Discovery and Preclinical Evaluation of Novel Single-Molecule Dual PI3K-CDK4/6 Inhibitors for the Treatment of Breast Cancer

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ABSTRACT

Breast cancer remains a leading cause of cancer-related mortality worldwide. While the simultaneous inhibition of PI3Kα and CDK4/6 has recently been clinically validated for advanced breast cancer, combination regimens of distinct targeted agents are often limited by overlapping toxicities and complex pharmacokinetics. To address these clinical hurdles and overcome therapeutic resistance, we developed a novel series of single-molecule dual PI3K/CDK4-6 inhibitors by structure-based drug design strategy. Through extensive SAR studies, lead compounds 11 and 13 were identified, exhibiting nanomolar potency against PI3Kα and CDK4/6, high kinase selectivity, and potent antiproliferative activity in breast cancer cell lines. Compound 13 demonstrated robust in vivo efficacy in a T47D xenograft model, comparable to the Palbociclib–BKM120 combination with a good safety profile. This work highlights single-molecule dual PI3K/CDK4-6 inhibition as a highly promising therapeutic strategy for breast cancer.

Biochemical Characterization

Based on systematic SAR optimization, compounds 11 and 13 were selected for advanced profiling. Both leads displayed favorable target binding affinity (Figure 1): compound 11 exhibited Kd values of 0.31 nM for PI3Kα and 56.1 nM for CDK4, while compound 13 showed corresponding Kd values of 1.32 nM and 247 nM.

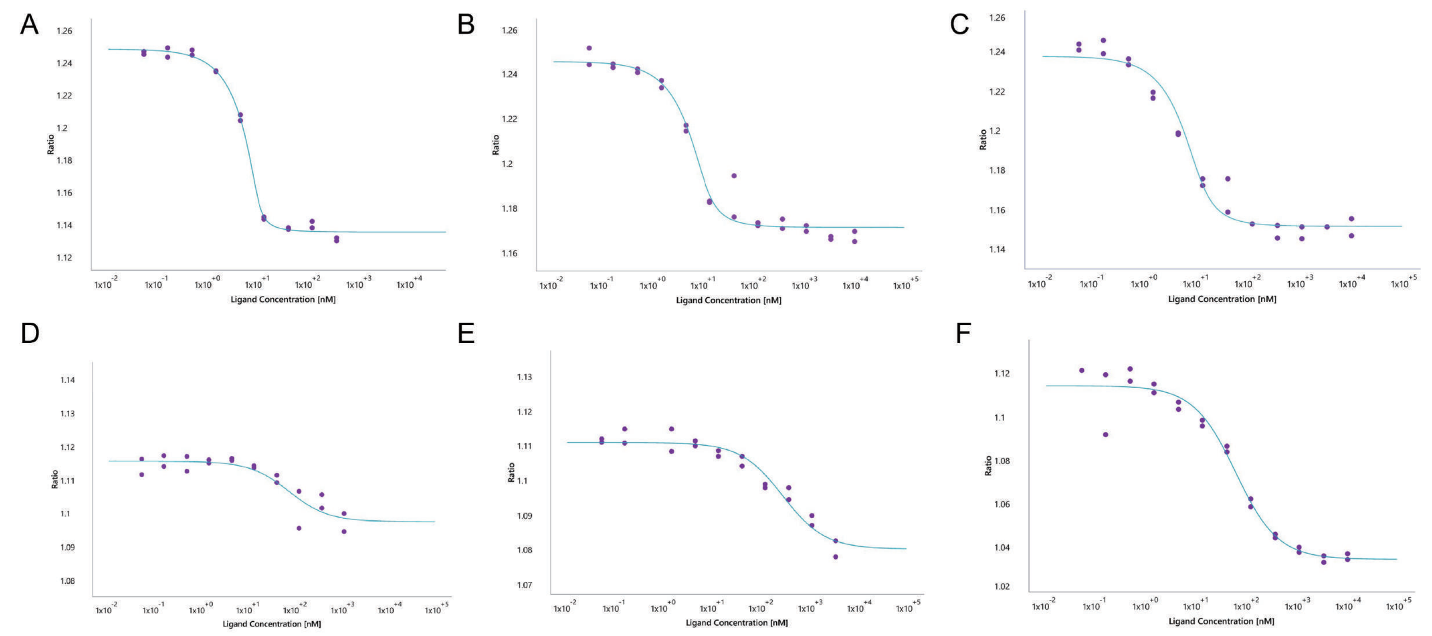

Figure 1. Binding affinity detemination of compounds to the target protein using Spectral Shift(SpS).Dose-response curves for the binding of (A) compound 11 to PI3Kα, (B) compound 13 to PI3Kα, (C) BKM120 to PI3Kα, (D) compound 11 to CDK4, (E) compound 13 to CDK4, and (F) Palbociclib to CDK4. The y-axis represents the response signal ratio, and the x-axis represents the ligand concentration (nM). The dissociation constants (Kd) were calculated by non-linear fitting of the data and are indicated in each panel.

