Discovery of a mammalian FASN inhibitor against xenografts of non-small cell lung cancer and melanoma

Dear Editor,

Cancer cells need to reprogram fatty acid (FA) metabolism to promote cell growth and survival through exogenous lipid uptake and FA biosynthesis catalyzed by the multidomain containing mammalian FA synthase (FASN).\textsuperscript{1,2} The bidirectional relationships of oncogenic signaling and nonlipogenesis (DNL) suggest that FASN is a druggable target in many cancers. Although FASN inhibitors including Fasnall, GSK2194069, IPI-9119, orlistat, TVB-2640, TVB-3166, and TVB-3664 have shown promise in preclinical cancer models or early-phase clinical trials, none have been approved for the treatment of cancers (Supplementary Table S1).\textsuperscript{3}

Here we report a unique inhibitor targeting the ketosynthase (KS) domain of FASN, which shows superior cytotoxicity and selectivity over orlistat and TVB-3166, as well as strong antitumor effects in both non-small-cell lung cancer (NSCLC) and melanoma mouse xenografts.

Platensimycin was originally discovered as a bacterial FabF/B inhibitor by competition with their substrate malonyl-acyl carrier protein (ACP), while later it showed potent inhibitory activity (IC_{50} = 0.3 \mu M) against mammalian FASN.\textsuperscript{4} To discover selective FASN inhibitors against NSCLC, we screened a focused library of platensimycin derivatives against KRAS-positive A549 and NCI-H1299 cells (Fig. 1a and Supplementary Figs. S1–S6). Several 6-aryl platensimycin derivatives showed inhibitory activity from 54.3% to 91.2% against both cell lines (Supplementary Fig. S7), among which 6p exhibited an IC_{50} of 16.95 ± 1.96 \mu M, superior to TVB-3166, orlistat, and cerulenin (Fig. 1b and Supplementary Table S2). The selectivity of 6p may protect the less fatty acid-dependent normal cells upon its exposure. The 6p-treated cells showed notable morphological changes, reduced colony number and sizes either alone or in combination with cis-platinum (CDDP) (Fig. 1c and Supplementary Fig. S8a–b), as well as G2/M cell cycle arrest and apoptosis (Supplementary Fig. S8c–e). Furthermore, 6p appreciably inhibited cell migration and invasion (Fig. 1d and Supplementary Fig. S9). In contrast, 6-(4-bromophenyl)-platensic acid and platensimycin showed no cytotoxicity, suggesting that 3-amino-2,4-dihydroxybenzoic acid (ADHBA) and 6-(4-bromophenyl) group are critical for the cytotoxicity of 6p (Supplementary Fig. S10).

To study if 6p interacts with FASN, it was docked to the KS-malonyltransferase (MAT) domain of FASN, showing hydrogen bonding interactions of ADHBA with H299 and H331 of KS active site (Fig. 1e and Supplementary Fig. S10c). The molecular dynamic simulation revealed that 6p likely bound to KS-MAT more tightly than platensimycin, with 26.76 kcal/mol less non-bond energy (Fig. 1f and Supplementary Fig. S10). The IC_{50} values of 6p were reduced by 9- or 4-fold in A549 and NCI-H1299 cells cultured with 1% fetal bovine serum to decrease free FAs uptake, confirming the target specificity of 6p against FASN (Fig. 1g and Supplementary Fig. S11b). Surprisingly, the cell growth inhibition could only be partially rescued by the addition of oleate and palmitate, while exogenous palmitate could rescue cell inhibition by orlistat (Fig. 1h and Supplementary Fig. S11).

FASN is highly expressed in A549 and NCI-H1299 cells, and 6p markedly decreased its expression in a dose-dependent manner (Figs. 1i, j and Supplementary Fig. S12). Orlistat treatment only modestly affected FASN expression, while platensimycin had no obvious effects (Supplementary Fig. S13). Immunofluorescence confirmed that 6p crucially decreased the expression of FASN in the cytoplasm of treated NCI-H1299 cells (Fig. 1k). In contrast, Fasnall or TVB-3166/IPI-9119 treatment led to either no change of FASN expression or significantly induced FASN expression, suggesting that 6p may bypass the FASN-overexpression compensatory mechanism.\textsuperscript{5} RNA sequencing of 6p-treated NCI-H1299 cells showed that 6p significantly downregulated the expression of key genes involved in lipogenesis, cell cycle, DNA replication, mitosis, and steroid biosynthesis, and upregulated genes in apoptosis, ferroptosis, and amino acid metabolism pathways, in comparison with the control group (Fig. 1l and Supplementary Figs. S14–S15). Subsequent RT-qPCR confirmed that 6p treatment resulted in pronounced downregulation of FASN, sterol regulatory element-binding transcription factor 1 (SREBF1), stearoyl-CoA desaturase-1 (SCD1), and acetyl-CoA carboxylase (ACC) in both A549 and NCI-H1299 cells (Fig. 1m).

