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Article

Computer-aided Discovery of Novel COX-2 Inhibitors for Anti-inflammatory Therapy Using Pharmacophore Modelling, Molecular Docking, ADMET, and Virtual Screening

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Abstract

Cyclooxygenase-2 (COX-2) is a central enzyme in inflammation and a validated target for anti-inflammatory drug development. However, many existing COX-2 inhibitors cause gastrointestinal and cardiovascular side effects, necessitating safer alternatives. This study employed a computer-aided drug discovery approach integrating pharmacophore modeling, molecular docking, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis to identify natural COX-2 inhibitors from 72 sesquiterpene lactones. A validated pharmacophore model (AUC = 0.77) screened 33 potential inhibitors, and molecular docking revealed 13 compounds with strong binding affinities. Among them, Tanshinone IA showed the highest binding affinity (-10.6 kcal/mol), surpassing celecoxib (-9.5 kcal/mol). ADMET profiling indicated favorable pharmacokinetic properties, high gastrointestinal absorption, and low toxicity for most compounds. Overall, Tanshinone IA emerged as a promising, naturally derived COX-2 inhibitor with potential for safer anti-inflammatory therapy. These findings provide a computational foundation for future experimental validation and preclinical development of natural anti-inflammatory agents.

Keywords

Tanshinone IA, COX-2 Inhibitors, Pharmacophore Modelling, Molecular Docking, Anti-inflammatory therapy

Article History

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1. Introduction

Cyclooxygenase (COX), also known as prostaglandin G/H synthase, COX is an enzyme that plays a vital role in the body by converting arachidonic acid, (a crucial fatty acid) into a family of potent signaling molecules called prostanoids [1]. The essential prostanoids are Prostaglandins, thromboxanes, and prostacyclins, each influencing various physiological processes. The COX enzyme has two well-known isoforms COX-1 and COX-2 [2]. COX-1 is constitutively expressed, functioning as an essential housekeeping enzyme. It continuously catalyzes the production of prostanoids [3], a diverse group of signaling molecules, that contribute to maintaining physiological homeostasis through processes like platelet aggregation, kidneys protection, gastric mucosal protection, and blood flow regulation . In contrast to COX-1, COX-2 expression is typically undetectable under basal conditions. However, during an inflammatory response, inflammatory cytokines and other signaling molecules stimulate COX-2 production [4], which further catalyzes the production of additional prostanoids, such as prostaglandins, that act as local messengers, perpetuating the inflammatory response. Furthermore, the researchers highlighted the role of COX-2 in the development of a wide spectrum of diseases including respiratory illnesses, rheumatoid arthritis, osteoarthritis, cancer, neuropsychiatric disorders, major depressive disorder, and even viral infections like dengue fever [5]. Due to its specific role in inflammation and pain, COX-2 is a feasible target for the development of targeted anti-inflammatory and analgesic drugs [6].

Researchers over the past few decades developed various drugs which can be used to inhibit COX-2, targeting it as a therapeutic target for many inflammatory diseases. Structurally, COX-2 is composed of 604 amino acids (UniProt ID P35354) [7]. These amino acids are organized into three distinct regions and major domains. The first domain is called the epidermal growth factor (EGF) domain, which helps in the interaction of COX-2 with other molecules within the cell (amino acids 34-72). The second domain is the membrane binding domain which ensures COX-2 is positioned correctly within the cell membrane for proper functioning (amino acids 73-116). The third domain is the largest, and it is called the catalytic domain, where the real work happens. It contains the active sites for COX-2's enzymatic activities, allowing it to produce signaling molecules (Contains COX and peroxidase active sites) [UniProt- P35354]. Two specific amino acids, tyrosine at position 371 and serine at position 516, are located near the active site of COX-2 and are essential for its catalytic activity [8]. Additionally, a valine residue at position 509 creates a hydrophobic pocket within the active site [9]. This pocket plays a key role in the selective activation of the enzyme, ultimately influencing the inflammatory response. The COX-2 catalyzes the reaction in two steps: (i) COX reaction in this step the arachidonic acid (a fatty acid) is converted into a molecule called PGG2 [10,11]. This reaction happens within a hydrophobic channel (HYD channel) located at the protein's core and (ii) peroxidase reaction COX-2 transforms PGG2 into PGH2. This crucial molecule acts as a precursor for prostacyclin, a substance involved in inflammation, and the reaction occurs at a site near the protein surface where a heme molecule present [12].

Non-steroidal anti-inflammatory drugs (NSAIDs) are a common class of medications widely used to relieve pain and inflammation. NSAIDs have two main classes (i) non-selective NSAIDs[13]. These drugs inhibit both COX (COX-1 and COX-2) enzymes[14]. COX-1 is responsible for the production of prostaglandins that help the protection of normal physiological functions (the mucosal lining of the stomach and kidneys). Inhibiting COX-1 can led to gastrointestinal side effects like ulcers and bleeding. Examples of non-selective NSAIDs include ibuprofen, naproxen, aspirin, and diclofenac. (ii) COX-2 selective inhibitors these drugs are used to inhibit only COX-2 and reduces inflammation by inhibiting the PG synthesis via forming a strong bond (covalent interaction) with a specific amino acid (Serine-516) located within the active site of COX-2 [15]. This class of enzymes has the minimal to no effect on COX-1 [16]. Although these drugs reduced gastric toxicity, they were later associated with cardiovascular complications, particularly when administered at high doses or over prolonged periods [17] (Figure 1).

Thus, the search for novel, safe, and effective COX-2 inhibitors remains an important therapeutic goal. In recent years, the interest in natural product–based drug discovery has grown rapidly due to their structural diversity, biocompatibility, and lower toxicity profiles[18]. Natural compounds have historically served as invaluable leads for drug development across multiple therapeutic areas, including inflammation and cancer [19]. To develop the natural drug for COX-2 Traditional Chinese Medicine (TCM) is selected because of its long history and use in treating inflammatory diseases[20]. TCM offers a vast array of herbal remedies, and sesquiterpene lactones (SQTLs) are a group of natural compounds emerging as promising candidates for treating inflammatory diseases While the scientific evidence is still evolving, TCM offers a unique approach to managing inflammation, often focusing on restoring balance within the body [21].

