STM JOURNALS

International Journal of
Molecular Biotechnological Research

Volume 3, Issue 1, 2025
January–June
DOI (Journal): *10.37591/IJMBR*

https://journals.stmjournals.com/ijmbr/

Research IJMBR

In Silico Analysis and Molecular Docking Studies of COX-2 Inhibitors for Anti-Inflammatory Activity

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Abstract

Although nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to treat pain, lower fevers, and control inflammation, they frequently cause gastrointestinal side effects because they inhibit the COX-1 and COX-2 enzymes. Despite their effectiveness and lack of gastrointestinal side effects, selective COX-2 inhibitors have been linked to cardiovascular risks. To find new COX-2 inhibitors that are safer and more effective, this study uses in silico techniques, such as molecular docking and virtual screening, to find promising anti-inflammatory substances. SwissADME and Protox tools were utilized to evaluate drug-likeness, pharmacokinetics, bioavailability, and toxicity after key compounds were obtained from the PubChem database. Molecular docking analysis were used to assess the binding affinities between possible ligands and COX-2 after the protein was prepared and its structure was examined using the RCSB Protein Data Bank. Apigenin, Cucurbitacin S, Curcumin, Gedunin, and Kaempferol were found to be the best candidates. The compounds showed low levels of toxicity, good gastrointestinal absorption, and compliance with Lipinski's Rule of Five. The results of this study indicate that these substances might function as efficient COX-2 inhibitors with fewer adverse effects, promoting the development of anti-inflammatory medications. To maximize these candidates and confirm their therapeutic potential for chronic inflammatory conditions, more in vitro and in vivo research is advised.

Keywords: Molecular docking, cyclooxygenase-2 (COX-2) inhibition, anti-inflammatory compounds, in silico drug discovery, SwissADME, binding affinity

INTRODUCTION

The manufacture of several prostanoids, including prostaglandins, prostacyclin, and thromboxane, which are implicated in the physiological and pathological mechanisms of inflammation, depends on cyclooxygenase (COX) enzymes. COX-1 and COX-2 are the two isoforms of COX enzymes that are expressed [1, 2]. Headaches, sprains, severe menstrual cramps, arthritis, and other inflammatory disorders can all be effectively treated with nonsteroidal anti-inflammatory drugs (NSAIDs) [3], which are commonly used to relieve pain, lower fever, and reduce inflammation. These medications work by preventing the production of prostaglandins, a class of chemicals that cause fever, pain, and inflammation at injury sites, by the Cyclooxygenase (COX) enzymes. NSAIDs that block both COX-1 and COX-2 can lead to gastrointestinal problems like stomach, intestinal, or esophageal ulcers. This happens because prostaglandins, Bleeding, stomach ulcers, and kidney damage are among the adverse effects of long-term NSAID use. The three powerful and gastrointestinal-safe anti-inflammatory medications are celecoxib, valdecoxib, and rofecoxib [4] which shield the stomach lining from the damaging effects of gastric acids, are also produced by COX-1. Because there are fewer protective prostaglandins when COX-1 is inhibited, stomach acids can harm the stomach lining and cause gastrointestinal issues. Coxibs, or

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Received Date: December 27, 2024
Accepted Date: January 11, 2025
Published Date: February 12, 2025

Citation: Debosmita Banerjee. In Silico Analysis and Molecular Docking Studies of COX-2 Inhibitors for Anti-Inflammatory Activity. International Journal of Molecular Biotechnological Research. 2025; 3(1): 38–55p.

selective COX-2 inhibitors, were created to lessen this issue. These medications lower the risk of gastrointestinal side effects by specifically targeting COX-2, the enzyme linked to pain and inflammation. Nevertheless, a higher risk of heart attacks and strokes has been associated with selective COX-2 inhibitors. Some COX-2 inhibitors, such as Vioxx, were eventually taken off the market because of these cardiovascular risks [5].

A key method in computer-aided drug design and structural molecular biology is molecular docking. Predicting the most likely binding modes of a ligand interacting with a protein with a known three-dimensional structure is the main objective of ligand-protein docking. Efficient navigation of high-dimensional search spaces and the use of a scoring function that precisely ranks possible docking configurations are prerequisites for effective docking techniques [6]. To optimize lead compounds, docking is especially helpful in virtual screening, where it can be used to assess sizable compound libraries, rank the results, and produce structural hypotheses about how ligands might inhibit the target protein [7] significantly influenced by chronic inflammation. Therefore, one of the main goals in the hunt for potent therapeutic agents is to comprehend and target the molecular mechanisms that cause inflammation. Cyclooxygenase-2 (COX-2) is a key modulator of inflammatory responses. The synthesis of pro-inflammatory prostaglandins depends on the enzyme COX-2, which is activated during inflammation. It has been demonstrated that inhibiting COX-2 is a successful method of treating inflammation and associated illnesses. NSAIDs frequently work by targeting COX-2 to reduce inflammation and relieve pain, but they can have negative side effects, especially if used for an extended period. The initial phases of drug discovery have been transformed by molecular docking and virtual screening. Virtual screening makes it possible to quickly evaluate large compound libraries to find those that might interact with biological targets. By predicting a compound's ideal orientation within a target protein's binding site and estimating the strength of their interaction, molecular docking further refines these findings. The purpose of this study is to find new anti-inflammatory substances that can inhibit COX-2 by using docking and virtual screening techniques [8]. The study looks for candidates with high binding affinities and advantageous interaction profiles with these important targets by screening a large library of compounds and running thorough docking simulations. The findings of this study may result in the creation of novel, less harmful anti-inflammatory drugs that have substantial therapeutic advantages for the treatment of long-term inflammatory diseases [8].

Following an initial virtual screening of 14 ligands from PubChem, we narrowed down our selection to 5 ligands that, according to their docking scores, showed a significant association with the target protein, COX-2 (Cyclooxygenase-2). Indomethacin, Apigenin, Celecoxib, Rofecoxib, Valdecoxib, Curcumin, Meloxicam, Nimesulide, Gedunin, Resveratrol, Cucurbitacin S, Quercetin, Ibuprofen, and Kaempferol were among the 14 ligands that were first evaluated. These ligands were chosen because of their well-established analgesic and anti-inflammatory qualities, which are consistent with COX-2's crucial function in inflammatory pathways. Out of the 14 ligands, Apigenin, Curcumin, Gedunin, Cucurbitacin S, and Kaempferol had the highest docking scores and the most promising binding interactions with the COX-2 active site. Strong binding affinities were demonstrated by these ligands, which formed important hydrophobic interactions, hydrogen bonds, and π-π stacking with important residues in the COX-2 binding pocket. Their structural properties, such as terpenoid and polyphenolic compounds, help them to block COX-2 enzymatic activity [9].

The flavonoids apigenin and kaempferol stood out for having high docking scores, which suggested that they interacted well with the catalytic region of COX-2, most likely because of their hydroxyl groups creating hydrogen bonds. Strong binding was demonstrated by curcumin, a well-known polyphenol with multi-target anti-inflammatory qualities, which is consistent with its documented function as a COX-2 inhibitor in the literature. The capacity of the limonoid gedunin and the triterpenoid cucurbitacin S to create hydrophobic interactions – which are essential for maintaining the ligand-protein complex – made them stand out. These findings provide a good framework for additional exploration, including molecular dynamics simulations to examine the stability of these ligand-COX-2 complexes under physiological settings. Furthermore, in vitro and in vivo tests can be used to confirm

these ligands' anti-inflammatory properties and investigate their potential therapeutic uses for COX-2-mediated illnesses [10].

METHODS

Retrieval of Anti-Inflammatory Compound

In this study, we aimed to retrieve the raw data using the in-silico process for investigational anti-inflammatory drugs for targeting Cox-2 proteins. The selection of these drugs was carried out using the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/), which provides a comprehensive repository of detailed drug information, including pharmacological properties and therapeutic uses. Following the identification of suitable anti inflamatory candidates, their structural files were obtained from the PubChem database, a reliable source for chemical structures and bioactivity data. These structural files were essential for conducting precise molecular docking simulations [11].

Retrieval of the Proteins

The crystal structures of Cyclooxygenase-2, was retrieved from the RCSB PDB repository (https://www.rcsb.org/), with a resolution of 2.45 Å. The structure was resolved through Xray diffraction methodologies. The protein structure was subjected to purification in DS Biovia Discovery Studio. The non-structural components including water molecules, hetero atoms and ligands were removed from the crystal structure of the protein. To reduce the structural complexity only the A chains were retained in the protein and the additional chains were removed. The protein structure was prepared by adding polar hydrogen atoms and the purified structure was saved in .pdb format [12].

Identifying the pharmacological properties

SwissADME (https://www.swissadme.ch/) is a versatile online resource utilized in drug discovery to analyze the physicochemical characteristics, pharmacokinetics, drug-likeness, and medicinal chemistry compatibility of small molecules. It offers crucial insights into a compound's drug potential by predicting important aspects, such as lipophilicity, solubility, gastrointestinal absorption, and cytochrome P450 enzyme interactions. SwissADME also evaluates drug-likeness using Lipinski's Rule of Five and provides bioavailability visualization through tools like the Bioavailability Radar and the Boiled-Egg Model. By delivering comprehensive predictions of absorption, distribution, metabolism, and excretion (ADME) properties, SwissADME supports researchers in refining lead compounds, identifying suitable candidates, and uncovering potential challenges early in the drug development process [13].

Protox

Protox (https://tox.charite.de/protox3/) is an online tool used in drug discovery to predict the toxicity of small molecules, aiding in the assessment of their safety as potential drug candidates. It estimates key toxicological parameters, such as the median lethal dose (LD50), and classifies compounds into toxicity classes based on the Globally Harmonized System (GHS). Protox also predicts organ-specific toxicity and evaluates the likelihood of compounds being carcinogenic, mutagenic, or immunotoxic. By providing early insights into the potential risks associated with chemical compounds, Protox helps researchers optimize lead compounds, minimize toxicity, and ensure regulatory compliance, ultimately supporting the development of safer drugs [14].

Molecular Docking and Visualization

Molecular docking is a crucial technique in computational drug discovery, offering insights into the interaction between small molecules and their protein targets. For this study, PyRx was employed to facilitate the docking processes of various antiviral drugs with the protein; multitargets. This protein was prepared for a blind docking procedure, which does not assume prior knowledge of the ligand's binding site. The docking space was defined by grid dimensions to encapsulate the entire protein structure. The docking simulations treats protein as a rigid while allowing the ligands to remain flexible. An exhaustiveness parameter set to 8 ensured a thorough search, and the outcomes were primarily

evaluated based on the binding affinities computed at zero RMSD (Root Mean Square Deviation) [15].

RESULT

Top Phytocompounds

The COX-2 is a FDA-approved agent for the treatment of Inflammation. The standard structural file of this compound was procured from the PubChem database, and the structure was visualised with DS Biovia Discovery Studio Visualizer. The COX-2 was prepared by adding the universal force filed (_uff) and the ligand structural files were converted to PDBQT format using PyRx for docking simulations. After conducting an initial screening, we narrowed down our selection from 15 ligands to 5 ligands. These 5 ligands demonstrated significant association with our target protein, Cyclooxygenase-2, based on their docking scores.

Apigenin

Apigenin, also known as 4′, 5, 7,-trihydroxyflavone, is a naturally occurring compound in the flavone category and serves as the aglycone for various glycosides. It has the molecular formula C15H10O5 and a molecular weight of 270.24. Flavones, including their synthetic variants, have demonstrated a range of biological activities, such as antioxidant, anti-inflammatory, antitumor, antigenotoxic, anti-allergic, neuroprotective, cardioprotective, and antimicrobial effects. Apigenin appears as a yellow crystalline solid and has historically been used for dyeing wool (Figure 1).

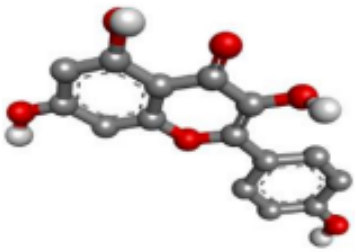

Figure 1. Apigenin (5280443).

Cucurbitacins

Cucurbitacins and their derivatives, a group of highly oxidized tetracyclic triterpenoids, are found in the Cucurbitaceae family and possess a broad spectrum of biological and pharmacological properties. These compounds have been utilized in traditional medicine for centuries, where plants containing cucurbitacins are recognized for their antipyretic, analgesic, anti-inflammatory, antibacterial, and antitumor effects. Cucurbitacins B and E, which are abundant in the traditional Chinese herb GuaDi, are commonly prescribed by Chinese doctors for liver-related ailments. Consequently, during the 1970s and 1980s, some Chinese researchers explored the anti-hepatitis and anti-hepatic cancer effects of these cucurbitacins, focusing primarily on clinical studies (Figure 2).

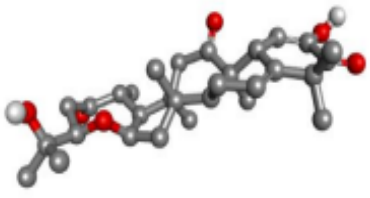

Figure 2. Cucurbitacin.

Curcumin

Curcumin is a primary compound found in the rhizome of Curcuma longa (L) and other Curcuma species. Commercially, curcumin makes up about 77% of the mixture, along with two related compounds, demethoxycurcumin and bis-demethoxycurcumin. These compounds are part of the diarylheptanoid group and are collectively known as curcuminoids. Curcumin itself is a crystalline substance with a vibrant orange-yellow hue and is widely used as a coloring agent and food ingredient (Figure 3).

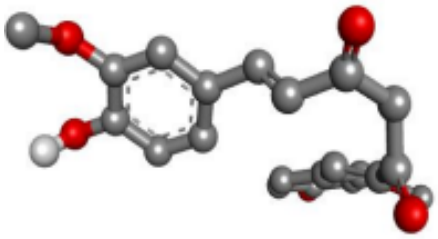

Figure 3. Curcumin (969516).

Gedunin

Gedunin is a significant limonoid found primarily in the seeds of various genera within the Meliaceae family. It is known for its wide range of biological activities, including antibacterial, insecticidal, antimalarial, antiallergic, anti-inflammatory, anticancer, and neuroprotective properties. The identification of gedunin as an inhibitor of heat shock proteins (Hsp) marked a crucial advancement in its potential use as a therapeutic agent (Figure 4).

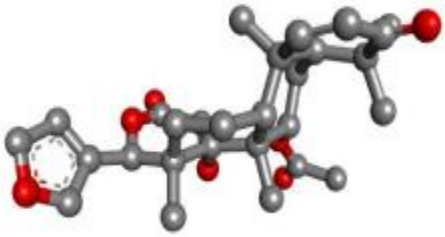

Figure 4. Gendunin (12004512).

Kaempferol

Kaempferol is a commonly found aglycone flavonoid in the form of glycosides, characterized as a tetrahydroxyflavone with hydroxyl groups at positions 3, 5, 7, and 4′. This yellow compound is present in various parts of plants, including seeds, leaves, fruits, flowers, and vegetables. Kaempferol, along with its glycosylated forms, has been shown to exhibit cardioprotective, neuroprotective, anti-inflammatory, antidiabetic, antioxidant, antimicrobial, antitumor, and anticancer properties (Figure 5).

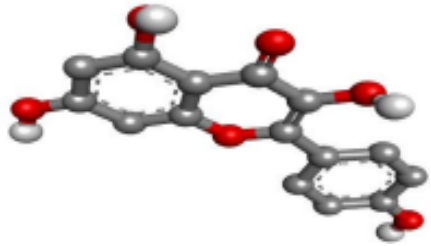

Figure 5. Kaempferol (5280863).

Retrieval of Proteins

The following proteins were selected as multitarget for anti-inflammatory drug discovery in the present study.

Cyclooxygenase-2

Cyclooxygenase-2 (COX-2) is an enzyme that plays a crucial role in the body's inflammatory response by converting arachidonic acid into prostaglandins, which are key mediators of pain and inflammation. Unlike Cyclooxygenase-1 (COX-1), which supports normal physiological processes, COX-2 is usually activated in response to inflammation, making it an important target for developing anti-inflammatory drugs [7]. This project focuses on investigating the molecular interactions between COX-2 and various drug ligands to assess their potential as selective COX-2 inhibitors. Given COX-2's critical involvement in conditions, like arthritis, cancer, and cardiovascular diseases – where inflammation plays a significant role – targeting COX-2 presents a promising approach for therapeutic intervention. The study will employ molecular docking techniques to examine how different compounds bind to COX-2, potentially leading to the identification or repurposing of drugs that are both more effective and have fewer side effects [8].

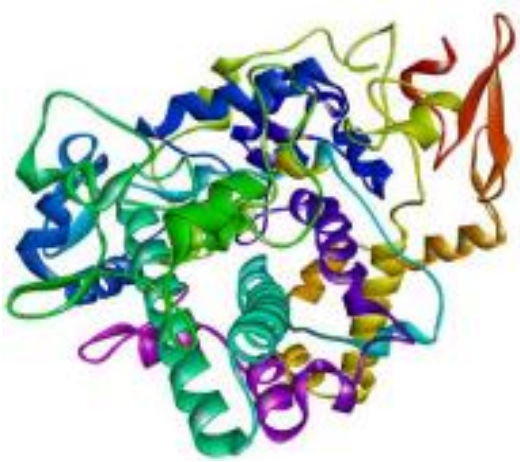

Figure 6. Cyclooxygenase-2.

By centering on COX-2, this project aims to contribute to the development of more efficient treatments for inflammatory conditions, with a particular focus on reducing the risks associated with COX-2 inhibition, such as cardiovascular issues. The findings from this research could help guide the design of new COX-2 inhibitors that offer improved therapeutic outcomes for patients (Figure 6).

Molecular Docking and Visualization

The docking study focused on the evaluation of binding affinities between a suite of sesquiterpenoids and the protein target 5KIT, providing critical insights for potential drug discovery. Binding affinities were measured in kilocalories per mole (kcal/mol), with lower values indicative of stronger and more energetically favourable ligand-protein interactions. A range of affinities was observed, from –8.4 to –9.4 kcal/mol (Table 1).

Table 1. Molecular docking of Sesquiterpenoids against 5KIT.

Drug Compound	Pubchem ID	Binding Affinity with 5KIT in kcal/mol
Kaempferol	5280863	–9.4
Apigenin	5280443	–9.3
Cucurbitacin S	119287	–9
Curcumin	969516	–8.5
Gedunin	12004512	–8.4

INTERACTION

Apigenin (5280443)

Tryptophan, tyrosine, lysine, arginine, proline, and threonine are among the essential amino acids that interact with apigenin, a naturally occurring flavonoid. These interactions emphasize the potential therapeutic effects of apigenin in a variety of biochemical processes and are essential for preserving the structural integrity of proteins, such as fibrinogen, preventing their dislocation or disintegration (Figure 7).

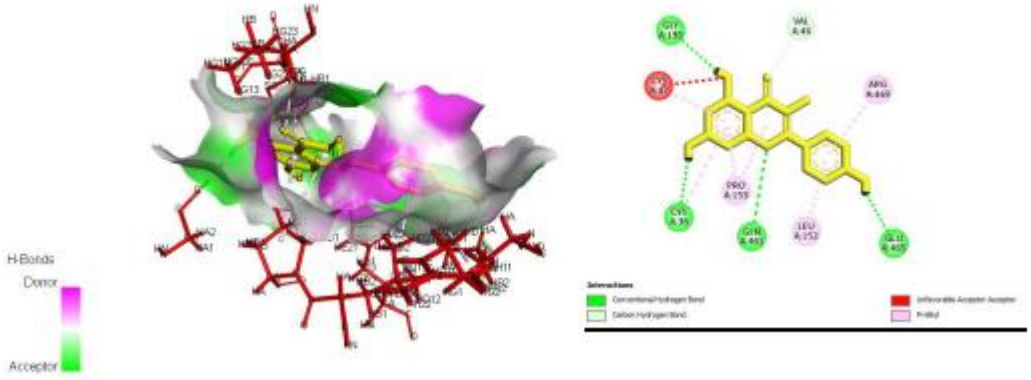

Figure 7. Bonds and Hydrophobic interactions.

Cucurbitacin S (119287)

A bioactive substance presents in many plants, cucurbitacin S particularly interacts with amino acids, such as glutamic acid (GLU) and aspartic acid (ASP). Its biological activity and potential for therapeutic use depend on these interactions, which mostly occur through hydrogen bonds and carbon-hydrogen bonds (Figure 8).

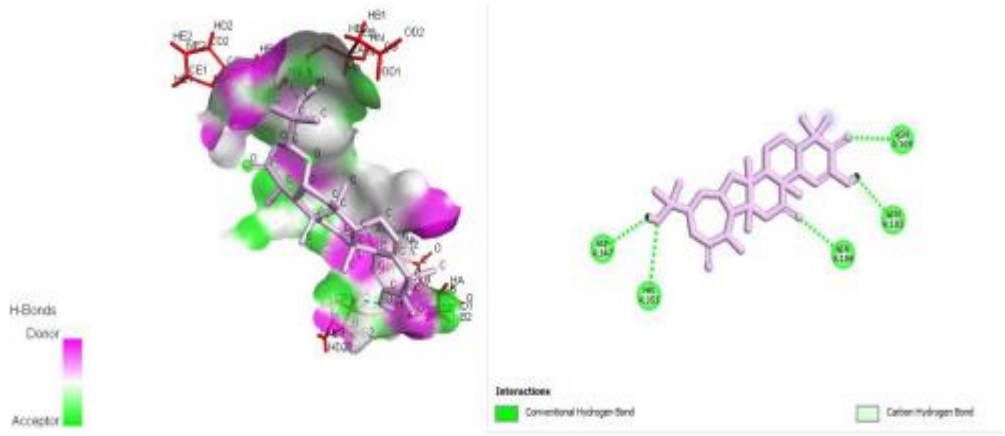

Figure 8. Molecular docking interaction of the Cucurbitacin ligand with the target protein, highlighting hydrogen bonds and hydrophobic interactions.

Curcumin (969516)

Turmeric contains a polyphenolic molecule called curcumin, which interacts with aspartic acid, histidine, arginine, and lysine, among other amino acids. Hydrogen bonds and other molecular interactions are involved in these interactions, which help explain curcumin's diverse biological activities and medicinal benefits (Figure 9).

