In silico validation of anti-inflammatory activities of compounds isolated from *Avena sativa* (Oat Meal) extract

Onyeloni Sunday Onyemali*[1], Uzochukwu Chijioke Ikemefuna[2], Akachukwu Ibezim[2], Omeje Edwin Ogechukwu[3],

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria
[2]Department of Pharmaceutical/Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria
[3]Department of Pharmaceutical/Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

Submitted: 10th Sept., 2025; Accepted: 24th Oct., 2025; Published online: 31st Oct., 2025
DOI: https://doi.org/10.54117/jcbr.v5i5.1
**Corresponding Author: Onyeloni Sunday O;* onyeloniso@gmail.com*; +2348062725284.*

Abstract

Oat (*Avena sativa*) Fam. Poaceae is a cereal originally used as animal feed but now serves as a functional food, as a result of its robust nutritional content such as carbohydrate, protein, lipids, fibres and reported therapeutic uses including anti-inflammatory and anti-oxidant activities. This study is aimed at a systematic molecular docking evaluation of fifteen (15) phytocompounds derived from Oat meal against the active site of cyclooxygenase-2 (COX-2) which is a well-known enzyme implicated in inflammatory responses and a key target in non-steroidal anti-inflammatory drugs (NSAID) development. This procedure has the core objective of predicting the binding affinity and assess the binding mode stability of each ligand in complex with COX-2, with the aim of identifying novel or optimized scaffolds for selective COX-2 inhibitor. The physicochemical properties of the studied compounds were determined to further assess their drug likeness and pharmacokinetic feasibility of the tested compounds with key indicators of logP, aqueous solubility, (logS), molecular weight, number of hydrogen bond donor (HBD), hydrogen bond acceptors (HBA), rotatable bonds (b-rotN) and polar surface area (ASA-P). This study employs the technique of molecular docking simulation using the 10-crystalized ligand of COX-2, S58 a well characterized selective COX-2 inhibitor with the validation done using the molecular operating environment (MOE) software suite. The result shows that 3 compounds; Decanamide, N-(2-hydroxyethyl)- (-4.39±0.33 kcal/mol), Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy (-16.21±0.04 kcal/mol), 2-Octylcyclopropene-1-heptanol (-10.46±0.03 kcal/mol) show good promise as potential drug candidate compared to Celecoxib (-11.48±0.03) with Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy having the most favourable docking score among drug like compounds and zero Lipinski's violation.

Keywords: Docking, cyclooxygenase-2, Oat meal, anti-inflammatory, inflammation.

Introduction

Oat meal formulated from whole oat grain is a functional food with both nutritional and reported medicinal use. *Avena sativa* commonly called Oat is reported to have anti-inflammatory, anti-microbial and anti-pruritic activities (Zhang *et al*., 2021).

Inflammation is a complicated response to cell injury, trauma or toxins that can last for a few days (Acute inflammation) to longer duration (chronic inflammation) and are mediated by inflammatory response such as tumor necrosis factor and interleukin 1b and interleukin 6 as well as inflammatory target such as macrophages and immune cells (neuro immune response).

This study investigates molecular docking of 15 structurally diverse small molecules isolated by Onyeloni *et al*. (2025) as shown in Table 1, from Oat meal against the active site of cyclooxygenase-2. The primary objective of this study is to predict the binding affinities and evaluate the stability of the ligand-protein complexes with COX-2 with the ultimate aim of identifying novel or optimized scaffolds for selective COX-2 inhibition. This procedure evaluates two critical parameters (binding energy and root mean square deviation (RMSD) to assess potential drug candidates for further investigation and development. Although work have been done on phytochemical analysis of oat meal (Onyeloni et al., 2025) but molecular docking to evaluate teir anti-inflammatory activities is lacking.

Materials and methods

Sample Collection

Two (2) tins of Quacker Oat^{TM} containing 840 g whole oat grain packed by Pepsico Foods Nigeria Limited, Lagos, Nigeria was purchased from a supermarket in Abraka, Delta State, Nigeria on the 10th of June, 2024.

Sample Extraction

All solvents used in this study were of AR grade. About 800g of Quacker Oat^{TM} meal was weighed using a weighing balance and extracted in 70% methanol using soxhlet apparatus at a temperature of 40ºC for 24 hours. The extract was filtered using Whatman No. 1 filter paper under suction and lyophilized and stored at 4°C for further analysis.

Phytochemical Analysis

Qualitative and quantitative phytochemical analysis was done using standard method for flavonoids, phenolics, alkaloids, tannins and saponins (Gown Rajkumar *et al*., 2022).

Fractionation and Purification of Oat Meal Extract

The Oat meal extract was subjected to column chromatography to separate it into its component fractions using a column size of diameter 4.0 cm and length 39.0 cm, using silica gel a highly hygroscopic substance as stationary phase and varying solvent combination of increasing polarity as the mobile phase. The solvent system include: hexane: ethyl acetate 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. The same ratio was applied to ethyl acetate methanol combination. The eluted fractions were further analyzed using TLC with silica gel as stationary phase and hexane: methanol as mobile phase for further separation. Fractions with similar TLC mobility and band formation pattern were bulked into a beaker and subjected to another round of TLC analysis to determine the number of spots. The pooled fractions were concentrated using rotary evaporator at 40°C at high pressure. The oily fractions were then dissolved in 5 ml of n-hexane and stored in a refrigerator for GC-MS spectrophotometric analysis (Gomathi *et al*., 2015).

