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# Molecular Docking Analysis of Phytocompounds from Hyptis Verticillata as Potential Inhibitors of Human Cyclooxygenase-2 (COX-2)

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## ABSTRACT

Cyclooxygenase-2 (COX-2) is a key inducible enzyme in the inflammatory pathway, catalyzing the conversion of arachidonic acid into prostaglandins that mediate pain, fever, and inflammation. Selective COX-2 inhibitors such as celecoxib and rofecoxib have demonstrated strong therapeutic effects but are associated with adverse cardiovascular and gastrointestinal complications, underscoring the need for safer alternatives. Medicinal plants represent a valuable source of novel bioactive compounds with promising anti-inflammatory properties. Hyptis verticillata, a member of the Lamiaceae family, has been widely used in ethnomedicine for treating fever, colds, and inflammatory conditions, and is known to contain diverse phytochemicals including terpenoids, flavonoids, sterols, and essential oils. This study aimed to evaluate the molecular docking interactions of phytocompounds from H. verticillata with human COX-2 (PDB ID: 6COX) as potential natural anti-inflammatory agents. Seven phytochemicals reported in previous phytochemical profiling of the plant were docked against the COX-2 active site using AutoDock Vina implemented in PyRx, and their interactions compared with reference inhibitors celecoxib and rofecoxib. Binding affinities ranged from −3.7 to −8.2 kcal/mol. Squalene demonstrated the strongest affinity (−8.2 kcal/mol), comparable to rofecoxib (−8.2 kcal/mol), while aliphatic hydrocarbons such as 1-octadecyne (−6.9 kcal/mol) and 1-fluorodecane (−6.1 kcal/mol) showed moderate activity. Celecoxib, unexpectedly scoring −3.7 kcal/mol, highlighted potential docking protocol limitations that warrant revalidation. Interaction analysis revealed that hydrophobic contacts dominated ligand binding, consistent with the structural hydrophobicity of the COX-2 catalytic tunnel. Although squalene showed high docking affinity, ADMET predictions indicated poor solubility and oral bioavailability, limiting its drug-likeness. In contrast, smaller hydrocarbons displayed more favorable pharmacokinetic profiles but weaker binding energies. These findings suggest that H. verticillata harbors compounds with structural potential for COX-2 inhibition, though optimization and experimental validation are required. The study provides a computational foundation for developing safer plant-derived anti-inflammatory agents.

**Keywords:** *Hyptis verticillata*, cyclooxygenase-2, molecular docking, phytochemicals, anti-inflammatory, ADMET.

## INTRODUCTION

Inflammation is a central biological process that plays both protective and pathological roles in the human body. While acute inflammation is essential for host defense and tissue repair, chronic inflammation contributes to the pathogenesis of various diseases, including cancer, cardiovascular diseases, diabetes, rheumatoid arthritis, and

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neurodegenerative disorders (Medzhitov, 2008). One of the key enzymes in the inflammatory cascade is cyclooxygenase (COX), which exists in two isoforms: COX-1 and COX-2.

COX-1 is constitutively expressed and maintains physiological functions such as gastric mucosal protection and platelet aggregation, whereas COX-2 is inducible and upregulated in response to pro-inflammatory stimuli like cytokines and endotoxins (Chandrasekharan et al., 2002). COX-2 catalyzes the conversion of arachidonic acid to prostaglandin H2, a precursor for pro-inflammatory prostaglandins and thromboxanes (Rouzer & Marnett, 2009). The structural substitution of isoleucine-523 in COX-1 with valine-523 in COX-2 creates a secondary pocket, enabling selective inhibition of COX-2 by coxibs such as celecoxib and rofecoxib (Kurumbail et al., 1996).

Despite the therapeutic potential of COX-2 inhibition, conventional non-steroidal anti-inflammatory drugs (NSAIDs) and coxibs are associated with adverse effects including gastrointestinal toxicity, renal impairment, and cardiovascular complications (Hinz & Brune, 2002). This has driven the search for natural COX-2 inhibitors with safer pharmacological profiles. Medicinal plants are a promising source of novel bioactive compounds, and ethnopharmacological knowledge has guided the discovery of many therapeutic agents.

*Hyptis verticillata* Jacq., commonly known as John Charles, is a perennial herb belonging to the family Lamiaceae. The plant is widely distributed in tropical regions, including Africa and the Caribbean, and has been traditionally used in folk medicine for managing fever, colds, gastrointestinal disorders, and inflammatory conditions (Adesina, 1982; Lans, 2006). Phytochemical studies have revealed that *H. verticillata* contains essential oils, flavonoids, terpenoids, fatty acid esters, and alkaloids with diverse biological activities (Ajiboye et al., 2018). GC-MS profiling of its leaf extracts from Nigeria confirmed the presence of hydrocarbons, fatty acid esters, and sterols, some of which have reported anti-inflammatory and antimicrobial properties (Ajiboye et al., 2018).

Given its ethnomedicinal relevance and phytochemical diversity, *H. verticillata* represents a potential source of novel COX-2 inhibitors. However, the molecular interactions between its bioactive compounds and the COX-2 active site remain unexplored. Computational approaches such as molecular docking provide valuable insights into ligand–receptor interactions, binding affinity, and structure-activity relationships, thereby accelerating drug discovery (Kitchen et al., 2004).

### Statement of the Problem

Conventional NSAIDs and selective COX-2 inhibitors are effective anti-inflammatory agents but pose risks of gastrointestinal and cardiovascular toxicity. There is a need for safer, plant-derived alternatives that can inhibit COX-2 effectively while minimizing adverse effects. *Hyptis verticillata* is traditionally used in treating inflammation-related conditions, but its molecular potential as a COX-2 inhibitor has not been systematically evaluated.

### Aim of the study

This study aims to evaluate the molecular docking interactions of phytocompounds derived from Hyptis verticillata with the human cyclooxygenase-2 (COX-2) enzyme (PDB ID: 6COX). The goal is to determine their potential as natural anti-inflammatory agents by analyzing binding affinities and molecular interactions. Selected phytochemicals will be compared against standard reference inhibitors such as celecoxib and rofecoxib. This research seeks to highlight promising compounds that may serve as leads in drug discovery for COX-2 inhibition. Ultimately, the study provides a computational foundation for future pharmacological validation.

## MATERIALS AND METHODS

### Study Design

This study employed an in-silico design involving molecular docking simulations of phytochemicals from *H. verticillata* against COX-2. Comparative docking with reference inhibitors was used for validation.

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**Insilico analysis**

The databases used includes:

1. PubMed Database (https://pubmed.ncbi.nlm.nih.gov/)
2. PubChem (https://pubchem.ncbi.nlm.nih.gov/)
3. RCSB-Protein Data Bank (https://www.rcsb.org/)
4. Chemspider (http://www.chemspider.com/)
5. Swissadme (http://www.swissadme.ch/)
6. ADMET lab 2.0 (https://admetmesh.scbdd.com/)

Softwares used includes:

1. OpenBabel in build in PyRx 0.8
2. Discovery Studio 2024
3. AutoDock Vina in built in PyRx 0.8

**Protein Preparation**

The 3D crystal structure of human cyclooxygenase-2 bound to the selective inhibitor SC-558 was retrieved from the RCSB Protein Data Bank (PDB ID: **6COX**) at 2.8 Å resolution (Kurumbail et al., 1996). Protein preparation involved:

- Removal of co-crystallized ligand (SC-558) and water molecules.
- Retention of the heme cofactor essential for enzyme activity.
- Addition of polar hydrogens and assignment of Kollman charges using AutoDock Tools 1.5.6.
- Energy minimization and conversion to PDBQT format.

**Table 1 Selected receptors in PCOS**

| **TARGET PROTEIN** | **ID NUMBER** |
|---|---|
| COX-2 | **6COX** |

**Ligand Preparation**

7 bioactive phytochemicals in structured Data Format (SDF) from *H. verticillata*, were retrieved from the PubChem database, PubMed Database and the Ligand molecules were further converted to the dockable PDBQT format using AutoDock Tools.

Referenced drugs

**Celecoxib** (PDB ligand ID: **CLX**) – a selective COX-2 inhibitor, clinically used as an anti-inflammatory and analgesic (arthritis, pain, etc.).

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**Rofecoxib** (Vioxx) – another selective COX-2 inhibitor, widely studied though withdrawn from the market due to cardiovascular risk.

**Table 2 List of phytocompounds and referenced drugs**

| Ligands |
|---|
| 3a.4,5,6,7,7a-hexahydro-4,7-methanoindene |
| 4,7- methanon-1H-indene |
| R-R,R-E- trans-Phytol |
| Squalene |
| 9,12,15-octadecatrien-1-ol |
| 1-octadecyne |
| 1-fluorodecane |
| Rofecoxib |
| Colecoxib |

**Docking Procedure**

Docking was performed using AutoDock Vina integrated in PyRx 0.8.

- The grid box was centered at the SC-558 binding site with dimensions of 25 Å × 25 Å × 25 Å.
- Exhaustiveness was set at 8 for accurate conformational sampling.
- Each ligand was docked to generate up to 10 binding poses ranked by binding affinity (kcal/mol).

**Interaction Analysis**

Discovery Studio Visualizer 2024 was used to analyze protein–ligand interactions, focusing on:

- Hydrogen bonding
- Hydrophobic contacts
- π–π interactions

**ADMET and Drug-Likeness Analysis**

SwissADME (Daina et al., 2017) and ADMETlab 2.0 were used to predict pharmacokinetic properties of the top docked phytocompounds, assessing:

- Lipinski's rule of five compliance
- Absorption and distribution
- Toxicity predictions (hepatotoxicity, mutagenicity)

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# RESULTS AND DISCUSSION

**Molecular docking results**

The results of molecular docking against the selected receptor are shown below as represented by the docking scores. The docking scores of the compounds range from -3.7 to -8.2.

**TABLE 3** Docking score of phytochemicals from *Hyptis Verticillata* with receptor

| **Ligands** | **Binding Affinity** |
|---|---|
| | **1CX2** |
| 3a.4,5,6,7,7a-hexahydro-4,7-methanoindene | -4.3 |
| 4,7- methanon-1H-indene | -4.3 |
| R-R,R-E- trans-Phytol | -5.3 |
| Squalene | -8.2 |
| 9,12,15-octadecatrien-1-ol | -5.2 |
| 1-octadecyne | -6.9 |
| 1-fluorodecane | -6.1 |
| Rofecoxib | -8.2 |
| Colecoxib | -3.7 |

**Drug-likeness screening result**

**TABLE 4** Drug-likeness screening result of phytocompounds from *Hyptis Verticillata*

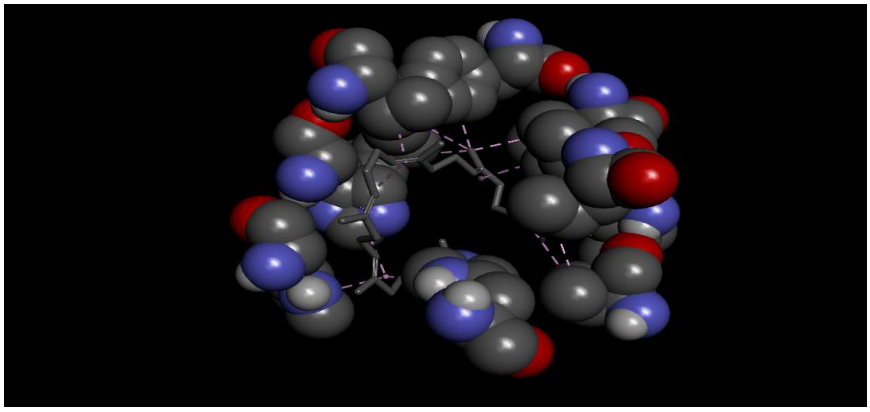
| Compounds | Lipinski | Ghose | Veber | Egan | Muegge | Remark |
|---|---|---|---|---|---|---|
| 3a.4,5,6,7,7a-hexahydro-4,7-methanoindene | Yes | No | Yes | Yes | No | No |
| 4,7- methanon-1H-indene | Yes | No | Yes | Yes | No | No |
| R-R,R-E- trans-Phytol | Yes | No | Yes | No | No | No |
| Squalene | Yes | No | Yes | No | No | No |
| 9,12,15-octadecatrien-1-ol | Yes | Yes | Yes | Yes | No | Passed |
| 1-octadecyne | Yes | No | No | No | No | No |
| 1-fluorodecane | Yes | Yes | Yes | Yes | No | Passed |

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**Evaluation of high binding affinity in comparison with selected REF drug**

**TABLE 5** Docking results of compound with high binding affinity with rofecoxib

| Ligands | Binding Affinity |
|---|---|
| Squalene | -8.2 |
| Rofecoxib (Referenced drug) | -8.2 |



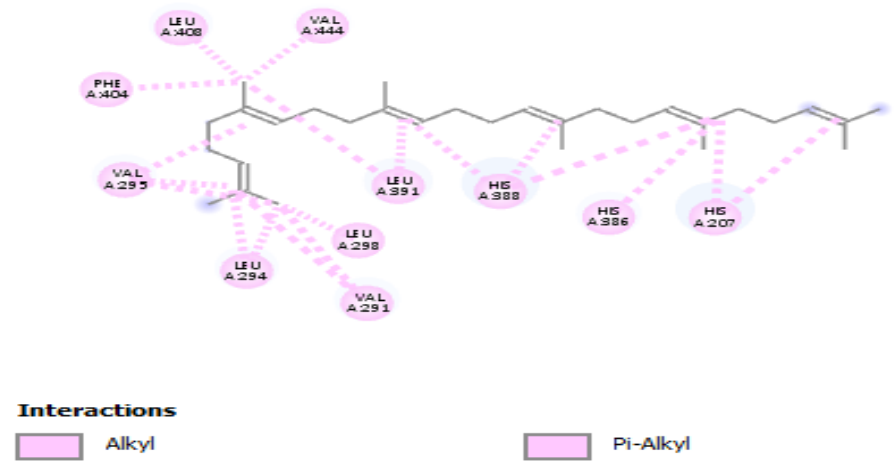
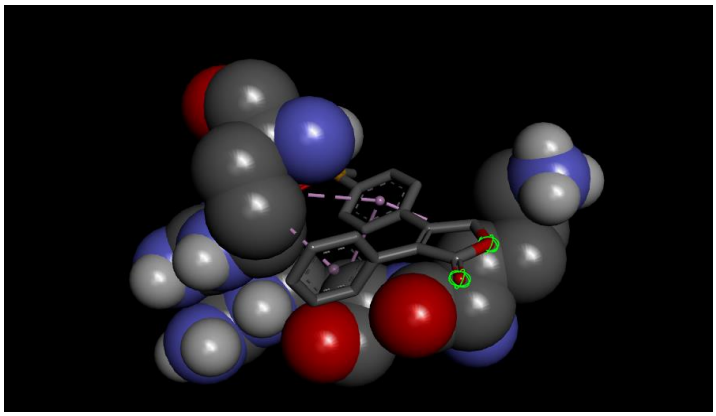



Figure 1. COX2 - Squalene interaction: (2D) & (3D) surface view

Schematic representation of main interaction of squalene interaction with COX2, purple color represents hydrophobic bond.



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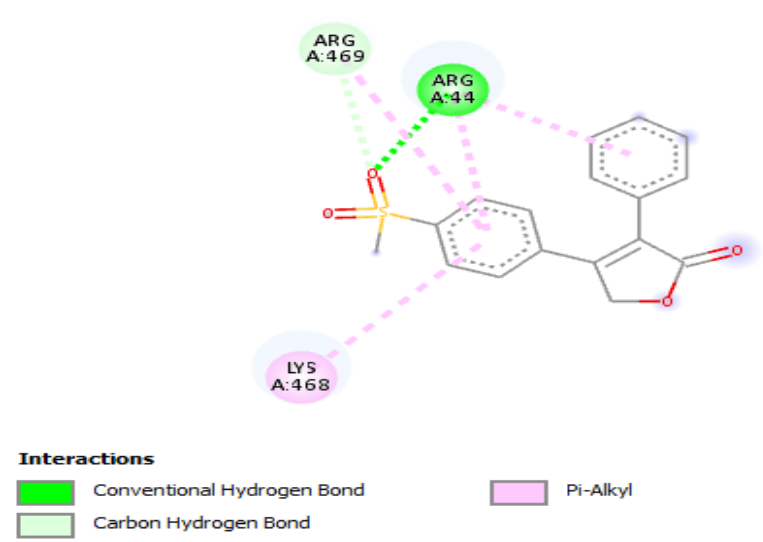



Figure 2. COX2 - Rofecoxib interaction: (2D) & (3D) surface view

Schematic representation of main interaction of rofecoxib squalene interaction with COX2, purple represents hydrophobic bond.

