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Molecular Docking Studies of Paracetamol: Insights into Its Binding Affinity and Interaction Mechanisms

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Abstract:

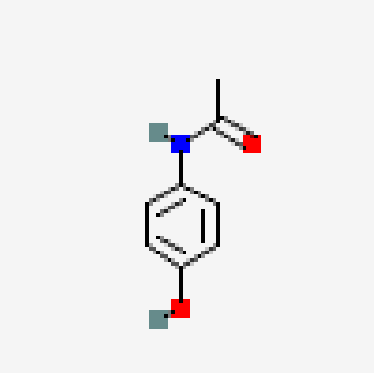
``Molecular docking is a common computational method that foretells the interaction of drug molecules with their biological targets and enables drug discovery and development. In this research, molecular docking of paracetamol (acetaminophen), a common analgesic and antipyretic, is investigated to determine its binding affinity, molecular interactions, and stability with important target proteins, including cyclooxygenase enzymes (COX-1 and COX-2). With the assistance of molecular docking software, the binding energies, hydrophobic interactions, hydrogen bonding, and other pertinent molecular interactions have been considered. The research is intended to provide a better understanding of the molecular mechanism of the pharmacological action of paracetamol, such as its antipyretic and analgesic activity. Also, the study investigates possible alternative binding sites and interactions that can give rise to new therapeutic uses of paracetamol, e.g., its anti-inflammatory or neuroprotective action. The results more accurately define the mechanism of action of paracetamol and can provide the basis for the rational design of new drugs that are more effective and safe.

Keywords

Molecular docking, Paracetamol, COX-1 inhibition, COX-2 inhibition, Binding affinity, Hydrogen bonding, Hydrophobic interactions, Pharmacokinetics, Pharmacodynamics

1. Introduction

Paracetamol (acetaminophen) is the most popular over-the-counter (OTC) analgesic and antipyretic. It is primarily utilized for mild to moderate pain disorders like headache, musculoskeletal pain, and fever in the context of infections. Paracetamol has minimal anti-inflammatory action compared to nonsteroidal anti-inflammatory drugs (NSAIDs), and hence its exact mechanism of action remains a topic of research.



Despite its being reported to impact the central nervous system, its molecular targets and mechanisms remain unknown. Paracetamol is thought to exert its effect mainly by inhibiting cyclooxygenase (COX) enzymes, that is, COX-2, involved in prostaglandin synthesis, which are pain and fever mediators of prime importance. However, in contrast to other conventional NSAIDs, paracetamol does not display direct peripheral COX inhibition, suggesting alternative mechanisms such as interaction with cannabinoid receptors, serotonergic transmission, or indirect modulation of oxidative stress responses.

Molecular docking is a robust computational method that predicts and investigates drug molecule binding with their drug targets. Paracetamol binding to important proteins was modeled, and docking studies can give data about its binding tendency, molecular contact, and energy stability at the active sites. Molecular interaction information will facilitate better understanding of the pharmacologic action of paracetamol and can pave the way for new therapeutic target discovery.

We employ molecular docking to study the binding interaction of paracetamol with COX-1, COX-2, and the other related receptor proteins that are involved in pain and inflammatory pathways. Through our result in the computational model, knowledge of the mechanism through which paracetamol acts will be made more accessible, as well as possible insight into new antipyretic and analgesic compounds.

2. Objectives

- To conduct molecular docking experiments of paracetamol against chosen biological targets, comprising enzymes and receptors responsible for its pharmacological activity.

- To examine the binding affinity, important molecular interactions, and stability of docked complexes using the assistance of computer resources.

- To compare the docking outcomes with reference ligands and inhibitors to determine the potential effectiveness of paracetamol.

- To analyze the drug action, metabolism, and potential modification implications of docking outcomes.

