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Computational thermodynamics of Spilanthol as a COX-2 inhibitor: Docking, dynamics, and binding energy analysis

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Spilanthol, a bioactive compound from *Acmella oleracea*, has been evaluated for its potential as a COX-2 inhibitor through computational methods. The study utilizes Lipinski's rule of 5, Molinspiration scoring, molecular docking, MM-GBSA/MM-PBSA calculations, and molecular dynamics simulations to assess Spilanthol's drug-likeness, binding affinity, and interaction stability with COX-2. Spilanthol meets several criteria of the Lipinski rule, indicating favorable drug-like properties. The Molinspiration analysis reveals that Spilanthol has significant enzyme inhibition potential, though it is less effective against other targets such as GPCRs and kinases. Molecular docking results demonstrate a strong binding affinity with COX-2, evidenced by a binding energy of –7.50 kcal/mol and an inhibition constant of 3.21 µM. MM-GBSA/MM-PBSA calculations further support these findings with negative binding energies, indicating stable interactions. Molecular dynamics simulations highlight significant conformational changes in COX-2 upon Spilanthol binding, as reflected by alterations in RMSF values. These results suggest that Spilanthol effectively binds to and inhibits COX-2, offering it as a potential natural anti-inflammatory agent. The compound's interaction profile and stability within the COX-2 active site emphasizes its promise for development as an alternative to synthetic COX-2 inhibitors, and which may be offered as an alternative to synthetic COX-2 inhibitors, though further experimental validation is needed.

Keywords: Spilanthol, COX-2, Molecular docking, MM-GBSA, Molecular dynamics, Lipinski rule

Pain is a complex sensation that serves as a protective mechanism to alert the body to potential or actual harm. It typically results from noxious stimuli, which are harmful or potentially harmful agents that can damage tissues. These stimuli can be mechanical, thermal, or chemical in nature, and they activate nociceptors, specialized sensory neurons responsible for detecting pain. When a noxious stimulus is detected, nociceptors transmit signals through the peripheral nerves to the spinal cord and brain, where the sensation of pain is perceived. This process involves the release of various cellular mediators, such as prostaglandins, bradykinin, and cytokines, which enhance the pain signal and promote inflammation[1]. These mediators increase the sensitivity of nociceptors, a phenomenon known as sensitization, making the affected area more responsive to subsequent stimuli. Inflammation plays a crucial role in pain perception, as it involves the recruitment of immune cells to the site of injury. These cells release additional mediators that sustain and amplify the pain response. Understanding the complex interplay between noxious stimuli, cellular mediators, and the nervous system is essential for developing effective pain management strategies and treatments aimed at alleviating pain and improving the quality of life for individuals experiencing chronic or acute pain[2].

Cyclooxygenase-2 (COX-2) plays a crucial role in the pain process by being a key enzyme in the synthesis of prostaglandins, which are lipid compounds that mediate inflammation and pain. COX-2 is an inducible enzyme, meaning its expression is upregulated in response to inflammatory stimuli, such as injury or infection.At the molecular level, COX-2 converts arachidonic acid, a fatty acid released from cell membrane phospholipids, into prostaglandin H2 (PGH2). PGH2 is then further converted into various prostaglandins, including prostaglandin E2 (PGE2). PGE2 is particularly significant in pain perception as it sensitizes nociceptors, the sensory neurons responsible for detecting painful stimuli, thereby lowering their threshold for activation[3]. This sensitization leads to hyperalgesia, an increased sensitivity to pain. The pathway involving COX-2 begins with an inflammatory stimulus that activates signaling molecules such as cytokines (*e.g.*, interleukin-1) and

growth factors. These molecules, in turn, activate transcription factors like nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), which increase the expression of the COX-2 gene. The upregulated COX-2 then catalyzes the production of prostaglandins, perpetuating the inflammatory response and pain sensation[4]. Inhibiting COX-2, through nonsteroidal anti-inflammatory drugs (NSAIDs) or selective COX-2 inhibitors (coxibs), can effectively reduce the production of prostaglandins, thereby alleviating pain and inflammation. This makes COX-2 a significant target for pain management therapies[5].

COX-2 inhibitors, while effective in reducing pain and inflammation, can cause adverse effects. These include an increased risk of cardiovascular events such as heart attack and stroke, as well as gastrointestinal issues like ulcers and bleeding, though they are generally less harmful to the stomach lining than non-selective NSAIDs[6,7]. The need for COX-2 inhibitors from natural sources arises to potentially mitigate these side effects. Natural compounds, like those found in certain herbs and foods, may offer safer alternatives with fewer adverse effects, providing anti-inflammatory benefits while reducing the risk of cardiovascular and gastrointestinal complications[8]. Bioinformatics and computer-aided drug discovery (CADD) are transforming the field of drug development. These approaches leverage computational tools to identify and optimize potential drug candidates efficiently. Bioinformatics enables the analysis of vast biological data, facilitating target identification and validation. CADD uses techniques like molecular docking and virtual screening to predict how drug molecules interact with targets, significantly speeding up the discovery process. The advantages include reduced time and cost, improved accuracy in identifying promising candidates, and the ability to explore a wider chemical space[9]. These methods enhance the precision and efficiency of developing new, effective therapeutics.

The need for analgesics from natural sources stems from the desire to find safer, more sustainable, and potentially less harmful alternatives to synthetic drugs. Synthetic analgesics, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, can cause significant side effects, including gastrointestinal issues, cardiovascular risks, and addiction potential. Natural analgesics, derived from plants, fungi, and other sources, may offer effective pain relief with fewer adverse effects[10].

Plant and plant products have emerged as a promising source of COX-2 inhibitors. Several species of plants contain bioactive compounds that exhibit anti-inflammatory and analgesic properties. These natural COX-2 inhibitors could offer a complementary or alternative approach to conventional pain management strategies. By harnessing the medicinal properties of mushrooms, it is possible to develop new analgesic formulations that provide effective pain relief with a lower risk of adverse effects, contributing to overall health and well-being[11]. Spilanthol, a bioactive compound found in the plant *Acmella oleracea* (commonly known as the toothache plant), has been identified as a potential COX-2 inhibitor. Research indicates that Spilanthol exhibits significant anti-inflammatory and analgesic properties. By inhibiting the activity of COX-2, Spilanthol reduces the production of pro-inflammatory prostaglandins, which play a key role in pain and inflammation[12]. This makes Spilanthol, a promising natural alternative for managing pain and inflammatory conditions, with the potential to offer fewer side effects compared to synthetic COX-2 inhibitors. Its natural origin and efficacy make it a valuable addition to pain management strategies.

The present work was aimed to evaluate the interaction of Spilanthol with COX-2, utilizing both computational and experimental approaches. Molecular docking studies are employed to predict the binding affinity and interaction sites of Spilanthol on the COX-2 enzyme. Following this, molecular dynamics simulations are conducted to assess the stability and behavior of the Spilanthol-COX-2 complex over time.

Experimental Section

Lipinski's Rule of Five Criteria

Lipinski's Rule of Five was evaluated using the Sanjeevni server available at IIT Delhi. The SMILES of the compound was input into the server. The server's algorithm was used to analyze the compound's properties. Results were reviewed to determine adherence to Lipinski's criteria[13].

Bioactivity score

The drug-likeness properties of Spilanthol was assessed using the online tool Molinspiration (https://www.molinspiration.com/cgi-bin/properties) [14]. Spilanthol was provided in SMILES format. Following this evaluation, Spilanthol was chosen for further protein-ligand interaction studies

based on their favorable drug-likeness and bioavailability characteristics. This step aims to identify compounds with promising pharmaceutical properties for potential use in drug discovery.

Docking Studies

The software utilized for the study included Python 2.7, obtained from www.python.com, Molecular Graphics Laboratory (MGL) tools, and AutoDock 4.2 downloaded from www.scripps.edu. Discovery Studio Visualizer 4.1 was acquired from www.accelerys.com. The Python 2.7 language served as the foundation for the subsequent molecular docking experiments[15].

Molecular docking investigations were conducted on Spilanthol and Celecoxib against COX-2. The molecular structureofSpilantho lwas sourced from PubChem, while the COX-2 [PBD ID: 4COX] was downloaded from the Protein Data Bank (PDB) at www.rcsb.org/pdb. Pre-processing steps involved editing the COX-2, including the removal of heteroatoms and addition of C-terminal oxygen. Gasteiger-Marsili partial charges were assigned to ligands, non-polar hydrogen atoms were merged, and torsions were allowed during docking. Docking employed the Lamarckian Genetic Algorithm for energy minimization with default parameters, and Discovery Studio was employed for result visualization. An additional docking study of Spilanthol against COX-1 (PDB ID: 4PGE) was performed to identify notable interactions.

MM-GBSA/MM-PBSA Calculations

The MM-GBSA/MM-PBSA calculations were conducted using the fastDRH server, an online tool designed for efficient free energy calculations (http://cadd.zju.edu.cn/fastdrh/submit). Initially, the protein-ligand complex structures were uploaded to the server. The server then performed energy minimization to prepare the complexes. Following this, the MM-GBSA and MM-PBSA methodologies were applied to compute the binding free energies. The server utilized molecular mechanics energies combined with generalized Born (GB) and Poisson-Boltzmann (PB) solvation models to estimate the free energy of binding. The results provided detailed insights into the stability and affinity of the ligand within the protein's active site, based on calculated binding energies.