Unlike previous computational studies that predominantly investigated synthetic NSAIDs or flavonoid-based derivatives, the present work focuses on the discovery of natural COX-2 inhibitors from sesquiterpene lactones (SLs) using an integrated pharmacophore modeling, molecular docking, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) pipeline. This approach not only enhances predictive accuracy but also introduces a novel exploration of structurally diverse and biocompatible plant-derived compounds with potentially reduced toxicity. By combining ligand-based pharmacophore modeling with virtual screening and pharmacokinetic evaluation, this study provides a unique

computational framework for identifying safer, nature-inspired COX-2 inhibitors that could serve as promising leads for next-generation anti-inflammatory drug development.

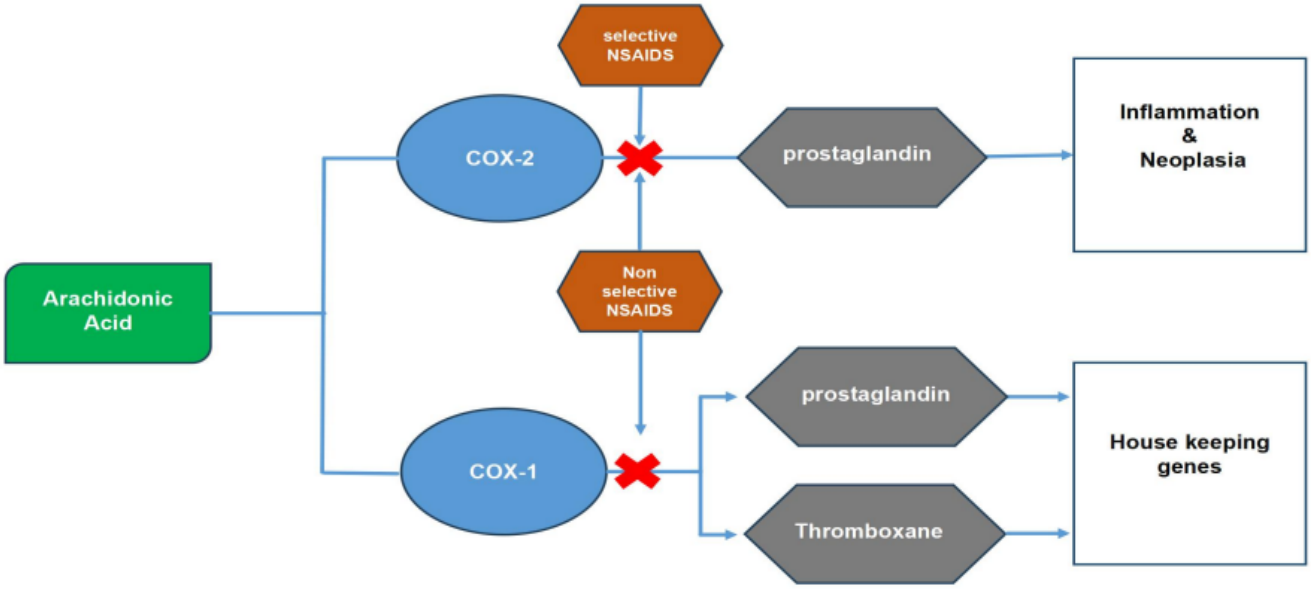


Figure 1. Mechanism of COX-2 inhibition by selective and non-selective NSAIDs.

Selective NSAIDs specifically interact with the COX-2 active site, reducing inflammation while minimizing gastrointestinal side effects. In contrast, non-selective NSAIDs inhibit both COX-1 and COX-2 enzymes, leading to decreased prostaglandin synthesis and a higher risk of gastric toxicity.

SLs are compounds found in many genera within the Asteraceae family. The extract of these plants were traditionally used for the treatment of inflammatory diseases [22]. Over the past two decades, researchers have identified and isolated more than 500 sesquiterpene lactones from various higher and lower plant species They are recognized as the key components in numerous medicinal plants traditionally employed in treating inflammatory ailments. SLs have emerged as exciting candidates for developing new anti-inflammatory drugs due to their unique ability to reduce inflammation and regulate the immune system. This promising potential has attracted researchers to delve deeper into their biological mechanisms. Computer-aided drug discovery (CADD) has improved therapeutic research by enabling early predictions of molecular interactions, pharmacokinetics, and toxicity. In this study, a CADD-based approach was applied to identify natural sesquiterpene lactones as potential COX-2 inhibitors. Unlike earlier in silico investigations that mainly examined synthetic NSAIDs or flavonoid derivatives, this work focuses on sesquiterpene lactones naturally occurring compounds with diverse chemical frameworks and reactive centers that influence inflammatory signaling. A validated pharmacophore model was constructed and used for virtual screening, molecular docking, and ADMET evaluation. Of the 72 screened compounds, 13 showed strong binding affinities, with Tanshinone IA exhibiting the best docking score compared to celecoxib. These findings underline the promise of sesquiterpene lactones as plant-based COX-2 inhibitors and demonstrate an effective computational workflow for designing safer anti-inflammatory agents.

2. Methodology

The methodological flow chart of the research paper are shown in Figure 2.

