

Yihua Chen, Ph.D. Prof.
+86 21 24206647 (Phone)
+86 21 54344922 (Fax)
E-mail: yhchen@bio.ecnu.edu.cn

Aug 22nd, 2022.

Dear Dr. Bell,

We sincerely thank you and the reviewers for the constructive comments on our manuscript entitled “Discovery of 1,5-diaryl-1,2,4-triazole derivatives as MYOF inhibitors and its antitumor effects in pancreatic cancer (Manuscript ID: FMC-2022-0168)”, as well as the opportunity to submit the revised manuscript for further consideration. In the revised manuscript, we have made corresponding changes according to the reviewers’ and editor’s comments and the revisions have been highlighted in green. Therefore, we believe that the quality of the manuscript has been significantly improved and qualified for publication in *Future Medicinal Chemistry*. Please find below a point-to-point response to the reviewers’ comments:

Response to reviewer #1:

Comments:

This manuscript reported the synthesis of a series of 1,5-diaryl-1,2,4-triazole derivatives and evaluated the binding activities with MYOF. They designed and synthesized the 1,5-diaryl-1,2,4-triazole derivative (E4) that was directly bound to MYOF-C2D. Compound E4 effectively restrained the proliferation and invasiveness of pancreatic cancer cells in vitro. The water solubility of E4 was improved by about 22 times than that of WJ460 and it was expected to have better metabolic stability. This article revealed for the first time the underlying binding mechanism of the inhibitor and MYOF, which laid the scientific foundation for further research on the binding pattern of the ligand and MYOF. I thus recommend publication after minor revision.

1. Page 3, line 13: “At previous studies” should be changed to “In previous studies”.

Author’s Response: Thanks for the reviewer’s helpful suggestion. We have replaced “At previous studies” with “In previous studies” in the revised manuscript.

2. Page 4, line 18: “were” should be changed to “was”.

Author’s Response: Thanks for the reviewer’s helpful suggestion. We have replaced “were” with “was” on page 4, line 18.

3. Page 5, line 12: “were” should be changed to “was”.

Author’s Response: Thanks for the reviewer’s helpful suggestion. We have replaced “were” with “was” on page 5, line 12.

4. In its NMR data, there are many formatting errors. For example, the “J” for the coupling constant should be written in italics; “¹H NMR (500 MHz, DMSO-d₆)” and “¹³C NMR (126 MHz, DMSO-d₆)” should be changed to “¹H NMR (500 MHz, DMSO-d₆)” and “¹³C NMR (126 MHz, DMSO-d₆)”.

Author’s Response: Thanks for the reviewer’s helpful suggestion. We have carefully modified the format of NMR data in the revised manuscript based on the beneficial comments from the reviewer.

5. The format of references should be consistent. Like reference 3, a maximum of three Author’s names should be displayed. Like references 6 and 27, periodical title format should be uniform. Like reference 26, “N heteroaromatic” should be changed to “N heteroaromatic”.

Author’s Response: Thanks for the reviewer’s helpful suggestion. We have rearranged the format of the corresponding references in revised manuscript based on the beneficial comments from the reviewer and Author’s guideline.

Reviewer: 2

Comments to the Author’s

This manuscript reported on the design, synthesis and evaluation of 1,5-diaryl-1,2,4-triazole derivatives as inhibitors by targeting MYOF.

The Author’s presented a work optimizing their WJ460 with moderate binding affinity and poor druglike properties and confirmed the direct binding of E4 to MYOF by using surface plasmon resonance (SPR). E4 effectively inhibited the proliferation and migration of pancreatic cancer cells and had approximately 22 times improvement in

water solubility. Hence, the druglike ability of E4 was expected to be improved. In general, the experiments were carefully designed and executed, and the conclusions were sound and consistent with the results.

In my opinion, the article is particularly noteworthy because the novelty protein (MYOF) could enable future efforts toward the development of a new therapeutic strategy to suppress the metastasis of pancreatic cancer by regulating vesicle trafficking, as well as against other cancers with overexpression of MYOF. Therefore, I recommend the manuscript for publication in the “Future Medicinal Chemistry” after addressing a few minor issues:

1: The Authors can add a “Graphical abstract” to make the work more visible.

