Turkish Computational and Theoretical Chemistry

Turkish Comp Theo Chem (TC&TC)

Volume(Issue): 9(2) – Year: 2025 – Pages: 19-32

e-ISSN: 2602-3237

https://doi.org/10.33435/tcandtc.1442939

Received: 28.02.2024 ***Accepted***: 27.08.2024 *Research Article*

Identification of Indole Derivatives as Selective Cyclooxygenase-2 Inhibitors by Virtual Screening and Molecular Dynamic Simulation

Mohammed Abdulaali Sahib[a,b,1], Monther Faisal Mahdi[a]

[a]Department of Pharmaceutical Chemistry, College of Pharmacy, Mustansiriyah University, Baghdad, 10001, Iraq

[b]Department of Pharmaceutical Chemistry, College of Pharmacy, Kerbala University, Kerbala, 56001, Iraq

Abstract: Selective COX-2 inhibitors present a promising strategy in pain management due to their reduced adverse effects compared to non-selective COX inhibitors targeting COX-1. This study focuses on the design and virtual exploration of 8 indole derivatives using molecular docking, ADMET, and molecular dynamics (MD) simulations. Molecular docking highlighted the significance of hydrophobic and hydrophilic amino acid residues for ligand stability, with compounds 4 and 5 exhibiting notably strong in silico affinities to COX-2 (docking scores of -11.349 kcal/mol and -10.872 kcal/mol, respectively). Molecular dynamics simulations confirmed the stability of the COX-2-compound 4 complex over 50 ns, revealing consistent ligand-protein interactions through Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and ligand binding analysis. ADMET analysis of the active compounds showed favorable drug-like profiles and desirable pharmacokinetic properties. This comprehensive computational approach sheds light on the structural dynamics and pharmacological characteristics of these indole derivatives, underscoring their potential as selective COX-2 inhibitors for pain management and paving the way for further experimental validation and clinical exploration.

Keywords: Indole Derivatives, Molecular dynamic simulation, Compound 4, molecular docking, 4-Imidazolidinone, COX-2.

1. Introduction

One explanation proposes that acute inflammation is a continuous process triggered by producing pro-inflammatory chemicals such as prostaglandins (PGs) and leukotrienes (LTs). This process is regulated by pro-resolving promoters, omega-3 polyunsaturated fatty acid (PUFA)-derived molecules [1]. Several factors often trigger These inflammatory pathways, such as contact with pathogenic organisms, cellular damage, and toxic substances [2]. Vasoactive chemicals such as prostaglandins (PGs) and prostacyclin (PGE2, PGF2a, PGD2, and PGI2) cause blood vessels to dilate in an inflammatory reaction [3]. (PGs) are produced through the arachidonic acid (AA) pathway, which is catalyzed by cyclooxygenase (COX) isoenzymes, specifically COX-1 and COX-2. These PGs are known to play a vital role in the primary symptoms of acute inflammation and are significantly increased in inflamed tissues [4–7].

The COX isoenzyme is classified into two subtypes, namely COX-1 and COX-2. These subtypes have a little over 60% similarity in their amino acid composition. The enzymes' expression appears on the inner and outer nuclear envelope membranes and surfaces facing the endoplasmic reticulum (ER) lumen [8]. They differ in their preference for interacting with enzymes located up or downstream in the CNS and their distribution throughout the body [9]. Although COX-1 is fundamentally expressed in most tissues and cells, it can be induced in specific cell types when conditions are met. It is responsible for generating PGs, essential for maintaining physiologic

[1] Corresponding Authors

e-mail: mohamed_a.alalli@uomustansiriyah.edu.iq

processes and homeostasis, most notably the regulation of Kidney activities. PGs are also linked to healthy functions of the gastric and renal systems [10,11]. In contrast, COX-2 activation is mainly triggered by signals produced during inflammation. COX-2 inhibition shows the potential to reduce pain and edema without interfering with the normal functions of PGs derived by COX-1, which is a positive indication [12]. A selective COX-2 inhibitor can confer anti-inflammatory benefits, including alleviating inflammatory, fever-inducing, blood clotting, neurological, and cancer-related conditions, without the adverse effects associated with blocking COX-1 [13,14]. Therefore, there has been widespread promotion and development of nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit COX-2 specifically as compared to COX-1 [15]. COX-1 and COX-2 are homo-dimers of 576 and 581 amino acids, respectively. Both varieties of COXs contain three mannose oligosaccharides, one of which facilitates the folding of proteins. The decomposition process is regulated by an exclusive oligosaccharide found only in COX-2 [16]. The enzyme's central pillar contains a hydrophobic region that facilitates the cyclooxygenase process [17].

Each COX monomer contains three structural domains: a membrane-binding domain (MBD), a spherical catalytic domain, and a compact N-terminal epidermal growth factor domain [18–21]. Most COX monomers are entirely synthesized in the catalytic domain, which is also responsible for NSAID activity and substrate binding. A lengthy hydrophobic pathway is created by connecting the lowest of the MBD to the active site of COX, which provides entry to the catalytically active region [22,23]. By cooperating, an active center "lounge" is produced by the MBD and catalytic compartment of the COX circuit. This is primarily owing to the precise arrangement of three residues at this location: Tyr-355, Arg-120, and Glu-524. Despite the virtually identical nature of the active domains of COX-1 and COX-2, COX-2 possesses an extra area above the Tyr-355/Arg-120/Glu-524 framework [24–26]. The investigation of various chemical group-containing COX-2 antagonist families is currently underway. The primary factor underlying the molecules' preference for COX-2 is the presence of two alterations in the structure that distinguish COX-1 from COX-2. To begin with, COX-2 replaces the more substantial Ile523 with the more streamlined Val523, thereby facilitating access to The addition of a hydrophilic side region and the substitution of histidine with arginine at position 513 in COX-1 [27–30]. In the context of two isoforms, there is a conserved Leu384 present in the upper part of the channel whose positioning varies. COX-1 isoform has a phenylalanine at position 503, which compels Leu384 to orient towards the active site. In contrast, the COX-2 isoform has a slight Leucine residue at the same position, facilitating the displacement of the Leu384 side chain from the site of activity. This creates a conveniently accessible area at the top of the COX-2 interaction area [31]. As mentioned earlier, two significant differences might be used to develop specific COX-2 inhibitors since they result in a 20% increase in the size of the COX-2 active site compared to COX-1 [32,33].

Diverse NSAID frameworks interact with COX-2 in different ways. Indomethacin, naproxen, and flurbiprofen are NSAIDs; they all contain a carboxylic acid group that creates hydrogen bonds and ion pairs with Arg-120 and Tyr-355 residues placed at the active site's basis [19,34,35]. Conversely, inhibitors (e.g., diclofenac and lumiracoxib) participate in inverted interactions in the apex of the interacting center, where hydrogen bonds are formed between their carboxylates and Ser-530 and Tyr-385 [36–38]. NSAIDs such as celecoxib and rofecoxib contain sulfonamide or sulfone moieties, which exhibit more excellent activity for COX-2. These moieties bind to an additional region near the active binding site of COX-2, which is adjacent to Val-523 [39,40].

The emergence of COX-2 inhibitory ligands, such as celecoxib and rofecoxib, was precipitated by research revealing structural dissimilarities between the two varieties of COXs. Significantly fewer adverse gastric effects occur when COX-2 inhibitors are administered. Regrettably, cardiovascular (CV) events arise as a result of prolonged use of these medications. Moore et al. (2010) found a correlation between higher doses of celecoxib and myocardial infarction (MI) [41,42]. Also, rofecoxib was withdrawn from the marketplace because of its cardiovascular hazard [43]. Because of these negative consequences, a more recent anti-inflammatory medication that exhibits selectivity for COX-2 is relevant. Recent

investigations have revealed that in-silico techniques, including molecular docking and molecular dynamic stimulation, are being extensively investigated.

The indole core has been recognized as a "privileged scaffold" in medicinal chemistry. Therefore, significant research and analysis have focused on producing and characterizing indole derivatives. Compounds that include the indole nucleus are of great importance in medicinal chemistry as they serve as crucial healing agents with various therapeutic properties, including antioxidant [44], anticancer [45], anti-rheumatoid [46], and anti-HIV [47]. Additionally, these compounds play a vital role in the immunological and immune-modulatory systems [48]. Indole molecules have been identified as very efficient scavengers of free radicals [49]. The presence of the imidazole moiety is a very significant synthetic approach in the field of pharmaceutical research and development. Imidazole compounds have various applications in clinical medicine, including Antimalarial, anti-inflammatory, anticancer, antimicrobial, antifungal, antiviral, and antitubercular agents [50,51]. The imidazole nucleus is found in several natural compounds, such as metronidazole (an anti-parasitic drug), histidine (an essential amino acid), dacarbazine (anti-cancer drug), cimetidine (an antihistaminic), and losartan (An antihypertensive drug agent) [52,53]. Based on the information above, the current research was designed to explore novel indole derivatives containing a 4-imidazolidinone ring, as shown in figure 1, and assess their potential anti-inflammatory properties. Several functional groups were included in the target molecules to explore the development of a new category of anti-inflammatory medicines with enhanced selectivity towards the COX-2 enzyme.

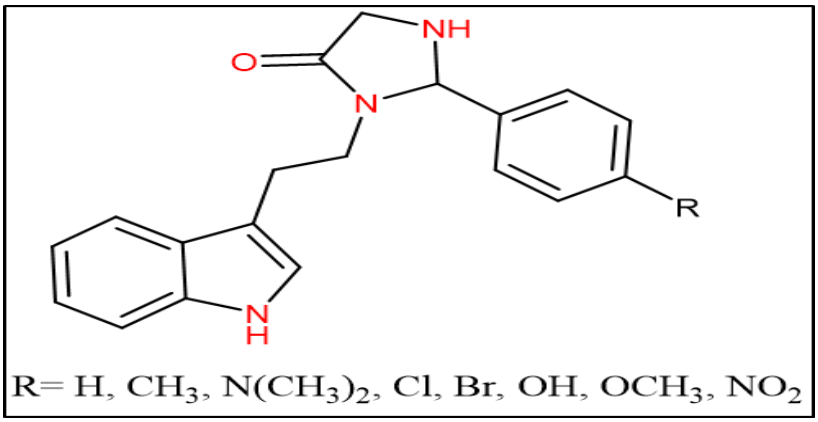

Figure 1. The general structure of proposed compounds.

The current study examined eight indole compounds' binding affinity and selectivity. In addition, in silico analyses were conducted to assess the selectivity and interaction of these compounds with the COX-2 protein. The most appropriate compounds were virtually screened using QikProp, using various physicochemical filters such as ADMET characteristics and Lipinski's rule of five. The compounds and reference ligands were subjected to docking analysis inside the binding site of cyclooxygenase-2. This analysis aimed to determine the specific residues contributing to their reported affinity and selectivity. Subsequently, molecular dynamics simulations lasting 50 nanoseconds were conducted on the complex between ligand and COX-2 protein to examine the dynamic nature of the interactions between the ligand and the target. This work enables us to comprehend the structural interactions of the molecule that may have the capacity to suppress COX-2.

2. Computational Method

2.1. Ligand Preparation

The LigPrep tool transforms 2D into 3D structures. Once integrated into the initial stage, ligand activity's 3D shapes and values were used to refine and generate conformers utilizing the OPLS force field for each minimized ligand [54].

2.2. ADMET Prediction

Schrodinger's QikProp software version 2020.3 assessed the pharmacokinetic features of eight indole derivatives, including absorption,

distribution, metabolism, and excretion (ADME) [55]. Identify characteristics that may match cell permeability and bioavailability. The pharmacokinetics of the designed compounds were also analyzed using the freely available tool Swiss-ADME (http://www.swissadme.ch), including factors such as the degree of passive absorption from the gastrointestinal tract and the blood-brain barrier [56].

2.3. Molecular Docking

Computational computations were conducted using The Schrodinger Suite for Small Molecule Drug Discovery. The protein's atomic coordinates were obtained through X-ray crystallography analysis of COX-2 (PDB: 4M11) [57]. The COX-2 enzyme was set up for calculations using the Protein Preparation Wizard from Schrodinger [58]. The central point of the grid box was determined based on the position of co-crystallized reference compounds and then adjusted and established at a distance of 20 A°. To produce three-dimensional structures with minimal energy consumption, the Lig-Prep module was set up within the OPLS-AA force field [59]. Glide was used to conduct molecular docking experiments. Glide identifies the optimal ligand binding position within the grid space of proteins by assessing their energy interactions [60].

2.4. Molecular Dynamics Simulation

To determine the stability of the complex molecular dynamic simulation and the mode of ligand-receptor binding, a 50-ns simulation was performed. The experiment uses the Desmond program in Schrodinger software on a Linux platform. Initially, the interaction between a simple point charge (SPC) water simulation was employed to solvate the receptor and ligand, ensuring compliance with an orthorhombic box boundary condition. A 50 mM solution was prepared by adding sodium and chloride ions to neutralize the system. The NPT ensemble was executed at a consistent temperature of 300 K and a pressure of 1.01325 bar. A recording interval of 50 picoseconds was employed, with an energy of 1.2. The entire investigation was conducted using the force field of OPLS3e in MD simulation.

After analyzing the dynamic simulation, the Simulation Interaction Diagram was used to generate trajectories. This trajectory, in conjunction with the root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and protein-ligand contacts, was employed to interpret the stability and interaction of the protein-ligand complex [61].

3. Results and discussion

3.1. Molecular Docking Analysis

We conducted molecular docking simulations to analyze the possible binding mechanisms of promising ligands to COX-2 as potential inhibitors at a molecular level. The docking findings analysis helped to rationalize the COX-2 inhibition expectations. All investigated compounds were effective inhibitors with binding solid affinities to the target protein COX-2, ranging from -11.349 to -9.940 Kcal/mol, as shown in table 1. We were studying the interaction of compounds 1-8 with COX-2 by determining the binding affinities of the proposed compounds towards COX-2. According to molecular docking research, compounds 4 and 5 had the most excellent docking scores of (-11.349 and -10.872 kcal/mol, respectively). Figure 2 (A and B) clearly illustrates that compound 4 forms H-bonds with amino acid residues ALA527, ARG120, TYR355, and LYS360. Further, molecule 4 has a hydrophobic interaction with MET535, LEU534, LEU531, ALA527, LEU117, VAL116, MET113, LEU359, and PHE361.

Indole derivatives compounds contain an imidazolidinone ring, which contributes significantly to the binding affinity through its ability to form hydrogen bonds with critical amino acid residues within the COX-2 active site. This ring structure enhances the overall stability of the ligand-protein complex by providing additional sites for interaction. The indole moiety, a core structure in the investigated compounds, is recognized for its planar and aromatic nature, which allows it to participate effectively in π-π stacking interactions with aromatic residues such as TYR355 and PHE361. This interaction is essential for anchoring the ligand within the active site and maintaining the orientation necessary for inhibitory activity. The indole ring's hydrophobic character also facilitates strong interactions with hydrophobic residues, further stabilizing the ligand within the COX-2 binding pocket. Compound 4 contains the chlorine substituent, particularly when attached to

the indole or other aromatic rings, enhancing the binding interactions through both electronic and steric effects. Chlorine atoms can bond halogen with amino acid residues, adding another interaction layer to the binding affinity. Moreover, chlorine's electron-withdrawing nature can increase the aromatic ring's overall electrophilicity, making it more reactive towards hydrogen bonding and other electrostatic interactions with the active site residues. These structural features of imidazolidinone, indole, and chlorine moieties significantly enhance the binding interactions with COX-2, contributing to the high binding affinities observed in the docking studies. This detailed understanding of the molecular interactions provides valuable insights into designing more potent and selective COX-2 inhibitors.

