Title: Exploration of potential inhibitors of poxin protein in monkeypox virus through molecular docking techniques: An in-silico drug repurposing study

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Reviewer D: Sharadindu Sinha, ORCID: 0009-0006-6758-4655, COI: None, AI disclosure: None

Please provide your comments on the following points. [Author should respond to these comments including the line numbers where the changes have been done in the revised manuscript]

1. Comment **Completeness and accuracy of the Abstract.**

Line 26: an abbreviation should not appear at first without prior introducing. Line 28: it would be fine if the authors would write molecular docking plays instead of playing. In method section, the authors should specify the name of target and software used for.

Response: Thank you for your comment to improve the manuscript. we revised the manuscript based on your comment. (Page: 02 ; Line: 26-28)

2. Comment Clarity of the rationale for conducting the study is given in the Introduction section. The rationale of the study is written clearly. The target and action of target should be written sequentially. e. g; Page 85-86. It would be fine if the authors rewrite the sentence. Target validation is important. The authors should cite some journals where the targets are validated or not.

Response: Thank you for your comment to improve the manuscript. We updated the manuscript based on your comment.

3. Comment Quality, clarity and appropriateness of the Table(s). Yes, tables are clear and self-explanatory. All the tables (table 1&2) should contain only the important variables instead of all the variable.

Response: Thank you for your comment to improve the manuscript. We revised the manuscript based on your comments.

4. Comment Pertinence of the Discussion section whether it justify the main message of the manuscript without repeating the results. The discussion should be started with main finding of the study rather than reviewing the previous results.

Response: Thank you for your comment to improve the manuscript. We revised the discussion part based on your comments. (Page: 08; Line: 175-180)

5. Comment Whether Strength(s) and Limitation(s) are well described. Not described

Response: Thank you for your comment. We described the strength and limitations of our study. (Page: 10; Line: 233-237)

6. Comment Whether the manuscript is supported by appropriate and up-to-date References.
Some references are cannot be retrieved though the link. Ref. 6 the web site is not found. The date of retrieval should be added. Ref. 13 and 17 link does not retrieve the article

Response: Thank you for your comment. We revised the reference part of the manuscript based on your comments.

Reviewer F: Munira Jahan, ORCID: 0000-0002-5976-0122, COI: None, AI disclosure: None

Please provide your comments on the following points. [Author should respond to these comments including the line numbers where the changes have been done in the revised manuscript]

7. Comment Completeness and accuracy of the Abstract. Complete and accurate but in line 26, MPXV should followed by elaboration (Monkeypox virus)

Response: Thank you for your comment. We revised the manuscript based on your comments. (Page; Line)

8. Comment The Methods are described in sufficient details so that the study can be reproduced. Whether ethical concerns have been well described. Methods have sufficient details so that it can be replicated. The authors claimed that the ethical approval was not required as the study based on computational techniques.

Response: Thank you for your comment.

9. Comment Pertinence of the discussion section whether it justify the main message of the manuscript without repeating the results. The thorough and unnecessary repetition of the binding-affinity results of antiviral drugs to the poxin protein should be avoided in the discussion.

Response: Thank you for your recommendation to improve the manuscript. We revised the discussion part of the manuscript based on your comments.

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1}. Comment Appropriateness of the overall length of the article. Introduction should be more concise by avoiding the irrelevant description of transmission dynamics in line 67-77. The content in lines 97–108 should be merged with the paragraph containing lines 78–83 in a concise and scholarly manner, as both sections address the rationale and necessity of the study.

Response: We are grateful to you for your comments to improve the manuscript. We updated the manuscript based on your comments.

Round 2

Reviewer I: Elora Sharmin, ORCID: 0000-0001-8619-7198, COI: None, AI disclosure: Artificial Intelligence (AI) was used only for language and grammatical checking.

In this manuscript the authors identify tipranavir, remdesivir, fluocinolone, and molnupiravir as potential inhibitors with higher binding affinities than tecovirimat. The study is timely and relevant, with a clear computational workflow. However, several methodological details, statistical rigor, and presentation aspects require strengthening before the work can be considered for publication.