Gas Chromatography – Mass spectroscopic Analysis of Oat Meal Fractions

GC-MS analysis of n-hexane fractions was performed using a Perkin Elmer GC Clarus 500 system with a gas chromatograph interfaced with a mass spectrometer. Helium gas was used as a carrier gas at a constant flow rate of 1mL/min and an injection volume of 2 µL was employed. Turbo mass Gold Perkin Elmer was used as mass detector and the software adopted to handle mass spectra and chromatogram was Turbo mass version 5.2. The injection was operated at 250°C and the oven temperature was moderated at 60°C for 15mins and gradually increased to 300°C at 3mins. The identification of compounds was based on leedex libraries as well as comparison of their retention indices. The compound was identified after comparison with those in nature using computer library attached to the GC-MS instrument (Onyeloni *et al*., 2025).

In-silico anti-inflammatory activity determination of bioactive compounds in extract of oat meal

Compounds identified from Oat meal extract was used for molecular docking evaluation to the binding site of COX-2 enzyme, a key mediator that catalyze the production of prostaglandins responsible for inflammatory responses. The crystal structure of COX-2 was obtained from RCSB protein data bank and processed with MOE program. The hardware used in this study is the ASUS vivo book X53OUN S15 with an intel core i7 8550 8th Gen and window 10 64-bit operating system. The software employed in the molecular docking procedure is Vina and AutoDock tools 15.6 software (Forli *et al.*, 2016). The validation study was done using co-crystalized ligands extracted from the corresponding proteins and used as a reference molecular (Ali *et al*., 2023).

Ligand preparation

Target compound used were structured using the builder module of MOE. The compounds were then collected from Pubchem database and prepared by adding hydrogen, calculating partial charges and energy minimization using force field MMFF94X.

Docking protocol

The receptor was generated using MOE-site finder, and the ligand was docked using MOE within the active site. The docking method was validated using Celecoxib as the reference ligand which was extracted and used repeatedly for the docking procedure after being charged, set torque and then saved as Pdbqt (Ali *et al*., 2023). Appropriate RMSD threshold for Celecoxib was 1.978A calculated using profit results outsource. RMSD determines the techniques validity quantitatively which determines higher probability and liability of a valid docking result.

Results

Table 1: Qualitative and Quantitative Phytochemical Analysis of Oat Meal Extract

Phytochemicals	Qualitative analysis	Quantitative analysis
Flavonoids	+	198.85
Phenolics	+	60.51
Alkaloids	+	60.36
Tannins	+	20.30
Saponins	+	60.15

Table 2: GC-MS Analysis of n-hexane Fractions of Oat Meal

S/N	Rt (min)	Name of compound	Molecular formula	Molecular weight (g/mol)	Peak
1.	16.653	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	10.92
2.	17.954	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	1.75

3.	20.056	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721	22.81
4.	20.104	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.4876	20.31
5.	20.201	11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320.5093	1.31
6.	20.481	Methyl stearate	$C_{19}H_{38}O_2$	298.50	0.97
7.	21.017	Decanamide, N-(2-hydroxyethyl)-	$C_{12}H_{25}NO_2$	259.3850	1.55
8.	21.238	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721	5.11
9.	21.270	9-Octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310.5145	3.03
10.	23.273	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}C_{10}$	298.89	4.41
11.	24.012	Octadecanoic acid, 2-hydroxy-1,3-propanediyl e	$C_{39}H_{76}O_2$	625.02	2.06
12.	24.529	Octanamide, N-(2-hydroxyethyl)-	$C_{10}H_{21}NO_2$	187.28	18.62
13.	26.184	Hexadecanoic acid, 1-[(2-aminoethoxy)hydro	$C_{35}H_{70}NO_8P$	663.9	0.79
14.	26.663	Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy	$C_{10}H_2O$	156.26	2.14
15.	28.125	2-Octylcyclopropene-1-heptanol	$C_{18}H_{34}O$	266.5	4.21
					100.00

Table 3: ***In-silico*** **Anti-inflammatory Activity Determination of Bioactive Compounds in Oat Meal Extracts (Cyclooxygenase-2)**

Ligands	**Binding energy (kCal/mol)**				**Mean±SD**
	1	**2**	**3**	**4**	
Celecoxib	-11.51	-11.49	-11.43	-11.49	-11.48±0.03
Etoricoxib	-9.88	-8.21	-9.94	-9.86	-9.47±0.84
Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy	-16.16	-16.18	-16.24	-16.26	-16.21±0.04
2-Octylcyclopropene-1-heptanol	-10.47	-10.40	-10.47	-10.49	-10.46±0.03
9,12-Octadecadienoyl chloride, (Z,Z)-	-7.69	-7.83	-7.68	-7.82	-7.76±0.08
Octadecanoic acid ethyl ester	-7.49	-7.43	-7.58	-7.30	-7.45±0.12

9,12-Octadecadienoic acid (Z,Z)-, methyl ester	-7.17	-7.58	-6.85	-7.19	-7.20±0.30
Methyl stearate	-7.26	-7.15	-7.09	-7.21	-7.17±0.07
11,14,17-Eicosatrienoic acid, methyl ester	-7.04	-7.32	-7.42	-6.62	-7.10±0.36
Octadecanoic acid, 2-hydroxy-1,3-propanediyl e	-6.83	-6.94	-6.84	-6.85	-6.86±0.05
9-Octadecenoic acid (Z)-, methyl ester	-7.22	-6.53	-6.53	-6.95	-6.81±0.34
2-Octylcyclopropene-1-heptanol	-6.72	-6.74	-6.76	-6.83	-6.76±0.05
Hexadecanoic acid, 1-[(2-aminoethoxy)hydro	-6.64	-6.62	-6.62	-6.80	-6.67±0.09
Octanamide, N-(2-hydroxyethyl)-	-5.47	-5.49	-5.61	-5.63	-5.55±0.08
Decanamide, N-(2-hydroxyethyl)-	-4.45	-4.96	-4.11	-4.03	-4.39±0.33

Key: Data are present in Mean±SD n=4

Table 4. Physicochemical Properties and Drug-Likeness Assessment

Compound	**Weight (Da)**	**logP**	**logS**	**HBA**	**HBD**	**RotB**	**ASA_P**	**Rule Violations**
1	271.47	6.88	-6.71	2	0	15	56.59	1 (logP)
2	285.49	7.22	-7.04	2	0	16	75.22	1 (logP)
3	295.49	4.94	-4.92	2	0	17	110.76	0
4	297.50	6.17	-6.17	2	0	17	80.97	1 (logP)
5	321.53	4.58	-4.71	2	0	19	142.66	0
6	299.52	7.77	-7.74	2	0	17	57.22	1 (logP)
7	217.35	3.23	-3.02	3	3	11	132.81	0
8	295.49	4.94	-4.92	2	0	17	113.34	0
9	311.53	6.52	-6.50	2	0	18	101.91	1 (logP)
10	299.91	5.57	-5.55	1	0	16	180.13	1 (logP)
11	956.59	21.80	-23.49	8	2	57	186.85	4
12	189.30	2.34	-1.99	3	3	9	132.31	0
13	693.99	10.14	-13.75	9	5	39	252.56	4
14	161.25	1.46	-0.95	3	3	7	135.56	0

15	268.49	6.52	-7.54	1	1	14	88.12	1 (logP)

Table 5. Best Performing Compounds Based on Binding Affinity and RMSD

Compound No.	Best Binding Affinity (kcal/mol)	Corresponding RMSD (Å)	Remarks
13	-16.1636	3.7425	Strong binding but RMSD above ideal limit; may need re-evaluation.
14	-10.4743	1.4905	Good affinity and stable pose with RMSD within allowed limit.
7	-4.4553	3.9809	Decent affinity, but with RMSD above the limit of reliability.

Table 6: Summary of Ligand–Receptor Binding Highlights

Compound	Key Interactions	Remarks
Native Ligand	Arg120, Ser353, Arg513	Validation of docking method; canonical COX-2 contacts
Compound 7	Arg513, Glu524, Lys83	Moderate affinity; similar to native profile
Compound 13	Arg120, Glu524, Val523	High affinity, poor drug-likeness (violates Lipinski)
Compound 14	Arg513, Ser353, Leu352	Best compromise of affinity and drug-likeness

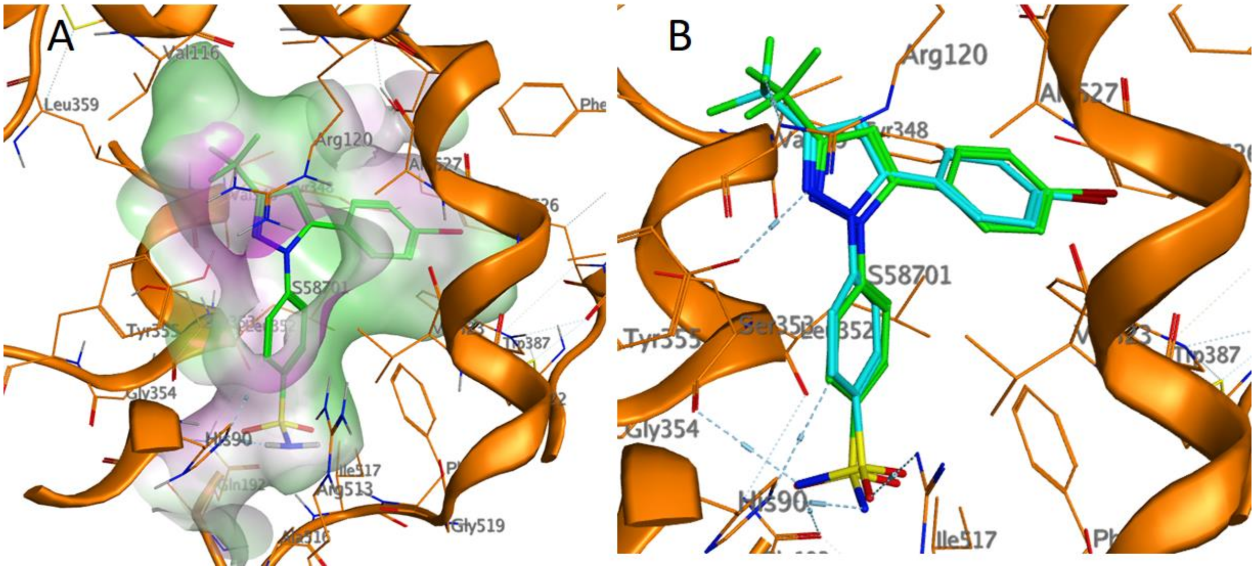

Figure 1. (A) Native ligand (S58) in its crystallographic binding pocket. (B) Superposition of the co-crystallized ligand and its corresponding docked pose within COX-2, illustrating the accuracy of redocking. Hydrogen bonds are shown in blue.

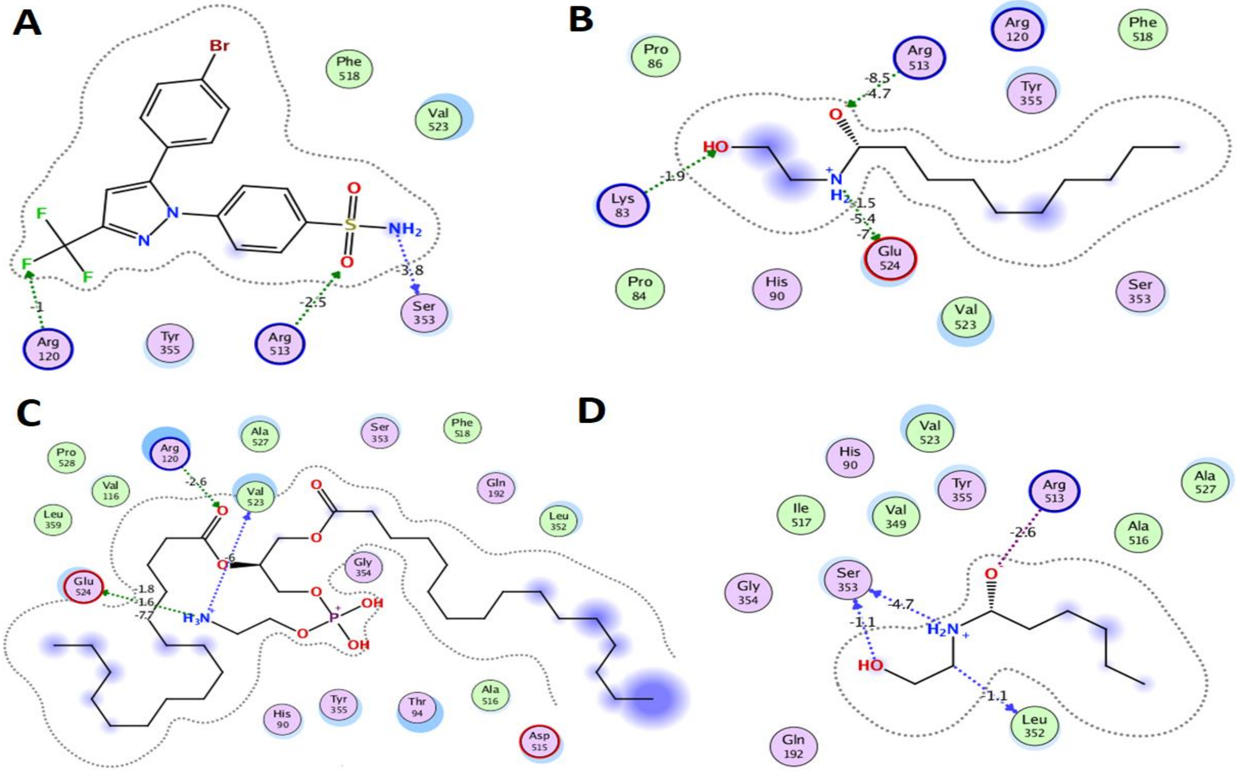

Figure 2. Interaction diagrams of selected top-ranking compounds with COX-2 based on molecular docking results. (A) Binding interactions of re-docked native ligand. (B) Binding interactions of Compound 7 (C) Binding interactions of Compound 13 (D) Binding interactions of Compound 14.

Key interactions such as hydrogen bonds, hydrophobic contacts, and π-π stacking are highlighted to illustrate crucial ligand–receptor contacts. Ligand poses were selected based on the lowest binding energy and optimal root mean square deviation (RMSD) values obtained from docking simulations.

Discussion

Docking Protocol Validation and Application of Docking Protocol to Test Compounds

The reliability and predictive accuracy of the molecular docking protocol used in this study were validated through a redocking experiment using the co-crystallized ligand of COX-2, S58, a well-characterized selective COX-2 inhibitor (Kurumbail *et al*., 1996). The validation was conducted using the Molecular Operating Environment (MOE) software suite (MOE, 2022), a trusted and widely adopted platform for receptor–ligand interaction modelling and structure-based drug discovery.

In the redocking procedure, the co-crystallized ligand was first extracted from the original protein–ligand complex and subsequently re-docked into the active site of COX-2. This method assesses the docking algorithm's ability to reproduce the experimentally determined binding pose, a key step for confirming the protocol's accuracy and predictive capability (Meng *et al*., 2011).

The docking workflow employed the Triangle Matcher algorithm for pose generation, which aligns ligand atom triplets with corresponding pharmacophoric features in the binding site using geometric hashing (Labute, 2001). This placement strategy has been recognized for its robust balance between computational efficiency and pose accuracy, especially in complex binding environments (Thomsen and Christensen, 2006). Following placement, each pose was refined through force-field-based energy minimization, improving the accuracy of predicted interactions (Wang *et al*., 2003; Meli *et al*., 2022).

