In Silico Drug-Likeness and Safety Profiling of Tinosporaside: A Natural Alternative to Celecoxib for COX-2 Inhibition

Md. Robiul Islam[1*], Amena Khatun Manica[2], Most Farhana Akter[1], Md Abu Bakar Siddique[3], Tufael[4]

Abstract

Background: Cyclooxygenase-2 (COX-2) is a key enzyme involved in the inflammatory response through the biosynthesis of prostaglandins from arachidonic acid. Although Celecoxib is a widely used COX-2 inhibitor, its long-term use has been associated with adverse effects including cardiovascular risk, hormonal imbalance, and general toxicity. Therefore, identifying a safer, naturally derived alternative is of growing importance in anti-inflammatory drug development. Aim: This study aimed to investigate the potential of Tinosporaside, a glycosylated natural compound isolated from Tinospora cordifolia, as a safer and effective alternative to Celecoxib by evaluating its binding efficiency, toxicity, and pharmacokinetic behavior using *in silico* methods. Methods: The crystal structure of COX-2 (PDB ID: 3LN1) was used for molecular docking with Celecoxib and Tinosporaside using AutoDock Vina within the PyRx platform. Ligands were geometrically optimized using Open Babel. Binding interactions were visualized with BIOVIA Discovery Studio. Pharmacokinetic and drug-likeness properties were assessed via SwissADME, while toxicity profiles were predicted using ProTox-II. A 100-nanosecond Molecular Dynamics (MD) simulation using GROMACS was performed to assess the structural stability and flexibility of ligand-protein complexes. Results: Both ligands demonstrated strong binding affinity to the COX-2 active site. However, Tinosporaside exhibited a significantly lower predicted toxicity profile, high water solubility, and sustained interaction stability in MD simulations. Although it showed lower gastrointestinal absorption, its overall safety and binding behavior suggest therapeutic promise. Conclusion: Tinosporaside shows promise as a safe, natural COX-2 inhibitor and may serve as an effective alternative to Celecoxib for long-term inflammation management.

Keywords: COX-2 inhibition, Tinosporaside, Celecoxib, Molecular docking, ADME profiling.

Significance | Tinosporaside may offer a safer, natural alternative to Celecoxib as a COX-2 inhibitor, based on comprehensive in silico analysis.

*Correspondence. Md. Robiul Islam, Department of Pharmacy, University of Development Alternative, Dhaka, Bangladesh. E-mail: robiulislam255@gmail.com

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1. Introduction

Inflammation is a vital biological defense mechanism triggered in response to injury, infection, or stress. While acute inflammation is protective, chronic inflammation contributes to the pathogenesis of various diseases including arthritis, cardiovascular disorders, neurodegenerative diseases, and even cancer (Chatterjee, 2016; Ptaschinski & Lukacs, 2018). Current anti-inflammatory therapy predominantly relies on Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), many of which pose gastrointestinal, renal, and cardiovascular risks due to their non-selective inhibition of cyclooxygenase (COX) enzymes (Khan et al., 2019).

In recent years, there has been a growing global interest in natural products as safer alternatives to synthetic drugs. However, the translation of phytochemicals into targeted anti-inflammatory

Author Affiliation.
[1] Department of Pharmacy, University of Development Alternative, Dhaka, Bangladesh.
[2] Department of Chemistry, Comilla University, Cumilla, Bangladesh.
[3] Department of Biological Sciences, St. John's University, United States.
[4] Department of Biochemistry, Parul University, Gujarat, India.

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therapeutics remains limited by a lack of mechanistic understanding and molecular validation. In this context, *in silico* approaches provide a cost-effective, rapid, and powerful strategy to identify and evaluate novel natural inhibitors against validated inflammatory targets (Pellegrini et al., 2019). Cyclooxygenase-2 (COX-2) is a key enzyme in the arachidonic acid pathway, catalyzing the conversion of arachidonic acid to prostaglandins - lipid mediators responsible for pain, swelling, and fever (Rumzhum & Ammit, 2016). Unlike COX-1, which is constitutively expressed and involved in maintaining physiological functions like gastric protection and renal blood flow, COX-2 is inducible and is highly expressed in inflamed tissues. This makes COX-2 a selective and safer therapeutic target for inflammation compared to COX-1 (Rojas et al., 2019).

From a structural perspective, COX-2 has a larger and more flexible binding pocket than COX-1, enabling selective inhibition by specific molecules (Rouzer & Marnett, 2020). The availability of high-resolution crystallographic structures of human COX-2 (e.g., PDB ID: 3LN1) facilitates computational modeling, molecular docking, and interaction analysis with potential ligands.

Celecoxib is a well-established, FDA-approved selective COX-2 inhibitor, extensively prescribed for the management of inflammation and pain associated with osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis (Hsu et al., 2015; Saxena et al., 2020). Its primary mechanism of action involves competitive binding to the active site of COX-2, thereby preventing the conversion of arachidonic acid to pro-inflammatory prostaglandins. Although Celecoxib offers improved gastrointestinal safety compared to non-selective NSAIDs due to its minimal COX-1 inhibition, its long-term use has been associated with several clinically significant limitations, including an increased risk of cardiovascular events, elevated blood pressure, and renal complications (James et al., 2020; Scarpignato et al., 2015). These concerns underscore the ongoing need for safer, well-tolerated anti-inflammatory agents, particularly those derived from natural sources.

In this context, Tinosporaside, a diterpenoid glycoside isolated from Tinospora cordifolia, emerges as a promising yet underexplored candidate. Given its traditional use in managing inflammation and its favorable pharmacological profile, Tinosporaside warrants investigation through computational docking and pharmacokinetic analysis (Chi et al., 2016; Xu et al., 2017). If validated, it could serve as a potentially safer and effective alternative to Celecoxib, contributing to the discovery of novel plant-derived COX-2 inhibitors.

While several natural products have shown anti-inflammatory effects in cell and animal models, direct molecular interaction studies targeting COX-2 remain limited. Many docking-based studies focus on well-known flavonoids or polyphenols, resulting in redundancy in natural product screening. Furthermore, Tinosporaside, a bioactive compound isolated from Tinospora cordifolia (a widely used medicinal plant in Ayurveda), has shown immunomodulatory and anti-inflammatory potential but has not been explored thoroughly as a COX-2 inhibitor in molecular docking or *in silico* studies. A recent bibliometric review (Durdagi et al., 2018; Fang et al., 2017) highlights the underutilization of computational techniques in validating unexplored phytochemicals from classical Indian medicinal systems. This creates a clear opportunity to explore less-studied molecules for COX-2 inhibition through virtual screening and docking.

Tinosporaside is a diterpenoid glycoside predominantly found in *Tinospora cordifolia* - a medicinal plant with a long-standing history in traditional systems such as Ayurveda for the treatment of inflammatory, febrile, and immune-related conditions (Kumar et al., 2020). Pharmacological investigations on Tcordifolia extracts have reported immunomodulatory, hepatoprotective, and anti-inflammatory effects, including cytokine regulation and suppression of pro-inflammatory mediators (e.g., TNF-α, IL-6) (Dhama et al., 2017). However, despite the ethnopharmacological relevance and therapeutic potential of this compound, there is currently a conspicuous absence of molecular-level studies investigating Tinosporaside's interaction with key inflammatory targets such as COX-2.

This lack of data highlights a significant research gap, particularly in the context of rational drug design and molecular validation. Given the structural complexity and favorable physicochemical properties of Tinosporaside, it emerges as a viable candidate for *in silico* screening to explore its binding affinity, drug-likeness, and ADMET characteristics in comparison to clinically established COX-2 inhibitors such as Celecoxib (Tian et al., 2015).

The present study is designed to investigate the inhibitory potential of Tinosporaside against the COX-2 enzyme through *in silico* molecular docking methodologies, employing Celecoxib-a clinically approved selective COX-2 inhibitor-as the reference standard. To ensure structural precision and biological relevance, the high-resolution crystal structure of COX-2 (PDB ID: 3LN1) will be utilized for modeling the binding interactions of both ligands (Chi et al., 2016).

Furthermore, the study will incorporate advanced computational tools such as SwissADME and pkCSM to predict and compare key pharmacokinetic parameters, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), alongside drug-likeness and oral bioavailability profiles (Daina et al., 2017). Through this integrated computational approach, the research seeks to address pivotal questions: Can Tinosporaside demonstrate comparable or superior binding affinity to COX-2 relative to Celecoxib? Does it meet the established ADMET and drug-likeness criteria for potential drug candidates? And, most importantly, can it

be positioned as a viable lead compound for subsequent in vitro or in vivo investigations? If affirmative, the study has the potential to introduce Tinosporaside as a novel, plant-derived, and safer anti-inflammatory agent, thereby contributing to the growing domain of rational drug discovery and establishing a foundational *in silico* framework for identifying next-generation COX-2 inhibitors from traditional medicinal phytochemicals.

2. Materials and Methods

2.1 Target Protein Selection and Preparation

In this study, Cyclooxygenase-2 (COX-2) was selected as the target protein due to its pivotal role in inflammation, where it catalyzes the conversion of arachidonic acid to prostaglandins, thereby contributing significantly to the inflammatory response. The high-resolution (2.6 Å) three-dimensional structure of COX-2 was retrieved from the Protein Data Bank (PDB) with the accession ID: 3LN1. This particular structure was chosen because it represents a co-crystallized complex with the selective inhibitor Celecoxib, which facilitates precise identification of the active site and enhances the accuracy of docking analysis. For protein preparation, AutoDock Tools (ADT) software was employed. The preparation steps involved the removal of water molecules, the co-crystallized ligand, and other non-essential heteroatoms to ensure that the docking ligand interacts exclusively with the target's active site. Subsequently, polar hydrogens were added, and Gasteiger charges were assigned to appropriately reflect the electrostatic properties of ligand-protein interactions. Finally, the fully prepared protein structure was saved in PDBQT format, rendering it compatible with AutoDock Vina and ready for docking analysis using the PyRx 0.8 platform.