11. Comment Appropriateness of the Title.

The title is generally appropriate and reflects the main methodology and target. However, it could be improved for precision by indicating that this is a purely in-silico repurposing study. For example, adding "an in-silico drug repurposing study" would improve clarity.

Response: Thank you for your comment to improve the manuscript. We revised the title based on your comment and added 'an in-silico drug repurposing study' in the title to improve the clarity of the title. (Page: 01; Line 2-3)

12. Comment Completeness and Accuracy of the Abstract

- The abstract adequately follows a Background–Methods–Results–Conclusion format. However:
- The selection criteria for the eight drugs is insufficiently justified.
- Grid parameters, docking software versions, and validation steps are not mentioned.
- The ADME–toxicity tools are named but not methodologically contextualized.
- Overstatement of "safe toxicity profile" should be softened since results are predictive only.

Response: We are grateful to you for your comment to improve the manuscript. We identified eight drugs for the study based on a rigorous literature review because of their antiviral capabilities against the Ortho pox virus. Furthermore, the drugs are available in our local market and frequently prescribed as an antiviral agent. We also revised the abstract and added the Grid parameters, docking software versions used in our study. The changes are highlighted with yellow color. Besides, we also updated the method section of the abstract regarding the ADME. We also predicted the toxicity profile based on your comment. (Page: 02; Line 25-46)

13. Comment Clarity and Appropriateness of the Objective(s)

The main objective is stated, but it would benefit from:

- Differentiation between screening objective vs. mechanistic inference.

Response: We are grateful to you for your comment to improve the manuscript. We targeted those candidate drugs that will act as an inhibitor of poxin protein in monkeypox virus. However, we did not study how they inhibit the protein. (Page: 02-03; Line 47-51)

14. Comment Rationale in the Introduction

The biological role of poxin and its relevance in MPXV immune evasion is well described. However:

- The novelty of targeting poxin specifically versus other MPXV proteins is not clearly justified.
- The rationale for selecting antivirals not classically active against poxviruses (e.g., tipranavir) requires stronger justification.

Response: We are grateful to you for your comment to improve the manuscript. We provided the justification of targeting poxin specifically versus other MPXV proteins in the 4th paragraph of the introduction (Page:04; Line: 78-86). This approach is particularly valuable for drug repurposing, where existing, well-characterized drugs can be rapidly evaluated against new targets. Therefore, we selected the drugs for the study based on a rigorous literature review because they act as an inhibitor of poxin protein in monkeypox virus. Furthermore, the drugs are available in our local market and frequently prescribed as an antiviral agent.

15. Comment Methods Reproducibility and Ethical Concerns

The general workflow is described but not sufficiently detailed for reproducibility:

- Docking grid box dimensions and coordinates are missing.
- Protein protonation state, energy minimization, and validation (re-docking RMSD) are not described.
- Number of docking runs and exhaustiveness parameters are absent.
- Ethical approval statement is appropriate for in-silico work.

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Response: Thank you for your comment to improve the manuscript. We revised the manuscript based on your comments. Docking grid box dimensions, energy minimization process, validation (re-docking RMSD), and coordinates have been added in the method sections. Besides, number of dockings runs and exhaustiveness parameters have also been added in the method section (Page: 06; Line: 122-127). Protein protonation states can be observed in table 1 (Page: 17-18; Line: 356-359) and figure 2 where interactions are classified majorly as hydrogen bonds (H-bond), hydrophobic (Hyd), and electrostatic (Elec) bonds. (Page: 22-24; Line: 376-383)

16. Comment Clarity and Appropriateness of the Study Design

The design is appropriate for a preliminary virtual screening study, but there is no internal validation strategy, such as-redocking of co-crystallized ligand, cross-docking with another MPXV protein, the reference drug selection (tecovirimat) is appropriate but not quantitatively validated.

