Reviewer's report

Title: Synergistic effect of acyclovir and 3,19-isopropylideneandrographolide on herpes simplex virus wild types and drug resistant strains

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Reviewer: Laura B Talarico

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The manuscript “Synergistic effect of acyclovir and 3,19-isopropylideneandrographolide on herpes simplex virus wild types and drug resistant strains” by Priengprom et al. studies the antiviral activity of 3,19-isopropylideneandrographolide (IPAD) against herpes simplex virus types 1 and 2, including wild type as well as drug-resistant strains, in Vero cells. The synergistic effect of IPAD and acyclovir was evaluated against HSV strains by a plaque reduction assay, inhibition of viral DNA synthesis and viral protein (glycoprotein D) synthesis.

Major compulsory revisions

-I strongly recommend reviewing the English grammar and style of the manuscript. The manuscript is not well-written and it has a tremendous amount of syntax errors (lines 41-44, 80-81, 83-86, 100-101, 113-114, 120-122, 123-125, 163-165, 172-173, 209-211, 211-215, 216-223, 248-251, to mention some). It becomes difficult to understand what the authors mean to say in many sentences.

-The manuscript has also important concept mistakes, such as the use of units (uM) in selectivity indexes (SI) (line 173 and Table 1). SI is the ratio CC50/IC50 and does not have units.

-The viral entry assay that the authors used is in fact a virucidal assay, since there is an incubation between the virus and the compound before adding the mixture to the cells. The entry assay should be performed separately to evaluate if there is an effect on virus binding (adsorption of virus to Vero cells at 4°C for 1 h in the presence of compound) and internalization (infection of Vero cells at 37°C for 1 h in the presence of compound) to the cells.

-The MOI used in the plaque reduction assays do not make sense. If the assays were performed in 24-well plates (this is not stated in the manuscript), in each well there are approximately 2.5x10^5 cells, so an MOI of 0.01 means that the authors are able to count 2.5x10^3 PFU in a well.

-The selectivity indexes of IPAD against HSV strains are very low (around 2), which means that this compound is not a good antiviral agent against HSV. A desirable SI for an antiviral agent should be >10. The authors performed the antiviral assays at a concentration of 22.5 uM (where they see 50-60% inhibition) which is very near the CC50 (39.71 uM), so it is difficult to distinguish antiviral from cytotoxic effects. This important issue makes the compound a very poor
antiviral agent. The authors do not mention this important limitation of the study.

- A good antiviral agent should have a selectivity index >10, so that the antiviral assays could be performed at a concentration near the IC90 (so as to have 90% inhibition of viral plaques).

- For the antiviral assays against drug-resistant strains of HSV, the authors used a concentration of ACV of 2220 uM which is higher than the CC50 (>1000 uM). The authors should evaluate that the range of concentrations that they are using for the antiviral assays are non-cytotoxic.

- The CC50 of the combination of IPAD and ACV was not determined and should be included in the manuscript.

Minor Essential Revisions

- The references should be revised, since specific studies are cited as the source of general information regarding HSV epidemiology and pathogenesis (line 54).

- The authors do not mention the strain of HSV type 2 that they used in the study.

- The authors do not mention the staining method used to count plaques (crystal violet).

- The method used for the calculation of the potential synergistic effect should be described in more detail and clearly.

- In Figure 2, the legend for ACV2 is missing.

- In Figure 3, the authors mention that IPAD reduced the expression of viral protein, but there is gD expression of drug-resistant HSV strains determined by western-blot.

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

**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