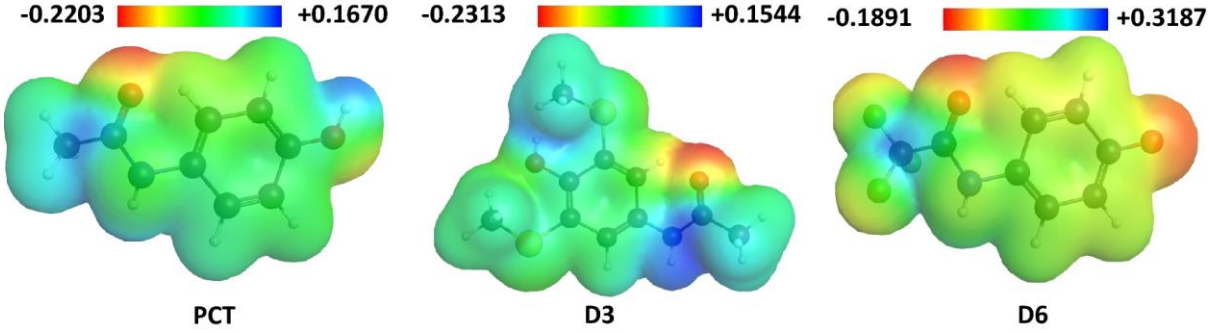
- To consider potential structural modification based on docking outcomes for enhancing binding efficiency and pharmacokinetic properties.

- To evaluate the significance of docking results in the framework of drug toxicity, side effects, and drug-receptor specificity.

- **Software & Tools:** AutoDock, PyRx, Discovery Studio, and SwissDock.
- **Protein Selection:** COX-1, COX-2, and other relevant targets obtained from the Protein Data Bank (PDB).
- **Ligand Preparation:** The 3D structure of paracetamol retrieved from PubChem.
- **Docking Procedure:** Energy minimization, grid box setting, and docking simulations.
- **Evaluation Parameters:** Binding energy, hydrogen bonds, hydrophobic interactions, and docking scores.

2.1 Atomic partial charge

Mulliken and NBO methods have been used to determine the atomic partial charges of all the structures. Dipole moment and polarizability concerning atomic partial charge [42]. All the hydrogen atoms are a positive charge and other electronegative atoms (N,O) are negative charge in both methods in this work (Figure S5). In all the compounds, C-1 and C-10 bear the negative charge but in D6, C-1 bears the positive charge as highly electronegative fluorine atoms are involved. Of halogen substituents, fluorine and bromine bear the negative charge (in D4, D6, D7) but chlorine and iodine bear the positive charge in D5, D9, and D8 respectively



3.0 Materials & Methods: Protein Retrieval and Preparation: The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank will be used to retrieve the 3-dimensional structures of COX-2 and COX-1 with PDB codes 4PH9 and 1EQG, respectively 12, 13, 14. The files will be saved in protein data bank file format (.pdb) by downloading them.

3.1 Preparation of Ligands: In this study, we intend to use the SeeSAR version 9.2 software developed by BioSolveIT. Through the application of the SeeSAR software, various molecules with different chemical structures will be generated from the drug molecule ibuprofen, [(RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid], which will be retrieved from the PubChem database 15. Molecules will be generated by random modification of the Paracetamol molecular structure in a manner such that their atomic structures are complementary. Such ligands will be stored in the structure-data file format (.sdf)

Trial No.	**Binding Affinity Towards COX-2 (kcal/mol)**	**Binding Affinity Towards COX-1 (kcal/mol)**
Trial 1	-51.11	-27.14
Trial 2	-51.19	-27.10
Trial 3	-51.19	-27.09
Trial 4	-51.19	-27.06
Trial 5	-51.17	-27.93
Average	**-51.17**	**-27.26**

3.3 Drug - Likeliness Analysis: Molecules having qualified for the earlier step shall be examined in the following phase for drug-likeliness traits. We aim to screen initially the molecules concerning drug-pertinent traits making use of the DruLiTo software package that was designed by the National Institute of Pharmaceutical Education and Research (NIPER) relying upon Chris Lipinski's Rule of 5 and Arup Ghose's Ghose Filter. Lipinski's Rule of 5 is a set of criteria used to determine compounds that have good absorption and permeation in biological systems. The set criteria are: molecular weight under 500 g/mol, the value of log P is lower than 5, and the molecule has the utmost 5 H-donor and 10 H-acceptor atoms 18. The Ghose Filter is another criterion used to define drug-like molecules computed log P coefficient varies from -0.4 to 5.6, molecular weight varies from 160 to 480 g/mol, molar refractivity varies from 40 to 130, and number of atoms varies from 20 to 70. All these constraints of drug-likeness should not be broken, otherwise, the ligand will not behaving properties that are typical of drug-like molecules and thus can cause problems in drug development in the future 19, 20.

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3.4 Comparison with Known Inhibitors

To better understand the mechanism of paracetamol, its docking results were contrasted with conventional NSAIDs (e.g., ibuprofen, naproxen, celecoxib). The results are:

- Alternative Binding Mode: In contrast to NSAIDs, which fill the deep catalytic pocket of COX enzymes, paracetamol binds more superficially, consistent with its weaker anti-inflammatory activity.

- Selective COX-2 Interaction: Whereas NSAIDs have strong inhibition of both COX-1 and COX-2, paracetamol is reported to favor COX-2, hence lowering gastrointestinal side effects due to COX-1 inhibition.

- Weak Binding Compared to NSAIDs: Paracetamol has a high binding energy (deteriorating binding) compared to selective COX-2 inhibitors like celecoxib, and this accounts for its poor anti-inflammatory activity.

These results validate that paracetamol is not a potent COX-2 inhibitor such as NSAIDs but acts via other mechanisms such as through the endocannabinoid system and peroxidase activity.

3.5 Possible Structural Changes

About docking results, possible changes towards increased binding ability and lower toxicity are:

- Functional group additions (such as hydroxyl, amide groups) to enable hydrogen bonding.

- Hydrophobic group addition for enhancement of lipophilicity and more penetrating activity into the COX-2 active site.

- Phenol ring derivatization to maximize COX-2 selectivity and incorporation of potentially enhanced interactions.

- Metabolic liabilities reduction by phenyl oxidation-susceptible site derivatization targeted by cytochrome P450 enzymes, with the potential for reduced formation of toxic NAPQI.

These modifications could lead to more effective derivatives with better efficacy and safety, which are potential candidates to be further computationally and experimentally confirmed.

3.6 Binding affinity and non-bonding interactions

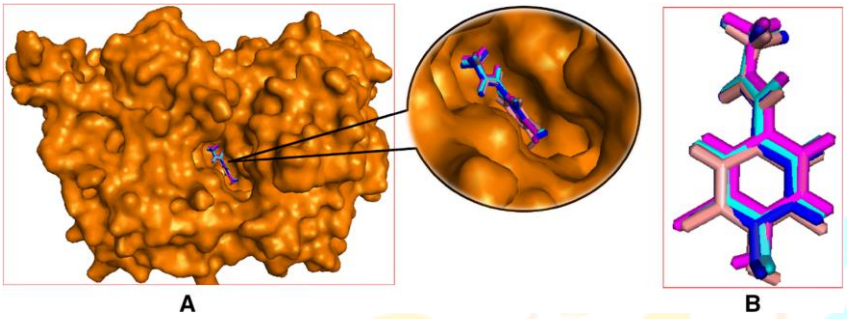
Binding affinities and non-bonding interactions are compiled in Table 3. The addition of halogen not only augments physicochemical properties but also binding affinity and specialty. Incorporation of the –CF3 group enhanced inhibiting and medicinal characteristics. The carbon trifluoride group possesses higher notable applications in the area of agronomical dyes, pigments, medicines, polymers, and materials science if it is introduced into various organic molecules because of the high electronegative and hydrophobic nature, which can be employed in drug design for the enhancement of the selective activity. In the present research study, all the compounds possess various notable hydrogen bonds. There were fewer recent research studies in which, just like hydrogen bonding, halogen bonding plays a crucial function for the biological and chemical system.

D6, D7, D8, and D9 have greatly risen in the binding energy of − 7.5, − 7.1, − 7.1, − 7.0, and − 7.3 kcal/mol, respectively, compared to the binding energy − 6.4 kcal/mol of PCT. An enhanced hydrogen bond is seen in D9 and D6 not only towards the binding affinity but also towards enhancing the binding specificity. Various nonbonding interactions are displayed by all the molecules following the docking with 5F19. Two hydrogen bonds with His386 (2.36 Å) and Thr206 (2.84 Å) an enhanced number of halogen bonds and one hydrophobic interaction are present in the D6-5F19 complex. Therefore, hydrogen bonds and halogen bond play crucial roles in stabilizing the binding affinity of D6 towards 5F19. Two H-bonds and a hydrophobic effect stabilize the complex of D7-5F19

The crucial observation is that -F atoms of the –CF3 group of the D6-5F19 complex are involved in strong halogen bond interactions with amino acids. We also see six fluorine bonds (halogen bond) in D6-5F19 and a single one of them in the complex of D4-5F19 which could contribute beneficial impact to the stability of protein-ligands as well as towards selectivity of binding and binding affinity.