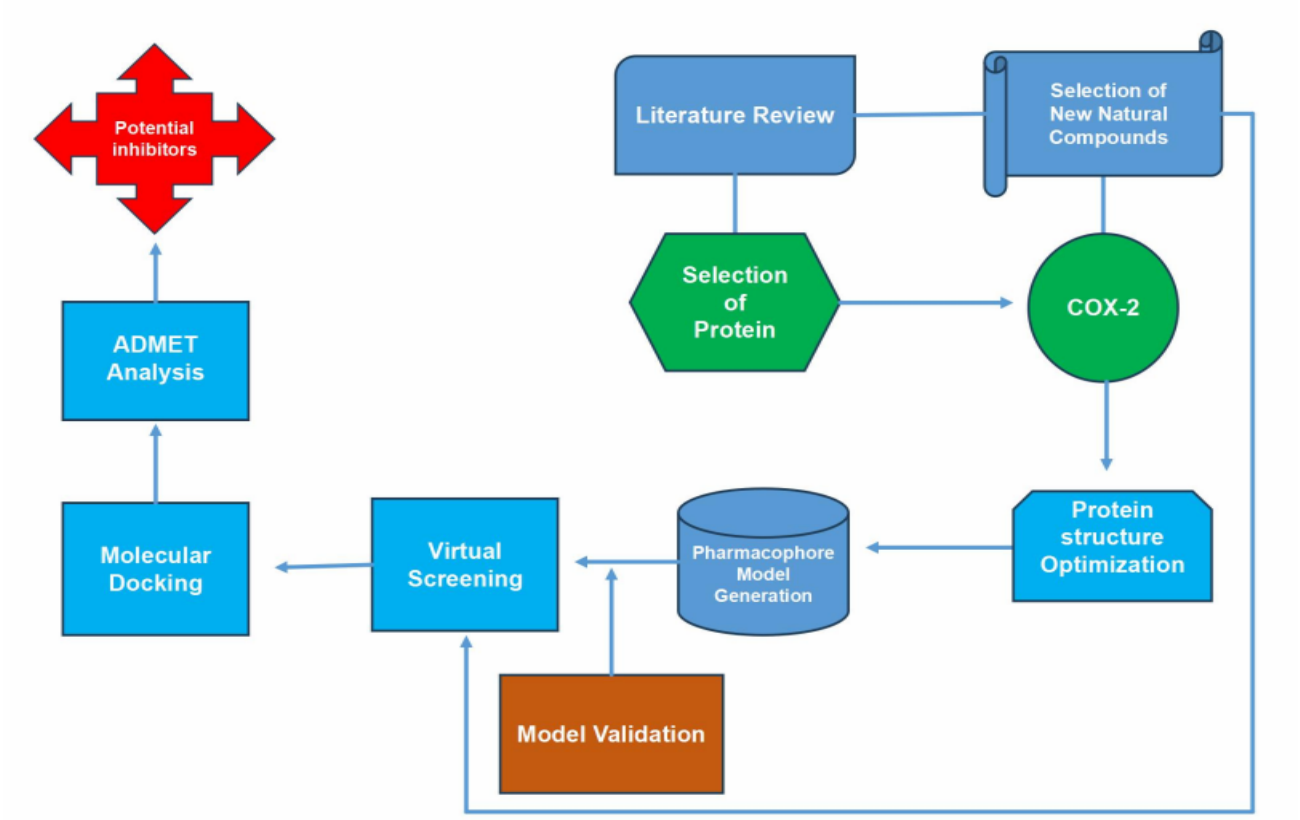


Figure 2. Methodological workflow of the study. The diagram summarizes the key steps of this research, including compound selection, pharmacophore modeling, virtual screening, molecular docking, ADMET evaluation, and identification of potential COX-2 inhibitors.

2.1 Protein Structural Optimization

The structural analysis of COX-2 was conducted by retrieving its crystal structure from the protein data bank (PDB) using the identifier 3LN1 (https://www.rcsb.org) [23]. This specific structure was obtained through crystallization in the presence of celecoxib, a widely used selective COX-2 inhibitor for treating inflammatory conditions. The complex structures of COX-2 were meticulously refined and optimized using BIOVIA Discovery Studio [24]. This optimization process involved several key steps, including the removal of associated ligands and water molecules to focus on the COX-2 structure examination. Moreover, the addition of polar hydrogens further improved molecular accuracy, enhancing the precision of the analysis.

2.2 Data Set Generation

Sesquiterpene lactones are terpenes that have in common a basic structure of 15 carbons. This structure results from biosynthesis involving three isoprene units with a cyclical structure along with a fused α-methylene-γ-lactone ring. Some sesquiterpene lactones have been reported in the literature to possess anti-inflammatory and anti-cancer activities [25]. A comprehensive literature review led to the identification of 72 compounds which are used for screening purposes. For this study, a total of 72 sesquiterpene lactones was compiled from the literature, focusing on compounds identified in plant extracts with reported anti-inflammatory activity. These compounds were selected based on their reported pharmacological relevance and potential to interact with the COX-2 enzyme [12,26]. The molecular structures of these selected compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the SDF format [27]. Five conformers for each compound were generated using the Meiler Lab web server (https://meilerlab.org/), resulting in the creation of a diverse library comprising 360 distinct molecular structures for screening purposes.

The selection of sesquiterpene lactones was based on predefined criteria, including documented anti-inflammatory or COX-related biological activity in peer-reviewed literature, availability of experimentally validated 3D structures in the PubChem database, and chemical diversity across the sesquiterpene lactone scaffold (e.g., variations in lactone rings, α-methylene-γ-lactone groups, and hydroxylation patterns). These criteria were applied to ensure biological relevance and structural diversity while minimizing bias. Future work will expand the compound library to include a broader range of natural and synthetic analogs to further reduce selection bias.

2.3 Pharmacophore Model Generation

Pharmacophore modeling is a widely used approach to analyze the chemical features of active sites and the spatial arrangement of ligand substituents in three-dimensional space [28]. To construct and validate the pharmacophore model, 26 known active COX-2 inhibitors with IC_{50} values were collected as datasets through literature review. The selected inhibitors were divided into two sets: a training set and a test set. Five highly potent inhibitors (with low IC_{50} values) were selected for the training set to construct the pharmacophore model, and the remaining inhibitors were used as the test set to evaluate the accuracy of the constructed model. LigandScout 4.5 was employed to generate a ligand-based pharmacophore model [29]. The software produced several pharmacophore models. The model with the highest pharmacophore fit score (0.76) and key features was selected for further analysis [30]. After selecting the pharmacophore model, the screening feature of LigandScout was used to conduct virtual screening of a library containing 360 compounds. The screening conditions were set as satisfying at least 2 of the 3 pharmacophore features, and finally, 33 compounds meeting the pharmacophore fit criteria were selected from the library for further molecular docking analysis.