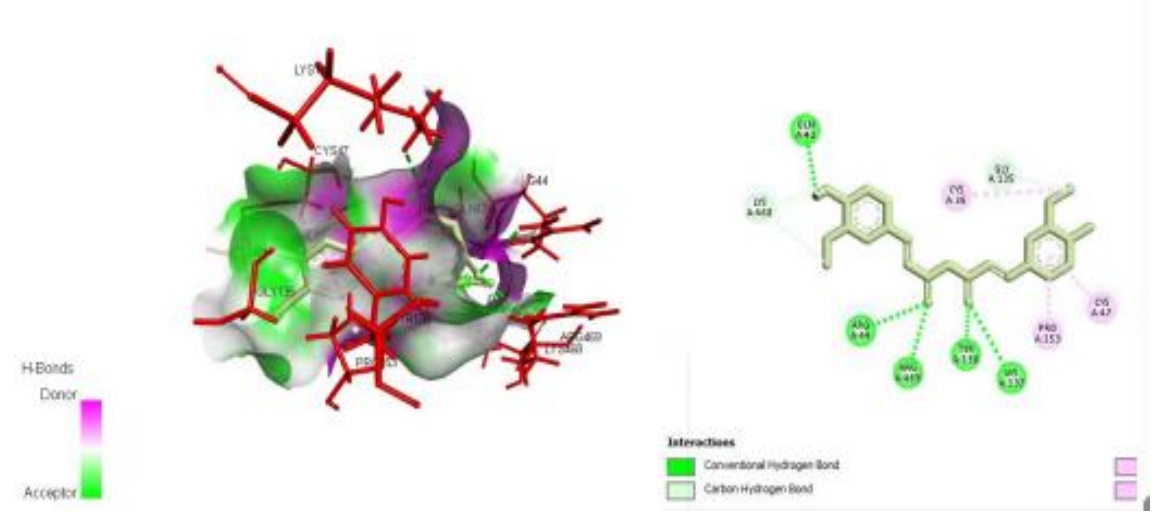

Figure 9. Molecular docking interaction of the Curcumin ligand with the target protein, highlighting hydrogen bonds and hydrophobic interactions.

Gedunin (12004512)

A limonoid found in neem trees, gedunin interacts with amino acids, like tyrosine and tryptophan, to produce hydrophobic and hydrogen bonding interactions that are essential to its biological activity and possible medicinal benefits (Figure 10).

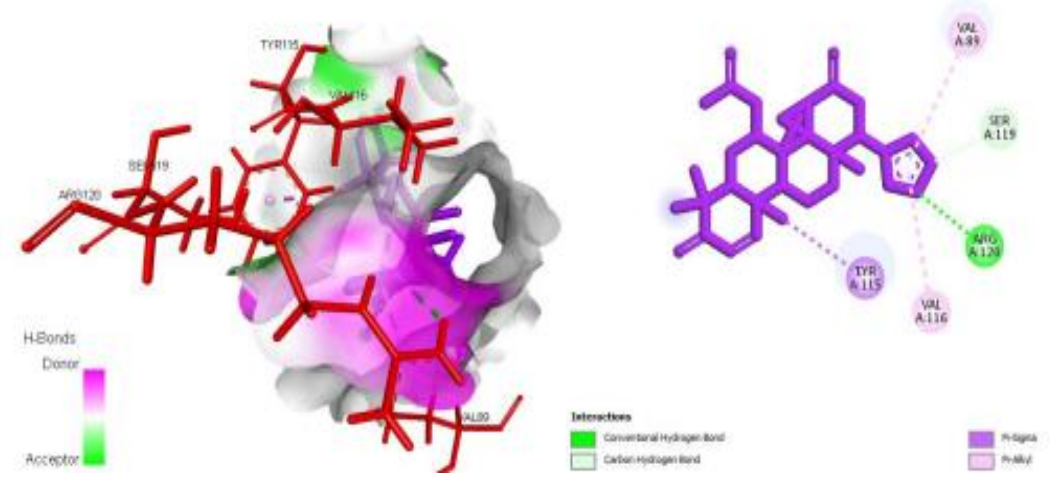

Figure 10. Molecular docking interaction of the Gedunin ligand with the target protein, highlighting hydrogen bonds and hydrophobic interactions.

Kaempferol (5280863)

Numerous fruits and vegetables contain kaempferol, a flavonoid that interacts with amino acids such glutamic acid, leucine, proline, arginine, and valine. The anti-inflammatory and antioxidant qualities of kaempferol are influenced by these interactions, which include hydrogen bonds and hydrophobic interactions (Figure 11).

Toxicity Compound

The picture displays (Figure 12) Apigenin's physiochemical characteristics with an emphasis on its potential toxicity. A Table 2 with several toxicity classes, their targets, abbreviations, predictions (active or inert), and corresponding probability is included. The categories include toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, and nutritional toxicity) as well as organ toxicity (hepatotoxicity and nephrotoxicity). According to the table, Apigenin is predicted to be inactive in

nephrotoxicity, carcinogenicity, and nutritional toxicity with probabilities of 0.90, 0.62, and 0.74, respectively, and active in hepatotoxicity, immunotoxicity, and mutagenicity with probabilities of 0.69, 0.96, and 0.97. A radar graphic that displays the probability of activity and inactivity across various toxicity endpoints beneath the table gives a thorough picture of Apigenin's possible toxicological consequences.

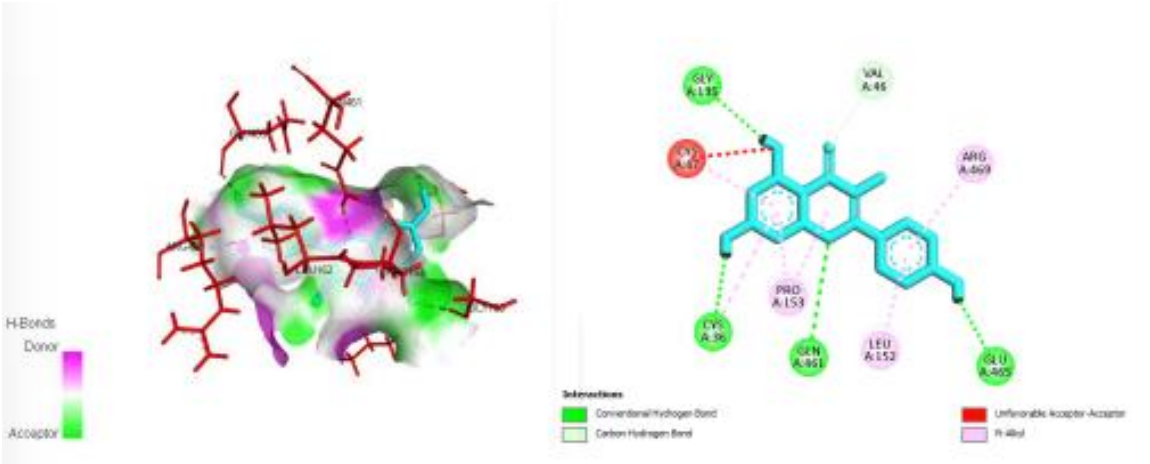

Figure 11. Molecular docking interaction of the Kaempferol ligand with the target protein, highlighting hydrogen bonds and hydrophobic interactions.