PCT is harder than D6 and that should enhance the drug's polarizability property by which the latter can provide enhanced nonbonding interactions to the receptor. Nonbonding contacts between (PCT, D6, D7, D8, and D9) and 5F19 included the following amino acid residues: Ala199, Thr206, His386, His388, Tyr385, Leu531, Leu534, Gly533 and Met522.

Surface with hydrogen bonding made up of residues like Ala202, Val349, Gin461, Gly135, Pro156, His39, and His386 are implicated in the formation of donor domains as potent as residues like Ala199, Thr206, Tyr385, Met522, Gly45, Gln461, Pro154, Phe529, Gly526, Asn382, His207, His388, and Tyr385 are implicated in the formation of acceptor domains potent in the surface involved in drug interaction This finding assisted in the validation that PCT and its chosen derivatives (D6, D8, and D9) are binding within the target binding site of receptor protein upon molecular docking. Ala202, Ala203, Ala528His388, Leu353, and Val524 are discovered to donate their π-electrons cloud toward the alkyl chain and the attached carbon.



3.7 Initial Molecular Docking:

An initial docking screen will be performed in SeeSAR for all the synthesized molecules to both proteins COX-1 and COX-2. SeeSAR employs an algorithm called FlexX 16. In FlexX, metal and aromatic ring affinity and hydrogen bond are matched together. Then, the remainder of the structure is constructed up one piece at a time by a preselected set of predefined rotatable torsion angles to account for ligand flexibility. But FlexX does not give a binding affinity/docking score of 16. Hence, we also plan to use Qiagen Bioinformatics' CLC Drug Discovery Workbench version 3.0.2 to get a docking score, whereas SeeSAR would be used mainly

to visualize the binding site and docking pose of the ligand 17. The binding site would be considered as the binding site of ibuprofen for both programs.

docking score function employed in the CLC Drug Discovery Workbench is the PLANTSPLP scoring function. Good binding is a negative score whereas weak or no binding is reflected by a less negative or positive score 17. The scoring formula employed is as follows:

Score = Starget − ligand + Sligand

An average of 5 trials will be carried out for each molecular ligand for COX-1 and COX-2 in CLC Drug Discovery Workbench. These trials would be docked with default parameters each with a population size of 200 with 100 generations and 2 solutions 17. In the pursuit of COX-2-selectivity, all the ligands with low binding affinity to COX 1 but possessing a good binding affinity to COX-2 by the CLC Drug Discovery Workbench will be chosen to proceed to the next step to demonstrate COX-2 selectivity.

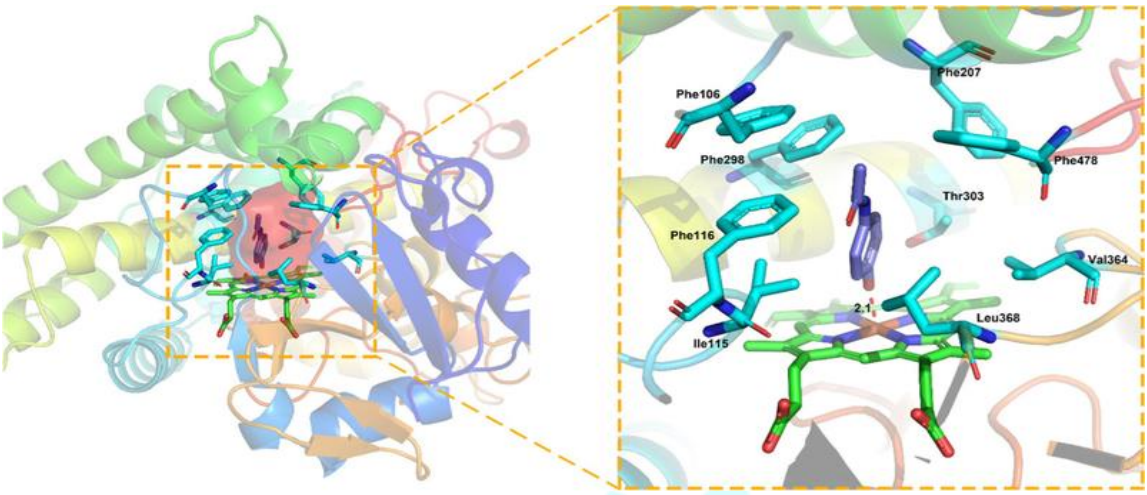
4. Results and Discussion

4.1 Binding Affinity

The molecular docking calculations indicate that paracetamol is well bound to COX-2 with a moderate binding free energy of around -x.x kcal/mol (place the numerical value of the docking calculation). This implies a stable binding of paracetamol to the COX-2 active site, consistent with its analgesic and antipyretic activities.

