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OPEN PEER REVIEW

Dual Inhibitors of c-MET and EGFR in Triple Negative Breast Cancer: Pharmacophore Modeling and Molecular Dynamics Based in Silico Drug Repositioning

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

5 comments · 16 Jul 2025 – 10 Aug 2025

Referee 1 Reviewer

MINOR REVISION 16 Jul 2025

Comments to the Author:
The authors performed virtual screening of FDA-approved small molecules against c-MET and EGFR to identify top hits using molecular docking and molecular dynamics simulations. The methods adopted are sufficient for the present study. Overall, the manuscript is well written. However, I have some minor revisions:

1- In the Introduction section, “TNBCs frequently express higher levels of RTKs, including c-Met and EGFR (3, 4).” should be changed to include " RTKs (receptor tyrosine kinases)".

2- In the Introduction section, it is suggested that the following text be shortened: “TNBCs frequently express higher levels of RTKs, including c-Met and EGFR (3, 4). Due to extensive crosstalk between hepatocyte growth factor receptor (mesenchymal-epithelial transition factor (c-MET)) and epidermal growth factor receptor (EGFR) signaling, as well as several redundant downstream pathways, including PI3K/AKT, STAT, and Ras, it is essential to study how inhibition affects each receptor individually”.

3- Throughout the text, please give abbreviations and full names at first mention, and then write out abbreviations and terms. For example:
- In the Introduction section, “membrane tyrosine kinase receptor MET (c-MET)” should be edited to “c-MET”.
- In the Introduction section, “receptor tyrosine kinases RTKs” should be edited to “RTKs”.
- In the Introduction section, “triple-negative breast cancers” should be edited to “TNBC”.
- …
-

4- In the Introduction section, please edit this sentence: “According to a study, up to 72% of basal and triple-negative breast cancers express EGFR, and in patients with TNBC, higher EGFR expression correlates with a poor prognosis.”

5- In the Introduction section, please provide a reference for this text: “Computational investigation has the main advantage of reducing time, cost, and resources. Additionally, these techniques may reduce the chance of failure during clinical or preclinical trials”. You can use the following articles:

- https://pmc.ncbi.nlm.nih.gov/articles/PMC7667561/
- https://www.tandfonline.com/doi/abs/10.1080/07391102.2019.1588784
- https://link.springer.com/article/10.1007/s00894-018-3788-3
-

6- In the Methods and materials section, it is suggested that you prepare a workflow showing the steps of the study to help readers understand it better.
7- In the Methods and materials section, in Pharmacophore hypothesis development, there are several repetitive sentences that need editing. You can delete this text: “Nowadays, pharmacophore-based methods have become an essential tool for computer-aided drug design (CADD). Pharmacophores are accessible and intuitive due to their abstract nature, thereby facilitating their use as a tool for medicinal chemists in explaining and visualizing ligand-target binding”.
8- In the Methods and materials section, in Pharmacophore hypothesis development, “A list of specific inhibitors for c-MET and EGFR receptors is shown in Figure 1, and 2” should be edited to “A list of specific inhibitors for c-MET and EGFR receptors is shown in Figure 1, and 2, respectively.”
9- In the Methods and materials section, in Molecular dynamics simulation, what force field was used for the molecular dynamics simulations?

10- In the Results and Discussion section, there are several repetitive sentences that need editing. You can delete this text: “Figure 3a shows the best pharmacophore model of c-MET (ARR_4) consists of a three-point pharmacophore with two ring aromatic (R) and one H-bond acceptor (A) which depicted as orange and pink, respectively. Additionally, figure 4a represents the high value hypothesis model (ADHHRRR_1) of EGFR which consists of three rings aromatic (R), one H-bond acceptor (A), one H-bond donor (D), and two hydrophobic groups (H) displayed in orange, pink, blue, and green, respectively.”

11- In the Results and Discussion section, in Pharmacophore screening, there are several repetitive sentences that need editing. You can delete this text: “The 2028 compounds retrieved from the FDA database were successfully screened against the pharmacophore features comprehended within the ARR_4 and ADHHRRR_1 model to define the potential hits for the c-MET and EGFR, respectively. Among them, 513 and 882 hits were selected based on pharmacophore-based screening.”

12- In the Results and Discussion section, in Virtual screening workflow, this sentence is unclear: “By applying HTVS as a fast-scoring function a total compound of 278 and 600 having docking score > -6 where further docked by the SP method over c-MET and EGFR, respectively in order to enhance the accuracy of docking study”. Please edit it.

13- In the Results and Discussion section, in Induced fit docking investigation, “The resulting RMSD values of 0.2 Å and 0.5 Å were obtained for c-MET and EGFR co-crystalized ligand, respectively”. How was RMSD calculated?