Molecular Dynamics

Molecular dynamic simulation was performed using CABS Flex 2.0 (http://biocomp. chem.uw. edu.pl/CABSflex2/) which is an open access web server with 100 cycles and 100 trajectory frames[16,17]. The boundaries for atom pairing within the defined space during simulation were set using default parameters. The webserver uses coarse-grained modeling approach. No additional distance constrains with respect to the process were modified. The solvent probe radius was set at 1.4 Å, minimum atomic radius 1 Å and temperature was 1.4 K in order to analyze the interaction between the complex of respective proteins Spilanthol. The fluctuations of each residue of the hit complex could be explained using the root mean square fluctuation (RMSF) values obtained[18].

ADMET Studies

The ADMET properties of Spilanthol were evaluated using the ADMETlab 3.0 web server (https://admetmesh.scbdd.com/). The SMILES notation of the compound was submitted, and predictions were generated for absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles. Parameters such as Caco-2 permeability, plasma protein binding, CYP enzyme inhibition, and hepatotoxicity were assessed. All results were derived from machine learning-based predictive models integrated within the platform. The compound's pharmacokinetics and organ toxicity profiles were analyzed to estimate its drug-likeness and safety[19].

Results

Lipinski Rule of 5 assessments

The Lipinski rule of 5 assessments for Spilanthol reveals several key characteristics. The compound has a molecular mass of 312.00 Da, which is within the acceptable range. It has 5 hydrogen bond donors and 6 hydrogen bond acceptors, indicating potential for significant interactions with biological targets. The Log P value of –0.053 suggests Spilanthol is hydrophilic, which may affect its membrane permeability. The molar refractivity of 77.15 is also noted (Table 1). Overall, Spilanthol exhibits optimal drug-like properties, particularly in its Log P value and hydrogen bonding capacity, which could influence its pharmacokinetic profile.

Molinspiration score

The Molinspiration scores for Spilanthol against various cellular targets reveal its potential interactions with multiple protein classes. As a GPCR ligand,

Spilanthol scores –0.15, indicating weak affinity. Its score of 0.08 as an ion channel modulator suggests modest potential in this role. However, Spilanthol shows a notably low score of –0.70 as a kinase inhibitor, implying minimal effectiveness in this capacity. For nuclear receptor ligands, the score of –0.26 indicates weak binding affinity. As a protease inhibitor, Spilanthol scores –0.16, again reflecting limited potential. Importantly, the enzyme inhibition score of 0.12 stands out, highlighting Spilanthol's relative effectiveness as an enzyme inhibitor (Table 2). This positive score suggests that Spilanthol may have a more significant role in inhibiting enzymatic activity, making it a compound of interest for further investigation in therapeutic applications targeting enzyme-related pathways.

Table 1 — Lipinski rule of 5 assessments for Spilanthol

Feature	Value
Molecular mass	312.000000
Hydrogen bond donor	5
Hydrogen bond acceptors	6
Log P	–0.053101
Molar Refractivity	77.145782

Table 2 — Molinspiration score for Spilanthol against various cellular tergets

Target	Molinspiration score
GPCR ligand	–0.15
Ion channel modulator	0.08
Kinase inhibitor	–0.70
Nuclear receptor ligand	–0.26
Protease inhibitor	–0.16
Enzyme inhibitor	0.12

Molecular docking

Molecular docking results revealed that both Spilanthol and Celecoxib exhibit significant binding affinities toward the COX-2 active site. Spilanthol showed a binding energy of –7.50 kcal/mol with an estimated inhibition constant of 3.21 µM, while Celecoxib demonstrated a stronger binding energy of –8.03 kcal/mol and an inhibition constant of 18.82 nM, indicating higher affinity. Spilanthol formed several key interactions within the COX-2 binding pocket. These included Pi-Sigma interactions with TYR385, PHE381, and TRP387, along with Van der Waals contacts involving residues such as GLY526, LEU384, and MET522. It also formed conventional hydrogen bonds with LEU352 and SER353, Pi-Alkyl bonding with ARG513, and alkyl or carbon-hydrogen bonds with ALA516 and ILE517. Celecoxib, a known selective COX-2 inhibitor, formed multiple strong interactions including Pi-Sigma and Pi-Cation interactions with TYR385 and ARG120, respectively, and amide–Pi stacking with TYR355. It exhibited a broader range of hydrophobic contacts with residues such as VAL523, LEU359, ALA516, and PHE518. Additionally, Celecoxib formed hydrogen bonds with HIS90 and GLN192 (Fig. 1, Table 3).

In terms of interactions with **COX-1**, the molecular docking results suggest that Spilanthol has relatively weak interactions with COX-1, with a binding energy of –4.33 Kcal/mol and an inhibition constant of 4.02 µM. While there are interactions with key residues such as LEU, PHE, TYR, and SER, the overall binding affinity is moderate compared to

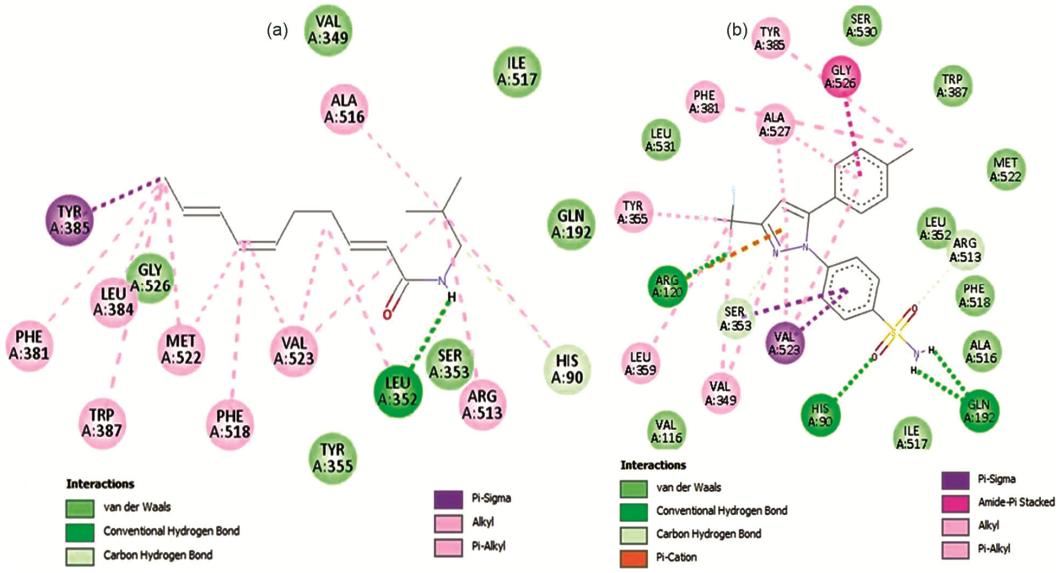

Fig. 1 — Docking interactions of (a) Spilanthol and (b) Celecoxib with COX-2

Table 3 — Molecular docking interactions of Spilanthol with COX-2

Compd	Binding Energy (Kcal/mol)	Inhibition constant	Interacting residues		
			Interaction Type	Amino Acid	Residue
Spilanthol	–7.50	3.21 μM	Pi-Sigma bond	TYR	A:385
				PHE	A:381
				TRP	A:387
			Van der Waals interaction	GLY	A:526
				LEU	A:384
				MET	A:522
				VAL	A:523
				VAL	A:349
				ALA	A:516
				ILE	A:517
				GLN	A:192
				TYR	A:355
				HIS	A:90
			Conventional Hydrogen Bond	LEU	A:352
				SER	A:353
			Pi-Alkylbond	ARG	A:513
			Alkyl bondsbond	`ALA	A:516
				ILE	A: 517
			Carbon Hydrogen Bond	LEU	A:352
Celecoxib	–8.03	18.82 nM	Pi-Sigma	TYR	A:385
			Pi-Cation	ARG	A:120
			Amide–Pi Stacked	TYR	A:355
			Pi-Alkyl	VAL	A:523
				ALA	A:527
				LEU	A:359
				VAL	A:349
			Alkyl	VAL	A:349
				HIS	A:90
				VAL	A:116
				ILE	A:517
				GLN	A:192
				MET	A:522
				ARG	A:513
				PHE	A:518
				ALA	A:516
				LEU	A:352
				TRP	A:387
				SER	A:530
				GLY	A:526
				PHE	A:381
				SER	A:353
			Carbon Hydrogen Bond	LEU	A:352
			Conventional Hydrogen Bond	HIS	A:90
				GLN	A:192

stronger inhibitors of COX-1. The weak interaction profile implies that Spilanthol may not be a potent inhibitor of COX-1, especially when compared to well-known NSAIDs. Given its relatively poor binding affinity, Spilanthol is less likely to cause the significant inhibition of COX-1 that is typically associated with ulcerogenic effects. NSAIDs often induce gastrointestinal ulcers through strong, irreversible binding to COX-1, particularly through the acetylation of Ser530 (Fig. 2, Table 4). Since Spilanthol does not exhibit strong binding to the active site of COX-1, it may not trigger the same

Table 4 — Molecular docking interactions of Spilanthol with COX-1

Compd	Binding Energy (Kcal/mol)	Inhibition constant (μM)	Interacting residues		
			Interaction Type	Amino Acid	Residue
Spilanthol	–4.33	4.02	Pi-Alkyl	LEU	B:352
				TRP	B:387
				LEU	B:384
				PHE	B:518
				MET	B:522
				TYR	B:348
				ILE	B:523
				VAL	B:349
			`Alkyl	LEU	B:531
			Van der Waals	PHE	B:381
				SER	B:353
				TYR	B:355
				TYR	B:385
				SER	B:530
				GLY	B:526
				ALA	B:527

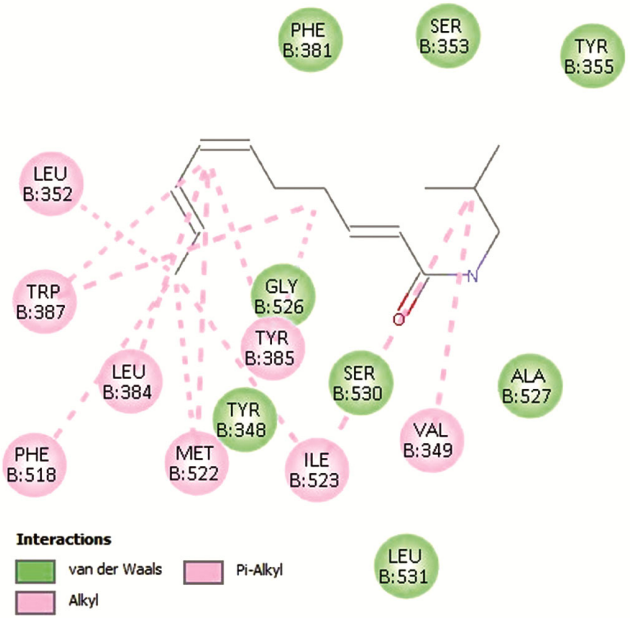

Fig. 2 — Docking interactions of Spilanthol with COX-1

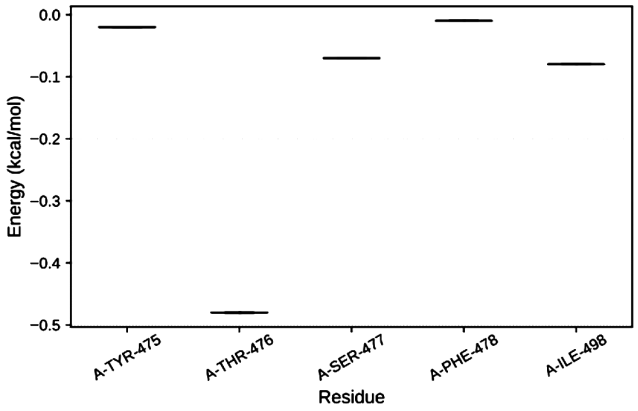

Fig. 3 — Energy boxplot of the potential hotspot residues of COX-2 with Spilanthol

ulcerogenic side effects commonly observed with potent COX-1 inhibitors.

MM-GBSA/MM-PBSA Calculations

The MM/PB(GB)SA analysis provides insights into the binding free energies of Spilanthol with COX-2, using different solvation models to evaluate the stability and affinity of the binding poses. The Poisson-Boltzmann (PB) and Generalized Born (GB) methods yield varying total binding energies (PBTOT/GBTOT) for different binding poses. For the Poisson-Boltzmann (PB) method, the binding energies are as follows: PB1 (2.45 kcal/mol), PB3 (–3.02 kcal/mol), and PB4 (–1.75 kcal/mol). The positive value for PB1 indicates an unfavorable binding pose, suggesting less stability. Conversely, PB3 and PB4 exhibit negative values, indicating more favorable binding interactions and higher stability. PB3, with the most negative value (–3.02 kcal/mol), represents the most stable binding pose among the PB evaluations.The Generalized Born (GB) method provides more uniformly negative binding energies: GB1 (–1.23 kcal/mol), GB2 (–0.89 kcal/mol), GB5 (–0.88 kcal/mol), GB6 (–0.95 kcal/mol), GB7 (–1.19 kcal/mol), and GB8 (–1.07 kcal/mol). These consistently negative values suggest that, in general, Spilanthol forms stable complexes with COX-2 across multiple binding poses (Fig. 3, Table 5). Among these, GB1 (–1.23 kcal/mol) and GB7

(–1.19 kcal/mol) show the most favorable interactions, indicating strong binding affinities. MM/PB(GB) SA analysis highlights the potential of Spilanthol as a COX-2 inhibitor, with several binding poses demonstrating favorable binding energies. The negative binding energies observed, particularly in PB3 and the GB series, reinforce the compound's potential efficacy and stability within the COX-2 active site.

Molecular Dynamics Simulation

The table provides data on the interactions between COX-2 residues and Spilanthol, comparing COX-2 alone to COX-2-Spilanthol complex interactions. Notable interactions are observed where there is a significant change in the values between the two conditions. Residue A33 shows a marked increase from 3.243 to 4.792, suggesting a strong interaction with Spilanthol. Similarly, residues A34 and A36 also exhibit notable increases, indicating enhanced binding or interaction when Spilanthol is present (Fig. 4). Conversely, residues such as A40 and A53 show a decrease in values, suggesting a potential decrease in interaction or a different conformational change upon Spilanthol binding. These significant changes in interaction values for specific residues highlight the key areas where Spilanthol may be exerting its effects on COX-2, potentially leading to altered enzyme activity or stability.

