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In silico testing of $C_9H_{12}ClNO_2$ and $C_6H_5Cl_2NO$ as derivatives of acetaminophen using molecular docking method

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Abstract

Paracetamol, a commonly used analgesic and antipyretic medication, is well-known for its ability to relieve pain and reduce temperature. However, there is a constant push to improve its therapeutic efficacy, especially towards increasing its oral bioavailability. The increase in bioavailability will lead to a better reception of the drugs by the body. This research aims to provide valuable insights into the molecular mechanisms underlying paracetamol's mode of action and propose novel strategies for enhancing its therapeutic effectiveness. We investigated the notion of functional group alteration by molecular docking as a strategy to increase the efficacy of paracetamol in this work. Using modern computational approaches, it could be conducted through the examination of the structural characteristics and active regions of paracetamol and its target receptors. Additionally, molecular docking simulations were used to examine the binding interactions between paracetamol and its target receptors, offering insights into the essential functional groups required for ligand-receptor recognition. Tests of several molecular docking techniques and scoring functions allowed the researchers to find potential alterations that might improve its pharmacological characteristics. By integrating structural analysis, molecular docking studies, and computational screening, the uncovering of promising modifications that can significantly improve paracetamol's efficacy was expected. Ultimately, this work may lead to the development of next-generation analgesics with superior pharmacological profiles, providing enhanced pain relief and fever reduction for patients.

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Keywords: active regions; computational approach; fever reduction; functional group alteration; ligand-receptor recognition; molecular docking; paracetamol; pharmacological characteristics; therapeutic efficacy

Introduction

Paracetamol or acetaminophen is one of the most commonly used analgesic and antipyretic drugs due to its wide availability and low cost (Ferreira *et al*., 2021). It is frequently used to treat mild-to-moderate pain, such as headaches, toothaches, and menstrual cramps, as well as to decrease body temperature during fevers. In the current situation, the management of more severe pain requires a conjunction pair of this drug with other medicines such as codeine (Hannibal *et al*., 2018). On an average therapeutic dose, an 80% bioavailability eases paracetamol absorption using passive diffusions in the small intestine (Ayoub, 2021). The lipid-soluble weak organic acid properties also render the blood-brain barrier and cell membranes penetrable.

From a molecular perspective, arachidonic acid conversion to prostaglandin is a two-stage process performed by the cyclooxygenase (COX) involving its two active sites: the conversion of arachidonic acid to prostaglandin G2 (PGG2) at the COX site and the conversion of that intermediate into prostaglandin H2 (PGH2) at the POX site (Sharma and Mehta, 2014). Paracetamol can interact with ferryl protoporphyrin IX radical cation (Fe^{4+}= OPP^{*+}) that is involved in the electron transfer to form tyrosine-385 radical (Tyr385*) at the POX site, which is necessary to convert arachidonic acid to PGG2 by the COX enzyme, thus decreasing its substrate in the process. Paracetamol functions as an analgesic by inhibiting the conversion of arachidonic acid into prostaglandin, which is responsible for inflammatory responses in the body (Grosser *et al*., 2017; Ghlichloo and Gerriets, 2019; Van Rensburg and Reuter, 2019).

Paracetamol is generally thought to be safe and effective when used as directed, however, it can cause liver toxicity and other side effects when taken in high doses or in combination with other drugs that affect liver function (Pacifici *et al*., 2015; Moriarty and Carroll, 2016; Tzankova *et al*., 2017). As a result, there is continued interest in producing safer and more effective versions of paracetamol, particularly for patients requiring long-term pain relief (Ralapanawa *et al*., 2016).

Following numerous other types of drugs, there have been attempts to modify the structure of paracetamol to increase the bioavailability of the drug (Oloyede *et al*., 2023), especially those targeting oral consumption as the route has the lowest bioavailability when compared to intravenous and parenteral routes (Atkinson *et al*., 2015). This research therefore aims to improve the efficacy of paracetamol by modifying the structures using molecular docking to affirm the efficacy of the change in the physical and chemical structure of paracetamol (Wang *et al*., 2018).

The proposed molecule used will be a substitution of the longer sidechain found in the acetaminophen structure. The first is $C_9H_{12}ClNO_2$ where the addition of methyl and halogen groups in the alkyl side chain was done, as it may decrease the toxicity of acetaminophen (Dahlhoff *et al*., 2014). The second, $C_6H_5Cl_2NO$, substitutes the side chain with halogen, particularly chloride, as it can increase the affinity of drug-target binding and modulate the pharmacokinetic properties of the drug (Zhang *et al*., 2017).

Materials and Methods

3D structure retrieval and preparation of the target protein

The 3D structure of the cyclooxygenase (COX) site was imported from the protein data bank using the code 5F19 (Schrödinger, 2010). The structure contained impurities as well as ligands which were then cleared by editing the structure and removing molecules other than the base protein (Aini *et al*., 2023a). After that, the file was saved into a PDB file format. Following that, energy minimization of the protein was done using Swiss

PDB viewer. The minimization of the protein changed the file's properties to ensure that it was as accurate as possible for the docking procedure.