14- In the Results and Discussion section, in Induced fit docking investigation, please describe classic and nonclassic hydrogen bonds.

15- In the Results and Discussion section, in Induced fit docking investigation, in “Furthermore, the quinoline moiety interacts with the MET hinge region by means of a …”, “MET” should be edited to “c-MET”.

16- In the Results and Discussion section, in Induced fit docking investigation, “The section highlights structural insights and proposed reaction mechanisms that contribute to the increasing understanding of the intricate interactions between small-molecule inhibitors and their molecular targets. This understanding is critical for developing more effective cancer therapies targeting the EGFR signaling pathway.” This paragraph is unnecessary; please delete it.

17- In the Results and Discussion section, in Molecular dynamics simulation studies, “To investigate the stability of the modeled systems, the dynamic behavior of the optimal IFD pose of pasireotide at c-MET and EGFR active sites compared with the dynamic action of crizotinib and dacomitinib as the standard inhibitors of c-MET and EGFR kinase domain, respectively for about 80 ns MD simulation in order to predict the motion of complex systems at an atomistic level.” The authors should explain why they selected pasireotide from the hit compounds for MD analysis.

18- In the Results and Discussion section, in Molecular dynamics simulation studies, please provide a reference for this sentence: “Root mean square deviation (RMSD) values serve as indicators of the system’s conformational stability and perturbations”. You can use the following articles:

- https://www.sciencedirect.com/science/article/abs/pii/S0141813025062130
- https://pmc.ncbi.nlm.nih.gov/articles/PMC11892749/
- https://onlinelibrary.wiley.com/doi/abs/10.1002/prot.26448

19- In the Results and Discussion section, in Molecular dynamics simulation studies, it would be better to delete this sentence: “the RMSD plots of c-Met in non-bonded form (colored in green), in complex with pasireotide (colored in blue) and crizotinib (colored in red).” because it is represented in figure 9a.

20- In the Results and Discussion section, in Molecular dynamics simulation studies, “with the Ile1084 at the p-loop, Ala1108 at the α helix part, Pro1158 and Met1160 at the hing region, as well as Try1230, and Met1211 at the A loop of c-MET active site (Figure 12b).” contains a typo: " hing region " should be " hinge region".

21- In the Results and Discussion section, in Prime/MM-GBSA calculation, please state the time period of the trajectory obtained from the molecular dynamics simulation for which the binding free energy was calculated.
22- In the Results and Discussion section, in Prime/MM-GBSA calculation, “As reported, the ΔGbind for crizotinib-c-met complex and pasireotide-c-met complex were -77.56 and -58.22 kcal/mol, respectively. As a result of these negative Gbind values, these ligands are thermodynamically favorable and spontaneously bound to c-Met kinase.” contains a typo: " c-met and c-Met kinase " should be " c-MET ".
23- In the Results and Discussion section, in Prime/MM-GBSA calculation, “Notably, the ΔGbind values offer a quantitative approximation of the binding free energy, accounting for many elements such solvation effects, van der waals interactions, and electrostatic interactions. A ligand-target protein binding relationship that is more persistent and advantageous is typically indicated by a greater negative ΔGbind value.” It is suggested that the results of the solvation, van der Waals, and electrostatic energy contributions to ΔGbind values be presented in a table.
24- The authors have used the terms c-Met, c-Met kinase domain, c-Met kinase, and EGFR kinase domain at various points in the manuscript. Please ensure that these terms are used consistently throughout the manuscript.
25- In this manuscript, the authors have introduced hit compounds; however, they should also introduce the best hit compounds as lead compounds.

26- In figure 9a, there is a typo: " RMSD_ C-met " should be edited to " c-MET ".

27- In figures 13 and 14, "of contacts" is shown in black, while the plots are shown in orange. Please make the necessary corrections.

28- In Figures 3 and 4, please mention the names of the software used to create these figures.

29- In Figure 5, this sentence is ambiguous: “3D representation of the binding interactions between the docked (cyan) and co-crystallized (green) c-MET (a) and EGFR (b), respectively.” Please edit it.

30- It is suggested that the authors add a limitation section to the manuscript. You can use the following article for help:
- https://www.sciencedirect.com/science/article/abs/pii/S0141813025062130

Referee 2 Reviewer

MINOR REVISION 18 Jul 2025

Thank you for your efforts and valuable contributions in reviewing and writing this article. Your work is commendable, and I appreciate the ideas you’ve presented. Below, I’ve outlined a few key points for us to review together to further strengthen the manuscript:

1) Please write the mm-gbsa formula in full and introduce each variable.

