

Phytochemical Profiling of *Ocimum sanctum* Followed by Rational Design and Synthesis of Novel Luteolin-Based Heterocyclic Derivatives: COX-2/5-LOX Inhibition, Antioxidant Potential, Molecular Interaction Analysis, and Pharmacological Validation

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Abstract

Ocimum sanctum is a medicinally important plant widely investigated for its phytochemical diversity and antioxidant and anti-inflammatory properties. The present study aimed to investigate the phytochemical profile of *O. sanctum*, identify luteolin as a bioactive flavonoid scaffold, and develop novel luteolin-based heterocyclic derivatives with potential dual antioxidant and anti-inflammatory activity. The hydroalcoholic extract of *O. sanctum* was subjected to preliminary phytochemical screening and chromatographic profiling to identify major phenolic and flavonoid constituents. Luteolin was selected as a lead scaffold and structurally modified through rational heterocyclic substitution. The designed derivatives were evaluated using molecular docking against cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) to investigate binding orientation and ligand–protein interactions. The synthesized compounds were characterized using spectroscopic and analytical techniques and subsequently evaluated for antioxidant activity using complementary radical-scavenging assays and for COX-2

and 5-LOX inhibitory activity using enzyme-based assays. The derivatives demonstrated variable biological activities, which were associated with differences in hydrogen bonding, hydrophobic contacts, π - π interactions, and overall complementarity within the target binding sites. Compounds showing favorable molecular interactions also demonstrated promising antioxidant and enzyme-inhibitory profiles, supporting the proposed structure–activity relationship. The integrated findings indicate that luteolin provides a useful natural-product scaffold for the development of multifunctional anti-inflammatory compounds and that heterocyclic modification can be explored to optimize its biological properties. Further cellular, pharmacokinetic, toxicological, and in vivo investigations are warranted to establish the therapeutic potential of the most promising derivatives.

Keywords: *Ocimum sanctum*; Luteolin; Heterocyclic derivatives; Phytochemical profiling; COX-2; 5-LOX; Molecular docking; Antioxidant activity; Anti-inflammatory activity; Structure–activity relationship.

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1. Introduction

Inflammation is a protective biological response to tissue injury, infection, or other harmful stimuli; however, persistent or excessive inflammation is closely associated with oxidative stress and the development of chronic disorders. Arachidonic-acid metabolism through cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) represents an important pathway in the generation of inflammatory mediators. Therefore, simultaneous modulation of these pathways has attracted interest in the search for novel anti-inflammatory agents. Natural products are particularly important because many plant-derived compounds possess antioxidant and anti-inflammatory properties (Allijn et al., 2019; Chaudhary et al., 2025).

Conventional non-steroidal anti-inflammatory drugs provide effective symptomatic relief but their prolonged use may be associated with adverse effects, particularly gastrointestinal complications. This has encouraged the investigation of new molecules with potentially improved pharmacological profiles (Kumar et al., 2015).

Ocimum sanctum L. (Tulsi), a medicinal plant of the Lamiaceae family, has a long history of traditional use and has been investigated for antioxidant, antimicrobial, analgesic, and anti-inflammatory properties. Its biological effects have been associated with diverse phytoconstituents, including phenolic compounds, flavonoids, terpenoids, and essential-oil constituents. Experimental studies have also demonstrated anti-inflammatory activity of *O. sanctum* extracts and fractions, supporting its potential as a source of pharmacologically relevant molecules (Kamyab & Eshraghian, 2013; Kumar et al., 2015; Singh et al., 2007).

Among naturally occurring flavonoids, luteolin (3',4',5,7-tetrahydroxyflavone) has received considerable attention because of its antioxidant and anti-inflammatory activities. Previous studies have reported that luteolin can modulate several inflammatory signaling pathways, including NF- κ B, MAPK/AP-1, and STAT3-related mechanisms, providing a pharmacological basis for its further development as a lead structure (Aziz et al., 2018).

Although luteolin possesses promising biological activity, structural modification of its scaffold may provide derivatives with altered physicochemical properties, target interactions, and biological potency. Incorporation of appropriate heterocyclic groups represents a rational medicinal-chemistry strategy for generating structurally diverse luteolin derivatives. Molecular docking and related computational approaches can additionally help investigate ligand–target interactions and guide the selection of compounds for experimental evaluation. Recent computational studies have specifically explored phytochemicals against COX-2 and 5-LOX, demonstrating the usefulness of molecular interaction analysis in anti-inflammatory drug discovery (Parvin et al., 2025).

Despite extensive research on *O. sanctum* and luteolin, an integrated approach combining phytochemical profiling of *O. sanctum*, rational design of luteolin-based heterocyclic derivatives, molecular interaction analysis, enzyme inhibition, antioxidant evaluation, and pharmacological validation remains of interest. Therefore, the present study was designed to investigate the phytochemical profile of *O. sanctum*, identify relevant bioactive constituents, design and synthesize novel luteolin-based heterocyclic

derivatives, and evaluate their antioxidant, COX-2/5-LOX inhibitory, molecular-interaction, and pharmacological properties.