Author’s Response: Thanks for the reviewer’s kindly suggestion. We have added a “Graphical abstract” to the revised manuscript.

2: The first reference (5) recommended do not seem to match the brief explaining sentence – “Overview the emerging therapeutic approach by targeting Myoferlin to inhibit the proliferation and migration of malignant tumors”

Author’s Response: Thanks for the reviewer’s comment. We have changed the location of the brief explanation sentence from reference (5) to reference (6) for matching.

3: The Authors should check the article carefully to iron out some language glitches.

For example, “The medium was supplemented with 10% fetal bovine- serum (FBS), 1% penicillin-streptomycin” and “the latest research shows that lysosomal retargeting of MYOF mitigates membrane stress to enable pancreatic cancer growth”

Author’s Response: Thanks for the reviewer’s beneficial suggestion. We have checked the article carefully to iron out several language glitches and highlighted the corrections in the revised manuscript.

4: Some important references should be added, such as Page 23, line 2 “In vitro model of the Caco-2 monolayer cell is widely used to predict the absorption of orally administered drugs.”, the applied softwares (The Schrodinger software 5 (Version 2019-1), etc.)

Author's Response: Thanks for the reviewer's beneficial suggestion. We have added several important references on page 23, line 2 and the applied softwares were presented in the revised manuscript.

5: The default parameters of performing in Ligand preparation, protein preparation, Receptor grid generation, Docking and analysis, should be added, especially for the parameters of method, grid box in docking.

Author's Response: Thanks for the reviewer's beneficial suggestion. We have added the default parameters of performing in Ligand preparation, protein preparation, Receptor grid generation, Docking and analysis and grid box in docking. The default parameters were added in the corresponding parts and highlighted in the revised manuscript.

6: What is the meaning of different color dotted lines in Figure 2A? Additionally, the properties of bonds of "In addition, the distances between the ethyl terminus and the residues of amino acids T-1126, N-1125, I-1123 and E-862 were 2.44, 2.40, 2.16 and 3.01 Å" should be detailed, H-bond or the others?

Author's Response: Thanks for the reviewer's beneficial suggestion. We have described the meaning of different color dotted lines in Figure 2A. Additionally, we predicted the **spatial distance** between the ethyl terminus and the amino acids T-1126, N-1125, I-1123 and E-862 were 2.44, 2.40, 2.16 and 3.01 Å, respectively. Based on the docking model and our previous findings, it was reasonable to speculate that the ethyl terminus might form certain hydrophobic interactions with these amino acids, which might be the reason why the activity of ethyl substitution was much better than that of methyl (**D2 vs D3** and **E1 vs E3**).

The following new descriptions have been added and are also highlighted in the article. "The hydrogen bonds were shown in yellow and the pi-cation interaction was shown in green. The distances between the ethyl terminus and the residues of several amino acids were shown in purple." "The ethyl terminus occupied a narrow cavity formed by several nearby amino acids and may form certain hydrophobic interactions which might be the reason why the activity of ethyl substitution was much better than that of methyl (**D2 vs D3** and **E1 vs E3**)."

7: From Figure 2B, it is hard to find that ethyl terminus is in the narrow cavity. It can be presented using more clear visual type.

Author's Response: Thanks for the reviewer's beneficial suggestion. We have presented Figure 2B in a clearer visual type in the revised manuscript (Figure 5B).

Editorial comments:

1. Please check that all abbreviations present are commonly used and all are defined in their first instance in the manuscript. These should also be redefined in the abstract & figure/table legends if the abbreviation is used.

Author's Response: Thanks for the editor's kindly reminder. We have checked all abbreviations and defined them in their first instance in the revised manuscript. These abbreviations also were redefined in the abstract & figure/table legends if the abbreviations were used.