To incorporate ligand flexibility and adequately explore conformational space, 30 low-energy conformations were generated for each ligand. These were then subjected to induced-fit refinement, which optimizes both ligand poses and nearby receptor side chains. This step introduces a limited but biologically meaningful level of receptor flexibility, allowing for better accommodation of ligands within the active site and more realistic binding interactions (Sherman *et al*., 2006).

Post-placement and refinement, binding poses were evaluated using the London ΔG scoring function, an empirical scoring method. The London ΔG scoring function was employed to estimate binding free energy in kcal/mol. This scoring function approximates the enthalpic contribution to binding based on empirical parameters such as van der Waals contacts, hydrogen bonding potential, metal binding, and desolvation effects. A more negative score reflects stronger predicted binding affinity, corresponding to a more energetically favourable ligand–protein interaction. The London ΔG function is a standard in MOE due to its efficiency and reasonable correlation with experimental binding data (Vilar *et al*., 2008; Labute, 2008).

From the 30 generated conformations, the top five poses with the most negative binding free energy values were selected for each ligand. These were further evaluated for geometric consistency, stability, and molecular interactions with key active site residues. The redocking yielded a close match between the docked and crystallographic pose of the native ligand, indicating that the docking protocol successfully reproduces experimental binding orientations and thus is reliable for further virtual screening.

Figure 1 illustrates the validation of the docking protocol. Panel A shows the native (co-crystallized) ligand bound within the COX-2 active site, while Panel B presents the superposition of the original ligand and its re-docked pose. The close alignment between the two confirms the protocol's reliability, demonstrating its ability to accurately reproduce the experimentally observed binding conformation.

With the docking protocol validated through redocking of the co-crystallized COX-2 ligand, the same approach was extended to the 15 test compounds to assess their potential as selective COX-2 inhibitors. The simulations utilized the MOE platform under identical conditions, ensuring methodological consistency and reliability in comparative analysis.

Each compound was docked into the COX-2 active site, and 30 conformations were generated to explore flexibility and binding mode diversity. From these, the five lowest-energy poses per compound were retained for detailed evaluation. Binding affinities were estimated using the London ΔG scoring function, with more negative values indicating stronger predicted interactions.

In addition to docking scores, root mean square deviation (RMSD) values were calculated for each pose relative to the lowest energy conformation. RMSD serves as a measure of pose stability and structural convergence, where lower RMSD values (typically < 2.0 Å) indicate a consistent and reliable binding orientation within the active site (Morris and Lim-Wilby, 2008). RMSD analysis also assists in validating the reproducibility of predicted poses across different conformers, which is crucial when comparing structurally dissimilar ligands.

The combined evaluation of binding affinity (ΔG in kcal/mol) and pose reliability (RMSD in Å) provides a multidimensional approach to assess ligand performance. Through this methodology, the study aims to prioritize ligands not only for their binding strength but also for their potential binding stability, specificity, and alignment with known pharmacophoric features of COX-2 selective inhibitors (Warner *et al*., 1999).

This streamlined assessment allowed for the prioritization of compounds not only based on their binding strength but also on the structural stability of their docked conformations. Results from this phase informed subsequent structure–activity analysis and highlighted compounds with favourable characteristics for further experimental investigation.

Binding Affinity Evaluation

Molecular docking simulations provide two critical parameters for evaluating potential drug candidates: binding affinity (in kcal/mol) and root mean square deviation (RMSD) of the ligand pose relative to a known or optimal binding conformation. The RMSD is a crucial metric to validate the reliability of predicted ligand conformations. An RMSD < 2.0–2.5 Å typically indicates that the binding pose is likely to be biologically relevant and consistent across conformers (Warren *et al*., 2006). In this study, binding affinities ranged from –16.16 kcal/mol (indicative of strong and spontaneous interactions) to over +300 kcal/mol (suggestive of poor, unstable, or even artifactual interactions). Typically, more negative binding scores correspond to higher binding affinity and better thermodynamic favourability of ligand–protein interactions (Kitchen *et al*., 2004). Conversely, very high positive scores may arise from steric hindrance, poor fit within the active site, excessive ligand flexibility, or limitations of the scoring function in the MOE platform.

Among the 15 docked compounds, Hexadecanoic acid, 1-[(2-aminoethoxy)hydro displayed the most negative binding score at –16.1636 kcal/mol, suggesting excellent thermodynamic compatibility with the COX-2 active site. However, the reliability of this pose is tempered by its high RMSD of 3.74 Å, which exceeds the commonly accepted cutoff of 2.5 Å for docking reliability. RMSD values above this threshold generally indicate poor pose convergence, structural instability, or significant divergence from energetically viable conformations. In the case of Hexadecanoic acid, 1-[(2-aminoethoxy)hydro, while the energy score is appealing, the high RMSD calls for caution and may necessitate re-docking, MD simulations, or experimental confirmation to validate binding accuracy.