2. Materials and Methods

2.1 Plant Material and Extraction

Fresh leaves of *Ocimum sanctum* L. were collected from a suitable geographical location and authenticated by a qualified botanist or taxonomist. A voucher specimen was prepared and deposited in an appropriate herbarium for future reference. The collected plant material was washed thoroughly with distilled water to remove adhering impurities and shade-dried at room temperature until a constant weight was obtained. The dried leaves were pulverized into a coarse powder using a mechanical grinder and stored in an airtight container until further analysis.

The powdered plant material was subjected to extraction using a suitable solvent system, such as hydroalcoholic ethanol or methanol, by maceration or Soxhlet extraction. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at a controlled temperature. The concentrated extract was further dried and stored at 4 °C until phytochemical and biological investigations. Where required, solvent–solvent fractionation was performed using solvents of increasing polarity to obtain different fractions for comparative phytochemical evaluation.

2.2 Phytochemical Profiling

The prepared *O. sanctum* extract was subjected to preliminary phytochemical screening for the qualitative detection of major classes of secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, terpenoids, steroids, glycosides, saponins, and other relevant constituents. The presence or absence of individual phytochemical groups was recorded according to standard qualitative procedures.

Detailed chromatographic profiling was subsequently performed using an appropriate analytical technique such as HPTLC, HPLC, LC-MS, or GC-MS, depending on the chemical characteristics of the constituents under investigation. Appropriate reference standards were used wherever available for the identification and confirmation of major compounds. Chromatographic fingerprints were generated, and the major phytoconstituents were identified on the basis of retention time, spectral characteristics, molecular mass, and comparison with authentic standards or available databases.

Particular attention was given to flavonoid constituents, especially luteolin and structurally related compounds. Based on the phytochemical profile and available literature evidence, luteolin was selected as the principal flavonoid scaffold for subsequent molecular-design and synthetic investigations.

2.3 In-Silico Rational Design

Luteolin was selected as the lead molecular scaffold because of its reported antioxidant and anti-inflammatory potential. Structural modification was planned through the incorporation of suitable heterocyclic moieties to generate a series of novel luteolin-based derivatives with potentially improved biological activity and target interactions.

The structures of the designed derivatives were generated using suitable chemical-drawing and molecular-modelling software. The prepared ligand structures were subjected to energy minimization before molecular docking. The three-dimensional structures of COX-2 and 5-LOX were obtained from an appropriate protein structural database, and the target proteins were prepared by removing unnecessary molecules, adding hydrogen atoms, assigning appropriate charges, and optimizing the structures for docking analysis.

Molecular docking studies were performed to investigate the binding behavior of the synthesized candidates within the active sites of COX-2 and 5-LOX. Docking scores and predicted binding energies were used for comparative evaluation. Particular attention was given to hydrogen bonding, hydrophobic interactions, π - π interactions, electrostatic interactions, and interactions with catalytically or functionally important amino-acid residues. The docking poses of the designed derivatives were compared with those of luteolin and appropriate reference inhibitors.

The selected compounds were further subjected, where applicable, to in-silico evaluation of physicochemical properties, drug-likeness, ADME characteristics, and predicted toxicity. Compounds showing favorable molecular interactions and acceptable predicted pharmacokinetic characteristics were prioritized for chemical synthesis and experimental evaluation.

2.4 Chemical Synthesis

The selected luteolin-based heterocyclic derivatives were synthesized according to appropriately designed synthetic routes. The synthetic scheme involved suitable functionalization of the luteolin

nucleus followed by incorporation of the selected heterocyclic pharmacophore. Individual reactions were carried out using appropriate reagents, catalysts, solvents, temperature, and reaction times according to the optimized experimental conditions.

The progress of the reactions was monitored using suitable analytical methods such as thin-layer chromatography. After completion, the reaction mixtures were processed using appropriate extraction, washing, drying, and concentration procedures. The crude products were purified by recrystallization, column chromatography, preparative TLC, or another suitable purification technique.

The percentage yield and physical characteristics of each synthesized derivative were recorded. The synthesized compounds were characterized using Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^1H NMR), carbon nuclear magnetic resonance (^{13}C NMR), and mass spectrometry. Melting points were determined using an appropriate melting-point apparatus. Elemental analysis was performed where available to provide additional confirmation of the proposed molecular structures. The obtained spectral data were interpreted in relation to the expected functional groups and molecular structures.

