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In-silico design, molecular docking, molecular dynamic simulations, Molecular mechanics with generalised Born and surface area solvation study, and pharmacokinetic prediction of novel diclofenac as anti-inflammatory compounds

Mazen Mohammed Jwaid[a,1], Abdulmohaimen Amjed Adnan[b]

[a]Department of Pharmaceutical chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
[b]Department of pharmaceutical chemistry, College of pharmacy, Al-Turath University, Baghdad, Iraq.

Abstract: The prostaglandins inside inflamed tissues are produced by cyclooxygenase-2 (COX-2), making it an important target for improving anti-inflammatory medications over a long period. Adverse effects have been related to the traditional usage of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of inflammation, mainly centered around gastrointestinal (GI) complications. The current research involves the creation of a virtual library of innovative molecules showing similar drug properties via a structure-based drug design. A library that includes five novel derivatives of Diclofenac was designed. Subsequently, molecular docking through the Glide module and determining the binding free energy implementing the Prime-MMGBSA module by the Schrödinger software package was used to identify compounds that showed marked specificity towards the COX-2 isoform. In addition, the ligands are subject to evaluation of their drug-like properties and ADMET (absorption, distribution, metabolism, excretion, and toxicity) characteristics using the QikProp module. Finally, molecular dynamics simulation has been calculated for the best molecule. The docking results indicated that all compounds own a predictive capability for specific binding to the COX-2 enzyme compared to the standard drug with a docking score range from -10.07 to -10.66 Kcal/mole, thus potentially overcoming the limitations imposed previously by the drugs currently used in clinical use. The ADMET analysis of the virtually active compounds demonstrated an acceptable drug-like profile and desirable pharmacokinetics properties. MM/GBSA calculation revealed that all the suggested compounds exhibited favorable free binding energies (-49.150 to - 60.185 Kcal/mole), indicating their strong potential to fit well into the COX-2 receptor. Finally, the MD simulation study revealed that compound 1 had perfect alignment with COX-2 receptor.

Keywords: Cyclooxygenase-2, Diclofenac derivatives, structure-based drug design, MMGBSA module, ADMET and molecular dynamic simulation.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the classical and most effective pharmaceutical entities for relieving inflammation and pain by inhibiting prostaglandin synthesis. However, using them is linked to many side effects. NSAIDs, such as diclofenac(Fig.1), are recommended to improve various levels of inflammation associated with a range of conditions which include such as acute musculoskeletal disorders, arthritic disorders, and other illnesses brought on by physical trauma. The use of NSAIDs has been associated with gastrointestinal bleeding, ulceration, or perforations, which can result in fatal consequences. Occurrences of serious gastrointestinal complications can arise during treatment, regardless of a patient's medical history. In cases where an individual experiences gastrointestinal bleeding or ulceration while taking diclofenac, the treatment will discontinue [1,2]. Both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes play

[1] Corresponding Authors
e-mail: mazen.m@copharm.uobaghdad.edu.iq

significant roles in inflammation. COX is a type of integral membrane protein that exists in two different forms, known as COX-1 and COX-2. The majority of tissues contain COX-1, which is regarded as a fundamental enzyme responsible for the regulation of many essential cellular processes. Conversely, COX-2 is activated in response to specific stimuli and is mainly connected to inflammation, fever, and pain processes [3]. Due to the similar structures and sequences of both isoforms, NSAIDs like Diclofenac behave nonselectively (Fig. 2) [4].

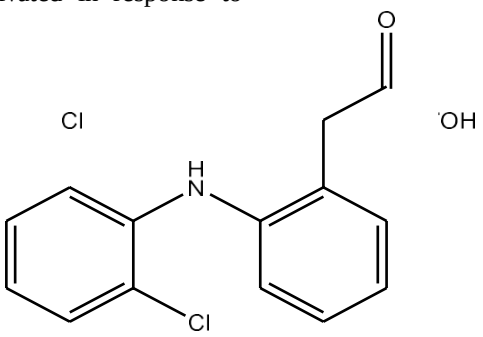


Figure 1. 2D representation of Diclofenac structure.

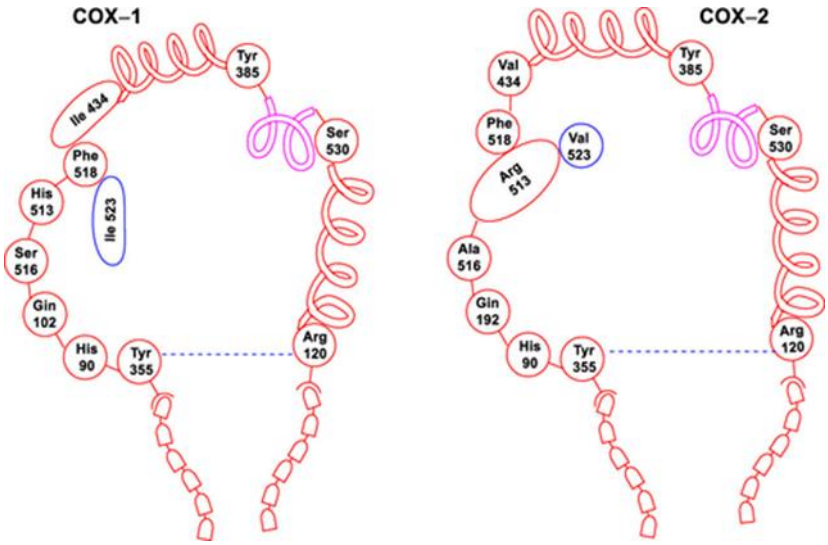


Figure 2. Structures of COX-1 and COX-2.

