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Peer Review

Review of: "*In Silico* Evaluation of the Predicted Inhibitory Potential of p-Hydroxybenzoic Acid and Cyclohexane-1,2,3,4,5-pentol Isolated from *Hyphaene coriacea* Gaertn. Toward Human Maltase-Glucoamylase and Pancreatic α-Amylase"

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The manuscript presents a molecular-docking study of p-hydroxybenzoic acid and cyclohexane-1,2,3,4,5-pentol, compounds reported as isolated from *Hyphaene coriacea*, against human maltase-glucoamylase and pancreatic α-amylase, using miglitol and acarbose as reference ligands. The topic is relevant to natural-product research and postprandial glucose regulation. However, important issues concerning **ligand identity and stereochemistry, protonation states, docking-protocol validation, pose selection, interpretation of very small docking-score differences, and extrapolation from docking to enzyme inhibition/therapeutic potential** should be addressed before the conclusions can be considered robust.

1. Title

1. The title appropriately identifies the study as **in silico**, which is important.
2. The phrase **"Predicted Inhibitory Potential"** remains somewhat stronger than what molecular docking alone demonstrates. Docking predicts binding poses and scores, not actual enzyme inhibition.

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3. Consider replacing "inhibitory potential" with **"predicted binding potential"** or **"molecular docking interactions."**
4. The statement that the compounds were **"isolated from *Hyphaene coriacea*"** relies on previous work rather than isolation performed in the present study. This should be made clear.
5. A possible title is:

   "Molecular Docking Evaluation of p-Hydroxybenzoic Acid and Cyclohexane-1,2,3,4,5-pentol Reported from *Hyphaene coriacea* against Human Maltase-Glucoamylase and Pancreatic α-Amylase."

2. Abstract

1. The Abstract clearly describes the targets, docking software, reference compounds, and principal docking scores.
2. The statement that the two compounds possess **"promising inhibitory potential"** should be moderated because no enzyme-inhibition experiment was performed.
3. The phrase **"natural therapeutic candidates for type 2 diabetes"** is premature for compounds assessed solely by molecular docking.
4. A more appropriate conclusion would be that the compounds **show predicted active-site binding and warrant experimental enzyme-inhibition testing**.
5. The Abstract should explicitly indicate that Vina values are **docking scores/predicted affinities**, not experimentally determined binding free energies.
6. The differences for maltase-glucoamylase are very small: −6.1 and −5.9 kcal/mol versus −5.8 kcal/mol for miglitol. Such differences should not be interpreted as evidence that the phytochemicals bind more strongly than the reference drug.
7. The Abstract should mention the important limitation that the stereochemical configuration of cyclohexane-1,2,3,4,5-pentol was not defined.

3. Introduction

1. The Introduction provides an appropriate general rationale for targeting carbohydrate-digesting enzymes in postprandial glucose regulation.
2. The hypothesis that the compounds **"may act as competitive inhibitors"** is too specific. Molecular docking cannot establish a competitive mechanism.

3. The hypothesis should instead state that the compounds may **interact with the catalytic sites** of the selected enzymes.
4. The rationale for selecting exactly these two compounds should be strengthened. Were they major constituents of *H. coriacea*, compounds previously associated with antidiabetic activity, or simply compounds previously identified by the authors?
5. The manuscript should distinguish more carefully between **binding, enzyme inhibition, postprandial glucose modulation, and antidiabetic efficacy**.
6. The novelty of performing docking for these compounds against these two specific human enzymes should be stated more explicitly.

4. Materials and Methods

1. The most important issue concerns **cyclohexane-1,2,3,4,5-pentol stereochemistry**. Table 1 explicitly states **"without stereochemical configuration."**
2. Cyclohexane-1,2,3,4,5-pentol can have multiple stereochemical configurations, which may produce substantially different three-dimensional conformations and protein interactions. Which exact stereoisomer was experimentally isolated from *H. coriacea*?
3. If the stereochemistry of the isolated natural product has not been established, docking a single unspecified/arbitrarily generated 3D structure cannot provide a compound-specific binding prediction. This limitation must be resolved or explicitly acknowledged.
4. The authors should provide stronger evidence for the identity of the compounds reportedly isolated from *H. coriacea*. Reference 9 appears to be a symposium contribution rather than a detailed peer-reviewed chemical-characterization paper.
5. Were NMR, MS, optical rotation, or other analytical methods originally used to establish the structure and stereochemistry of cyclohexane-1,2,3,4,5-pentol?
6. Ligand protonation states are not adequately described. This is particularly important for **p-hydroxybenzoic acid**, whose carboxyl group can be ionized under physiologically relevant conditions.
7. What protonation state of p-hydroxybenzoic acid was used for docking?
8. Were alternative ionization/protonation states evaluated?
9. The Methods should describe ligand geometry optimization or energy minimization before docking. Conversion from SDF/PDB to PDBQT alone is insufficient methodological information.