Furthermore, both leads exhibited potent nanomolar inhibition across all four class I PI3K isoforms (α, β, γ, δ) and strong activity against CDK6. Crucially, 11 and 13 displayed remarkable selectivity for CDK4/6 over other CDK isoforms (Figures 2A, 2B) and maintained a highly favorable overall selectivity profile when evaluated against a diverse panel of 80 kinases (Figure 2C).

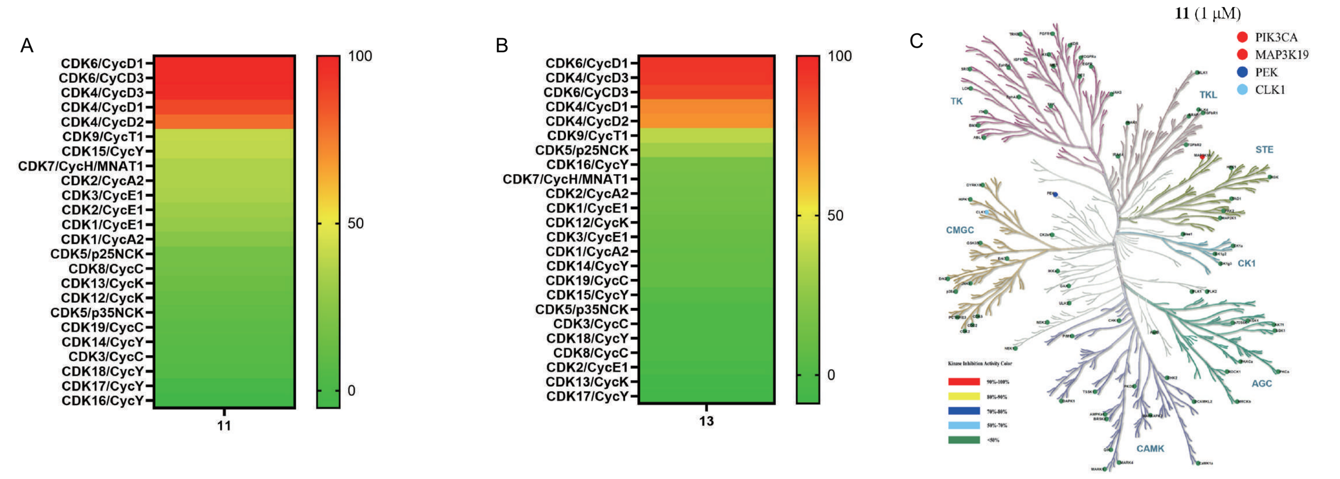

Figure 2. The selectivity profile of compound 11 and 13. (A)The selectivity profile of compounds 11 across CDK isoforms. (B)The selectivity profile of compounds 13 across CDK isoforms .(C)The selectivity profile of compound 11 against a panel of 80 kinases.

In Vitro Study

Compounds 11 and 13 demonstrated superior activity at 90nM compared to the combination of Palbociclib and BKM120 in ZR75-1 cells.

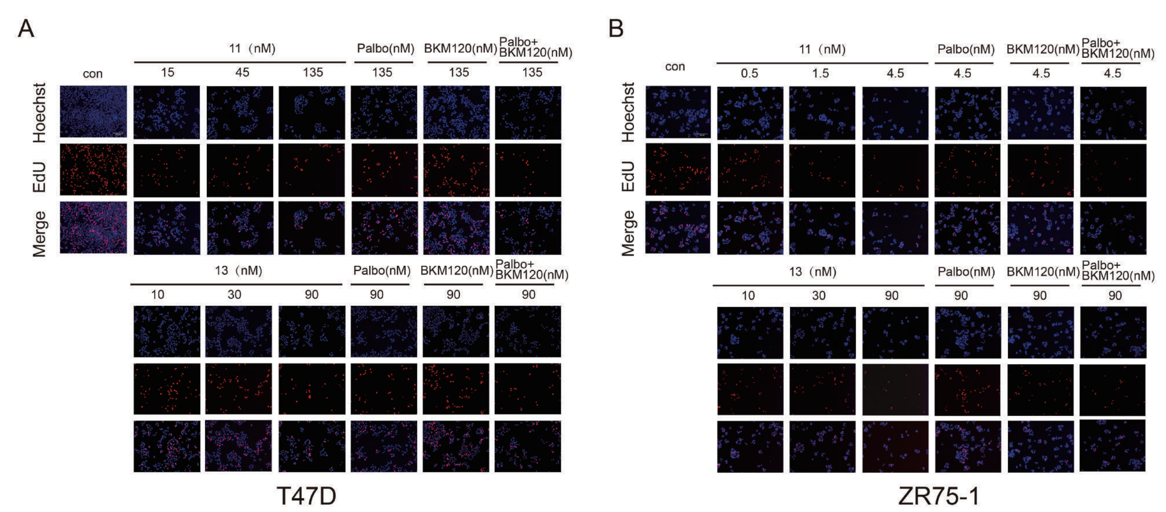

Figure 3. Effects of compounds 11 and 13 on cell proliferation assessed by EdU assay. (A) Representative EdU staining images of T47D cells following 72 h of treatment with the indicated concentrations of compounds 11 and 13. (B) Representative EdU staining images of ZR75-1 cells following 72 h of treatment with the indicated concentrations of compounds 11 and 13. *P < 0.05, ** P < 0.01, *** P < 0.001, ****P < 0.0001 compared to the control.

Compounds 11 and 13 displayed inhibitory effects comparable to the Palbociclib/BKM120 combination at high concentrations, as demonstrated by Western blot analysis of key downstream effectors in T47D and ZR75-1 breast cancer cells..

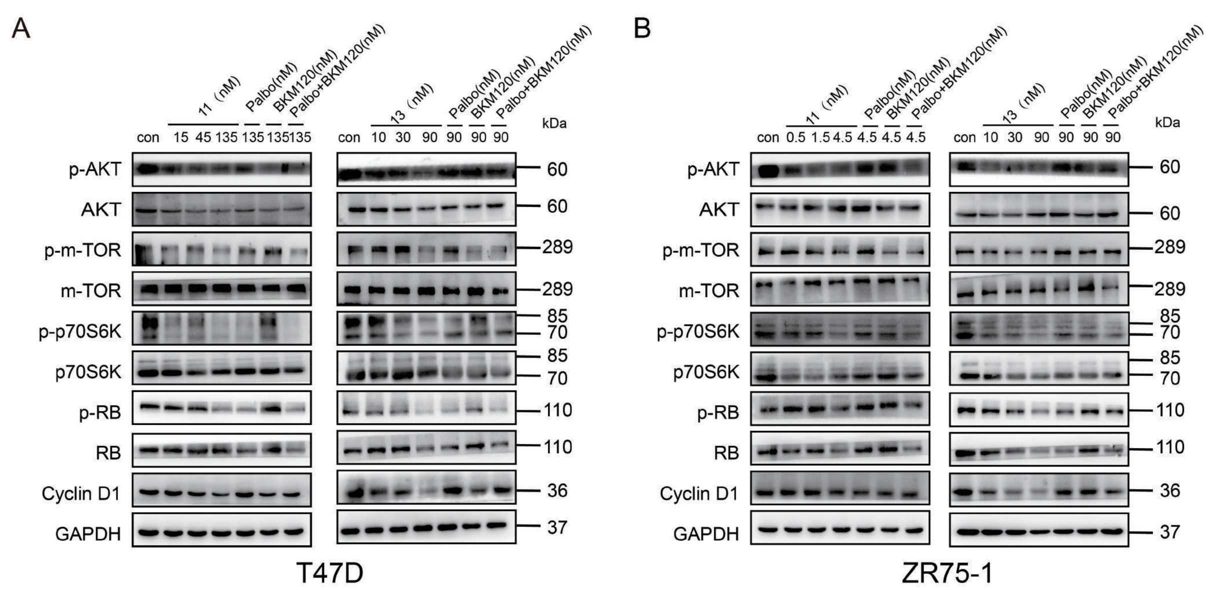

Figure 4. Western blot analysis of compounds 11 and 13 on phosphorylation of PI3K and CDK4/6 pathway effectors in T47D (A) and ZR75-1 (B) breast cancer cells.

At the concentration of 450nM, compounds 11 and 13 induced G0/G1 arrest to an extent comparable to the combination of Palbociclib (450nM) and BKM120 (450nM) in both T47D and ZR75-1 cell lines.

