Reviewer’s report

Title: Benzofuroxan derivatives N-Br and N-I induce intrinsic apoptosis in melanoma cells by regulating AKT/BIM signaling and display anti metastatic activity in vivo.

Version: 3
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Reviewer: Ravi P Sahu

Reviewer’s report:

The manuscript entitled "Benzofuroxan derivatives N-Br and N-I induce intrinsic apoptosis in melanoma cells by regulating AKT/BIM signaling and display anti metastatic activity in vivo" by Farias et al is an interesting and nicely written manuscript. Most of the data presented are convincing. I have several comments to improve this manuscript.

Major Compulsory Revisions:
1. Authors should show the B16F10-Nex2 tumor volume data with N-Br/N-I compounds in C57BL/6 mice. This data will complement the lung metastasis data with these compounds.
2. Since N-Br/N-I derivative compounds mediated effects (increased cytotoxicity/apoptosis etc) on B16F10-Nex2 cells are due to the generation of ROS, vitamin C (quencher of ROS) should be used to confirm the effects of derivative compounds in in vitro experiments instead of NAC which works by replenishing depleted glutathione levels in the cells. Since, NAC supplementation prevented N-Br/N-I induced decreased viability of B16F10-Nex 2cells (Figure 4), these data also indicate the possible role of glutathione in this effect.
3. Were cells pretreated, simultaneously treated or post-treated with NAC to observe NAC effects on derivative compounds mediated effect (cell viability) on B16F10-Nex2 melanoma cells? Please state this in the method section for the clarity.
4. What is the rationale of using IC100 dose of N-Br/N-I compounds for apoptosis assay (Figure 3A)? Usually at higher doses agents with potent cytotoxic activity kill cancer cells and thus are not considered optimal doses. IC25 and IC50 doses should be considered to test the efficacy of anti-cancer drugs.

Minor Essential Revisions:
1. Table 2, the IC50 concentration of different benzofuroxan compounds ranges between 6.9µM to 25.4µM, instead 9.7µM to 25.4µM. Please change this.
2. At various places “Nac” is used instead of “NAC”. Please fix this.
3. Please state the level of significance for figure 2C and figures 4.

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.

**Declaration of competing interests:**

I declare that I have no competing interests.