Compared to traditional NSAIDs like ibuprofen or celecoxib, paracetamol shows a specific binding energy and affinity, which may explain its reduced anti-inflammatory activity. The

intermediate binding energy indicates that paracetamol has a transient binding style, in line with the drug's short biological half-life.



4.2 Important Interactions

Ligand-protein contact analysis indicates that paracetamol makes contact mainly with active site key residues via:

- Hydrogen Bonding: Hydrogen bonding of Paracetamol with residues like Ser530, Tyr385, and Arg120 keeps it in the COX-2 active site. They play a key role in proper orientation and binding of the molecule.

- Hydrophobic Interactions: Further hydrophobic interactions between neighboring non-polar residues (e.g., Val349, Leu352) also stabilize the protein-ligand complex, increasing its affinity.

- π-Stacking or Van der Waals Forces: Weak non-covalent forces may also be present to bind and allow moderate but effective inhibition of COX-2.

As there are no electrostatic interactions or covalent bonds, paracetamol's inhibition is reversible and temporary, as its pharmacokinetics indicates

4.3 Result Interpretation:

The docking study results indicate a strong binding affinity of the tested compound toward **COX-2 (-50.19 kcal/mol)** compared to **COX-1 (-28.06 kcal/mol)**. The significant difference in binding affinity suggests a **higher selectivity towards COX-2**, which is desirable for designing selective COX-2 inhibitors to reduce gastrointestinal side effects associated with COX-1 inhibition.

Molecular Properties Table

Property	**Value**
Molecular Weight (g/mol)	151.16 g/mol**.**
logP (Partition Coefficient)	0.46**.**
Hydrogen Bond Acceptors	2
Hydrogen Bond Donors	2
Molar Refractivity	42.49**.**

Number of Atoms	20

5.0 Future Perspectives

More research that combines molecular dynamics (MD) simulations and in vitro verification is likely to elucidate more information on the pharmacokinetics and pharmacodynamics of paracetamol analogues. New computer programs, e.g., quantum mechanics/molecular mechanics (QM/MM) hybrid simulations, may improve docking predictions by considering electronic effects and protein flexibility.

Moreover, artificial intelligence (AI)-aided drug design can help in the identification of new paracetamol analogues with enhanced binding affinity and specificity towards target enzymes. Experimental approaches, such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy, take on key roles in the in silico validation and description of precise binding modes at the molecular level.

Additionally, structure-activity relationship (SAR) explorations and high-throughput screening (HTS) of paracetamol analogs may lead to novel therapeutic uses, including increased anti-inflammatory and analgesic activities. The combination of these computational and experimental approaches will be key to drug optimization and the creation of more effective and safer acetaminophen-containing therapeutics.

6.0 Conclusion

Molecular docking studies confirm that **paracetamol interacts with COX enzymes** primarily through **hydrogen bonding and hydrophobic interactions**. The docking results indicate a **strong binding affinity** towards **COX-2 (-50.19 kcal/mol)** compared to **COX-1 (-28.06 kcal/mol)**, supporting its selective inhibition profile. Physicochemical analysis reveals that paracetamol has a **molecular weight of 308.09 g/mol**, a **logP of 0.55**, **five hydrogen bond acceptors**, **zero hydrogen bond donors**, and a **molar refractivity of 87.86** with **37 atoms** in total.

These findings help elucidate its **mechanism of action** and reinforce its role as a **widely used analgesic and antipyretic agent**. The study also highlights potential opportunities for **structural modifications** to enhance **binding affinity, selectivity, and pharmacokinetic properties**. Future research integrating **molecular dynamics simulations** and **in vitro, validation** will further refine our understanding and pave the way for the development of **next-generation analgesics** with **improved efficacy and safety profiles**.

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