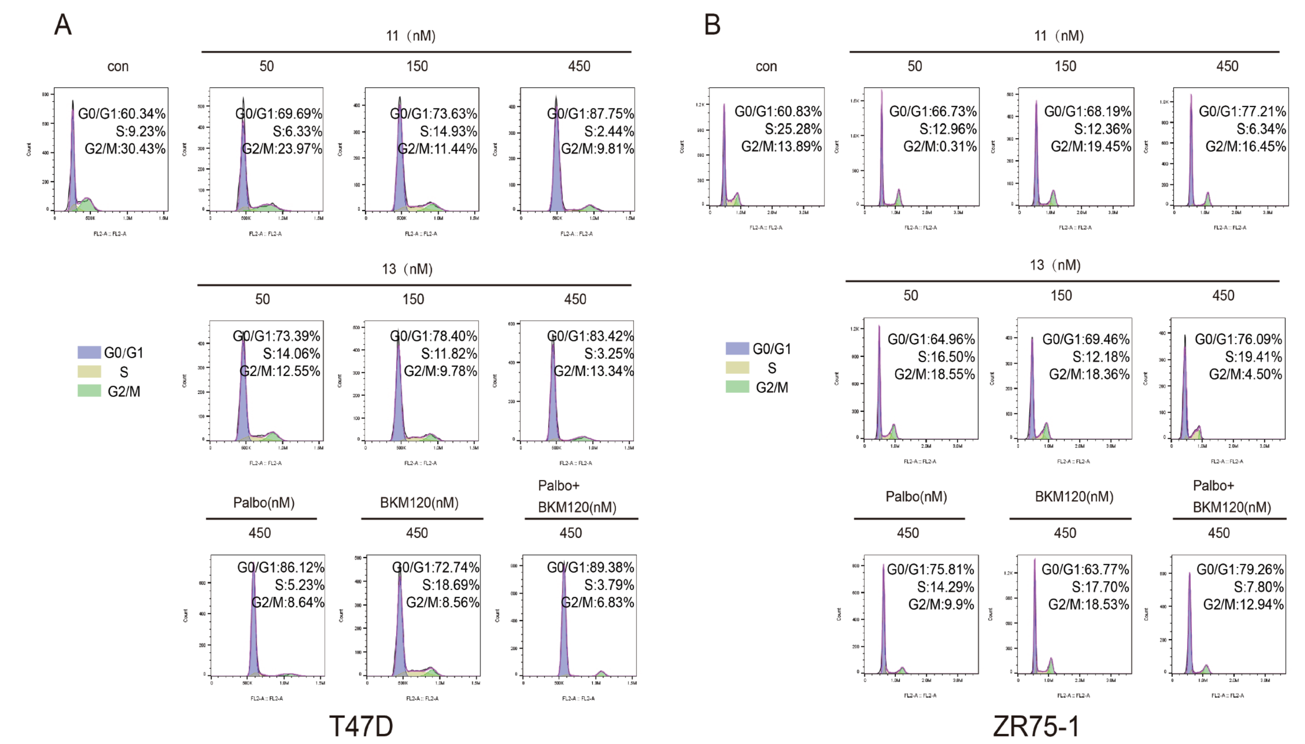

Figure 5. Effects of compounds 11 and 13 on cell cycle. (A) Flow cytometry analysis of the cell cycle in T47D cells. (B) Flow cytometry analysis of the cell cycle in ZR75-1 cells. *P < 0.05, ** P < 0.01, *** P < 0.001, ****P < 0.0001 compared to the control.

In Vivo Study

Compound 13 was evaluated in a T47D xenograft model. It showed potent antitumor activity (Figure 6B and 6C), with tumor growth inhibition (TGI) exceeding 70% at all tested doses, comparable to the positive control combination of Palbociclib (25 mg/kg) and BKM120 (25 mg/kg).

Furthermore, no obvious compound-related toxicity was observed in heart, liver, spleen, lung, and kidney tissues by H&E staining. IHC analysis revealed that compound 13 markedly reduced the phosphorylation of Akt and Rb, confirming effective dual inhibition of the PI3K and CDK4/6 signaling pathways.

