Reviewer's report

Title: Kinesin Eg5 inhibition by 3,4-dihydropyrimidin-2(1H)-one (or thione) derivatives impairs multiple tumorigenic properties inducing breast cancer cells to apoptosis

Version: 4
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Reviewer: Marzia Pennati

Reviewer's report:

The manuscript is aimed at evaluating the effects of newly synthesized 3,4-dihydropyrimidin-2(1H)-one (or thione) derivatives (DHPMs) in breast cancer (BC) cells. A dose- and time-dependent inhibition of cell growth was observed in two BC cell lines after treatment with the different compounds. In addition, at concentrations up to 1 mM, DHPMs did not alter the growth of normal fibroblasts. The authors reported that DHPMs inhibited kinesin Eg5 activity causing a mitotic catastrophe, induced apoptosis, caused cell cycle perturbation and inhibited angiogenesis both in vitro and in vivo. Moreover, DHPMs modulated the CD44+/CD24- phenotype leading to a decrease in the CSC population in BC cells.

The manuscript is clearly written and, even if the question posed by the authors is scientifically sound and the study is, at least in principle, characterized by a certain level of originality, some data presented are not consistent and sufficient to support the authors' conclusions. From my standpoint, the manuscript cannot be therefore accepted in the present form. It is however my feeling that, when presented in an appropriate manner, findings reported could be of potential interest.

Here follow my major concerns, which need to be fully addressed to make the manuscript eligible for publication.

General comments

- The main weakness of the paper is the selection of the doses used in the experiments reported in Figures 5 and 6 that are considerably higher than IC50 values (1.0 mM for 4m, 0.8 mM for 4bt, 0.4 mM for 4p, 1.0 mM for 4bc, and 0.8 mM for 4x). How were selected the doses used for these experiments?

To draw their conclusions, the authors have to perform the analysis of the effects of DHPMs on cell cycle perturbation and induction of apoptosis at increasing EQUITOXIC concentrations (i.e. IC20, IC50 and IC80) and/or at different time-exposure.

- The authors stated that “Minor doses and shorter treatment periods were sufficient to induce a high number of MDA-MB-231 cells to apoptosis (Fig 1A and 1C)” (see page 11, lines 7-9). However, they treated cells only for 72h with a single dose of DHPMs, and data reported in Figure 1 REFERRED to the cytotoxic effect of DHPMs but not to the induction of apoptosis. The authors
should clarify this point.

Minor points

• Add standard deviation in Table 1.
• Table S1 should be moved to the main text from supporting information.

**Level of interest:** An article of importance in its field

**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