Research Article

Novel Coumarin-Indole Hybrids as Dual COX-2 And 5-LOX Inhibitors: *In Silico* Analysis and Molecular Docking Studies

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ABSTRACT

Inflammation is a hallmark of various chronic diseases, often involving the overactivation of enzymes such as cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX). Targeting both pathways simultaneously offers an improved anti-inflammatory strategy with reduced side effects compared to traditional nonsteroidal anti-inflammatory drugs. In this study, a novel series of coumarin–indole hybrid molecules were designed and evaluated through computational methods for their potential as dual COX-2/5-LOX inhibitors. The designed compounds were subjected to silico screening using various softwares, confirming good drug-likeness and favorable pharmacokinetic profiles. Molecular docking was performed using AutoDock Vina. Among the designed molecules, compound IC18 demonstrated the highest binding affinity, with docking scores of –10.2 kcal/mol (COX-2) and –10.6 kcal/mol (5-LOX), surpassing standard drugs Indomethacin and Zileuton. These findings provide a basis for further synthesis and biological evaluation to confirm vitro efficacy.

Keywords: Coumarin–indole hybrids, Anti-inflammatory agents, Dual inhibition, Cyclooxygenase-2, 5-lipoxygenase, AutoDock Vina.

INTRODUCTION

Inflammation serves as a fundamental protective mechanism in the body, but when improperly regulated, it can become detrimental, contributing to the development and persistence of chronic illnesses such as rheumatoid arthritis, asthma, atherosclerosis, and neurodegenerative disorders like Alzheimer's disease.[1] Central to the inflammatory cascade is the metabolism of arachidonic acid, mediated by key enzymes including cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX), which generate pro-inflammatory compounds like prostaglandins and leukotrienes. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly employed to manage inflammation, their non-selective inhibition of cyclooxygenase enzymes often leads to undesirable gastrointestinal and cardiovascular side effects. As a result, recent research has focused on identifying compounds that simultaneously inhibit both COX-2 and 5-LOX, offering a more comprehensive and safer anti-inflammatory approach.[1]

Heterocyclic compounds such as coumarins and indoles have long been recognized for their diverse pharmacological properties, including antioxidant, antimicrobial, anticancer, and anti-inflammatory activities.[2, 3] Coumarins are known to inhibit inflammatory mediators and modulate oxidative stress, while indole derivatives exhibit strong binding affinity toward a wide range of enzymes and receptors due to their electron-rich, planar aromatic systems. The hybridization of these two scaffolds represents a promising strategy to enhance biological activity through synergistic mechanisms of action.

The present study focuses on the *in silico* design and molecular docking analysis of novel coumarin–indole hybrid derivatives as potential anti-inflammatory agents targeting both COX-2 and 5-LOX enzymes. The designed compounds were evaluated for drug-likeness using Lipinski's Rule of Five, while AutoDock Vina was employed to predict their binding affinities and interaction profiles within the active sites of COX-2 and 5-LOX (PDB ID: 6N2W**)**. The findings offer a computational rationale for further synthesis and biological validation of these dual-target inhibitors.[2, 3]

MATERIALS AND METHODS

Materials

A combination of cheminformatics and molecular modelling software was utilized to design and evaluate novel coumarin–indole hybrid compounds. Table 1 lists the utilized software programs together with associated utilities.

Table 1. List of softwares and their applications

Sl. No	Softwares	Applications
1	ChemSketch	Used to create and modify 2D and 3D chemical structures of designed ligands.
2	Molinspiration	Predicts drug-likeness and calculates physicochemical properties based on Lipinski's Rule of Five.
3	AutoDock Vina	Performs molecular docking and estimates binding affinities of ligand–protein complexes.
4	BIOVIA Discovery Studio	Visualizes protein–ligand interactions and analyzes docking poses and hydrogen bonds.