By contrast, Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy emerged as a strong candidate, exhibiting a binding affinity of –10.47 kcal/mol. Although this value is slightly less favorable than that of Hexadecanoic acid, 1-[(2-aminoethoxy)hydro, it still reflects a substantial interaction potential with the COX-2 active site. When compared to the co-crystallized ligand, which showed a binding affinity of –8.17 kcal/mol and an RMSD of 0.72 Å, Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy demonstrates superior binding strength. More importantly, it maintains a low RMSD of 1.49 Å, indicating a reliable and reproducible binding pose within the enzyme's active site. This combination of favourable energetics and geometric stability makes Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy particularly noteworthy. Its robust binding is likely driven by optimal steric complementarity and stable hydrogen bonding with critical active site residues, as illustrated in the interaction diagram (see Figure 2). In addition to Hexadecanoic acid, 1-[(2-aminoethoxy)hydro and Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy, Decanamide, N-(2-hydroxyethyl)- exhibited a moderate binding affinity of –4.46 kcal/mol. However, unlike Propan-2-ol, 1-(2-isopropyl-5-

methylcyclohexy, it showed a high RMSD of 3.98 Å, pointing to significant pose instability and suggesting a less reliable interaction profile.

These patterns support a key principle in structure-based drug design: strong binding alone does not guarantee ligand viability, pose reliability and chemical feasibility must also be considered. RMSD, in particular, serves as an important structural checkpoint alongside energetic parameters.

As seen in the table, Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy stands out as potential candidate with both good energetics and docking reliability. Hexadecanoic acid, 1-[(2-aminoethoxy)hydro and Decanamide, N-(2-hydroxyethyl)-, while displaying excellent affinity, may require molecular dynamics simulations or re-docking to verify pose stability due to their higher RMSD.

Structure–Activity implications (expanded discussion)

The docking results reveal meaningful structure–activity relationships (SARs) among the tested compounds, which can inform rational design strategies for selective cyclooxygenase-2 (COX-2) inhibitors. Several key patterns emerged when comparing docking scores and RMSD values to structural features:

Substituent polarity enhances binding affinity

Compounds with polar functional groups demonstrated stronger binding affinities and more reliable conformational poses (see figure 1). For example, Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy, which yielded a binding energy of -10.4743 kcal/mol and RMSD of 1.49 Å, benefited from multiple hydrogen bonding interactions with the hydrophilic residues lining the COX-2 binding tunnel (e.g., Ser353, Arg513, Leu352). These interactions mimic those exploited by known selective COX-2 inhibitors such as celecoxib, which contain diaryl heterocycles capable of targeting the enzyme's secondary binding pocket (Kurumbail *et al*., 1996).

Furthermore, the presence of hydrophilic substituents on aromatic rings may increase electrostatic complementarity with polar residues near the entrance of the active site (e.g., Arg120), further stabilizing the ligand orientation.

Steric Hindrance and Molecular Flexibility Affect Affinity

Compounds that scored poorly, exemplified by high positive binding energies (e.g., some conformers of Hexadecanoic acid, 1-[(2-aminoethoxy)hydro and others scoring >100 kcal/mol), typically share traits such as excessive molecular bulk, multiple flexible bonds, or lack of spatial complementarity. Bulky, non-planar ligands may encounter steric clashes within the narrow COX-2 binding channel or fail to achieve the correct orientation for effective interaction with critical residues, thus destabilizing the binding pose and inflating RMSD values.

Similarly, high conformational flexibility, which increases the entropic penalty upon binding, could explain suboptimal docking scores and inconsistent RMSDs across poses for compounds with rotatable bonds or long aliphatic chains (Kellenberger *et al*., 2004).

Pharmacophoric Correlation

The observed trends strongly align with one or more of known pharmacophores for selective COX-2 inhibitors, which typically include:

a) A central aromatic or heteroaromatic scaffold,

b) Hydrophobic substituents that extend into the selectivity pocket,

c) One or more hydrogen bond acceptors/donors, and

d) A geometry that mimics the orientation of arachidonic acid or selective COXIBs.

Several test compounds, especially Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy, exhibit features c and d above, reinforcing the biological relevance of the docking results and suggesting these ligands could serve as viable scaffolds for further development.

The SAR analysis underscores the importance of balanced molecular design, incorporating polar groups for key hydrogen bonding, to achieve high-affinity and reliable COX-2 binding. In contrast, oversized ligands, regardless of favourable functional groups, may lose affinity due to steric mismatch or entropic penalties. These insights can be leveraged to guide lead optimization strategies and in vitro follow-up studies.

Physicochemical Property Analysis and Drug-Likeness Evaluation

To further assess the drug-likeness and pharmacokinetic feasibility of the tested compounds, a comprehensive evaluation of their physicochemical properties was performed. The key descriptors examined include logP, aqueous solubility (logS), molecular weight, number of hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), rotatable bonds (b_rotN), and polar surface area (ASA_P). Table 4 summarizes the key properties of all 15 compounds in relation to these criteria.

Lipinski's Rule of Five Compliance

Lipinski's Rule of Five is a widely used guideline to predict oral bioavailability. The rules state that a compound is more likely to have good permeability and absorption when:

- Molecular weight ≤ 500 Da
- logP ≤ 5
- H-bond donors ≤ 5
- H-bond acceptors ≤ 10
- Rotatable bonds and polar surface area are also considered in extended guidelines (Veber *et al*., 2002).