Table 5 — MM/PB(GB) SA scores (kcal/mol) for the binding poses of Spilanthol with COX-2

Procedure	PBTOT/GBTOT
PB1	2.45
PB3	–3.02
PB4	–1.75
GB1	–1.23
GB2	–0.89
GB5	–0.88
GB6	–0.95
GB7	–1.19
GB8	–1.07

Pharmacokinetics and Toxicity

The toxicological screening of Spilanthol (Table 6) indicates a favorable safety profile with no predicted risks. The compound showed negative results for hepatotoxicity, cardiotoxicity (hERG), mutagenicity (AMES), and carcinogenicity, suggesting no potential for liver damage, cardiac arrhythmias, genetic mutations, or cancer development. Additionally,

Table 6 — Toxicological screening of Spilanthol

Organ/System	Toxicity Prediction	Interpretation
Hepatotoxicity	Negative	No expected liver toxicity
Cardiotoxicity (hERG)	Negative	Low risk of causing cardiac arrhythmia
Mutagenicity (AMES)	Negative	Not predicted to be mutagenic
Carcinogenicity	Negative	Not predicted to be carcinogenic
Skin Sensitization	Negative	No potential for skin sensitization

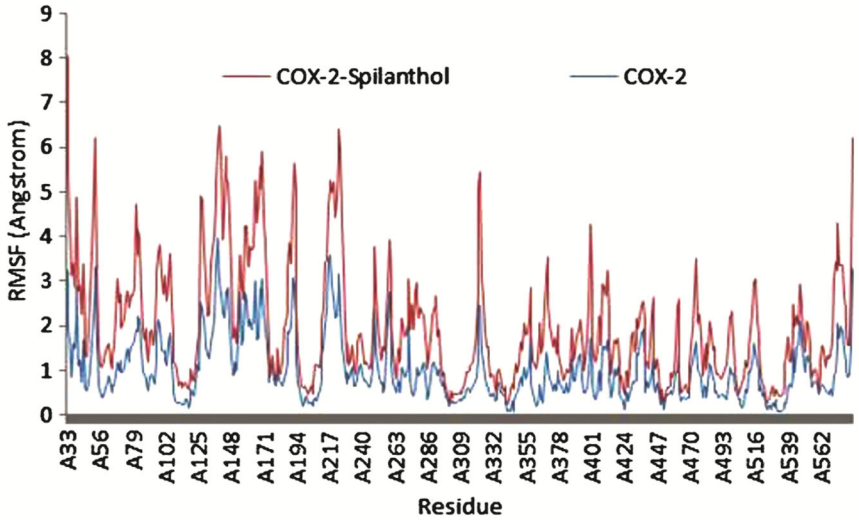

Fig. 4 — RMSF plot of Spilanthol with COX-2

Spilanthol did not show any potential for skin sensitization. In terms of pharmacokinetics (Table 7), Spilanthol demonstrates promising absorption characteristics, with good Caco-2 permeability (−4.51) and positive predictions for human intestinal absorption, although MDCK permeability was low (−4.74), suggesting limited cell permeability. The compound shows moderate oral bioavailability (F50% negative) and high plasma protein binding (95.7%). It has limited blood-brain barrier penetration and a low volume of distribution (−0.538). Regarding metabolism, Spilanthol is not an inhibitor of most major CYP enzymes (CYP2C9, CYP2C19, CYP2D6, CYP3A4), and it exhibits good stability in human liver microsomes. Its plasma clearance is moderate (9.576 mL/min/kg), with an ultra-short half-life of 0.75 h, which may influence its dosing regimen. Overall, Spilanthol's pharmacokinetic and toxicological properties support its potential for therapeutic development.

Discussion

Computer-aided drug discovery (CADD) is an essential tool in identifying new drug leads, revolutionizing traditional methods with its efficiency and precision. CADD leverages computational power to quickly screen vast chemical libraries, predicting potential drug candidates within a fraction of the time required for experimental methods. This rapid identification process significantly accelerates the early stages of drug development[20]. CADD's reliability stems from its ability to model interactions at atomic and subatomic levels, providing detailed insights into how potential drugs interact with their targets. Techniques like molecular docking, molecular dynamics simulations, and quantitative structure-activity relationship (QSAR) models allow researchers to visualize and predict the binding affinities and stability of drug-target complexes[20]. These computational methods not only save time and resources but also enhance the accuracy of predictions, leading to more effective and targeted drug design.

