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COMMENT

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Reply to the Comment on "Design, synthesis, anticancer activity and molecular docking of quinoline-based dihydrazone derivatives" by R. Weiskirchen, *RSC Adv.*, 2025, 15, https://doi.org/10.1039/D5RA00388A

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In this reply, Lu *et al.* address the cell line contamination concerns raised in the comment on their original paper (*RSC Adv.*, 2025, **15**, 231–243). To ensure the reliability of their conclusions, the key experiments were repeated using authenticated cell lines (Huh-7 and HUVEC), confirming the reproducibility and validity of the reported results.

Our reply for comment

We sincerely appreciate the thoughtful feedback on our manuscript (**https://doi.org/10.1039/D4RA06954D**). In their comment, the author raised concerns about potential contamination in the cited cell lines (BGC-823, BEL-7402 and HL-7702) used in our manuscript. After receiving this comment, we paid more attention to the questions raised by the author and acquired new cell models (Huh-7 and HUVEC) to conduct relevant experiments again to ensure the rigor and accuracy of the research conclusions. The details are as follows:

In our study, four tumor cell lines—MCF-7, A549, BGC-823, and BEL-7402—were used to evaluate the antitumor activity of quinolinopyrimidine bishydrazone derivatives. While concerns regarding potential HeLa contamination in certain cell lines are acknowledged, it should be emphasized that all mechanistic investigations were conducted exclusively with MCF-7 cells, which have been verified to be free of HeLa contamination.[1–3] Therefore, any potential contamination in BGC-823 and BEL-7402 does not impact the mechanistic conclusions or molecular docking analyses presented in our study. To further substantiate the broad-spectrum antitumor potential of these compounds, additional MTT assays were performed using human hepatocellular carcinoma cells (Huh-7 and HUVEC). The results (Table 1) demonstrated significant inhibitory effects, consistent with observations in other tumor cell lines, thereby reinforcing our findings.

Regarding biosafety assessment, our toxicity evaluation extended beyond cellular-level assays to include comprehensive *in vivo* studies in murine models, incorporating blood biochemical parameter analysis and histopathological examination (H&E staining), all of which confirmed the favorable biosafety profile of the tested compounds. To ensure experimental rigor, additional toxicity testing was conducted using a new batch of human umbilical vein endothelial cells (HUVECs), with the new data (Table 1)[4] corroborating our original conclusion of minimal toxicity toward normal human cells.

We fully recognize the critical importance of using authenticated cell lines in biomedical research. In future work, we will strengthen our experimental management protocols by implementing routine cell line identity verification through STR profiling and by regularly consulting updated databases such as Cellosaurus to prevent potential cross-contamination issues.[5]

Conflicts of interest

All authors declare that they have no relevant conflict of interest.

Table 1 The IC_{50} (μM) values of **3a–d** against the selected cells for 48 h

Compound	Huh-7	HUVEC
3a	11.49 ± 0.35	51.73 ± 2.34
3b	16.79 ± 0.21	33.59 ± 3.43
3c	12.79 ± 0.27	>100
3d	15.49 ± 0.42	>100

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Data availability

Data will be available on request.

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Notes and references

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