Interpretation and lead identification

Most compounds demonstrated good compliance with Lipinski's rules. Notably:

- Compounds 3, 5, 7, 8, 12, and 14 had zero violations, indicating high drug-likeness potential.
- Compound 14, which also had the most favourable docking score (-10.47 kcal/mol), is highly promising due to its small size (161.25 Da), moderate logP (1.46), and excellent synthetic accessibility.
- Compounds 11 and 13, by contrast, violated multiple rules and displayed extreme values in logP, solubility, and molecular weight, making them poor candidates for oral delivery or further development.

Compounds with slightly elevated logP (e.g., 1, 2, 4, 6, 9, 10, 15) may still be viable but could suffer from poor solubility, which may limit bioavailability unless formulation strategies are applied (Amidon *et al*., 1995).

Aqueous solubility (logS)

Solubility, as reflected by logS, is a critical property for absorption. Values below -5 often indicate poor solubility. Compounds 3, 5, 7, 8, 12, and 14 again performed favorably, while compounds such as 1, 2, 6, 9, and 15 fell below this threshold, flagging potential absorption issues despite promising docking scores.

Implications for optimization

The physicochemical analysis provides crucial guidance for lead optimization:

- Compound 14, due to its compliance with drug-likeness filters, strong binding affinity, and chemical tractability, stands out as a lead candidate.
- Future analogs could aim to retain polar moieties, incorporate aromatic scaffolds while improving solubility and metabolic stability.
- Large or highly lipophilic compounds (e.g., 13, 11) should either be deprioritized or redesigned to reduce molecular weight and logP, aligning

them with acceptable pharmacokinetic profiles.

Protein–Ligand interaction analysis of top-performing COX-2 binders

To provide mechanistic insights into ligand binding and to rationalize docking scores, detailed 2D interaction diagrams were generated for the top-performing compounds (Figure 1). These visualizations highlight key ligand–protein contacts such as hydrogen bonds, salt bridges, and hydrophobic interactions within the COX-2 binding pocket. The selected compounds (7, 13, and 14) demonstrated high binding affinities comparable to that of the co-crystallized ligand (-8.1745 kcal/mol), reinforcing their binding consistency and relevance. Table 3 summarizes the ligand–receptor binding interactions observed in the selected compounds.

Native ligand (Figure 2A)

The redocked native ligand served as a reference, showing robust polar and hydrophobic interactions within the COX-2 active site. Key interactions include:

- Hydrogen bonds with residues Arg120, Ser353, and Arg513, which are well-established as crucial anchoring points for NSAID binding (Kurumbail *et al*., 1996).
- Fluorine-mediated interaction, enhancing binding orientation and specificity.
- The RMSD from the crystal structure is low, validating the docking protocol's reliability.

These interactions confirm that the docking methodology correctly identifies canonical COX-2 binding features.

Decanamide, N-(2-hydroxyethyl)- (Figure 2B)

Compound 7 binds to the active site with a strong hydrogen-bonding network:

- Notably forms hydrogen bonds with Arg513, mimicking native ligand interactions.
- Engages Lys83, and Glu524 via polar contacts, potentially contributing to binding stabilization.

Despite its moderate docking score (–4.46 kcal/mol), compound 7 exhibits a favorable interaction pattern resembling the native ligand, suggesting room for optimization of affinity without sacrificing specificity.

Hexadecanoic acid, 1-[(2-aminoethoxy)hydro (Figure 2C)

Compound 13 demonstrated a high binding affinity (-16.164 kcal/mol), and its interaction map reveals a highly extensive binding profile:

- Forms multiple hydrogen bonds with Val523, Arg120, and Glu524 contributing significantly to its high binding energy.
- The extended conformation and large molecular size allow engagement with both the main pocket and the secondary channel (side pocket), potentially accounting for its superior score.

However, the compound violates multiple Lipinski rules (e.g., MW = 693.99, logP = 10.14), suggesting poor drug-likeness, despite favourable binding.

Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy (Figure 2D)

Compound 14, which had the most favourable docking score among drug-like compounds (–10.47 kcal/mol) and zero Lipinski violations, exhibited:

- Strong hydrogen bonds with Arg513, and Ser353, recapitulating interactions critical for COX-2 inhibition.
- Polar contact with Leu352 potentially enhanced ligand anchorage and orientation.
- The relatively compact structure fits snugly within the COX-2 active site,

minimizing steric clashes while maximizing polar and van der Waals interactions.

This combination of favourable interactions and a compliant physicochemical profile confirms compound 14 as the most promising lead molecule from both docking and ADMET perspectives.

Conclusion

The docking study, reinforced by physicochemical profiling and drug-likeness evaluation, identifies Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy as a top candidate for COX-2 inhibition, combining a strong binding profile with favourable ADME-relevant properties, adhering fully to Lipinski's Rule of Five, offering a superior pharmacokinetic profile compared to bulkier alternatives such as Hexadecanoic acid, 1-[(2-aminoethoxy)hydro. This strengthens its candidacy as a lead molecule for further *in vitro* and *in vivo* evaluation. Structure–activity relationships suggest that hydrogen bonding potential, modest polarity, and conformational compactness are crucial in achieving high-affinity binding to COX-2. Compounds violating multiple drug-likeness criteria (e.g., Octadecanoic acid, 2-hydroxy-1,3-propanediyl e and Hexadecanoic acid, 1-[(2-aminoethoxy)hydro) are less likely to succeed in downstream development without substantial modification. This integrative analysis provides a strong foundation for rational lead optimization in COX-2 targeted drug discovery. Further study needs to be carried out on Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy to evaluate its potential oral anti-inflammatory agents devoid of GIT side effects associated with NSAIDs, since it is isolated from a functional food. Furthermore, Hexadecanoic acid, 1-[(2-aminoethoxy)hydro needs to be further investigated as topical anti-inflammatory drug. *In-silico* validation of Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexy have not been previously reported as COX-2 inhibitor.

