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OPEN PEER REVIEW

Synthesis, Antioxidant, Cytotoxicity, Induce Apoptosis Investigation and Docking Study of New Halogenated Dihydropyrano[3,2-*b*]Chromene-3-Carbonitrile Derivatives on MCF-7 Breast Cancer Cell Line

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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

4 comments · 6 Dec 2022 – 20 Dec 2022

Referee 1 Reviewer

MAJOR REVISION 6 Dec 2022

Dear AE,

The manuscript was reviewed carefully, and it needs some major revisions, as outlined below.
1- Please add a scheme to show the structures of the lead compounds and explain more about the SAR of the designed compounds.
2- What is the mechanism of action of the synthesized compounds? Which enzyme is the target of these compounds?
3- One of the more important aspects of identifying effective compounds is performing a docking study. Please add this section to the study to improve understanding of the mechanism of action of the synthesized compounds.
4- The text of the manuscript has many grammatical errors; please recheck and correct these errors.
5- These compounds have a cytotoxic effect on the MCF-7 cell line, but did you test the cytotoxic effect of these compounds on the normal cell line? These compounds must show a low cytotoxic effect on normal cells to be valuable.
6- The most important revision is correcting the "title"! Please correct the word "Opoptisis" in the title!

Referee 2 Reviewer

MINOR REVISION 19 Dec 2022

Dear AE,

The study is interesting and comprehensive. However, some minor revisions are needed:
1- It is better to add a standard (reference) drug for comparison in the biological evaluation.
2- For the MTT test, using a normal cell line (fibroblast) is critical. Then the authors can claim that their compounds selectively affect the cancerous cell line, not the normal cell line.

Associate Editor

MAJOR REVISION 20 Dec 2022

Dear EIC,

Based on the reviewer's comments, the manuscript needs major revision.
Regards,

Editor-in-Chief

MAJOR REVISION 20 Dec 2022

Dear Author,

Your article has been reviewed carefully by the reviewers. According to their comments, this document needs major revision. Please follow their comments as requested.
Thanks,

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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.

Dear Editor,

We would like to thank you and the reviewers for their valuable comments. We paid attention to the comments, revised the sections in the text, and highlighted the changes in yellow. We hope that the revised manuscript meet your high standards. We look forward to hearing from you.

It is of the importance that the title has been changed due to the addition of Docking study and correcting the wrong spelling of Apoptosis. The new title is:

Synthesis, Antioxidant, Cytotoxicity, Induce Apoptosis Investigation and Docking Study of New Halogenated Dihydropyrano[3,2-*b*]Chromene-3-Carbonitrile Derivatives on MCF-7 Breast Cancer Cell Line

Answering Reviewers

The manuscript has been improved according to the suggestions of reviewers. We have carefully addressed the criticisms point-by-point, and our response is indicated below.

We appreciate the reviewers for their kindly comments about the manuscript.

Reviewer 1:

All English writings and typos corrected. Technical question addressed and Table 1 was corrected. In the HNMR section of compounds 6d and 6j we have 7 aromatic hydrogens totally, while it is obvious that NH_2 hydrogens are located in this region also. To sum up 9 hydrogens are in this region which are presented separately. HNMR for compound 6j was corrected.

If 6a and 6f show higher antioxidant capacity than FeSO4, IC50 for these compounds should be lower than IC50 for FeSO4. Different results of Figure 1 with Table 1?

Response: Table 1 was corrected. Mistakenly the IC50 were for 60%.

What is relation between 0.01-100 mg/L and umol/Fe(II) concentrations of the compounds?

Response: It was corrected (the unit converted µM).

Comparing Fig 1 with Table 1, it seems that IC50 for 6f is less than 280. How did you calculate IC50 for the compounds shown in Table1?

Response: All of data related to Table 1 in the text were corrected.

It is not true. All 6a, e,f,g,h,i showed LDH secretion (according to your results, Table 2).

Response: The text corrected as follow: According to the results, all of the HDCCD (**6b, 6d, 6c, 6j**) were safe and did not damage the cell membrane. However, **6a**, **6e, 6f, 6g, 6h,** and **6i** showed disturbing effect on the cell membrane and secreted LDH enzyme.

How do you know it? Have you done any statistic calculation or analysis?

Response: The control figure is completely in the range of treatment. The word “significant” was changed to “notable”.

What do you mean by the acceptable results? You are referring to what table or figure?

Response: It was Corrected. According to table 2, 6d and 6g have the best results in terms of cytotoxicity.

According to the scheme shown in Figure2, 6i does not show 100% toxicity. Apparently, compounds 6j,c,d,b show 100% toxicity.

Response: It was Corrected. 6i was changed to 6d.

How did you find such results, and for what compounds?

Response: The flow cytometry instrument gives us percentages: According to figure 3, Q4 is early apoptosis and Q2 is late apoptosis the sum of these two quadrant is nearly 50%.

Reviewer 2

It is better to add a standard (reference) drug to compare the results with for the biological evaluation.

Response: We add the Doxorubicin data to table 2 as a reference.

For the MTT test, using the normal cell line (fibroblast) is critical. Then you can claim that your compounds selectively affect the cancerous cell line, not the normal cell line.

Response: In the present study the effect of the synthesized compound on MCF-7 cell line was investigated. Specifically we mention the name of the cell line in the title and cytotoxicity was solely done on this line as this can be observed in some articles, following are some as a sample. Although for the betterment of judgement one can evaluate cytotoxicity on the normal cells, we believe it is not compulsory particularly when the name of cell line is mentioned in the title. Moreover, nowhere in the article did we claim our compound selectively affect cancerous cell line. But we decide to put this to consideration in our future studies.

1. Jose JO, Sudhakaran SU, Kumar S, Jayaraman SO, Variyar EJ. A comparative evaluation of anticancer activities of flavonoids isolated from Mimosa pudica, Aloe vera and Phyllanthus niruri against human breast carcinoma cell line (MCF-7) using MTT assay. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(2):319-22.

2. Abubakar AN, Achmadi SS, Suparto IH. Triterpenoid of avocado (Persea americana) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells. Asian Pacific Journal of Tropical Biomedicine. 2017 May 1;7(5):397-400.

3. Ramar M, Manikandan B, Marimuthu PN, Raman T, Mahalingam A, Subramanian P, Karthick S, Munusamy A. Synthesis of silver nanoparticles using Solanum trilobatum fruits extract and its antibacterial, cytotoxic activity against human breast cancer cell line MCF 7. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015 Apr 5;140:223-8.

4. Ali A, Banerjee S, Kamaal S, Usman M, Das N, Afzal M, Alarifi A, Sepay N, Roy P, Ahmad M. Ligand substituent effect on the cytotoxicity activity of two new copper (ii) complexes bearing 8-hydroxyquinoline derivatives: validated by MTT assay and apoptosis in MCF-7 cancer cell line (human breast cancer). RSC advances. 2021;11(24):14362-73.

Reviewer 3

Response: With the thorough search of relevant articles on chromene we proposed CDK6 as the main biological target and perform docking study on this enzyme (pdb:4EZ5). Surprisingly, interesting results achieved which can pave road for further investigation of these molecules (refer to docking part added). In addition, all typos were corrected.

Reviewer 4

Please add a scheme to show the structure of the lead compounds and explain more about the SAR of the designed compounds.

Response: The SAR was added to the disscussion part.

What is the mechanism of synthesized compounds? Which enzyme is the goal of these compounds?

Response: CDK6 was proposed as probable biological target (pdb: 4EZ5) and docking study was added to the article (refer to docking part).

One of the more important parameters in recognizing effective compounds is performing a docking study. Please add this section to your work for more understanding of the mechanism of synthesized compounds.

Response: Docking study was done and added to the article.

The text of your manuscript has many grammatical errors; please recheck and correct these errors.

Response: All spelling errors, grammatical errors and typos were corrected and highlighted.

These compounds have a cytotoxic effect on the MCF-7 cell line, but did you test the cytotoxic effect of these compounds on the normal cell line? These compounds must show a low cytotoxic effect on the normal cell to be valuable.

Response: In the present study the effect of the synthesized compound on MCF-7 cell line was investigated. Specifically we mention the name of the cell line in the title and cytotoxicity was solely done on this line as this can be observed in some articles, following are some as a sample. Although for the betterment of judgement one can evaluate cytotoxicity on the normal cells, we believe it is not compulsory particularly when the name of cell line is mentioned in the title. Moreover, nowhere in the article did we claim our compound selectively affect cancerous cell line. But we decide to put this to consideration in our future studies.

1. Jose JO, Sudhakaran SU, Kumar S, Jayaraman SO, Variyar EJ. A comparative evaluation of anticancer activities of flavonoids isolated from Mimosa pudica, Aloe vera and Phyllanthus niruri against human breast carcinoma cell line (MCF-7) using MTT assay. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(2):319-22.

2. Abubakar AN, Achmadi SS, Suparto IH. Triterpenoid of avocado (Persea americana) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells. Asian Pacific Journal of Tropical Biomedicine. 2017 May 1;7(5):397-400.

3. Ramar M, Manikandan B, Marimuthu PN, Raman T, Mahalingam A, Subramanian P, Karthick S, Munusamy A. Synthesis of silver nanoparticles using Solanum trilobatum fruits extract and its antibacterial, cytotoxic activity against human breast cancer cell line MCF 7. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015 Apr 5;140:223-8.