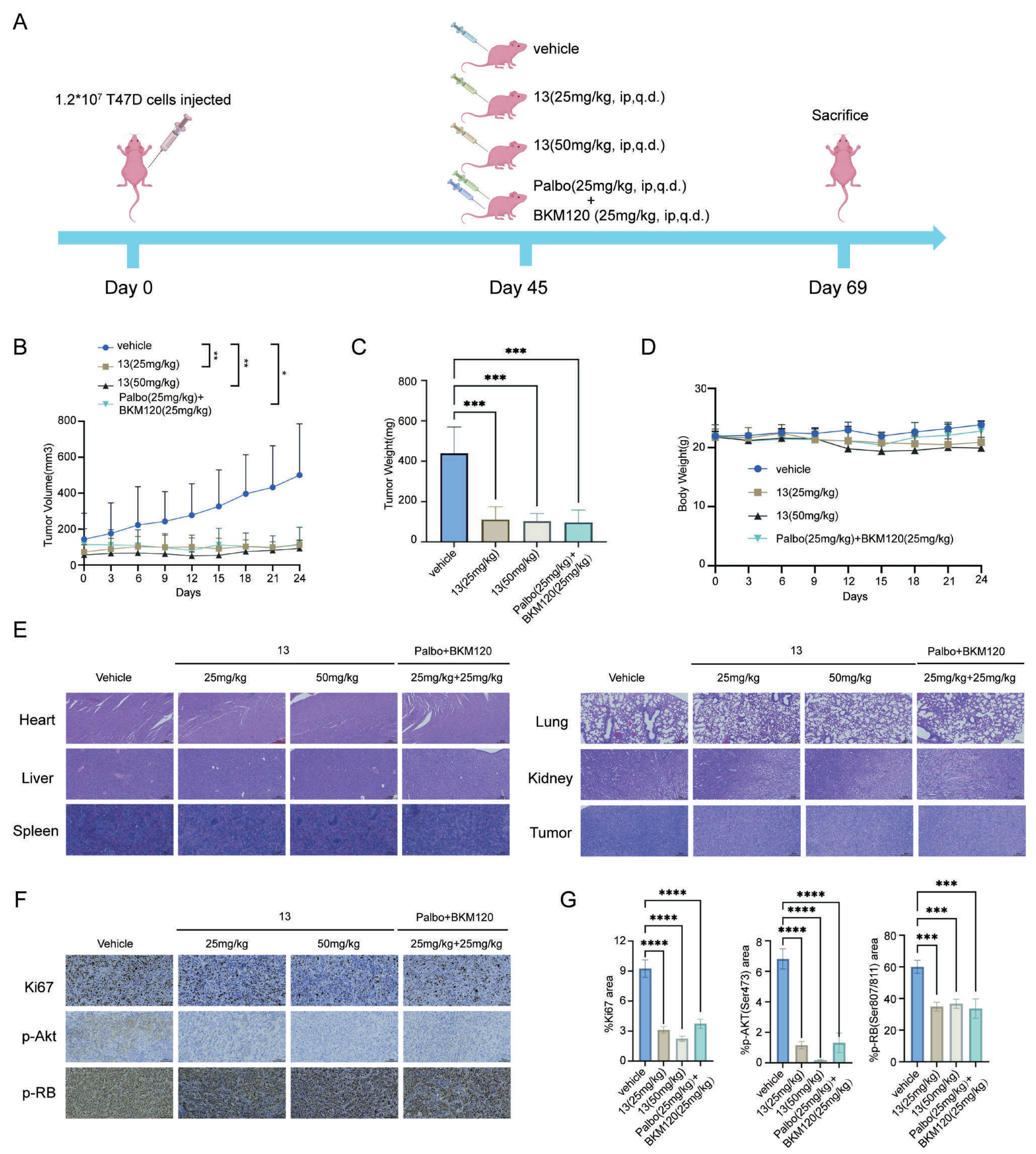

Figure6. In vivo anticancer efficacy of compound 13 in a T47D xenograft model. (A) Schematic diagram of constructing the xenograft tumor model in nude mice. (B) Body weight curves. (C) Tumor growth curves. (D) Tumor weights at the end of treatment. (E) Representative H&E stained images of heart, liver, spleen, lung and kidney at the end of treatment. (F) Representative immunohistochemistry images of Ki67, p-Akt, and p-RB in tumors at the end of treatment. (G) Quantitative analysis for F. *P < 0.05, ** P < 0.01, *** P < 0.001, **** P <0.0001 compared to the vehicle group.

Conclusion

Despite the clinical success of dual PI3Kα and CDK4/6 blockade in breast cancer, the co-administration of distinct agents is frequently hampered by cumulative adverse effects and pharmacokinetic complications. In this study, we utilized rational design to discover a novel class of single-molecule dual PI3K/CDK4-6 inhibitors. The optimized lead, compound 13, demonstrated potent dual-target engagement and excellent selectivity in vitro, which translated to robust anti-tumor activity in vivo. Crucially, monotherapy with compound 13 in a T47D xenograft model achieved tumor suppression comparable to the Palbociclib/BKM120 combination, while maintaining excellent tolerability. This confirms that integrating dual inhibitory activity into a single agent is a highly viable and translatable strategy for management of breast cancer.