2.4 Molecular Docking

After pharmacophore-based screening, 33 compounds with high pharmacophore fit scores were subjected to geometry optimization and docking. The molecular docking studies were conducted using PyRx 0.8, an open-source software designed for virtual screening. The chemical structure of selected compounds was obtained from the ligand Scout in SDF format [31].

A cutoff binding energy of ≤ -8.5 kcal/mol was used to select top-performing compounds. Using this threshold, 13 sesquiterpene lactones demonstrated strong predicted affinity toward the COX-2 active site, including Tanshinone IA (-10.6 kcal/mol) and Dihydroartemisinin (-9.4 kcal/mol), surpassing the reference inhibitor celecoxib (-9.5 kcal/mol). We changed these ligands' MDL SDF format to a PDBQT file using the the PyRx tool to get their atomic coordinates. Then, we used the mmff94 force field set on PyRx to minimize their energy through optimization. The structure of the compounds extracted from the receptor's active area was transformed to PDBQT file using PyRx tool to produce atomic coordinates, and energy was minimized via optimization using the mmff94 force field set on PyRx. After preparing the protein and ligand, molecular docking analysis was performed using PyRx, with AutoDock Vina selected due to its reliable scoring function [32]. For our examination, we utilized the PyRx, AutoDock Vina exhaustive search docking capability. After the minimization process, the grid box resolution was centered, the grid box was created around the whole protein and the docking protocol was executed using AutoDock Vina within the PyRx environment. The BIOVIA Discovery studio Visualizer is utilized for the diagrammatic presentation of molecular complexes [33].

2.5 ADME and Toxicity Analysis

ADME is a quantitative discipline that investigates the processes (absorption, distribution, metabolism, and excretion) of drugs within the biological system, elucidating the dynamic principles governing drug behavior in the body[34]. The SwissADME server and ProTox-II webserver were used in this work for predicting the principal ADME properties and compounds' toxicity, with the subsequent assessment of the reactions associated with absorption, distribution, metabolism, and excretion[35].

Solubility predictions were based on the estimated solubility (ESOL) model implemented in SwissADME. Solubility categories were interpreted according to SwissADME criteria: highly soluble (log S > -2), soluble (-4 ≤ log S ≤ -2), moderately soluble (-6 ≤ log S < -4), and poorly soluble (log S < -6). These thresholds were used to assign solubility categories in Table 3.

3. Results

3.1 Pharmacophore Model Generation

Ligand-based pharmacophore modeling was carried out to identify the essential structural features needed to inhibit COX-2, an enzyme central to the inflammatory response. Five selective COX-2 inhibitors (coxibs), were chosen as the training set to generate the pharmacophore model The model, consisting of hydrogen bond acceptor, aromatic, and hydrophobic features, was successfully generated and rigorously validated through receiver operating characteristic (ROC) curve analysis The best pharmacophore model was made up of eight features, including four hydrogen bond acceptors, two hydrophobic groups, and two aromatic rings as shown in Figure 3 the model was validated and the compounds were screened out of 360 compounds 32 were selected on the bases of pharmacophore fit score and matching features. All the compounds used for screening are shown in Table S1.

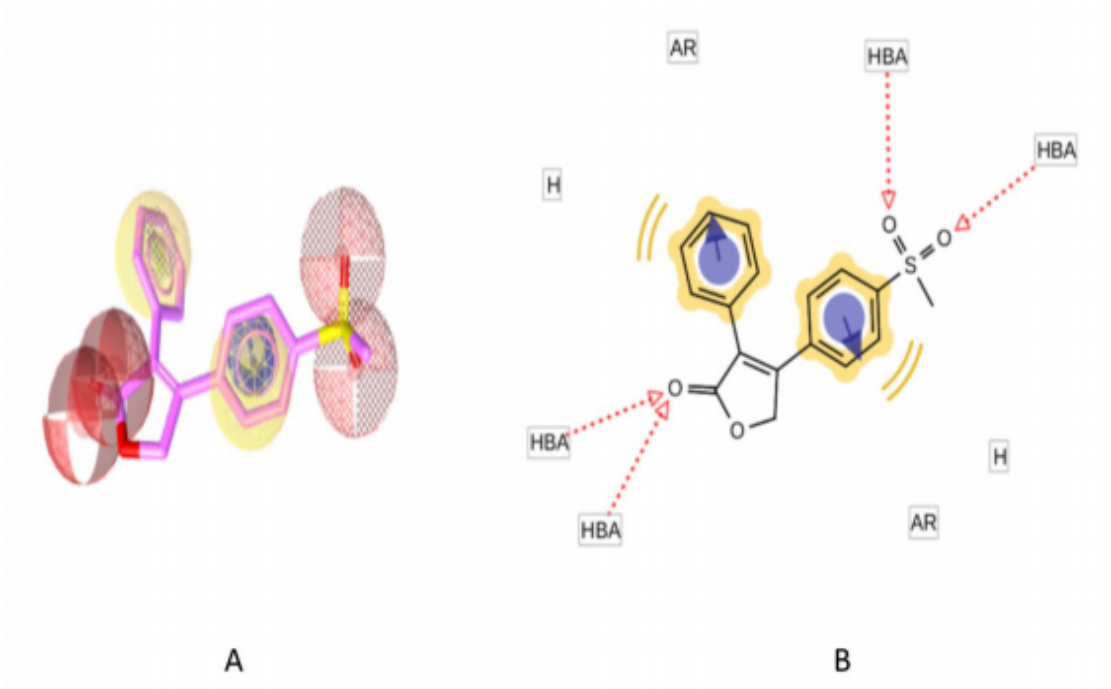


Figure 3. (A) Pharmacophore model for COX-2; (B) The compound with the highest pharmacophore score.