Table 2. Toxicity predictions for Apigenin.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.69
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	immuno	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74

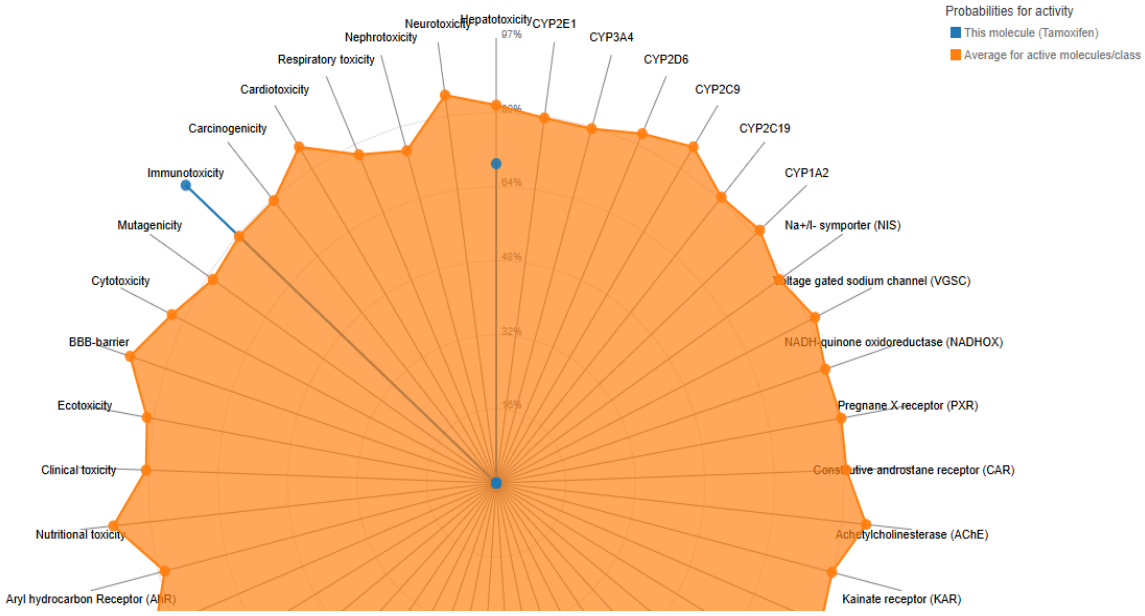

Figure 12. Apigenin's physiochemical characteristics.

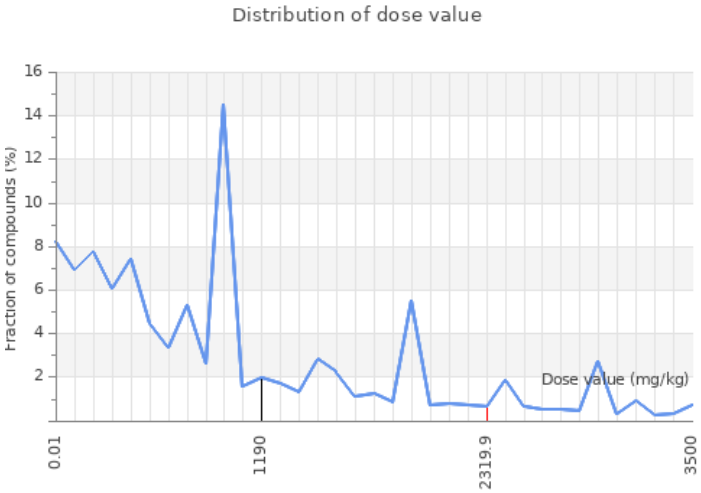

Figure 13. Apigenin Distribution of dose value.

The "Distribution of dose value" (Figure 13) graph displays the dose value (mg/kg) on the x-axis and the fraction of compounds (%) on the y-axis. Significant spots in the distribution are indicated by the blue line, which has prominent peaks around 1190 mg/kg and smaller peaks about 2319 mg/kg. At 15%, the highest peak is reached. comprehension the toxicity distribution of Apigenin, which is categorized as toxicity class 4, requires a comprehension of this graph.

Table 3. Toxicity predictions for Cucurbitacin S.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.69
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	Immune	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74

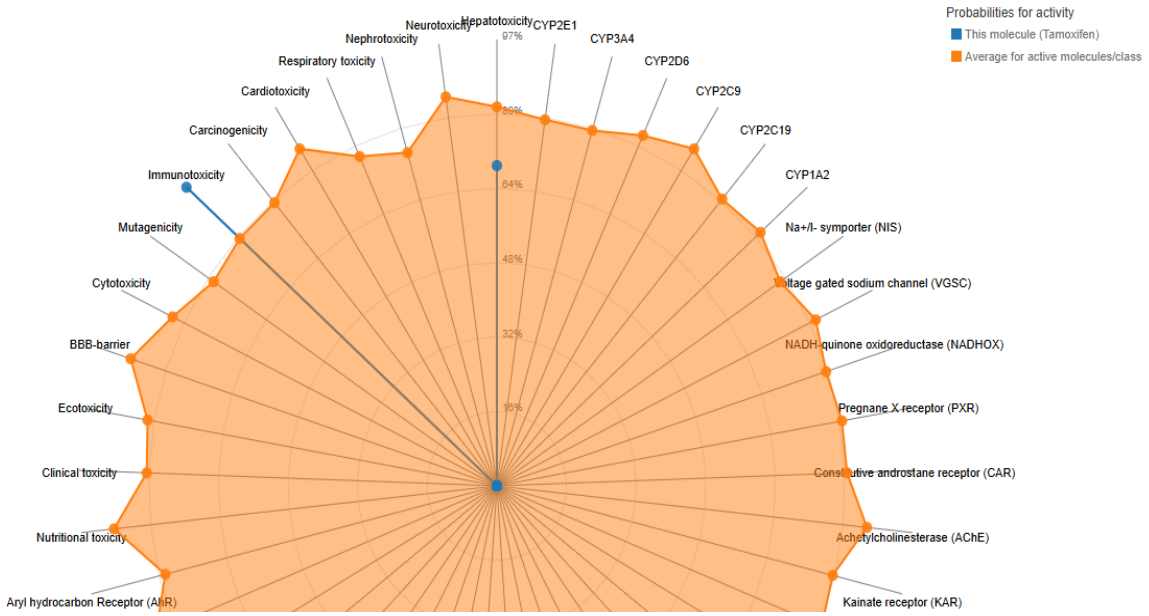

Figure 14. Cucurbitacin S physiochemical characteristics.

A thorough examination of the toxicity projections (Figure 14) for Cucurbitacin S is included in the graphic. The Table 3 shows whether the substance is predicted to be active or inactive for each category, along with corresponding probabilities ranging from 0.62 to 0.97. It lists different toxicity classifications, such as organ toxicity (hepatotoxicity and nephrotoxicity) and toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, and nutritional toxicity). The radar chart, which shows the expected activity of Cucurbitacin S across many biological targets, including CYP enzymes, ion channels, and receptors, is displayed beneath the table. Understanding the possible toxicological consequences of Cucurbitacin S on different biological systems is made easier by this thorough analysis.

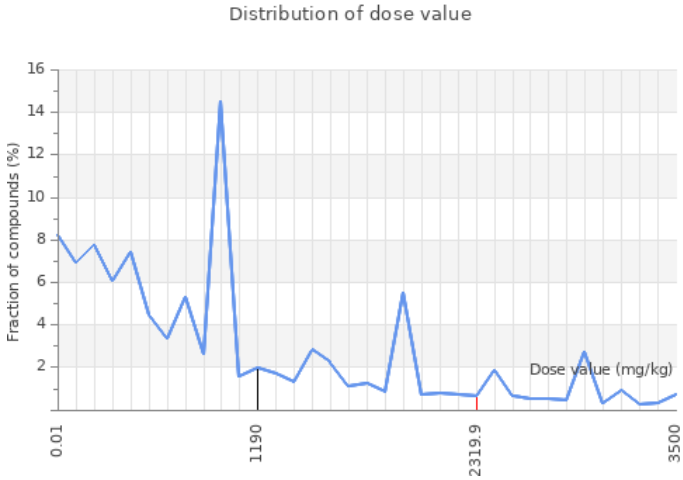

Figure 15. Cucurbitacin S distribution of dose value.