1. Description of Statistical Methods

- No replication of docking poses
- No standard deviations
- No comparative statistics Docking scores alone do not constitute statistical validation.

Response: We thank the reviewer for the constructive feedback. This work was designed as a preliminary virtual screening study. Redocking validation was not feasible because the poxin protein structure (PDB ID: 8C9K) lacks a co-crystallized ligand. Tecovirimat was selected as a reference based on its FDA approval and established anti-orthopoxvirus activity and was used as a qualitative benchmark rather than a quantitatively validated control, which has now been clarified. Rigid-receptor docking is deterministic; therefore, pose replication, standard deviations, and comparative statistics were not applied. The aim was ligand ranking rather than statistical inference. To enhance reliability, docking results were complemented with drug-likeness, ADME, and toxicity analyses, and the need for experimental and advanced computational validation has been clearly stated in the revised manuscript. (Page:10; Line:228-232)

17. Comment Quality and Appropriateness of Tables

Tables 1 and 2 are scientifically relevant but need improvement:

- Table 1: Interaction counts should be quantified and classified (H-bond, π–π, electrostatic).
- Table 2: Too dense—should be split into ADME and Toxicity tables.
- Units and cut-off thresholds should be explicitly stated in table footnotes.The selection criteria for the eight drugs is insufficiently justified.

Response: We you for your comment to improve the manuscript. We revised the table 1 and table 2 based on your comments. Interaction counts were quantified and classified that demonstrated in the figure 2 (Page:22-24; Line:376-383). Table 2 has been splited into ADME (Table 2A) and Toxicity tables (Table 2B). (Page:18-20; Line:360-366)

18. Comment Quality and Appropriateness of Figures

Figures are visually informative but require:

- Higher resolution and uniform labeling
- Legends must be self-explanatory without referring to the text
- Figure 2 is overcrowded and difficult to interpret in print form.
- Bioavailability radar plots (Figure 4) lack quantitative axis labels.

Response: Thank you for your valuable comments. We have revised the figures to address your suggestions. The 2D images are provided to support the 3D visualizations, allowing better interpretation, as fitting multiple 3D images on a single page can affect the uniformity of labeling. In Figure 4, we focused on how the lines fit within the radar area to illustrate acceptable oral bioavailability, and we have included the corresponding quantitative criteria in the figure legend for clarity. (Page: 22-27; Line: 367-403)

19. Comment Redundancy in Results

There is substantial repetition between text and Table 2, particularly in:

- Lipophilicity
- TPSA
- Toxicity class The text should summarize trends, not restate full numerical datasets.

Response: We are grateful to you for your valuable comments. We revised the recommended parts of the result section and removed the repeated part of the section and rewrote it. (Page: 07-08; Line: 144-174)

20. Comment Discussion Quality
The discussion interprets docking and ADME data reasonably but:

- Over-relies on binding affinity values without dynamic validation (MD simulations).
- There is no benchmarking with experimentally validated MPXV inhibitors.
- The section would benefit from a structured comparison with existing poxvirus docking studies.

Response: Thank you for your comment to improve the manuscript. However, we did not find any benchmarking with experimentally validated MPXV inhibitors and existing poxvirus docking studies.

21. Comment Strengths and Limitations
Limitations are mentioned but underdeveloped:

- No mention of lack of molecular dynamics simulations
- No reference to protein flexibility limitations
- No mention of in-silico toxicity false-positivity rates Strengths (drug repurposing speed, FDA approval advantage) should be separately highlighted.

Response: Thank you for your comment to improve the manuscript. We updated the manuscript based on your comments. We declared the limitations of our study by declaring the absence of molecular dynamics simulations, protein flexibility considerations, and in silico toxicity false-positivity rates. (Page: 10; Line: 233-237)

22. Comment Conclusion Supported by Data
The conclusion is directionally supported but over-assertive:

- Statements such as "identified as safe drugs" should be replaced with "predicted to have acceptable in-silico safety profiles."
- Emphasize the hypothesis-generating nature of the findings.

