Reviewer's report

Title: Identification of a Novel CK2 Inhibitor - Hematein (3, 4, 10, 6a-tetrahydroxy-7, 6a-dihydroindeno [2, 1-c] chroman-9-one) from a Natural Product Library

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Reviewer: Lorenzo A Pinna

Reviewer's report:

General Comments

The work presented adds a new member (hematein) to the growing list of compounds that are inhibitors of protein kinase CK2 and display cytotoxic effect on tumour cell lines. No attempt is done to demonstrate a cause-effect relationship between the two effects.

Major compulsory revision

1. The authors adopt kinase assays different from those commonly applied in the literature, apparently relying only on the manufacturer’s protocols, without any bibliographic support. It looks especially strange that one kit exploits p53 serine-46 phosphorylation to quantify CK2 activity, whereas the CK2 site in p53 is not serine-46. The authors shouldn’t blindly trust the manufacturer’s claims and instead they should support the reliability of their methods with appropriate references. In any case experimental conditions (notably source and concentration of the protein kinase) should be better defined.

2. The crucial experiment showing the decrease of Akt S129 phosphorylation (Fig. 6A) is biased by an ambiguous DMSO control (irregular intensity of the band). This should be replaced by a better experiment with statistically significant quantification of the outcome.

3. Higher efficacy of hematein toward cancer cells as opposed to “normal” cells is an interesting outcome of this work. It rises however the issue of selectivity which has not been touched by the authors, while the selectivity of most other CK2 inhibitors (included TBB here used for sake of comparison) has been thoroughly examined on panels of numerous kinases. This gap should be filled at least by testing hematein on a few protein kinases (notably those belonging to the DYRK, PIM, HIPK, PKD and CDK families) which have been reported to be particularly susceptible to CK2 inhibitors.

4. The 100 uM concentration of effective compounds used to perform the in vitro experiment shown in Fig. 2 is exceedingly high, especially considering that the concentration of compounds used to perform the in cell experiment of Fig. 1 is lower (6-27 uM). This experiment should be repeated at 10 uM concentration.

Minor essential revisions.

1. In Fig. 1 only 11 compounds out of the 400 compounds of the library are
considered, only 3 of which display significant inhibition of cell proliferation. I presume that all the other compounds were devoid of any effect. If so, this should be clearly stated and the negative data mentioned as “not shown”.

2. Fig. 3 (hematein structure) should be incorporated into Fig. 2, together with the structures of the other two library compounds which display significant CK2 inhibitory activity (4A6 and 4A9).

Discretionary revisions.

1. Given the difference in M.W: among the library compounds (ranging between 183-832) it is not quite accurate to test all of them at a fixed 5 ug/ml concentration (corresponding to quite variable molar concentrations). It would be preferable to test all compounds a fixed molar concentration, I suggest 30 uM.

2. To make sure that the pro-apoptotic efficacy of hematein is mediated by inhibition of CK2 rather than of other kinases the authors could use mutants refractory to inhibition (e.g. V66/I174AA) to overcome the effect of hematein. All the more so considering the present lack of information about hematein selectivity (see above MCR-3).

**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 interest.