Reviewer’s report

Title: 2-Ethoxystypandrone, A Novel Small-Molecule STAT3 Signaling Inhibitor from Polygonum Cuspidatum, Inhibits Cell Growth and Induces Apoptosis of HCC cells and HCC Cancer Stem Cells

Version: 1 Date: 02 Oct 2018

Reviewer: Yin Quan Tang

Reviewer's report:

General comments:
Overall is good, but minor correction is needed.
Suggest to use "the root ethyl acetate (EtOAc) extract of P.cuspidatum" instead of "the ethyl acetate (EtOAc) extract of the roots of P.cuspidatum"
Please include standard error mean (SEM) value for each IC50.
Please define "NMR, MS, UV and IR analysis" as can't find inside the text.
Need explanation on how 2-ethoxystypandrone (1) can block cell cycle progression at the S and G2/M phases, and how this blockage can led to led to apoptosis.

Abstract:
Generally, the abstract is good but minor corrections are needed for clarity.
Suggest to use "the root ethyl acetate (EtOAc) extract of P.cuspidatum" instead of "the ethyl acetate (EtOAc) extract of the roots of P.cuspidatum"
Please change "...by flow cytometry measurement, respectively." to "flow cytometric analysis."
Isn't it cell proliferation and cell survival are the same? Please recheck the write up of "...inhibited cell proliferation and cell survival of HCC...."
Please recheck the sentence ".....fits the Lipinski' rule of five....." as this no meaning without further explanation.

Background:
Introduction is good and sufficient.
Please standardize either using "HCC CSCs' or "CSCs in hepatocellular carcinoma" as both meaning are the same.
Suggest to amend "......target for novel anti-cancer drugs." to "......target for development of novel anti-cancer drugs."
Please include standard error mean (SEM) value for each IC50.
IC50 has an exact value ± SEM, and not the range (IC50 = 3.69-20.36uM). Please recheck the IC50 of 2-ethoxystypandrome stated here.

Methods:
Please state the brand and model of machines/equipment like microscope used in this study.
Incomplete sentence: "2-Ethoxystypandrone (1): yellow acicular crystal in acetone. mp: 153-154ºC."
Author mentioned in abstract that "….including NMR, MS, UV and IR analysis and….", but author didn't explain much about these analysis except putting the some numbering and ions which hard to understand (under section "Identification of 2-ethoxystypandrone (1)"). Please define "FCS" and indicate the brand as well. Author mentioned tumorspheres were photographed and counted. How author define/count one tumorsphere? Based on size? Because tumorsphere size can vary sometimes. Under the "Flow Cytometric Analysis" section,

- Author define upper left quadrant is damages cells. Please define these damaged cells.
- Author showed the results of cell cycle analysis, but never mentioned how this assay was conducted. Please add the methodology for this part.

Results:
Please include standard error mean (SEM) value for each IC50.
Please remove "...(3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, Thiazolyl Blue Tetrazolium Bromide)…" from the sentence.
Please include standard deviation value for apoptosis percentage derived from three independent experiments using flow cytometry.

Author proposed the linkage of cell cycle arrest and apoptosis by stating (…. 2-ethoxystypandrone (1) can induce apoptosis of HCC CSCs by blocking cell cycle progression at the S and G2/M phases." But author didn't further explain on this in discussion section. Please explain on this phenomenon.

No statistical analysis on IC50 across different hepatoma cell lines (HepG2, HepG3b, SK-HEP-1, Li-7 and Huh-7).
Are the IC50 of 2-Ethoxystypandrone on HepG2, HepG3b, SK-HEP-1, Li-7 and Huh-7 cell lines are significant different each other? If yes, the IC50 on Li-7 (IC50=5.58uM) and Huh-7 (IC50=3.36uM) cells are different despite both cell lines are constitutively activated STAT3? Any explanation regarding this statement.

No indication (asterisk) for significant statistical differences among the groups in Figure 2 (A and B), Figure 3 (A) and Figure 4 (C)

Discussion:
Discussion is acceptable with strong support from the data. But need further clarification on some findings.

What does author mean "….to clarify SAR and ….."?

Authors should explain what is Lipinski's rule of five instead of stating it in text without explanation.

Authors stated "2-Ethoxystypandrone (1) prefers to inhibit cell growth and induce apoptosis of HCC CSCs compared to regular HCC Huh-7 cells". Isn't the stemness of HCC CSCs make these cells less susceptible to any treatment or "stronger" than regular HCC Huh-7 cells?

"……2-ethoxystypandrone (1) might be proposed to block cancer stem cell activity and suppress the tumorsphere...... " What does author mean block CSC activity?
No discussion for the differences between the IC50 on Li-7 (IC50=5.58uM) and Huh-7 (IC50=3.36uM) cells despite both cell lines are constitutively activated STAT3.

No explanation on how 2-ethoxystypandrone (1) can block cell cycle progression at the S and G2/M phases.

No explanation on how the blocking cell cycle progression at the S and G2/M phases by 2-ethoxystypandrone (1) can led to apoptosis of HCC CSCs.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable
**Declaration of competing interests**

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal