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: 0 Date: 27 Jul 2018

Reviewer: Tomoharu KUBOYAMA

Reviewer's report:

You identified 2-ethoxystypandrone, which inhibited STAT3 activation and induced apoptosis in HCC cells. Although the effects of the compound are interesting, I have following questions and suggestions.

1. You mentioned HepG2, Hep2, and HeG2 cells. Are they the same cell line? If so, what is real name? Why are HepG2/STAT3 cells resistant to 2-ethoxystypandrone? Are HepG2/STAT3 cells also resistant to pyridone 6?

2. Is STAT3 inhibition really needed for the effects of 2-ethoxystypandrone? Have you ever investigated effects of pyridone 6 in Figs. 3 and 4?

3. Please write catalog numbers of each antibody.

4. Which methods did you use for statistical analysis? Please mention it in each figure legend. What kind of software did you use for statistical analysis?

5. Please mention details of the plasmid construct expressed in HepG2/STAT3 cells.

6. In Fig. 2C,D, inhibitory concentrations of 2-ethoxystypandrone are different from IC50 in Table 2. Why?

7. Your discussion about structure-activity relationship looks imagination because you hide detail information. Is it possible to discuss SAR by referring your previous studies?
8. You discuss that 2-ethoxystypandrone (1) prefers to inhibit cell growth and induce apoptosis of HCC CSCs compared to regular HCC Huh-7 cells. Is it caused by difference of culture condition? Have you ever compared the effects of conventional anti-cancer drugs between HCC CSCs and regular HCC Huh-7 cells? Do your HCC CSCs show drug resistance?

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

No

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

No

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

No

**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 recommend additional statistical review

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

Acceptable
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