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Integrated network pharmacology, molecular docking and biological validation revealed the inhibitory effect of a benzoxazinone derivative ZAK-I-57 in hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide[1]. Although targeted therapies, such as sorafenib, have demonstrated improved survival benefits, they are modest[2]. Therefore, there is an urgent need to discover novel therapeutic agents for the treatment of HCC. Amidst this landscape, the development of novel therapeutic agent is crucial.

Benzoxazinone derivatives were recognized for their broad pharmacological properties, which have garnered significant attention as potential candidates for targeted cancer therapy.

Therefore, this study investigated the therapeutic potential of benzoxazinone derivative ZAK-I-57, which have emerged as a promising candidate owing to its' impressive efficacy and safety profiles.

Materials and Methods

- Benzoxazinone-derived compound ZAK-I-57 was designed and synthesized through a *De novo* method;
- The HCC-related target interactions and mechanistic pathways were predicted using network pharmacology analysis;
- The cytotoxicity of ZAK-I-57 was assessed by MTT assay;
- The interaction of ZAK-I-57 and HCC-related targets were validated by molecular docking and western blotting methods;
- PLC/PRF/5 tumor-bearing mouse (**Fig. 1a**) and patient-derived xenografted models (**Fig. 1b**) were employed to evaluate the anti-tumor effects of ZAK-I-57 in HCC;

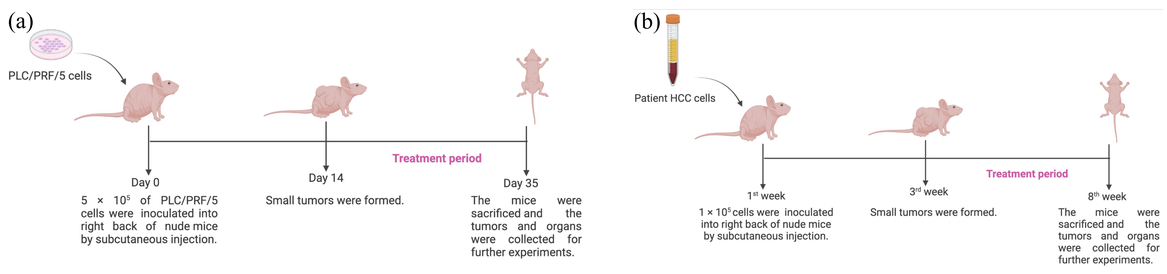

Fig. 1 (a) Schematic diagram of PLC/PRF/5 tumor-bearing mouse model; (b) Schematic diagram of patient-derived xenografted (PDTX#1) mouse model.

Results

◆ Network pharmacology analysis of benzoxazinone-derived compound ZAK-I-57 on HCC

Fifty candidate targets were in common with those predicted targets from ZAK-I-57 and HCC (**Fig. 2c**). In PPI network comprised of these targets, EGFR and MYC exhibited the highest degrees, suggesting these targets play major roles on anti-cancer activity of ZAK-I-57 in HCC (**Fig. 2d**). ZAK-I-57 exhibited strong binding affinity with EGFR and MYC proteins (**Fig. 2e&f**).

