Reviewer's report

**Title:** Jacarelhyperol A induced apoptosis in leukaemia cancer cell through inhibition the activity of BCL2 Proteins

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**Reviewer:** Bernhard Gillissen

**Reviewer's report:**

In their manuscript entitled “Jacarelhyperol A induced apoptosis in leukaemia cancer cell through inhibition the activity of Bcl-2 Proteins.” Zhang et al. present solid and interesting data regarding an anti-cancer effect of Jacarelhyperol A (Jac-A). Jacarelhyperol A is a characteristic constituent of the herb Hypericum japonicum, which is used in Chinese herbal medicine for the treatment of some bacterial diseases, infectious hepatitis and tumors. In this study Jac-A was identified as a potential Bcl-2 inhibitor via high throughput screening. A potential function of Jac-A as an inhibitor of anti-apoptotic Bcl-2 proteins is supported by computational modeling which predicted binding of Jac-A into the hydrophobic groove of Bcl-xL. Furthermore the authors demonstrated that Jac-A inhibits proliferation of leukemia cell lines, induces apoptosis in the human leukemia cell line K562 and inhibits tumor growth in a K562 mouse xenograph model.

In general data are clearly presented and all experiments are performed in a thorough manner. However, I would recommend some additional experiments to strengthen the author’s hypothesis that Jac-A induces apoptosis in leukemia cells by inhibiting anti-apoptotic Bcl-2 proteins.

**Major Compulsory Revisions**

1. The authors used a fluorescence polarisation assay to show competitive binding of a BH3-peptide and Jac-A to anti-apoptotic Bcl-2 proteins. To confirm that Jac-A binds to anti-apoptotic Bcl-2 family members and competes with binding of pro-apoptotic proteins in vivo, co-immunoprecipitation of e.g. Mcl-1/Bak should be performed to analyze if these interactions are disrupted by Jac-A.

2. To demonstrate involvement of the mitochondrial apoptosis pathway, the authors should analyze breakdown of the mitochondrial membrane potential and/or release of cytochrome c upon Jac-A treatment.

3. The authors state that Jacarelhyperol A induces apoptosis in leukemia cancer cells. However, analysis of Jac-A-induced apoptosis by Annexin/PI staining was done only for one of the cell lines (K526 cells in figure 3). For all the other cell lines only a proliferation assay is shown. Annexin/PI staining should at least also be done for the other leukemia cell lines. To conclude on a general role of Jacarelhyperol A as a potential inducer of apoptosis it is also recommended to perform Annexin/PI staining with the solid cancer cell lines.
4. The authors showed caspase activation upon Jac-A treatment. To show that cell death induction by Jac-A depends on caspase activation they should also analyze whether inhibition of caspase activity abrogates Jac-A-induced cell death.

Minor Essential Revisions

1. In table 1 the affinity of gossypol to Bcl-xL, Bcl-2 and Mcl-1, determined by a fluorescence polarisation assay, is shown. However, gossypol is neither mentioned in the legend nor main text. Furthermore, gossypol has been shown to induce caspase-dependent apoptosis in the absence of Bak and Bax by converting Bcl-2 from an inhibitor to an activator of apoptosis. The authors should discuss these results in consideration of Jac-A induced apoptosis.

2. In table 2 Doxorubicin was used as a positive control but neither mentioned in the legend nor main text. In addition it would be more appropriate and interesting to compare Jac-A with Obatoclax, a BH3 mimetic, which binds to a broad spectrum of Bcl-2 family members, including Bcl-2, Bcl-xL, and Mcl-1.

3. In figure 3 the “PI-AV+” quadrant Q4 is labeled “PI-AV-“.

4. In figure 5 D labeling of the y-axis is confused.

5. In figure 5D partitioning for the quadrants differs compared to figure 5A-D.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

I declare that I have no competing interests