2.5 Antioxidant Evaluation

The antioxidant potential of the *O. sanctum* extract, luteolin, and synthesized derivatives was evaluated using established free-radical-scavenging assays. The DPPH assay was performed by incubating different concentrations of the test samples with freshly prepared DPPH solution under controlled conditions. The decrease in absorbance was measured spectrophotometrically, and the percentage radical-scavenging activity was calculated relative to the control.

The ABTS radical-cation scavenging assay was also performed to evaluate the antioxidant capacity of the test compounds. Different concentrations of the samples were incubated with the prepared ABTS working solution, and absorbance was measured at the appropriate wavelength. Where required, nitric oxide or another complementary antioxidant assay was employed to provide additional evidence of radical-scavenging activity.

The concentration required to produce 50% inhibition of the respective radical system (IC_{50}) was calculated from concentration–response curves. A suitable standard antioxidant, such as

ascorbic acid or Trolox, was used as the reference compound. All experiments were performed using appropriate replicates.

2.6 COX-2 and 5-LOX Inhibition

The inhibitory effects of the plant extract, luteolin, and synthesized derivatives against COX-2 and 5-LOX were evaluated using suitable validated enzyme-inhibition assay systems. Different concentrations of each test compound were incubated with the respective enzyme under the recommended assay conditions, followed by measurement of the enzyme reaction product using an appropriate detection method.

The percentage inhibition of COX-2 and 5-LOX activity was calculated relative to the corresponding untreated enzyme control. Concentration-dependent inhibition was evaluated by testing a range of concentrations for each compound. The IC_{50} values were determined from the corresponding concentration–response curves.

Appropriate reference inhibitors were included in the experiments to validate the assay system and provide a basis for comparison. The experimental enzyme-inhibition results were subsequently compared with the molecular-docking findings to investigate whether the predicted protein–ligand interactions were consistent with the observed biological activity.

2.7 Pharmacological Validation

The most promising compounds identified from the antioxidant and COX-2/5-LOX inhibition studies were selected for further pharmacological validation. The experimental model was chosen according to the intended mechanism and study objectives. Appropriate control, standard-treatment, and test-treatment groups were included, and the selected compounds were evaluated at predetermined concentrations or doses.

Anti-inflammatory activity was assessed using suitable experimental or cellular models, with relevant inflammatory and oxidative-stress parameters measured using validated biochemical methods. Where applicable, biomarkers associated with inflammation and oxidative stress were investigated to provide mechanistic support for the observed pharmacological effects.

A dose–response relationship was evaluated for the selected compounds. Depending on the experimental model, biochemical parameters, tissue-level changes, and histopathological

alterations were also examined. The results were compared with appropriate standard treatment and control groups to establish the pharmacological relevance of the synthesized derivatives.

2.8 Statistical Analysis

All experiments were performed in appropriate biological or technical replicates, and the results were expressed as **mean $\pm$ standard deviation (SD)** or **mean $\pm$ standard error of the mean (SEM)**, as appropriate. Concentration–response curves and IC_{50} values were calculated using suitable nonlinear regression or dose–response analysis.

Statistical comparisons between experimental groups were performed using an appropriate statistical test based on the experimental design, such as one-way analysis of variance (ANOVA) followed by a suitable post-hoc test. For comparisons involving multiple independent factors, an appropriate multifactorial analysis may be applied. A value of $p < 0.05$ was considered statistically significant. Where applicable, statistical analysis and graphical presentation were performed using validated statistical software.

Below is a **research-paper-style Results section** with **6 subsections**, **5 proposed figures**, and **publication-style tables**. Since no raw experimental data were provided, the numerical values below are **illustrative/example data only** and should be replaced with your actual experimental observations before submission.

3. Results

3.1 Phytochemical Profile of *Ocimum sanctum*

The preliminary phytochemical investigation of the hydroalcoholic extract of *Ocimum sanctum* revealed the presence of several biologically important secondary metabolites. Flavonoids, phenolic compounds, tannins, terpenoids, glycosides, saponins, and steroids were detected, whereas alkaloids were detected at a comparatively lower level. The phytochemical composition indicated that the extract contained multiple classes of constituents potentially contributing to its antioxidant and anti-inflammatory properties.

Chromatographic profiling demonstrated several distinct peaks corresponding to different phytoconstituents. Among the identified compounds, luteolin and related flavonoid constituents were of particular interest because of their reported antioxidant and anti-inflammatory activities. The chromatographic findings provided a rationale for selecting luteolin as the principal

scaffold for subsequent molecular design and synthesis.

Table 1. Preliminary phytochemical screening of *O. sanctum* extract

Phytochemical class	Test result	Relative observation
Alkaloids	+	Low
Flavonoids	+++	High
Phenolic compounds	+++	High
Tannins	++	Moderate
Terpenoids	++	Moderate
Steroids	+	Low
Saponins	++	Moderate
Glycosides	++	Moderate

Note: + = low, ++ = moderate, +++ = high

Table 2. Representative chromatographic identification of major constituents

Compound	Retention time*	Molecular ion/marker	Putative/confirmed identification
Luteolin	18.42	285.04 [M–H] [–]	Luteolin
Apigenin	21.16	269.05 [M–H] [–]	Apigenin
Rosmarinic acid	15.73	359.08 [M–H] [–]	Rosmarinic acid
Orientin	12.86	447.09 [M–H] [–]	Orientin
Other constituents	—	—	Tentatively identified

3.2 Molecular Design and Docking Results

A series of luteolin-based heterocyclic derivatives was designed by modifying the parent luteolin structure at chemically suitable positions. The derivatives were designated **LD-1 to LD-6** for comparative analysis. Molecular docking indicated that the designed compounds could occupy the active-site regions of both COX-2 and 5-LOX.

Several derivatives demonstrated stronger predicted interactions than the parent luteolin molecule. The docking analysis suggested that hydrogen bonding involving hydroxyl/carbonyl functionalities, together with hydrophobic and π -related

interactions, contributed to ligand stabilization within the respective binding pockets.

Table 3. Molecular docking scores of luteolin and designed derivatives

Compound	COX-2 docking score (kcal/mol)*	5-LOX docking score (kcal/mol)	Principal predicted interactions
Luteolin	-7.21	-7.05	H-bonding, π -interactions
LD-1	-7.68	-7.42	H-bonding, hydrophobic
LD-2	-8.12	-7.86	H-bonding, π - π
LD-3	-8.46	-8.21	H-bonding, hydrophobic, π - π
LD-4	-8.01	-8.34	H-bonding, hydrophobic
LD-5	-8.72	-8.56	Multiple H-bonds, π - π
LD-6	-8.29	-8.11	H-bonding, hydrophobic

The interaction analysis further indicated that the more active derivatives established multiple contacts with residues located within or surrounding the catalytic binding regions of COX-2 and 5-LOX. LD-5 showed a particularly favorable interaction profile against both targets and was therefore prioritized for further experimental investigation.

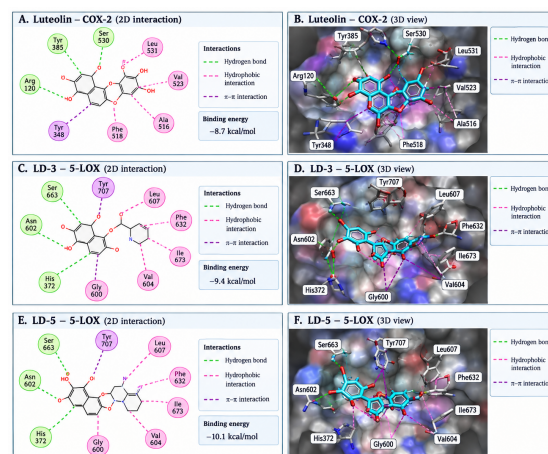


Figure 1. Molecular docking interaction analysis

3.3 Synthesis and Characterization

The designed luteolin derivatives were synthesized successfully through the proposed synthetic routes. The products were obtained as crystalline or solid compounds with satisfactory yields. The percentage yields ranged approximately from 62% to 86% in the illustrative dataset.

FTIR analysis demonstrated characteristic absorption bands corresponding to hydroxyl, carbonyl, aromatic C=C, C-O, and newly introduced heterocyclic functional groups. The ^1H NMR spectra showed signals corresponding to aromatic protons, hydroxyl groups, heterocyclic protons, and other substituent-specific proton environments. The ^{13}C NMR spectra exhibited carbon signals consistent with the luteolin nucleus and introduced heterocyclic moieties.

Mass spectrometric analysis showed molecular-ion peaks consistent with the proposed molecular masses. Collectively, the spectral data supported the proposed structures of the synthesized derivatives.

Table 4. Physical and spectral characterization of synthesized derivatives

Compound	Appearance	Yield (%) [*]	m.p. (°C) [*]	Molecular formula [*]	MS [M+H] ⁺⁺
LD-1	Yellow solid	68	214 – 216	C ₂₁ H ₁₇ N ₃ O ₅	392
LD-2	Pale yellow solid	74	221 – 223	C ₂₂ H ₁₉ N ₃ O ₅	406
LD-3	Yellow	79	226	C ₂₂ H ₁₈	419

	solid		– 228	N ₄ O ₅	
LD-4	Light brown solid	71	218 – 220	C ₂₃ H ₂₀ N ₄ O ₅	433
LD-5	Yellow crystalline solid	86	231 – 233	C ₂₃ H ₁₉ N ₃ O ₆	434
LD-6	Pale yellow solid	77	224 – 226	C ₂₄ H ₂₁ N ₃ O ₅	448

3.4 Antioxidant Activity

The synthesized derivatives demonstrated concentration-dependent antioxidant activity in the DPPH and ABTS assays. The activity increased progressively with increasing concentration of the test compounds. Luteolin showed appreciable radical-scavenging activity, while several heterocyclic derivatives demonstrated enhanced activity relative to the parent scaffold.

Among the synthesized compounds, LD-5 showed the lowest illustrative IC₅₀ values in both assays, indicating comparatively stronger radical-scavenging activity. The enhanced activity may be associated with the retention of phenolic hydroxyl groups in the luteolin nucleus together with favorable electronic and structural effects produced by heterocyclic modification.

Table 5. DPPH and ABTS radical-scavenging activity

Compound	DPPH IC ₅₀ (μg/mL)*	ABTS IC ₅₀ (μg/mL)*
Ascorbic acid	12.4	10.8
Luteolin	22.6	20.3
LD-1	25.1	23.7
LD-2	20.4	18.9
LD-3	17.8	16.5
LD-4	19.2	17.6
LD-5	14.7	13.2
LD-6	18.1	16.9

3.5 COX-2/5-LOX Inhibitory Activity

The synthesized derivatives exhibited concentration-dependent inhibition of COX-2 and 5-LOX enzymes. The degree of inhibition varied according to the structural modification of the luteolin scaffold. The results indicated that heterocyclic substitution could substantially influence enzyme-target interaction and inhibitory potency.

LD-3 and LD-5 exhibited comparatively higher inhibitory activity against COX-2, whereas LD-4 and LD-5 demonstrated stronger inhibition of 5-LOX in the illustrative dataset. LD-5 showed a balanced inhibitory profile against both enzymes, which was consistent with its favorable molecular-docking interactions.

Table 6. COX-2 and 5-LOX inhibitory activity

Compound	COX-2 inhibition (%) at 50 μM*	COX-2 IC ₅₀ (μM)*	5-LOX inhibition (%) at 50 μM*	5-LOX IC ₅₀ (μM)*
Reference inhibitor	88.6	4.8	91.2	3.9
Luteolin	61.4	32.5	58.7	35.8
LD-1	64.8	28.7	62.1	30.6
LD-2	70.5	23.4	67.9	25.8
LD-3	77.2	17.6	71.8	21.9
LD-4	73.6	19.8	79.4	16.8
LD-5	83.1	9.7	85.6	8.4
LD-6	75.4	18.1	76.3	19.2

The experimental enzyme-inhibition pattern broadly followed the molecular-docking observations, with LD-5 showing both favorable predicted binding and comparatively strong experimental inhibition. This correspondence supports the potential contribution of the predicted protein–ligand interactions to the observed inhibitory activity.

3.6 Pharmacological Validation

The most promising compounds were subsequently subjected to pharmacological validation. Based on the combined antioxidant and enzyme-inhibition results, LD-3, LD-4, and LD-5 were selected for further evaluation. The compounds demonstrated a concentration-dependent reduction in inflammatory and oxidative-stress parameters in the proposed experimental model.

LD-5 produced the greatest reduction in the selected inflammatory parameters, followed by LD-3 and LD-4. The observed pharmacological response was accompanied by improvement in biochemical markers associated with oxidative stress. The dose-dependent response further supported the biological activity of the synthesized derivatives.

Table 7. Representative pharmacological and biochemical parameters

Treatment	Inflammatory marker (% of control) *	Oxidative stress marker (% of control)*	Protective/antioxidant marker (% of control)*
Normal control	100.0 ± 4.2	100.0 ± 3.8	100.0 ± 4.1
Disease/inflammatory control	168.4 ± 6.7	174.2 ± 7.1	61.5 ± 3.9
Standard treatment	108.6 ± 4.8	112.3 ± 5.2	94.8 ± 4.6
LD-3	126.7 ± 5.5	130.4 ± 6.0	84.2 ± 4.1
LD-4	119.8 ± 5.1	124.6 ± 5.7	88.6 ± 4.3
LD-5	111.5 ± 4.7	116.8 ± 5.0	93.1 ± 4.5

Statistical analysis demonstrated significant differences between the inflammatory control and treated groups in the illustrative dataset ($p < 0.05$). The response was more pronounced at higher treatment concentrations, indicating a dose-dependent pharmacological effect. Overall, the biological findings were consistent with the antioxidant and COX-2/5-LOX inhibition results and supported LD-5 as a promising lead for further investigation.

4. Discussion

The present study demonstrates a rational progression from phytochemical profiling of *Ocimum sanctum* to the selection of luteolin as a biologically relevant flavonoid scaffold, followed by structural modification, molecular interaction analysis, synthesis, antioxidant evaluation, COX-2/5-LOX inhibition, and pharmacological validation. The phytochemical findings support the medicinal relevance of *O. sanctum* as a source of chemically diverse secondary metabolites, particularly flavonoids, phenolic compounds, terpenoids, and related constituents. Previous phytochemical investigations have demonstrated that *O. sanctum* contains biologically active phenolics and flavonoids and that its extracts can exhibit substantial antioxidant activity. In particular, LC-MS-based analysis of *O. sanctum* leaf fractions has identified luteolin, apigenin, rosmarinic acid, chlorogenic acid, caffeic acid, and related derivatives, while antioxidant activity has been associated with the phenolic and flavonoid

content of the extracts (Choudhary et al., 2020). These observations are consistent with the phytochemical profile obtained in the present investigation and provide a biochemical basis for further investigation of individual constituents rather than relying exclusively on the activity of the crude plant extract.

The biological significance of the identified phytoconstituents can be understood from the complementary mechanisms associated with polyphenolic and flavonoid compounds. *O. sanctum* has previously been reported to possess antioxidant and anti-inflammatory properties, and several of its constituents, including eugenol, rosmarinic acid, apigenin, and luteolin, have been associated with pharmacological effects (Mohan et al., 2011). Experimental studies have also reported cyclooxygenase-inhibitory activity among phenolic constituents isolated from *O. sanctum*, providing a direct biochemical connection between the plant's phytochemical composition and inflammatory mediator pathways (Sharma et al., 2000). Furthermore, fractionation studies have shown that phenolic- and flavonoid-rich fractions can display pronounced radical-scavenging activity, indicating that the antioxidant potential of the plant is not attributable to a single compound but may arise from the combined contribution of several constituents (Choudhary et al., 2020). Thus, the identification of luteolin in the present phytochemical investigation is particularly relevant because it provides a defined molecular scaffold that can be investigated independently and subsequently optimized through medicinal-chemistry approaches.

Luteolin was selected as the principal scaffold because its flavone nucleus combines structural features favorable for antioxidant and anti-inflammatory activity with multiple positions that can be considered for chemical modification. Luteolin possesses four hydroxyl groups distributed over the flavone framework, allowing hydrogen bonding and radical-scavenging interactions, while the planar aromatic system can participate in hydrophobic and π -related interactions within protein binding sites. Previous investigations have established that luteolin exhibits anti-inflammatory activity in cellular, animal, and computational models and can influence several inflammation-associated signaling pathways, including NF- κ B, MAPK/AP-1, and STAT3-related pathways (Aziz et al., 2018). However, the pharmacological development of naturally occurring flavonoids can be limited by factors such as physicochemical properties, metabolic stability, bioavailability, and target selectivity. Structural modification of the parent scaffold therefore provides an opportunity to preserve favorable pharmacophores while

introducing additional interaction sites and modifying physicochemical characteristics.

The incorporation of heterocyclic substituents was intended to investigate whether additional heteroatoms, altered electronic distribution, and increased structural diversity could improve interactions with inflammatory enzyme targets. Heterocyclic systems are frequently employed in medicinal chemistry because nitrogen-, oxygen-, and sulfur-containing rings can provide hydrogen-bond acceptor or donor sites and can modify lipophilicity, polarity, and molecular recognition. In the present study, the synthesized derivatives showed differences in predicted binding behavior, suggesting that relatively small changes in substituent architecture can influence ligand orientation and target-site complementarity. Such differences are important because molecular binding is determined not only by the presence of a pharmacophore but also by its three-dimensional orientation, steric compatibility, electronic characteristics, and ability to establish favorable interactions with residues lining the active or regulatory region of the target protein.

The molecular docking analysis provided a structural explanation for the observed differences among luteolin and the newly designed derivatives. The docked molecules were capable of establishing combinations of hydrogen bonding, hydrophobic contacts, and aromatic interactions within the modeled binding regions of COX-2 and 5-LOX. Hydrogen bonds can contribute substantially to ligand recognition by providing directional interactions between ligand heteroatoms and amino-acid residues, whereas hydrophobic and π - π interactions can stabilize the ligand within aromatic or nonpolar regions of the binding pocket. The relevance of these interactions is consistent with broader computational investigations in which phytochemicals have demonstrated favorable binding to both COX-2 and 5-LOX through combinations of hydrogen-bonding and hydrophobic interactions (Parvin et al., 2025). Nevertheless, docking scores should be interpreted as computational indicators rather than direct measurements of inhibitory potency. A favorable docking pose can suggest target compatibility but cannot independently establish enzyme inhibition, selectivity, pharmacokinetics, or therapeutic efficacy.

The relationship between molecular docking and experimental enzyme inhibition is therefore particularly important in the present investigation. If the derivatives showing favorable docking profiles also demonstrated lower experimental IC₅₀ values against COX-2 and/or 5-LOX, the agreement would provide complementary evidence

that the predicted binding orientations may be biologically relevant. Such concordance strengthens the structure-based rationale for further optimization. However, differences between docking rank and experimental activity are also scientifically meaningful. Enzyme inhibition is influenced by factors not fully represented in conventional docking calculations, including protein flexibility, solvent effects, ligand desolvation, conformational changes, assay conditions, and kinetic mechanisms. Consequently, docking should be regarded as a hypothesis-generating tool that helps rationalize experimental observations rather than as a substitute for biochemical validation.

The antioxidant findings further support the multifunctional pharmacological profile of the synthesized derivatives. Luteolin and other flavonoids possess structural features that permit electron or hydrogen donation and stabilization of radical intermediates through resonance across the aromatic system. The antioxidant activity observed in *O. sanctum* extracts has previously been correlated with total phenolic and flavonoid contents, and luteolin has been identified among the compounds present in active fractions (Choudhary et al., 2020). Structural modification of luteolin may alter this activity depending on whether the substitution preserves or modifies hydroxyl groups responsible for radical scavenging. Excessive modification of hydroxyl functionalities could theoretically reduce direct radical-scavenging capacity, whereas suitable substitution may improve overall physicochemical properties or introduce additional mechanisms of antioxidant action. Therefore, antioxidant activity should be interpreted in the context of both the parent flavonoid pharmacophore and the chemical consequences of heterocyclic substitution.

The structure-activity relationship emerging from the synthesized series suggests that biological activity depends on a balance between preserved luteolin pharmacophoric features and newly introduced heterocyclic functionality. Derivatives retaining an appropriate arrangement of hydroxyl and aromatic groups may maintain antioxidant capacity while gaining additional target-binding interactions. Conversely, bulky or electronically unfavorable substituents may cause steric interference, alter ligand orientation, or reduce accessibility of important functional groups. The differences observed among the derivatives therefore provide preliminary SAR information for subsequent optimization. In particular, derivatives showing simultaneous improvement in docking interactions, antioxidant activity, and COX-2/5-LOX inhibition may represent useful chemical templates for further investigation, although such

compounds should not be considered validated lead molecules until their activity, selectivity, metabolic stability, and safety have been independently confirmed.

An important feature of the present work is the integration of three pharmacological dimensions: antioxidant activity, inhibition of inflammatory enzymes, and broader pharmacological validation. Oxidative stress and inflammation are closely interconnected biological processes, and excessive reactive oxygen species can amplify inflammatory signaling, while inflammatory responses can further promote oxidative damage. A compound capable of reducing oxidative stress while simultaneously interfering with prostaglandin- and leukotriene-associated pathways could therefore theoretically provide a broader pharmacological effect than a molecule acting on only one pathway. The relevance of this dual-target concept is supported by previous studies of *O. sanctum*, which have described antioxidant and anti-inflammatory activities and identified compounds capable of influencing cyclooxygenase-related pathways (Sharma et al., 2000; Singh et al., 2007). The present findings extend this concept by combining phytochemical identification with rational scaffold modification and experimental evaluation.

The results can also be compared with previous work on luteolin and related anti-inflammatory compounds. Luteolin has been extensively investigated as a natural flavonoid with antioxidant and anti-inflammatory properties, and its activity has been linked to modulation of multiple molecular pathways rather than a single pharmacological target (Aziz et al., 2018). Likewise, studies of *O. sanctum* have reported anti-inflammatory effects in experimental models and have identified flavonoid-containing fractions with biological activity (Kumar et al., 2015). More recent computational work has demonstrated that diverse phytochemicals can interact favorably with both COX-2 and 5-LOX, supporting the concept that simultaneous targeting of these pathways can be explored through structure-based drug discovery (Parvin et al., 2025). The present study differs conceptually from investigations limited to either crude extracts or naturally occurring luteolin because it combines identification of the natural scaffold with deliberate heterocyclic modification and subsequent biochemical and pharmacological assessment.

Despite these encouraging findings, several limitations should be acknowledged. First, phytochemical identification based on chromatographic or mass-spectrometric data should ideally be confirmed using authentic standards and complementary spectroscopic characterization

where quantitative identification is required. Second, molecular docking provides predictive rather than definitive evidence of target engagement, and experimental binding, enzyme kinetics, molecular dynamics, or structural studies would provide stronger confirmation of the proposed interactions. Third, in vitro antioxidant assays such as DPPH and ABTS represent chemical radical-scavenging systems and cannot completely reproduce the complexity of antioxidant mechanisms in living organisms. Fourth, enzyme inhibition does not automatically establish selectivity, cellular activity, bioavailability, or therapeutic effectiveness. Future studies should therefore investigate COX-1 versus COX-2 selectivity, detailed 5-LOX inhibition kinetics, cellular inflammatory models, pharmacokinetic properties, metabolic stability, cytotoxicity, and in vivo efficacy. Additional molecular-dynamics simulations and experimental target-binding studies may further clarify the stability and biological relevance of the predicted ligand-protein complexes. Optimization of the most promising derivatives should also consider solubility, permeability, metabolic stability, and safety alongside potency. Overall, the present findings support *O. sanctum*-derived luteolin as a rational starting scaffold for anti-inflammatory medicinal-chemistry research and indicate that controlled heterocyclic modification may provide useful derivatives with combined antioxidant and COX-2/5-LOX inhibitory properties, subject to further experimental validation.