The "Distribution of dose value" (Figure 15) graph shows the dose value (mg/kg) on the x-axis and the fraction of compounds (%) on the y-axis. The blue line prominently displays a dosage value of 231.99 mg/kg, with major peaks at approximately 1190 mg/kg and lesser peaks at different places. At 15%, the highest peak is reached. Understanding the toxicity distribution of Cucurbitacin S, which is categorized under toxicity class 4, requires knowledge of these data.

Table 4. Toxicity predictions for Curcumin.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.69
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	Immune	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74

The picture (Figure 16) offers a thorough examination of the toxicity forecasts for curcumin, complete with a radar chart and Table 4. Organ toxicity (hepatotoxicity and nephrotoxicity) and toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, and nutritional toxicity) are among the toxicity classifications included in the table. Table 4 shows the associated probability for each categorization along with whether Curcumin is expected to be active or inert. For instance, hepatotoxicity has a probability of 0.69 to be active, whereas nephrotoxicity has a probability of 0.90 to be inactive. By contrasting the expected activity of curcumin with the mean for active compounds, the radar chart illustrates these probabilities. Understanding Curcumin's safety profile and its toxicological consequences is made easier by this thorough explanation.

The "Distribution of dose value" (Figure 17) graph displays the logarithmically scaled dose value (mg/kg) on the x-axis and the fraction of compounds (%) on the y-axis. The distribution of dose values

for curcumin, which is categorized as toxicity class 4, is shown in the graph. To determine the toxicity of curcumin and safe dosage levels, significant peaks are seen about 1190 mg/kg, with the biggest peak reaching almost 14%. These peaks show important points in the distribution and frequency of various dose values.

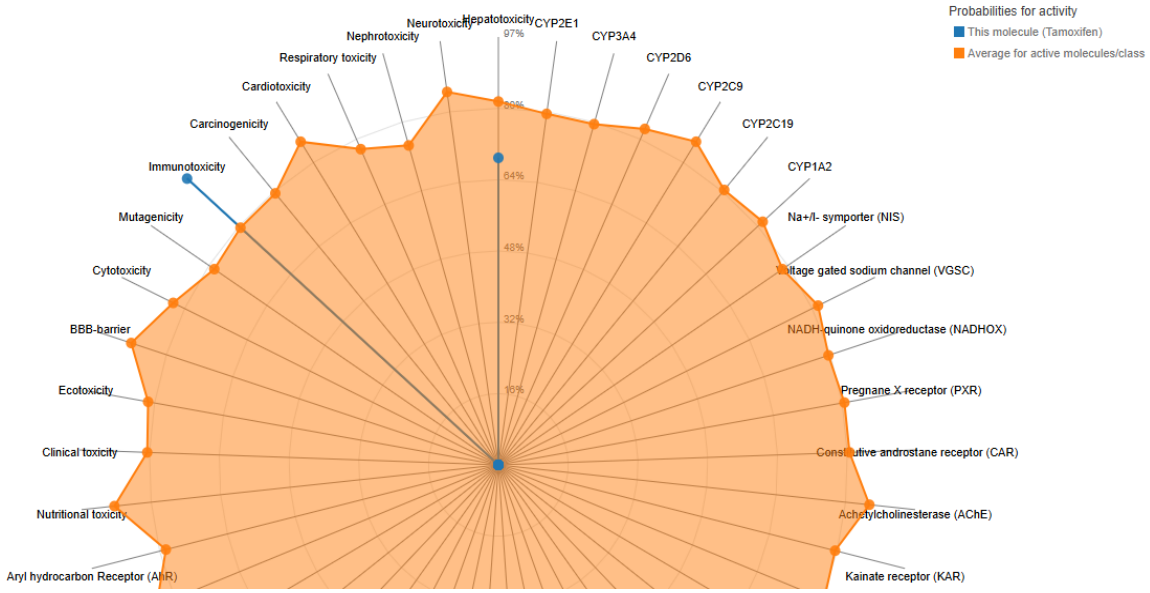

Figure 16. Curcumin physiochemical characteristics.

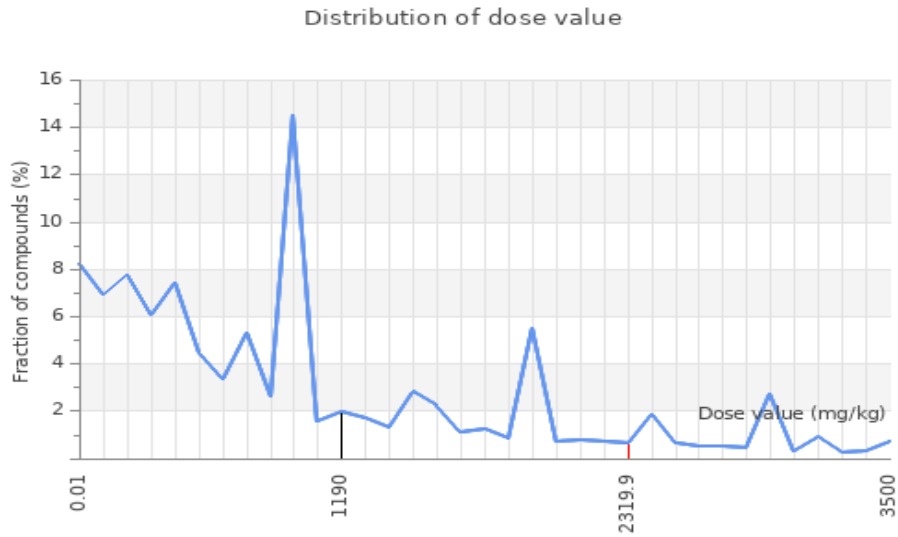

Figure 17. Curcumin Distribution of dose value.

Table 5. Toxicity predictions for Kaempferol.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.69
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	Immune	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74

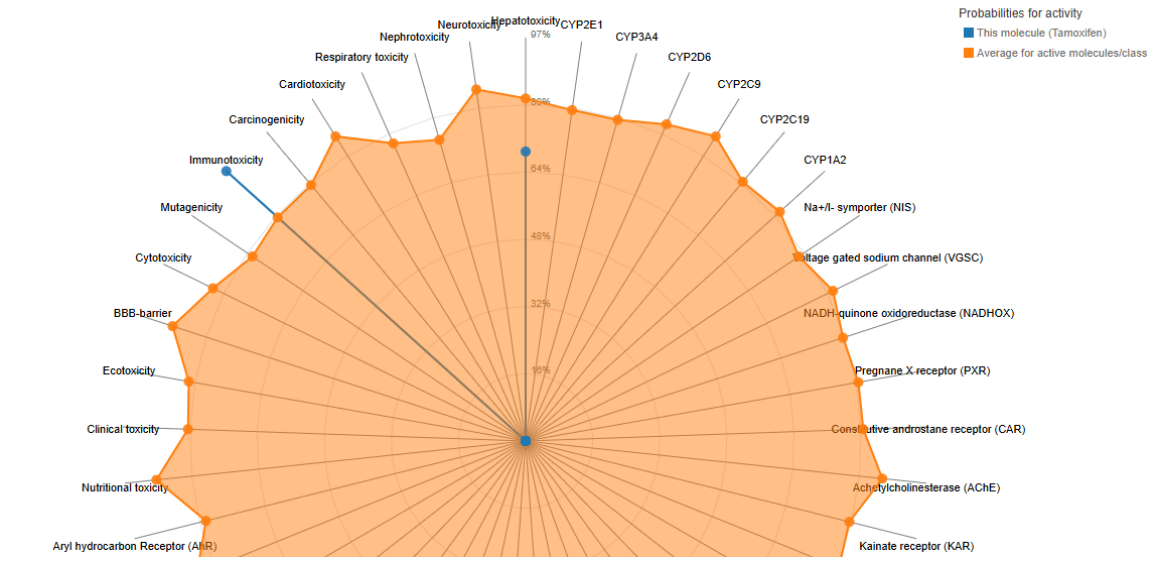

Figure 18. Kaempferol physiochemical characteristics.

The picture (Figure 18) includes a thorough examination of the toxicity projections for kaempferol. Numerous toxicity classifications are listed in Table 5, such as toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, and nutritional toxicity) and organ toxicity (hepatotoxicity and nephrotoxicity). The table lists the corresponding probabilities, which range from 0.62 to 0.97, for each categorization and shows whether Kaempferol is expected to be active or inert. A radar chart that contrasts Kaempferol's anticipated activity with the mean for active molecules is displayed beneath the table to graphically depict these possibilities. The possible toxicological effects and safety profile of kaempferol are better understood thanks to this thorough study.

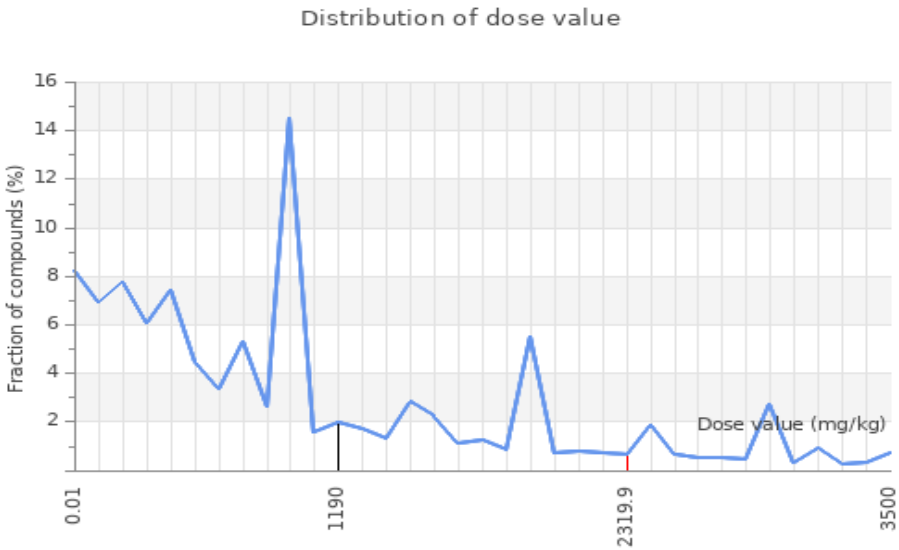

Figure 19. Kaempferol Distribution of dose value.

"Distribution of dose value" (Figure 19) is a graph that shows the fraction of compounds (%) on the y-axis and the dose value (mg/kg) on the x-axis. The graph shows prominent peaks for kaempferol, which is categorized as toxicity class 4, especially around 1190 mg/kg, with the largest peak rising to almost 15%. This distribution provides crucial information about the safe dosage ranges and toxicity levels of kaempferol by highlighting important dosage points and frequency.

Table 6. Toxicity predictions for Gedunin.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Active	0.69
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.90
Toxicity end points	Carcinogenicity	carcino	Inactive	0.62
Toxicity end points	Immunotoxicity	Immune	Active	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.97
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.74

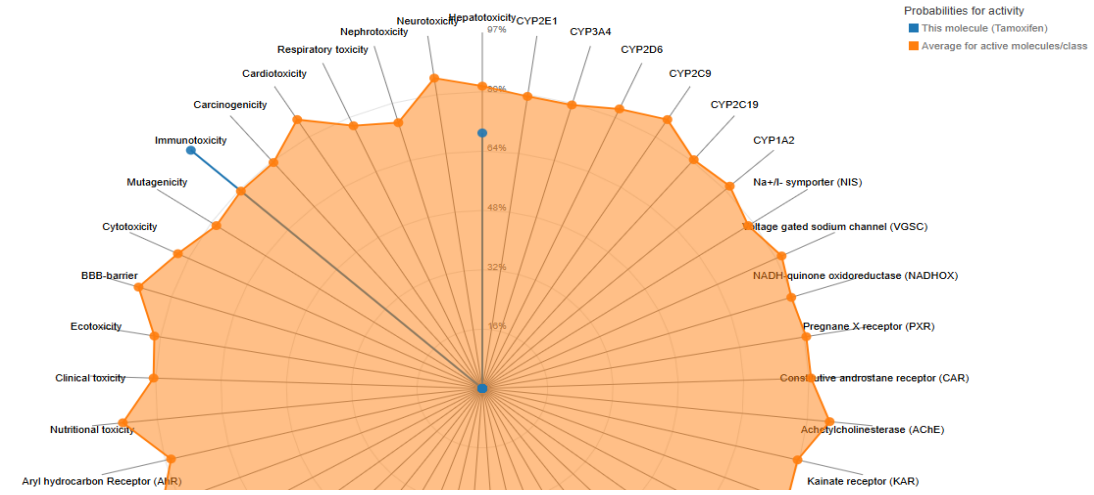

Figure 20. Gedunin's physiochemical characteristics.

Gedunin's (Figure 20) toxicity projections are thoroughly examined in the image. The Table 6 lists several toxicity classifications, such as toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, and nutritional toxicity) and organ toxicity (hepatotoxicity and nephrotoxicity). When it comes to hepatotoxicity (0.69 likelihood), immunotoxicity (0.96 probability), and nephrotoxicity (0.90 probability), carcinogenicity (0.62 probability), mutagenicity (0.97 probability), and nutritional toxicity (0.74 probability), Gedunin is shown to be inactive. The radar chart, which contrasts Gedunin's anticipated activity with the mean for active molecules, provides a visual representation of these probabilities beneath the table. Gedunin's safety profile and possible toxicological effects are explained in detail in this overview, which is essential for both safety evaluation and medication development.

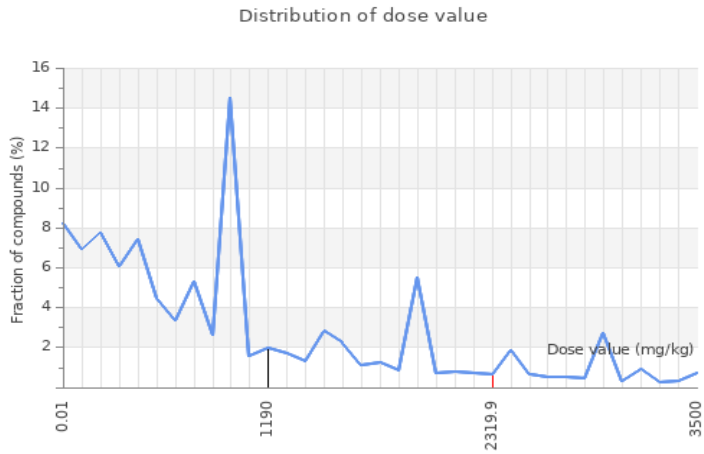

Figure 21. Gedunin Distribution of dose value.

The "Distribution of dose value" (Figure 21) graph displays the dose value (mg/kg) on the x-axis and the fraction of compounds (%) on the y-axis. There is a prominent peak at about 1190 mg/kg, which reaches 15%. Gedunin is categorized under toxicity class 4, and this distribution helps to understand its toxicity levels and acceptable dose ranges by revealing its frequency and critical dosage points.

SwissADME

The SwissADME results indicate favorable drug-like properties for the four selected compounds, with all showing acceptable lipophilicity, solubility, and good gastrointestinal absorption according to Lipinski's Rule of Five. Additionally, the Bioavailability Radar and Boiled-Egg Model confirm their potential for high oral bioavailability.

Table 7. Pharmacokinetic properties of Drug Compounds.

