Author's response to reviews

Title: A novel naproxen derivative capable of displaying anti-cancer and anti-migratory properties against human breast cancer cells

Authors:

Jolly Deb (bcjd@iacs.res.in)
Joydeb Majumder (ocjm@iacs.res.in)
Siddhartha Sankar Jana (bcssj@iacs.res.in)

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Author's response to reviews: see over
Reviewer: Anthony Lucci

Minor Essential Revisions:

1. Grammatical issues
   In the abstract: Increasingly needs a comma after it. Introduction: change the wording “a lot”, human needs to be plural, (last sentence) in murine should be in a murine, excessive use of commas throughout manuscript.

#Ans: As per learned reviewers suggestion we have scrutinized the manuscript and revised the manuscript thoroughly to address the grammatical issues.

Major Compulsory Revisions:

1. It is impossible to calculate significance for some of the experiments because the experiments (MTT, Apo-TRACE, flow cytometry, and migration) were only performed two times. These experiments should be repeated one more time so that p values and therefore, statistical significance, can be calculated. Even though statistical analyses could not be performed, within the results section the authors report IC50 values and “better killing property” for derivatives 1, 2, and 4 compared to the parent compound.
   Similarly, the figure legends the authors use terms such as “enhanced killing effect”, “no significant cell cycle arrest”. These statements cannot be adequately supported without calculating p values.

#Ans: We agree with the suggestion of learned reviewer. We have performed t-test and mentioned the p values in figures.

Reviewer: Daotai Nie

Major Compulsory Revisions:

1. The title and conclusions are not fully supported by the data presented. In vivo data are needed to claim anti-metastatic properties.

#Ans: We agree with learned reviewers view and in our current version of the manuscript, we have changed the title from “A novel naproxen derivative capable of displaying anti-cancer and anti-metastatic properties against human breast cancer cells” to “A novel naproxen derivative capable of displaying anti-cancer and *anti-migratory* properties against human breast cancer cells.”

2. PGE2 inhibition by the compound 4 was presented at a single concentration (6 mM). They need to test multiple concentrations to determine IC50 in inhibiting PGE2 synthesis.

#Ans: It was not intended to assess the inhibitory concentration of compound 4 against PGE2 synthesis. We wanted to study the effect of Naproxin and its derivatives on cell
survival/proliferation of MDA-MB-231. We used 6 mM dose of compound 4 on PGE$_2$ synthesis status of MDA-MB-231 as this dose proved to be the IC$_{50}$ dose for these cells.

3. Wound healing assay to monitor tumor cell migration has limitations especially after overnight treatment. The reduced wound healing may be due to inhibition of cell proliferation and/or induction of apoptosis. Other assays such as Boyden chamber assay (transwell assay) can be used to assess cell migration by the treatment especially at shorter durations.

#Ans: Wound healing assays are widely used by the researchers to study migration, where as transwell assays are useful to study invasion of cancer cells (Lambertini et al., 2012; Manu et al., 2011). In the present manuscript, our aim was to monitor the effect of 4 on migratory property of MDA-MB-231 as migration is the first step towards invasion and metastasis of cancer cells (Timoshenko et al., 2003). Along with capturing images at designated times (0 and 22 hours), we have also monitored the wound closure using time lapse videography for up to 24hr. The figures (photomicrographs) presented in the manuscript includes both short duration (8h) and longer duration (16h and 22h).

Minor essential revisions:
4. The concentrations for the compound 4 to induce apoptosis, inhibit wound closure, and inhibit PGE$_2$ synthesis varies. Are the observed effects related to the inhibition of COX-2?

#Ans: The IC$_{50}$ value of compound 4 for MDA-MB-231 was found to be at around 6 mM. As mentioned before, we wanted to assess the effect of the similar dose on PGE$_2$ synthesis status of MDA-MB-231. For the same reason, we also carried out apoptosis assay at the same concentration, which gave us a clear indication of cellular death due to apoptosis rather than necrosis. For wound healing assay, we opted for a much lower concentration (1 mM) since exposing the cells to its IC$_{50}$ dose for a longer duration would not only kill 50% of the cells but also interfere with the assay. Naproxin is a known Cox inhibitor and PGE$_2$ synthesis is closely related to cellular Cox status. Currently we are evaluating the effect of compound 4 on specific Cox enzymes which is included in a manuscript in preparation. This points are addressed in result and discussion section now for more clarity.

5. Statistical analysis needs to be done in some figures such as Figure 4.

#Ans: As per the suggestion of learned reviewer we have performed t-test to assess the statistical significance of the experimental data and revised and modified the figures and legends accordingly.

Discretionary revisions:
6. Does compound 4 have off-target effects? Does it induce apoptosis in breast cancer cells with COX-2 expression knocked down? The off-target effects can be addressed in the discussion of the limitations of the compound.
As per the suggestion of the learned reviewer following two paragraphs are included in the result/discussion and conclusion section.

“A hallmark of breast cancer cells is up-regulated COX-2, which in turn result in an increase in PGE$_2$ synthesis (Timoshenko et al., 2003). In our study, we have documented a significant reduction in PGE2 synthesis after treatment with 4. The observed phenomenon could be due to inhibition of COX-2, as the parent compound Naproxen is a well known COX inhibitor.”

“However, it is also important to note the difference in the IC50 vales of 4 against p53 wild type MCF-7 (~3 mM) and p53 null MDA-MB-231 (~6 mM) cells. The highly skewed killing of MCF-7 cells by this compound might indicate the involvement of p53 mediated apoptotic pathway. One of the major off target effects of 4 may include p53 targets such as bax. Further experimentation is needed to elucidate these potential off target effects of this novel anti-cancer compound. Potential limitation of this drug could be the fact that it may include p53 as one of its targets, thus the dose needed to induce significant killing may be relatively elevated for p53 muted cancer cells compared to p53 wild type cancer cells.”

References:

