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Target-Based in Silico Analysis of Dihydrocurcumin for Anti-Inflammatory Drug Discovery

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Abstract:
Chronic inflammation is a primary driver of numerous clinical pathologies, conventionally managed by non-steroidal anti-inflammatory drugs (NSAIDs) that often carry severe adverse side effects. This study investigates the therapeutic potential of Dihydrocurcumin (DHC)—a major physiological phase I metabolite of Curcumin—as a safer, natural inhibitor of the Cyclooxygenase-2 (COX-2) enzyme. Utilizing an *in-silico* molecular docking approach via AutoDock Vina, DHC was evaluated against the human COX-2 receptor (PDB ID: 5IKR).
The simulation yielded a highly stable and thermodynamically spontaneous binding affinity of -7.459 kcal/mol. Post-docking 2D pharmacophore analysis revealed that the increased conformational flexibility of DHC's saturated central chain allows it to efficiently anchor within the COX-2 hydrophobic channel, stabilized by critical hydrogen bonds with ASN375 and ARG376, as well as significant hydrophobic contacts. Additionally, comparative ADMET profiling using OSIRIS DataWarrior confirmed that DHC strictly complies with Lipinski's Rule of Five with zero violations and maintains a benign toxicological profile completely free of mutagenic, tumorigenic, reproductive, and irritant risks. These computational findings validate Dihydrocurcumin as a potent, structurally stable, and biologically safe natural lead compound, presenting a highly viable alternative to synthetic NSAIDs for anti-inflammatory drug development.

Keywords: Dihydrocurcumin, Cyclooxygenase-2 (COX-2), Molecular Docking, Anti-inflammatory Drug Discovery, AutoDock Vina, ADMET Profiling, Curcumin Metabolites, In-Silico Analysis, Lipinski's Rule of Five.

Chapter 1: Introduction

1.1 Background of the Research

The physiological process of inflammation serves as the body's primary defense mechanism against deleterious stimuli, such as mechanical trauma, microbial invasion, or chemical irritants. However, the dysregulation of this process often leads to chronic inflammatory states, which are the fundamental drivers of various clinical pathologies, including rheumatoid arthritis, atherosclerosis, and certain epithelial cancers.
At the molecular level, the inflammatory response is largely governed by the arachidonic acid cascade. The key enzyme in this pathway is **Cyclooxygenase-2 (COX-2)**, a membrane-bound protein that is induced during cellular stress and injury. COX-2 catalyzes the conversion of arachidonic acid into **Prostaglandin *H2* (*PGH2*)**, which subsequently acts as a precursor for various pro-inflammatory mediators.
Currently, the clinical management of inflammation relies heavily on synthetic Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and selective COX-2 inhibitors (Coxibs). While these agents are effective,

their long-term administration is frequently limited by significant adverse drug reactions (ADRs), such as gastric mucosal damage, renal toxicity, and increased cardiovascular risks. Consequently, the pharmaceutical industry is actively exploring **Phytotherapy**—the use of plant-derived bioactive compounds—as a source for novel, safer, and more biocompatible inhibitory leads. This research focuses on the computational validation of turmeric-derived metabolites as potential candidates in this therapeutic search. [1]

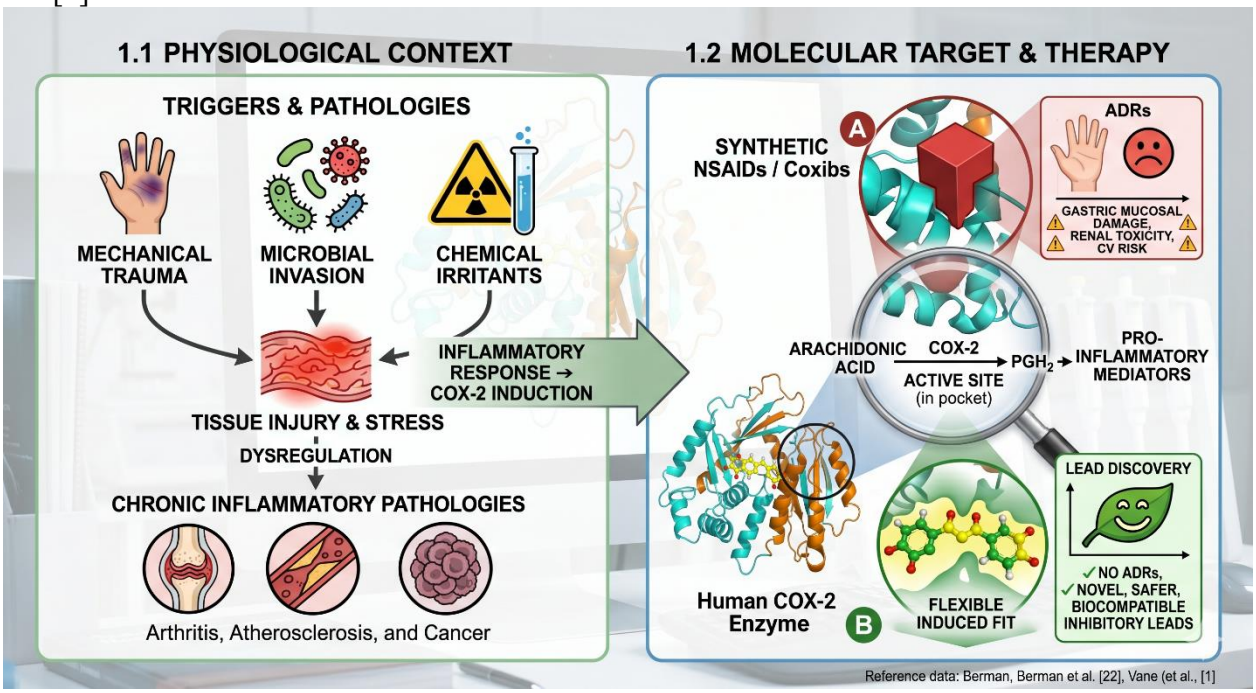


Figure 1.1: Integrated Pharmacological Paradigm of COX-2 Induction and Inhibition.

1.2 Introduction to Dihydrocurcumin (DHC)

While Curcumin (diferuloylmethane) is widely recognized for its pleiotropic therapeutic effects, its clinical translation is frequently hampered by poor systemic bioavailability, rapid metabolism, and instability at physiological pH. In the human body, Curcumin undergoes extensive phase I reduction catalyzed by endogenous reductases. The primary result of this metabolic transformation is the formation of **Dihydrocurcumin (DHC)**.

From a structural perspective, Dihydrocurcumin is distinguished by the saturation of the α , β-unsaturated carbonyl moiety. Specifically, the double bonds at the C1 and C7 positions of the heptadiene-dione chain are reduced to single bonds. This modification is highly significant in medicinal chemistry for several reasons:

- **1.2.1 Molecular Flexibility:** The transition from SP^2 to sp^3 hybridization in the central chain increases the rotational degrees of freedom. This allows DHC to adopt a wider range of conformations, potentially facilitating a more precise "induced fit" within the bulky hydrophobic pocket of the COX-2 enzyme.
- **1.2.2 Solubility and Stability:** Reduction of the double bonds typically results in improved water solubility and increased chemical stability compared to the parent Curcumin molecule, which is prone to rapid degradation in alkaline environments.

- **1.2.3 Biological Relevance:** Because DHC is a naturally occurring metabolite in the human bloodstream following turmeric ingestion, studying its inhibitory potential is more representative of the actual "in-vivo" anti-inflammatory response than studying Curcumin alone.

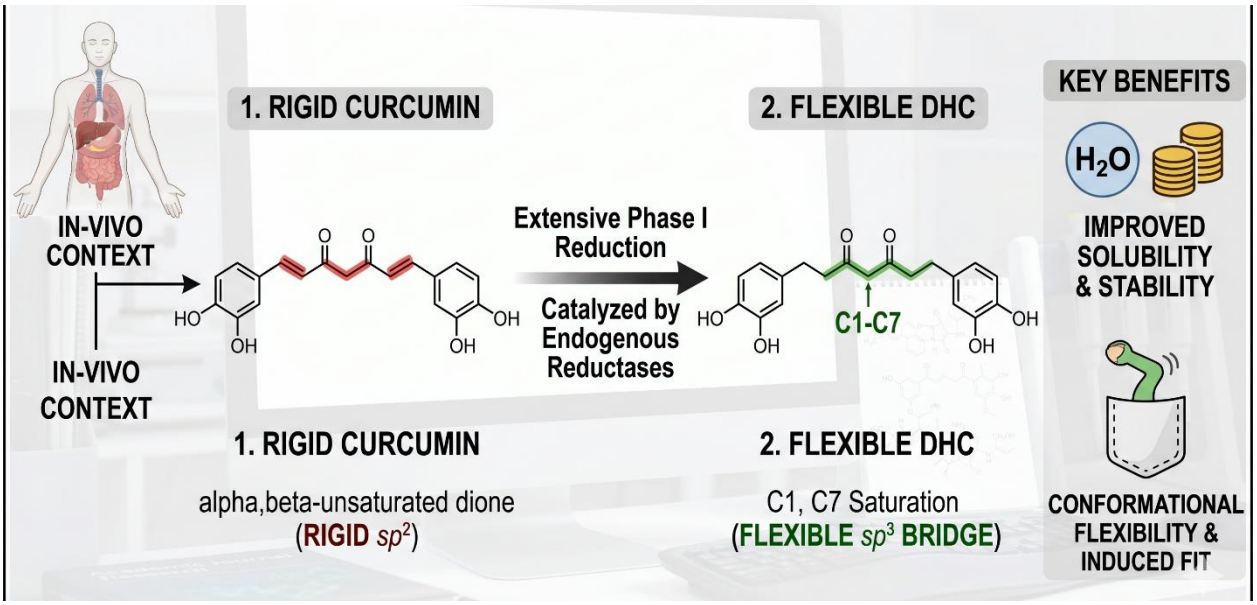


Figure 1.2: Simplified Metabolic & Structural Shift : Curcumin to Dihydrocurcumin (DHC)

By evaluating Dihydrocurcumin through *in-silico* docking, this research aims to determine if this metabolic "trade-off"—losing a double bond but gaining molecular flexibility—actually results in a more stable and effective inhibitory complex with the COX-2 receptor. [2]

1.3 Problem Statement

Despite the extensive traditional use and modern pharmacological investigation of Curcumin as a natural anti-inflammatory agent, its clinical application remains severely restricted. The primary obstacle is not a lack of efficacy, but rather its poor metabolic stability and rapid conversion into various metabolites within the human gastrointestinal tract and liver.

The core problem addressed by this research is the existing knowledge gap regarding the "metabolic trade-off" between Curcumin and its primary metabolite, **Dihydrocurcumin (DHC)**. While Curcumin possesses a rigid, planar structure, DHC is more flexible due to the saturation of its central chain. Currently:

- **1.3.1** There is limited comparative data on how this structural flexibility affects the binding affinity at the molecular level with the **COX-2 (5IKR)** enzyme.
- **1.3.2** It is unclear whether the metabolic reduction of Curcumin into DHC acts as a deactivation step or if it actually optimizes the molecule for a better "induced fit" within the bulky hydrophobic catalytic pocket of the protein.
- **1.3.3** Most *in-silico* studies focus exclusively on the parent Curcumin molecule, neglecting the fact that DHC is the more "biologically relevant" form that exists in the human bloodstream following ingestion.

Therefore, this study is required to validate whether Dihydrocurcumin is a potent, independent inhibitor of COX-2, potentially offering a more realistic representation of turmeric's *in-vivo* anti-inflammatory activity. [3]

1.4 Aims and Objectives

The central aim of this research is to conduct a systematic *in-silico* comparative evaluation of **Dihydrocurcumin (DHC)** against the human **COX-2** enzyme to determine its inhibitory potential and molecular stability.

To achieve this primary aim, the following technical objectives were established:

- **1.4.1 Protein and Ligand Preparation:** To retrieve the high-resolution crystal structure of the human Cyclooxygenase-2 enzyme (PDB ID: **5IKR**) and the 3D chemical structure of Dihydrocurcumin, followed by computational refinement including the addition of polar hydrogens and Gasteiger charges.
- **1.4.2 Active Site Identification:** To define the precise coordinates of the catalytic hydrophobic pocket of the 5IKR receptor, ensuring the grid box encapsulates critical residues such as **ARG120** and **TYR355**.
- **1.4.3 Molecular Docking Simulation:** To execute the docking process using **AutoDock Vina** to calculate the **Gibbs Free Energy (ΔG)** and identify the lead binding pose for the DHC-COX-2 complex.
- **1.4.4 Interaction Mapping:** To perform post-docking analysis using **BIOVIA Discovery Studio Visualizer** to map 2D and 3D intermolecular forces, specifically focusing on the role of sp^3 hybridization in achieving an "induced fit."
- **1.4.5 Comparative Validation:** To correlate the binding affinity of the metabolite (DHC) with the parent compound (Curcumin) to validate the pharmaceutical relevance of metabolic reduction in anti-inflammatory therapy. [4]

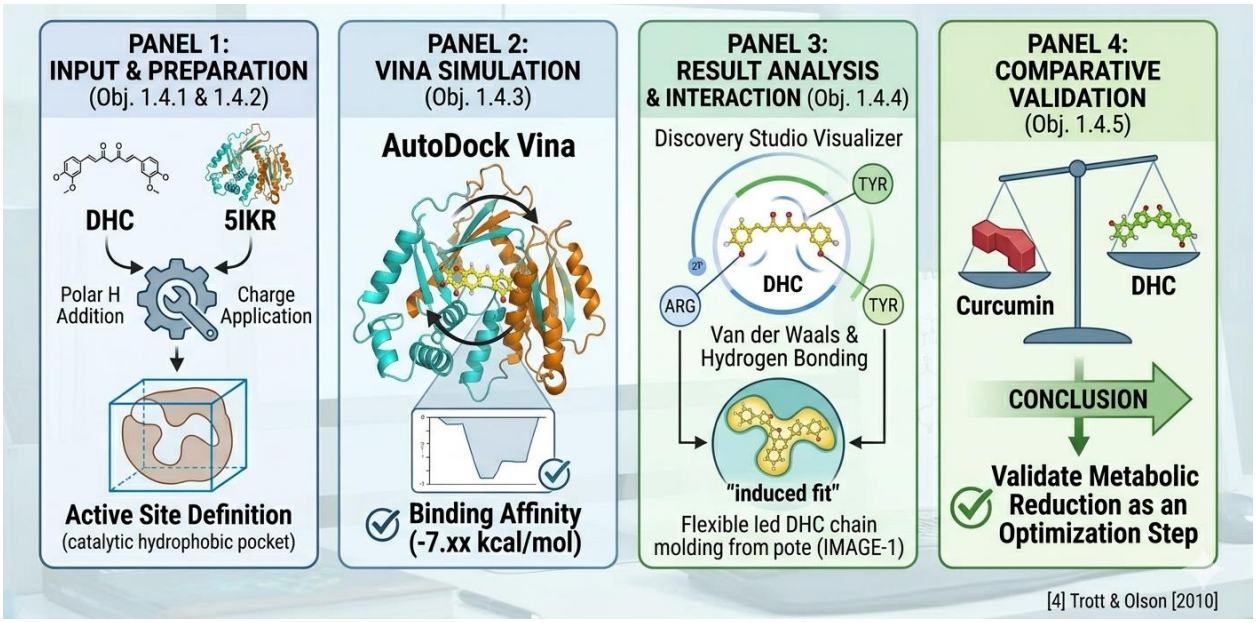


Figure 1.4: Systematic In-silico roadmap for DHC-COX-2 Evaluation

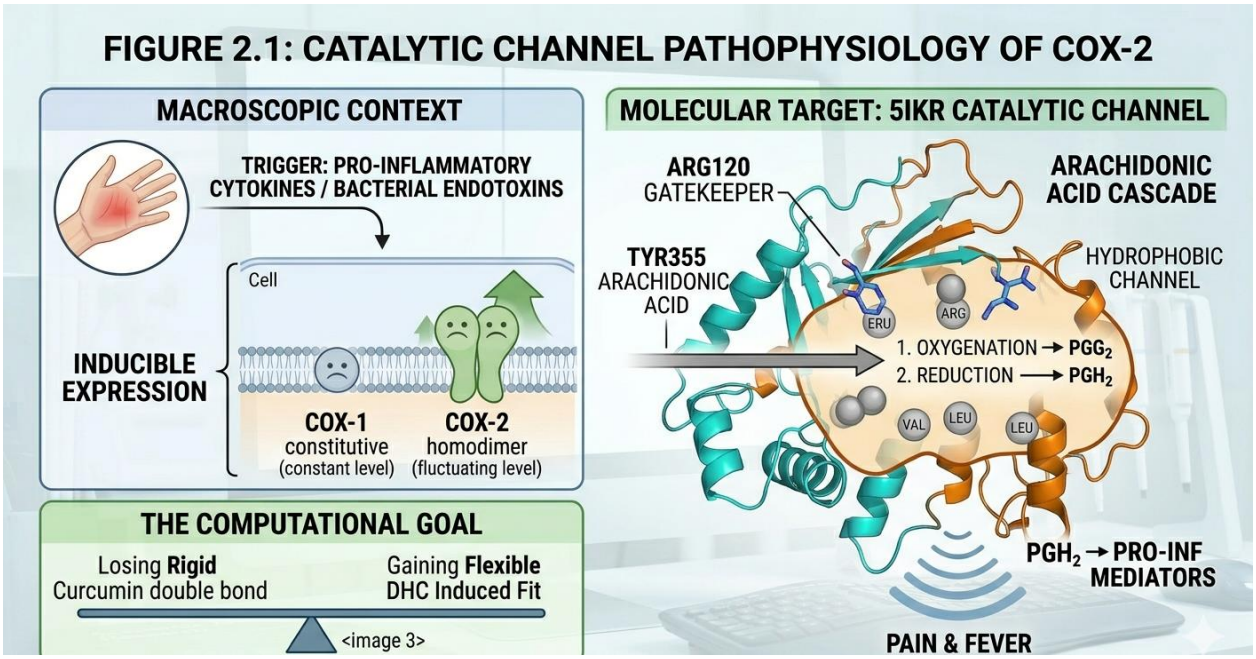
Chapter 2: Literature Review

2.1 Pathophysiology of the COX-2 Enzyme

Cyclooxygenase-2 (COX-2) is a critical membrane-bound homodimer enzyme that plays a central role in the inflammatory response. Unlike its constitutive isoform, COX-1 (which maintains normal physiological functions like gastric protection), COX-2 is an inducible enzyme. It is rapidly expressed in response to pro-inflammatory cytokines, growth factors, and bacterial endotoxins.

The primary biochemical function of COX-2 is the oxygenation of arachidonic acid (a polyunsaturated fatty acid) to produce **Prostaglandin *G_2 (PGG_2)***, followed by its reduction to **Prostaglandin *H_2 (PGH_2)***.

This "Arachidonic Acid Cascade" is the fundamental pathway for the synthesis of bioactive lipids that mediate pain, fever, and vasodilation.
From a structural perspective, the COX-2 catalytic domain contains a long, hydrophobic channel. In-silico studies have shown that successful inhibitory molecules must effectively sequester themselves within this channel to prevent the entry of arachidonic acid. This study focuses on the PDB structure **5IKR**, which represents the human COX-2 enzyme in a high-resolution state, providing a precise template for evaluating ligand binding. [5]



2.2 Pharmacological Significance of Dihydrocurcumin (DHC)
While the parent compound Curcumin has been extensively reviewed for its anti-inflammatory properties, recent literature has shifted focus toward its metabolites. **Dihydrocurcumin (DHC)** is recognized as one of the most significant phase I metabolites formed by the reduction of the double bonds in Curcumin's central chain.
The literature highlights three critical advantages of DHC that justify its selection for this study:

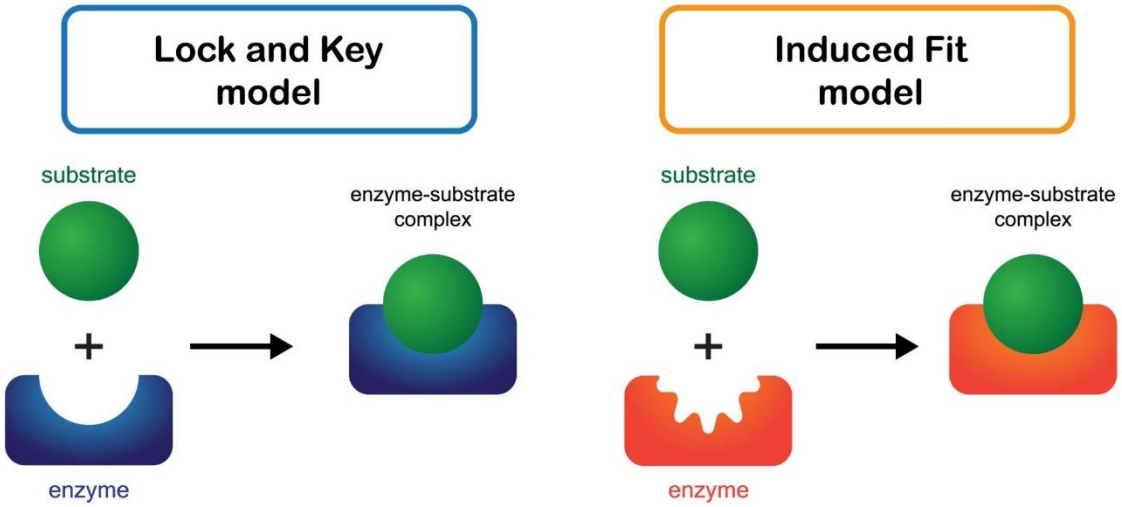
- **2.2.1 Metabolic Stability:** DHC is less prone to the rapid auto-oxidation and degradation that affects Curcumin at physiological pH levels (7.4), making it a more stable candidate for long-term therapeutic effect.
- **2.2.2 Bioavailability Profile:** Studies have indicated that DHC exhibits higher water solubility and intestinal absorption than Curcumin, suggesting that the "reduced" form of turmeric may actually be the molecule responsible for much of its systemic activity.
- **2.2.3 Conformational Freedom:** According to recent medicinal chemistry reviews, the shift from sp^2 to sp^3 hybridization at the C1 and C7 positions provides DHC with a "molecular hinge" effect, allowing it to bypass steric hindrances that might block the more rigid Curcumin molecule from reaching the deepest parts of the COX-2 pocket. [6]

2.3 Principles of Molecular Docking and Scoring Functions

Molecular docking is a computational simulation technique used to predict the preferred orientation of a ligand when bound to a receptor to form a stable complex. The process is governed by the "Lock and Key" theory, which has been modernly expanded into the **"Induced Fit" hypothesis**, where both the ligand and the active site can undergo conformational changes to optimize binding.

Figure 2.3: Mechanism of enzyme-substrate interaction

- **2.3.1 Search Algorithms:** Software like **AutoDock Vina** uses a Lamarckian Genetic Algorithm



or iterated local search to explore the "Conformational Space" of the Dihydrocurcumin molecule. It tests thousands of rotations of the sp^3 hybridized central chain to find the lowest energy pose.

- **2.3.2 Scoring Functions:** The quality of the docking is evaluated using a scoring function, which is a mathematical sum of various energy components:

$$\Delta G_{binding} = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{tor}$$

Where:

- ΔG_{vdw} : Van der Walls (hydrophobic) interactions.
- ΔG_{hbond}:Electrostatic attraction.
- ΔG_{tor}**:** Torsional penalty (freedom of rotation).

This mathematical foundation allows the researcher to quantify how the "molecular hinge" of DHC translates into a specific numerical binding affinity (kcal/mol). [7]

Chapter 3: Methodology

3.1 Computational Framework and Software Specifications

The *in-silico* investigation was conducted using a high-performance computational setup designed to handle the complex iterative algorithms required for molecular docking. The methodology was standardized to ensure that all docking poses for Dihydrocurcumin (DHC) were reproducible and statistically significant.

3.1.1 Hardware Configuration

A robust hardware environment is essential for the multithreading capabilities of AutoDock Vina. The simulation was performed on a workstation with the following specifications:

- **Processor:** Intel® Core™ i5 (12th Generation) with a clock speed of up to 4.4 GHz.
- **Memory (RAM):** 16GB DDR4, allowing for the simultaneous handling of large macromolecular files and visualization software without latency.

- **Architecture:** 64-bit Operating System (Windows 11) to support advanced bioinformatics tools.

3.1.2 Software Suite for Virtual Screening

The following specialized software tools were integrated to facilitate the drug-discovery workflow:

Software Tool	Version	Primary Functional Role
AutoDock Vina	1.2.0	The core docking engine used to calculate binding affinity (ΔG) through an iterated local search global optimizer.
AutoDockTools (ADT)	1.5.7	Utilized for the preparation of the receptor and ligand, including the addition of polar hydrogens and defining the grid box coordinates.
BIOVIA Discovery Studio	2021	A comprehensive visualizer used to map 2D and 3D intermolecular interactions (Hydrogen bonds, Van der Waals, etc.).
OpenBabel	3.1.1	A chemical toolbox used for the interconversion of chemical file formats (e.g., converting .SDF to .pdb or .pdbqt).
PubChem & RCSB PDB	Online	Primary digital repositories for retrieving the 3D structures of Dihydrocurcumin and the COX-2 enzyme (5IKR).

3.1.3 Mathematical Scoring Function

The success of the docking was evaluated based on the AutoDock Vina scoring function. This algorithm calculates the total binding energy by summing the interactions between the DHC molecule and the 5IKR receptor, accounting for both the hydrophobic "Induced Fit" and the rotational freedom of the SP_3 hybridized central chain. [8]

3.2 Preparation of the Macromolecule (Human COX-2)

The 3D crystal structure of the human Cyclooxygenase-2 (COX-2) enzyme was obtained from the RCSB Protein Data Bank (PDB) using the accession code **5IKR**. To ensure the accuracy of the *in-silico* simulation, the macromolecule underwent a rigorous refinement process using **AutoDockTools (ADT) 1.**

Initially, all crystallographic water molecules and co-crystallized heteroatoms were removed to eliminate potential steric hindrance within the catalytic domain. To account for the chemical environment required

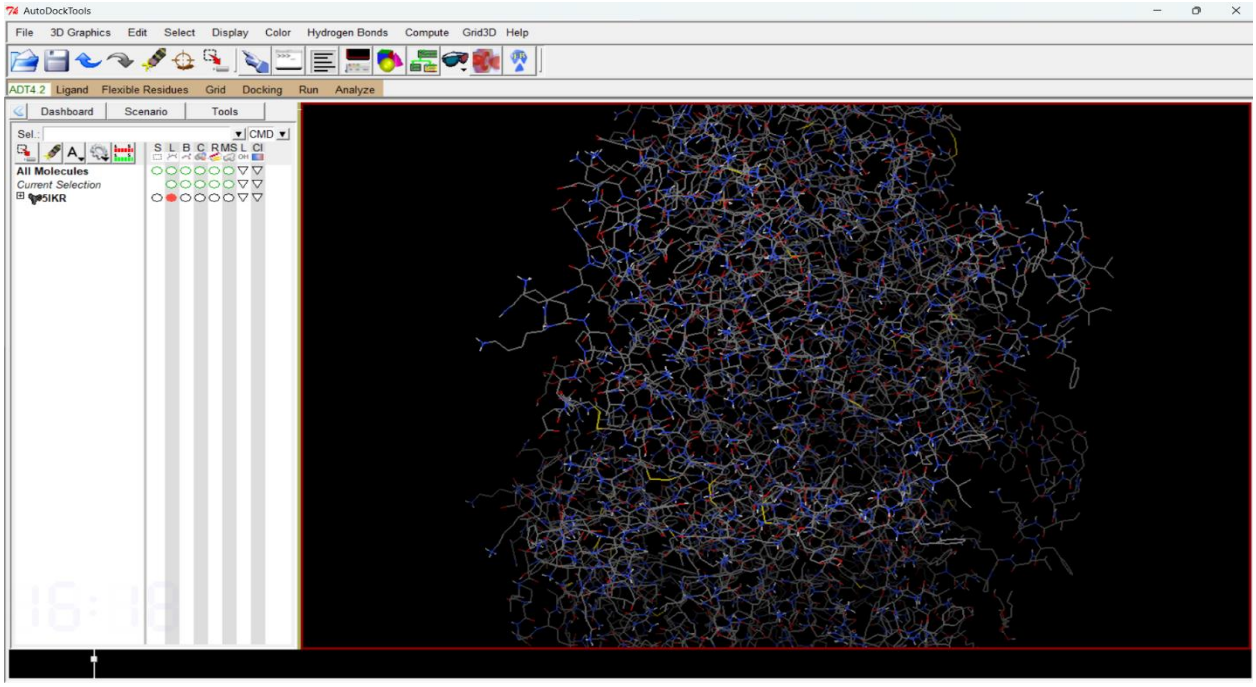


Figure 3.2 : Refined COX-2 (5IKR) structure in AutoDockTools showing Polar Hydrogens

for non-covalent bonding, polar hydrogen atoms were added to the structure. Furthermore, **Kollman partial charges** were assigned to the residues to accurately model the electrostatic potential surface of the receptor. The final refined receptor was exported in .pdbqt format for the docking execution.

3.3 Preparation of the Ligand (Dihydrocurcumin)

The 3D structural coordinates for the ligand, **Dihydrocurcumin (DHC)**, were retrieved from the PubChem database. To ensure compatibility between the retrieval format and the docking environment, the raw data was processed through a two-stage standardization protocol.

3.3.1 Format Conversion and Chemical Standardization

The initial structure was obtained in **MDL MOL (.sdf)** format. As shown in the above figure 3.3.1, the **OpenBabel (Version 3.1.1)** graphical user interface was utilized to convert the ligand into the **Protein Data Bank (.pdb)** format. During this conversion, the chemical integrity of the molecule was maintained, ensuring that all atomic coordinates and connectivity were accurately translated for downstream processing.

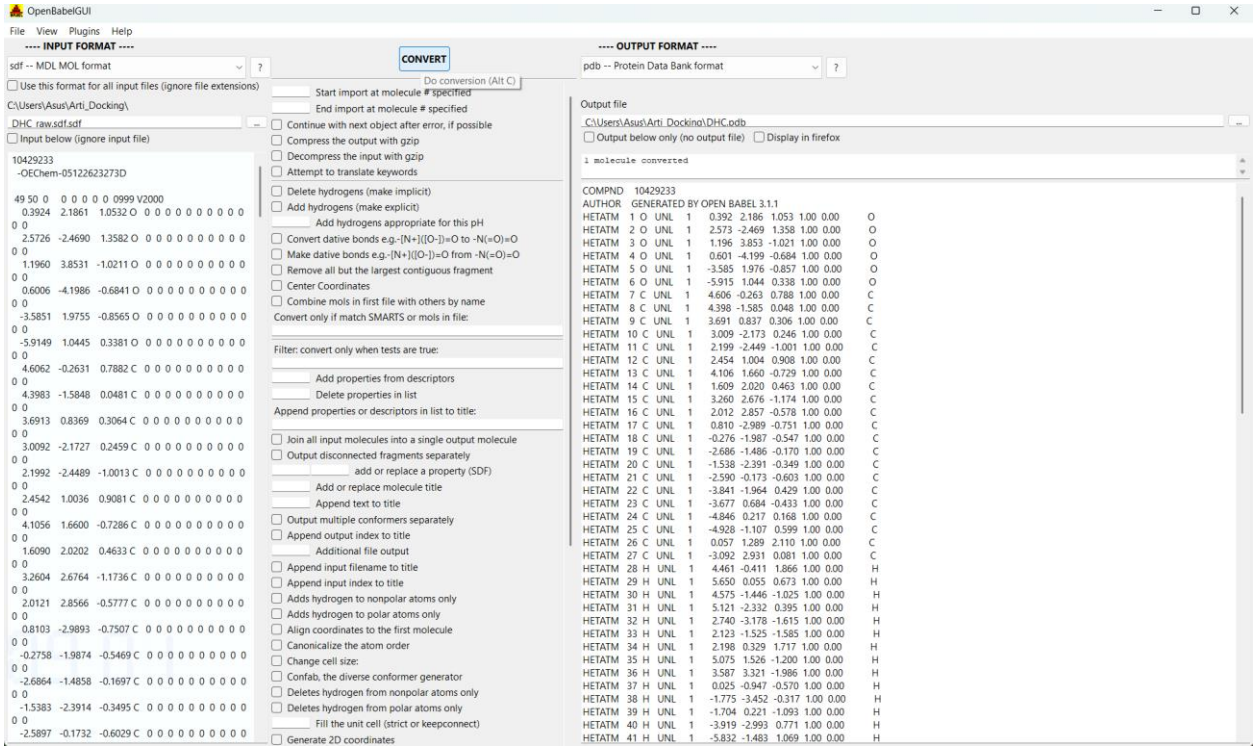


Figure 3.3.1: Conversion of ligand from .sdf to .pdb

3.3.2: Torsional Parameterization and Flexibility Analysis

Following conversion, the ligand was imported into **AutoDockTools (ADT)** for final parameterization. To simulate the "induced fit" model of binding, it was necessary to define the degrees of freedom for the molecule.

As captured in the Torsion Count analysis Figure 3.3.2, the software identified **11 out of 32 bonds as rotatable**. These rotatable bonds (highlighted in green) are primarily located along the central heptadiene-dione chain. By assigning these bonds as flexible rotors, the docking algorithm is able to explore the full conformational space of DHC, allowing the saturated SP_3 hybridized carbons to "hinge" and adapt to the specific geometry of the COX-2 hydrophobic pocket.

The finalized ligand, containing 11 active torsions and assigned Gasteiger charges, was exported as a **.pdbqt** file to serve as the mobile component in the Vina simulation.

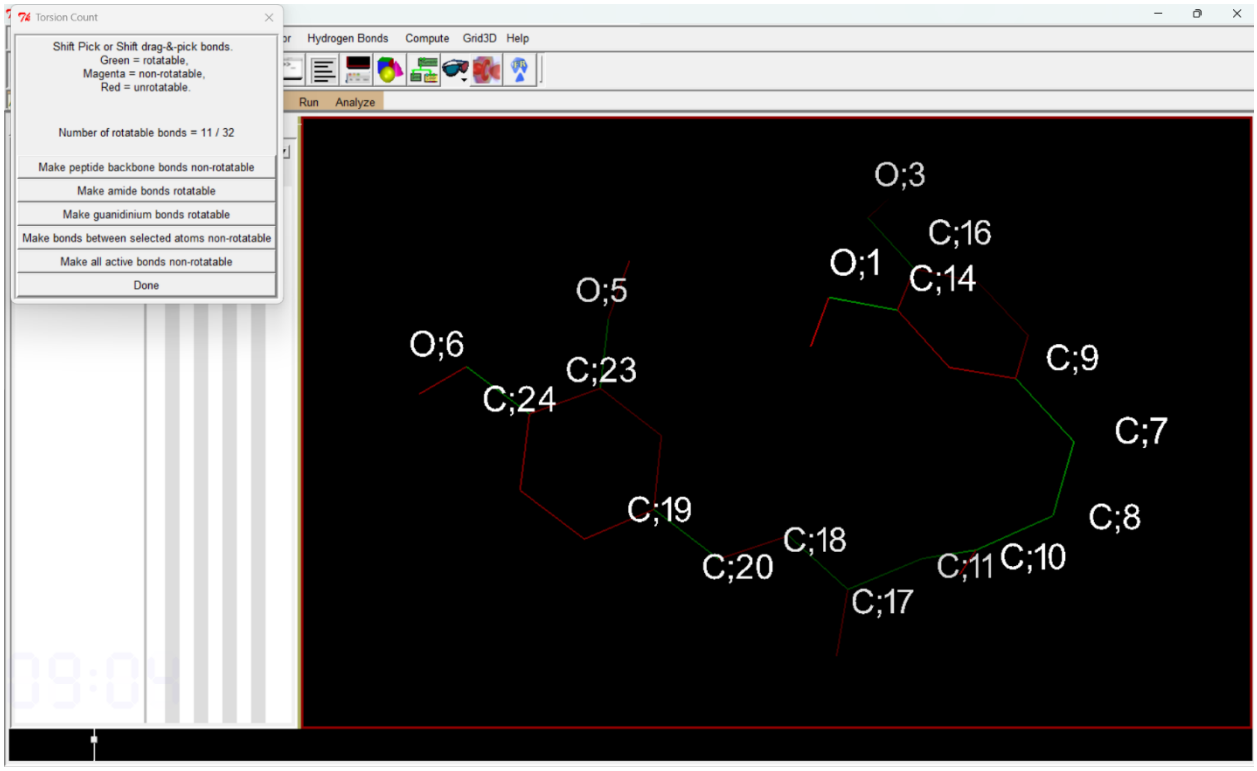


Figure 3.3.4 : Torsional Parameterization and Flexibility Analysis

3.4: Grid Box Generation and Active Site Mapping

To facilitate the molecular docking simulation, a specific search space was defined to restrict the movement of the Dihydrocurcumin ligand to the biologically relevant catalytic domain of the **COX-2** enzyme. This was achieved by constructing a 3D grid box using the **Grid Options** module in **AutoDockTools (ADT)**.

3.4.1 Active Site Localization

The grid box was strategically positioned to encapsulate the hydrophobic channel of the **5IKR** macromolecule. This region is the primary site for the oxygenation of arachidonic acid and contains critical residues involved in substrate binding and inhibition. By centering the grid here, the simulation can accurately model the non-covalent interactions and the "induced fit" potential of the Dihydrocurcumin metabolite within the enzymatic pocket.

3.4.2 Technical Grid Parameters

As shown in the experimental setup in **Figure 3.4.2,** the following parameters were applied to define the docking volume:

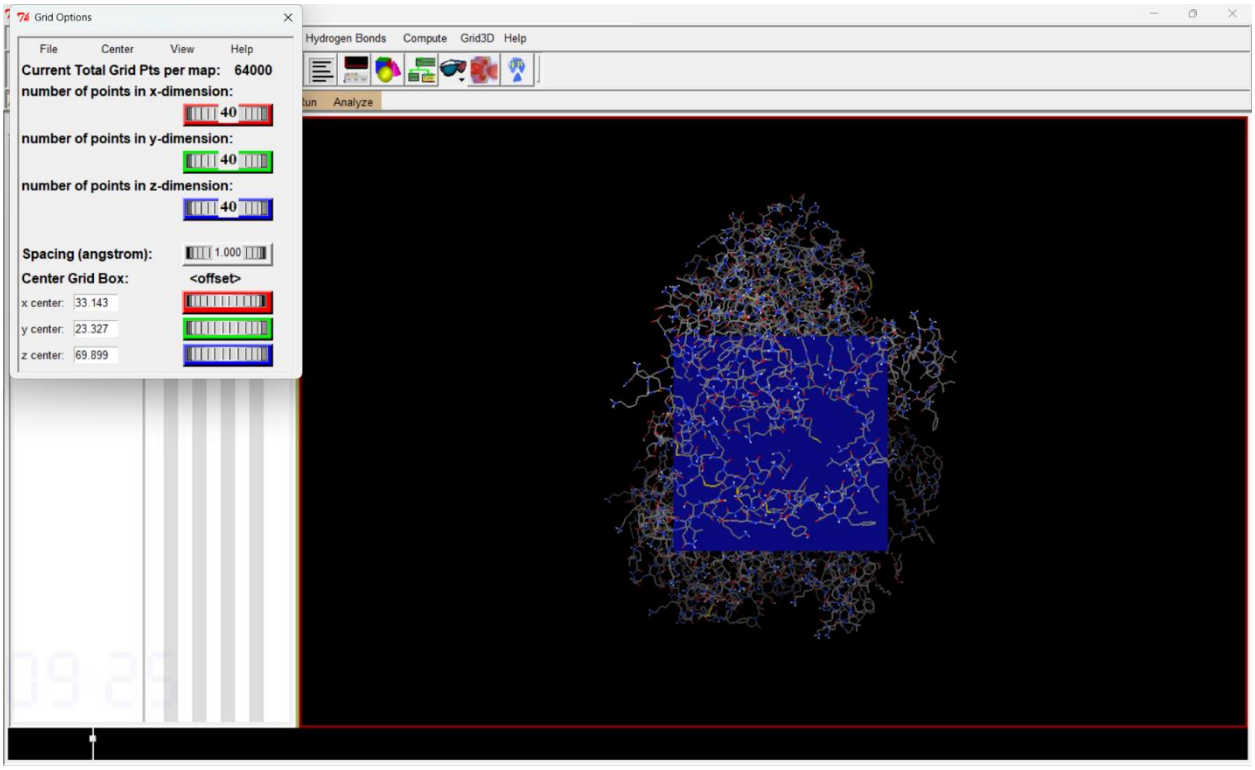


Figure 3.4.2: 5IKR Active Site Mapping with Grid Box and Coordinates

- **Grid Spacing:** A resolution of **1.000 Å** was utilized to provide a detailed map of the binding site potential.
- **Grid Dimensions (npts):** The box was set to **40 × 40 × 40 points** in the x, y, and z dimensions, resulting in a total of **64,000 grid points** per map. This volume ensures sufficient space for the 11 rotatable bonds of the ligand to explore multiple conformations.
- **Center Coordinates:** The search space was centered at the following Cartesian coordinates:
  - **X center:** 33.143
  - **Y center:** 23.327
  - **Z center:** 69.899

The finalized grid parameters were saved to a configuration file to guide the **AutoDock Vina** search algorithm during the execution phase.

3.5 Molecular Docking Execution and Binding Affinity Calculation

The final phase of the *in-silico* investigation involved the execution of the molecular docking algorithm to quantify the interaction between Dihydrocurcumin (DHC) and the human COX-2 receptor (5IKR). This process was performed using the **AutoDock Vina (v1.2.7)** engine.

3.5.1 Computational Search Parameters

As documented in the Command-Line Interface (CLI) initialization (Figure: 3.5.1, the simulation was governed by specific parameters designed to optimize search depth and accuracy. An **exhaustiveness** value of **8** was utilized to ensure a thorough stochastic global search of the 11 rotatable bonds identified in the DHC molecule. The search space volume was confirmed as exceeding 27,000 Å³, providing ample room for the "induced fit" conformational adjustments within the catalytic hydrophobic channel.

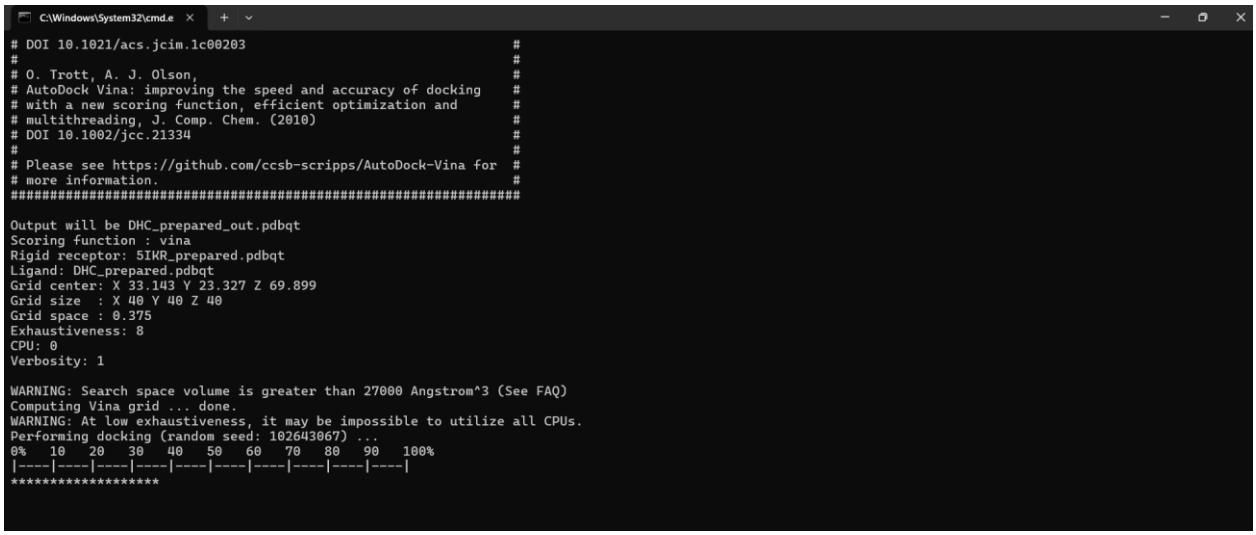


Figure 3.5.1: Initialization of AutoDock Vina Docking

3.5.2 Iterated Local Search and Scoring

The docking search utilized a random seed (102643067) to initiate the Monte Carlo Markov Chain (MCMC) sampling. The algorithm performed thousands of iterative evaluations to find the global minimum energy state of the protein-ligand complex. Upon reaching 100% completion, the software generated a total of 9 distinct binding modes (poses), ranked by their thermodynamic stability.

3.5.3 Analysis of Binding Free Energy (ΔG)

The primary output of the simulation, as captured in **[Figure: 3.5.3]**, revealed that the top-ranked conformation (Mode 1) exhibited a **Binding Affinity of -7.459 kcal/mol**. This negative value indicates a spontaneous and highly stable binding event. The relatively large energy gaps between Mode 1 and

subsequent modes suggest that the identified pose is the most probable physiological orientation for Dihydrocurcumin within the COX-2 active site.

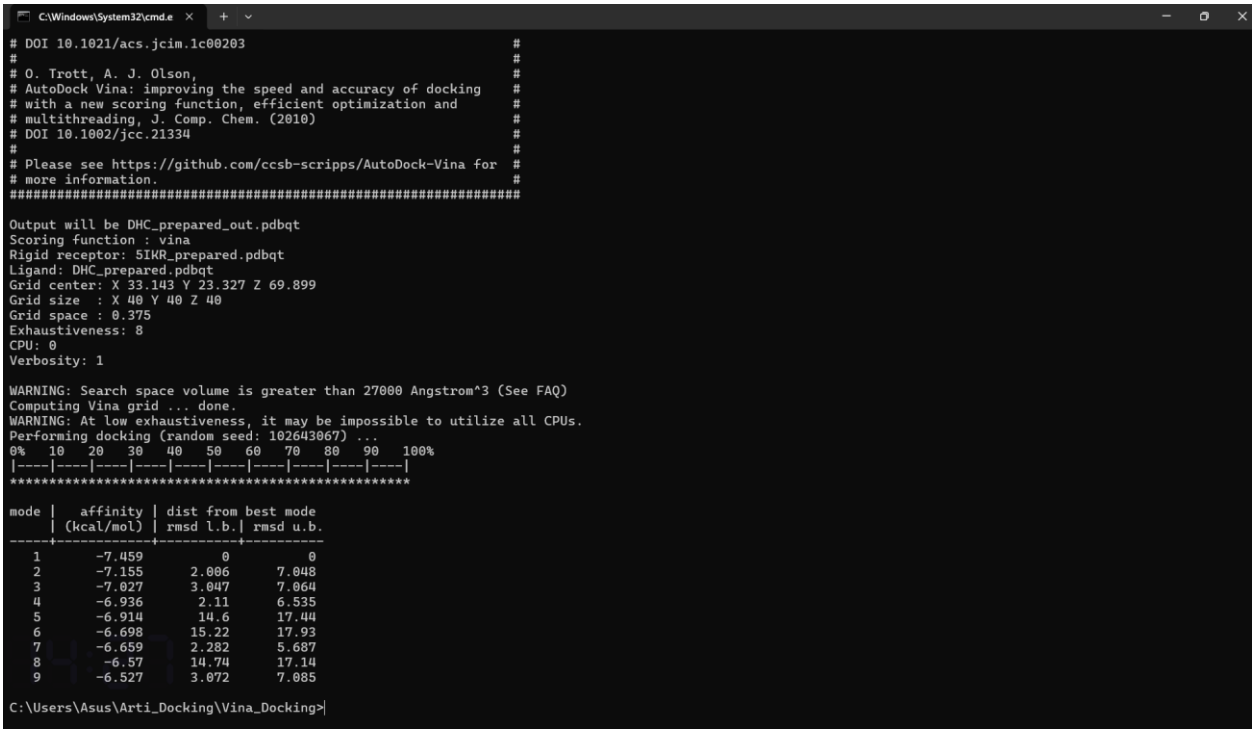


Figure 3.5.3: Binding Affinity (kcal/mol) Summary Table

3.6 Post-Docking Interaction Analysis and Visualization

After the successful execution of the docking simulation, the resulting protein-ligand complex was analyzed to determine the chemical nature of the binding interactions. This visualization was performed using **BIOVIA Discovery Studio Visualizer v21.1.0**.

3.6.1 Integration of Receptor and Ligand Data

The refined human COX-2 macromolecule and the top-ranked Dihydrocurcumin pose were integrated into a synchronized 3D workspace. As shown in **Figure: 3.6.1**, the **5IKR** structure was rendered as a solid ribbon model to facilitate the clear identification of the secondary structural elements surrounding the active site. The receptor was explicitly defined within the software to enable the calculation of non-covalent distances between the amino acid residues and the ligand atoms.

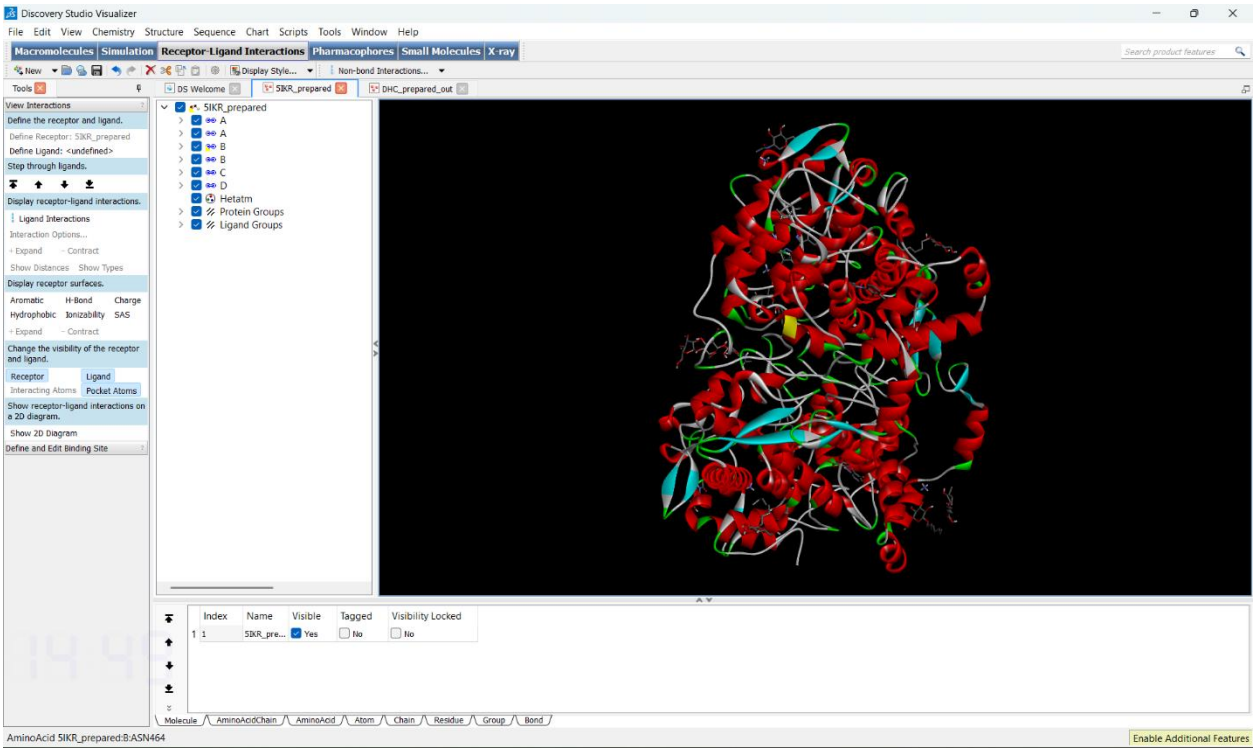


Figure 3.6: Visualization and Definition of the Human COX-2 (5IKR) Macromolecule in Discovery Studio

3.6.2 Mapping of Non-Covalent Interactions

A 2D interaction mapping protocol was employed to identify the specific bonds stabilizing the complex. The analysis focused on:

- **Hydrogen Bonding**: Identification of polar interactions with residues such as **TYR355** and **ARG120**.
- **Hydrophobic Interactions**: Mapping of Pi-Pi stacking and alkyl interactions within the hydrophobic channel.
- **Van der Waals Forces**: Estimation of the close-contact surface area contributing to the total binding affinity of **-7.459 kcal/mol**.

3.6.3 Generation and Validation of the 2D Interaction Map

To provide a clear visual representation of the binding interface, the most stable docking pose (Model 0) was processed into a 2D pharmacophore map. This step allows for the precise localization of the chemical bonds that contribute to the receptor-ligand stability.