Long-term COX-1 enzyme-targeting medication use increases the risk of liver or kidney damage as well as gastrointestinal (GI) problems like ulceration [5]. Consequently, in an effort to lessen these side effects, researchers have worked to identify selective NSAIDs [6]. Specifically, diclofenac (DCF) is a well-known and often prescribed nonsteroidal anti-inflammatory drug (NSAID) that is used to treat fever, rheumatoid arthritis (RA), postoperative pain, and migraine headaches. Like other NSAIDs, diclofenac inhibits prostaglandin synthesis by blocking the COX-1 and COX-2 enzymes. Thromboxane-receptor protein inhibition and control over arachidonic acid uptake and release [7], protection against leukocyte-endothelium interactions, suppression of lipoxygenase enzymes, and stimulation of the nitric oxide (NO)/cyclic guanosine monophosphate (GMP) pathway are just a few of the mechanisms by which DCF has anti-inflammatory effects [8,9]. Molecular docking is an effective drug discovery tool. This technique is effective at evaluating a lot of ligands, which makes it a useful tool in searching for novel effective compounds [10-17]. Additionally, the results of docking studies yield results that are easily comparable using ligand scoring, which facilitates the identification of promising candidates for synthesis and experimental validation [18,19]. Studies conducted in silico have many benefits, including new

perspectives on various molecular aspects. These include studying molecular electrostatic potential, performing molecular dynamic simulations, and comprehending solvation effects. All of these contribute to a better understanding of how compounds behave in different environments [20,21]. We created a number of Diclofenac (DCF) derivatives using computational molecular docking techniques in order to assess how well they bonded to target proteins [22,23].

The study aims to achieve the following objectives. Investigate the impact of newly designed Diclofenac (DCF) derivatives on their target proteins using computational molecular docking methods. Determine the binding energies of five novel ligands (1, 2, 3, 4, and 5) when interacting with both COX-1 and COX-2 receptors for assessing the way of interaction between ligands and the target receptors. Predict the relative ligand-receptor complex binding free energies using the primary MM-GBSA technique, which allows for a comprehensive assessment of the thermodynamic aspects of binding. Analyze the various energy components contributing to the overall binding energies, including the individual energies of the ligand, COX-2 receptor, and the complex formed between them. This analysis can help identify the key factors driving the binding interactions. Conduct an in silico ADME prediction study To estimate the designed compounds' pharmacokinetic characteristics and drug-likeness. To further confirm the interaction, compound with the best docking in complex with COX 2 was subjected to MDS analysis. This research aims to understand how novel DCF derivatives interact with COX-1/COX-2 receptors. The study will use computational molecular docking and energy calculation methods to gain insights into the binding properties of these compounds and their potential as drug candidates.

2. Computational Method

2.1. Molecular Docking studies

Molecular docking studies performed using the Schrödinger Maestro software version to examine the interactions between newly designed diclofenac derivatives (1–5) and the COX-1 and COX-2 receptors (PDB ID: 4O1Z and 5IKR, respectively). The study also examined the interactions of Diclofenac, Meloxicam, and Mefenamic acid with the same receptors. This study's primary goal was to comprehend the molecular mechanisms underlying these substances' binding to the COX-1 and COX-2 receptors. This would offer important details regarding their binding styles and possible applications as medicinal substances. LigPrep was utilized in the research process to prepare the ligands for the molecular docking study. LigPrep uses the Build Panel to generate 3D ligand representations and optimizes ligand structures for docking simulations.

The crystallographic structures of receptors obtained from Protein Data Bank, so that we could perform protein docking. Specifically, we retrieved structures with PDB IDs 4O1Z(COX-1) and 5IKR(COX-2). The Protein Preparation Wizard was used to prepare these structures for the docking investigation. For the purpose of creating a clean and appropriate environment for the docking simulations, the preparation steps involved adding hydrogen atoms and eliminating solvent molecules. In cases where the protein structures had bound ligands, such as Meloxicam and Mefenamic acid, additional steps were taken to ensure accurate simulation of ligand binding interactions and assessment of binding affinities. The protein-ligand complexes were minimized to ensure that the bound ligands were in energetically favorable conformations within the protein binding site. Molecular docking was produced by referencing the cocrystallized bound ligands. These grids helped define the potential binding sites within the catalytic sites of the target proteins (COX-1 and COX-2), which was crucial for guiding the docking simulations.