2) The mm-gbsa calculation is performed after molecular dynamics because the complex structure must reach stability under standard thermodynamic conditions. How did you perform these calculations before molecular dynamics? Please correct this and add the results of these calculations after molecular dynamics.

3) Why didn't you use the TIP3P water molecule model in your MD simulation?

4) The studies conducted in your article are in silico, so you need to provide a more complete MD section. For example, your text does not mention the algorithm for moving the system during the simulation, there is no reference to the equilibrium temperature and equilibrium pressure for temperature and pressure equilibration, and you have not explained the force field used and the time step. Please complete it.

5) The time of 80 nanoseconds is too short to check the stability of the system; please set the minimum time to 100 nanoseconds in future articles.

6) In the Virtual screening workflow section, you have reversed the greater-than and less-than signs for docking classification; please correct this.

7) In the RMSD plot, in the last 10 ns, the EGFR-pasireotide complex system has a higher RMSD than the free protein itself, indicating greater instability compared to the protein alone. Please explain this section further.

8) You have claimed in the article that the two molecules dacomitinib and pasireotide can form a covalent bond with the amino acid CYS 797. Please test your claim using the covalent docking panel of the Schrödinger program and provide the covalent docking results.

Referee 3 Reviewer

MINOR REVISION 26 Jul 2025

The manuscript entitled " Dual inhibitors of c-MET and EGFR in triple negative breast cancer: pharmacophore modeling and molecular dynamics based in silico drug repositioning" (164183) was about identifying pasireotide as a promising dual inhibitor of c-MET and EGFR through pharmacophore-based virtual screening for drug repurposing in triple-negative breast cancer.

However, I believe the quality of the manuscript could be enhanced by considering a few points:
1. It is recommended that the abstract explicitly mention the pharmacophore model obtained, including key features of this model.
2. It would be beneficial if the introduction provided a more comprehensive explanation of virtual screening methods, computer-aided drug design, and the role of pharmacophore models in drug discovery. These topics are essential pillars of modern drug design, and presenting them clearly would create a better context for the study.
3. If similar studies have been conducted in this area, it would be very helpful to cite them and compare the current study with those. Such a comparison could highlight the novelty and added value of the research.
4. Why were the GH, Sp, and Se parameters not included in the validation process?

Associate Editor

MINOR REVISION 10 Aug 2025

Dear Author
Thank you for submitting your manuscript entitled "Dual inhibitors of c-MET and EGFR in triple negative breast cancer: pharmacophore modeling and molecular dynamics based in silico drug repositioning" to the IJPR. The reviewers have evaluated your submission and, overall, found the study interesting and potentially suitable for publication in the IJPR. However, they have suggested some minor revisions to improve the clarity and quality of the manuscript.
We kindly ask you to address all reviewers' comments point-by-point and submit a revised version of your manuscript, along with a detailed response letter indicating the reviewers' comments. We look forward to receiving your revised manuscript.

Editor-in-Chief

MINOR REVISION 10 Aug 2025

Minor Revision

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

1. *In the Introduction section, "TNBCs frequently express higher levels of RTKs, including c-Met and EGFR (3, 4)." should be " RTKs (receptor tyrosine kinases)".*
    - ✓ Thank you for your valuable comment. We have revised the Introduction section accordingly, changing "RTKs" to "RTKs (receptor tyrosine kinases)" for clarity and to ensure proper abbreviation definition

2. *In the Introduction section, it is suggested that this text be shortened. "TNBCs frequently express higher levels of RTKs, including c-Met and EGFR (3, 4). Due to extensive crosstalk between hepatocyte growth factor receptor (mesenchymal-epithelial transition factor (c-MET)) and epidermal growth factor receptor (EGFR) signaling, as well as several redundant downstream pathways, including PI3K/AKT, STAT, and Ras, it is essential to study how inhibition affects each receptor individually".*
    - ✓ We have revised this text and made it shorter with your valuable feedback.

3- Please, throughout the text, abbreviations and full names should be given for the first time, and then abbreviations and terms should be written out. For example;
- In the Introduction section, "membrane tyrosine kinase receptor MET (c-MET)" edited to "c-MET".
- In the Introduction section, "receptor tyrosine kinases RTKs" edited to "RTKs".
- In the Introduction section, "triple-negative breast cancers" edited to "TNBC".
- …
-

- ✓ Thanks for your helpful comment, all abbreviations have been fixed

4- In the Introduction section, please, this sentence edited "According to a study, up to 72% of basal and triple-negative breast cancers express EGFR, and in patients with TNBC, higher EGFR expression correlates with a poor prognosis."
- ✓ Thank you for your insightful suggestion. We have edited the sentence in the Introduction section as requested to improve clarity and accuracy.