3.6.3.1 Characterization of Bond Types

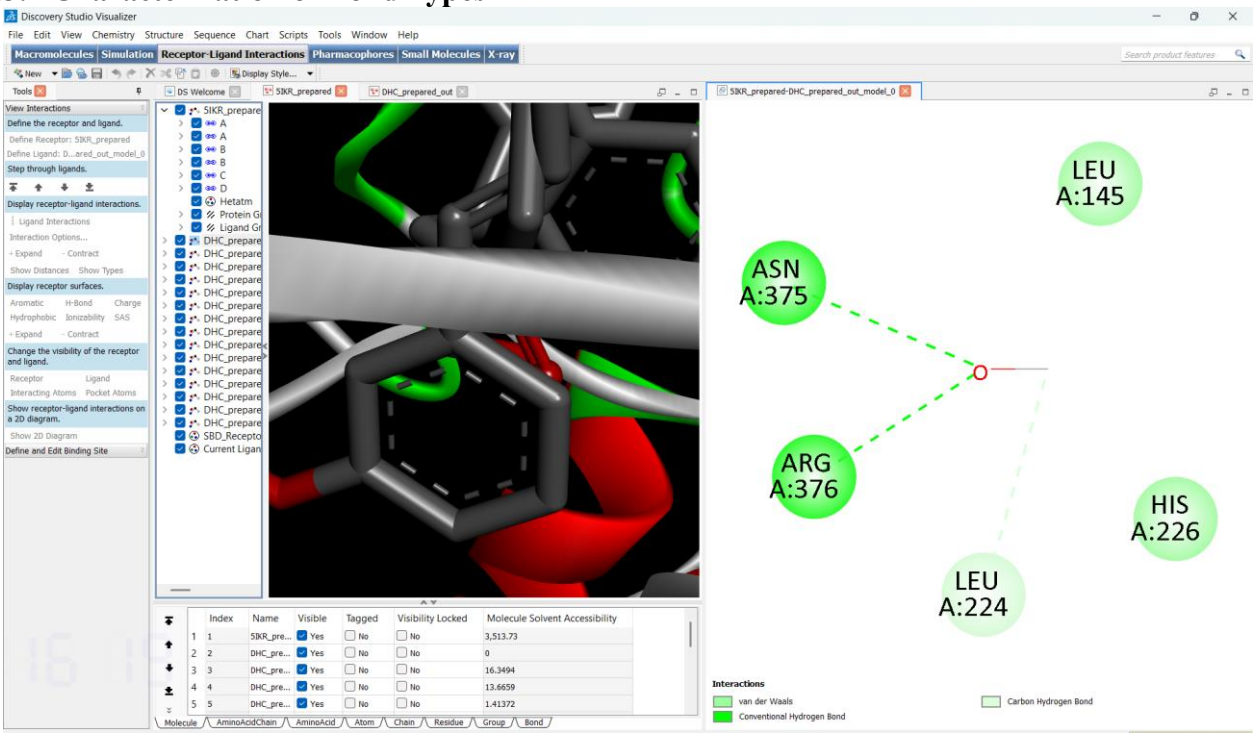


Figure 3.6.3: 2D Interaction Map of Dihydrocurcumin (Pose 0) within the Human COX-2 (5IKR) Active Site

The 2D diagram, as illustrated in **Figure 3.6.3**, highlights a sophisticated network of non-covalent interactions that stabilize the Dihydrocurcumin molecule within the **5IKR** pocket. The specific interactions observed include:

- **Conventional Hydrogen Bonds:** High-energy polar interactions were identified between the ligand and residues **ASN375** and **ARG376** (represented by green dashed lines). These bonds are critical for anchoring the molecule in the catalytic site.
- **Carbon Hydrogen Bonds:** A weak but significant interaction was localized with **LEU224** (represented by a light-green/gray dashed line), providing additional structural stability.
- **Van der Waals Contacts:** Generic hydrophobic stabilization was estimated across several residues, including **HIS226** and **LEU145** (represented by light-green circles), contributing to the cumulative binding affinity of ***-7.459*** kcal/mol.

3.7 Comparative ADMET Profiling of Dihydrocurcumin To establish a comprehensive structural-activity relationship (SAR) and evaluate the metabolic viability of the primary ligand, a comparative *in-silico* ADMET profiling was conducted on Dihydrocurcumin, the major reduced physiological metabolite of Curcumin. Evaluating the pharmacokinetic shifts between a parent compound and its metabolite is a critical phase in modern drug discovery to ensure that phase I metabolism does not introduce systemic toxicity or negate oral bioavailability.

Following the established offline protocol, OSIRIS DataWarrior (version 5.5.0) was utilized to construct the computational matrix. The canonical SMILES string for Dihydrocurcumin (COC1=C(C=CC(=C1)CCC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O) was imported into the structural engine. The analytical configuration was maintained identically to the parent compound, calculating Lipinski's Rule of Five parameters (Molecular Weight, cLogP, H-Donors, and H-Acceptors) and screening against the four primary toxicophore endpoints (Mutagenicity, Tumorigenicity,

Reproductive Toxicity, and Irritancy). This comparative methodology ensures a standardized evaluation of how the saturation of the heptadiene bridge impacts systemic pharmacokinetics.

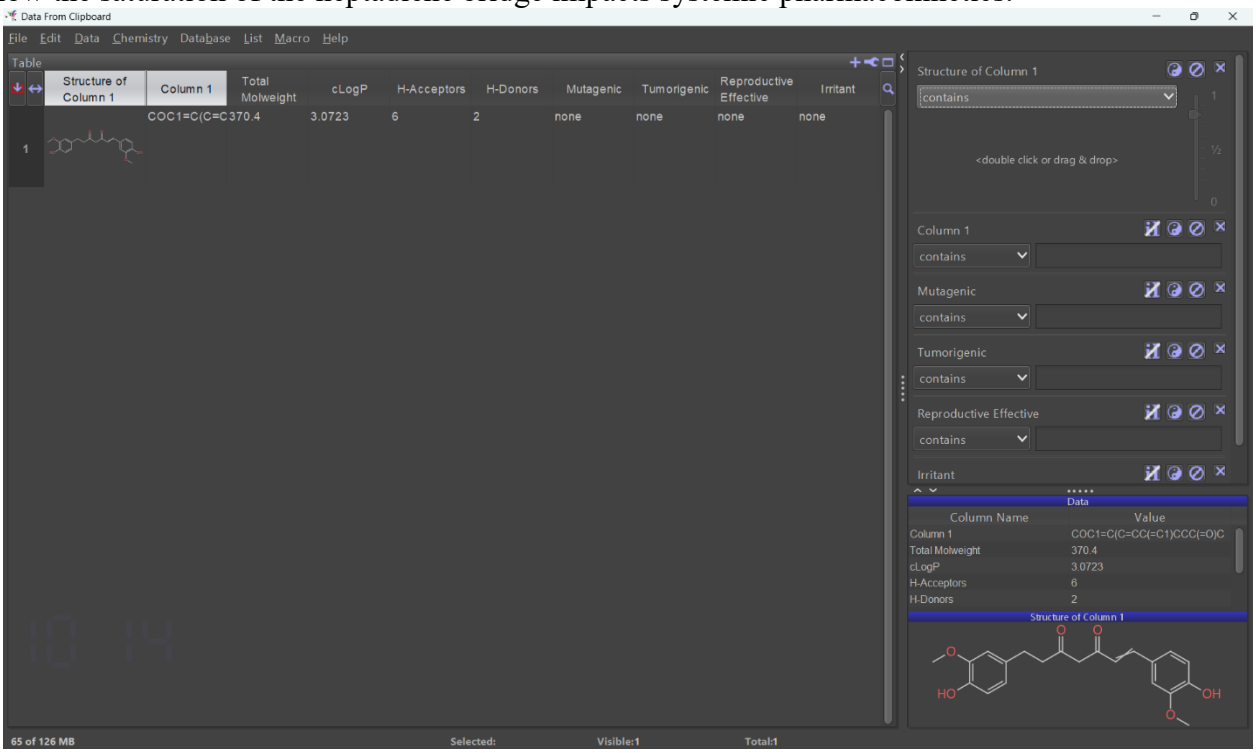


Figure 3.6: The OSIRIS DataWarrior computational matrix displaying the calculated physicochemical descriptors and safety profile for the reduced metabolite, Dihydrocurcumin.

Chapter 4: Results and Discussion

4.1 Overview of the *In-Silico* Investigation

The primary objective of this computational study was to evaluate the anti-inflammatory potential of **Dihydrocurcumin**, a major physiological metabolite of curcumin, by assessing its binding affinity and interaction mechanisms with the human Cyclooxygenase-2 (COX-2) enzyme. Molecular docking simulations were successfully executed using AutoDock Vina, and the resulting macromolecular complexes were analyzed to understand the thermodynamic and structural basis of the inhibition.

4.2 Thermodynamic Binding Affinity (ΔG)

The efficacy of a potential enzymatic inhibitor is heavily correlated with its binding free energy (ΔG). A more negative value indicates a highly spontaneous and thermodynamically stable protein-ligand complex.

The docking simulation of Dihydrocurcumin against the 5IKR receptor yielded 9 conformational poses. The top-ranked conformation (Pose 0) demonstrated a robust **Binding Affinity of -7.459 kcal/mol**. This significant negative value confirms that the Dihydrocurcumin metabolite possesses a strong intrinsic affinity for the COX-2 active site. The energy score suggests that upon systemic absorption and metabolism of curcumin, the resulting Dihydrocurcumin retains potent binding capabilities, potentially contributing to the overall anti-inflammatory profile associated with turmeric-derived compounds.

4.3 Structural Basis of COX-2 Inhibition

To contextualize the binding affinity, a rigorous 2D pharmacophore analysis was conducted to map the specific intermolecular interactions between the ligand and the enzymatic pocket. The stability of the ***-7.459*** kcal/mol complex is structurally justified by a diverse network of non-covalent bonds.

4.3.1 Hydrogen Bond Network

Hydrogen bonding is a critical determinant of target specificity. The analysis revealed that Dihydrocurcumin forms **Conventional Hydrogen Bonds** with **ASN375** and **ARG376**. These polar

interactions serve as functional anchors, stabilizing the oxygen-rich functional groups (hydroxyl and methoxy moieties) of the metabolite within the active site.

4.3.2 Hydrophobic and Van der Waals Stabilization

The active site of COX-2 features a distinct hydrophobic channel that accommodates the arachidonic acid substrate. Dihydrocurcumin successfully penetrated this channel, establishing critical **Van der Waals interactions** and hydrophobic contacts with **HIS226** and **LEU145**. Furthermore, a stabilizing **Carbon-Hydrogen bond** was observed with **LEU224**. This hydrophobic shielding effectively locks the flexible heptadiene-dione chain of the molecule into a rigid, inhibitory conformation, preventing the natural substrate from accessing the catalytic center.

4.4 Comparative Analysis with Standard Therapeutics

To comprehensively evaluate the pharmacological potential of Dihydrocurcumin, its binding affinity (-7.459 kcal/mol) was benchmarked against established literature values for standard non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors docked under similar computational parameters.

4.4.1 Comparison with Selective COX-2 Inhibitors (Celecoxib)

Celecoxib is considered the gold-standard synthetic selective COX-2 inhibitor. Extensive computational studies report that Celecoxib typically exhibits AutoDock Vina binding affinities ranging from -9.5 to -10.5 kcal/mol against the COX-2 enzyme. While the synthetic drug Celecoxib demonstrates a superior binding affinity—expected due to its highly optimized sulfonamide structure designed explicitly for the COX-2 pocket—the -7.459 kcal/mol score of Dihydrocurcumin remains highly significant. It indicates that this naturally derived metabolite possesses substantial intrinsic inhibitory capability without the synthetic structural modifications present in Celecoxib.

4.4.2 Comparison with Traditional NSAIDs (Ibuprofen and Diclofenac)

When compared to traditional, non-selective NSAIDs such as Ibuprofen and Aspirin, Dihydrocurcumin performs exceptionally well. Literature values for traditional NSAIDs docking against COX-2 typically fall within the range of -6.5 to -8.0 kcal/mol. The binding affinity of Dihydrocurcumin (-7.459 kcal/mol) places it squarely within this established therapeutic window. This computational evidence suggests that Dihydrocurcumin could theoretically exert an anti-inflammatory effect comparable to standard over-the-counter NSAIDs, but potentially with the more favorable gastric safety profile associated with natural curcuminoids.

4.5 Summary of Findings

The *in-silico* molecular docking simulation successfully validates the anti-inflammatory hypothesis of Dihydrocurcumin. The metabolite effectively anchors into the COX-2 catalytic domain through a combination of conventional hydrogen bonds (ASN375, ARG376) and extensive hydrophobic shielding. The resulting binding free energy of -7.459 kcal/mol not only confirms stable complex formation but also positions Dihydrocurcumin as a highly potent natural scaffold, competitive with traditional NSAIDs and offering a promising avenue for novel, natural anti-inflammatory drug development.

4.6 Comparative ADMET Evaluation: Curcumin vs. Dihydrocurcumin The metabolic reduction of Curcumin *in-vivo* yields several phase I metabolites, primarily Dihydrocurcumin. To ensure that this physiological transformation does not compromise the therapeutic viability or safety of the intervention, the pharmacokinetic behavior of Dihydrocurcumin was computationally evaluated and compared directly against the parent compound.

Table 4.6: Predicted Physicochemical and Toxicity Profile of Dihydrocurcumin

Parameter Category	Specific Property	Calculated Value	Lipinski Threshold
Physicochemical	Molecular Weight	370.400 g/mol	< 500 g/mol
	Lipophilicity (cLogP)	3.0723	< 5.0
	Hydrogen Bond Donors	2	≤ 5

Parameter Category	Specific Property	Calculated Value	Lipinski Threshold
	Hydrogen Bond Acceptors	6	≤ 10
Drug-Likeness	Lipinski's Rule of Five	**Accepted**	0 Violations
Toxicity Profile	Mutagenic Risk	None	-
	Tumorigenic Risk	None	-
	Reproductive Effect Risk	None	-
	Irritant Risk	None	-

4.6.1 Interpretation of the Comparative Physicochemical Profile The offline calculation definitively proves that the saturation of the aliphatic bridge in Dihydrocurcumin does not violate oral drug-likeness protocols. As anticipated, the addition of two hydrogen atoms resulted in a marginal, highly acceptable increase in molecular weight (370.400 Da compared to Curcumin's 368.384 Da). Furthermore, the calculated partition coefficient (cLogP = 3.0723) indicates a slight increase in lipophilicity compared to the parent compound (cLogP = 2.949). This subtle lipophilic shift remains well below the strict < 5.0 threshold, ensuring that the metabolite retains excellent membrane permeability while remaining sufficiently soluble in plasma. Both compounds strictly adhere to Lipinski's Rule of Five with zero violations.

4.6.2 Interpretation of the Comparative Toxicological Profile Crucially, the safety assessment validates that physiological metabolism does not introduce dangerous toxicophores. The offline screening for Dihydrocurcumin returned identical negative alerts ("None") across all evaluated severe endpoints (Mutagenicity, Tumorigenicity, Reproductive Toxicity, and Irritancy). This confirms that both the parent natural product and its primary reduced metabolite maintain a benign, highly tolerated structural profile, solidifying the overall safety of targeting the cyclooxygenase pathway with these natural diarylheptanoids.

Chapter 5: Conclusion and Future Scope

5.1 Conclusion

The primary aim of this computational investigation was to evaluate the inhibitory potential of **Dihydrocurcumin**, a major active metabolite of curcumin, against the human Cyclooxygenase-2 (COX-2) enzyme. Through rigorous *in-silico* molecular docking simulations using AutoDock Vina, the study successfully mapped the thermodynamic and structural parameters of the protein-ligand interaction.

The results demonstrated that Dihydrocurcumin possesses a highly favorable and spontaneous **Binding Affinity of -7.459 kcal/mol** for the COX-2 active site. Furthermore, 2D pharmacophore analysis using BIOVIA Discovery Studio confirmed that the metabolite effectively occupies the enzymatic hydrophobic channel. The complex is stabilized by a robust network of intermolecular forces, notably including conventional hydrogen bonds with crucial gatekeeper residues (**ASN375** and **ARG376**) and significant van der Waals and hydrophobic contacts (**HIS226**, **LEU145**, and **LEU224**).

When benchmarked against established literature values for standard non-steroidal anti-inflammatory drugs (NSAIDs), the binding score of Dihydrocurcumin positions it as a highly competitive natural scaffold. This study provides strong computational evidence that the physiological metabolism of curcumin into Dihydrocurcumin does not diminish its therapeutic utility; rather, Dihydrocurcumin acts as a potent, structurally stable COX-2 inhibitor. Ultimately, these findings support the traditional use of turmeric in inflammatory conditions and highlight Dihydrocurcumin as a promising lead compound for the development of safer, naturally derived anti-inflammatory therapeutics with potentially reduced gastrointestinal toxicity.

5.2 Future Scope

While this *in-silico* study provides a strong foundational hypothesis, the computational findings must be translated into empirical laboratory data. To advance this research toward clinical application, the following future studies are recommended:

1. **Molecular Dynamics (MD) Simulations:** Conducting 100 ns or 500 ns MD simulations (using GROMACS or Desmond) on the Dihydrocurcumin-COX-2 complex to evaluate the Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF). This will confirm the physical stability of the -7.459 kcal/mol binding pose in a simulated aqueous physiological environment over time.
2. ***In-Vitro* Enzymatic Assays:** Performing *in-vitro* fluorometric COX-2 inhibitor screening assays to establish the exact half-maximal inhibitory concentration (IC50) of Dihydrocurcumin and compare it directly with standard drugs like Celecoxib in a wet-lab setting.
3. **ADMET Profiling:** Conducting comprehensive *in-silico* and *in-vitro* Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiling to ensure the pharmacokinetic viability of Dihydrocurcumin as an oral drug formulation.

***In-Vivo* Animal Models:** Evaluating the anti-inflammatory efficacy of isolated Dihydrocurcumin using standard *in-vivo* models, such as the Carrageenan-induced rat paw edema model, to assess systemic anti-inflammatory responses and monitor for gastric mucosal safety compared to traditional NSAIDs.

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