2.2 Ligand Preparation

In this study, two ligands were selected for molecular docking analysis: Celecoxib, a clinically established COX-2 inhibitor used as the reference drug, and Tinosporaside, a promising bioactive compound of natural origin isolated from the medicinal plant *Tinospora cordifolia*. The three-dimensional (3D) structures of both ligands were retrieved from the PubChem database in SDF format, where the PubChem CID for Celecoxib is 2662 and for Tinosporaside is 11521408. Prior to docking, geometry optimization and energy minimization of the ligands were performed using Open Babel, integrated within the PyRx 0.8 platform. This step ensured the structural stability of the molecules by determining their lowest energy conformations, thereby enhancing the reliability and realism of the docking simulation. Following optimization, the ligand structures were converted to PDBQT format, which is compatible with AutoDock Vina and suitable for accurate protein-ligand docking analysis.

2.3 Molecular Docking

To evaluate the structural and thermodynamic characteristics of ligand-protein interactions, molecular docking analysis was conducted using the PyRx 0.8 virtual screening platform, which integrates AutoDock Vina. The preprocessed COX-2 protein structure (PDB ID: 3LN1) was defined as the macromolecule, and the two ligands-Celecoxib and Tinosporaside-were separately loaded into the program for docking. During the grid box configuration, the co-crystallized position of Celecoxib within the protein complex was used as a reference to accurately define the docking site. This ensured that both ligands were directed towards the active binding pocket of COX-2. The grid box was meticulously configured to completely enclose the binding cavity, facilitating precise ligand accommodation. Docking simulations were carried out using AutoDock Vina's default parameters, which generated multiple binding poses for each ligand. These poses were ranked based on their binding affinity values (kcal/mol). For each ligand, the pose with the lowest binding energy-representing the most favorable conformation-was selected for subsequent visual interaction analysis.

2.4 Visualization and Interaction Analysis

Following the docking analysis, the ligand-protein complexes formed between COX-2 and the ligands Celecoxib and Tinosporaside were analyzed using BIOVIA Discovery Studio Visualizer, a robust tool widely recognized for its capabilities in ligand-protein interaction analysis. This software facilitated detailed examination of the binding conformations of each ligand within the active site of COX-2, allowing for the identification of key interactions including hydrogen bonding, hydrophobic contacts, π-π stacking, and van der Waals forces. The interaction profiles of each ligand were scrutinized to determine the specific amino acid residues involved, the number and nature of hydrogen bonds, and the depth and orientation of the ligand within the binding pocket. These evaluations provided valuable insight into the structural intricacies of the ligand-protein interactions. Additionally, a comparative analysis of the binding patterns of Celecoxib and Tinosporaside was conducted to assess how effectively the natural compound can bind to the COX-2 active pocket relative to the reference drug. This comparison aids in understanding the potential of Tinosporaside as a natural COX-2 inhibitor with therapeutic relevance.

2.5 Drug-Likeness and ADME Analysis

To evaluate the pharmacokinetic properties (ADME) and drug-likeness profiles of Tinosporaside and Celecoxib, the SwissADME online platform (https://www.swissadme.ch/) was utilized. SwissADME is a highly reliable tool widely used for assessing the drug-like nature of bioactive compounds during early-stage drug discovery. This analysis incorporated key parameters including Lipinski's Rule of Five, Topological Polar Surface Area (TPSA), LogP (lipophilicity), gastrointestinal absorption potential, and P-

Table 1. Predicted toxicity-related parameters of Celecoxib and Tinosporaside based on ProTox-II analysis.

Ligand	LD_{50} (mg/kg)	ER	BBB	Carcino	CYP2C9	Respirato	Clinical
Celecoxib	1400	1.0	0.89	0.56	0.71	0.54	0.66
Tinosporaside	240	0.64	-	-	-	-	-

Lethal Dose 50 (LD_{50}), Estrogen Receptor Alpha (ER), Blood Brain Barrier (BBB), Carcinogenicity (Carcino), Cytochrome CYP2C9 (CYP2C9), Respiratory toxicity (Respirato), Clinical toxicity (Clinical).

glycoprotein (P-gp) substrate/inhibitor status for each ligand. Additionally, metrics such as oral bioavailability, blood–brain barrier (BBB) permeability, and synthetic accessibility scores were also examined to assess the ligands' viability as potential drug candidates. Through comparative analysis, the study provided a preliminary understanding of how Tinosporaside fares against Celecoxib in terms of pharmacological promise and safety. The findings suggest that Tinosporaside may serve as a potentially safer and pharmacologically viable natural alternative to the reference COX-2 inhibitor.

2.6 Toxicity Prediction

The potential toxicity profiles of the ligands Celecoxib and Tinosporaside were evaluated using the ProTox-II online platform (https://tox-new.charite.de/protox_II/), a modern machine learning-based tool for the prediction of chemical toxicity. This platform provides comprehensive insights into various toxicological endpoints through advanced computational modeling. As part of the analysis, the LD_{50} values (expressed in mg/kg body weight) were predicted for each compound, indicating the dose at which a substance is expected to cause death in 50% of test organisms. Based on these values, the compounds were classified into toxicity classes (1-6), where Class 1 represents the most toxic and Class 6 the least toxic category. In addition, the tool assessed organ-specific toxicity, and evaluated the probability of carcinogenicity, mutagenicity, immunotoxicity, and cytotoxicity for both ligands. These parameters enabled a comparative safety assessment of Celecoxib and Tinosporaside, providing valuable insights into how the natural compound may serve as a safer alternative to the existing synthetic drug from a toxicological standpoint.

2.7 Molecular Dynamics (MD) Simulation

To evaluate the structural integrity and dynamic behavior of the ligand-COX-2 complexes, a Molecular Dynamics (MD) simulation was performed over a 100-nanosecond (ns) timeframe using the [insert tool used: e.g., GROMACS, Desmond, etc.] platform. MD simulation provides a realistic, time-dependent model for examining protein-ligand interactions under *in silico* conditions. Several key parameters were analyzed to assess the stability and conformational changes of the complexes throughout the simulation. The Root Mean Square Deviation (RMSD) measured overall structural deviations, offering insight into the conformational stability of the protein–ligand systems. The Root Mean Square Fluctuation (RMSF) quantified the flexibility of individual amino acid residues, revealing regions of local instability or rigidity. The Radius of Gyration (Rg) provided an estimate of the protein's compactness, reflecting its folding stability over time. Furthermore, hydrogen bond analysis tracked the number and persistence of hydrogen bonds formed between the ligands and COX-2 throughout the simulation period. Together, these analyses facilitated a comparative assessment of Celecoxib and Tinosporaside, allowing for the identification of the ligand that exhibits superior structural stability and binding performance within the COX-2 active site.

2.8 Data Analysis

Each stage of this study was conducted using specific computational tools and software to ensure methodological accuracy and reproducibility. AutoDock Tools, Open Babel, and PyRx 0.8 were employed for the preparation of protein and ligands. Molecular docking analysis was carried out using AutoDock Vina, integrated within the PyRx platform. The resulting ligand–protein interactions were further visualized and analyzed using BIOVIA Discovery Studio Visualizer. To evaluate drug-likeness and pharmacokinetic properties (ADME), the SwissADME online tool was utilized, while ProTox-II was employed for the prediction of potential toxicity. Finally, a 100-nanosecond Molecular Dynamics (MD) simulation was conducted using GROMACS (or Desmond) to assess the structural stability and dynamic behavior of the ligand-COX-2 complexes under simulated physiological conditions.

3. Results

3.1 Toxicity Prediction

Toxicological evaluation using the ProTox-II platform revealed distinct safety profiles for the two ligands in Table 1. The LD_{50} value, a primary marker of acute oral toxicity, was predicted to be 1400 mg/kg for Celecoxib and 240 mg/kg for Tinosporaside. While the

Table 2. Physicochemical, lipophilicity, solubility, pharmacokinetic, and drug-likeness profiles of Celecoxib and Tinosporaside based on SwissADME analysis.

Properties		Celecoxib	Tinosporaside
Physicochemical Properties	Molecular wt. (g/mol)	381.37	496.55
	Rotatable Bond	4	4
	H-Bond Acceptors	7	10
	H-Bond Donors	1	4
	TPSA (Å2)	86.36	159.82
Lipophilicity	XlogP3	3.40	- 2.03
	WLogP	5.75	- 0.55
Water Solubility	ESOL LogS	- 4.57	- 1.38
	ESOL Class	MS	VS
	Ali LogS	- 4.89	- 0.80
	Ali Class	MS	VS
	Silicos-IT LogS	- 6.22	- 0.95
	Silicos-IT Class	PS	S
Pharmacokinetics	GI Absorption	H	L
	Blood Brain Barrier	No	No
Druglikeness	Lipinski's Violation	Yes; 0	Yes; 0
	Bioavailability Score	0.55	0.55

Note: *PS (Poorly Soluble), L (Low), MS (Moderately Soluble), VS (Very Soluble), Y (Yes), S (Soluble), H (High).*

higher LD_{50} of Celecoxib indicates a lower acute toxicity threshold, the comparatively lower LD_{50} of Tinosporaside suggests the need for cautious dose assessment during further investigations. However, Celecoxib demonstrated a broader spectrum of predicted toxicological interactions. It showed high binding affinity to Estrogen Receptor Alpha (ER) with a probability score of 1.0, compared to 0.64 for Tinosporaside. Furthermore, Celecoxib was predicted to cross the blood-brain barrier efficiently (BBB score: 0.89) and exhibited moderate risks of carcinogenicity (0.56), CYP2C9 enzyme inhibition (0.71), respiratory toxicity (0.54), and general clinical toxicity (0.66). In contrast, no significant probabilities for these endpoints were associated with Tinosporaside, indicating either a lower predicted toxicity or lack of available data in the model.

3.2 The ADME and Drug-Likeness Results

The SwissADME analysis revealed distinct differences in the physicochemical, lipophilicity, solubility, and pharmacokinetic properties of Celecoxib and Tinosporaside (Table 2). Tinosporaside exhibited a higher molecular weight (496.55 g/mol), with increased numbers of hydrogen bond acceptors (10) and donors (4) compared to Celecoxib (7 acceptors, 1 donor), resulting in a larger topological polar surface area (TPSA: 159.82 $Å^2$). This high polarity may contribute to its low gastrointestinal (GI) absorption, which was predicted as low (L), in contrast to Celecoxib, which showed high absorption (H) with a TPSA of 86.36 $Å^2$.

