Author's response to reviews

Title: A novel cytosporone 3-Heptyl-4,6-dihydroxy-3H-isobenzofuran-1-one: synthesis; toxicological, apoptotic and immunomodulatory properties; and potentiation of mutagenic damage.

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Date: 9 June 2015

Author's response to reviews: see over
June 9, 2015.

Dear Editors of the BMC Cancer Journal,

We appreciate the opportunity to resubmit our article “A novel cytosporone 3-Heptyl-4,6-dihydroxy-3H-isobenzofuran-1-one: synthesis; toxicological, apoptotic and immunomodulatory properties; and potentiation of mutagenic damage”.

All comments by reviewers have been addressed, with corresponding changes made directly to the manuscript where appropriate. Accompanying this letter, please find a revised version of our manuscript. Detailed responses to the reviewers are included in a separate document.

Yours Sincerely,

Rodrigo Juliano Oliveira.

Send correspondence to Rodrigo Juliano Oliveira. Faculdade de Medicina, Universidade Federal do Mato Grosso do Sul. Cidade Universitária, S/N. 79070-900 Campo Grande, MS, Brazil. Phone: +55 (67) 3345-3068 / +55 (67) 3345-7671 Fax: 0800-6470710 E-mail: rodrigo.oliveira@ufms.br
Dear Editor-in-Chief,

BMC Cancer.

The manuscript “A novel cytosporone 3-Heptyl-4,6-dihydroxy-3H-isobenzofuran-1-one: synthesis; toxicological, apoptotic and immunomodulatory properties; and potentiation of mutagenic damage.” (ID 1087389032146929) was revised and all the corrections were carefully considered. We are sending the manuscript to be resubmitted to your Journal, BMC Cancer.

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<th>Reviewer's report:</th>
<th>We appreciate your consideration of our manuscript.</th>
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<tr>
<td>I don't have any comments.</td>
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<th>Level of interest:</th>
<th>We appreciate your recognition of our work and its importance to the field.</th>
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<td>An article of importance in its field</td>
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<th>Statistical review:</th>
<th>We appreciate your consideration of our manuscript. Statistical methods and tests used are utilized in several international papers.</th>
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<td>No, the manuscript does not need to be seen by a statistician.</td>
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Reviewer Maria Stasiuk

Reviewer's report:
I don't have any comments.

Level of interest:
An article of importance in its field

Statistical review:
No, the manuscript does not need to be seen by a statistician.

Reviewer Bin Su

Reviewer's report:
Major compulsory revision
The authors described the synthesis and in vivo evaluation of several new compounds for their toxicity. The chemistry work is OK and the toxicity study is solid as well. However, the compounds by designing are anti-cancer agents. The authors missed the very important study, anti-cancer activity of the compounds. I did not see any cell growth inhibition or apoptosis inducing study of the compounds in cancer cells, neither with animal study. Even the compounds did not cause toxic effects to the animals, what is the point to have these compounds if they don't inhibit tumor growth?

A minor comment, Table 9, the structure 4 side chain is not consistent to other structures

We would like to clarify that our work is focused in the toxicological screening of a new compound. Thus, neither in the title nor in the objectives we presented anticarcinogenic assays. On the other hand we utilized toxicological assays (biomarkers; comet assay and micronucleus test). Both tests allow us to infer that the compound has anticancer activities because there is a strict relation between carcinogenicity and mutagenicity (MANTOVANI et al., 2008)

1. (...) The authors missed the very important study, anti-cancer activity of the compounds. I did not see any cell growth inhibition or apoptosis inducing study of the compounds in cancer cells, neither with animal study.
The available literature also reports that experimental studies with rodents are adequate to screening tests. Assays that evaluate mutagenic and genotoxic activity are acceptable by international agencies in predicting the (anti)carcinogenic/(anti)mutagenic activity of a compound (Zeiger, 1987; Ishidate Jr et al., 1988; Ashby and Tennant, 1991; Camargo et al., 1994; OECD, 2014). Thus, we assume that the proposed assays are acceptable tests as predictors of possible anticancer activity of the tested compound.

We agree with the reviewer that we did not use any assay/protocol of study in tumor cellular lineages or in vivo carcinogenic studies. However, in the other hand, the studies are focused in the toxicological screening of the new compound.

2. Even the compounds did not cause toxic effects to the animals, what is the point to have these compounds if they don’t inhibit tumor growth?

The focus of the paper was to establish if the compound is toxic and if there is any interesting biological activity. Data obtained from our research shows that the compound isn’t genotoxic nor mutagenic (the compound is safe for use in the tested protocols). Moreover, when the compound is administered alone, the apoptotic events are increased and this is a process that is essential to anticancer therapies. The fact that there is no DNA damage caused by the compound (observed in the micronuclei and comet assays) allows us to infer that the apoptotic events are being caused by the interaction between the compound and the NUR77 receptor (as discussed in the main text). Thus, when associated with the commercial chemotherapy Cyclophosphamide, the compound increase the frequency of mutagenic and apoptotic events. Both activities are desirable in a combined
therapy with the tested compound and cyclophosphamide.

Based on the above, we suggest that the compound has anticancer activity.

3. A minor comment, Table 9, the structure 4 side chain is not consistent to other structures

Changed as per learned referee’s comments. The structure has been changed. Table 9 was updated.

References


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<th>those with closely related research interests</th>
<th>importance of the research in the field.</th>
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<td><strong>Statistical review:</strong></td>
<td>We appreciate your consideration of our manuscript. Statistical methods and tests used are utilized in several international papers.</td>
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<td>Yes, but I do not feel adequately qualified to assess the statistics.</td>
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