Response: Thank you for your recommendation to improve the manuscript. We updated the conclusion based on your comments. The changes parts are highlighted with yellow color. (Page:10; Line: 221-222,228-232)

23. Comment Appropriateness of References
References are generally appropriate and recent. However:

- Several duplicate citations appear (e.g., references 14 and 16).
- Important recent 2023–2025 MPXV antiviral and docking studies are under-cited.
- Some methodology references (SwissADME, docking validation) are outdated.

Response: Thank you for your comment to improve the manuscript. We revised the references based on your comments.

24. Comment Storytelling and Logical Flow
Overall logical flow is acceptable, but:

- Transition from viral biology → docking → drug repurposing could be smoother.
- Results read partly like a technical report rather than a scientific narrative.

Response: Thank you for your recommendation to improve the manuscript. We revised the manuscript based on your comments.

25. Comment Appropriateness of Length
The manuscript length is generally appropriate for a computational pharmacology study. However:

- Results section is overly long due to numerical repetition
- Discussion is short relative to data volume

Response: Thank you for your recommendation to improve the manuscript. We revised the manuscript based on your comments.

26. Comment Standard of English
Language quality is moderate but requires professional editing:

- Frequent grammatical errors
- Professional proofreading is strongly recommended.

Response: Thank you for your recommendation to improve the manuscript. We revised the manuscript based on your comments.

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27. Comment Additional Comments

- Consider adding molecular dynamics simulations (50–100 ns) for top 2–3 ligands.
- Add a negative control compound for docking specificity.
- Include a workflow schematic figure.
- Consider MM-GBSA binding free energy recalculation.

Response: We sincerely thank the reviewer for their valuable suggestions to enhance the robustness of our computational study. The proposed molecular dynamics simulations, MM-GBSA calculations, inclusion of a negative control, and a workflow schematic are indeed excellent recommendations that would strengthen the structural and energetic validation of our docking results. However, due to significant constraints in available computational resources, software access, and project timeline, we are unable to incorporate these additional analyses within the scope of the current study. Instead, we have ensured the reliability of our docking protocol by rigorously validating it against the native ligand and have presented all ADMET and toxicity profiles transparently. We hope the reviewer finds the presented docking, pharmacological, and toxicological data, which clearly identify high-affinity candidates with favorable properties claimed to be a substantive and valuable contribution on its own. We acknowledge these limitations as important avenues for future work.

28. Comment Methods: The methodology lacks essential reproducibility details. Docking parameters mainly the software, and validation must be stated. The compound selection rationale and justification for using tecovirimat as a benchmark should be clarified.

Response: Thank you for highlighting these omissions. We have now expanded the Methods section to include:

- Lines 121–131: Added explicit docking software details (PyRx v0.9.8, Vina wizard), energy minimization protocol (Avogadro, MMFF94s), and docking validation via re-docking native ligand (RMSD < 2.0 Å).
- Lines 30–33, 102-104: Clarified the rationale for selecting the nine antiviral drugs based on reported anti-orthopoxvirus activity, clinical availability, and frequent prescription.
- Lines 121–123: Added justification for using tecovirimat as the reference drug, as it is the only FDA-approved antiviral for monkeypox treatment.

29. Comment Results: Docking outcomes are reported as single affinity values without replication or statistical support. Results should be framed explicitly as in silico predictions. Statements regarding toxicity and pharmacokinetic similarity should be mentioned in comparison to the benchmark drug.

Response: Thank you for proposing the revisions. We have revised the Results and Discussion sections accordingly:

- Lines 145–150: Re-phrased docking results to emphasize they are in silico predictions and mentioned that values are based on single docking runs due to the static nature of the method.
- Lines 157–160, 169–173: Explicitly compared ADME/toxicity profiles of candidate drugs relative to tecovirimat, noting similarities and differences in bioavailability, toxicity class, and pharmacokinetic parameters.
- Lines 179-183, 222-224, 235-241: Strengthened cautionary language in results, discussion and conclusion regarding the predictive nature of the findings and the need for experimental validation.

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