Ligand retrieval and preparation

The proposed drug was first thought up and designed using SwissADME as the basis to see if it was possible or not from the given statistical value in the site. The proposed molecules are $C_9H_{12}ClNO_2$ (proposed molecule 1) and $C_6H_5Cl_2NO$ (proposed molecule 2), with their SMILES value being imported for them to be changed into an SDF file format using biotech fyicenter website. The next ligand was the normal acetaminophen molecule. The file for this was found in the NCBI database, specifically the acetaminophen compound page in PubChem, and was saved with the format SDF. The acetaminophen was designated as the control to see if there were any advantages regarding the binding affinity of the proposed molecules

Molecular docking

Using PyRx, specifically the Vina Wizard, blind docking of the protein and ligands was done to predict the binding (Padmi *et al*., 2022). COX protein was imported as the macromolecule while the proposed structure as well as control was imported into the binding ligand section. The binding site set was for the whole molecule rather than a specific part to ensure the best binding for the ligands. Then, software was started to produce the value needed.

3D and 2D visualization

After the docking, the structure of the docked protein was saved before it was imported into PyMOL for 3D structure visualization using a cartoon-style animation for ease of visualization before it was changed into an image file format (Wahyuni *et al*., 2022). The 2D visualization used Protein Plus from the University of Hamburg. After all was imported, the pose view menu was then chosen as it would create the 2D binding site structure. Each docking with the ligand was then saved into a separate file for 2D visualization.

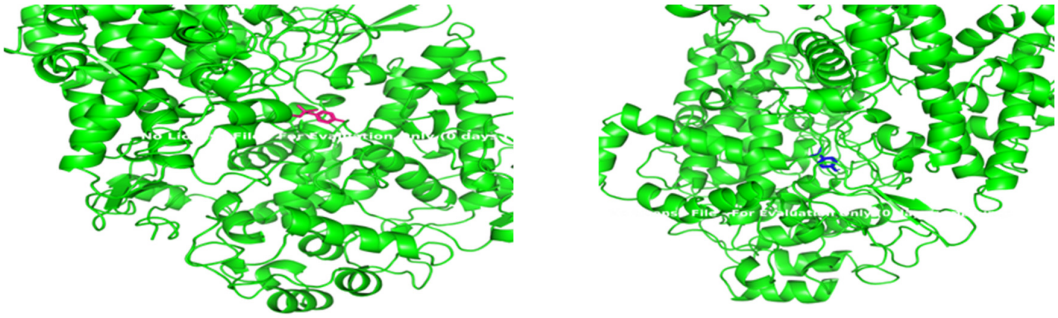
SwissADME bioactivity analysis

The bioactivity value of the ligands was analyzed by importing the SMILES (Aini *et al*., 2023b). The value consisted of the several parameters that are commonly found in drug development. Next, SMILES of the ligands were also imported into the target prediction menu in SwissADME. The *Homo sapiens* was used as the target for the ligands before the probability of binding against the possible target was calculated. Finally, the resulting target molecules were checked using SwissProt/Uniprot to ascertain the possible effects of the drug through the corresponding functions of its target molecules.

Results

Molecular docking

The molecular docking results show that there were possible binding sites for the proposed molecules, as can be seen in Figure 1. Such figure illustrates the 3D structure of the possible binding between the proposed molecule (coloured in red in Figure 1A and blue in Figure 1B) and the COX enzyme.



(A) (B)

Figure 1. 3D structure of proposed drugs 1 and 2 in the protein

The figure contains the 3D structure of the drug after it has been combined with the target molecule in PyMOL; (A) The binding ligand i.e $C_9H_{12}ClNO_2$, can be seen in the pink color after it has bound to a specific part of the COX site; (B) The binding ligand i.e $C_6H_5Cl_2NO$, can be seen in the blue color after it has bound to a specific part of the COX site

Though it may not be clear, the specific binding site can be observed in Figure 2 using the 2D structure as the basis. From the figures, we found that both bind to different areas in the protein with their specific binding affinity. The binding site for the first ligand closely followed the number of bindings the control had while the second one seemed to be different with both.

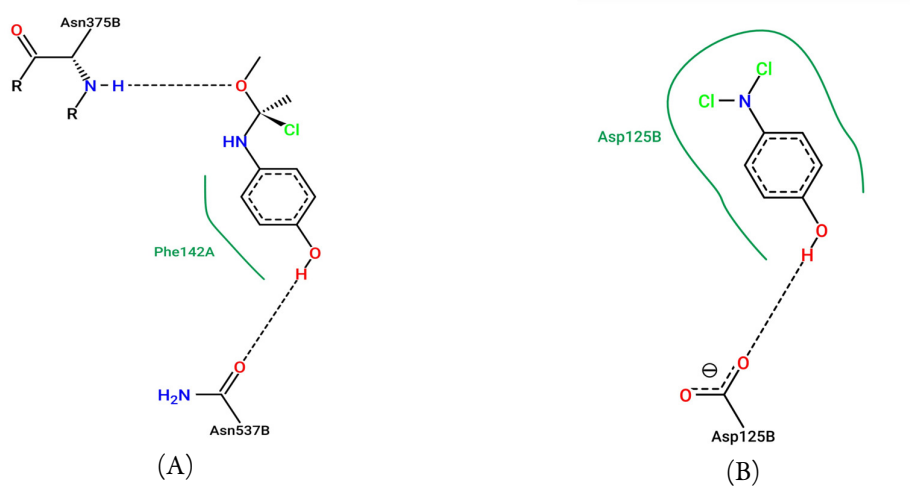


Figure 2. 2D structure of proposed drug 1 and 2 binding site location

The figure contains the 2D structure of the drugs when binding to their specific target sites; (A) The binding ligand i.e. $C_9H_{12}ClNO_2$, can be seen binding to two different molecules and an amino acid; (B) The binding ligand i.e. $C_6H_5Cl_2NO_2$, can be seen inbound to a molecule and an amino acid

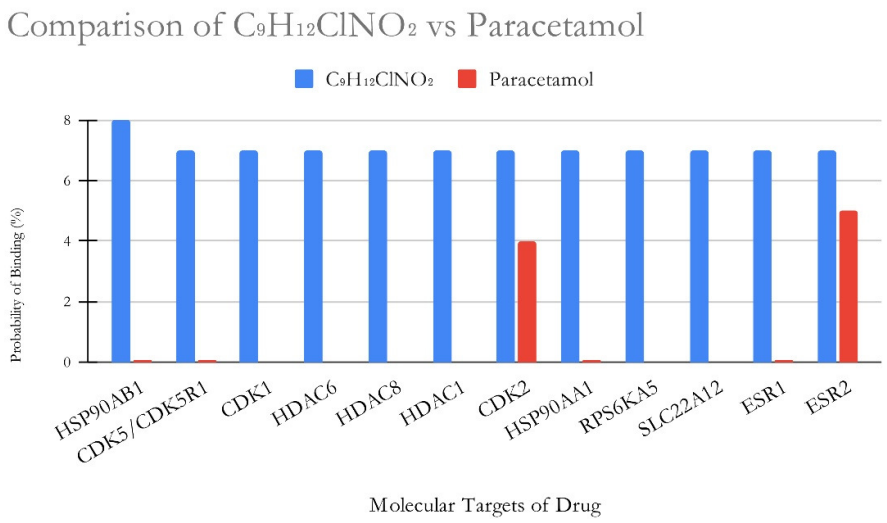
The specific binding affinity of each ligand differed from each other. The molecule with the least binding affinity to the binding site was $C_9H_{12}ClNO_2$ with its value of -5.8 which makes it lower against the control value of -5.9, making the control have a better binding affinity. Meanwhile, the best binding affinity comes from $C_6H_5Cl_2NO$ with a value of -6.1. This would signify that the combination for a variant of the acetaminophen came from the second proposed drug rather than the first (Table 1).

The SwissADME value showed multiple indicators to consider as they are used as parameters when designing a drug. Some of the parameters were not met either by the control and the proposed ligands. This indicated that some definition is allowed such as the lead likeness where all violate the same specification. One of the most important aspects of all when designing the drug is the bioavailability score, which shows the same

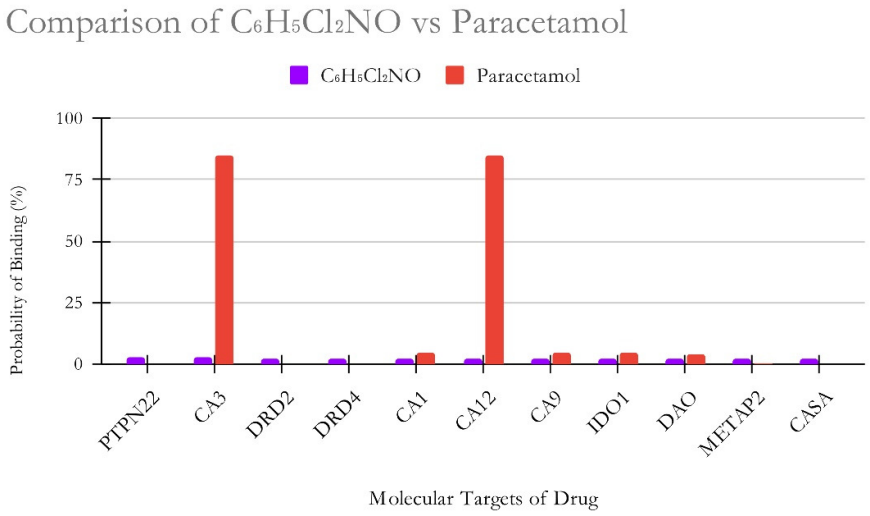
value. While Table 1 shows the parameters, Figures 3a and 3b indicate the probability of the binding sites according to SwissADME. We found that against the control, $C_9H_{12}ClNO_2$ showed higher binding affinity to some other targets specified in SwissADME (Table 1). However, the inverse happened to $C_6H_5Cl_2NO$, where paracetamol had a significantly higher probability, especially for Carbonic Anhydrase III (CA3) and Carbonic Anhydrase XII (CA12).

Table 1. SwissADME parameters of the ligands tested

Parameters	Acetaminophen	$C_9H_{12}ClNO_2$	$C_6H_5Cl_2NO$
Average Lipophilicity Prediction	0.94	1.94	1.94
Water Solubility			
ESOL Solubility	6.93e+00 mg/mL; 4.59e-02 mol/L	3.42e-01 mg/mL; 1.69e-03 mol/L	2.29e-01 mg/mL; 1.29e-03 mol/L
Solubility	Very Soluble	Soluble	Soluble
Ali Solubility	1.30e+01 mg/mL; 8.62e-02 mol/L	2.24e-01 mg/mL; 1.11e-03 mol/L	4.19e-01 mg/mL; 2.36e-03 mol/L
Solubility	Very Soluble	Soluble	Soluble
SILICOS-IT Solubility	9.72e-01 mg/mL; 6.43e-03 mol/L	1.76e-01 mg/mL; 8.73e-04 mol/L	7.27e-01 mg/mL; 4.08e-03 mol/L
Solubility	Soluble	Soluble	Soluble
Pharmacokinetics prediction			
GI absorption	High	High	High
BBB permeant	Yes	Yes	Yes
P-gp substrate	No	No	No
CYP1A2 inhibitor	No	No	Yes
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Log Kp (skin permeation)	-6.90 cm/s	-5.80 cm/s	-5.62 cm/s
Druglikeness			
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose	No; 1 violation: MW<160	Yes	No; 1 violation: #atoms<20
Veber	Yes	Yes	Yes
Egan	Yes	Yes	Yes
Muegge	No; 1 violation: MW<200	Yes	No; 1 violation: MW<200
Bioavailability Score	0.55	0.55	0.55
Medicinal Chemistry			
PAINS	0 alert	0 alert	0 alert
Brenk	1 alert: hydroquinone	4 alerts: N-C-halo, alkyl_halide, het-C-het_not_in_ring, hydroquinone	1 alert: N-halo
Lead Likeness	No; 1 violation: MW<250	No; 1 violation: MW<250	No; 1 violation: MW<250
Synthetic accessibility	1	2.44	1.14



(A)



(B)

Figure 3. Binding site probability for the first and second proposed drug
The figure contains a bar chart for the probability of the ligands binding to a specific target site; (A) The binding ligand i.e. $C_9H_{12}ClNO_2$, binds to several different targets for *Homo sapiens*; (B) The binding ligand i.e. $C_6H_5Cl_2NO$ binds to several different targets for *Homo sapiens*

Discussion

Control and ligands

As stated, before for the first proposed drug, the addition of methyl and halogen groups could decrease the toxicity of the drug. This notion was proved through the formulation of the structure in SwissADME, which implies that there was no cytochrome P450 (CYP) enzyme inhibition (Daina *et al.*, 2017). Such inhibition may cause dangerous adverse effects such as liver toxicity since the CYP enzyme plays an important role in catalysing the reaction for phase 1 metabolism of drugs and other toxic compounds from the

environment (Hakkola *et al*., 2020). Additionally, high GI absorption indicates that the modification enhances the oral bioavailability of acetaminophen (Daina *et al*., 2017; Salah *et al*., 2020). Moreover, the synthetic accessibility of the structure reached 2.44, which indicates that it is relatively easy for the drug to be synthesized, however, it is harder compared to the previously proposed acetaminophen structure (Skoraczyński *et al*., 2023).

Meanwhile, the second proposed drug showed synthetic accessibility of 1.14, suggesting that it would be very possible to be produced (Daina *et al*., 2017). Furthermore, it also had physicochemical properties, indicating a low lipophilicity score with high GI absorption as a result, hence making it possible to be easily absorbed via oral administration, similar to one of the administration methods of paracetamol (Daina *et al*., 2017; Freo *et al*., 2021; Morak-Młodawska *et al*., 2023).

For the control, the normal acetaminophen was used as a comparison as it is required to provide retrospective information regarding the optimum performance in terms of the precedent and readily binding ligands (Bender *et al*., 2021). In addition, systematic and stochastic alteration in the original paracetamol structure enables different modification points to converge into the most optimum capability (Ferreira *et al*., 2015). Paracetamol possesses type I and type II structures that differ in terms of monocetamol orientation and structures as their isomer (Liu *et al*., 2020). In addition, the presence of a more elusive and thermodynamically unstable type III paracetamol has also been reported (Ehmann and Werzer, 2014).

The differences from each form are summarized as follows (Ehmann and Werzer, 2014). Form I paracetamol contains rings, shaped as a herringbone with delocalized π orbitals, and methyl-phenyl orientation interconnected to the next paracetamol molecules. Similarly, form II paracetamol is an orthorhombic unit with more contact to the surface along with more rings and polymorphic to form I paracetamol (Agnew *et al*., 2017). In contrast, form III has a distinct needle-like structure which is reduced in terms of the surface area, compensated with a greater height of 450 nm (Agnew *et al*., 2016).

PyMOL and protein plus

PyMOL was used in this study due to its clear and publishable 3D visualization of the binding, it is also able to identify the amino acids involved in the binding proposition between the COX enzyme and the proposed drug model (Yuan *et al*., 2017). The results of molecular docking through PyMOL showed a favourable outcome, in which both proposed drugs have a binding site corresponding to the COX2 molecular structure (Figure 1).

Proteins Plus was used to exactly name and identify the binding site's amino acids and the structure it binds to (Fährrolfes *et al*., 2017). PyMOL was unable to specifically determine the binding site of paracetamol by its amino acids (Mooers, 2016). Protein Plus generates a 2D structure, giving clarity to the specification of the binding site's contents (Figure 2). The difference between the two proposed drug models became more palpable through the generated 2D structure. It can be observed that the first alternative model for the drug, $C_6H_5Cl_2NO$, had a similar binding pattern to the control paracetamol with three binding sites, however, these sites interacted with different amino acids. On the contrary, the second model, $C_9H_{12}ClNO_2$, had a different binding pattern to the control, as the molecule only bound to two amino acids instead of three (Figure 2). Such results indicate that the first drug model is the same, if not more effective compared to paracetamol, while the second drug model is less likely to be more effective than the control. This is because the number of binding sites is attributable to the binding affinity of the drug molecule towards the enzyme (Wakefield *et al*., 2020).

Energy minimization and binding affinity

Energy minimization is done to reduce the protein's potential energy (Bhattacharya and Cheng, 2013). This process is done using Swiss PDB software. By doing this process, decreases the protein's likelihood to undergo structural changes, thus maintaining the integrity of the protein, leading to increased stability and prolonged shelf life (Yang *et al*., 2020). It also increases the drug's chances of being effectively absorbed by the human body and eases the distribution process to the target, thus minimizing the drug's immunogenicity while

also improving its bioavailability and therapeutic efficacy (Dayer, 2020). This is all achieved through geometry optimization and steric clash removal done with the software. As a result of the Swiss PDB software, the energy has been successfully minimized.

To further determine the efficacy of the alternative paracetamol structures, assessing their binding affinity was necessary to ensure that the structures will bind selectively and specifically to their desired target, with the target receptor being the COX enzyme (Lonsdale and Ward, 2018). One way to investigate binding affinity is by molecular docking using PyRx as it provides an auto dock feature, namely AutoDock Vina, that provides the output of ten best binding modes between the ligand and enzyme macromolecule (Dallakyan and Olson, 2015).

The first proposed molecule, $C_9H_{12}ClNO_2$, had a binding affinity that was lower compared to the control molecule which indicates a weaker binding. This indicates that the first proposed molecule has a weaker affinity to the COX enzyme compared to the original paracetamol (Dallakyan and Olson, 2015).

On the other hand, the second proposed molecule, $C_6H_5Cl_2NO$, had a higher binding affinity toward the COX enzyme compared to the control molecule, suggesting that the proposed molecule would bind better to the COX enzyme as its binding affinity value was more negative than paracetamol (Dallakyan and Olson, 2015; Jain *et al*., 2021). Such a result is also in agreement with a study conducted by Uzzaman *et al*. (2019) as they have also discovered alternative paracetamol structures that have higher binding affinities compared to the original.

Parameter analysis

Predictions of parameter analysis are done to determine the predicted molecules' properties; this is done to determine if there would be any improvements in drug processing inside the body. The first parameter is lipophilicity that indicates the affinity of the molecule for lipophilic environments which corresponds to its ability to cross cell membranes as well as the resulting binding to proteins. Higher lipophilicity is generally indicative of higher absorption, distribution, metabolism, and excretion (ADME) while immense lipophilicity is a sign of potential toxicity in live model tests (Lobo, 2019). Both proposed alternatives had an increased lipophilicity compared to paracetamol, indicating higher ADME predictions with possible toxicity. Water solubility affects the oral bioavailability of a drug, with reduced water solubility resulting in the reduction of an oral drug's efficacy (Sanches and Ferreira, 2019). Both theorized molecules were slightly less soluble than paracetamol but were still considered water-soluble drugs, signifying a weaker but still viable drug oral efficacy compared to paracetamol. Pharmacokinetics are all parameters that affect ADME the most (Grogan and Preuss, 2021). Both molecules showed very little deviation from the original paracetamol indicating that the alternative molecules have a similar ADME with paracetamol. The singular deviation is that Molecule 2 is predicted to have a possibility of inhibiting CYP450. CYP450 plays an important role in removing non-self-biological agents, metabolism of cells, and homeostasis. Inhibition of the protein might lead to unwanted adverse effects or unintended drug-drug interactions such as reducing clearance of tizanidine (Manikandan and Nagini, 2018; Villa-Zapata *et al*., 2022). Skin permeability is included in this section, and it is essential as it affects transdermal drug delivery (Supe and Takudage, 2020). The results show that the new molecules had a lower log Kp, indicating a slightly better skin permeability. The last two key parameters are the bioavailability score and synthetic accessibility. Bioavailability is indicative of how much of the drug in dosage can circulate in the body and then effectively bind to its intended receptor (Price and Patel, 2020; Kharisma *et al*., 2023). As seen, the bioavailability scores of all three molecules were the same. This result means that both new molecules are just as effective as paracetamol in terms of bioavailability. Synthetic accessibility or the SA Score was calculated to check difficulty in synthesizing the drug, from 1 being easy and 10 being hard (Skoraczyński *et al*., 2023). Molecule 1 is harder to synthesize compared to paracetamol with a SA score of 2.44, while molecule 2 is only slightly harder to synthesize compared to paracetamol with a score of 1.14. Overall, molecule 2 is predicted to have a higher efficacy as a possible alternative to paracetamol, however, it is also predicted to inhibit CYP450 which may cause adverse effects.