3.2 Pharmacophore Model Validation

The effectiveness of a pharmacophore is commonly assessed based on its capacity to discern inactive compounds from those that are active. This evaluation reflects the pharmacophore's ability to differentiate between substances that lack biological activity and those that exhibit pharmacological effects. A dataset consisting of 21 active COX-2 inhibitors and 20 decoys was used to assess this. The ROC curve was used to assess the predictive performance of the pharmacophore model, representing the relationship between the true positive rate (sensitivity) and the false positive rate (1-specificity). The model achieved an AUC value of 0.76, approaching the ideal value of 1 and indicating strong discriminatory power. These results suggest that the pharmacophore model is sufficiently reliable for early-stage virtual screening and can effectively guide the selection of potential COX-2 inhibitors for subsequent docking and ADMET analyses, as illustrated in Figure 4 and Table 1.

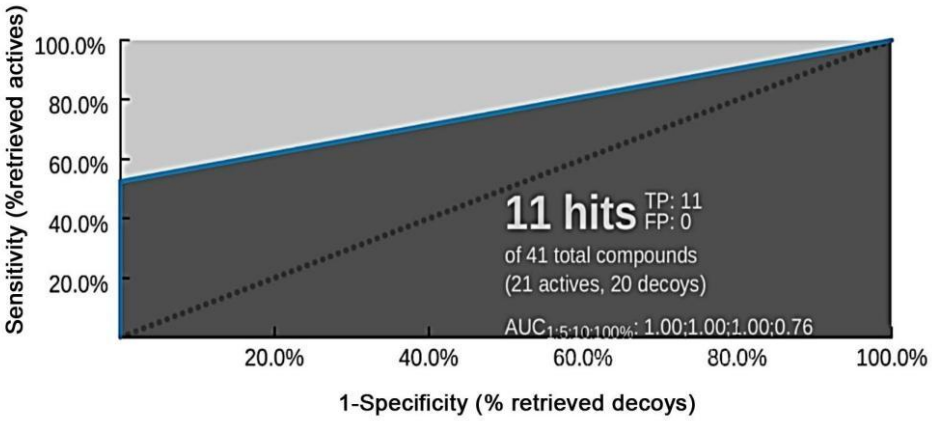
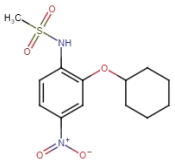
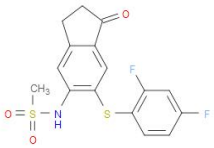
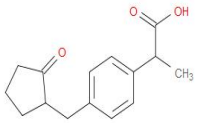
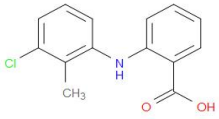
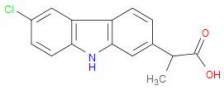
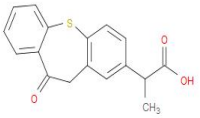
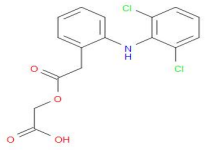
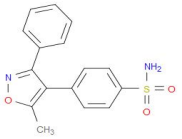
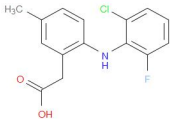
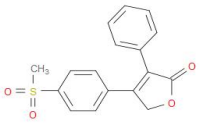
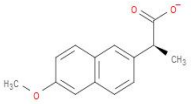
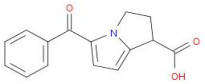
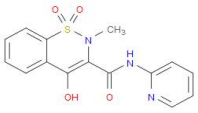
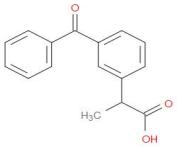
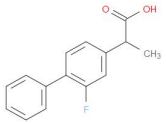
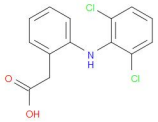
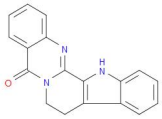
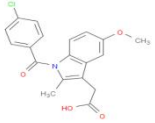
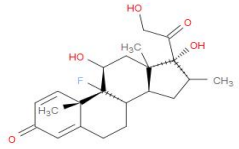
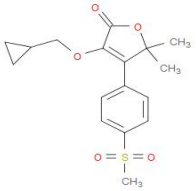


Figure 4. The ROC curve used to validate the pharmacophore model.

Table 1. Chemical structures and IC_{50} values of known COX-2 inhibitors used as reference compounds in this study.

No.	PubChem Id	Structure	IC_{50} Value	No.	PubChem Id	Structure	IC_{50} Value
1.	4553		1.77	14.	9799453		2.3
2.	3965		13.5	15.	610479		0.2
3.	2581		3.9	16.	5720		0.34
4.	71771		0.77	17.	119607		0.005
5.	151166		0.14	18.	5090		0.34
6.	23681059		5.2	19.	3826		3.50
7.	54676228		4.4	20.	3825		2.33
8.	3394		0.47	21.	3033		0.63
9.	65752		0.28	22.	3715		0.15
10.	5743		0.0073	23.	208910		0.13

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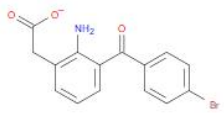
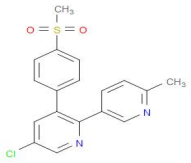
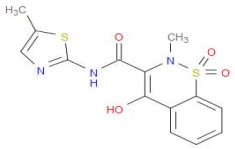
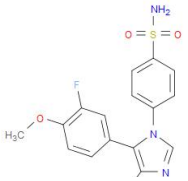
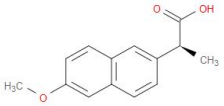
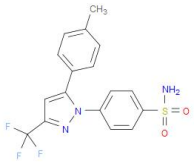
11.	23693301		7.45	24.	123619		1.1
12.	54677470		13.28	25.	213053		14.1
13.	156391		5.2	26.	2662		40

3.3 Molecular Docking

The molecular docking analysis revealed that all complexes exhibited acceptable binding affinities toward COX-2. Among the screened molecules, 13 compounds with the lowest binding energies were selected for detailed analysis. Tanshinone IA showed the most favorable docking score (-10.6 kcal/mol), surpassing the reference inhibitor celecoxib (-9.5 kcal/mol). Dihydroartemisinin also demonstrated strong affinity (-9.4 kcal/mol), while Tanshinone IIA and Isopimaric acid ($C_{20}H_{30}O_2$) displayed notable binding energies of -9.0 and -8.9 kcal/mol, respectively (Table 2). Several newly identified compounds, including Pimaric acid ($C_{20}H_{30}O_2$), Ainsliaside D, Cryptomeridiol, and Eupalinolide O ($C_{22}H_{32}O_8$), exhibited significant interactions with COX-2, and their binding interactions including hydrogen bonds, hydrophobic contacts, and π-π stacking were examined to evaluate stability and affinity within the active site (Figure 5 and Figure 6). The binding conformations of the top ten compounds are provided in Supplementary Figure S1 (Eupalinolide with COX-2), Figure S2 (Ainsliaside D with COX-2), Figure S3 (Sugiol with COX-2), Figure S4 (Pimaric with COX-2), Figure S5 (Tanshinone IIA with COX-2), Figure S6 (Artemether with COX-2), Figure S7 (dihydroartemisinin with COX-2), Figure S8 (Cryptoneribion with COX-2), Figure S9 (Artactylenolide III| with COX-2), and Figure S10 (Artemisinic acid with COX-2). The docking protocol was validated by re-docking the co-crystallized ligand celecoxib into the COX-2 active site, reproducing key interactions with Arg120, Tyr355, and Ser530, thereby confirming the reliability of the docking approach.

Table 2. Docking binding energy of prioritized compounds with COX-2.

S. No	Complex	Binding Energy (kcal/mol)
1	Eupalinolide O	-7.5
2	Ainsliaside D	-8.2
3	Sugiol	-8.8
4	Isopimaric Acid	-8.9
5	Pimaric Acid	-8.5
6	Tanshinone IIA	-9
7	Tanshinone IA	-10.6
8	Artemether	-8
9	Dihydroartemisinin	-9.4
10	Cryptomeridiol	-6.9
11	Atractylenolide III	-8
12	Artemisinic Acid	-8
13	Celecoxib	-9.5

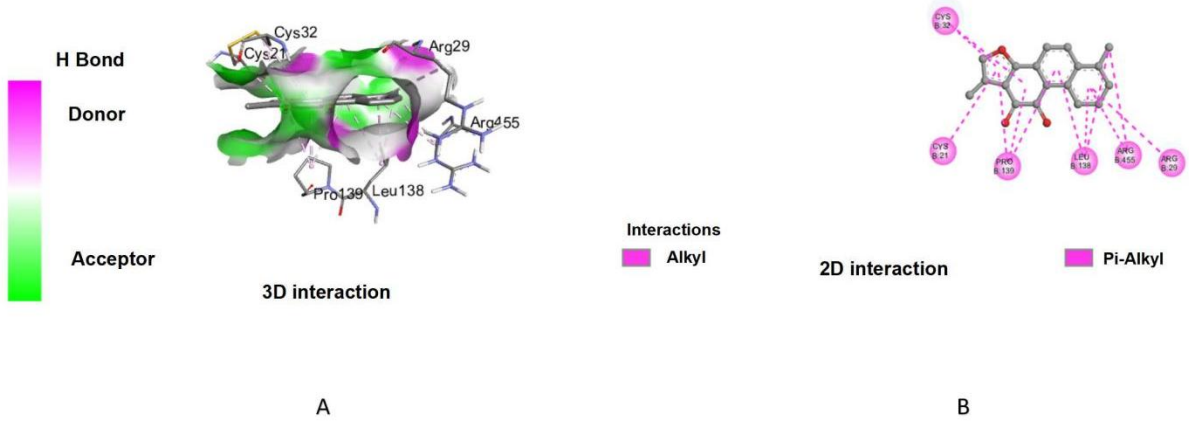


Figure 5. Binding between COX-2 and Tanshinone IA. (A) 3D interaction between COX-2 and the ligand; (B) 2D interaction of the ligand with COX-2.

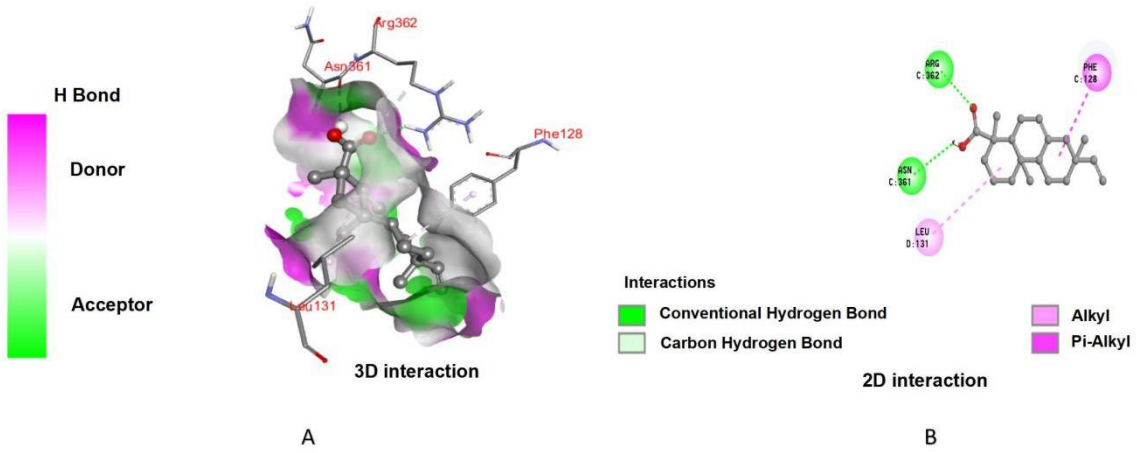


Figure 6. Binding between COX-2 and Isopimaric acid. (A) 3D interaction between COX-2 and the ligand; (B) 2D interaction of the ligand with COX-2.

3.4 ADMET Analysis

Understanding the absorption, distribution, metabolism, elimination, and toxicity (ADMET) characteristics is crucial for assessing the effectiveness and safety of potential drug candidates. All the compounds underwent ADMET analysis using the SwissADME server demonstrated adherence to Lipinski's rules and exhibited favorable drug probability across various properties. The results of ADMET analysis for novel compounds are shown in Table 3. The logS value reflects the solubility of the drug. The smaller the value, the less soluble the compound is in water. The SL compounds exhibit properties ranging from moderate solubility to full solubility in water. The SwissADME prediction parameters showed that Isopimaric acid, Tanshinone IA, Cryptomeridiol, pimaric acid and Eupalinolide O have high gastrointestinal absorption. ADMET analysis reveals Isopimaric acid, Tanshinone IA, and Cryptomeridiol as BBB penetrants, unlike celecoxib. Consistently, all compounds in our study exhibited commendable bioavailability scores, indicating favorable prospects for systemic absorption and distribution. In the context of P-glycoprotein interactions, Eupalinolide O and Cryptomeridiol emerged distinct, showcasing non-substrate behavior, in contrast to most tested compounds. Our research has rigorously assessed toxicity across six domains, including Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, and LD_{50} predictions. The predicted toxicity class of all compounds range from 4 to 6 as shown in Table 3-4. Solubility classifications (“soluble” and “moderately soluble”) were assigned based on SwissADME ESOL log S thresholds.

Table 3. ADMET properties of the prioritized compounds Physicochemical and Lipinski properties.

Properties	Celecoxib	Ainsliaside D	Isopimaric Acid	Tanshinone IA	Cryptomeridiol	Pimaric Acid	Eupalinolde O
Molecular weight	381.37 g/mol	418.52 g/mol	307.49 g/mol	276.29 g/mol	240.38 g/mol	307.49 g/mol	426.50 g/mol
Log $P_{o/w}$	2.65	-0.26	4.74	2.3	2.88	4.74	1.55
Num. rotatable bonds	4	4	2	0	1	2	8
Num. H-bond acceptors	7	8	2	3	2	2	8
Num. H-bond donors	1	6	2	0	2	2	1
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 4. Pharmacokinetic and toxicity profile.

Properties	Celecoxib	Ainsliaside D	Isopimaric Acid	Tanshinone IA	Cryptomeridiol	Pimaric Acid	Eupalinolide O
Formula	$C_{17}H_{14}F_3N_3O_2S$	$C_{21}H_{38}O_8$	$C_{20}H_{35}O_2$	$C_{18}H_{12}O_3$	$C_{15}H_{28}O_2$	$C_{20}H_{35}O_2$	$C_{22}H_{34}O_8$
Log S (ESOL)	(-4.57)	(-2.34)	(-5.86)	(-2.91)	(-3.12)	(-5.86)	(-3.39)
Class	Moderately Soluble	Soluble	Moderately Soluble	Soluble	Soluble	Moderately Soluble	Soluble
GI absorption	High	Low	High	High	High	High	High
BBB permeant	No	No	Yes	Yes	Yes	Yes	No
Log K_p (skin permeation)	(-6.21 cm/s)	(-8.66 cm/s)	(-3.39 cm/s)	(-6.62 cm/s)	(-5.67 cm/s)	(-3.39 cm/s)	(-7.29 cm/s)
P-gp substrate	No	Yes	Yes	Yes	No	Yes	No
Bioavailability Score	0.55	0.55	0.55	0.55	0.55	0.85	0.55
Hepatotoxicity	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive
Carcinogenicity	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Immunotoxicity	Inactive	Active	Active	Inactive	Inactive	Inactive	Active
Mutagenicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Predicted Toxicity Class	4	6	4	4	5	6	4
Predicted LD_{50}	1400 mg/kg	23000 mg/kg	5000 mg/kg	1655 mg/kg	5000 mg/kg	20000 mg/kg	400 mg/kg

4. Discussion

This study employed an integrated computational pipeline combining pharmacophore modeling, molecular docking, and ADMET profiling to identify sesquiterpene lactones as potential COX-2 inhibitors. Tanshinone IA emerged as the lead compound, demonstrating a binding affinity stronger than celecoxib and exhibiting favorable pharmacokinetic properties. Several additional compounds, including eupalinolide, isopimaric acid, and dihydroartemisinin, also showed strong binding interactions with key COX-2 residues [36]. While most prioritized molecules demonstrated favorable ADMET profiles, a few exhibited predicted hepatotoxicity or immunotoxicity alerts, indicating the need for careful optimization and

experimental validation. The pharmacophore model (AUC = 0.77) and docking results are consistent with previous studies reporting natural products as potential COX-2 inhibitors [insert citation here], supporting the utility of natural scaffolds for anti-inflammatory drug discovery. Although this computational approach provides valuable early-stage insights, *in vitro* and *in vivo* validation will be required to confirm biological efficacy and safety. Overall, these findings highlight the potential of sesquiterpene lactones as promising templates for developing safer COX-2 inhibitors[37]. Tanshinone IA stands out as a promising lead due to its combination of favorable attributes beyond binding affinity. As a naturally derived compound [38], it is likely to exhibit lower overall toxicity and better biocompatibility compared to conventional synthetic NSAIDs. Its predicted pharmacokinetic profile supports effective systemic exposure, while natural sourcing may provide cost-effective and sustainable production. These features, together with its structural complementarity within the COX-2 active site, underscore the potential of Tanshinone IA as a selective, plant-derived anti-inflammatory candidate suitable for further preclinical investigation [39].

Molecular docking revealed that Tanshinone IA achieved the lowest binding energy (-10.6 kcal/mol), outperforming celecoxib (-9.5 kcal/mol) [40,41]. Importantly, the docking interactions of Tanshinone IA demonstrated strong hydrogen bonding and hydrophobic interactions with critical COX-2 active site residues, suggesting that this compound could effectively block the catalytic conversion of arachidonic acid to prostanoids. Other compounds, including isopimaric acid, dihydroartemisinin, and eupalinolide O, also demonstrated favorable binding affinities, broadening the potential pool of candidate inhibitors. These results align with previous studies reporting the anti-inflammatory activity of sesquiterpene lactones and reinforce their therapeutic relevance.

