Author's response to reviews

Title: Screening of protein kinase inhibitors identifies PKC inhibitors as inhibitors of osteoclastic acid secretion and bone resorption

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Author's response to reviews: see over
Dear Editor of BMC musculoskeletal diseases

Thank you for this second positive review of our manuscript entitled “Screening of protein kinase inhibitors identifies PKC inhibitors as inhibitors of osteoclastic acid secretion and bone resorption”.

We wish to thank the reviewers for their constructive criticism after the second revision.

The issues raised by the reviewers are answered in the paper and has been marked in green, and in the point-by-point comments in the rebuttal letter below.

Reviewer: Fraser Coxon

Comment: The authors have satisfactorily addressed all of the concerns raised in the original submission.

Answer: We thank the reviewer for the positive feedback

Comment: One minor point is that in the discussion (page 16), concerning potential mechanisms of toxicity of rottlerin, it would be helpful to stress that toxicity of rottlerin was only seen at 10µM, whereas the inhibition of acid influx occurred at lower doses. This suggests that the latter may be mediated via PKC inhibition, and the former by non-specific mechanisms, such as acting as a protonophore.

Answer: We thank the reviewer for this important comment, and we have added a section in the discussion stating that the toxicity of Rottlerin in the bone resorption assay was only seen at 10µM, while the inhibitory effect of Rottlerin on bone resorption, as well as acid influx occurred at lower doses than 10µM (page 17, line 5-10). As the reviewer suggests this can be because inhibition of the acid influx through PKC inhibition occurs at doses less than 10µM, while at the 10µM dose non-specific side-effects i.e. due to protonophore effects are seen.

Reviewer: Teun de Vries

Comment: I critically assessed the answers of the reviewers and I think they have answered them appropriately.

Answer: We thank the reviewer for the positive feedback
Major Compulsory Revisions:

Comment: In the revised manuscript, the authors concluded “acid secretion by osteoclast is specifically regulated by PKC in osteoclasts” (Abstract). Unfortunately, however, this conclusion is currently not supported by functional data and depends solely on the yet unclear specificity of the inhibitors. Mere detection of PKC alpha by Western blotting is apparently not sufficient. Therefore, the inhibitory effects of Rottlerin and GF109203X on acid influx and bone resorption, which are convincing and interesting, should not be generalized to PKC inhibitors in the absence of functional data supporting PKC involvement.

Answer: We thank the reviewer for this comment. We have changed the conclusion line in the abstract to “acid secretion by osteoclasts may be specifically regulated by PKC in osteoclasts”. In general we agree that the inhibitors have dubious specificities, which we have clearly stated in the discussion; however, the fact that most of the “speculated” PKC inhibitors work the same way, albeit with varying potencies in our opinion supports that the effect is mediated through PKC. We have still moderated the conclusion to be less “hard”. We do realize that si or shRNA would provide far more specific data; however this has yet to be performed consistently in the literature.

Discretionary Revisions:

Comment: The principle of acridine orange assays should briefly be described.

Answer: We have now included a sentence in the results section about the assay. We hope that it is satisfactory.

Reviewer: Jean-Pierre David

Comment: I do not think that the authors have answered to my main of comments.

Answer: We are sorry that the reviewer does not think we have answered his main comments in the revision of the manuscript, and we have now done our best to further explain our answers, especially relating to the IC50 value of the compounds in various tests (see below).

Comment: The choice of 50 microM is as artificial than 10 microM dose, each compound has its own IC50 that should be used as reference.

Answer: We have now included the commercially available IC50 values in table 1, and as can be seen most of the compounds were tested at concentrations, which by far exceeded their expected IC50 values, and thus the negative results should be valid at least as far as the inhibitor specificity can be trusted (discussed in the paper). However, we would like to emphasize that an IC50 value is highly context dependent, and as we have previously published IC50 values for given compounds vary greatly between assays, such as the cell-based acridine orange, acid influx and bone resorption, and thus any comparison to an in vitro
enzymatic IC50 value should be done with caution. We have now included this in the limitations section of the paper.

Comment: There is still no evidence that the inhibitors working or inefficient have been inhibiting any of their potential targeted kinases and I do believe that it is a major drawback.

Answer: As we also comment in the manuscript this is true; however, since we now have included the expected IC50 values, and since the concentrations we have used are either higher or in the right range we feel that these data can be trusted as far as the compounds can be trusted. We realize that these data should be strengthened by specific knock-downs of a given kinase, such as PKC; however, since this at present is virtually impossible to do in human osteoclasts, as no reliable transfection techniques are available this will be a future study. Furthermore, we have openly discussed the limitations of the inhibitors in article, and we have not made any final conclusions, only indications, based on our findings.