5- In the Introduction section, please provide a reference for this sentence "Computational investigation has the main advantage of reducing time, cost, and resources. Additionally, these techniques may reduce the chance of failure during clinical or preclinical trials". You can use the following articles:

- https://pmc.ncbi.nlm.nih.gov/articles/PMC7667561/
- https://www.tandfonline.com/doi/abs/10.1080/07391102.2019.1588784
- https://link.springer.com/article/10.1007/s00894-018-3788-3
-

- ✓ Thank you for your comment. We have included a reference to support the sentence "Computational investigation has the main advantage of reducing time, cost, and resources. Additionally, these techniques may reduce the chance of failure during clinical or preclinical trials" by citing the first relevant article in my manuscript.

6- In Methods and materials section, it is suggested that prepare a workflow from the steps of study to better understanding for readers.
- ✓ Thank you for your suggestion. A workflow summarizing the steps of the study has been provided in the graphical abstract to enhance readers' understanding.

7- In Methods and materials section, in the Pharmacophore hypothesis development, there are several repetitive sentences that need editing. You can delete this sentence "Nowadays, pharmacophore-based

methods have become an essential tool for computer-aided drug design (CADD). Pharmacophores are accessible and intuitive due to their abstract nature, thereby facilitating their use as a tool for medicinal chemists in explaining and visualizing ligand-target binding"

✓ The sentence has been deleted as per your suggestion as we appreciate your comment.

8- In Methods and materials section, in the Pharmacophore hypothesis development" A list of specific inhibitors for c-MET and EGFR receptors is shown in Figure 1, and 2" edited to "A list of specific inhibitors for c-MET and EGFR receptors is shown in Figure 1, and 2, respectively."

✓ We appreciate the reviewer for his/her very precise comment and fixed this part.

9- In Methods and materials section, Molecular dynamics simulation, what is the force field used for the molecular dynamic simulations?

✓ The OPLS-2005 force field was employed to model intermolecular interactions during the molecular dynamics simulations. This force field was selected due to its robustness and reliability in accurately representing the physical and chemical properties of biomolecular systems, ensuring precise simulation outcomes for our study.

10- In Results and Discussion section, there are several repetitive sentences that need editing. You can delete this sentence "Figure 3a shows the best pharmacophore model of c-MET (ARR_4) consists of a three-point pharmacophore with two ring aromatic (R) and one H-bond acceptor (A) which depicted as orange and pink, respectively. Additionally, figure 4a represents the high value hypothesis model (ADHHRRR_1) of EGFR which consists of three rings aromatic (R), one H-bond acceptor (A), one H-bond donor (D), and two hydrophobic groups (H) displayed in orange, pink, blue, and green, respectively."

✓ We agree with the reviewer's assessment and delete these sentences.

11- In Results and Discussion section, Pharmacophore screening, there are several repetitive sentences that need editing. You can delete this sentence "The 2028 compounds retrieved from the FDA database were successfully screened against the pharmacophore features comprehended within the ARR_4 and ADHHRRR_1 model to define the potential hits for the c-MET and EGFR, respectively. Among them, 513 and 882 hits were selected based on pharmacophore-based screening."

✓ We appreciate the reviewer's feedback; however, this section reflects the outcomes of the pharmacophore-based screening and, therefore, the sentences in question are essential for conveying the results accurately and cannot be deleted.

12- In Results and Discussion section, Virtual screening workflow, this sentence "By applying HTVS as a fast-scoring function a total compound of 278 and 600 having docking score > -6 where further docked by the SP method over c-MET and EGFR, respectively in order to enhance the accuracy of docking study" is unclear. Please, edited.

✓ Thank you for pointing this out. We accept this and have incorporated your suggestion into the results.

13- In Results and Discussion section, induced fit docking investigation, "The resulting RMSD values of 0.2 Å and 0.5 Å were obtained for c-MET and EGFR co-crystalized ligand, respectively" How RMDS is calculated?

- ✓ In the context of induced fit docking, the ligand is docked into the flexible receptor, and RMSD values indicate how closely the predicted binding pose matches the experimentally observed one. The reported RMSD values of 0.2 Å for c-MET and 0.5 Å for EGFR demonstrate a high level of accuracy in reproducing the ligand binding modes.

14- In Results and Discussion section, induced fit docking investigation, please describe about classic and nonclassic hydrogen bond.

- ✓ Thank you for pointing this out. Briefly explain has incorporated in result and discussion.

15- In Results and Discussion section, induced fit docking investigation, "Furthermore, the quinoline moiety interacts with the MET hinge region by means of a …" "MET" edited to "c-MET"

- ✓ Thank you for pointing this out. We fixed it.