Methods

Preparation of protein

The three-dimensional crystal structures of cyclooxygenase-2 (COX-2, PDB ID: 6BL4) and 5-lipoxygenase (5-LOX, PDB ID: 6N2W) were retrieved from the Protein Data Bank in PDB format. These proteins were selected due to their critical roles in the arachidonic acid pathway, making them validated therapeutic targets for the development of anti-inflammatory drugs. The structures were prepared using BIOVIA Discovery Studio Visualizer, where the following steps were performed:

- Removal of water molecules, heteroatoms, and co-crystallized ligands.
- Addition of polar hydrogen atoms to ensure accurate modeling of hydrogen bonding interactions.
- Assignment of appropriate Kollman charges.
- Energy minimization was done.
- Active site identification was based on the coordinates of the co-crystallized ligand.

The cleaned and minimized protein structures were saved in PDBQT format using AutoDock Tools for subsequent docking analysis.

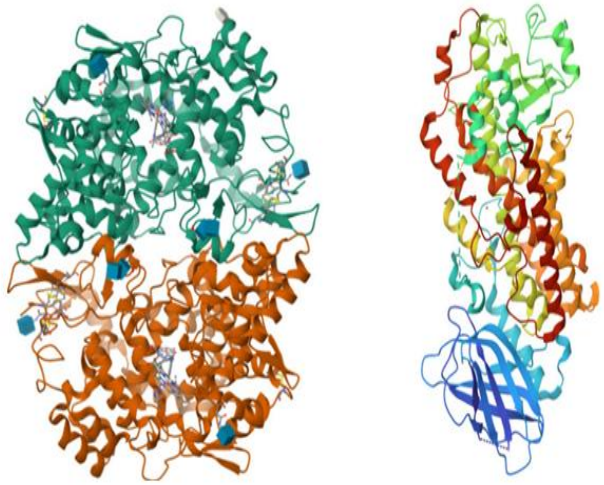
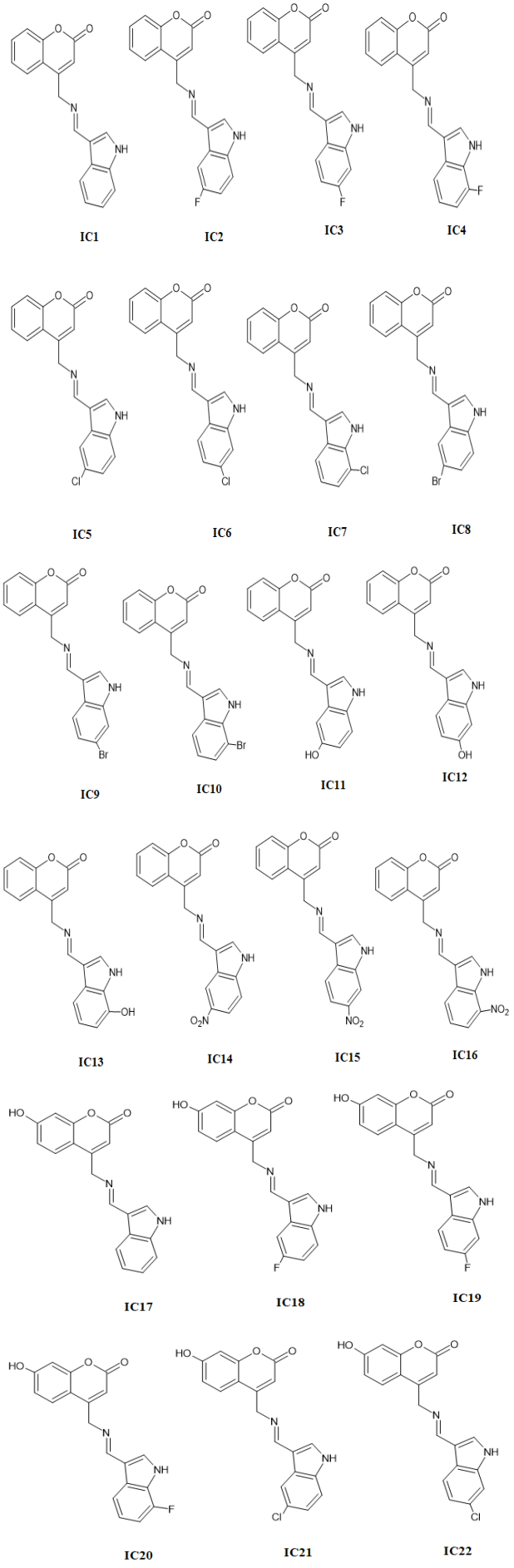


Figure 1: 3D Structures of 6BL4 and 6N2W.