In the present study, molecular docking and dymanics studies were utilized to determine the interactions of Spilanthol with COX-2. The Lipinski rule of 5 provides a framework for assessing the drug-likeness of compounds, focusing on molecular mass, hydrogen bond donors and acceptors, Log P (partition coefficient), and molar refractivity. For Spilanthol, the molecular mass of 312.00 Da is within the acceptable range, suggesting suitable size for oral bioavailability. The compound has 5 hydrogen bond donors and 6 acceptors, which indicates a high potential for interaction with biological targets but might also pose challenges for permeability and membrane crossing. The Log P value of –0.053 is notably low, suggesting Spilanthol is highly hydrophilic. This could impair its ability to cross lipid membranes effectively, potentially limiting its oral bioavailability. The molar refractivity of 77.15 falls within a reasonable range, supporting its potential as a drug candidate. Despite some deviations from optimal drug-like properties, Spilanthol's interaction potential and size suggest it

Table 7 — Pharmacokinetics of Spilanthol

Parameter	Value/Description
Absorption	
Caco-2 Permeability	−4.51 (Good; above −5.15 threshold)
MDCK Permeability	−4.74 (Low)
Human Intestinal Absorption (HIA)	Negative (Good absorption predicted)
F20% (Oral Bioavailability >20%)	Positive
F50% (Oral Bioavailability >50%)	Negative (Moderate bioavailability)
Distribution	
Plasma Protein Binding (PPB)	95.7% (High)
Volume of Distribution (VDss)	−0.538 (Low)
Blood-Brain Barrier (BBB) Penetration	Negative
Fraction Unbound (Fu)	5.2% (Low–moderate)
Metabolism	
CYP2C9, CYP2C19, CYP2D6, CYP3A4 Inhibition	Non-inhibitor
Human Liver Microsomal (HLM) Stability	Stable (+++)
Excretion	
Plasma Clearance (CLplasma)	9.576 mL/min/kg (Moderate)
Half-Life (T1/2)	0.75 h (Ultra-short)

could be developed further with modifications to enhance its pharmacokinetic profile[21].

The Molinspiration scores for Spilanthol across different cellular targets provide insights into its potential biological interactions[22]. As a GPCR ligand, Spilanthol shows weak affinity, suggesting limited interaction with this protein class. Its role as an ion channel modulator is marked by modest potential, indicating that while it may have some effect, it is not highly significant. The compound's performance as a kinase inhibitor is notably poor, reflecting minimal efficacy in this area. Similarly, its activity as a nuclear receptor ligand and protease inhibitor is weak, suggesting limited binding and inhibition capabilities. However, Spilanthol demonstrates a relatively strong performance as an enzyme inhibitor. This positive score points to a more pronounced ability to affect enzymatic activity, highlighting its potential value for therapeutic applications targeting enzyme-related pathways. While Spilanthol's interactions with several targets are modest, its enzyme inhibition capability is a noteworthy aspect warranting further research.

The molecular docking analysis against COX-2 (PDB ID: 4COX) revealed that both Spilanthol and Celecoxib effectively interact with the enzyme's active site, which is critical for its cyclooxygenase activity. The COX-2 active site is a hydrophobic channel that accommodates ligands through a combination of van der Waals forces, hydrogen bonds, and π-interactions. Notably, key residues such as TYR385, SER530, ARG513, and HIS90 play crucial roles in substrate recognition and inhibitor binding. Spilanthol demonstrated a binding energy of –7.50 kcal/mol and interacted with several critical residues. Pi-Sigma interactions with TYR385, PHE381, and TRP387 suggest anchoring within the active site's hydrophobic pocket. Van der Waals contacts with residues like GLY526, LEU384, and MET522 further stabilized the complex. Importantly, hydrogen bonding with LEU352 and SER353, along with Pi-Alkyl and alkyl interactions, indicate multi-faceted binding, enhancing its overall affinity. These interactions occur within the same regions targeted by selective COX-2 inhibitors, highlighting Spilanthol's potential as a competitive binder. Celecoxib, a reference drug, showed stronger binding energy (–8.03 kcal/mol) and formed extensive hydrophobic and polar interactions. It interacted with nearly all key active site residues, including hydrogen bonds with HIS90 and GLN192, and π-interactions with TYR385 and ARG120, confirming its well-established selective inhibition. Comparatively, while Celecoxib binds more tightly, Spilanthol mimics many of its key interactions, particularly within the hydrophobic cleft and catalytic residues.