Drug Compound	Pubchem ID	GI	BBB	Lipinski	Solubility	Synthetic Accessibility
Kaempferol	5280863	High	No	Yes:0 violation	1.40e-01 mg/ml; 4.90e-04 mol/l	3.14
Apigenin	5280443	High	No	Yes:0 violation	3.07e-02 mg/ml; 1.14e-04 mol/l	2.96
Curcubitacin S	119287	High	No	Yes:0 violation	6.27e-03 mg/ml; 1.26e-05 mol/l	6.56
Curcumin	969516	High	No	Yes; 0 violation	4.22e-02 mg/ml; 1.15e-04 mol/l	2.97
Gedunin	12004512	High	No	Yes;0 violation	1.93e-03 mg/ml; 4.00e-06 mol/l	6.48

ADME Property

The "ADME Property" chart highlights the gastrointestinal absorption (GI), blood-brain barrier permeability (BBB), Lipinski rule violations, solubility, and synthetic accessibility of five medicinal compounds: Kaempferol, Apigenin, Cucurbitacin S, Curcumin, and Gedunin. None of the substances break Lipinski's rule of five, and all exhibit high GI absorption and no BBB permeability. The most soluble substance is kaempferol, whereas the least soluble substance is gedunin. The range of synthetic accessibility scores, which indicate different levels of ease of synthesis, is 2.96 to 6.56. When developing new drugs, this information is crucial for assessing the pharmacokinetic characteristics of these substances.

Drug Property

Numerous drug compounds, such as kaempferol, apigenin, cucurbitacin S, curcumin, and gedunin, are listed in the "Drug property" Table 7 along with their corresponding characteristics. The table provides information on each compound's PubChem ID, bioavailability (all 0.55), PAINS (all 0 alerts), BRENK alerts (all 0 to 2 warnings with concerns indicated), and PGP status (either "Yes" or "No"). The pharmacological characteristics and possible problems associated with these substances are well summarized in this Table 8, which also highlights their applicability and possible difficulties in medication development.

Table 8. Pharmacological Properties and Development Challenges of Drug Compounds.

Drug Compound	Pubchem ID	Bioavailability	Pains	Brenk	PGP
Kaempferol	5280863	0.55	0 alert	0 alert	No
Apigenin	5280443	0.55	0 alert	0 alert	No
Curcubitacin S	119287	0.55	0 alert	1alert: isolated_alkene	Yes
Curcumin	969516	0.55	0 alert	2 alerts:beta_keto_anhydride, michael_acceptor_1	No
Gedunin	12004512	0.55	0 alert	2 alerts:Three-membered_heterocycle, more_than_2_esters	Yes

DISCUSSION

An essential biological reaction, inflammation protects the body from damaging stimuli like infections, poisons, and tissue damage. Nonetheless, several illnesses, including as cancer, heart disease, arthritis, and neurological conditions, like Alzheimer's, are linked to persistent inflammation. The arachidonic acid route, NF-κB signaling, and cytokine-mediated processes are some of the pathways involved in the inflammatory process. Cyclooxygenase-2 (COX-2), an inducible isoform of cyclooxygenase, is a crucial component of the arachidonic acid pathway, which is particularly important among them. In contrast to constitutive COX-1, which carries out homeostatic tasks such protecting the stomach, COX-2 is increased in reaction to inflammatory stimuli. It produces prostaglandins, which mediate inflammation, fever, and pain, from arachidonic acid. Because of its structurally hydrophobic active site, which promotes substrate binding and enzymatic activity, COX-2 is a desirable target for the development of anti-inflammatory drugs. Numerous inflammatory conditions, such as rheumatoid arthritis, inflammatory bowel disease, and even cancer, are strongly associated with COX-2 overexpression, which promotes tumor growth via inflammation-driven pathways [15].

This study examined 14 ligands with established analgesic and anti-inflammatory qualities to find possible COX-2 inhibitors. Indomethacin, Apigenin, Celecoxib, Rofecoxib, Valdecoxib, Curcumin, Meloxicam, Nimesulide, Gedunin, Resveratrol, Cucurbitacin S, Quercetin, Ibuprofen, and Kaempferol were among these ligands. Based on their docking scores and binding interactions with the COX-2 active site, molecular docking simulations reduced this list to the top five candidates: Apigenin, Curcumin, Gedunin, Cucurbitacin S, and Kaempferol. These ligands showed strong hydrogen bonding, π-π stacking, and hydrophobic interactions with important residues in the COX-2 binding pocket. Their structural characteristics, especially the terpenoid and polyphenolic groups, improve their capacity to suppress the enzymatic activity of COX-2, which lowers inflammation and prostaglandin formation [16].

According to ADME (Absorption, Distribution, Metabolism, Excretion) research, the top five ligands all shown characteristics of drugs. High gastrointestinal absorption and adherence to Lipinski's Rule of Five, which forecasts oral bioavailability, were demonstrated by them. Of the substances, kaempferol was the most soluble, whereas gedunin had a slightly lower solubility but still had good pharmacokinetics. The ratings for synthetic accessibility varied from 2.96 to 6.56, indicating different degrees of synthesis ease. These results provide credence to the compounds' suitability for medication development [17].

The ProTox-II research shed light on these chemicals' safety characteristics. Apigenin, Curcumin, and Cucurbitacin S were deemed relatively safe for therapeutic application due to their low acute toxicity, with LD50 values of approximately 1190 mg/kg. However, some ligands, especially Apigenin and Gedunin, were warned for hepatotoxicity and immunotoxicity with probability more than 0.69. None of the ligands exhibited notable nephrotoxicity or carcinogenicity in spite of these dangers. These forecasts show where lead development must be further optimized to strike a balance between safety and efficacy [18].

In this case Apigenin, Curcumin, Gedunin, Cucurbitacin S, and Kaempferol were found to be promising COX-2 inhibitors using the combination of molecular docking, ADME profiling, and toxicity predictions. Their promise as selective COX-2 inhibitors with little off-target effects are suggested by their significant binding affinities and advantageous pharmacokinetic characteristics. These results highlight the need for additional experimental validation and optimization to address potential safety concerns and offer a solid basis for the development of safer and more effective treatments for inflammatory illnesses.

This study's main drawback is its reliance on in silico techniques, such as toxicity predictions and molecular docking, which, although useful, are unable to accurately capture the complexity of

biological systems. The capacity to verify the anticipated interactions and therapeutic effectiveness of the ligands is restricted by the absence of experimental validation, such as in vivo testing or enzyme tests. Selectivity between COX-2 and COX-1 isoforms, which is essential for preventing possible adverse effects, is also not taken into consideration in this study. Although instructive, the toxicity study is also predicated on computer forecasts and does not account for all potential negative consequences. Additionally, issues with medication solubility, bioavailability, and formulation were not addressed, and the study's narrow emphasis on 14 ligands may have excluded other interesting compounds.

CONCLUSIONS

In conclusion, this study offers important new information about the potential of a number of ligands as selective COX-2 inhibitors for the management of inflammatory illnesses, such as kaempferol, epigenin, curcumin, gedunin, and cucurbitacin S. Molecular docking simulations revealed that the ligands have significant hydrophobic contacts, hydrogen bonds, and π-π stacking with the COX-2 active site, which might effectively limit COX-2 enzymatic activity. These substances appear to have favorable pharmacokinetic characteristics, such as good gastrointestinal absorption and no violations of Lipinski's Rule of Five, according to the ADME analysis. Confirming their therapeutic potential and evaluating their safety profile, however, requires additional experimental validation, including in vitro and in vivo research. This study establishes the foundation for upcoming medication development that targets COX-2 and emphasizes the potential of both natural and synthetic chemicals as anti-inflammatory medicines.

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