Preparation of ligands

The ligands were first designed using ChemSketch software, and the structures were saved in **.**mol format. These files were then imported into BIOVIA Discovery Studio Visualizer**,** where hydrogens were added to complete valency and the structures were saved in Protein Data Bank (PDB) format. Further, the ligands were prepared for docking using AutoDock Tools by converting the 3D structures into PDBQT format.



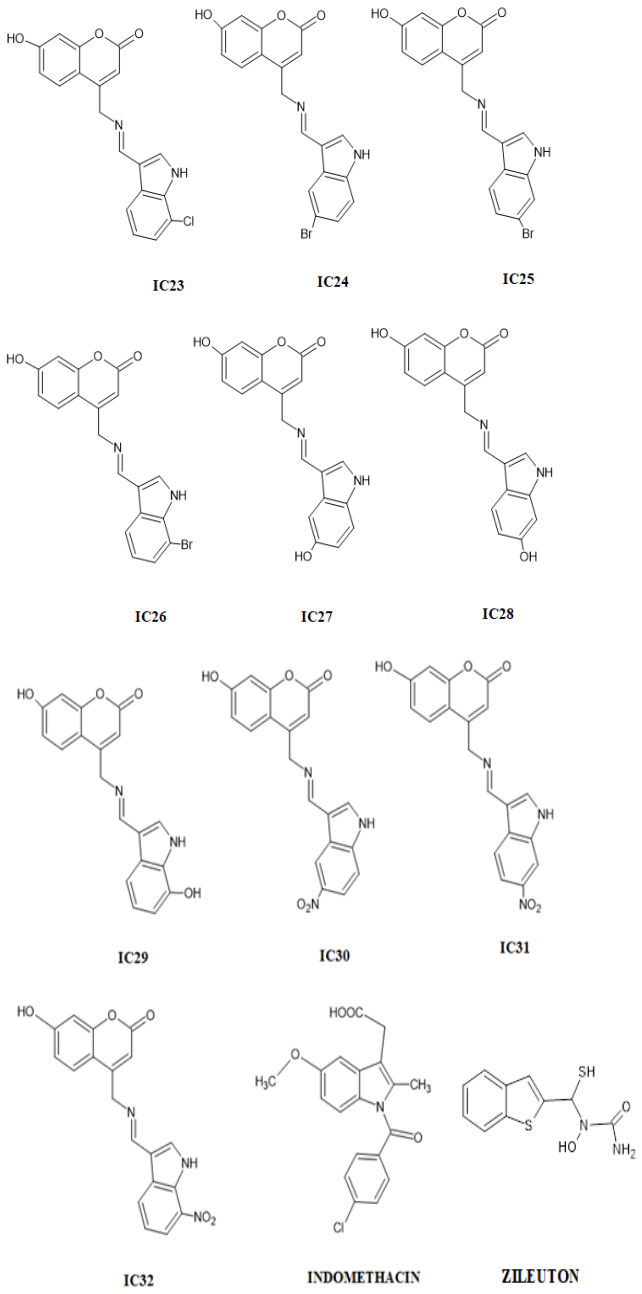


Figure 2: 2D structures of the ligands.

Lipinski's Rule of Five

Lipinski's Rule of Five is a widely accepted guideline for evaluating the drug-likeness of small molecules based on their physicochemical properties. According to this rule, a compound is more likely to be orally active if it does not violate more than one of the following parameters: molecular weight ≤ 500 Da, log P ≤ 5, hydrogen bond donors ≤ 5, and hydrogen bond acceptors ≤ 10. Compounds failing more than one of these criteria may show poor permeability or absorption potential *in vivo*.[4]

Molecular docking studies

Molecular docking is a core computational approach in drug discovery, utilized to predict how small molecules (ligands) orient and interact within the active site of a target protein. By simulating these interactions, docking aids in understanding the molecular basis of biological activity and facilitates the design of more effective therapeutic candidates.