**ADMET RESULT**

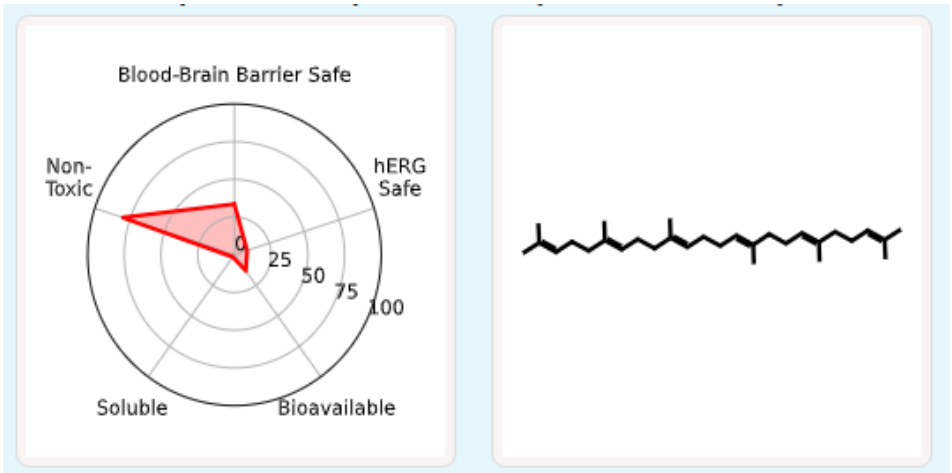



Figure 3. ADMET result of Squalene

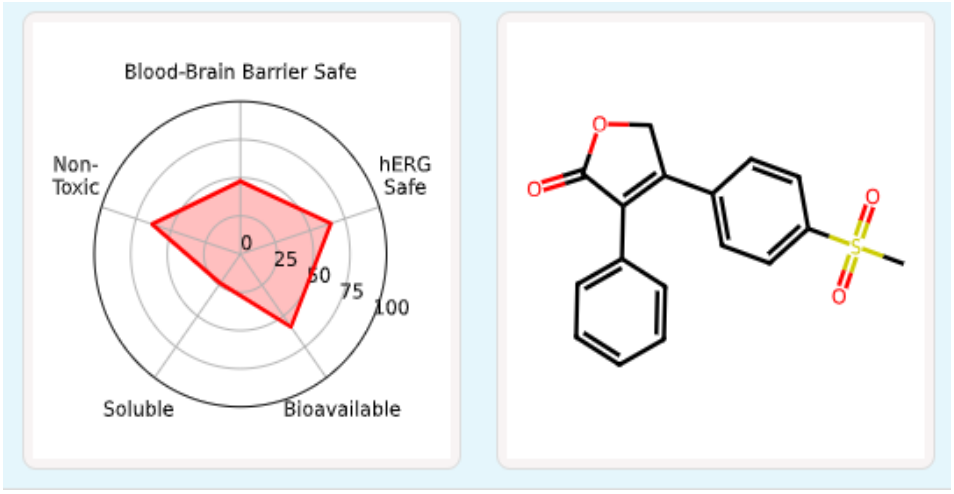



Figure 4. ADMET result of Rofecoxib

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# RESULTS AND DISCUSSION

## Docking Outputs

Molecular docking of seven *Hyptis verticillata* phytocompounds against the COX-2 binding site produced binding affinities ranging from −3.7 to −8.2 kcal/mol. The top-scoring ligand was squalene (−8.2 kcal/mol), which tied with the reference inhibitor rofecoxib (−8.2 kcal/mol). Intermediate scores were observed for 1-octadecyne (−6.9 kcal/mol) and 1-fluorodecane (−6.1 kcal/mol), whereas R-R,R-E-trans-phytol (−5.3 kcal/mol), 9,12,15-octadecatrien-1-ol (−5.2 kcal/mol), and the bicyclic indene derivatives (−4.3 kcal/mol) were lower. The draft table lists "Colecoxib" at −3.7 kcal/mol, which we interpret as celecoxib; this unexpectedly weak score is evaluated below in the context of known structural pharmacology (Kurumbail et al., 1996; Wang et al., 2010). The docking was performed using PDB 6COX, a murine COX-2 crystal structure co-crystallized with the selective inhibitor SC-558 at 2.8 Å resolution. Although 6COX is a widely used structural surrogate for human COX-2, the species difference should be kept in mind when interpreting absolute scores and residue-level contacts (Kurumbail et al., 1996).

