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Original Research Article

Computational docking study of naproxen binding to COX-2 (6COX) using AutoDock and visualization tools

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Abstract

Purpose: *To investigate the molecular interaction of Naproxen and cyclooxygenase (COX) using a docking approach.*
Methods: *Naproxen structure was built and energy minimized in HyperChem to attain its stable conformation. The COX receptor (PDB ID: 6COX) was prepared by stripping water molecules and co-crystal ligands, adding polar hydrogens and assigning charge prior to performing molecular docking. AutoDock Vina was used to dock Naproxen to predict its orientation within the COX active site.*
Results: *The docking yielded five binding modes with the best-ranked pose having a binding affinity of −7.643 kcal/mol, which indicates favorable binding. Visualization by PyMOL indicates that Naproxen fits well inside the COX binding pocket, where the aromatic core is buried deep in the hydrophobic channel, while the carboxylic group is directed toward the pocket entrance, supporting stabilizing interactions.*
Conclusion: *Naproxen binds within the COX active site with a predicted binding affinity of −7.643 kcal/mol. The ligand is stabilized by hydrophobic interactions and hydrogen bonding.*

Keywords: *AutoDock Vina, COX-2, Cyclooxygenase, HyperChem, Molecular Docking, Naproxen, PyMOL*

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INTRODUCTION

The important role of cyclooxygenase (COX) enzymes in initiating inflammation is particularly associated with the COX-2 variant. These enzymes catalyze the conversion of arachidonic acid into prostaglandins, which are important mediators of inflammation, pain and fever [1]. In many parts of the body, COX-1 is constitutively expressed in normal tissues. However, COX-2 is not typically present except temporarily during and after inflammation, where it tends to be expressed to a greater extent [2]. Cyclooxygenase-2 (COX-2) has become an important target for pharmacological intervention as the basis for anti-inflammatory therapies. Naproxen is one of the most widely used NSAIDs, used for the treatment of a good number of inflammatory conditions, including arthritis, pain in muscles, and menstrual cramps. Naproxen inhibits the activity of COX enzymes, thereby reducing the formation of the prostaglandins whose action leads to pain and inflammation [3]. Although proven useful clinically, understanding the detailed behavior of Naproxen and COX-2 interactions at a molecular level will give additional insight into its action, and may assist drug design efforts aimed at safer and more selective agents.

In recent years, various computational approaches based on modeling and docking have been developed for studying interactions between a drug and target protein. This yields predictions of how the bound drug will orient itself, along with estimates of binding energy and details of important stabilizing interactions, mode of action, and effect of binding [4]. Therefore, this study examined the molecular interaction of Naproxen with its target enzyme, COX-2, using following computational workflow from initial building and molecular orientation to visualization. The structure of Naproxen was built and optimized using HyperChem, while docking of Naproxen into COX-2 binding site was performed using AutoDock Vina, and PyMOL was used to visualize the best binding pose and examine how Naproxen fits inside the COX binding pocket. Many researchers have recently been drawn to how non-steroidal anti-inflammatory drugs (NSAIDs) interact with the COX-2 enzyme, due to the advancement in drug discovery approaches using computers. Docking and visualizing how NSAIDs bind into the COX active site and how binding occurs relative to other inhibitors are valuable for both selectivity, predicting the strength of binding, and designing better anti-inflammatory drugs.

Ruslin *et al* [5] analyzed the interaction of (R)-Naproxen with COX-2 using a framework computational strategy, which first performed pharmacophore modelling of the ligand in LigandScout and subsequent docking simulations with AutoDock and iDock, and reported specific amino acid interactions. Flores *et al* [6] compared similar docking analyses of popular NSAIDs (aspirin, ibuprofen, diclofenac and naproxen) to selective COX-2 inhibitors (e.g., celecoxib and etoricoxib) and saw evidence of higher binding affinities and better selectivity toward COX-2. Hanjal *et al* [7] constructed a virtual library of potential COX-2 inhibitors and, ultimately, applied molecular docking and molecular dynamics simulations to screen them, highlighting two interestingly active compounds and the power of terrain predictions alongside ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling. Also, Shinu *et al* [8] designed and evaluated a compound (MAS-1696), which potentially inhibited COX-1 and COX-2, and under docking instructions, concluded that MAS-1696 showed good selectivity towards COX-2 compared to S-Naproxen. These studies displayed the importance of molecular docking and comparison within inhibitors to accurately study drug/enzyme interactions at atomic level. However, many of the previous studies merely took a docking score or looked at how the structures compared against each other, and were not holistic and illustrative. This current study combined ligand optimization (HyperChem), docking prediction (AutoDock Vina), and 3D visualization (PyMOL) to better understand Naproxen interaction with COX-2.

EXPERIMENTAL

Molecular modelling using HyperChem

The 2D chemical structure of Naproxen was generated in HyperChem 8.0.10 software. It was then converted into a three-dimensional model and optimized. Using a simple MM+ minimization followed by a semi-empirical optimization with PM3, a conformer was obtained (which has minimal potential energy) before docking and calculations.

Protein preparation

The 3D crystal structure of the target protein cyclooxygenase-2 (COX-2) was retrieved from Protein Data Bank [9]. Protein preparation was done with AutoDock Tools (ADT) [10]. Water molecules and any irrelevant ligands were removed to avoid interference with the docking process. Polar hydrogen atoms were then added, and Kollman charges were assigned to the receptor. The prepared protein structure was finally saved in PDBQT format.

Ligand preparation

The optimized Naproxen structure was saved in PDB format and imported into AutoDock Tools. Polar hydrogens were added and Gasteiger charges were calculated. The ligand was converted into PDBQT format, which was required for docking in AutoDock Vina.

Molecular docking using AutoDock Vina

Using AutoDock Vina (1.2.5) [11], docking simulations were undertaken on a Windows OS. A configuration file was created to specify docking parameters (for example, size of the grid box centered upon the active site region of the COX-2 enzyme). Docking procedure was executed using the command prompt. Several poses were generated while docking Naproxen to COX-2 enzyme, and the docking pose with the lowest binding affinity (kcal/mol) was chosen.

Visualization and interaction analysis using PyMOL

The constructed docking poses were evaluated and modeled using PyMOL 2.5 [12] to visualize

these interactions. In the visualization, the protein backbone was illustrated as a cartoon, and naproxen was represented using stick representations so that the user could quickly identify naproxen's position within the binding pocket. Key binding site residues were defined as those amino acid residues within 5 Å of Naproxen. Surface visualization and distance measurements of Naproxen were evaluated to understand how well the surfaces fit against those of the proteins surrounding them.

Ligand preparation using HyperChem

Naproxen was built in HyperChem and optimized [13]. The 2D structure of the ligand was used throughout the molecular drawing. The full 3D structure was created by using the MM+ force field, and then semiempirical optimization was done with the PM3 method. A better structure visually lets the molecule relax into an ideal conformation for subsequent docking.

RESULTS

Drawing and optimizing the ligand (Naproxen) in HyperChem

Naproxen was built manually in HyperChem. It is known to have a fused aromatic naphthalene core structure, a methoxy group in the C6-position, and a propionic acid side chain (Figure 1). Atom types were manually assigned (single, double, aromatic bond types) to ensure correct connectivity, and hydrogen atoms were added to fill all valences. The molecule was then optimized to yield a reasonably low-energy conformation (Figure 2). The molecule was saved in PDB format for use in docking studies (Table 1).

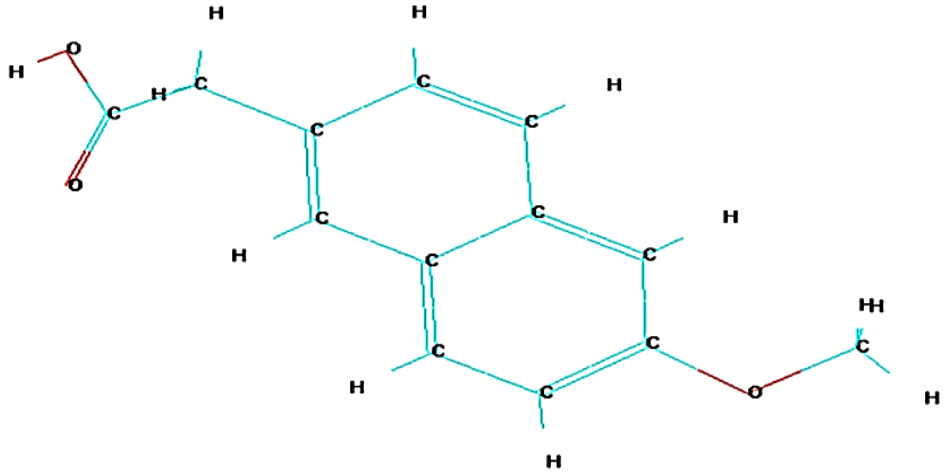

Figure 1: Structure of Naproxen with HyperChem, with the correct bonds and functional groups (before optimization)

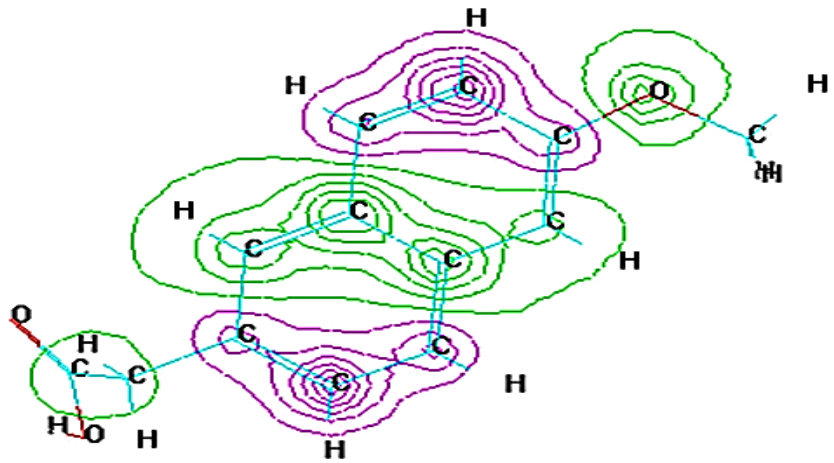

Figure 2: Total charge density distribution of Naproxen calculated using the HyperChem program by semi-empirical (PM3) method

Table 1: Physicochemical properties of Naproxen

Parameters	Value
Total Energy	-60100.9828 Kcal/mol
Binding Energy	-3119.7424 Kcal/mol
Heat of Formation	-94.2714 Kcal/mol
Electronic Energy	-349564.976 Kcal/mol
Nuclear Energy	289463.9939 Kcal/mol
Free Energy	-60101 Kcal/mol
Dipole Moment	2.518 Debyes
RMS Gradient	0.08764 Kcal/mol
HOMO Energy	-9.3294 ev
LUMO Energy	-0.11348 ev
(HOMO –LUOM) Energy	-9.21592 ev

Ligand and receptor in AutoDock Tools

The optimized Naproxen molecule was imported to AutoDock Tools, where polar hydrogens were added and Gasteiger charges assigned so that the correct molecular interactions were assumed during docking. The target protein (cyclooxygenase-2) was prepared by removing all water molecules and any co-crystallized ligands, adding polar hydrogens, and assigning charges to make the target protein compatible with AutoDock Vina (Figure 3).

Docking setup

A docking grid centered around 20, 25, and 30 with dimensions of 40 Å × 40 Å × 40 Å covered the entire length of the catalytic pocket to provide an accurate representation of binding sites while also minimising computation times by setting the exhaustiveness parameter to 8.

Docking results

The docking run yielded 5 possible binding poses. Best scoring pose (Mode 1) showed an affinity of −7.643 kcal/mol binding, which showed a strong and stable binding to target protein. The rest of the poses showed slightly less affinity (−7.583 to −7.154) kcal/mol. Root mean square deviation (RMSD) values showed that the poses were stable in the binding pocket. The small range of energies suggested that stable conformations of Naproxen existed in the active site, with Mode 1 preferentially having the lowest energy (Table 2).

Table 2: Binding affinities and RMSD values for Naproxen docked into COX (6COX)

Mode	Affinity (kcal/mol)	RMSD l.b.	RMSD u.b.
1.0	-7.643	0.000	0.000
2.0	-7.583	1.188	1.461
3.0	-7.516	1.148	2.125
4.0	-7.157	1.202	2.152
5.0	-7.154	1.668	2.643

Visualization of the best pose

It was observed that the naphthalene aromatic core of Naproxen was buried within the hydrophobic channel of COX, and the propionic acid group reached the entrance of the active site, allowing π–π stacking (aromatic residues) (Figure 4).

Hydrogen bonding interactions

Analysis of the hydrogen bonding network illustrated the potential for forming bonds with residues from both the carboxylic group in naproxen and a variety of surrounding polar side chains located in proximity to the catalytic region on the cyclooxygenase (COX) enzyme active site (Figure 5).

Identification of key interacting residues

Through the use of residue mapping, it was possible to elucidate specific residues located within the COX active site that participated in both hydrophobic and hydrogen bonds with Naproxen (Figure 6).

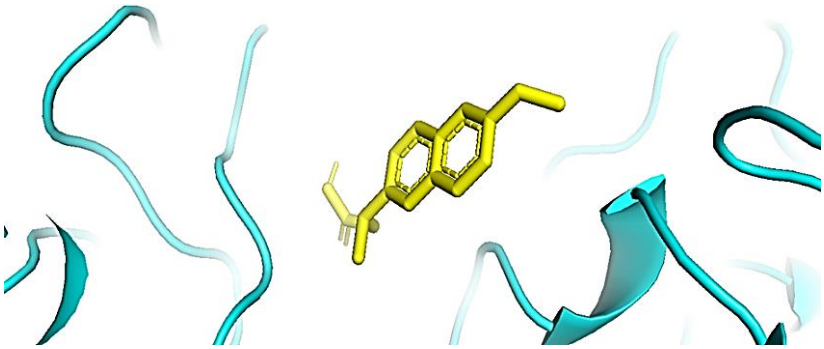

Figure 3: Optimized naproxen ligand (yellow sticks) near active site of cyclooxygenase (COX) (a cyan colored cartoon representation of the homodimer)

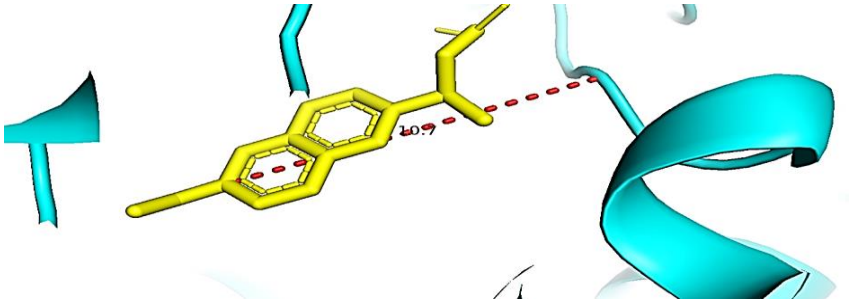

Figure 4: Best binding poses for Naproxen (yellow sticks) in COX active site (cyan cartoon). The aromatic core was deeply inserted

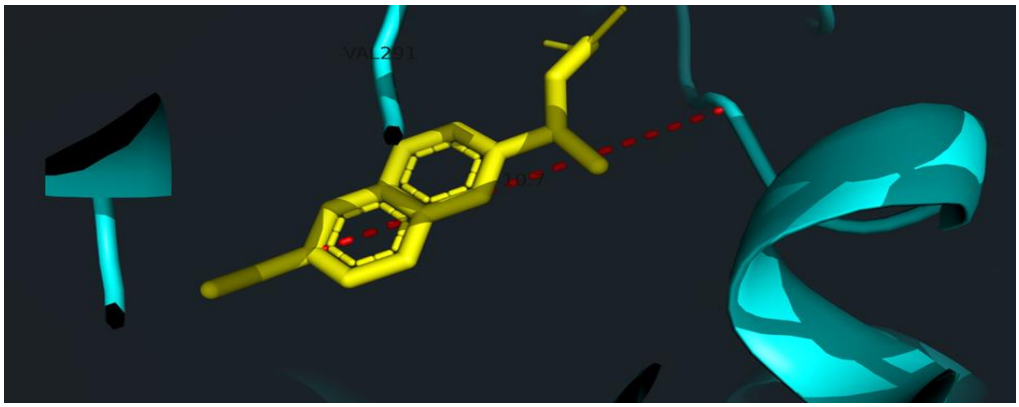

Figure 5: Interacting with polar residues in COX (cyan cartoon) active site via hydrogen bonding (highlighted) (red dashed lines)

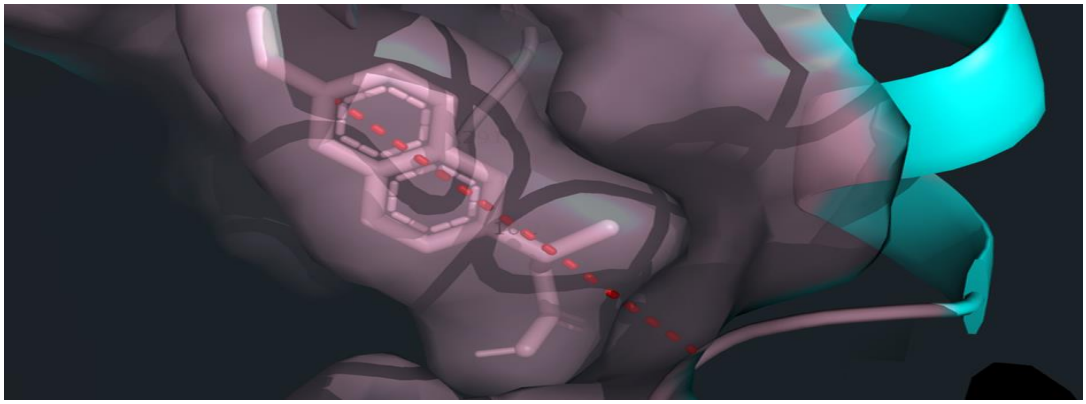

Figure 6: Hydrogen bonding / hydrophobic contacts with COX active site residues, seen engaging naproxen (yellow sticks) in its binding pocket

Surface representation of the binding pocket

A partially transparent molecular surface of the COX binding site was prepared to assess the extent to which flatter Naproxen was complementary to the catalytic pocket. Naproxen was deeply seated in a hydrophobic expanse and its aromatic moiety was largely buried and shielded from solvent exposure (Figures 7 and 8). Naproxen, being snugly fitted in the pocket, suggests excellent steric packing leading to lower degrees of freedom and higher binding energy.

DISCUSSION

Molecular modeling and in silico docking simulations are important methodologies in rational drug discovery, allowing researchers to evaluate the precise structural mechanisms of ligand-receptor interaction at an atomic level [14].

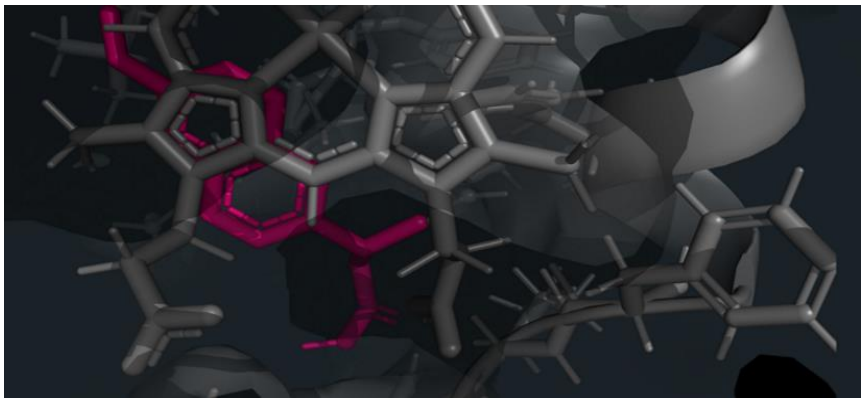

Figure 7: The binding pocket of COX, where Naproxen sits deep inside a hydrophobic cavity

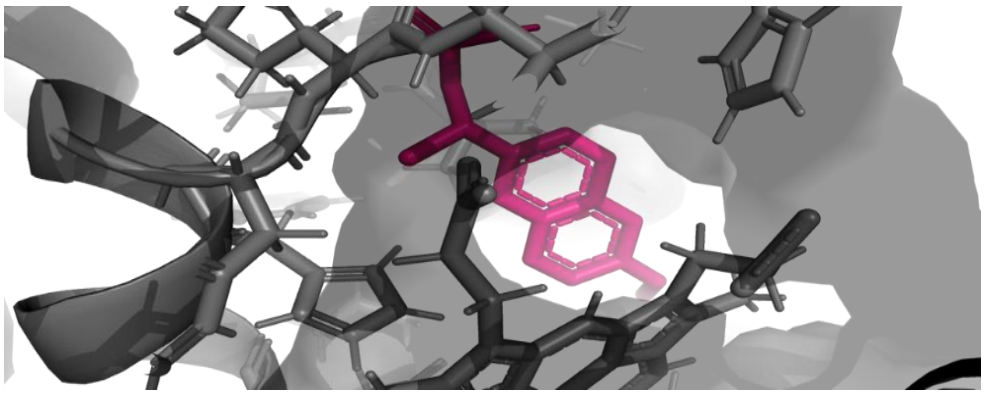

Figure 8: Another view of Naproxen (red) docked deeply within the binding site of COX active site

Cyclooxygenase-2 (COX-2) is a critical homodimeric, membrane-bound enzyme that catalyzes the conversion of arachidonic acid into pro-inflammatory prostaglandins [15]. Cyclooxygenase-2 (COX-2) is the principal therapeutic target for non-steroidal anti-inflammatory drugs (NSAIDs) designed to treat pain and swelling [15]. Naproxen is a classic, arylpropionic acid non-selective NSAID [15]. A rigid fused aromatic naphthalene core, a methoxy substituent, and a flexible propionic acid side chain characterize the structure of Naproxen.

Previous computational and structural studies revealed that acidic NSAIDs accumulate at inflammation sites and competitively block the active site of cyclooxygenase enzymes to inhibit prostaglandin biosynthesis [14]. Although traditional research primarily uses standard empirical frameworks to elucidate ligand-receptor interactions, integrating semi-empirical quantum chemistry provides an additional layer of precision. This study investigated the molecular interaction of Naproxen and cyclooxygenase (COX) using a docking approach. The docking revealed that Naproxen was buried inside the COX active-site cavity with a −7.643 kcal/mol binding pose affinity. The ligand was docked in the hydrophobic channel where the aromatic scaffold was stabilized and engaged in aromatic benzene π–π interactions while the carboxylic group was situated near the pocket entrance, forming polar contacts (i.e., hydrogen bonding). The flexible propionic acid tail projected outward towards the more polar opening of the active site. This distinct feature of the hydrophobic core anchoring deep in the channel and the polar tail interacting at the entrance is a classical structural signature of NSAID binding networks consistently documented in previous studies [14]. However, a slight difference was noticed in energy among predicted modes, indicating that the active site of the protein can also accommodate naproxen in more than one stable orientation, which may allow this ligand to stay docked under biological conditions. In a few words, the binding mode is similar to other NSAIDs that also invade the COX catalytic channel and block access to substrate.

Surface representation of the binding pocket revealed that Naproxen sat deeply within the hydrophobic channel, and the aromatic core was

completely shielded from surrounding water solvent. This steric effect maximizes Van Der Waals contacts while restricting the ligand's remaining degrees of freedom. This combination of structural locking and favorable thermodynamics explains the high binding energy and reliable binding stability observed in the simulations, consistent with previous professional drug-design workflows [14].