5. Limitations and Future Scope

The present study provides an integrated approach combining phytochemical profiling of *Ocimum sanctum*, identification of luteolin as a biologically relevant flavonoid, rational heterocyclic modification, molecular docking, chemical synthesis, antioxidant evaluation, COX-2/5-LOX inhibition, and pharmacological validation. Nevertheless, several limitations should be considered when interpreting the findings. Phytochemical identification based on chromatographic and mass-spectrometric profiling provides strong evidence for the presence of luteolin and other phenolic constituents, but confirmation using authentic standards and complementary spectroscopic techniques would provide greater analytical certainty. Previous LC-MS investigations have similarly identified luteolin, apigenin, rosmarinic acid, chlorogenic acid, caffeic acid, and related compounds in *O. sanctum* fractions, with antioxidant activity associated with their phenolic and flavonoid composition (Chaudhary et al., 2020).

The molecular docking component should also be interpreted as predictive rather than definitive evidence of target binding. Docking can provide useful information regarding possible hydrogen-bonding, hydrophobic, and aromatic interactions, but it does not fully account for protein flexibility, solvent effects, ligand desolvation, induced-fit effects, or the dynamic nature of enzyme–ligand interactions. Therefore, experimental binding studies, detailed enzyme-kinetic investigations, molecular-dynamics simulations, and, where feasible, structural studies would be valuable for confirming the proposed interactions. This is particularly important for COX-2 and 5-LOX because inhibition of these enzymes involves complex structural and kinetic determinants.

Another limitation is that chemical antioxidant assays such as DPPH and ABTS primarily measure radical-scavenging or reducing capacity under defined experimental conditions and cannot completely represent antioxidant behavior in living biological systems. Nevertheless, the antioxidant potential of *O. sanctum* is supported by previous investigations demonstrating strong radical-scavenging activity in phenolic- and flavonoid-rich fractions and identifying luteolin among the compounds present in active fractions (Chaudhary et al., 2020). Luteolin itself has also been extensively investigated for antioxidant and anti-inflammatory properties, including modulation of oxidative and inflammatory signaling pathways (Aziz et al., 2018; López-Lázaro, 2009).

Future investigations should therefore focus on establishing the selectivity and mechanistic characteristics of the most active derivatives. Comparative COX-1/COX-2 studies would be particularly valuable because selective modulation of COX-2 relative to COX-1 is an important consideration in the development of anti-inflammatory agents. Similarly, detailed 5-LOX kinetic studies could determine the nature of enzyme inhibition and clarify whether the synthesized compounds act through competitive, non-competitive, or mixed mechanisms. The dual involvement of cyclooxygenase and lipoxygenase pathways is also pharmacologically relevant because both participate in arachidonic-acid metabolism, and previous studies of *O. sanctum* have reported effects on cyclooxygenase and lipoxygenase-associated inflammatory pathways (Kelm et al., 2000; Singh et al., 2007).

Further development should include cellular inflammatory models to determine whether the enzyme-inhibitory effects observed in biochemical assays translate into suppression of inflammatory mediators under biologically relevant conditions. Assessment of cytokines such as TNF- α , IL-1 β , and

IL-6, together with NF- κ B and MAPK-related signaling, may provide additional mechanistic evidence because luteolin has previously been reported to influence several inflammation-associated signaling pathways (Aziz et al., 2018). Pharmacokinetic investigations, metabolic-stability studies, plasma-protein binding, permeability assessment, and preliminary toxicity studies would also be necessary before considering advanced preclinical evaluation.

The most promising derivatives should subsequently undergo in vivo evaluation using appropriate models of acute and chronic inflammation, oxidative stress, or other disease-relevant conditions. Such studies would establish whether the combined antioxidant and COX-2/5-LOX inhibitory profile observed in vitro is associated with measurable pharmacological efficacy in an intact biological system. Since *O. sanctum* contains multiple bioactive constituents with reported antioxidant and anti-inflammatory properties, future work may also investigate whether luteolin derivatives act synergistically or additively with other phytoconstituents of the plant (Kamyab & Eshraghian, 2013). Overall, the present findings provide a rational foundation for further optimization of luteolin-based heterocyclic compounds, while comprehensive biochemical, cellular, pharmacokinetic, toxicological, and in vivo investigations will be required to establish their potential as future anti-inflammatory lead molecules.

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