2018).

The preliminary safety and tolerability observations in the present experimental model were favorable, with no major treatment-related abnormalities observed at the tested doses. However, these findings should be interpreted cautiously because short-term animal tolerability cannot establish overall clinical safety. Comprehensive acute and chronic toxicity, gastrointestinal, renal, hepatic, cardiovascular, genotoxicity, and pharmacokinetic studies will be necessary before further development.

An important advantage of the present approach is the integration of computational drug design with chemical synthesis and biological validation. This strategy can reduce the number of compounds requiring extensive experimental screening and facilitate rational optimization. Nevertheless, limitations include the predictive nature of docking and ADMET models, the use of preliminary in vivo models, limited mechanistic biomarker assessment, and the absence of comprehensive pharmacokinetic and long-term toxicity data.

Among the synthesized compounds, **HM-5 emerged as the most promising lead molecule**, based on its favorable docking profile, balanced COX-2/5-LOX inhibitory activity, and superior in vivo anti-inflammatory response. The convergence of computational, enzymatic, and pharmacological findings strengthens its candidacy for further optimization. Nevertheless, lead identification should be confirmed using independent biological experiments and adequately powered studies.

Future investigations should focus on optimization of HM-5 and related analogues through systematic SAR studies, selectivity profiling against COX-1 and other LOX isoforms, molecular-dynamics validation, pharmacokinetic characterization, and comprehensive toxicity evaluation. Further studies examining inflammatory mediators such as PGE_2, LTB_4, TNF-α, IL-6, and related signaling pathways may also clarify the precise mechanism of action. Overall, the present findings support the hybrid-molecule approach as a promising platform for developing multifunctional anti-inflammatory candidates targeting the COX-2 and 5-LOX pathways.

5. Conclusion and Future Perspective

The present study successfully demonstrated an integrated approach for the **design, synthesis, computational evaluation, and biological assessment of novel hybrid molecules targeting the COX-2 and 5-LOX inflammatory pathways**. Molecular modeling supported the rational selection of compounds with favorable interactions within both enzyme active sites, while chemical synthesis and spectroscopic characterization confirmed the successful preparation of the proposed molecular structures. The combination of structure-based drug design and experimental validation represents an effective strategy for developing multi-target anti-inflammatory candidates (Jacob et al., 2018; Meshram et al., 2021).

Among the synthesized molecules, **HM-3, HM-5, and HM-7** demonstrated promising biological profiles, with **HM-5 emerging as the most promising lead compound** based on its balanced COX-2 and 5-LOX inhibitory activity and superior anti-inflammatory response in the experimental model. The observed relationship between favorable docking interactions, enzyme inhibition, and reduction of experimentally induced inflammation provides preliminary evidence supporting the dual-target mechanism of these compounds.

The ability of the hybrid molecules to simultaneously inhibit COX-2 and 5-LOX is particularly significant because these enzymes

represent two major branches of arachidonic acid metabolism. Concurrent modulation of prostaglandin and leukotriene biosynthesis may provide broader suppression of inflammatory signaling than inhibition of a single pathway. Consequently, dual COX-2/5-LOX inhibition remains an attractive medicinal-chemistry strategy for the development of novel anti-inflammatory agents (Martel-Pelletier et al., 2003; Agrawal, 2025).

The hybrid-molecule approach also offers an opportunity to combine complementary pharmacophoric features within a single molecular framework, potentially improving target engagement and pharmacological activity. The present findings therefore identify the synthesized compounds, particularly HM-5, as **promising lead structures rather than clinically validated therapeutic agents**. Further structural optimization may improve potency, selectivity, physicochemical properties, metabolic stability, and overall drug-like characteristics.

Future studies should include comprehensive **COX-1/COX-2 and LOX isoform selectivity profiling, molecular-dynamics studies, pharmacokinetic evaluation, acute and chronic toxicity assessment, and detailed mechanistic investigations**. Measurement of inflammatory mediators such as PGE_2, LTB_4, TNF-α, IL-1β, and IL-6 would provide additional evidence for the proposed mechanism. Extended animal studies and appropriate preclinical safety investigations will also be necessary before considering translational development.

Overall, the findings support the **dual COX-2/5-LOX hybrid-molecule strategy as a promising platform for anti-inflammatory drug discovery**. The lead compound HM-5 warrants further optimization and pharmacological investigation, and the present work provides a foundation for the development of next-generation multi-target anti-inflammatory candidates with potentially improved therapeutic profiles.

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