2. Please make sure that all figures, tables and boxes (including supplementary materials) are clearly and correctly titled, described and cited in the text. Please note that we do not use 'Scheme' as a figure title. Please rename your two 'Schemes' as 'Figures' and renumber accordingly.

Author's Response: We appreciate the editor's comments. We have double checked the whole manuscript and confirmed that all tables/figures/boxes were clearly titled and cited in the text. We have also deleted "Scheme 2. Synthesis of the compound C1" and added a corresponding reference (<https://doi.org/10.1007/s00044-022-02894-y>) in the revised manuscript.

3. All figures should be submitted in a high-resolution format (ideally JPEG/TIFF/Adobe Illustrator file types). Tables and boxes should be provided in an editable format such as word/excel files.

Author's Response: We appreciate the editor's comments. We have resubmitted the figures in a high-resolution format.

4. A combined limit of eight tables/figures/boxes in total is permitted with any additional made supplementary (online-only). Please either remove additional

tables/figures/boxes, or indicate those you would like to be made supplementary.

Author's Response: We appreciate the editor's comments. We have done our best to reduce the number of unnecessary tables/figures, but the total of tables/figures was still over 8. We reluctantly kept the number of existing tables/figures after referring to the articles published in *Future Medicinal Chemistry* which had a total number of tables/figures of more than 8. (<https://doi.org/10.4155/fmc-2022-0054>; <https://doi.org/10.4155/fmc-2022-0067>; <https://doi.org/10.4155/fmc-2021-0340>; <https://doi.org/10.4155/fmc.15.6>; <https://doi.org/10.4155/fmc-2022-0119>; <https://doi.org/10.4155/fmc-2022-0076>; <https://doi.org/10.4155/fmc-2022-0052>; <https://doi.org/10.4155/fmc-2022-0016>; <https://doi.org/10.4155/fmc-2022-0017>; <https://doi.org/10.4155/fmc-2021-0276>)

5. Articles can greatly benefit from a graphical abstract or infographic. If you would like to include one please feel free to upload it with your revised manuscript. Here you can find an example of a graphical abstract: <http://www.future-science.com/doi/full/10.4155/fsoa-2016-0066>

Here you can find an example of a video abstract: <https://www.youtube.com/watch?v=IUwKs56eAJc&feature=youtu.be>

Author's Response: We appreciate the editor's comments. We have added a "Graphical abstract" in the revised manuscript.

6. Please complete and upload an "Author's Disclosure Form". Please note that either a real or electronic signature is required. You can download the form here: <https://www.future-science.com/Author'sguide/submittrackarticle>

Author's Response: We appreciate the editor's comments. We added signature in the Author's Disclosure Form.

a) If you have indicated that "funded writing assistance was utilized in the production of this manuscript":

- Please provide additional information on this within the manuscript file, including sources of funding for this and any accompanying paperwork e.g. certificate provided

by the company used.

None.

b) If you have indicated that ‘financial and/or material support was received for this research and/or the creation of this work’:

- Please include a disclosure in your manuscript file indicating the sources and extent of involvement.

None.

c) If you have indicated that ‘one or more of the Authors’ have financial and/or nonfinancial relationships with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript’:

- Please include a disclosure in the manuscript file indicating the details of this financial involvement.

None.

d) Please provide any relevant ethical conduct of research information (for original research Author’s only) such as information on institutional review board approval, or for investigators without formal ethics review committees, indicate that the principles outlined in the Declaration of Helsinki have been followed). Please also provide information on how informed consent was obtained from human study participants (if applicable).

e) When completing the ‘Copyright status’ table, please ensure you list all tables as well as figures (including supplementary ones) along with their copyright status. Where copyright permission for reuse/adaptation has already been granted, please upload the relevant licence agreement.

Author’s Response: We appreciate the editor’s comments. We have completed and uploaded an “Author’s Disclosure Form”.

7. If you do not wish to pursue the fee-based open access option, please complete and upload a “Copyright Assignment form”. Please note that either a real or electronic signature is required. You can download the form here: <https://www.future-science.com/Author’sguide/submittrackarticle>

8. Future Medicinal Chemistry offers a selection of post-acceptance services for Author's; please indicate when you re-submit if you would be interested in any of the following:

a. Open access publication: Future Medicinal Chemistry offers an open access option, whereby for a fee, articles can be made freely available for all to read. Pricing varies by article type; for peer-reviewed content (i.e., Original Research or Review articles), the fee is \$2800; for content not reviewed externally (i.e., Editorials or Commentaries), the fee is \$980. For more information, visit our website here: <https://www.future-science.com/Author'sguide/openaccess>. If you are interested in taking this option, please let me know. Please note, if you would like to opt for Open Access, the OA form (available here: <https://www.future-science.com/Author'sguide/openaccess>) will be required in place of the Copyright Assignment form. If you have any concerns about your institution being able to pay the Open Access fee due to the current global situation, please do let me know and we can discuss appropriate arrangements.

b. Accelerated publication: We are also able to offer a fast-track production service, for a fee of \$980, providing guaranteed online publication within 3 weeks (subject to turnaround of proofs by the Author's within 3 working days). Should you be interested in this service, please let me know.

c. Colour printing: Colour Printing can enhance the impact of your article's figures with readers. Should you wish your figures to appear in colour in the print issue of the journal, there is a fee of \$220 for the first figure and \$135 for each subsequent figure. Please note that charges for colour figures only apply for print issues of the journal; all figures appear online in colour at no cost. If you are interested in having one or more of your figures printed in colour, please let me know.

d. Digital enhancements: We encourage Author's to enhance your article with digital

assets, such as graphical abstracts, infographics and videos, to help readers discover and learn about your research. More information is available at <https://www.future-science.com/Author'sguide/makingthemost>

e. Video Journal of Biomedicine: The Video Journal of Biomedicine (<https://www.biomedicine.video/>) is a new platform from the Future Science Group publishing videos that can be freely accessed and watched. The aim is to provide a platform for Author'ss to present their latest research in a short, high-quality video. Animated videos provide a ~90 second overview of a research paper where the article is converted into a bespoke animation with an engaging voiceover script. Publication Perspective videos are professionally produced videos, ~8 minutes in length that provide an opportunity for Author'ss to discuss their publication in full. Videos are produced in chapter-style format allowing the viewer to navigate between segments as required. By watching the video, viewers gain a comprehensive understanding of the subject. If you are interested developing in a video alongside your article, please email the Publisher of the Video Journal of Biomedicine, Joanne Walker (j.walker@biomedicine.video).

f. Plain language summary: We are keen to recognize the vital role of patients in medical and scientific research. I would therefore like to recommend that you add a plain language summary to your article. Plain language summaries are a summary of an article (of similar length to the main abstract) with any technical jargon removed – the aim of these is to make an article more accessible and discoverable by readers who might not be experts in the field but have an interest in the topic – this can include anyone, but plain language summaries are particularly useful for patients and patient advocates. Given the subject matter of your article, please consider including a plain language summary in your revision.

g. Tweetable abstract: Author'ss are encouraged to provide a tweetable abstract summarizing the key findings from the article and including any relevant hashtags. A

tweetable abstract is a short summary of your work used to share your article on Twitter, helping a wider audience discover your work. Tweetable abstracts can be shared directly on Twitter via the ‘Click to Tweet’ feature on the article page. Tweets can be up to a maximum of 240 characters, however we recommend ~200 characters for a tweetable abstract (shorter tweets generally receive better engagement).

Please find a link to the Future Medicinal Chemistry Author’s Guidelines which explain these sections in more detail: <https://www.future-science.com/Author'sguide>

Sincerely

Yihua Chen, Ph. D. Professor,

A handwritten signature in black ink, appearing to read 'Yihua' followed by a stylized flourish.

Yihua Chen, Ph. D., Professor

Shanghai Key Laboratory of Regulatory Biology, The Institute of Biomedical Sciences
and School of Life Sciences, East China Normal University.
500 Dongchuan Rd. Shanghai, China, 200241