ADMET profiling confirmed that most prioritized compounds adhered to Lipinski's "Rule of Five" and exhibited good oral bioavailability, high gastrointestinal absorption, and in several cases, blood-brain barrier (BBB) permeability [42]. Such properties are essential for ensuring systemic drug exposure and therapeutic efficacy. Notably, predicted toxicity profiles indicated that the compounds carried lower hepatotoxicity and mutagenicity risks compared to some synthetic NSAIDs, supporting their promise as safer alternatives [13,43]. However, variability in water solubility among the candidates suggests that formulation strategies may be required for certain compounds to achieve optimal bioavailability.

The pharmacophore model demonstrated an AUC of 0.77, which is considered indicative of good discrimination for ligand-based screening models in early-stage drug discovery. Although this value does not represent perfect predictive accuracy, it supports the model's suitability for prioritizing potential COX-2 inhibitors. The successful retrieval of known inhibitors and the identification of high-affinity candidates further reinforce its reliability. Nonetheless, future studies will aim to refine the model using larger datasets and complementary structure-based pharmacophore strategies to enhance predictive confidence.

Taken together, this study underscores the potential of sesquiterpene lactones as a natural reservoir for developing selective COX-2 inhibitors with reduced side-effect profiles compared to traditional NSAIDs. While in silico approaches accelerate the identification of promising leads, it is critical to recognize their inherent limitations. Computational predictions cannot fully substitute for empirical confirmation of biological activity, pharmacodynamics, and long-term safety. Therefore, experimental validation through *in vitro* enzyme inhibition assays, cellular models of inflammation, and ultimately *in vivo* pharmacological studies will be necessary to substantiate the therapeutic potential of these candidates.

The observed variability in solubility among some candidates could be addressed through formulation strategies or rational structural modifications to ensure optimal bioavailability without compromising COX-2 binding affinity. Predicted hepatotoxicity for specific compounds highlights the importance of careful evaluation, yet overall low immunotoxicity and favorable ADMET profiles support the potential of these natural products as safer alternatives. BBB predictions suggest possible neurological applications for most compounds, while those lacking CNS penetration may minimize associated risks. Structural optimization focusing on solubility, toxicity, and overall drug-likeness could further enhance the therapeutic promise of these molecules. This work provides a rational framework for the computer-aided discovery of natural COX-2 inhibitors and highlights Tanshinone IA as a lead compound with strong binding affinity and favorable pharmacokinetic attributes. The predicted BBB permeability of several top candidates suggests potential applications in neurological and neuroinflammatory disorders, given the documented involvement of COX-2 in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. While this property may broaden therapeutic relevance, CNS penetration also raises the possibility of off-target neurological effects. Therefore, future studies will incorporate *in vitro* BBB transport models and *in vivo* neurotoxicity and behavioral assessments to evaluate both therapeutic potential and CNS safety profiles. While In-silico approaches accelerate the identification of promising leads, computational predictions cannot fully substitute for empirical confirmation of biological activity, pharmacodynamics, and long-term safety. Therefore, experimental validation through *in vitro* enzyme inhibition assays, cellular models of inflammation, and *in vivo* pharmacological studies will be essential to substantiate the therapeutic potential of these candidates. Collectively, these findings lay the groundwork for subsequent preclinical investigations aimed at developing safer and more effective anti-inflammatory therapeutics derived from natural products.

5. Conclusion

This study identified sesquiterpene lactones, particularly Tanshinone IA, as promising natural COX-2 inhibitors through pharmacophore modeling, molecular docking, and ADMET analysis. Tanshinone IA demonstrated superior binding affinity compared to celecoxib and exhibited favorable pharmacokinetic and safety profiles, highlighting its potential as a lead compound for anti-inflammatory drug development. While these findings provide a strong computational basis, experimental validation is essential to confirm their therapeutic efficacy.

6. Limitations and Future Directions

Despite demonstrating promising computational evidence for sesquiterpene lactones as potential COX-2 inhibitors, this study has several limitations. First, the compound library was restricted to 72 sesquiterpene lactones retrieved from literature and public chemical databases, which may introduce selection bias toward compounds with pre-reported anti-inflammatory potential. Expanding the chemical space in future studies to include a larger and more diverse range of natural and synthetic analogs will improve chemical diversity and reduce selection bias. Second, although the pharmacophore model showed satisfactory predictive performance (AUC = 0.77) and successfully identified known COX-2 inhibitors, its predictive accuracy may be further strengthened through refinement with larger training datasets and incorporation of structure-based pharmacophore approaches. Third, molecular docking was conducted exclusively using AutoDock Vina; therefore, future work will implement cross-validation with additional docking tools and consensus scoring to enhance the reliability of binding predictions.

Furthermore, several compounds exhibited moderate solubility and isolated toxicity alerts, emphasizing the need for formulation strategies and rational structural modifications to enhance drug-likeness while minimizing adverse effects. Importantly, as this study is entirely based on in silico evaluations, the findings must be substantiated through experimental validation. Future work will involve systematic *in vitro* and *in vivo* studies, including COX-2 inhibition assays, hepatocyte and immunotoxicity assessments, and animal model evaluations to confirm anti-inflammatory efficacy and safety.

In addition, rational structural optimization will be pursued to improve the pharmacokinetic and toxicity profiles of lead compounds. Potential strategies include introducing polar or ionizable functional groups to increase aqueous solubility and absorption, fine-tuning hydrophobic moieties to balance lipophilicity, and applying bioisosteric substitutions to preserve COX-2 binding affinity while reducing predicted toxicity risks. Structure-activity relationship and quantitative structure-property relationship modeling will guide these modifications, and advanced formulation techniques, such as nano-delivery systems and prodrug strategies, will also be explored to enhance bioavailability. Collectively, these efforts will support the progression of the most promising candidates, such as Tanshinone IA and Isopimaric acid, toward preclinical development.

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Data Availability Statement

Overall data of this research paper is present in the manuscript and supplementary file.

Authors Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Osama Yousafzai and Najia Javed. The first draft of the manuscript was written, supervised and validate the results by Syed Luqman Ali, and Awais Ali. The review and analyzed the results by Amir Hamza. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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