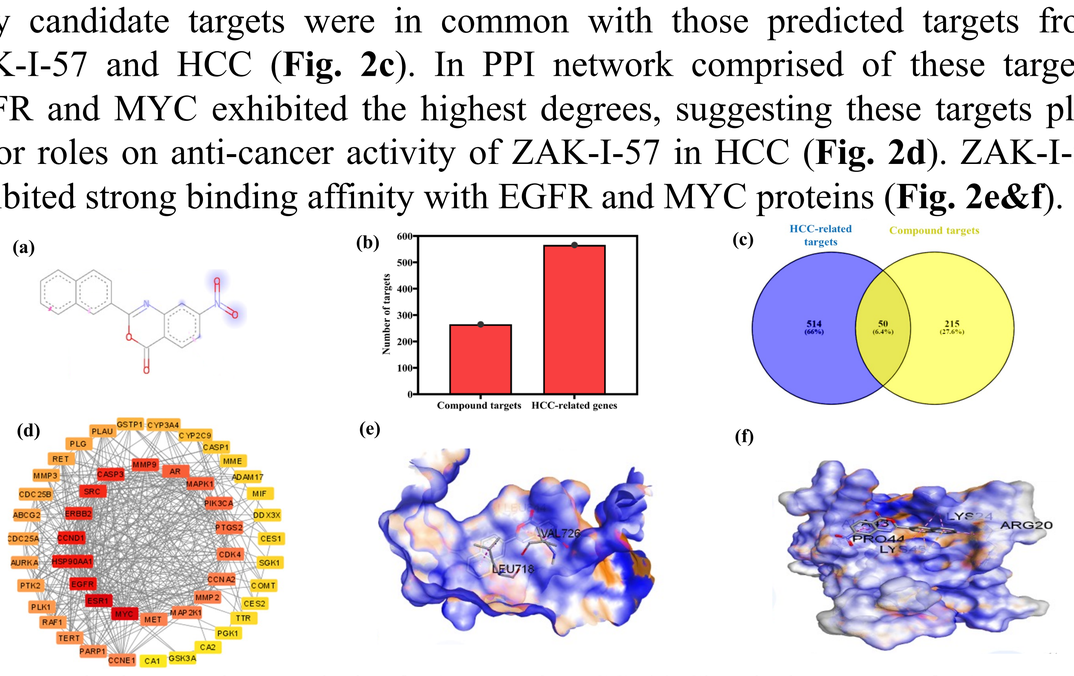

Fig. 2 Network pharmacology analysis of ZAK-I-57 in HCC. (a) Chemical structure of ZAK-I-57; (b) The target numbers of ZAK-I-57 and HCC selected from open public databases; (c) Venn plot of the targets of ZAK-I-57 and HCC; (d) Protein-protein interaction (PPI) networks; (e) Visual plot of ZAK-I-57 targeting to EGFR protein; (f) Visual plot of ZAK-I-57 binding to MYC protein.

◆ Cytotoxic activity of ZAK-I-57 on HCC cells by MTT assay

ZAK-I-57 exhibited the most sensitive cytotoxicity towards Huh7 (**Fig. 3a**) and PLC/PRF/5 cells (**Fig. 3b**).

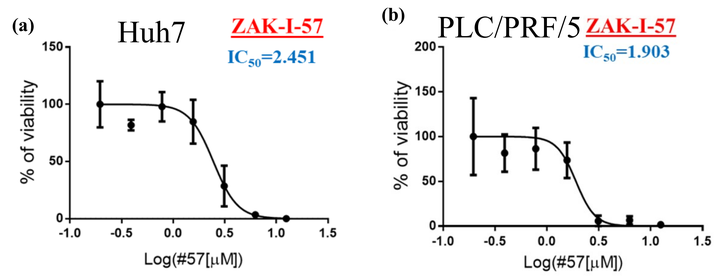

Fig. 3 Effect of ZAK-I-57 on cytotoxicity in HCC cells. (a) Huh7 cells; (b) PLC/PRF/5 cells (n = 3).

◆ Anti-tumor effect of ZAK-I-57 in PLC/PRF/5 tumor-bearing mice

Oral administration of ZAK-I-57 at 30mg/kg could significantly inhibit tumor growth in PLC/PRF/5 tumor-bearing mice (**Fig. 4a&b**).

The proliferation (Ki-67 expression, **Fig. 4c&d**) of tumor cells in tumors were suppressed after ZAK-I-57 (30 mg/kg) treatment.