Conflict of Interest

The authors declare that there is no conflict of interest

References

Ali, D. E., Gedaily, R. A. E., Ezzat, S. M., Sawy, M. A. E., Meselhy, M. R. and Abdel-Sattar, E. 2023. In silico and in vitro anti-inflammatory study of phenolic compounds isolated from Eucalyptus maculata resin. *Science Report*, 13(1):2093.

Amidon, G. L., Lennernäs, H., Shah, V. P. and Crison, J. R. 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical Research,* 12: 413-20.

Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K. and Uma, C. 2015. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. *Journal of Food Science Technology*, 52(2):1212-7.

Kellenberger, E., Rodrigo, J., Muller, P. and Rognan, D. 2004. Comparative evaluation of eight docking tools for docking and virtual screening accuracy. *Proteins*, 57: 225–242.

Kitchen, D. B., Decornez, H., Furr, J. R. and Bajorath, J. 2004. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Review Drug Discovery,* 3: 935–949.

Kurumbail, R. G., Stevens, A. M., Gierse, J. K., McDonald, J. J., Stegeman, R. A., Pak, J. Y., Gildehaus, D., Miyashiro, J. M., Penning, T. D., Seibert, K., Isakson, P. C. and Stallings, W.C. 1996. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, 384: 644–648.

Labute, P. 2001. A widely applicable set of descriptors. *Journal of Molecular Graphics & Modeling,* 18: 464–477.

Labute, P. 2008. The generalized Born/volume integral implicit solvent model: estimation of the free energy of hydration using London dispersion instead of atomic surface area. *Journal of Computational Chemistry,* 29: 1693–1698.

Meli, R., Morris, G. M. and Biggin, P. C. 2022. Scoring functions for protein–ligand binding affinity prediction using structure-based deep learning: A review. *Frontiers in Biology.* 2: 885983.

Meng, X. Y., Zhang, H. X., Mezei, M. and Cui, M. 2011. Molecular docking: a powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design,* 7: 146–157.

Molecular Operating Environment (MOE). 2022. *Chemical Computing Group ULC*. Montreal, QC, Canada. https://www.chemcomp.com.

Morris, G. M. and Lim-Wilby, M. 2008. Molecular docking. *Methods in Molecular Biology (Clifton, N.J.)*, 443: 365–382.

Onyeloni, S. O., Ikemefuna, C. U. and Ejiofor, I. 2025. Quantitative Phytochemical Analysis and Antioxidant Evaluation, Fractionation and Structural Elucidation of Oat Meal Extract. *Tropical Journal of Phytochemistry &. Pharmaceutical Sciences,* 4: 174 – 180.

Rajkumar, P., Sundari, S., Selvaraj, S., Natarajan, A., Suganya, R., Jayaprakash, R., Kasthuri, K. and Kumaresan, S. 2022. GC-MS, Phytochemical Analysis and In Silico Approaches of a Medicinal Plant

Acalypha indica. *Journal of Science Research*, 14(2): 671-684.

Sherman, W., Day, T., Jacobson, M. P., Friesner, R. A. and Farid, R. 2006. Novel procedure for modeling ligand/receptor induced fit effects. *Journal of Medicinal Chemistry,* 49: 534–553.

Thomsen, R. and Christensen, M. H. 2006. MolDock: A new technique for high-accuracy molecular docking. *Journal of Medicinal Chemistry*. 49: 3315–3321.

Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W. and Kopple, K.D. 2002. Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry,* 45: 2615-23. doi: 10.1021/jm020017n.

Vilar, S., Cozza, G. and Moro, S. 2008. Medicinal chemistry and the molecular operating environment (MOE): Application of QSAR and molecular docking to drug discovery. *Current Topics in Medicinal Chemistry,* 8: 1555–1572.

Wang, R., Lu, Y. and Wang, W. 2003. Comparative evaluation of 11 scoring functions for molecular docking. *Journal of Medicinal Chemistry,* 46: 2287–2303.

Warner, T. D., Giuliano, F., Vojnovic, I., Bukasa, A., Mitchell, J. A. and Vane, J. R. 1999. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proceedings of the National Academic of Sciences U.S.A.* 96: 7563–7568.

Warren, G. L., Andrews, C. W., Capelli, A. M., Clarke, B., LaLonde, J., Lambert, M. H., Lindvall, M., Nevins, N., Semus, S. F., Senger, S., Tedesco, G., Wall, I. D., Woolven, J. M., Peishoff, C. E. and Head, M.S. 2006. A critical assessment of docking programs and scoring functions. *Journal of Medicinal Chemistry,* 49: 5912–5931.

Zhang, K., Dong, R., Hu, X., Ren, C. and Li, Y. 2021. Oat-Based Foods: Chemical Constituents, Glycemic Index, and the Effect of Processing. *Foods*, 10: 1304.