Immunoblotting experiments showed that 6p dose-dependently decreased the expression of pSREBP-1c and mSREBP-1c in A549 and NCI-H1299 cells (Fig. 1n). In contrast, previous reports suggest that TVB-3166-treated tumor cells increased the expression of most of these genes.\textsuperscript{3} Since SREBP-1, SCD1, and ACC are all key nodes in DNL, their global down-regulation by 6p highlights its unique antiproliferative potential.

Although the oncogenic signal transduction pathways including PI3K-AKT-mTOR regulate fatty acid biosynthesis, and oncogene KRAS activates FASN, 6p treatment resulted in marked reduction of p-AKT and p-mTOR in the treated A549 and NCI-H1299 cells, since FA metabolism also regulates oncogenic signaling (Fig. 1o and Supplementary Fig. S16).\textsuperscript{5} The 6p- or TVB-3166-treated NCI-H1299 cells had distinct lipid profiles in the HR-EISI-MS/MS analysis, exemplified by their different lipid signature in the top ten lipid metabolites, while their overall lipid composition and distribution was similar (Supplementary Figs. S17–S19 and Supplementary Table S4). The decreased total abundance of saturated and monounsaturated fatty acids include C16:0 and C18:1 in various glycerolipids and glycerophospholipids, especially triglycerides as the main forms for fatty acid storage and transport, consistent with the inhibition of FASN by 6p (Fig. 1p, Supplementary Figs. S20–S22 and Supplementary Tables S5–S7). The significant accumulation of stearic acid (C18:0) may be caused by the down-regulation of SCD1 (Fig. 1m).

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We further explored the antitumor effect of 6p using the A549 xenograft mouse model. Subcutaneous injection of 6p or in combination with CDDP resulted in slower tumor growth and lower tumor weight than the control group (Fig. 1q, r and Supplementary Fig. S23). HE and Ki67 staining demonstrated that 6p effectively inhibited tumor growth with no obvious toxicity (Supplementary Fig. S23). Immuno-staining and immuno-blotting assay showed that 6p also decreased FASN and p-AKT...
levels in the treated tumors (Fig. 1s and Supplementary Fig. S23e). Although FASN is also highly expressed in melanoma, few FASN inhibitors except orlistat have been evaluated in a mouse melanoma model. Since 6p significantly inhibited clone formation and decreased FASN expression in A375 cell lines (Supplementary Fig. S24), we next evaluated its antitumor effects in A375 xenografts. 6p effectively inhibited melanoma growth without obvious toxicity, compared to orlistat and platensimycin (Fig. 1t, u). Although intravenous administration of 6p and CDDP inhibited tumor growth synergistically, two-way ANOVA test (q), One-way ANOVA test (r) (n = 5). s Western blotting showed that 6p increased the expression of p-AKT and FASN in A549 tumor xenografts (n = 5). t, u The tumor volume (t) and tumor weight (u) were significantly reduced when A375 tumor xenografts were treated with 6p. Two-way ANOVA test (t), One-way ANOVA test (u) (n = 5). v The proposed mode of inhibition of FASN by 6p, which decreased the expression of key lipogenic genes (labelled in green), and downregulated the PI3K-AKT-mTOR oncogenic signaling; exogenous polysaturated and odd-numbered FAs were transported to the cells as a compensating mechanism to sustain cell survival. Data were shown as means ± SD, *p < 0.05; **p < 0.01; ***p < 0.001

**DATA AVAILABILITY**

The online version of this article contains supplementary material available at https://doi.org/10.1038/s41392-022-01099-4.

**Competing interests:** The authors declare no competing interests.

**Ethics:** All animal studies were approved by the Institutional Animal Care and Treatment Committee of Central South University.

**AUTHOR CONTRIBUTIONS**

D-F.C., Y-W.D., and Y.H. designed the research and wrote the paper. D-F.C. and M.S. performed cytotoxicity screening experiments; J.Y. and Y-C.D. provided the reagents; D-F.C., Y-W.D., and Y.H. designed the research and wrote the paper. D-F.C. and M.S. performed gene expression, cellular analyses, and animal experiments with the guidance of X-Q.F., Y.X., and E-H.B. All authors have read and approved the article.

**ADDITIONAL INFORMATION**

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