As a part of the validation process for the docking protocol, Meloxicam (in COX-1) and Mefenamic acid (in COX-2) were redocked into the catalytic sites of the proteins. The successful occupation of the similar binding pockets by these reference ligands as seen in the crystallographic structures further supports the accuracy of the docking methodology.

The molecular docking simulations employed the Glide extra-precision (XP) mode, which is known for its high accuracy in predicting ligand binding poses and affinities. The docking process involved compounds 1-5, as well as Diclofenac, Meloxicam, and Mefenamic acid. For each molecule, the docking simulations generated and saved three

potential binding poses, utilizing the XP mode. This approach allowed for the exploration of different orientations and conformations of the ligands within the binding sites, helping to identify the most energetically favorable binding modes. It has been shown that this approach is helpful in anticipating how small molecule inhibitors will bind to their target proteins [24,25].

2.2. GBSA/MM Study

In this study, the Prime module within the Schrödinger molecular modeling package was used to perform energy calculations. The GBSA/MM (Generalized Born Surface Area/Molecular Mechanics) analysis was applied to determine the free energies of binding for all the designed compounds, as well as for the COX-2 cocrystallized ligand, Mefenamic acid, when it interacts with the COX-2 receptor (5IKR).

The energy calculations used the OPLS3 (Optimized Potentials for Liquid Simulations 3) force field. This force field is a set of mathematical functions that accurately describe the energy and geometry of molecular interactions, enabling precise estimation of binding energies and prediction of binding affinities.

The VSGB 2.0 model of solvation was used to account for the effects of solvent molecules on ligand-receptor binding energetics. This model is effective in considering the solvation effects in the calculations. The energy calculations were performed using complex structures representing the COX-2 receptor bound to the ligand. The purpose of these calculations was to understand the thermodynamics of ligand binding, specifically the free energy involved in binding. These free energies of binding are crucial in evaluating the strength of ligand-receptor interactions and their potential as drug candidates [24]. The binding free energies were calculated using specific equations [26].

The Prime MM-GBSA method is a computational technique that determines the energy of three main components: the optimized energy of the receptor alone, the optimized energy of the ligand alone, and the complex created when the ligand and receptor bind. In addition, it also estimates the ligand's strain energy through its simulation in a solution produced with the VSGB 2.0 suite. The Prime Energy Visualizer tool is then utilized to generate a visual representation of these energy calculations, which helps with the interpretation and analysis of energy-related data.

2.3. Molecular dynamic simulation

MDS was performed for the derivative with the best docking score using the Desmond modules of the Schrodinger 2023 with the OPLS4 force field [27]. To create a charge-neutral system for the protein-ligand complex, sodium ions were added, and 0.15 M sodium chloride NaCl was included to mimic the natural system. Utilizing the TIP3P solvent model, the system was produced [28]. The simulation was run for 50 ns, with recording intervals of 50 ps for the trajectory. The NPT ensemble class was utilized, and the system energy was set to 1.2. The simulation was set to operate at 1.01325 bar and 300 k. To create the simulation interaction diagram, the simulated system was evaluated after it was relaxed [29, 30].

2.4. ADMET Study

The study utilized the QikProp module of Maestro-Schrodinger to predict various pharmacokinetic and physicochemical properties with high accuracy. This software is well-known for being user-friendly, precise, and fast in predicting vital descriptors that help in understanding how drugs are absorbed, distributed, metabolized, excreted, and their potential toxicity (ADME-T) [31].

3. Results and discussion

In this study, we examined two distinct scoring systems: Glide DockingScore and MM-GBSA (Molecular Mechanics Generalized Born Surface Area) [32]. Prioritizing the created compounds and selected NSAIDs was the goal. Glide DockingScore is an empirical scoring function that has been calibrated for virtual screening to distinguish between molecules that bind and those that do not. These two approaches are very different from one another. However, Prime MM-GBSA is a physics-based method that computes the force field energies while accounting for the implicit solvent effects of the molecules involved in the binding event, both bound and unbound.

3.1. Molecular Docking Results

Molecular docking analysis can give a good insight into the binding modes of many active compounds [33–35]. In this research, molecular docking was

used to test five compounds and three anti-inflammatory medications (Diclofenac, Meloxicam, and Mefenamic acid) against COX-1 and COX-2 receptors. According to the findings, each molecule exhibited an analogous binding mode in the binding pocket of both COX receptors. The binding pockets were identified by their PDB IDs as 4O1Z (COX-1) and 5IKR (COX-2), as shown in Table 1 [24, 25].

The docking scores of the compounds were compared to those of Diclofenac and Mefenamic acid, which served as reference ligands. All of the compounds are superior to the reference ligands in terms of docking scores., indicating favorable binding. Moreover, the ligands showed a stronger affinity for COX-2 than for COX-1.

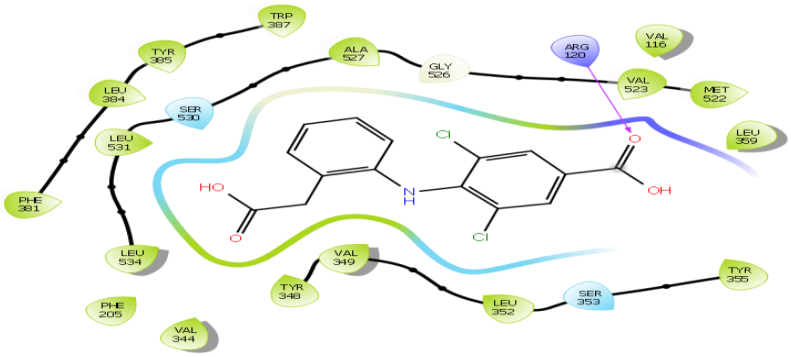


Figure 3. The interaction of compound 1 with COX-2 (5KIR) is illustrated.

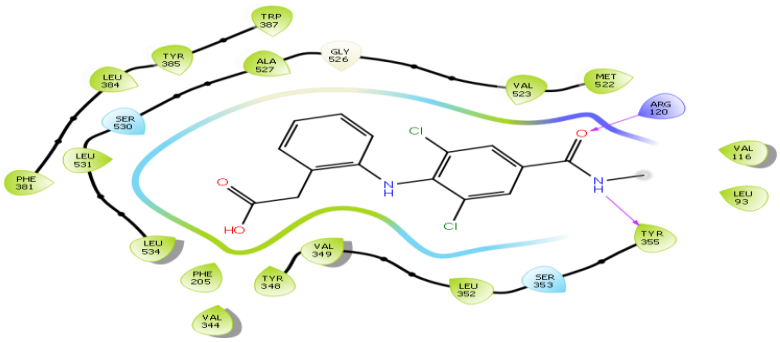


Figure 4. Ligand interaction of compound 2 with COX-2 (5KIR).

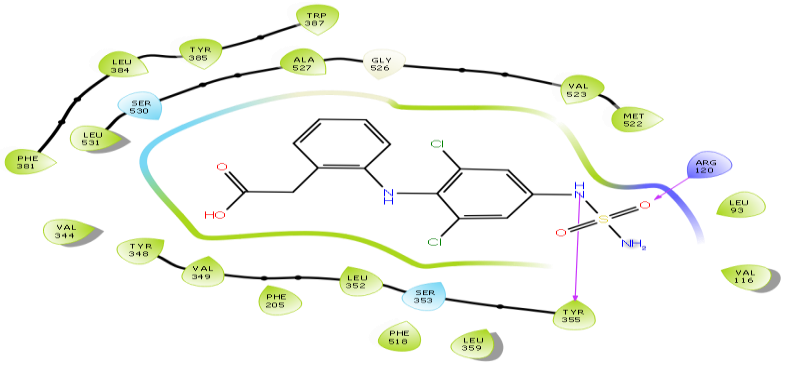
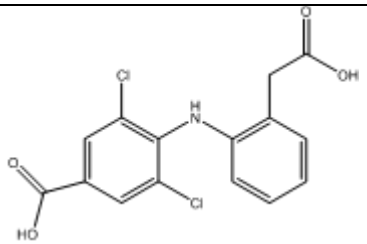
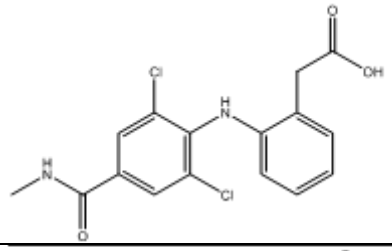
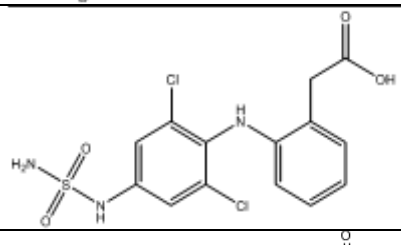
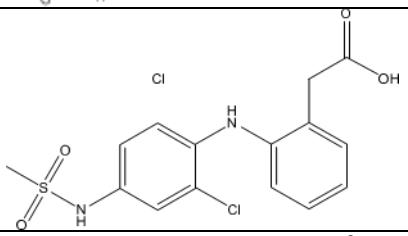
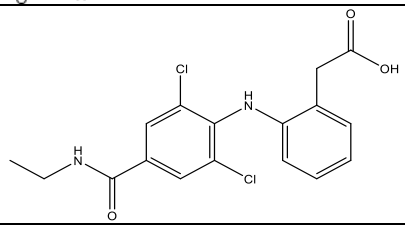
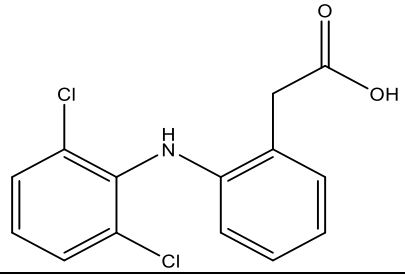
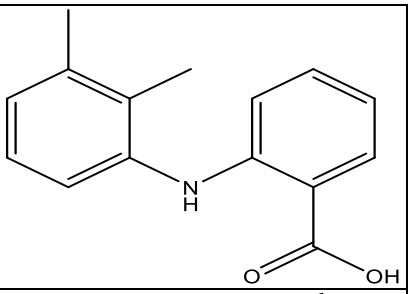
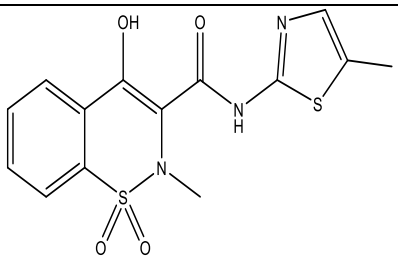


Figure 5. Ligand interaction of compound 3 with COX-2 (5KIR).

Comp 1 showed the highest docking score within the COX-2 receptor and had good binding interactions with the COX-2 receptor backbone. These interactions included favorable hydrophobic interactions with VAL349, TYR348, VAL344, PHE205, and LEU534, along with hydrogen bonding interactions involving ARG120. Notably, ARG120 is an important residue in both catalysis and binding processes (Fig. 3). Comp 5 showed a comparable pattern of interaction within the catalytic domain of the COX-2 receptor (Fig. 7).

Table 1. The molecular docking scores of all compounds at the active sites of COX-1. (PDB:4O1Z) and COX-2(5IKR) enzymes

Ligand	2D structure	COX-1 Docking score (Kcal/mole)	COX-2 Docking score (Kcal/mole)
1		-8.42	-10.66
2		-7.97	-10.25
3		-7.59	-10.09
4		-7.76	-10.01
5		-8.45	-10.07
Diclofenac		-8.83	-9.3

Mefenamic acid			-9.788
Meloxicam		-8.35	

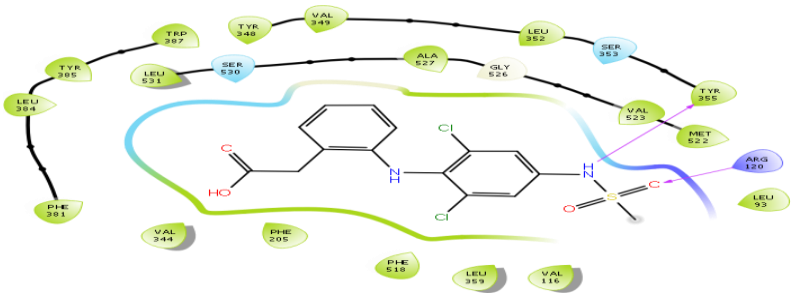


Figure 6. Ligand interaction of compound 4 with COX-2 (5KIR).

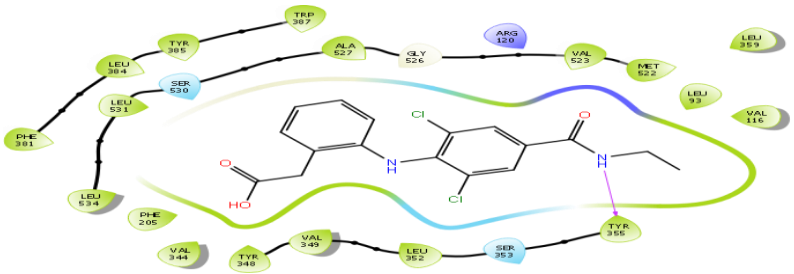


Figure 7. Ligand interaction of compound 5 with COX-2 (5KIR).

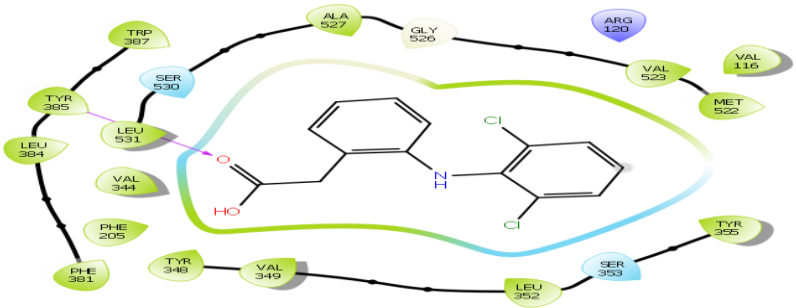


Figure 8. Ligand interaction of Diclofenac with COX-2 (5KIR).

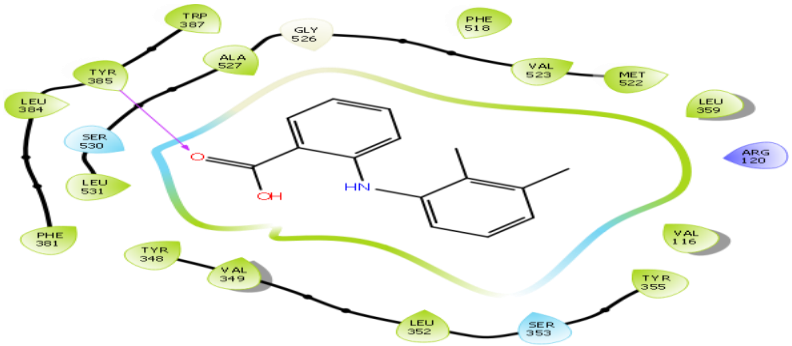


Figure 9. Ligand interaction of Mefenamic acid with COX-2 (5KIR).

Compounds 2, 3, and 4 had additional hydrogen bonding interactions with TYR355 within the COX-2 complex (Figures 4, 5, and 6).

For our docking studies, we chose to use the XP (extra-precision) mode of Glide. This mode exclusively utilizes high-quality ligand poses during the docking process and has been shown to produce better results than the SP (standard-precision) mode [36].

To calculate the docking score, many variables that affect the ligand-receptor binding affinity are taken into consideration. These factors include force fields like electrostatic and van der Waals forces, as well as terms that either penalize or reward specific interactions between the ligand and the receptor. The aim is to comprehensively account for all the parameters that affect the binding between the two molecules [37].

3.2. GBSA/MM Result

During molecular docking, an assessment was conducted using the MMGBSA (Molecular Mechanics Generalized Born Surface Area) free binding energy for the COX-2 target (PDB ID: 5IKR). This approach helps evaluate the binding interactions and energetics of ligands with the COX-2 receptor. The most accurate parameter for ligand-receptor complex stability assessment was Prime-MM/GBSA, which was computed. When calculating MM/GBSA, a number of factors affecting the complexes' overall stability are taken into consideration. This comprehensive evaluation provides insights into the strength and favorability of the binding interactions. The solvent effect is one of the critical parameters considered when calculating MM/GBSA for ligand-receptor complexes. Prime- MM/GBSA values for the COX-2 protein complexed with Mefenamic acid (positive control), Diclofenac, and five designed ligands were determined in the context of molecular docking studies.

Table 2. GBSA/MM values of Diclofenac and standard cocrystallized compound (Mefenamic acid) in COX-2 receptor (PDB ID: 5IKR).

Comp.	Prime Energy	ΔG bind (Kcal/mol)	ΔG bind Coulom	ΔG bind Covalent	ΔG bind Vander	ΔG bind Lipophilic	ΔG bind H Bond
1	-22857.7	-56.118	-16.965	7.835	-40.521	-22.241	-2.252
2	-22856.4	-60.185	-15.121	5.738	-39.565	-26.736	-2.130
3	-22903.4	-50.190	-37.194	9.088	-32.216	-25.451	-2.759
4	-22883.2	-49.150	-18.135	11.383	-24.779	-25.599	-2.370
5	-22859.2	-60.155	-16.322	8.235	-40.551	-27.915	-1.923
Diclofenac	-22827.1	-53.916	-10.978	5.513	-35.069	-25.169	-1.253
Mefenamic Acid	-22876.2	-52,812	-4.988	-1.824	-39.902	-20.299	-1.085

All the suggested analogues exhibited favorable free binding energies, indicating their strong potential to fit well into the COX-2 receptor. The binding energies ΔG of Compounds 2 and 5 to COX-2 are the highest, with a value of approximately -60 kcal/mol each (Table 2). Compared to the standard drug mefenamic acid, which has a ΔG binding energy of -52.812 kcal/mol, this value is much higher. The Van der Waals energy (ΔGvdW) and non-polar solvation

energy (ΔGLipo) are the energies that most significantly contribute to ligand binding within the COX-2 binding pocket, according to the MM-GBSA results. The extremely negative values that all compounds showed for these energy components demonstrate this. These results imply that non-polar solvation effects and favorable Van der Waals interactions are essential for the compounds' robust binding to COX-2. The other energy components, namely covalent energy (ΔGCov) and hydrogen bonding energy (ΔGHbond), are not important in receptor binding, in contrast to the Van der Waals energy (ΔGvdW) and non-polar solvation energy (ΔGLipo).

Based on this observation, we can conclude that the interaction between the COX-2 receptor and the compounds is mainly influenced by non-polar solvation effects and Van der Waals forces. However, hydrogen bonding and covalent bonding seem to have a relatively minor role in this particular context.

3.3. Molecular dynamic simulation(MDS)

Molecular dynamics simulations are now a well-established method that can be used to understand macromolecular ligand-receptor bindings [38]. The simulation's outcomes are similar to those that are relevant to biology. Furthermore, MD modeling takes into account the fact that proteins change over time, in contrast to the more static molecular docking approach [39].