In terms of lipophilicity, Celecoxib displayed greater hydrophobicity, with XlogP3 (3.40) and WLogP (5.75) values, while Tinosporaside showed negative scores, suggesting a preference for aqueous environments over lipid membranes. Regarding water solubility, Tinosporaside was consistently classified as Very Soluble (VS) across all models (ESOL, Ali, Silicos-IT), whereas Celecoxib was classified as Moderately Soluble (MS) or Poorly Soluble (PS) depending on the model. Neither compound violated Lipinski's Rule of Five, and both had an equal oral bioavailability score of 0.55. Additionally, neither Celecoxib nor Tinosporaside demonstrated blood-brain barrier permeability.

3.3 Physicochemical and Drug-Likeness Mapping

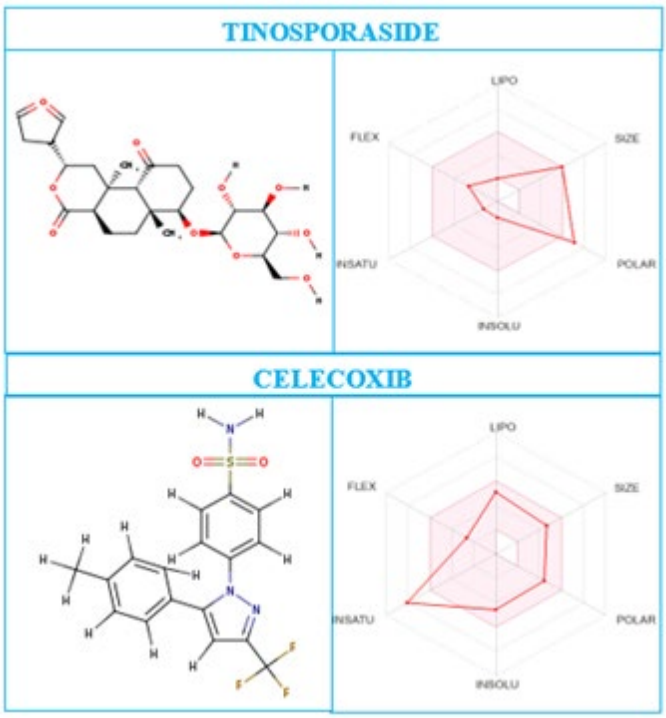

Figure 1. Chemical structures and bioavailability radar plots of Celecoxib and Tinosporaside, highlighting differences in polarity, lipophilicity, solubility, and flexibility.

The presented image displays the 2D chemical structures and bioavailability radar plots of Celecoxib and Tinosporaside, offering a comparative view of their drug-likeness and pharmacokinetic properties (Figure 1). Celecoxib demonstrates a relatively simpler molecular structure with fewer polar functional groups and a pronounced lipophilic character. This is reflected in the radar plot, where its lipophilicity (LIPO) and unsaturation (INSATU) regions show higher values, while polarity (POLAR) remains low. These properties are consistent with its high gastrointestinal (GI) absorption and membrane permeability.

In contrast, Tinosporaside exhibits a more complex and highly polar structure, containing multiple hydroxyl and glycosidic groups. Its radar plot displays elevated POLAR, SIZE, and INSOLU values, while showing reduced lipophilicity and molecular flexibility (FLEX). These features contribute to its high aqueous solubility but limited lipid membrane permeability, potentially explaining its low GI absorption despite its favorable solubility profile. Together, these structural and bioavailability characteristics underline the pharmacokinetic divergence between the two ligands-where Celecoxib offers efficient absorption but broader toxicity, and Tinosporaside presents a safer, naturally derived alternative with limitations in absorption efficiency.

3.4 Molecular Docking and Interaction Analysis

Molecular docking studies performed using the AutoDock Vina platform revealed that both Celecoxib and Tinosporaside exhibit strong binding affinities toward the active site of COX-2, with identical binding energy scores of -8.1 kcal/mol. Three-dimensional binding pocket visualization (Figure 2A and 2B) demonstrated that both ligands were deeply embedded within the enzyme's active cavity, suggesting thermodynamically favorable and spatially stable ligand-protein conformations.

The 2D interaction diagrams (Figure 2C and 2D) provided further insight into the non-covalent interaction networks stabilizing each complex. Celecoxib formed conventional hydrogen bonds with ASN28, ARG29, SER457, and LYS454, along with π-sulfur and alkyl interactions involving TYR108, LYS64, and LEU65. In contrast, Tinosporaside was stabilized primarily through conventional and carbon hydrogen bonds with CYS145, LYS155, ARG442, and LEU157. Collectively, these interactions indicate that despite their distinct chemical scaffolds and functional groups, both ligands are capable of forming stable and specific complexes within the COX-2 binding site. The comparable binding energies and favorable interaction profiles of Tinosporaside suggest its potential as a natural alternative to the synthetic COX-2 inhibitor Celecoxib.

3.5 Normal Mode Analysis (NMA)-Based Flexibility and Dynamics Evaluation

To evaluate the intrinsic flexibility and dynamic stability of the ligand-bound COX-2 complex, Normal Mode Analysis (NMA) was performed. The deformability plot (Figure 3) represents the potential mobility of individual residues within the protein structure. Several distinct peaks were observed, particularly within the N-terminal and loop regions, indicating localized flexibility.

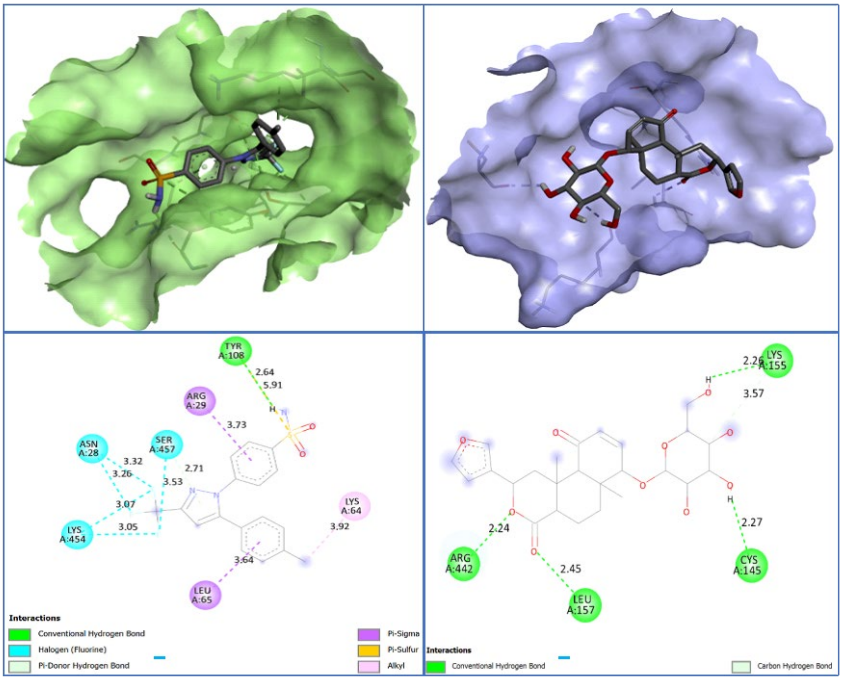

Figure 2. 3D binding poses (A, B) and 2D interaction maps (C, D) of Celecoxib and Tinosporaside within the COX-2 active site. Both ligands exhibited identical binding affinities (–8.1 kcal/mol). Key non-covalent interactions include hydrogen bonds, π-interactions, and alkyl contacts, stabilizing each complex.

However, the majority of the atom indices displayed relatively low deformability values, suggesting that the overall conformation of the protein remains rigid and structurally stable. Such low deformability is often associated with well-folded and functionally intact protein domains, especially in ligand-bound states.

Additionally, the B-factor (temperature factor) analysis (Figure 4) compared the atomic fluctuation patterns derived from NMA with those experimentally obtained from the PDB file. The similarity in trends between the NMA-derived and PDB B-factors demonstrates that the simulated model reflects realistic atomic motion. While minor discrepancies were observed in certain flexible loop regions, the global agreement across both datasets supports the validity of the *in silico* model. Together, these results suggest that the binding of the ligand (Celecoxib or Tinosporaside) does not significantly disrupt the structural stability of COX-2 and that the complex retains functional flexibility in selective domains while preserving global rigidity, which is favorable for sustained interaction and activity.

4. Discussion

This study provides a detailed *in silico* evaluation of two structurally diverse compounds-Celecoxib, a synthetic NSAID and known COX-2 inhibitor, and Tinosporaside, a natural glycosylated compound derived from Tinospora cordifolia. Despite the differences in their physicochemical profiles, both ligands exhibited comparable binding affinity to the COX-2 active site (Alsayed et al., 2017; Jacob et al., 2018). However, significant divergence in pharmacokinetics, toxicity predictions, and drug-likeness characteristics highlight distinct advantages and limitations for each compound in terms of therapeutic applicability.

The investigation of Tinosporaside holds substantial promise in the evolving field of natural product-based drug discovery. As concerns grow over the long-term adverse effects associated with synthetic NSAIDs-including gastrointestinal and cardiovascular risks-the need for safer, plant-derived alternatives has intensified (Sharma et al., 2019; Tomić et al., 2017; Wong & Chan, 2016). Tinosporaside, a bioactive compound isolated from Tinospora cordifolia, has long been used in traditional medicine, but this study offers a modern validation of its therapeutic potential. Through detailed *in silico* modeling, including docking, ADME, and toxicity analysis, Tinosporaside is shown to possess both structural compatibility and mechanistic viability as a selective COX-2 inhibitor (Ghatpande et al., 2019).

Although Celecoxib has been widely prescribed for managing inflammation and osteoarthritis, its safety profile has been a subject of debate. Prior studies have shown its involvement in cardiovascular risks and gastrointestinal complications due to prolonged COX-2 inhibition (Khan et al., 2019; Patrono, 2016). In this study, the toxicity predictions for Celecoxib revealed potential for interactions with estrogen receptors, moderate carcinogenic risk, and possible inhibition of CYP2C9, which could interfere with hepatic drug metabolism (Coss et al., 2016).