10. The protonation states of protein residues, especially histidines and catalytic acidic residues, should also be described.
11. The authors state that water molecules, co-crystallized ligands, and non-essential heteroatoms were removed while **"necessary structural ions"** were retained. Which ions were retained and on what basis?
12. Removing all crystallographic water molecules without examining whether any participate in ligand recognition should be justified.
13. The major methodological weakness is the absence of **docking-protocol validation by redocking the crystallographic ligands**.
14. Since 3L4W contains miglitol and 1B2Y contains acarbose, the authors should remove and redock the native ligand, then report the RMSD between the experimental and predicted pose.
15. A commonly acceptable redocking RMSD should be established before interpreting the phytochemical poses. Without this validation, there is no evidence that the selected grid/protocol can reproduce experimentally observed binding modes.
16. Were the reference-ligand poses shown in the figures obtained from **redocking** or simply taken from the crystallographic structures? This distinction must be stated.
17. Grid-box coordinates are provided, which improves reproducibility. However, the authors should show that each box adequately encloses the complete crystallographic ligand-binding region.
18. The exhaustiveness value of **8** is relatively modest. The authors should justify using the default value or demonstrate convergence using higher exhaustiveness.
19. Were docking calculations independently repeated with different random seeds? A single docking run provides no information concerning computational reproducibility.
20. The manuscript should report the AutoDock Vina version, not only PyRx version 0.8.
21. Most importantly, Table 3 shows that **rank 4**, rather than rank 1, was selected for both phytochemicals against α-amylase.
22. Why were rank-4 poses selected? What were the docking scores and locations of poses ranked 1–3?
23. Selecting a lower-ranked pose because it interacts with expected catalytic residues introduces a potential **post hoc selection bias**.
24. All pose scores should be provided, preferably in supplementary material, and the selection rule should be predefined and objectively justified.
25. If the most favorable Vina pose lies outside the expected binding site, this may itself indicate a problem with the docking box or binding-site definition and should not simply be bypassed by

choosing rank 4.

5. Results

1. The numerical docking results are clearly presented in Table 3.
2. The difference between −6.1 kcal/mol for cyclohexane-pentol and −5.8 kcal/mol for miglitol is only **0.3 kcal/mol**. This difference is too small to justify a meaningful claim of superior predicted binding.
3. Similarly, −5.9 versus −5.8 kcal/mol should essentially be interpreted as comparable within the uncertainty of the docking scoring function.
4. Vina scores should not be treated as experimentally meaningful quantitative binding affinities.
5. For pancreatic α-amylase, both test compounds show substantially less favorable docking scores (−5.0 and −5.2 kcal/mol) than acarbose (−9.7 kcal/mol). This should be emphasized more clearly.
6. The use of different molecular sizes also makes simple score ranking problematic because docking scores can favor larger ligands with more interaction opportunities.
7. The α-amylase interaction analysis is informative, but identifying contacts with catalytic residues does not demonstrate functional inhibition.
8. Cyclohexane-1,2,3,4,5-pentol shows an **unfavorable interaction with Asp300**, one of the catalytic residues. The possible significance of this unfavorable contact deserves more discussion.
9. Similarly, the unfavorable interaction with Asp542 in maltase-glucoamylase should not be overlooked when interpreting the pose.
10. For p-hydroxybenzoic acid, interaction with Asp197 via a π-anion interaction does not itself indicate inhibition or appropriate catalytic-site occupancy.
11. The manuscript should ideally report hydrogen-bond distances and relevant interaction distances rather than only naming residues.
12. Figures 1 and 2 provide useful visualization, but the panels are split across different pages, which reduces readability.
13. The caption **“Figure 2.3D and 2D...”** needs typographical correction to **“Figure 2. 3D and 2D...”**
14. The interaction figures should clarify whether they show the selected redocked pose, crystallographic reference pose, or another pose.

6. Discussion

1. The Discussion is generally more cautious than the Abstract and correctly acknowledges that docking cannot establish a competitive inhibition mechanism.
2. Nevertheless, greater emphasis should be placed on the uncertainty associated with AutoDock Vina scores.
3. Differences of only **0.1–0.3 kcal/mol** should not be used to infer stronger binding than miglitol.
4. The manuscript should discuss the lack of docking validation as a major limitation.
5. The undefined stereochemistry of cyclohexane-1,2,3,4,5-pentol should also be discussed as a major limitation rather than a minor structural detail.
6. The authors appropriately state that docking does not establish enzyme selectivity, magnitude of inhibition, physiological efficacy, or gastrointestinal tolerability. This cautious wording should also be adopted in the Abstract and Conclusion.
7. The suggestion that these compounds might provide **"more moderate enzymatic modulation with improved gastrointestinal tolerability"** is highly speculative and should be shortened or explicitly identified as a hypothesis for future research.
8. The comparison with D-pinitol is interesting but cannot substitute for experimental evidence because the compounds differ structurally and stereochemically. The authors already partly acknowledge this limitation.
9. The Discussion would benefit from explicitly stating that docking provides **structural hypotheses**, not proof of binding or inhibition.
10. Molecular dynamics and free-energy calculations could strengthen the computational component, but **in vitro enzyme assays are considerably more important** for validating the main biological hypothesis.
11. Network pharmacology is not necessary at this stage. Experimental validation of the two primary enzyme targets should take priority.

7. Conclusion

1. The conclusion is appropriately more cautious than some parts of the Abstract and states that experimental validation is required.

2. The statement that the compounds are **“capable of interacting”** with the enzymes should preferably be changed to **“were predicted to interact in the docking simulations.”**
3. The study cannot conclude that the compounds modulate the enzymes; only possible active-site binding was computationally predicted.
4. The conclusion should explicitly acknowledge that no experimental inhibitory activity was determined.
5. The undefined stereochemistry of cyclohexane-1,2,3,4,5-pentol substantially limits the biological interpretation and should be mentioned.
6. The conclusion should avoid suggesting therapeutic relevance until enzyme inhibition, potency, mechanism, and biological exposure are experimentally established.

Overall Opinion

1. The manuscript addresses a relevant subject and has the advantage of using **human enzyme crystal structures with appropriate clinically relevant reference ligands**.
2. The docking study is relatively straightforward and potentially useful as a preliminary hypothesis-generating analysis.
3. However, its present scientific strength is limited because **the docking protocol was not validated by redocking the native co-crystallized ligands**.
4. A particularly important concern is that cyclohexane-1,2,3,4,5-pentol is explicitly modeled **without defined stereochemistry**, although stereochemistry can substantially affect molecular recognition.
5. Protonation and ionization states, ligand optimization, receptor preparation, docking reproducibility, and treatment of crystallographic waters/ions require more detailed reporting.
6. The selection of **rank-4 α-amylase poses** instead of the best-ranked poses requires strong justification and complete reporting of all higher-ranked alternatives.
7. The small docking-score differences between the phytochemicals and miglitol are not sufficient to establish stronger binding, and the scores should not be interpreted as experimental binding affinities.
8. The manuscript appropriately acknowledges some limitations in the Discussion, but the Abstract and parts of the interpretation still overstate **inhibitory and therapeutic potential**.
9. At minimum, the authors should perform **native-ligand redocking validation, clarify stereochemistry and protonation states, rerun/fully document the docking protocol, and**

substantially moderate biological claims.

10. **Recommended decision: Major Revision.** If the authors cannot establish the actual stereochemical identity of the cyclohexane-1,2,3,4,5-pentol isolated from *H. coriacea* or cannot validate the docking protocol by reproducing the crystallographic reference poses, the scientific interpretation of the docking results would remain substantially weakened.

Declarations

Potential competing interests: No potential competing interests to declare.