Universidad Tecnológica de Ciudad Juárez

November 20, 2025

Dear Editors:

This letter is related to the reference ***molecules*-3955571**, entitled *"**Green contribution to the chemistry of perezone, oxidation of the double bond of the side chain: theoretical study, and cytotoxic evaluation in MDA-MB231 cells**"* by René Gerardo Escobedo-González, Joel Martínez, Adriana L. Rivera-Espejel, Claudia Vargas-Requena, María Inés Nicolás-Vázquez, and René Miranda-Ruvalcaba.

Below, please find a "*Memo*" that explains, point-by-point, the details of revisions and our responses to the referee's comments, along with a corrected version of the manuscript. The corresponding changes are highlighted in yellow color.

I hope you find this new version suitable for its publication in the prestigious *Molecules*.

Sincerely yours

Dr. René Gerardo Escobedo-González

e-mail: renegerardo.escobedo@gmail.com

Reviewer 1

Open Review

( ) I would not like to sign my review report

(x) I would like to sign my review report

Quality of English Language

( ) The English could be improved to more clearly express the research.

(x) The English is fine and does not require any improvement.

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	(x)	( )	( )	( )
Is the research design appropriate?	( )	(x)	( )	( )
Are the methods adequately described?	(x)	( )	( )	( )
Are the results clearly presented?	(x)	( )	( )	( )
Are the conclusions supported by the results?	( )	(x)	( )	( )
Are all figures and tables clear and well-presented?	(x)	( )	( )	( )

Comments and Suggestions for Authors

The present manuscript by René Gerardo Escobedo-González et al describes the preparation of eight compounds (i.e. four oxidation derivatives of perezone and four of isoperezone). These compounds were evaluated in vitro for their cytotoxicity against MDA-MB231 cells. The derivatives were also studied in silico (structural and docking). The manuscript could be of interest to researchers in the field. However, a number of revisions are necessary:

1) In lines 131 and 410 it is stated "…the most active compound (3)…" and "…Bearing in mind, that 3 presented experimentally the highest biological activity, followed by 6,…". However, this is wrong as the most active compound, based on molarity (which should be used for all comparisons of structurally deferent compounds), is compound 6. Thus, all the manuscript should be modified based on this fact.

2) The correct structure of 7 and 8, based on 1H-NMR, is most likely the C12-acetate and not the C13-acetate.

US: Sir, in a kindly manner, the position of the acetate group on C13 was correlated with the NMR theoretical data, calculated by GIAO, assigning to our knowledge appropriately this position.

3) A positive control should be included in Table 1.

US: Done, the cisplatin was employed as reference, displaying a mortality of 40.53±0.135% at 48 hours of exposure, **the concentration of cisplatin was 40 µM.**

4) The cytotoxicity of isoperezone should be included in Table 1.

US: Done, the cytotoxicity of isoperezone was included in Table 1: 19.31±0.05 µM.

5) All the reported new derivatives could exist in two diastereomeric configurations (8R-12R & 8R-12S). Were the studied compounds pure diastereomers (which ones?) or mixtures? This should be studied/reported.

US: Thank you for your comment. The molecules were mixtures. In this sense, this comment was included in lines 85-86. Consequently, the purification to obtain the pure diastereomer is in course in our lab.

6) The cytotoxicity of the compounds towards normal cells (especially the known for toxicity epoxides) should be studied/reported.

US: Sir, the mortality percentages against the primary dermal fibroblast normal were included in Table 1. Please see the new version of the manuscript.