Target prediction

Molecule 1 had very low percentages of binding, indicating that the molecule has a low specificity of binding (Figure 3A). Molecule 1 is predicted to bind to molecules that have low percentages of binding to paracetamol with some molecules having no binding likelihood to paracetamol. Overall, the results indicate that molecule 1 will not produce the same analgesic effects that paracetamol produces. Further research using SwissProt determined that the proteins predicted to bind to the molecule are linked to cell cycles, transcription factors, or estrogen hormones. This means that the molecule could cause adverse effects related to the production of unintended proteins via the activated transcription factors, adverse effects related to abnormal cell cycles, or adverse effects related to estrogen malfunction.

Molecule 2 also had very low percentages of binding, however, many of the molecules targeted by Molecule 2 may also interact with paracetamol, particularly CA3 and CA12 (Figure 3B). These two molecules showed incredibly high percentages of binding with paracetamol, which indicates that these two proteins are some of the main binding partners to paracetamol. Despite their low percentage of binding with the second molecule, the predicted proteins were similar to molecules that bind to paracetamol as most of the target proteins reversibly hydrate carbon dioxide. An important notice for further research is that the highest percentage binding prediction for Molecule 2 was PTPN22, a negative regulator of T-Cell activity indicating a possibility of immunosuppression by the drug. From the target prediction, it can be concluded that Molecule 2 is a better alternative compared to Molecule 1 as the results would be more in line with paracetamol with better parameters and lower possibility of adverse effects.

Limitations and future recommendations

As predictions, these results can be overturned during live tests and dosages still need to be determined before clinical testing. The best way to address this issue would be further research by synthesizing the drug and testing its effects in vivo. Other possible improvements could be made by changing the modified groups to groups previously known to have an impact on pain inhibition or inflammatory pathways. This methodology of using molecular docking to add alternative groups into paracetamol can also be used to ascertain the binding affinity and selectivity of changed molecules towards certain target receptors or enzymes (Wang *et al*., 2018). Furthermore, investigating structural changes and molecular interactions by using paracetamol and molecular docking might assist in understanding the underlying processes responsible for enhanced drug effectiveness (Daneshgar *et al*., 2009). Critical structural properties for increased activity can be determined through analysis of the binding modes and energetics of the changed molecules. It would be beneficial to explore the possible synergistic effects of mixing paracetamol with other functional groups or co-administered medications as molecular docking simulations can anticipate the binding affinities and potential cooperative interactions between paracetamol and these other drugs, giving useful information for combination treatment techniques and the creation of more effective and personalized pharmaceutical formulations (El-Shaheny *et al*., 2019). Regardless of the results, experimental validation such as in vivo and in vitro testing will be needed to verify the results.

Conclusions

Out of the two molecules proposed, Molecule 2 or $C_6H_5Cl_2NO$ is more viable than the other proposed molecule. The efficacy, dosage, and toxicity need to be researched further in vivo and compared to existing medication to determine the effects of the molecules on live subjects. This research has proven that bioinformatic software and programs can help in predicting alternative drug structures, however, the predictions still require further wet lab research and testing to determine if a molecule can be marketed commercially as well as to determine possible side effects.

Authors' Contributions

JL, KI: Data curation; KI, JPWT, M: Formal analysis; AAP: Funding acquisition; SJ, NRPG, OT, KA Investigation; JL, M: Methodology; AAP, FS: Project administration; JL, KI, M, JPWT: Software; AAP, FS: Supervision; KI, M Visualization; JL, KI, JPWT, SJ, M, OT, NRPG, KA: Writing - original draft; M, JL, KI, JPWT, SJ, OT, NRPG, KA, AAP, FS Writing - review and editing. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aini NS, Ansori ANM, Kharisma VD, Murtadlo AAA, Tamam MB, Sucipto TH, ... Zainul R (2023a). An *in silico* study: Phytochemical compounds screening of garcinia atroviridis griff. ex t. anders as anti-DENV. Journal of Pure and Applied Microbiology 17(4):2467-2478. *https://doi.org/10.22207/jpam.17.4.45*

Aini NS, Kharisma VD, Ansori A, Murtadlo AAA, Tamam MB, Turista DDR, ... Zainul R (2023b). Bioactive compounds screening of *Rafflesia* sp. and *Sapria* sp. (Family: Rafflesiaceae) as anti-SARS-CoV-2 via tetra inhibitors: An *in silico* research. Journal of Pharmacy and Pharmacognosy Research 11(4):611-624. *https://doi.org/10.56499/jppres23.1620_11.4.611*

Atkinson HC, Stanescu I, Frampton C, Salem II, Beasley CP, Robson R (2015). Pharmacokinetics and bioavailability of a fixed-dose combination of ibuprofen and paracetamol after intravenous and oral administration. Clinical Drug Investigation 35:625-632. *https://doi.org/10.1007/s40261-015-0320-8*

Ayoub SS (2021). Paracetamol (acetaminophen): A familiar drug with an unexplained mechanism of action. Temperature 8(4):351-371. *https://doi.org/10.1080/23328940.2021.1886392*

Bhattacharya D, Cheng J (2013). 3Drefine: Consistent protein structure refinement by optimizing hydrogen bonding network and atomic-level energy minimization. Proteins: Structure, Function, and Bioinformatics 81(1):119-131. *https://doi.org/10.1002/prot.24167*