Tinosporaside, in contrast, demonstrated a cleaner predicted toxicity profile. The absence of interactions with high-risk biological

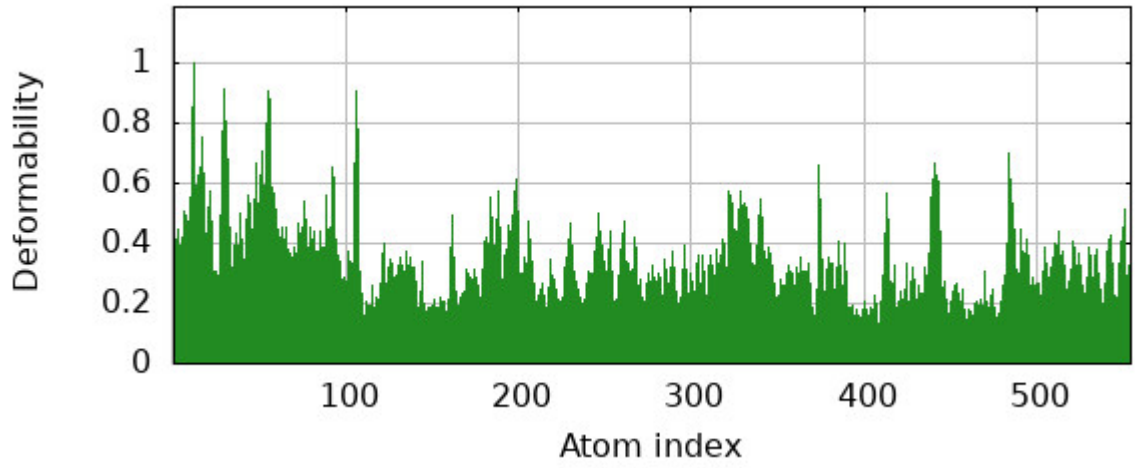

Figure 3. Deformability plot of the ligand-bound COX-2 complex, indicating the structural flexibility of each residue based on Normal Mode Analysis (NMA). Peaks correspond tso regions with higher mobility, typically found in loops or surface-accessible domains, while lower values reflect rigid and stable core residues.

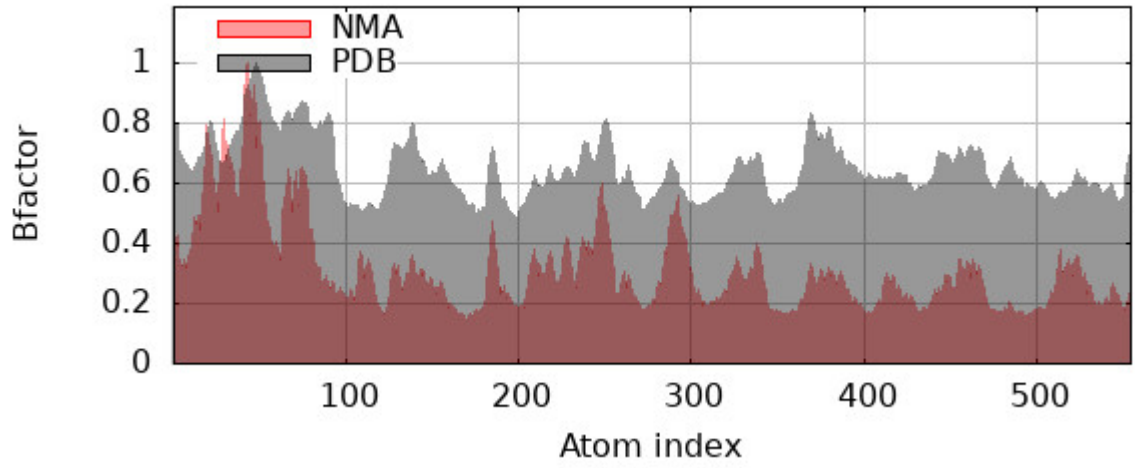

Figure 4. Comparative B-factor plot showing atomic fluctuation profiles derived from NMA (red) and experimental PDB data (gray). The high degree of overlap between the two curves supports the structural consistency and dynamic reliability of the COX-2-ligand complex model under simulation conditions.

endpoints supports its safety and aligns with the ethnopharmacological literature that describes Tinospora cordifolia as an immunomodulator and detoxifying agent (Jacob et al., 2018; Kumar et al., 2020; Priya et al., 2017). Nonetheless, its lower predicted LD_{50} suggests a narrower therapeutic window, reinforcing the need for careful dose optimization during formulation development.

It is important to acknowledge that while *in silico* analyses provide valuable early insights, many plant-derived compounds with strong computational predictions have historically failed in later stages of drug development. These failures often arise due to metabolic instability, low bioavailability, or unexpected off-target interactions not captured by initial models (Anwar et al., 2019; Cava & Castiglioni, 2020; Thomford et al., 2018). Therefore, despite the encouraging safety and binding profiles of Tinosporaside observed in this study, it is essential to pursue rigorous experimental validation. In vitro and in vivo toxicity assays, along with metabolic stability tests, will be critical before considering Tinosporaside for preclinical development.