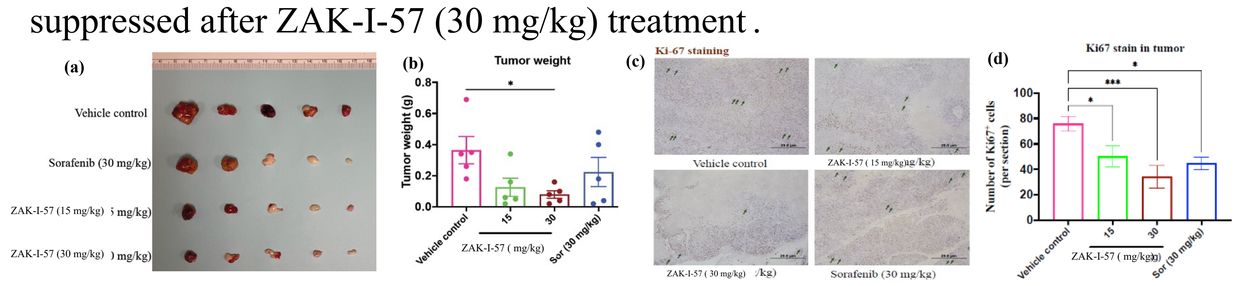

Fig. 4 Effect of ZAK-I-57 on tumor growth in PLC/PRF/5 tumor-bearing mice. (a) Representative tumor pictures; (b) The changes of tumor weight; (c) Representative ki-67 staining in tumors; (d) Ki-67 expression in tumors. $^{*}p < 0.05$, $^{***}p < 0.001$ compared to the control group (n = 5 in each group).

◆ Effect of ZAK-I-57 on tumor proteins expression in PLC/PRF/5 tumor-bearing mice

As shown in **Fig. 5**, the expression of EGFR, c-Myc and Bcl-2 were decrease, while the level of Bax was increased after ZAK-I-57 treatment in tumor samples of PLC/PRF/5 tumor-bearing mice.

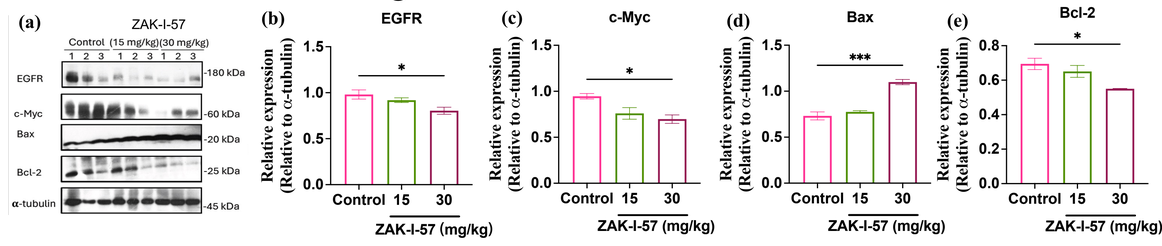

Fig. 5 Effect of ZAK-I-57 on proteins expression in PLC/PRF/5 tumor-bearing mice. (a) Representative blots of EGFR, c-Myc, Bax, Bcl-2proteins; (b) - (e) Histograms showed the quantitative results of target proteins expression. $^{*}p < 0.05$, $^{***}p < 0.001$ compared to the control group (n = 5).

◆ Anti-tumor effect of ZAK-I-57 in patient-derived xenografted mice

ZAK-I-57 at 30mg/kg could significantly inhibit tumor growth in patient-derived xenografted tumor-bearing mice (**Fig. 6a&b**).

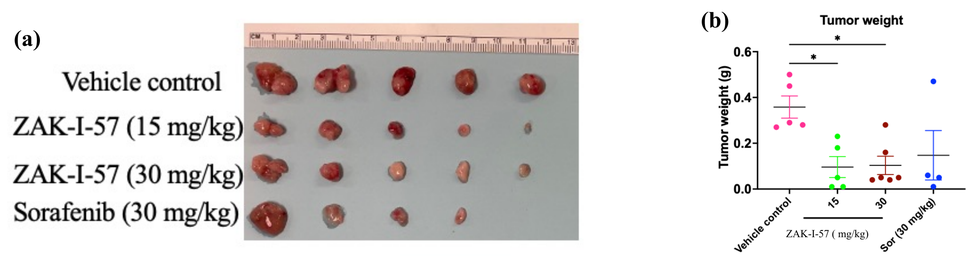

Fig. 6 Effect of ZAK-I-57 on tumor growth in patient-derived xenografted tumor-bearing mice. (a) Representative tumor pictures; (b) The changes of tumor weight. $^{*}p < 0.05$ compared to the control group (n = 5 in each group).

Conclusion

ZAK-I-57 presents a promising and innovative therapeutic option for HCC.

References

1.Josep M., *et al. Nat. Rev. Clin. Oncol*, 2024. 21: 294-311.

2.Shikun Jiang, *et al. Life Sci.*, 2020.258:118252.

Acknowledgement

Benzoxazinone-derived compound ZAK-I-57 has been applied for US-provisional patent (US63/657,193).