Chung CW, Dean AW, Woolven JM, Bamborough P (2012). Fragment-based discovery of bromodomain inhibitors part 1: inhibitor binding modes and implications for lead discovery. Journal of Medicinal Chemistry 55(2):576-586. *https://doi.org/10.1021/jm201320w*

Dallakyan S, Olson AJ (2015). Small-molecule library screening by docking with PyRx. Chemical Biology: Methods and Protocols 243-250. *https://doi.org/10.1007/978-1-4939-2269-7_19*

Daneshgar P, Moosavi-Movahed AA, Norouzi P, Ganjali MR, Madadkar-Sobhani A, Saboury AA (2009). Molecular interaction of human serum albumin with paracetamol: spectroscopic and molecular modeling studies. International Journal of Biological Macromolecules 45(2):129-134. *https://doi.org/10.1016/j.ijbiomac.2009.04.011*

Dayer MR (2020). Old drugs for newly emerging viral disease, COVID-19: Bioinformatic Prospective. arXiv preprint arXiv:2003.04524. *https://doi.org/10.48550/arXiv.2003.04524*

El-Shaheny R, Fuchigami T, Yoshida S, Radwan MO, Nakayama M (2019) Complementary HPLC, *in silico* toxicity, and molecular docking studies for investigation of the potential influences of gastric acidity and nitrite content on paracetamol safety. Microchemical Journal 150:104107. *https://doi.org/10.1016/j.microc.2019.104107*

Fährrolfes R, Bietz S, Flachsenberg F, Meyder A, Nittinger E, Otto T, ... Rarey, M (2017). Proteins Plus: a web portal for structure analysis of macromolecules. Nucleic Acids Research 45(W1):W337-W343. *https://doi.org/10.1093/nar/gkx333*

Ferreira GE, Dmitritchenko A, McLachlan AJ, Day RO, Saragiotto B, Lin C, ... Maher, C. G (2021). The efficacy and safety of paracetamol for pain relief: an overview of systematic reviews. The Medical Journal of Australia 214(7):324-331. *https://doi.org/10.5694/mja2.50992*

Ghlichloo I, Gerriets V (2019). Nonsteroidal anti-inflammatory drugs (NSAIDs). Europe PMC; StatPearls Publishings. *https://europepmc.org/article/nbk/nbk547742*

Grogan S, Preuss CV (2021). Pharmacokinetics. Nih.gov; StatPearls Publishing. *https://www.ncbi.nlm.nih.gov/books/NBK557744/*

Grosser T, Theken KN, FitzGerald GA (2017). Cyclooxygenase inhibition: pain, inflammation, and the cardiovascular system. Clinical Pharmacology and Therapeutics 102(4):611-622. *https://doi.org/10.1002/cpt.794*

Hannibal CG, Dehlendorff C, Kjaer SK (2018). Use of paracetamol, low-dose aspirin, or non-aspirin non-steroidal anti-inflammatory drugs and risk of ovarian borderline tumors in Denmark. Gynecologic Oncology 151(3):513-518. *https://doi.org/10.1016/j.ygyno.2018.09.022*

Jain AS, Sushma P, Dharmashekar C, Beelagi MS, Prasad SK, Shivamallu C, ... Prasad KS (2021). *In silico* evaluation of flavonoids as effective antiviral agents on the spike glycoprotein of SARS-CoV-2. Saudi Journal of Biological Sciences 28(1):1040-1051. *https://doi.org/10.1016/j.sjbs.2020.11.049*

Kharisma VD, Ansori ANM, Antonius Y, Rosadi I, Affan A, Jakhmola V, ... Purnobasuki H. (2023). Garcinoxanthones from *Garcinia mangostana* L. against sars-cov-2 infection and cytokine storm pathway inhibition: A viroinformatics study. Journal of Pharmacy and Pharmacognosy Research 11(5):743-756. *https://doi.org/10.56499/jppres23.1650_11.5.743*

Lobo, S (2019). Is there enough focus on lipophilicity in drug discovery? Expert Opinion on Drug Discovery 15(3):261-263. *https://doi.org/10.1080/17460441.2020.1691995*

Lonsdale R, Ward RA (2018). Structure-based design of targeted covalent inhibitors. Chemical Society Reviews 47(11):3816-3830. *https://doi.org/10.1039/C7CS00220C*

Manikandan P, Nagini S (2018). Cytochrome P450 Structure, Function and Clinical Significance: A Review. Current Drug Targets 19(1). *https://doi.org/10.2174/1389450118666170125144557*

McCrae JC, Morrison EE, MacIntyre IM, Dear JW, Webb DJ (2018). Long-term adverse effects of paracetamol–a review. British Journal of Clinical Pharmacology 84(10):2218-2230. *https://doi.org/10.1111/bcp.13656*

Mooers BH (2016). Simplifying and enhancing the use of PyMOL with horizontal scripts. Protein Science 25(10):1873-1882. *https://doi.org/10.1002/pro.2996*

Moriarty C, Carroll W (2016). Paracetamol: pharmacology, prescribing and controversies. Archives of Disease in Childhood-Education and Practice 101(6):331-334. *http://dx.doi.org/10.1136/archdischild-2014-307287*

Oloyede OO, Alabi ZO, Akinyemi AO, Oyelere SF, Oluseye AB, Owoyemi BCD (2023). Comparative evaluation of acetaminophen form (I) in commercialized paracetamol brands. Scientific African 19:e01537. *https://doi.org/10.1016/j.sciaf.2022.e01537*