Spilanthol's interaction with COX-1 appears to be relatively weak, as indicated by its moderate binding energy of –4.33 Kcal/mol and inhibition constant of 4.02 μM. A closer examination of its molecular binding mechanism reveals that while Spilanthol interacts with several key residues in the active site, these interactions are not as robust as those seen with more potent COX-1 inhibitors. The interaction primarily involves hydrophobic and Van der Waals forces, but these types of interactions are generally less favorable for strong, sustained enzyme inhibition. Unlike potent COX-1 inhibitors such as aspirin, which form stable covalent bonds (*e.g.*, acetylation of Ser530), Spilanthol does not appear to engage in such irreversible modifications. The lack of strong hydrogen bonding with critical residues, such as Tyr355 or Ser530, suggests that Spilanthol may not effectively lock the enzyme in an inactive conformation. Furthermore, Spilanthol's interactions with residues like Phe381, Leu531, and Ser530 indicate that while it might partially occupy the enzyme's active site, it does not form the key catalytic interactions necessary to significantly disrupt COX-1's enzymatic activity.

These findings suggest that Spilanthol may have limited efficacy as a COX-1 inhibitor, and its weak binding profile further implies that it is less likely to cause the common side effects associated with stronger inhibitors, such as gastrointestinal irritation or ulcer formation.

MM/PB(GB)SA data for Spilanthol binding to COX-2 provides valuable insights into the molecular interactions at the atomic level. The negative binding energies observed in both PB and GB methods, particularly PB3 (–3.02 kcal/mol) and several GB values (*e.g.*, GB1: –1.23 kcal/mol, GB7: –1.19 kcal/mol), indicate favorable and stable binding poses.At the atomic level, these interactions are likely driven by a combination of van der Waals forces, hydrogen bonding, and hydrophobic interactions. The binding site interactions reveal that residues such as TYR, PHE, TRP, LEU, MET, and VAL are critical for stabilizing Spilanthol within the COX-2 active site. The involvement of hydrogen bonds with residues like LEU (A:352) and SER (A:353) further

enhances binding affinity.The consistently negative GB values suggest that electrostatic solvation energies play a significant role in stabilizing the Spilanthol-COX-2 complex. The varying magnitudes of PB and GB scores reflect the differential impact of solvation models on binding affinity. Overall, the data highlights Spilanthol's potential as a COX-2 inhibitor through robust molecular interactions at the atomic level[23].

The analysis of RMSF values provides insight into the flexibility of COX-2 residues in the presence and absence of Spilanthol. Notable changes were observed in several residues, indicating alterations in their dynamic behavior upon binding with Spilanthol. Residues such as A33, A34, and A36 show increased RMSF values when complexed with Spilanthol, suggesting greater flexibility and possibly more significant conformational changes in these regions. This increased flexibility could be indicative of enhanced interactions or binding efficiency. On the other hand, residues A40 and A53 exhibit decreased RMSF values, implying reduced flexibility and potential stabilization of these regions by Spilanthol. Such differential effects on residue flexibility highlight specific areas where Spilanthol exerts its influence on COX-2 structure[24]. These findings are crucial as they suggest that Spilanthol binding may lead to significant conformational shifts, affecting COX-2's overall function and stability, which could have implications for its enzymatic activity and inhibition.

The pharmacokinetics and toxicity results presented for Spilanthol suggest a promising compound for therapeutic development, with a favorable safety profile and pharmacokinetic properties. However, several aspects of Spilanthol's ADMET (absorption, distribution, metabolism, excretion, and toxicity) data warrant further discussion. One notable feature not emphasized in the results section is the compound's metabolic stability, particularly its stability in human liver microsomes (HLM). The classification of Spilanthol as "stable (+++)" suggests that the compound is likely to have a consistent metabolic profile, reducing the risk of variability in its pharmacokinetic behavior. This can be especially beneficial in clinical settings, as it may ensure more predictable therapeutic outcomes. Additionally, Spilanthol's relatively low volume of distribution (−0.538) and low fraction unbound (5.2%) may imply that the compound is likely to remain confined to the bloodstream rather than being widely distributed in tissues, which could potentially limit side effects but also indicate a need for precise dosing strategies to achieve therapeutic concentrations at target sites. Although Spilanthol does not penetrate the blood-brain barrier, this is not necessarily a disadvantage depending on the intended therapeutic application, especially if targeting peripheral tissues. The compound's moderate clearance (9.576 mL/min/kg) and short half-life (0.75 h) suggest that it may require frequent dosing or formulation strategies to sustain effective therapeutic levels.

Conclusion

Spilanthol's potential as a COX-2 inhibitor is highlighted through various analyses. The compound adheres to some aspects of the Lipinski rule of 5, indicating its suitability for oral bioavailability, though its hydrophilicity may limit membrane permeability. Molinspiration scores reveal that Spilanthol has a notable enzyme inhibition capability but weaker interactions with other targets. The molecular docking and dynamics studies, supported by MM/PB(GB)SA data, demonstrate strong binding affinity and stable interactions with COX-2. RMSF analysis further shows significant conformational changes in COX-2 residues upon Spilanthol binding, suggesting altered enzyme flexibility. These findings collectively highlightSpilanthol's potential for development as an anti-inflammatory therapeutic.

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