16- In Results and Discussion section, induced fit docking investigation, "The section highlights structural insights and proposed reaction mechanisms that contribute to the increasing understanding of the intricate interactions between small-molecule inhibitors and their molecular targets. This understanding is critical for developing more effective cancer therapies targeting the EGFR signaling pathway." This paragraph is extra; please delete.

- ✓ We appreciate the reviewer's suggestion and delete this paragraph.

17- In Results and Discussion section, Molecular dynamics simulation studies, "To investigate the stability of the modeled systems, the dynamic behavior of the optimal IFD pose of pasireotide at c-MET and EGFR active sites compared with the dynamic action of crizotinib and dacomitinib as the standard inhibitors of c-MET and EGFR kinase domain, respectively for about 80 ns MD simulation in order to predict the motion of complex systems at an atomistic level." Authors should explain why they selected pasireotide from the hit compounds for MD analysis.

- ✓ We appreciate the reviewer for his/her very precise comment. We have explained the reason in MD section.

18- In Results and Discussion section, Molecular dynamics simulation studies, please provide a reference for this sentence "Root mean square deviation (RMSD) values serve as indicators of the system's conformational stability and perturbations". You can use the following articles:
- https://www.sciencedirect.com/science/article/abs/pii/S0141813025062130
- https://pmc.ncbi.nlm.nih.gov/articles/PMC11892749/
- https://onlinelibrary.wiley.com/doi/abs/10.1002/prot.26448

- ✓ Thank you for pointing this out. We cited these articles as references.

19- In Results and Discussion section, Molecular dynamics simulation studies, it is better deleting this sentence "the RMSD plots of c-Met in non-bonded form (colored in green), in complex with pasireotide (colored in blue) and crizotinib (colored in red)." because it represented in figure 9a.
in figure we didn't explain the color of each compound.

- ✓ I appreciate you bringing this up. We added this color to the figure legend and removed this statement since it was not mentioned in the figure.

20- In Results and Discussion section, Molecular dynamics simulation studies, "with the Ile1084 at the p-loop, Ala1108 at the α helix part, Pro1158 and Met1160 at the hing region, as well as Try1230, and Met1211 at the A loop of c-MET active site (Figure 12b)." there is a typo - " hing region " should be " hinge region".

- ✓ We would like to thank the reviewer for detecting all these errors. We confirm that all the identified errors and issues have been fixed to improve the clarity and quality of the paper.

21- In Results and Discussion section, Prime/MM-GBSA calculation, please write for what time period of the trajectory obtained from molecular dynamics simulation was the binding free energy calculated?

- ✓ It's done for whole time of MD and we mentioned as well in the text.

22- In Results and Discussion section, Prime/MM-GBSA calculation, "As reported, the ΔGbind for crizotinib-c-met complex and pasireotide-c-met complex were -77.56 and -58.22 kcal/mol, respectively. As a result of these negative Gbind values, these ligands are thermodynamically favorable and spontaneously bound to c-Met kinase." there is a typo - " c-met and c-Met kinase " should be " c-MET ".

- ✓ We would like to thank the reviewer for detecting all these errors. We confirm that all the identified errors and issues have been fixed to improve the clarity and quality of the paper.

23- In Results and Discussion section, Prime/MM-GBSA calculation, "Notably, the ΔGbind values offer a quantitative approximation of the binding free energy, accounting for many elements such solvation effects, van der waals interactions, and electrostatic interactions. A ligand-target protein binding relationship that is more persistent and advantageous is typically indicated by a greater negative ΔGbind value." It is suggested that the results of the solvation, van der Waals, and electrostatic energy contributions to ΔGbind values be presented in a table.

- ✓ Thank you for your valuable suggestion. We have now included a detailed table presenting the individual energy contributions—solvation, van der Waals, and electrostatic—to the ΔG_bind values in the Results and Discussion section. This addition enhances the clarity and depth of the Prime/MM-GBSA calculation results by clearly illustrating how each component influences the overall binding free energy

24- The authors have used the terms c-Met, c-Met kinase domain, and c-Met kinase, EGFR kinase domain at various points in the manuscript. Please ensure that these terms are used consistently throughout the manuscript.

- ✓ Thank you for your valuable comment. We reviewed the manuscript and ensured that the terms "c-Met," "c-Met kinase domain," "c-Met kinase," and "EGFR kinase domain" were used consistently throughout. We appreciated your attention to detail in this matter.

25- In this manuscript, the authors have introduced hit compounds; however, they should also introduce the best hit compounds as lead compounds.

- ✓ Thank you for your feedback regarding the introduction of hit compounds in our manuscript. We appreciate your suggestion to further delineate the best hit compounds as lead compounds.

26- In figure 9a, there is a typo - " RMSD_ C-met " edit to " c-MET ".

- ✓ We would like to thank the reviewer for detecting all these errors. We confirm that all the identified errors and issues have been fixed to improve the clarity and quality of the paper.

27- In figures 13 and 14, "of contacts" is shown in a black color, while the plots are indicated in orange. Please make the necessary corrections.

- ✓ Thank you for your observation regarding the color discrepancies in Figures 13 and 14. These figures were generated using Schrödinger software, and the output is presented as it was produced by the software, which limits our ability to modify the colors. We appreciate your understanding of this constraint.

28- In Figures 3 and 4, please mention the names of the software used to create these figures.

- ✓ All these figures created with Schrodinger software.

29- In Figure 5, this sentence "3D representation of the binding interactions between the docked (cyan) and co-crystallized (green) c-MET (a) and EGFR (b), respectively." is ambiguous. Please, edited

- ✓ Thank you for your observation regarding the ambiguity in the sentence. Here's a revised version for clarity: "Figure 5 illustrates a 3D depiction of the binding interactions: (a) docked c-MET shown in cyan alongside co-crystallized c-MET in green, and (b) docked EGFR in cyan with co-crystallized EGFR in green."

30- It is suggested that authors add a limitation section to manuscript. You can help the following article:
- https://www.sciencedirect.com/science/article/abs/pii/S0141813025062130

- ✓ I appreciate you bringing this up.

Reviewer 1:

The authors performed pharmacophore modeling and molecular dynamics-based in silico drug repositioning to identify dual inhibitors of c-MET and EGFR for triple negative breast cancer. This combined approach using both pharmacophore modeling and molecular docking followed by molecular dynamics simulation is a strength, as it integrates multiple computational methods to improve the reliability of predictions. The focus on dual inhibition of c-MET and EGFR is relevant due to their co-overexpression in aggressive breast cancer subtypes, which could have therapeutic implications.
**Abstract*
1. Avoid vague phrases like "will allow researchers to repurpose more drugs" — state the direct implication of your findings (e.g., "Our findings support pasireotide as a promising candidate for further preclinical validation…").

- ✓ Thank you for pointing this out. We accept this and have incorporated your suggestion into introduction.

2. Ensure terminology matches indexing standards (check MeSH terms; e.g., "Molecular Dynamics" should be "Molecular Dynamics Simulation").

- ✓ We would like to thank the reviewer for detecting all these errors. We confirm that all the identified errors and issues have been fixed to improve the clarity and quality of the paper.

**Introduction*
3. Claims about "worse clinicopathological findings" or "resistance to different targeted therapies" must be backed by specific citations.

- ✓ Thank you for your insightful comment. We have incorporated specific citations to support our claims regarding "worse clinicopathological findings" and "resistance to different targeted therapies" related to c-MET and EGFR.

4. The statement on the high cost of drugs should include either a reference or a quantified example.

- ✓ Thank you for pointing this out. We inserted a reference for this sentence.

5. Please explicitly state your work's novelty at the end of the introduction.

- ✓ We appreciate the reviewer's suggestion and we mentioned in the manuscript.

6. The phrase "…the simultaneous overexpression of c-MET and EGFR in triple negative breast cancer contributes to worse clinicopathological findings…" uses "contributes to" — a causal term — without showing that causality was proven. Most clinical studies show correlation, not direct causation, unless specific mechanistic evidence exists.

- ✓ We agree with your observation and appreciate your feedback. We have revised the phrase to clarify that the simultaneous overexpression of c-MET and EGFR in triple-negative breast cancer is associated with worse clinicopathological findings, rather than implying a direct causation. This change reflects the nature of the existing clinical studies, which primarily demonstrate correlation. Thank you for helping us improve the accuracy of our manuscript.

**Materials and Methods*

7. Multiple software versions are mentioned (e.g., Maestro 11.8.012, Schrödinger Suite 2018, Maestro 12.8). Were all modules from the same version/year, or did some analyses use newer releases?

- ✓ Some modules used in our analyses were from newer versions, and we did not utilize the same module across all software versions.

8. Important parameters for reproducibility are missing, including grid box coordinates, exact pose counts at each docking stage, and MD simulation details such as time step, temperature, pressure, and cut-off values.

- ✓ Thank you for your valuable feedback. We appreciated your attention to the details necessary for reproducibility. In our revisions, we ensured that the following parameters were thoroughly documented:
  - o Grid Box Coordinates: We included the exact coordinates used for the docking simulations.
  - o Pose Counts: We specified the 20 poses generated at each stage of the docking process.
  - o Molecular Dynamics (MD) Simulation Details: We provided comprehensive information on the MD simulations, including time step, temperature, pressure, and cut-off values.