4. Ali A, Banerjee S, Kamaal S, Usman M, Das N, Afzal M, Alarifi A, Sepay N, Roy P, Ahmad M. Ligand substituent effect on the cytotoxicity activity of two new copper (ii) complexes bearing 8-hydroxyquinoline derivatives: validated by MTT assay and apoptosis in MCF-7 cancer cell line (human breast cancer). RSC advances. 2021;11(24):14362-73.

The most important revision is correcting your "title"! Please correct the "Opoptisis" word in your title!

Response: The wrong spelling “opoptosis” was corrected in title.

2 Revision 1

5 comments · 28 Jan 2023 – 5 Feb 2023

Referee 2 Reviewer

MINOR REVISION 28 Jan 2023

Dear AE,

The manuscript is well-edited.
It is necessary to present the docking study under a separate heading in the method section. Also, the order of headings in the method section should be the same as in the results section.

Referee 1 Reviewer

MINOR REVISION 29 Jan 2023

Dear AE,

A few minor corrections are necessary:
1- Please, in the docking section, add the distances of important interactions between the more effective compound and the enzyme and show these important distances in the figure.
2- The stated reason for not evaluating the synthesized compounds in a normal cell line is, in my opinion, not acceptable; if it is possible for you, please evaluate your compounds in the normal cell line.

Referee 3 Reviewer

MINOR REVISION 1 Feb 2023

Dear AE,

The manuscript has been reviewed, and minor revisions are needed as follows.

1. Mahboubi Rabbani SMI, Zarghi A. Selective COX-2 inhibitors as anticancer agents: a patent review (2014-2018). Expert Opin Ther Pat. 2019 Jun;29(6):407-427. doi: 10.1080/13543776.2019.1623880. Epub 2019 May 27. PMID: 31132889.

2. Mahboubi-Rabbani M, Zarghi A. Lipoxygenase inhibitors as cancer chemopreventives: Discovery, recent developments and future perspectives. Current Medicinal Chemistry. 2021 Feb 1;28(6):1143-75.

Please add these references to the bibliography section in the manuscript.

Associate Editor

MINOR REVISION 4 Feb 2023

Dear EIC,

Considering the reviewers' comments, the manuscript needs minor revision before publication in the IJPR.
Regards,

Editor-in-Chief

MINOR REVISION 5 Feb 2023

Dear Author,

Your article has been reviewed by the reviewers. According to their comments, this document needs minor revision. Please follow their comments as requested.
Thanks,

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Revision 2

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.

Dear Editor,

We would like to thank you and the reviewers for their valuable comments. We paid attention to the comments, revised the sections in the text, and highlighted the changes in yellow. We hope that the revised manuscript meet your high standards. We look forward to hearing from you.

Answering Reviewers

The manuscript has been improved according to the suggestions of reviewers. We have carefully addressed the criticisms point-by-point, and our response is indicated below.

We appreciate the reviewers for their kindly comments about the manuscript.

1) The docking section was moved under a separate header. The order of method comply with the result part

2) The distance of important interactions were added in the text as well as figures.

Dear editor we do apologize, presently it isn't possible for us to test cytotoxicity of the compound on the normal cells but we promise to consider normal cells in our future work. We add this sentence to the text "Although for the better judgement one can evaluate cytotoxicity of the compounds on the normal cells, in this experiment we only claimed that our compounds affect cancerous cell line, MCF-7. But we decide to put the cytotoxicity evaluation to consideration in our future studies."

3) The highlighted words were corrected and added to the text. We included and highlighted the suggested sentence in the text.

Best Regards,

3 Revision 2

5 comments · 15 Feb 2023 – 22 Feb 2023

Referee 4 Reviewer

ACCEPT 15 Feb 2023

Dear AE,
I think the article could be accepted.
Thanks,

Referee 3 Reviewer

MINOR REVISION 19 Feb 2023

Dear AE,

The paper still requires some minor revisions because it lacks cytotoxicity test results on normal cells. The article is conditionally acceptable. But it is necessary to examine at least one of the compounds in terms of cytotoxicity and present a discussion on this matter.

Referee 2 Reviewer

ACCEPT 22 Feb 2023

Dear AE,
There are no more comments.
Regards,

Associate Editor

ACCEPT 22 Feb 2023

Dear EIC,

The paper is correctly revised and presented. Therefore, publication is recommended.
Regards,

Editor-in-Chief

ACCEPT 22 Feb 2023

Dear Author,

I am pleased to inform you that your manuscript has been accepted for publication in the Iranian Journal of Pharmaceutical Research.
Regards,