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: 01 Oct 2018

Reviewer: Tomoharu KUBOYAMA

Reviewer's report:

I yet cannot believe that 2-ethoxystypandrone shows anti-cancer effects via STAT3 inhibition. You did not show any evidences that STAT3 in HepG2/STAT3 cells were highly phosphorylated. You should cite appropriate references about HepG2/STAT3 cells, what kinds of plasmid expressed. In Fig. 2C, STAT3 was not phosphorylated without IL-6, but in Fig. 2D, STAT3 was constitutively phosphorylated. Please show that STAT3 is higher phosphorylated in HepG2/STAT3 cells than HepG2 cells. Are there correlation between STAT3 phosphorylation levels and effects of 2-ethoxystypandrone in HepG2, HepG3B, SK-HEP-1, Li-7, and Huh-7 cells? If pyridone 6 shows toxic effects in these cancer cells, please cite references in the manuscript. In HepG2/STAT3 cells, 2-ethoxystypandrone inhibited STAT3 phosphorylation within 2h and induced cell death within 6.5 h. However, in HepG2 cells, 2-ethoxystypandrone inhibited STAT3 phosphorylation within 2h, but did not induce cell death within 6.5 h, 24 h was needed to induce cell death. Why these different effects occurred?

What kinds of t-test did you use? Two-tailed unpaired t-test? If so, please write so.

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.

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 recommend additional statistical review

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

Acceptable

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