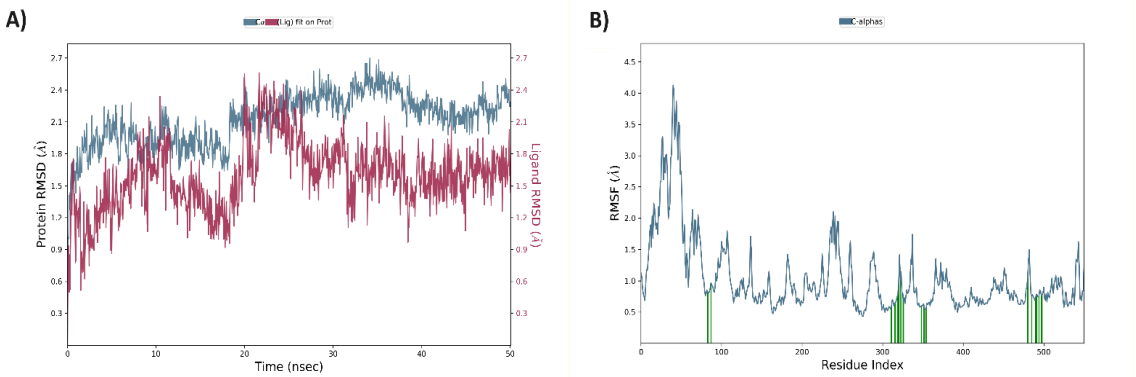


Figure 10. Root-mean-square deviation (A) and Root mean square fluctuations (B), during MDS analysis of compound 1 – COX 2 complex.

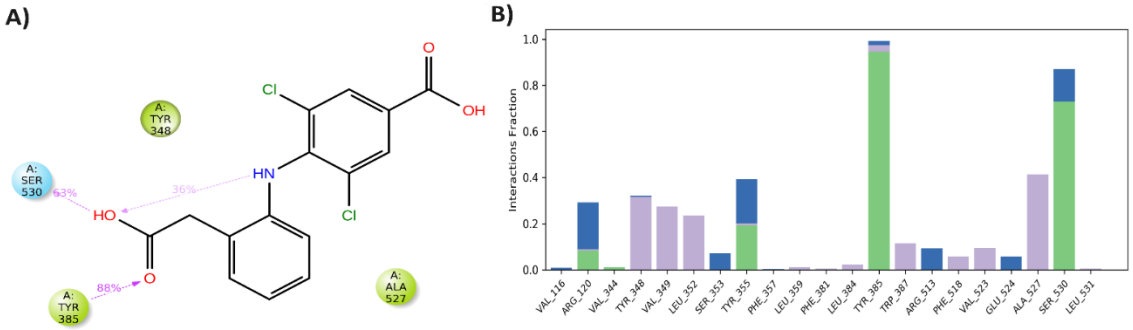


Figure 11. Interacting fraction (A), and interacting residues (B) in the COX 2-compound 1 complex during MDS analysis.

To further confirm the interaction, the comp 1 - COX 2 complex was subjected to MDS analysis. The results showed that the ligand had an RMSD of less than 2.4 Å, while the protein's RMSD was less than 2 A0 in the complex state. These findings suggest that the compound is relatively stable (Fig. 10A). Additionally, the majority of the protein had an RMSF of less than 2.0 A0, according to the RMSF analysis, which further demonstrates a stable protein structure (Fig. 10B). During more than 63% of the simulation time, Comp 1 and the COX 2 protein interacted at least twice (Fig. 11A). The specific residues involved in this interaction include ARG-120, TYR-348, VAL-349, LEU-352, TYR-355, TYR-385, ALA-527, and SER-350 (Fig. 11B).

The number of protein-ligand interactions made during the trajectory is displayed in the top panel of Figure 12. The protein residues that interacted with the ligands are listed in the bottom panel. These

results imply that comp 1 interacts with COX-2 amino acid residues that are catalytically active, like SER-530 and TYR-385, in an effective manner. The SSE composition for every trajectory frame during the simulation is shown in Figure 13. The graphic at the bottom shows the residues and their SSE assignments over time. The stable interaction is validated by the SSE, which remains relatively constant throughout the simulation.

3.4. ADMET predictions

The success of newly created pharmaceuticals depends on their ADME-T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics as poor pharmacokinetics and toxicity are frequently cited as signicant causes of costly late stage drug development failures [40]. Hence, assessing these characteristics is crucial in the later stages of drug development [41]. If a drug has unfavorable ADME-T attributes, it may not be considered for further development. Therefore, the newly designed ligands were evaluated using the QikProp module within the Schrödinger suite to determine their suitability for further development. The outcomes of the in-silico ADMET screening demonstrate that every new compound satisfies the suggested standards (Table 3). Every compound in the approved range of 4.637 to 6.753 has a dipole moment and a molecular weight less than 500.

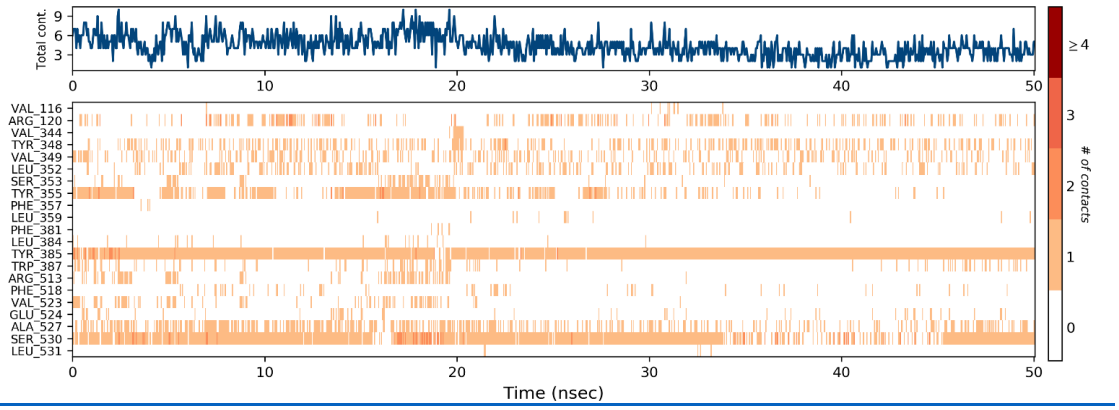


Figure 12. Representation of the interactions and contacts (H-bonds, Hydrophobic, Ionic, Water bridges) between compound 1 with COX-2 protein.

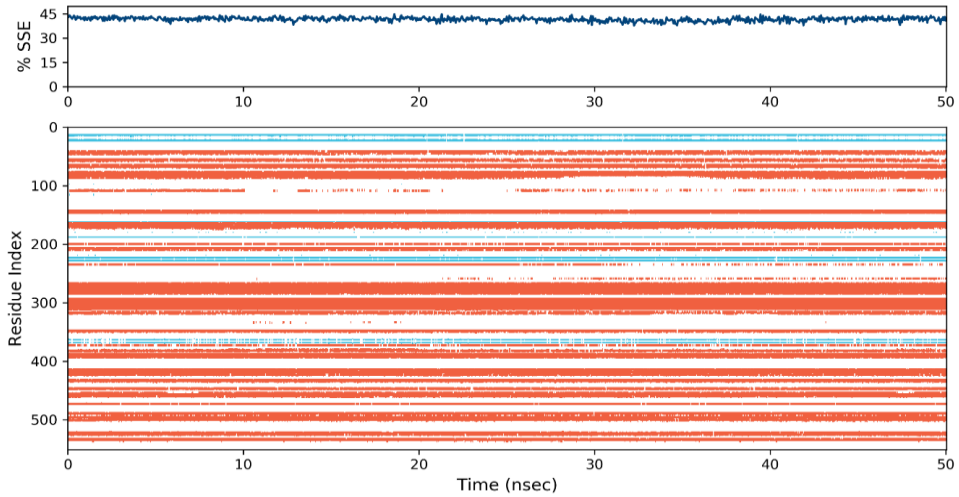


Figure 13. protein secondary structure elements (SSE) during simulation of COX 2 – compound 1 complex.

Furthermore, every compound satisfies Lipinski's Rule of Five, which states that all of the compounds' acceptable bounds for molecular weight, hydrogen bond donor and acceptor count, and Log P values are reached.

One tool that can help predict a drug's likelihood of reaching its target is Qikprop, which estimates the number of metabolic reactions a drug may experience (#metab). Here, every designed compound has a #metab that falls between the

recommended range of 1 and 8, suggesting a favorable metabolic profile.

Additionally, all of these compounds have a human oral absorption rate of more than 80%, which suggests that even if they do not adhere to Lipinski's Rule of Five, it is unlikely to have a significant impact on their bioavailability.

Table 3. Insilco ADMET screening for proposed compounds (1-5).

Comp	Mol.Wt.	Dipole	Donor HB	Accpt HB	QPlog o/w	#metab	Rule Of Five	% Human Oral Absorption
1	340.162	4.637	3	4.5	3.142	3	0	61.765
2	353.204	6.874	3	5	3.237	3	0	80.847
3	390.240	11.681	5	5.5	2.034	3	0	55.986
4	389.253	11.822	3	7	2.592	3	0	71.654
5	367.231	6.753	3	5	3.668	3	0	84.984
Recommend values	130-725	1-12.5	0– 6	2-20	-2-6.5	1 – 8	Max 4	>80% is high

4. Conclusions

Most of the medications currently available are capable of reducing inflammation. However, they often have significant side effects because they non-specifically inhibit both isoforms of the COX enzyme. This study used a structure-based computational drug design method to identify potential selective COX-2 inhibitors that are more likely to overcome the drawbacks associated with currently used drugs.

Our docking analysis results show that the designed compounds are more selective toward COX-2 than the standard drug(Diclofenac) and Comp 1 has the highest binding affinity docking score among the others (10.66). The ADMET analysis demonstrates that these compounds are drug-like molecules and have a good pharmacokinetic profile. The MD data revealed that the Compound 1-COX 2 complex was stable and that the critical protein-ligand interactions were preserved throughout the simulation time. Moreover, the results demonstrated that comp 1 had accepted RMSD and RMSF values, as well as good ligand interaction with COX-2 enzymes. Chemical synthesis of these compounds and in-vitro studies must be the next step.

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Declaration of Competing Interest

There are no conflicts to declare.

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