Meloxicam and celecoxib reference medicines were docked into the binding region to assess their binding position and interaction with indole derivatives. Meloxicam and celecoxib had total docking scores of (-7.890 and -9.516 Kal/mol, respectively) as shown in table 1. Interaction of compound 4 with the same amino acid of reference drug (meloxicam), as shown in figure 3 (A and B) and having a high docking score in the COX-2 active site propose that this compound might prevent the arachidonic acid from binding to COX-2 to facilitate oxygenation. Compound 4 demonstrated the standard binding mode typically employed by the reference inhibitor. However, it is essential to recognize the limitations inherent in these computational predictions. While docking simulations offer valuable estimates of potential binding interactions, they need to fully account for the dynamic and complex nature of biological systems where these interactions occur. An investigation into the molecular dynamics was conducted to evaluate the overall structural stability of the protein-ligand complex.

No.	R group	Docking score Kcal/mol	Interaction type		
			H bond	Hydrophobic	*Cation–π*
1	H	-10.395	SER530	LEU534, LEU531, ALA527, LEU352, TYR355, LEU117, VAL116, MET113, LEU534	
2	$N(CH_3)_2$	-10.675	SER530	VAL349, LEU352, TYR355, PHE357, LEU359, LEU534, LEU531, ALA527, MET522	ARG120
3	CH_3	-10.623	SER530	VAL523, MET522, VAL349, LEU352, TYR355, PHE357, LEU359, ALA527, LEU531	ARG120
4	CL	-11.349	ALA527, ARG120, TYR355, LYS360	MET535, LEU534, LEU531, ALA527, LEU117, VAL116, MET113, LEU359, PHE361	
5	Br	-10.872	SER530	LEU531, VAL523, MET522, VAL349, LEU352, TYR355, PHE357, LEU359, MET535	ARG120
6	OH	-10.453	SER530	LEU531, VAL523, MET522, VAL349, LEU352, TYR355, PHE357, LEU359, MET535	ARG120
7	OCH_3	-10.166	SER530	LEU534, LEU531, ALA527, MET522, VAL349, LEU352, TYR355, PHE357, LEU359	ARG120
8	NO_2	-9.940	SER530	VAL523, MET522, VAL349, LEU352, TYR355, PHE357, LEU359	
Reference drug	Meloxicam	-7.890	SER530, ARG120, TYR355	MET522, VAL523, ALA527, PRO528, LEU531, LEU534	
	Celecoxib	-9.516	VAL523	LEU359, VAL523, ALA527, LEU531, LEU534, MET535, MET113, VAL116, LEU117	

3.2. Molecular Dynamics Simulations

Considering the emphasis on conformational stability in theoretical research, it is interesting to investigate how ligands affect specific proteins using MD simulations. This article explores the conformational stability of COX-2 with Compound 4 and a reference drug (Meloxicam) over a 50 ns period. We studied the impact of these ligands on the COX-2 backbone over time by analyzing the RMSD of the protein core concerning conformation variations and interactions with ligands, which provides significant structural insights into the physical alterations.

The RMSD plot for compound 4 showed a consistent connection with COX-2, with the ligand's RMSD fluctuations staying close to 1.2 Å and the protein within 2.4 Å, achieving stability after ten ns during the MD simulation (figure 4-A).

In contrast, meloxicam's RMSD plot indicated that the system achieves stability also after approximately ten ns, with the ligand's RMSD values close to 1.5 Å and the protein RMSD stabilizing around 2.6 Å (figure 5-A). The RMSF for each residue near compound 4 was consistently below 0.4 Å, indicating a stable binding pocket throughout the MD simulation, as shown in figure (4-B, C). At the same time, meloxicam also showed low fluctuations, suggesting stable interactions within the binding pocket (figure 5-B, C).

During the MD simulation, COX-2 bound to compound 4 exhibited a strong binding relationship, forming hydrogen bond interactions with residues such as SER530, ALA527, VAL523, TYR355, and ARG120 and also, interacting with GLU524, VAL523, ARG513, TYR355, SER353, LEU352, ARG120, and HIS90 by bridging hydrogen bonds, with significant hydrophobic interactions involving amino acids such as LEU534, LEU531, ALA527, PHE518, TRP387, TYR385, LEU384, PHE381, LEU359, TYR355, LEU352, and VAL349 as shown in figure 6. That makes compound 4 strongly bind with the primary amino acid in COX-2, especially in the extra region and not in COX-1. Whereas meloxicam formed stable hydrogen bonds with SER530, ALA527, and TYR355, and engaged in significant hydrophobic interactions with PHE381, LEU534, and VAL523, maintaining consistent contact with critical active site residues, including ARG120, TYR355, and VAL523 as shown in figure 7.

Both meloxicam and compound 4 showed stable binding to COX-2, demonstrating similar RMSD and RMSF values. This suggests compound 4 is as stable and effective as meloxicam and supports its potential as a new COX-2 inhibitor with comparable stability and efficacy.

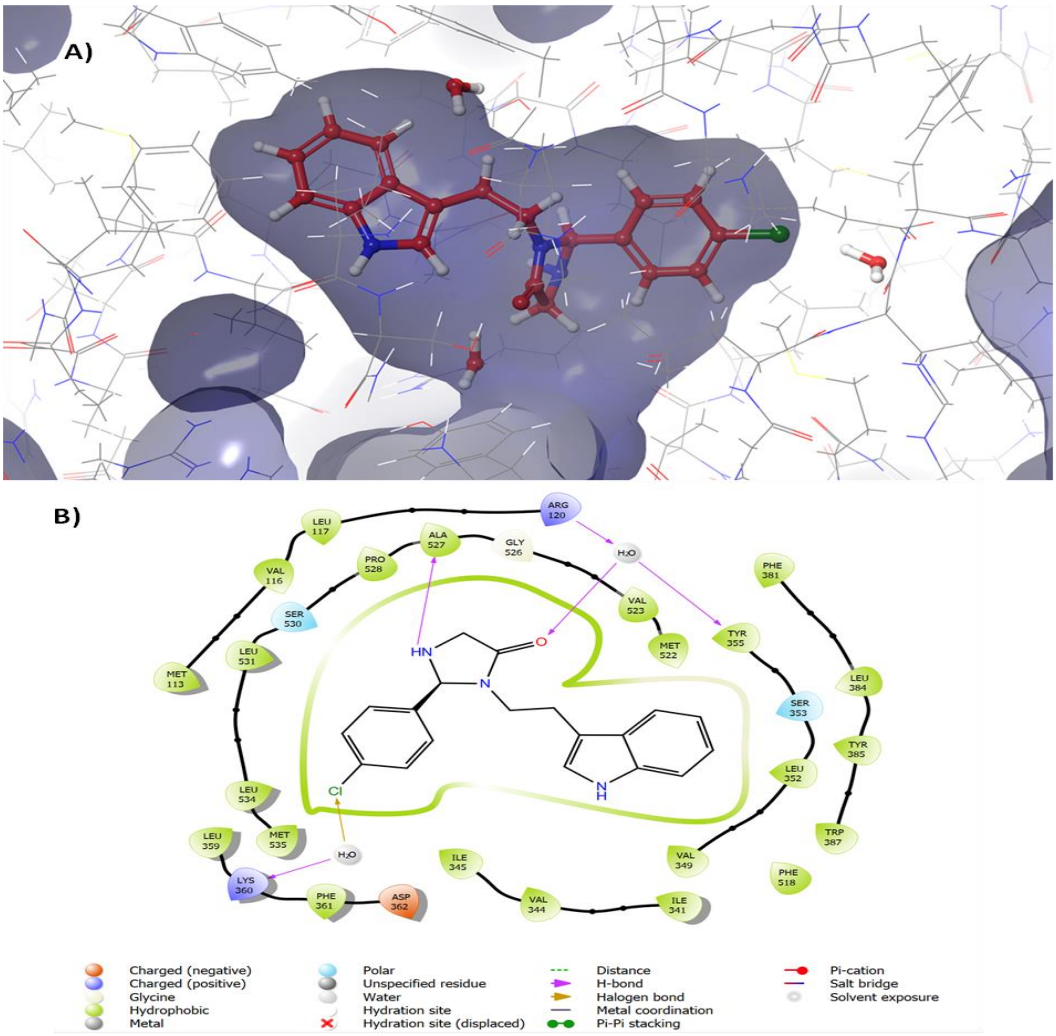

Figure 2. The interaction of compound 4 with the active binding site of COX-2. A) 3D structure docked inside the pocket, B) 2D structure interactions synchronized with 3D structure.

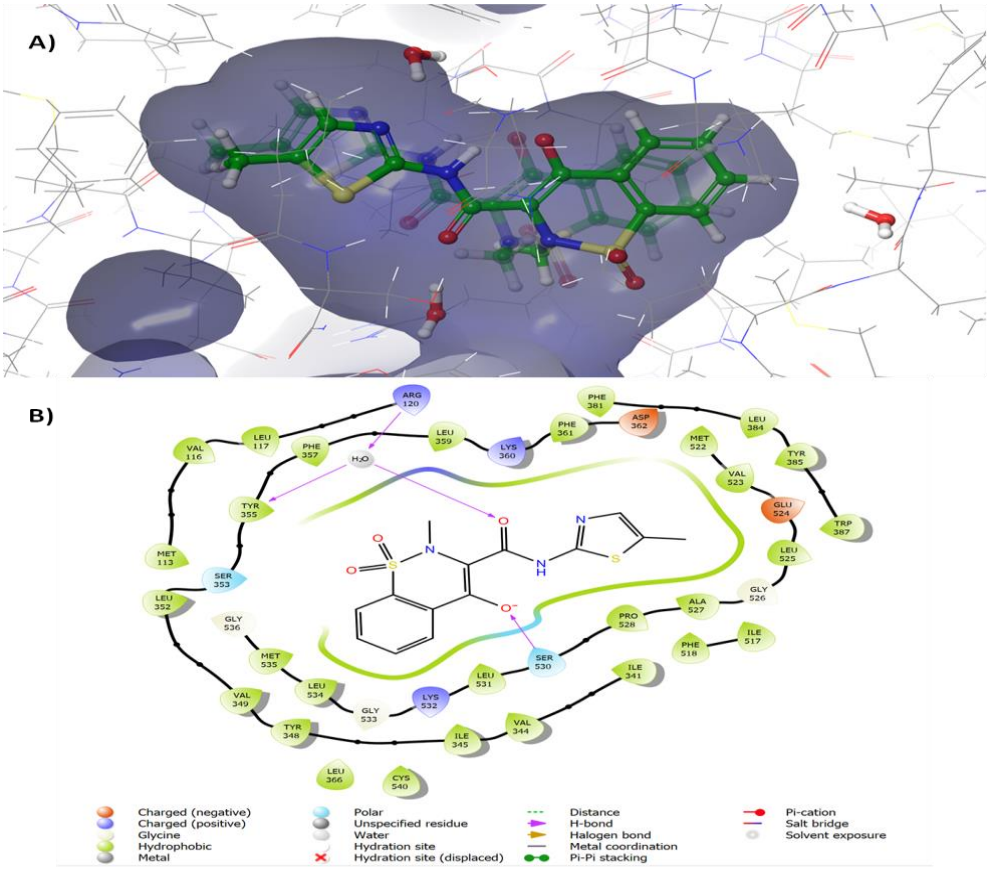

Figure 3. The interaction of meloxicam with the active binding site of COX-2. A) 3D structure docked inside the pocket, B) 2D structure interactions synchronized with 3D structure.

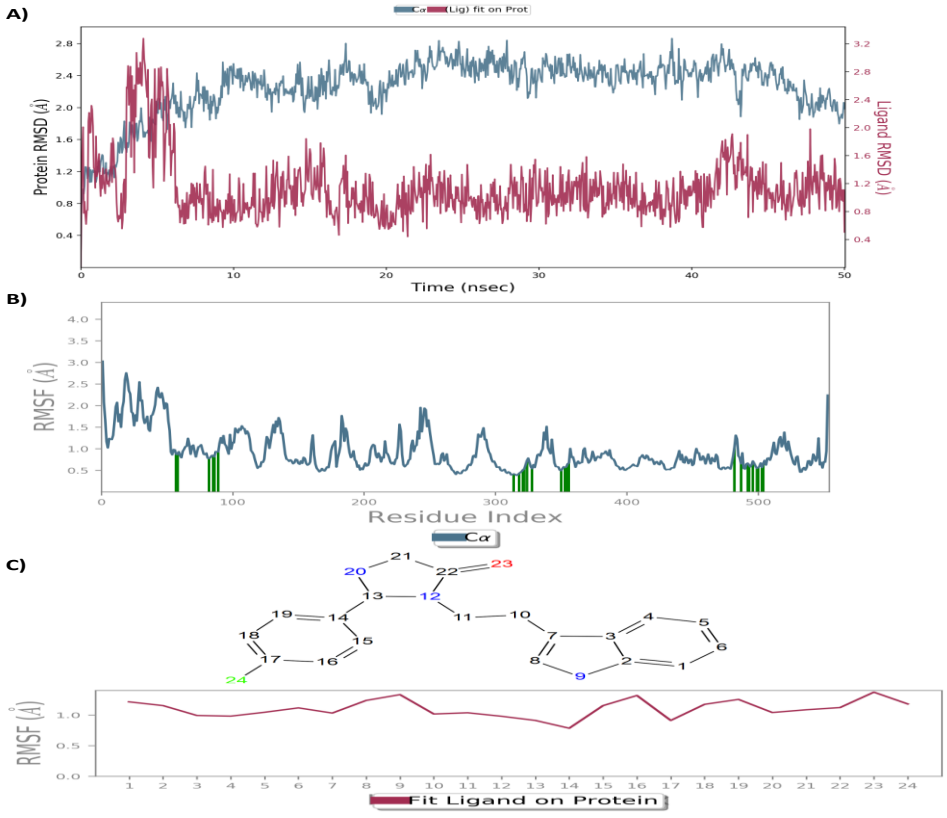

Figure 4: Compound 4 analysis: A) RMSD, B) protein-RMSF, C) ligand-RMSF.

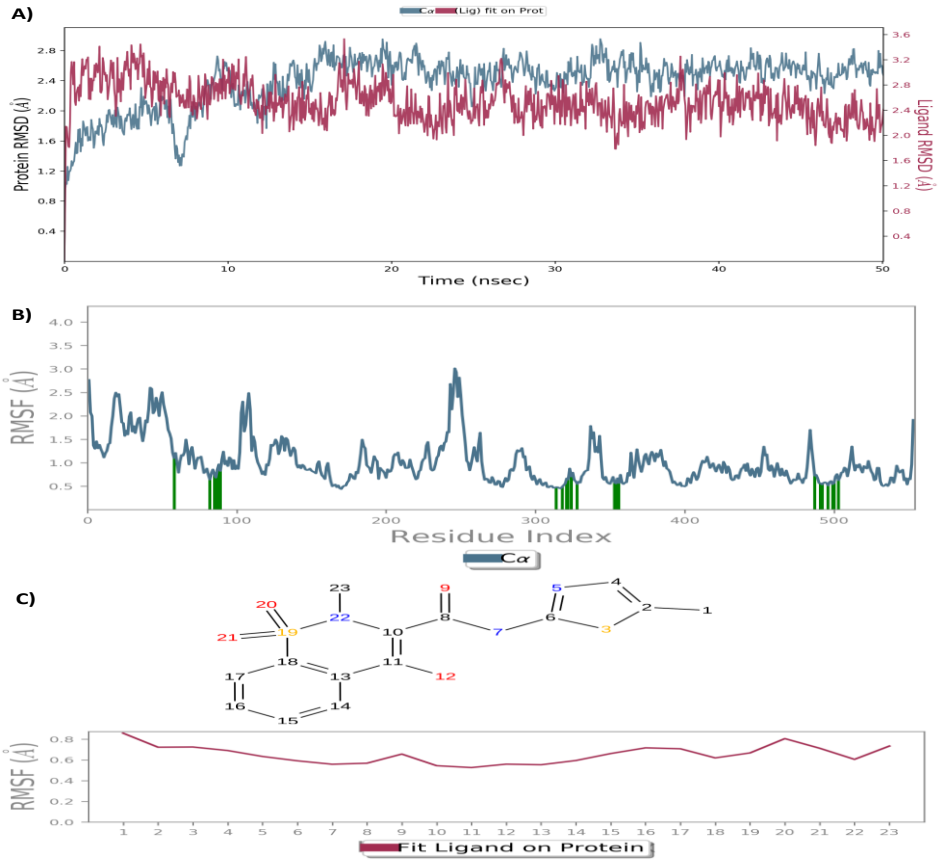

Figure 5. Reference drug (meloxicam) analysis: A) RMSD, B) protein-RMSF, C) ligand-RMSF.

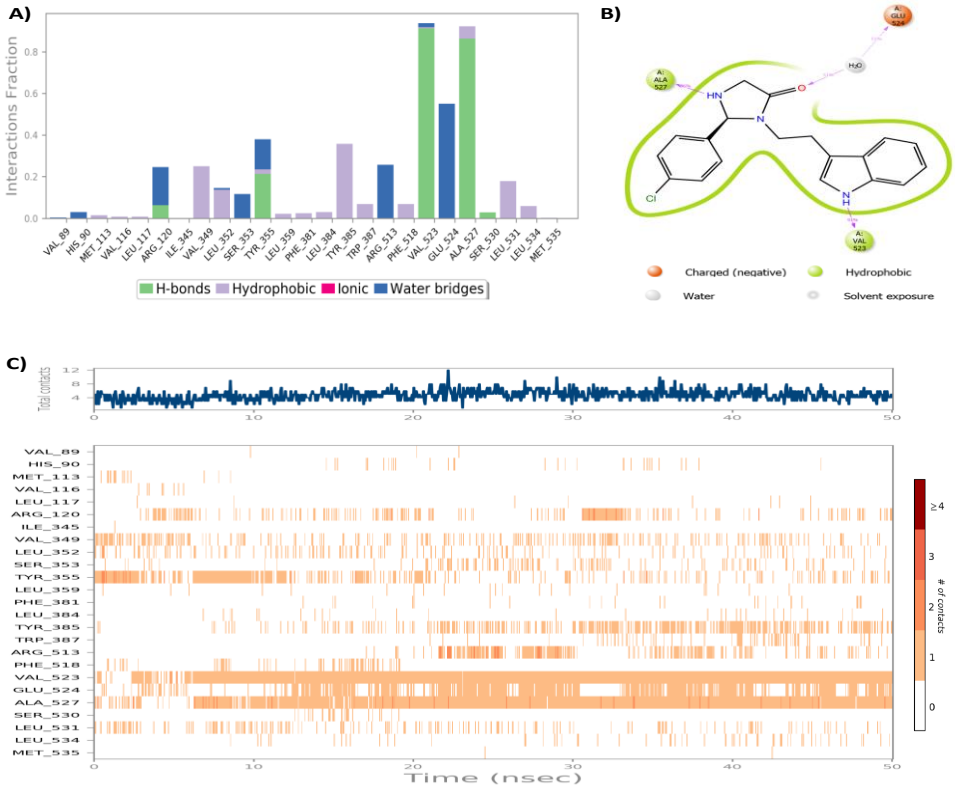

Figure 6. Scheme of interaction of compound 4 with COX-2. A) COX-2-compound 4 contacts explain the proportion of binding interactions. (B) and (C) Interaction of compound 4 with residues during all MD trajectories.

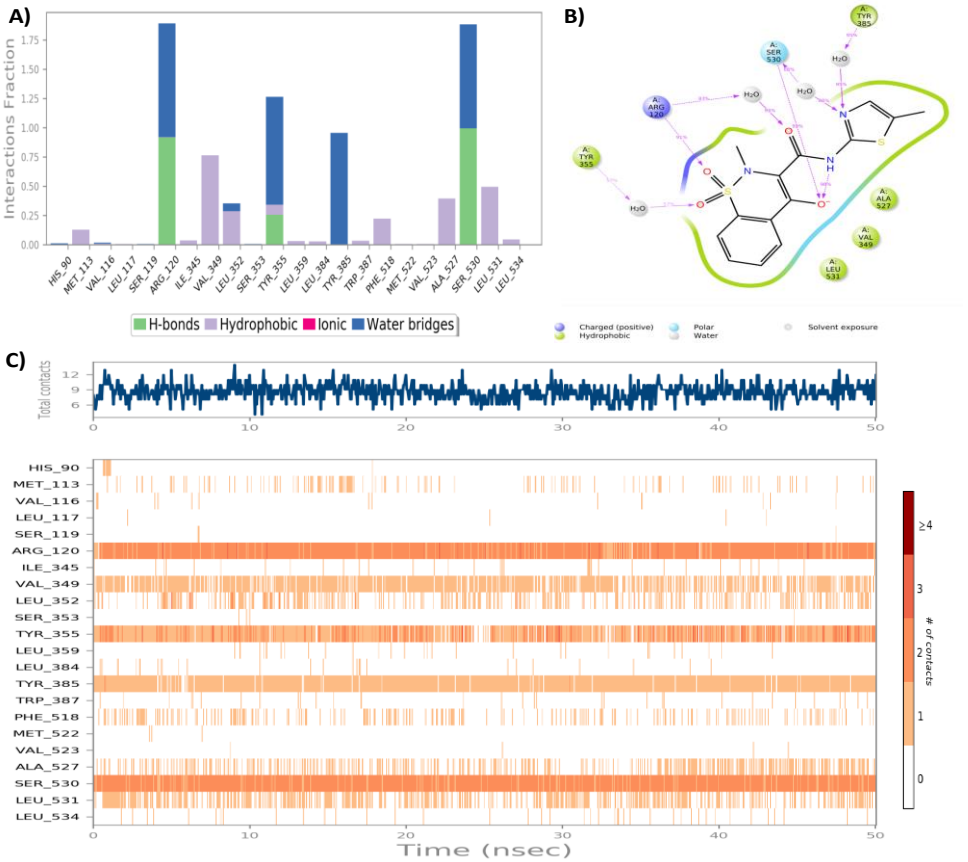

Figure 7. Scheme of interaction of meloxicam with COX-2. A) COX-2-meloxicam contacts explain the proportion of binding interactions. (B) and (C) Interaction of meloxicam with residues during all MD trajectories.

3.3. Drug-Likeness Evaluation

Computational estimation of pharmacokinetic features plays a significant role in expediting the identification of novel compounds with favorable qualities and identifying and rejecting those with unfavorable pharmacokinetic characteristics in the initial design phases. Table 2 provides data on molecular weight (mol_MW), number of hydrogen bond acceptors (accptHB), number of hydrogen bond donors (donorHB), Lipinski's Rule of Five violations (Rule of Five), octanol-water partition coefficient (QPlogPo/w), aqueous solubility (QPlogS), predicted IC50 value for HERG Kp channel blockage (QPloghERG), apparent Caco-2 cell permeability (OPPCaco), apparent MDCK cell permeability (OPPMDCK), and human oral absorption of eight compounds (1-8). The ADME parameters of proposed compounds obey within the approved range of most drug-likeness assessments.

Table 2. Computational estimations of pharmacokinetic characteristics of indole derivatives.

Compound	Accepted range	1	2	3	4	5	6	7	8
MOL_MW	<500	305.379	348.447	319.405	339.824	384.275	321.378	335.405	350.376
DONORHB	≤5	2	2	2	2	2	3	2	2
ACCPTHB	≤10	4.5	5.5	4.5	4.5	4.5	5.5	5.5	5.5
QPLOGPO/W	–2.0–6.5	2.145	2.720	2.297	2.740	2.707	1.262	2.235	1.314
QPLOGS	–6.5–0.5	-2.110	-3.280	-2.351	-3.097	-2.946	-1.548	-2.323	-1.993
QPLOGHERG	concern below –5	-5.117	-5.505	-4.879	-5.291	-5.098	-4.820	-5.064	-4.888
QPPCACO	<25 poor, >500 great	156.546	154.520	119.141	179.951	156.679	35.950	167.416	14.489
QPLOGBB	–3.0–1.2	-0.116	-0.272	-0.212	0.061	0.045	-0.760	-0.174	-1.185
QPPMDCK	<25 poor, >500 great	146.880	148.410	119.596	409.437	389.299	32.754	158.804	12.265

percent human oral absorption	>80% is high, <25% is poor	78.782	82.053	77.555	83.354	82.083	62.181	79.832	55.421
rule of five	maximum is 4	0	0	0	0	0	0	0	0

Brain permeability and gastrointestinal absorption are crucial factors in assessing the bioavailability of designed compounds. The two variables of the designed compounds were identified by utilizing the diagram of a boiled egg constructed using the parameters LogP and topological polar surface area (TPSA) [62,63]. The graphical depiction of the BOILED-Egg model is shown in figure 8. The results indicate that compounds 1 to 8 (shown by blue dots) exhibit a high degree of passive absorption from the gastrointestinal tract. Furthermore, it is expected that these compounds can cross the blood-brain barrier (BBB) and subsequently be eliminated by P-glycoprotein from the central nervous system (CNS), except for compound 8, which is unable to cross the blood-brain barrier.

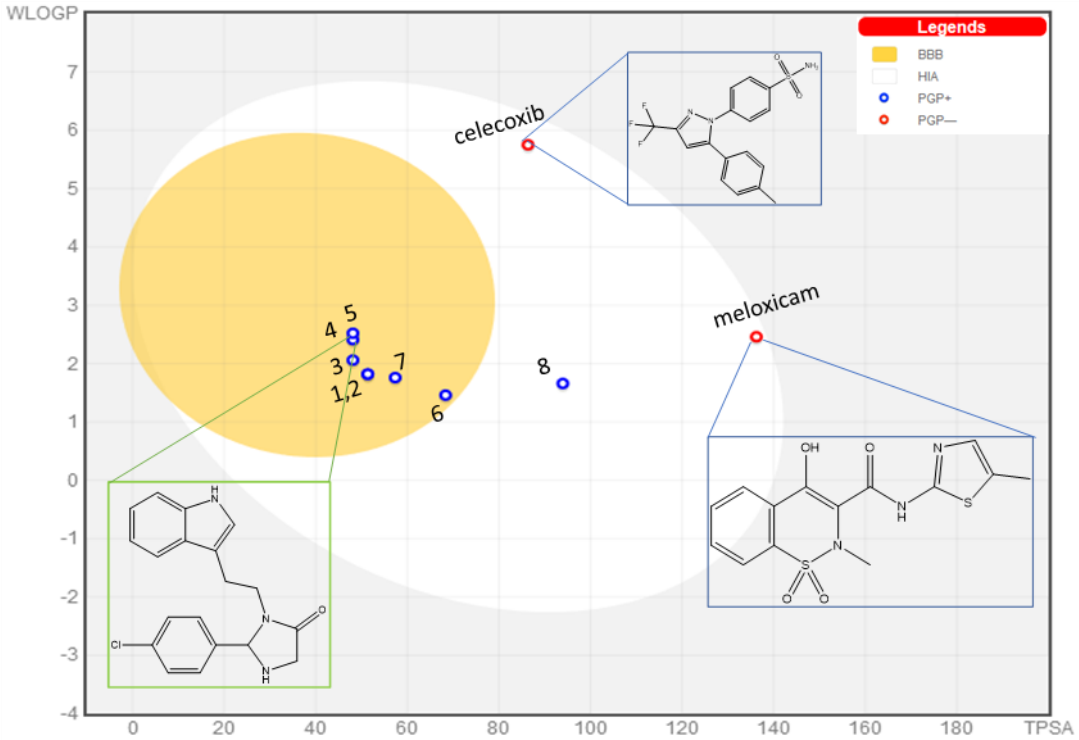

Figure 8. BOILED-Egg represented compounds (1-8) and reference drugs. Yellow yolk: molecules pass BBB. White: molecules absorbed by GIT. Blue dots: P-glycoprotein substrate. Red dots: No P-glycoprotein substrate. CNS is the central nervous system, GIT is the gastrointestinal tract, and BBB is the blood-brain barrier.

The ADMET profiles predicted through tools like QikProp and Swiss-ADME are based on established algorithms and known physicochemical properties, offering a preliminary assessment of a compound's drug-likeness. However, these predictions are not definitive and may not fully capture the compound's behavior in vivo. Factors such as metabolic stability, off-target effects, and the compound's ability to reach the target site in sufficient concentration are critical determinants of its actual efficacy and safety, which cannot be accurately predicted solely through in silico methods.

To translate the promising computational findings into clinical practice, initial preclinical studies should validate the efficacy and safety of the lead COX-2 inhibitors through in vitro and in vivo models. Subsequent early-phase clinical trials (Phase I) should focus on safety, tolerability, and optimal dosing in healthy volunteers. Phase II trials should evaluate the efficacy and safety in patients with inflammatory conditions, while Phase III trials should confirm these findings in larger populations

through randomized, double-masked, placebo-controlled studies. Post-marketing surveillance (Phase IV) is essential to monitor long-term safety and efficacy. These steps are crucial for developing safe and effective COX-2 inhibitors for clinical use.

4. Conclusions

This research investigated the possibility of new indole derivatives with a 4-imidazolidinone ring as anti-inflammatory drugs that target COX-2. Eight compounds were assessed for their binding affinity, selectivity, pharmacokinetic characteristics, and stability in complex with COX-2 using molecular docking, ADMET analysis, and molecular dynamics simulations. The molecular docking analysis showed strong interactions between designed compounds and COX-2, with compounds 4 and 5 demonstrating the most significant binding affinities. The compounds exhibited good interactions with critical amino acid residues in the COX-2 active site, indicating their potential as potent inhibitors. Molecular dynamics simulations verified the stability of the COX-2-compound 4 complex for 50 nanoseconds. The analysis of RMSD and RMSF data showed stable binding and low variations in the protein-ligand complex, suggesting the potential effectiveness of compound 4 as a COX-2 inhibitor. The ADMET analysis revealed the pharmacokinetic characteristics of the compounds, indicating favorable drug-like qualities and good pharmacokinetics. The BOILED-Egg model showed that most compounds exhibited high passive absorption in the gastrointestinal system, indicating they are suitable for oral delivery. Overall, this comprehensive computational study suggests that the designed indole derivatives have the potential to serve as promising candidates for further development as anti-inflammatory agents targeting COX-2.

Declaration of Competing Interest

There are no conflicts to declare

Acknowledgement

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) in Baghdad, Iraq, for their support in completing this work.

References

[1] Sachdeva S, Saluja H, Mani A, Phadnaik MB, Mani S. Lipoxins in inflammation. Clin Hemorheol Microcirc 2022;82:201–16.

[2] Chang MX, Xiong F. Astaxanthin and its Effects in Inflammatory Responses and Inflammation-Associated 2020:1–14.