In this study, molecular docking techniques were applied to evaluate the interaction potential of newly designed coumarin–indole hybrid compounds as possible anti-inflammatory agents. The inflammatory response is predominantly driven by enzymes such as cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX), which catalyze the formation of inflammatory mediators like prostaglandins and leukotrienes. Thus, simultaneous inhibition of both COX-2 and 5-LOX presents a valuable therapeutic strategy to improve anti-inflammatory outcomes and reduce side effects linked to conventional NSAID therapy.

Docking simulations were conducted using high-resolution crystal structures of COX-2 (PDB ID: 6BL4) and 5-LOX (PDB ID: 6N2W). The aim was to assess the binding orientations, interaction patterns, and predicted affinities of the hybrid molecules. Emphasis was placed on interactions with key residues in the catalytic domains of both enzymes to evaluate their inhibitory potential and inform future structural refinements.

RESULTS AND DISCUSSION

Lipinski's Rule of Five

Lipinski's Rule of Five provides a widely accepted framework to evaluate the oral bioavailability of drug candidates by examining key physicochemical properties such as molecular weight, lipophilicity (logP), the number of hydrogen bond donors (HBD), and hydrogen bond acceptors (HBA). In the present study, all designed compounds adhered to these guidelines without any rule violations. This suggests that the molecules possess favorable properties for oral absorption and reinforces their potential as promising drug-like agents for further pharmaceutical development.

Molecular Docking

To assess the binding potential of the synthesized coumarin–indole hybrids (IC1–IC32), molecular docking studies were performed against two key inflammatory targets: COX-2 (PDB ID: 6BL4) and 5-LOX (PDB ID: 6N2W). Binding affinities were calculated using AutoDock Vina, and the results were compared with standard inhibitors, Indomethacin for COX-2 and Zileuton for 5-LOX.

The docking scores, represented as binding free energy in kcal/mol, ranged from –9.2 to –10.3 for COX-2 and –8.4 to –10.6 for 5-LOX. Impressively, all the hybrid compounds outperformed the standard drugs, with Indomethacin docking at –8.9 kcal/mol (COX-2) and Zileuton at –7.4 kcal/mol (5-LOX).

Table 2. Lipinski's properties of the compounds

Sl. No	Compound	Mol.wt [g/mol]<500	LOG P <5	H-Donor <5	H-Acceptor <10	Violations
1	**IC1**	302.33	3.33	1	4	0
2	**IC2**	320.32	2.49	1	4	0
3	**IC3**	320.32	3.47	1	4	0
4	**IC4**	320.32	3.44	1	4	0
5	**IC5**	336.78	3.98	1	4	0
6	**IC6**	336.78	3.98	1	4	0
7	**IC7**	336.78	3.96	1	4	0
8	**IC8**	381.23	4.11	1	4	0
9	**IC9**	381.23	4.11	1	4	0
10	**IC10**	381.23	4.09	1	4	0
11	**IC11**	318.33	2.82	2	5	0
12	**IC12**	318.33	2.82	2	5	0
13	**IC13**	318.33	3.06	2	5	0
14	**IC14**	347.33	3.26	1	7	0
15	**IC15**	347.33	3.26	1	7	0
16	**IC16**	347.33	3.24	1	7	0
17	**IC17**	318.33	2.82	2	5	0
18	**IC18**	336.32	1.99	2	5	0
19	**IC19**	336.32	2.96	2	5	0
20	**IC20**	336.32	2.94	2	5	0
21	**IC21**	352.78	3.48	2	5	0
22	**IC22**	352.78	3.48	2	5	0
23	**IC23**	352.78	3.45	2	5	0
24	**IC24**	397.23	3.61	2	5	0
25	**IC25**	397.23	3.61	2	5	0
26	**IC26**	397.23	3.58	2	5	0
27	**IC27**	334.33	2.32	3	6	0
28	**IC28**	334.33	2.32	3	6	0
29	**IC29**	334.33	2.56	3	6	0
30	**IC30**	363.33	2.76	2	8	0
31	**IC31**	363.33	2.76	2	8	0
32	**IC32**	363.33	2.73	2	8	0
33	**Indomethacin**	343.77	4.13	1	5	0
34	Zileuton	236.30	2.46	3	4	0

Among the evaluated molecules:

- IC2, IC3, IC5, IC18, and IC30 exhibited outstanding binding strength toward both targets, with docking energies equal to or below –10.2 kcal/mol, indicating strong and stable interactions.
- IC18, in particular, emerged as the most promising candidate, showing binding energies of –10.2 kcal/mol with COX-2 and –10.6 kcal/mol with 5-LOX, making it superior to both reference drugs.

Several other candidates including IC6, IC7, IC13, IC15, IC22, and IC25 through IC32 also demonstrated robust dual-target binding profiles (docking scores ≤ –10.0 kcal/mol), highlighting their potential as dual inhibitors.

The favorable binding energies observed suggest that the coumarin–indole framework is well-suited for occupying the active sites of both enzymes. These interactions support the hypothesis that such hybrids could function as dual COX-2/5-LOX inhibitors, offering enhanced anti-inflammatory effects and possibly reducing adverse effects seen with selective inhibition.

Table 3. Binding affinities of designed derivatives

Compound	Binding energy (kcal/mol)	
	6BL4 (COX-2)	6N2W (5-LOX)
IC1	-9.8	-9.9
IC2	-10.2	-10.2
IC3	-10.2	-10.2
IC4	-10.0	-10.1
IC5	-10.2	-9.9
IC6	-9.7	-10.1
IC7	-9.4	-10.1
IC8	-9.6	-9.1
IC9	-9.7	-10.0
IC10	-9.8	-8.6
IC11	-10.1	-9.6
IC12	-9.8	-9.8
IC13	-9.3	-10.1
IC14	-9.9	-9.8
IC15	-9.3	-10.1
IC16	-9.9	-10.1
IC17	-9.7	-10.3
IC18	-10.2	-10.6
IC19	-10.0	-10.5
IC20	-9.8	-9.8
IC21	-10.0	-10.3
IC22	-9.7	-10.3
IC23	-9.5	-8.8
IC24	-10.1	-10.2
IC25	-9.7	-10.4
IC26	-9.4	-10.4
IC27	-9.9	-10.0
IC28	-9.9	-10.1
IC29	-9.2	-10.4
IC30	-10.3	-10.1
IC31	-9.7	-8.8
IC32	-9.6	-10.3
Indomethacin	-8.9	
Zileuton		-7.4

To assess the potential of the designed coumarin–indole hybrids as dual anti-inflammatory agents, the interaction profiles of **IC18** were compared with those of standard drugs **Indomethacin** (COX-2 inhibitor) and **Zileuton** (5-LOX inhibitor).

Indomethacin, the reference non-selective COX inhibitor, interacted with **TRP387** and **TYR348** via hydrogen bonding, along with π-interactions involving **PHE518**, **VAL523**, and **SER353**. While these interactions contribute to its anti-inflammatory effect, the interaction network is relatively limited. In contrast, **IC18** formed a more extensive and stronger binding network with COX-2. Major interactions include:

- **Hydrogen bonds** with **ASN382**, **THR212**, and **TYR148**
- **π–π stacking** with **HIS207**, **TRP387**, and **TYR385**
- Additional polar contacts with **GLN203** and **THR206**

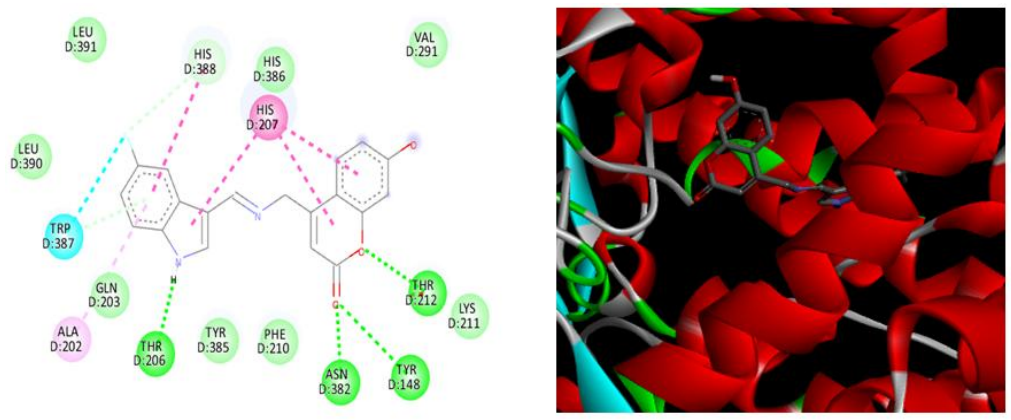
This rich interaction profile contributes to IC18's higher binding affinity (**–10.2 kcal/mol**) compared to Indomethacin (**–8.9 kcal/mol**), suggesting improved target occupancy and potential selectivity for COX-2.

Zileuton, a selective 5-LOX inhibitor, exhibited modest binding interactions centered primarily on **ARG457**, with auxiliary contacts involving **ALA453**, **PHE450**, and **LEU448**. The limited interaction profile correlates with its lower docking score of **–7.4 kcal/mol.**

Conversely, **IC18** demonstrated a significantly broader binding interface within the 5-LOX active site:

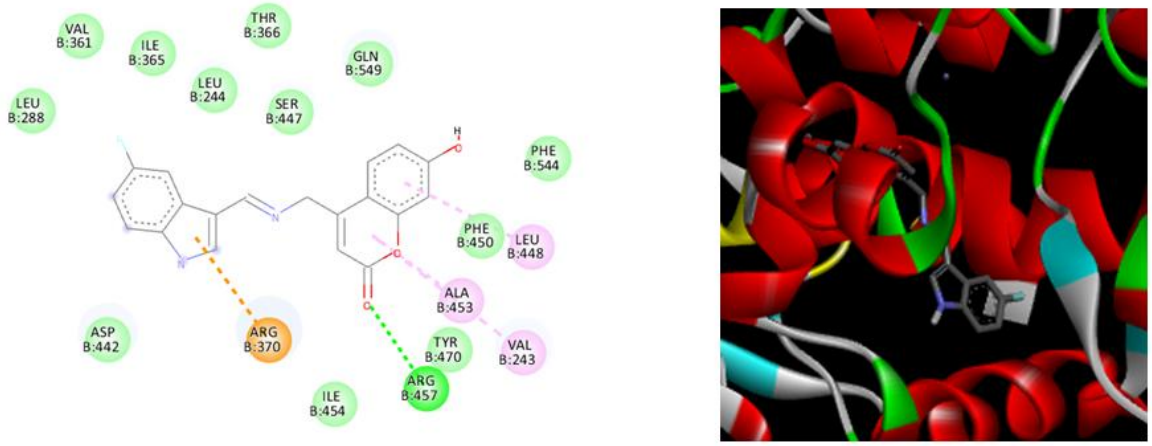
- **Hydrogen bonds** with **ARG457** and **TYR470**
- A strong **π–cation interaction** with **ARG370**
- Additional π–π and van der Waals contacts with **PHE450**, **PHE544**, and **ALA453**

These interactions explain IC18's much stronger docking score (**–10.6 kcal/mol**) and indicate its superior binding strength and stability within the 5-LOX binding pocket.



A B

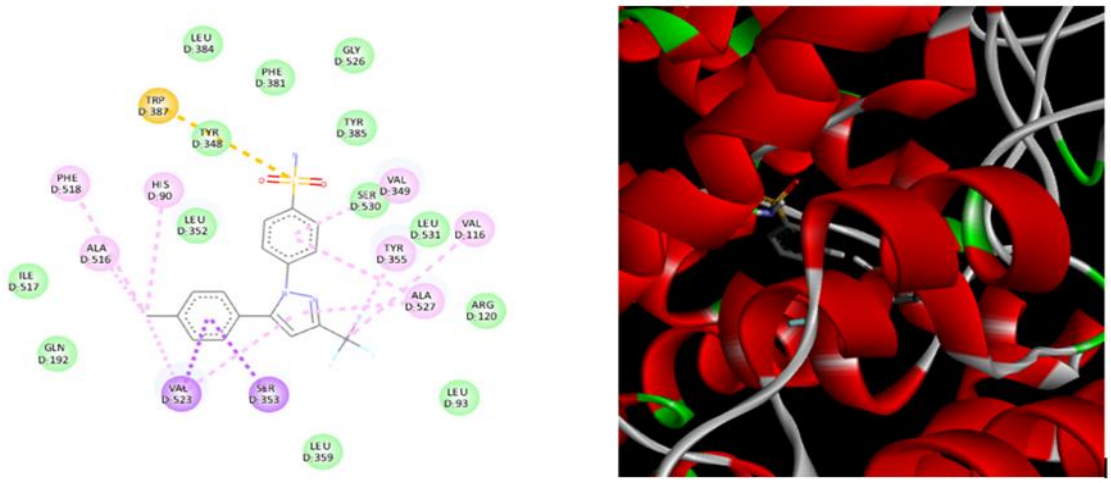
A) 2D structure of ligand IC18 interaction with binding site of protein 6BL4. B) 3D structure of ligand IC18 interaction with binding site of protein 6BL4.



C **D**

C) 2D structure of IC18 interaction with binding site of protein 6N2W. D) 3D structure of IC18 interaction with binding site of protein 6N2W.

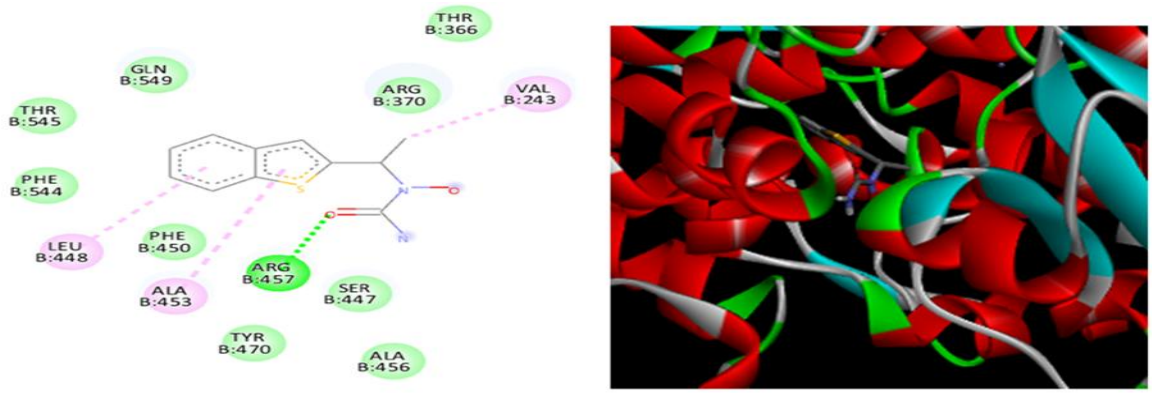
Figure 3: Binding interaction between the ligand and amino acids at the binding sites.



E **F**

E) 2D structure of Indomethacin interaction with binding site of protein 6BL4. F) 3D structure of Indomethacin interaction with binding site of protein 6BL4.

Figure 4: Binding interaction of standard drug Indomethacin at the binding sites.



G **H**

G) 2D structure of Zileuton interaction with binding site of protein 6N2W. H) 3D structure of Zileuton interaction with binding site of protein 6N2W.

Figure 5: Binding interaction of standard drug Zileuton at the binding sites.

CONCLUSION

This study focused on the *in silico* design and evaluation of a novel series of coumarin–indole hybrid compounds for their potential anti-inflammatory properties. All synthesized derivatives met Lipinski's Rule of Five, indicating favorable oral bioavailability and desirable drug-like characteristics.

Docking simulations were carried out against two central inflammatory mediators: COX-2 (PDB ID: 6BL4) and 5-LOX (PDB ID: 6N2W). A majority of the compounds exhibited stronger binding affinities than the benchmark drugs Indomethacin and Zileuton. Among them, IC18 emerged as the lead candidate, demonstrating the most potent dual-target binding with docking energies of –10.2 kcal/mol (COX-2) and –10.6 kcal/mol (5-LOX).

Interaction analysis revealed that IC18 formed stable complexes with both enzymes via multiple hydrogen bonds and π–π interactions involving critical active site residues, suggesting enhanced binding efficiency and specificity compared to the standards.

Overall, these computational results indicate that IC18 and structurally similar coumarin–indole hybrids may serve as promising dual inhibitors of COX-2 and 5-LOX, potentially offering improved anti-inflammatory efficacy with reduced side effects. Nonetheless, further biological evaluation through *in vitro* and *in vivo* studies is necessary to validate their therapeutic potential and safety profile.

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