## Rank Order and Comparison to Reference Drugs

Squalene matched rofecoxib (−8.2 vs −8.2 kcal/mol), placing both at the top of the ranking. This parity suggests that squalene can occupy the predominantly hydrophobic COX-2 cyclooxygenase channel with favorable van der Waals complementarity. However, docking energy alone is not a surrogate for drug potential because squalene violates several drug-likeness filters and lacks polar functionalities needed for specific interactions (Zarghi & Arfaei, 2011). The mid-tier ligands (1-octadecyne and 1-fluorodecane) performed better than oxygenated chains (phytol; octadecatrienol) and much better than the rigid bicyclic indenes, consistent with the notion that shape complementarity and lipophilicity drive affinity in the long, hydrophobic COX-2 channel. This trend aligns with the known structural pharmacology of COX-2, where a Val-523 substitution creates a side pocket that selectively accommodates bulkier, lipophilic motifs present in coxibs (Kurumbail et al., 1996).

The celecoxib score was unexpectedly weak relative to rofecoxib. Possible explanations include misassignment of the sulfonamide protonation state, grid misplacement, or the murine-human difference in binding pockets. Literature shows celecoxib consistently binds strongly in human COX-2 structures such as PDB 3LN1 (Wang et al., 2010).

## Binding-Mode Interpretation

Interaction depictions for squalene and rofecoxib show predominantly hydrophobic contacts, consistent with the non-polar interior of the COX-2 cyclooxygenase channel. Rofecoxib's sulfonyl-aryl motif engages the secondary pocket enabled by Val-523, a known determinant of selectivity (Kurumbail et al., 1996). Squalene, in contrast, relies on sheer hydrophobic surface coverage. The indenes scored poorly due to limited occupancy of the extended hydrophobic tunnel. Long aliphatic chains (e.g., octadecyne) align better but lack direct anchor points such as hydrogen bonds with Arg-120 and Tyr-355, residues often engaged by NSAIDs (Smith et al., 2000).

## Drug-Likeness and ADMET Analysis

Drug-likeness filtering showed that squalene passed Lipinski but failed multiple other filters, while smaller aliphatic molecules passed more rules. SwissADME predictions suggested squalene's poor solubility and GI absorption, whereas rofecoxib had more balanced oral drug properties but known cardiovascular risk (Daina et al., 2017; Mukherjee et al., 2001). This highlights that docking affinity must be complemented by pharmacokinetic evaluation.

## Practical Implications

- Squalene demonstrated high affinity but poor drug-likeness, making it unsuitable as a direct lead but useful as a chemotype inspiration.

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- Aliphatic hydrocarbons showed moderate potential and could be optimized through addition of polar anchor groups.

- Celecoxib and rofecoxib validate the docking system, though parameter refinement is necessary for accurate benchmarking (Zarghi & Arfaei, 2011).

## CONCLUSION

This study explored the molecular docking interactions of phytocompounds from *Hyptis verticillata* with cyclooxygenase-2 (COX-2) to assess their potential as natural anti-inflammatory agents. The docking analysis revealed that squalene exhibited the strongest binding affinity (−8.2 kcal/mol), comparable to the reference inhibitor rofecoxib, suggesting that hydrophobic terpenoids from *H. verticillata* can effectively occupy the COX-2 active site. Other compounds such as 1-octadecyne (−6.9 kcal/mol) and 1-fluorodecane (−6.1 kcal/mol) demonstrated moderate binding energies, while oxygenated derivatives and indenes showed weaker interactions.

Interaction profiling confirmed that hydrophobic contacts were the dominant stabilizing forces, consistent with the lipophilic architecture of the COX-2 catalytic tunnel. However, drug-likeness and ADMET analysis indicated that squalene, despite its strong binding affinity, may suffer from poor solubility and oral bioavailability, limiting its direct drug development potential. In contrast, smaller aliphatic compounds displayed more favorable pharmacokinetic properties but lower binding affinities, suggesting they could serve as starting points for optimization.

The study underscores the therapeutic promise of *H. verticillata* phytochemicals as potential COX-2 inhibitors while also highlighting the importance of balancing binding affinity with pharmacokinetic suitability. Overall, these findings provide a computational foundation for further structure-based drug design, molecular dynamics validation, and experimental studies to advance the development of safer plant-derived anti-inflammatory agents.

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