[3] Soares CLR, Wilairatana P, Silva LR, Moreira PS, Vilar Barbosa NMM, da Silva PR, et al. Biochemical aspects of the inflammatory process: A narrative review. Biomed Pharmacother 2023;168:115764.

[4] Ochiai T, Honsawa T, Sasaki Y, Hara S. Prostacyclin Synthase as an Ambivalent Regulator of Inflammatory Reactions. Biol Pharm Bull 2022;45:979–84.

[5] Kolawole OR, Kashfi K. NSAIDs and Cancer Resolution: New Paradigms beyond Cyclooxygenase. Int J Mol Sci 2022;23.

[6] Calder PC. Eicosanoids. Essays Biochem 2020;64:423–41.

[7] Kaur B, Singh P. Inflammation: Biochemistry, cellular targets, anti-inflammatory agents and challenges with special emphasis on cyclooxygenase-2. Bioorg Chem 2022;121:105663.

[8] Akhtar W, Nainwal LM, Khan MF, Verma G, Chashoo G, Bakht A, et al. Synthesis, COX-2 inhibition and metabolic stability studies of 6-(4-fluorophenyl)-pyrimidine-5-carbonitrile derivatives as anticancer and anti-inflammatory agents. J Fluor Chem 2020;236:109579.

[9] Famitafreshi H, Karimian M. Prostaglandins as the Agents That Modulate the Course of Brain Disorders. Degener Neurol Neuromuscul Dis 2020;10:1–13.

[10] Cheng H, Huang H, Guo Z, Chang Y, Li Z. Role of prostaglandin E2 in tissue repair and regeneration. Theranostics 2021;11:8836–54.

[11] Jara-Gutiérrez Á, Baladrón V. The role of prostaglandins in different types of cancer. Cells 2021;10.

[12] Jiahua C, Jinping J. Natural COX-2 Inhibitors as Promising Anti-inflammatory Agents: An Update. Curr Med Chem 2021;28:3622–46.

[13] El-Malah AA, Gineinah MM, Deb PK, Khayyat AN, Bansal M, Venugopala KN, et al. Selective COX-2 Inhibitors: Road from Success to Controversy and the Quest for Repurposing. Pharmaceuticals

2022;15:1–19.

[14] Lees P, Toutain P-L, Elliott J, Giraudel JM, Pelligand L, King JN. Pharmacology, safety, efficacy and clinical uses of the COX-2 inhibitor robenacoxib. J Vet Pharmacol Ther 2022;45:325–51.

[15] Mahesh G, Anil Kumar K, Reddanna P. Overview on the Discovery and Development of Anti-Inflammatory Drugs: Should the Focus Be on Synthesis or Degradation of PGE2? J Inflamm Res 2021;14:253–63.

[16] Krishnendu PR, Benny S, Kumar S, Jayan J, Bhaskar V, Pappachen LK, et al. A structure-based approach to explore novel COX-2 inhibitors using pharmacophore modelling, 3D-QSAR analysis, virtual screening and dynamics simulation study. J Mol Struct 2024;1295:136634.

[17] Hajhashemi V, Ghanadian M, Palizaban A, Mahnam K, Eshaghi H, Gheisari B, et al. Cycloarta-23-ene-3beta,25-diol a pentacyclic steroid from Euphorbia spinidens, as COX inhibitor with molecular docking, and in vivo study of its analgesic and anti-inflammatory activities in male swiss mice and wistar rats. Prostaglandins Other Lipid Mediat 2020;150:106473.

[18] Miciaccia M, Belviso BD, Iaselli M, Cingolani G, Ferorelli S, Cappellari M, et al. Three-dimensional structure of human cyclooxygenase (hCOX)-1. Sci Rep 2021;11:4312.

[19] Rouzer CA, Marnett LJ. Structural and Chemical Biology of the Interaction of Cyclooxygenase with Substrates and Non-Steroidal Anti-Inflammatory Drugs. Chem Rev 2020;120:7592–641.

[20] Sejdiu BI, Tieleman DP. COX-1 – lipid interactions: arachidonic acid, cholesterol, and phospholipid binding to the membrane binding domain of COX-1. BioRxiv 2020:2020.05.21.109363.

[21] Perrone MG, Miciaccia M, Vitale P, Ferorelli S, Araújo C da CB, de Almeida GS, et al. An attempt to chemically state the cross-talk between monomers of COX homodimers by double/hybrid inhibitors mofezolac-spacer-mofezolac and mofezolac-spacer-arachidonic acid. Eur J Med Chem 2021;209:112919.

[22] Dong L, Malkowski MG. Defining the Conformational Ensembles Associated with Ligand Binding to Cyclooxygenase-2. Biochemistry 2023;62:3134–44.

[23] Martínez-Puente DH, Garza-Morales R, Pérez-Trujillo JJ, Bernabé-Acosta F, Villanueva-Olivo A, García-García A, et al. Enhanced antitumor activity induced by a DNA vaccine encoding E7 antigen fused to an ERAD-targeting sequence. J Drug Target 2023;31:100–8.

[24] Uzzaman M, Mahmud T. Structural modification of aspirin to design a new potential cyclooxygenase (COX-2) inhibitors. Silico Pharmacol 2020;8:1.

[25] Araújo PHF, Ramos RS, da Cruz JN, Silva SG, Ferreira EFB, de Lima LR, et al. Identification of potential COX-2 inhibitors for the treatment of inflammatory diseases using molecular modeling approaches. Molecules 2020;25.

[26] Abdellatif KRA, Abdelall EKA, Elshemy HAH, Lamie PF, Elnahaas E, Amin DME. Design, synthesis of new anti-inflammatory agents with a pyrazole core: COX-1/COX-2 inhibition assays, anti-inflammatory, ulcerogenic, histopathological, molecular Modeling, and ADME studies. J Mol Struct 2021;1240:130554.

[27] Peregrym K, Szczukowski Ł, Wiatrak B, Potyrak K, Czyżnikowska Ż, Świątek P. In vitro and in silico evaluation of new 1,3,4-oxadiazole derivatives of pyrrolo[3,4-d]pyridazinone as promising cyclooxygenase inhibitors. Int J Mol Sci 2021;22:1–21.

[28] Naser NH, Abdul Aziz Alibeg A, Jawad AbdAl-Zahra A. Design, synthesis, in silico study and preliminary pharmacological evaluation of ibuprofen derivatives containing 1, 3, 4-Oxadiazole moiety. Mater Today Proc 2022;65:2669–75.

[29] Rosa FA, Gonçalves DS, Pianoski KE, da Silva MJ V, Ames FQ, Aguiar RP, et al. Discovery of a new pyrido[2,3-d]pyridazine-2,8-dione derivative as a potential anti-inflammatory agent through COX-1/COX-2 dual inhibition. RSC Med Chem 2024;15:1038–45.

[30] Ismail M, Hassan MHA, Mohamed EIA, Azmy AF, Moawad A, Mohammed R, et al. New insights into the anti-inflammatory

and anti-melanoma mechanisms of action of azelaic acid and other Fusarium solani metabolites via in vitro and in silico studies. Sci Rep 2024;14:14370.

[31] Garhwal S. THE DEVELOPMENT OF COX-1 AND COX-2 INHIBITORS: A REVIEW Rahul Kumar Vishwakarma and D. S. Negi * Department of Chemistry, H. N. B. Garhwal University (A Central University), Srinagar Garhwal - 249161, Uttarakhand, India. 2020;11:3544–55.

[32] Mohan M, Hussain MA, Khan FA, Anindya R. Symmetrical and un-symmetrical curcumin analogues as selective COX-1 and COX-2 inhibitor. Eur J Pharm Sci 2021;160:105743.

[33] Gomaa M, Gad W, Hussein D, Pottoo FH, Tawfeeq N, Alturki M, et al. Sulfadiazine Exerts Potential Anticancer Effect in HepG2 and MCF7 Cells by Inhibiting TNFα, IL1b, COX-1, COX-2, 5-LOX Gene Expression: Evidence from In Vitro and Computational Studies. Pharmaceuticals 2024;17.

[34] de Carvalho Bertozo L, Tadeu HC, Sebastian A, Maszota-Zieleniak M, Samsonov SA, Ximenes VF. Role for Carboxylic Acid Moiety in NSAIDs: Favoring the Binding at Site II of Bovine Serum Albumin. Mol Pharm 2024;21:2501–11.

[35] Deplano A, Karlsson J, Moraca F, Svensson M, Cristiano C, Morgillo CM, et al. Design, synthesis and in vitro and in vivo biological evaluation of flurbiprofen amides as new fatty acid amide hydrolase/cyclooxygenase-2 dual inhibitory potential analgesic agents. J Enzyme Inhib Med Chem 2021;36:940–53.

[36] C S S, Natarajan K. Nonsteroidal Anti-Inflammatory Drug Leads from Plant Sources BT - Drugs from Nature: Targets, Assay Systems and Leads. In: Haridas M, Abdulhameed S, Francis D, Kumar SS, editors., Singapore: Springer Nature Singapore; 2024, p. 179–203.

[37] Josiane CV, Joaquín RMC, Njogu KM, Silvana G, Cleydson dos SBR. The Role of Celecoxib as a Potential Inhibitor in the Treatment of Inflammatory Diseases - A Review. Curr Med Chem 2022;29:3028–49.

[38] Ganai SA, Rajamanikandan S, Shah BA, Lone A, Arwa F, Malik FA. Comparative structural study of selective and non-selective NSAIDs against the enzyme cyclooxygenase-2 through real-time molecular dynamics linked to post-dynamics MM-GBSA and e-pharmacophores mapping. J Mol Model 2023;29:192.

[39] Gedawy EM, Kassab AE, El Kerdawy AM. Design, synthesis and biological evaluation of novel pyrazole sulfonamide derivatives as dual COX-2/5-LOX inhibitors. Eur J Med Chem 2020;189:112066.

[40] Sağlık BN, Osmaniye D, Levent S, Çevik UA, Çavuşoğlu BK, Özkay Y, et al. Design, synthesis and biological assessment of new selective COX-2 inhibitors including methyl sulfonyl moiety. Eur J Med Chem 2021;209:112918.

[41] Moore AH, Bigbee MJ, Boynton GE, Wakeham CM, Rosenheim HM, Staral CJ, et al. Non-Steroidal Anti-Inflammatory Drugs in Alzheimer's Disease and Parkinson's Disease: Reconsidering the Role of Neuroinflammation. Pharmaceuticals 2010;3:1812–41.

[42] Mohsin N ul A, Irfan M. Selective cyclooxygenase-2 inhibitors: A review of recent chemical scaffolds with promising anti-inflammatory and COX-2 inhibitory activities. Med Chem Res 2020;29:809–30.

[43] Wang W, Wertheimer AI. History, status, and politicization of the FDA. Res Soc Adm Pharm 2022;18:2811–6.

[44] Kumar J, Kumar N, Sati N, Hota PK. Antioxidant properties of ethenyl indole: DPPH assay and TDDFT studies. New J Chem 2020;44:8960–70.

[45] Mehra A, Sharma V, Verma A, Venugopal S, Mittal A, Singh G, et al. Indole Derived Anticancer Agents. ChemistrySelect 2022;7:e202202361.

[46] Nisha, Singh S, Sharma N, Chandra R. The indole nucleus as a selective COX-2 inhibitor and anti-inflammatory agent (2011–2022). Org Chem Front 2022;9:3624–39.

[47] Qingtai C, Chongchong W, Jinjin Z, Enzhong L, Zhi X. Therapeutic Potential of Indole Derivatives as Anti-HIV Agents: A

Mini-review. Curr Top Med Chem 2022;22:993–1008.

[48] Fiore A, Murray PJ. Tryptophan and indole metabolism in immune regulation. Curr Opin Immunol 2021;70:7–14.

[49] Elkamhawy A, Oh NK, Gouda NA, Abdellattif MH, Alshammari SO, Abourehab MAS, et al. Novel Hybrid Indole-Based Caffeic Acid Amide Derivatives as Potent Free Radical Scavenging Agents: Rational Design, Synthesis, Spectroscopic Characterization, In Silico and In Vitro Investigations. Metabolites 2023;13.

[50] Fanjie H, Lina Z, Kutty NS, Kui C. Imidazole Scaffold Based Compounds in the Development of Therapeutic Drugs. Curr Top Med Chem 2021;21:2514–28.

[51] Siwach A, Verma PK. Synthesis and therapeutic potential of imidazole containing compounds. BMC Chem 2021;15:12.

[52] Rulhania S, Kumar S, Nehra B, Gupta GD, Monga V. An insight into the medicinal perspective of synthetic analogs of imidazole. J Mol Struct 2021;1232:129982.

[53] Tolomeu H V, Fraga CA. Imidazole: Synthesis, Functionalization and Physicochemical Properties of a Privileged Structure in Medicinal Chemistry. Molecules 2023;28.

[54] Agarwal S, Dixit A, Kashaw SK. Ligand and structure based virtual screening of chemical databases to explore potent small molecule inhibitors against breast invasive carcinoma using recent computational technologies. J Mol Graph Model 2020;98:107591.

[55] AL-Duhaidahawi D, AL-Zubaidy HFS, Al-Khafaji K, Al-Amiery A. Synthesis, anti-inflammatory effects, molecular docking and molecular dynamics studies of 4-hydroxy coumarin derivatives as inhibitors of COX-II enzyme. J Mol Struct 2022;1247:131377.

[56] Samer Tareq Jasim, Monther F. Mahdi. Molecular Modeling, Synthesis, and preliminary pharmacological evaluation of New Sulfonamide Derivatives as Selective Carbonic Anhydrase XII and IX inhibitors (Research). Al Mustansiriyah J Pharm Sci 2024;24:137–49.

[57] alqaysi yazen, Shakir M. Alwan, Ashour H. Dawood. In Silico Prediction of Binding Affinities of Hybrid Molecules of Benzothiazole cross-linked with Hydroxamic acid by certain linkers. Al Mustansiriyah J Pharm Sci 2024;24:59–67.

[58] Beura S, Chetti P. Identification of potential human COX-2 inhibitors using computational modeling and molecular dynamics simulations. J Mol Struct 2020;1216:128271.

[59] Pandi S, Kulanthaivel L, Subbaraj GK, Rajaram S, Subramanian S. Screening of Potential Breast Cancer Inhibitors through Molecular Docking and Molecular Dynamics Simulation. Biomed Res Int 2022;2022:3338549.

[60] Taslimi P, Erden Y, Mamedov S, Zeynalova L, Ladokhina N, Tas R, et al. The biological activities, molecular docking studies, and anticancer effects of 1-arylsuphonylpyrazole derivatives. J Biomol Struct Dyn 2021;39:3336–46.

[61] Wu N, Zhang R, Peng X, Fang L, Chen K, Jestilä JS. Elucidation of protein–ligand interactions by multiple trajectory analysis methods. Phys Chem Chem Phys 2024;26:6903–15.

[62] Anaridha S, Imran PKM, Meeran IS, Shabeer TK. Comparative theoretical analysis using DFT & MP2 calculations, ADMET profiling and docking studies of the drug telotristat ethyl. J Indian Chem Soc 2021;98:100248.

[63] alsalamy zaid, Ayad Kareem Khan, Mohammed Dheyaa Hamdi, Atheer Awad Mehde. Drug Design, In Silico-Profiling of New Pyrrole Derivatives: Promising Anticancer Agents Against Acute Myeloid Leukemia. Al Mustansiriyah J Pharm Sci 2024;24:252–63.