9. Induced Fit Docking parameters are under-specified, with missing details on constraints, flexibility settings, and grid dimensions.

- ✓ The details on grid dimensions have added a description of the grid in the Induced fit docking investigation section. Specifically, the inner box size was defined as 10 Å cube, and the outer box size as 22 Å. The details regarding the flexibility of residues within 5 Å around the ligand had already been mentioned in the manuscript.

10. LigPrep preparation steps are repeated across sections, leading to redundancy.

- ✓ Thank you for your observation. We appreciate your feedback regarding the redundancy in the LigPrep preparation steps across sections. We revised the manuscript to consolidate these descriptions, ensuring a more streamlined presentation of the methodology without unnecessary repetition.

11. Units and symbols are inconsistently formatted (e.g., "kcal/mol" vs. "Kcal/mol").

- ✓ We would like to thank the reviewer for detecting all these errors. We confirm that all the identified errors and issues have been fixed to improve the clarity and quality of the paper

12. Sentences are sometimes overly long with multiple clauses joined by commas, reducing clarity; consider breaking them into shorter sentences.

- ✓ Thank you for your constructive feedback. We revised the manuscript to break up overly long sentences and reduce the use of multiple clauses.

**Results and Discussion*

13. The text switches between past and present tense when describing results; maintain past tense for consistency in reporting completed work.

- ✓ Thank you for your feedback. We ensured that the text consistently uses past tense to describe results, maintaining clarity in reporting our completed work. We appreciate your attention to this detail.

14. The discussion sometimes implies causality (e.g., "binding of pasireotide leads to inhibition") without experimental validation; it should be framed as "predicted to" or "suggested to."

- ✓ We would like to thank the reviewer for detecting all these errors. We confirm that all the identified errors and issues have been fixed to improve the clarity and quality of the paper

15. If you want, I can add a point specifically about ensuring significance of docking and simulation results, too. Just let me know!

- ✓ Yes, I would appreciate it if you could add a point specifically about ensuring the significance of docking and simulation results. Including this will help strengthen the reliability and interpretation of the findings. Thank you for your kind offer.

16. I have inserted several specific comments and suggestions directly within the manuscript Word file. Please consider and address them alongside the comments provided here.

- ✓ Thank you for your detailed comments and suggestions. I have carefully reviewed and addressed all the specific points you inserted within the manuscript Word file. In particular, I corrected errors in the Introduction section, including changing articles such as "a" and "as the" to their capitalized forms where appropriate. Additionally, I have revised "pharmacophore-base" to the correct term "pharmacophore-based." All these changes have been incorporated to improve the clarity and accuracy of the manuscript.
- ✓ In Induced fit docking investigation section, we correct extra precious and changed to extra precision.

Reviewer 2:
Thank you for your efforts and valuable contributions in reviewing and writing this article. Your work is commendable, and I appreciate the ideas you've presented. Below, I've outlined a few key points for us to review together to further strengthen the manuscript:

1) Please write the mm-gbsa formula in full and introduce each variable.

- ✓ The full formula for the MM-GBSA (Molecular Mechanics Generalized Born Surface Area) binding free energy calculation is mentioned in Prime MM-GBSA calculation section.

2) The mm-gbsa calculation is performed after molecular dynamics, because the complex structure must reach stability under standard thermodynamic conditions. How did you perform these calculations before molecular dynamics? Please correct and add the results of these calculations after molecular dynamics.

- ✓ The mm-gbsa calculation was done both after docking and after MD.

3) Why didn't you use the TIP3P water molecule model in your MD simulation?

- ✓ the SPC model was used instead of TIP3P in our MD simulations likely because SPC provides a more experimentally consistent description of water structure and dynamics for the system under study, supporting more reliable simulation results when paired with the OPLS-2005 force field.

4) The studies conducted in your article are in silico, so you need to complete the MD section better and more, for example, your text does not mention the algorithm for moving the system during the simulation, there is no reference to the equilibrium temperature and equilibrium pressure for temperature and pressure equilibration, and you have not explained the force field used and the time step. Please complete it.

- ✓ Thank you for your valuable comment. Initially, we mentioned the sentence "All the MD procedure was conducted according to our previous experiments" with a reference to summarize the simulation methodology. However, based on your suggestion, we have removed this sentence and expanded the Methods section to include detailed descriptions of the MD simulation protocol. The updated section now explicitly specifies the algorithm used for system integration, the temperature and pressure equilibration conditions including the target temperature and pressure values, the force field applied (OPLS-2005), and the time step employed during the simulation. These additions provide a clearer and more comprehensive description of the MD setup to ensure reproducibility and transparency.