Pacifici GM, Allegaert K (2015). Clinical pharmacology of paracetamol in neonates: a review. Current Therapeutic Research 77:24-30. *https://doi.org/10.1016/j.curtheres.2014.12.001*

Padmi H, Kharisma VD, Ansori ANM, Sibero MT, Widyananda MH, Ullah ME, ... Zainul R (2022). Macroalgae bioactive compounds for the potential antiviral of SARS-CoV-2: An *in silico* study. Journal of Pure and Applied Microbiology 16(2):1018-1027. *https://doi.org/10.22207/jpam.16.2.26*

Price G, Patel DA (2020). Drug Bioavailability. PubMed; StatPearls Publishing. *https://www.ncbi.nlm.nih.gov/books/NBK557852/*

Qureshi MA, Khan TJ, Qureshi MS (2011). Molecular Docking helps in understanding the action of Paracetamol (acetaminophen): an approach towards finding a better COX2 inhibitor. RMJ 36(3):238-241. *https://www.rmj.org.pk/fulltext/27-1298307061.pdf*

Ralapanawa U, Jayawickreme KP, Ekanayake EMM, Dissanayake AMS (2016). A study on paracetamol cardiotoxicity. BMC Pharmacology and Toxicology 17(1):1-8. *https://doi.org/10.1186/s40360-016-0073-x*

Sanches BMA, Ferreira EI (2019). Is prodrug design an approach to increase water solubility? International Journal of Pharmaceutics 568:118498. *https://doi.org/10.1016/j.ijpharm.2019.118498*

Sharma CV, Mehta V (2014). Paracetamol: mechanisms and updates. Continuing Education in Anaesthesia, Critical Care and Pain 14(4):153-158. *https://doi.org/10.1093/bjaceaccp/mkt049*

Schrödinger LLC (2010). The PyMOL molecular graphics system. version 2.5.0

Skoraczyński G, Kitlas M, Miasojedow B, Gambin A (2023). Critical assessment of synthetic accessibility scores in computer-assisted synthesis planning. Journal of Cheminformatics 15(1). *https://doi.org/10.1186/s13321-023-00678-z*

Supe S, Takudage P (2020). Methods for evaluating penetration of drug into the skin: A review. Skin Research and Technology 27(3). *https://doi.org/10.1111/srt.12968*

Tzankova V, Aluani D, Kondeva-Burdina M, Yordanov Y, Odzhakov F, Apostolov A, Yoncheva K (2017). Hepatoprotective and antioxidant activity of quercetin loaded chitosan/alginate particles *in vitro* and *in vivo* in a model of paracetamol-induced toxicity. Biomedicine and Pharmacotherapy 92:569-579. *https://doi.org/10.1016/j.biopha.2017.05.008*

Uzzaman M, Shawon J, Siddique ZA (2019). Molecular docking, dynamics simulation and ADMET prediction of Acetaminophen and its modified derivatives based on quantum calculations. SN Applied Sciences 1:1-10. *https://doi.org/10.1007/s42452-019-1442-z*

Van Rensburg R, Reuter H (2019). An overview of analgesics: NSAIDs, paracetamol, and topical analgesics Part 1. South African Family Practice. *https://doi.org/10.1080/20786190.2019.1610228*

Villa-Zapata L, Gomez-Lumbreras A, Horn JR, Tan M, Boyce RD, Malone DC (2022). A Disproportionality Analysis of Drug–Drug Interactions of Tizanidine and CYP1A2 Inhibitors from the FDA Adverse Event Reporting System (FAERS). Drug Safety 45(8):863-871. *https://doi.org/10.1007/s40264-022-01200-4*

Wahyuni DK, Wacharasindhu S, Bankeeree W, Punnapayak H, Purnobasuki H, Junairiah, ... Prasongsuk S (2022). Molecular simulation of compounds from n-hexane fraction of *Sonchus Arvensis L.* leaves as SARS-CoV-2 antiviral through inhibitor activity targeting strategic viral protein. Journal of Pharmacy and Pharmacognosy Research 10(6):1126-1138. *https://doi.org/10.56499/jppres22.1489_10.6.1126*

Wakefield AE, Kozakov D, Vajda S (2022). Mapping the binding sites of challenging drug targets. Current Opinion in Structural Biology 75:102396. *https://doi.org/10.1016/j.sbi.2022.102396*

Wang Y, Lin W, Wu N, He X, Wang J, Feng Z, Xie XQ (2018). An insight into paracetamol and its metabolites using molecular docking and molecular dynamics simulation. Journal of Molecular Modeling 24:1-13. *https://doi.org/10.1007/s00894-018-3790-9*

Yang J, Anishchenko I, Park H, Peng Z, Ovchinnikov S, Baker D (2020). Improved protein structure prediction using predicted interresidue orientations. Proceedings of the National Academy of Sciences 117(3):1496-1503. *https://doi.org/10.1073/pnas.1914677117*

Yuan S, Chan HCS, Hu Z (2017). Using PyMOL as a platform for computational drug design. Wiley Interdisciplinary Reviews: Computational Molecular Science 7(2):e1298. *https://doi.org/10.1002/wcms.1298*

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