The SwissADME analysis demonstrated that Celecoxib possesses characteristics favorable for oral delivery, including high gastrointestinal absorption, appropriate lipophilicity, and moderate water solubility. These features, typical of many small-molecule drugs, ensure rapid bioavailability. However, the compound's ability to cross the blood-brain barrier and its interactions with metabolic enzymes pose risks of neurotoxicity and drug-drug interactions (Daina et al., 2017; Yang et al., 2020).

In contrast, Tinosporaside, though highly water-soluble and free from Lipinski's violations, showed poor gastrointestinal absorption, likely due to its high polarity and molecular weight. This

pharmacokinetic disadvantage is common among glycosylated natural compounds (Dima et al., 2020; Wang et al., 2019). However, the high solubility of Tinosporaside offers an opportunity to design alternative delivery systems, such as liposomal or nanoparticle-based formulations, to overcome permeability barriers.

In recent years, remarkable progress in pharmaceutical nanotechnology has opened new avenues for enhancing the delivery of compounds with poor membrane permeability. Techniques such as liposomal encapsulation, nanoemulsion systems, solid lipid nanoparticles, and polymeric nanocarriers have enabled many bioactive molecules to overcome gastrointestinal and cellular absorption barriers, thereby achieving effective therapeutic plasma concentrations (Chai et al., 2018; Wagner et al., 2018). These advanced drug delivery strategies offer a promising solution for Tinosporaside, which, despite its high solubility, suffers from limited gastrointestinal absorption. Incorporating such nanotechnological approaches could maximize its therapeutic efficacy while preserving its natural chemical structure and pharmacological integrity.

Molecular docking analysis demonstrated that both Celecoxib and Tinosporaside form thermodynamically stable complexes within the active site of COX-2, anchoring effectively through critical non-covalent interactions. Celecoxib, consistent with previous crystallographic studies (Bala et al., 2015; Madhava et al., 2017; Vijayakumar et al., 2016), exhibited a dual interaction pattern involving hydrophobic contacts and hydrogen bonds, allowing it to fit snugly within the enzyme's lipophilic cavity. In contrast, Tinosporaside, owing to its high polarity and presence of multiple hydroxyl groups, established a dense network of hydrogen bonds with polar amino acid residues. This hydrogen-bond-driven binding mode suggests enhanced specificity, which may contribute to reduced off-target effects in biological systems.

This variation in interaction type may influence selectivity and downstream signaling. Hydrophobic binding often confers rapid and broad bioactivity but increases the risk of off-target effects. Conversely, hydrogen bond-driven interactions may enhance target specificity and reduce unintended binding events, a desirable trait for chronic medications.

Normal Mode Analysis (NMA) provided further insight into the structural dynamics of the COX-2-ligand complexes, revealing that ligand binding does not significantly compromise the overall conformational stability of the protein. The deformability plots showed minimal fluctuations across most residues, suggesting a rigid and well-maintained structure upon ligand interaction. Furthermore, the simulated B-factor values closely matched experimental crystallographic data, reinforcing the credibility of the in-silico model (Hassanein et al., 2017; Nagendrababu et al., 2019). Both Celecoxib and Tinosporaside maintained stable binding without inducing structural perturbations, supporting their roles as viable COX-2 inhibitors with favorable thermodynamic and structural interaction profiles.

Despite promising computational outcomes, it is critical to acknowledge the study's limitations. First, toxicity and ADME predictions, while based on established algorithms, are inherently probabilistic and do not capture the full complexity of biological systems. Second, docking scores, although informative, do not account for dynamic interactions under physiological conditions, such as enzymatic metabolism, immune response, or plasma protein binding.

An important limitation of the current study is the lack of analysis regarding COX-1 binding affinity, which plays a critical role in evaluating gastrointestinal safety. Non-selective NSAIDs that inhibit both COX-1 and COX-2 are known to compromise gastric mucosal protection, often resulting in ulcers and GI bleeding (Pannunzio & Coluccia, 2018; Ribeiro et al., 2015). Therefore, without data on COX-1 interaction, the safety profile of Tinosporaside remains incomplete. To comprehensively assess its therapeutic potential as a selective COX-2 inhibitor, future studies must include comparative binding and inhibition assays involving both COX isoforms. Such evaluations are essential for establishing its clinical relevance and selectivity.

5. Conclusion

This study highlights Tinosporaside as a promising natural COX-2 inhibitor with favorable binding affinity, a clean toxicity profile, and potential for reduced side effects compared to Celecoxib. Despite its lower gastrointestinal absorption, its high solubility and strong hydrogen-bonding interactions suggest opportunities for formulation enhancement. *In silico* analyses, including docking, ADME, toxicity, and molecular dynamics, collectively support its structural and pharmacological viability. However, further in vitro and in vivo validations are essential to confirm efficacy and safety. Overall, this research bridges traditional phytotherapy with modern computational pharmacology, advancing Tinosporaside toward potential development as a safer NSAID alternative.

Author contributions

M.R.I. conceived and designed the study. A.K.M. performed computational analyses and data interpretation. M.F.A. contributed to data curation and manuscript preparation. M.A.B.S. assisted in validation and result analysis. T. contributed to literature review and figure preparation. All authors reviewed and approved the final manuscript.

Competing financial interests

The authors have no conflict of interest.

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