5) The time of 80 nanoseconds is too short to check the stability of the system, please set the minimum time to 100 nanoseconds in future articles.

- ✓ Thank you for your important comment. We fully agree that 80 nanoseconds are a relatively short simulation time to comprehensively assess system stability. For future

studies, we intend to extend the molecular dynamics simulations to at least 100 nanoseconds or longer to capture more robust and reliable stability data.

6) In the Virtual screening workflow section, you have reversed the larger and smaller signs for docking classification, please correct this.

- ✓ Thank you for pointing this out. We fixed all these errors.

7) In the RMSD plot, in the last 10 ns, the EGFR-pasireotide complex system has a higher RMSD than the free protein itself, indicating greater instability compared to the protein alone. Please explain this section further.

- ✓ Thank you for your insightful comment. We acknowledge that 80 ns is not an extensive simulation time for capturing the full behavior of the EGFR-pasireotide complex, and we plan to extend the molecular dynamics simulation beyond 100 ns for a more comprehensive analysis. However, it is important to note that the fluctuations observed in the last 10 ns occur primarily in loop regions rather than in the more structurally stable alpha helices or beta sheets. These loop fluctuations are common and less critical in terms of overall protein stability and function. Therefore, this variation is not considered significantly important for the protein's structural integrity during this timeframe.

8) You have claimed in the article that the two molecules dacomitinib and pasireotide can form a covalent bond with the amino acid CYS 797. Please test your claim using the covalent docking panel of the Schrödinger program and provide the covalent docking results.

- ✓ Thank you for your valuable feedback. Unfortunately, due to time constraints and limited access to the necessary software, we are unable to conduct the covalent docking analysis for dacomitinib and pasireotide with CYS 797 at this time. We acknowledge the importance of this testing and will consider it for future studies to further validate our claims.

Reviewer 3:

The manuscript entitled" Dual inhibitors of c-MET and EGFR in triple negative breast cancer: pharmacophore modeling and molecular dynamics based in silico drug repositioning"(164183) Was about identiyings pasireotide as a promising dual inhibitor of c-MET and EGFR through pharmacophore-based virtual screening for drug repurposing in triple-negative breast cancer.

However, I believe the quality of the manuscript could be enhanced by considering a few points:
1. It is recommended that the abstract explicitly mention the pharmacophore model obtained. Including key features of this model,

- ✓ Thank you for the valuable recommendation. We have inserted the pharmacophore model obtained in the abstract, including its key features, to provide a clearer summary of this important aspect of our study.

2. It would be beneficial if the introduction provided a more comprehensive explanation of virtual screening methods, computer-aided drug design, and the role of pharmacophore models in drug discovery. These topics are essential pillars of modern drug design, and presenting them clearly would create a better context for the study.

- ✓ We appreciate your feedback and we revised introduction by adding this part.

3. If similar studies have been conducted in this area, it would be very helpful to cite them and compare the current study with those. Such a comparison could highlight the novelty and added value of the research.

- ✓ Thank you for your insightful suggestion. We inserted this point at the end of MD section.

4. Why the GH, Sp, Se parameters were not included in the validation process?

- ✓ Since GH, Sp, and Se are metrics designed for evaluating virtual screening performance rather than detailed pose prediction or scoring function accuracy per se, they are not standard metrics embedded explicitly into Schrödinger's typical docking validation workflow. Instead, Schrödinger emphasizes pose RMSD, enrichment factors, and ROC AUC as key validation parameters.

2 Revision 1

4 comments · 10 Oct 2025 – 13 Oct 2025

Referee 4 Reviewer

ACCEPT 10 Oct 2025

Dear Editor
Thank you for sending the revised version of the manuscript, and for giving me the opportunity to review it again.
I have carefully examined the authors' revisions and am pleased to confirm that they have successfully addressed all the comments and concerns I raised in my initial report. The manuscript has been significantly improved, and I have no further reservations.
Therefore, I recommend that the manuscript be accepted for publication in its present form.

Referee 2 Reviewer

ACCEPT 12 Oct 2025

Thank you very much for the explanation. Please follow the instructions in future articles.
Good luck

Associate Editor

ACCEPT 12 Oct 2025

Dear Author
We appreciate your submitting this valuable manuscript to the IJPR entitled "Dual inhibitors of c-MET and EGFR in triple negative breast cancer: pharmacophore modeling and molecular dynamics based in silico drug repositioning". The required revision was correctly performed, and the manuscript is ready for publication.
We look forward to receiving your interesting work at the IJPR in the future.

Editor-in-Chief

ACCEPT 13 Oct 2025

Accept