Limitations of the study

Although this study presents an estimation of how Naproxen may bind within active site of cyclooxygenases (COXs) using only traditional molecular docking techniques that rely on simplistic scoring schemes and do not appropriately consider all aspects of protein flexibility, solvent effects and the evolution of protein structure over extended periods of time. As such, predictions for binding affinity are still only educated guesses and may differ from actual values obtained via experimental methods. Future studies involving the Naproxen-COX complex should be conducted to determine its long-term stability in the body, using molecular dynamics as well as realistic simulations or physiological conditions to predict long-term stability. Also, future work should include post-docking calculations to assess the binding free energies (MM-PBSA/MM-GBSA) associated with Naproxen-COX complex formation. Comparative docking studies for all available NSAIDs and selective COX-2 inhibitors will provide information regarding the relative selectivity or affinity of each inhibitor, and conducting *in vitro* COX inhibition assays will further strengthen the findings derived from computational methods.

CONCLUSION

Naproxen binds within the COX active site with predicted binding affinities of -7.643 kcal/mol, fitting inside the hydrophobic pocket and stabilized through hydrophobic contacts and hydrogen bonding across polar sites.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

Not applicable.

Use of Artificial intelligence / large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used in this study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. *Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. Inflamm Res 1998; 47 Suppl 2: S78-87. doi: 10.1007/s000110050284.*
2. *Patrignani P, Patrono C. Cyclooxygenase inhibitors: From pharmacology to clinical read-outs. Biochim Biophys Acta 2015; 1851(4): 422-432. doi: 10.1016/j.bbalip.2014.09.016.*
3. *Angiolillo DJ, Weisman SM. Clinical Pharmacology and Cardiovascular Safety of Naproxen. Am J Cardiovasc Drugs 2017; 17(2): 97-107. doi: 10.1007/s40256-016-0200-5.*
4. *Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 2011; 7(2): 146-157. doi: 10.2174/157340911795677602.*
5. *Ruslin R, Yamin Y, Kasmawati H, Mangrura S, Kadidae L, Alroem A, et al The search for Cyclooxygenase-2 (COX-2) inhibitors for the treatment of inflammation disease: an in-silico study. J Multidiscip Healthc 2022; 15: 783-791. doi: 10.2147/JMDH.S359429.*
6. *Oniga SD, Pacureanu L, Stoica CI, Palage MD, Crăciun A, Rusu LR, et al. COX inhibition profile and molecular docking studies of some 2-(trimethoxyphenyl)-thiazoles. Molecules 2017; 22(9): 1507. doi: 10.3390/molecules22091507.*

7. Ahmad V, Khan MI, Jamal QMS, Alzahrani FA, Albiheyri R. Computational molecular docking and simulation-based assessment of anti-inflammatory properties of Nyctanthes arbor-tristis Linn Phytochemicals. Pharmaceuticals (Basel) 2023; 17(1): 18. doi: 10.3390/ph17010018.
8. Chahal S, Rani P, Kiran, Sindhu J, Joshi G, Ganesan A, et al. Design and development of COX-II inhibitors: Current scenario and future perspective. ACS Omega 2023; 8(20): 17446-17498. doi: 10.1021/acsomega.3c00692.
9. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res 2000; 28(1): 235-242. doi: 10.1093/nar/28.1.235.
10. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009; 30(16): 2785-2791. doi: 10.1002/jcc.21256.
11. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function. J Comput Chem 2010; 31(2): 455–461
12. DeLano WL. The PyMOL molecular graphics system. San Carlos, CA, USA: DeLano Scientific, 2002
13. Hypercube Inc. HyperChem (Version 8.0). Gainesville (FL): Hypercube, Inc.; 2002
14. Shamsian S, Sokouti B, Dastmalchi S. Benchmarking different docking protocols for predicting the binding poses of ligands complexed with cyclooxygenase enzymes and screening chemical libraries. Bioimpacts 2024; 14(2): 29955
15. Ahmadi M, Bekeschus S, Weltmann KD, Von Woedtke T, Wende K. Non-steroidal anti-inflammatory drugs: recent advances in the use of synthetic COX-2 inhibitors. RSC Med Chem 2022; 13(5): 471-496.