Reviewer's report

Title: Screening of protein kinase inhibitors identifies Rottlerin as a potent inhibitor of osteoclastic acid secretion and bone resorption

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Reviewer: Fraser Coxon

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In this study the authors have utilised several assays that they have expertise in to screen numerous protein kinase inhibitors for their ability to inhibit acidification in osteoclasts, acid influx in isolated microsomes, and bone resorption. The striking finding is the ability of most of the PKC inhibitors to inhibit both bone resorption and acid influx, suggesting a mechanistic link. These compounds seemed to have different effects on cell viability, though, suggesting differing non-specific effects between them. These studies provide valuable new data regarding the regulation of vesicular acidification in osteoclasts, as well as identifying interesting potential novel anti-resorptive agents. However, there are a number of aspects of the study that require attention:

- Major Compulsory Revisions

1) In the acid influx assay, are the compounds acting through PKC inhibition or another effect, such as directly on the machinery responsible for acidification? Since these are completely cell-free experimental systems (and not incubated with cytosolic extracts), the latter seems quite likely, therefore the involvement of PKC needs to be demonstrated, or the conclusions from the data modified.

2) It is not clear why some compounds, which were extremely effective at inhibiting bone resorption and acid influx, had no discernible effect on acidification in intact osteoclasts. This conundrum should be given more attention in the discussion.

3) On page 11, it is stated that “however for GF109203X and Hypericin (figure 4A and B) and Palmitoyl-DLCarnitine DI (figure 4E) there was a clear distinction between inhibition of resorption and reduction of cell viability, although Ro31-8220 and Sphingosine exhibited toxic effects (Figure 4C and 4D)”. However, for Hypericin and Palmitoyl-DLCarnitine DI this difference was vastly smaller than for GF109203X, while sphingosine appeared to reduce viability at concentrations that had little effect on resorption. These subtle but important differences in the data should be clearly stated and discussed.

4) In the discussion it is stated that rottlerin was the most potent inhibitor, but from the data, GF109203X looks to be at least as potent, certainly in terms of inhibition of resorption. This needs to be clarified, while the title might be better describing the effects of PKC inhibitors more generally, rather than concentrating on Rottlerin.
5) Rottlerin decreases RANK expression in osteoclasts, most likely by a PKC-independent pathway (Kang et al 2004, Mol Cells 30, 438). This could therefore mediate some of the anti-resorptive effects of this compound and should be cited.

- Minor Essential Revisions

1) Background, line 11. “shown to have a very promising mode of action” would be better as “shown to have promising effects”.

2) Background, 2nd para, line 6: remove the word “indicated”.

3) How exactly were the Alamar blue assays carried out? Using the osteoclasts seeded on to the bone discs at the end of the culture period? This should be clarified.

4) Discussion, 2nd paragraph, last part of sentence on genistein “...however, in alignment with our assays...” is repetitive and should be removed.

5) Discussion, last paragraph. “Thus, indicating” should be changed to “This indicates”; “thus questioning” should be changed to “question”.

6) Legend to fig. 1 is repetitive and should be shortened.

7) Legend to Fig. 2 is the same as the Fig. 1 legend and therefore needs to be corrected.

8) It would be helpful to plot the anti-resorptive potency of the compounds against the potency in the acid influx assays. A good correlation would support this effect as a likely mechanism for inhibition of resorption. In this respect, a comparison with the ability of the compounds to inhibit their supposed target enzymes would also be beneficial.

9) HBDDE was able to inhibit acid influx but did not bone resorption. Why might this be the case? Is this compound less able to act in whole cells than the other compounds?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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