

A PI3K Inhibitor with Low Cardiotoxicity and Its Synergistic Inhibitory Effect with Gilteritinib in Acute Myelogenous Leukemia (AML) Cells

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Table S1. Pharmacokinetic profile of FD274 in rats.^[1]

iv (2 mg/kg) ^a		po (10 mg/kg) ^b	
T _{1/2} (h)	2.29	T _{1/2} (h)	4.48
V _z (mL/kg)	7260	T _{max} (h)	2.80
CL (mL h ⁻¹ kg ⁻¹)	2220	C _{max} (ng/mL)	15.5
AUC _{0-t} (h ng/mL)	898	AUC _{0-t} (h ng/mL)	104
AUC _{0-∞} (h ng/mL)	903	AUC _{0-∞} (h ng/mL)	121
MRT _{INF} (h)	0.593	F (%)	2.68

^a iv formulation: 10% DMSO in Water + 90% saline.

^b po formulation: 0.1% PEG400 + 0.5% CMC-Na in Water. Three SD male rats for iv and 5 SD male rats for po

Figure S1 Cardiac function parameters of mice on the fourth day after respectively treated with vehicle (control), FD274, and FD268 (n = 3). LVAWs: left ventricular anterior wall end-systolic dimension; LVAWd: LVAW-diastolic dimension; LVPWs: left ventricular posterior wall end-systolic dimension; LVPWd: LVPW-diastolic dimension.

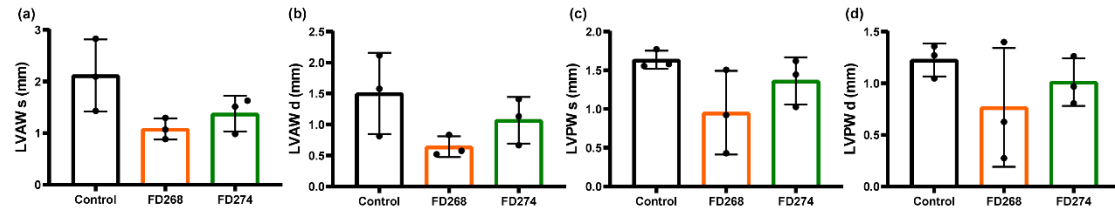


Figure S2 TUNEL staining of the heart sections of mice on the fourth day after respectively treated with vehicle (control), FD274, and FD268. Scale bar = 50 μ m

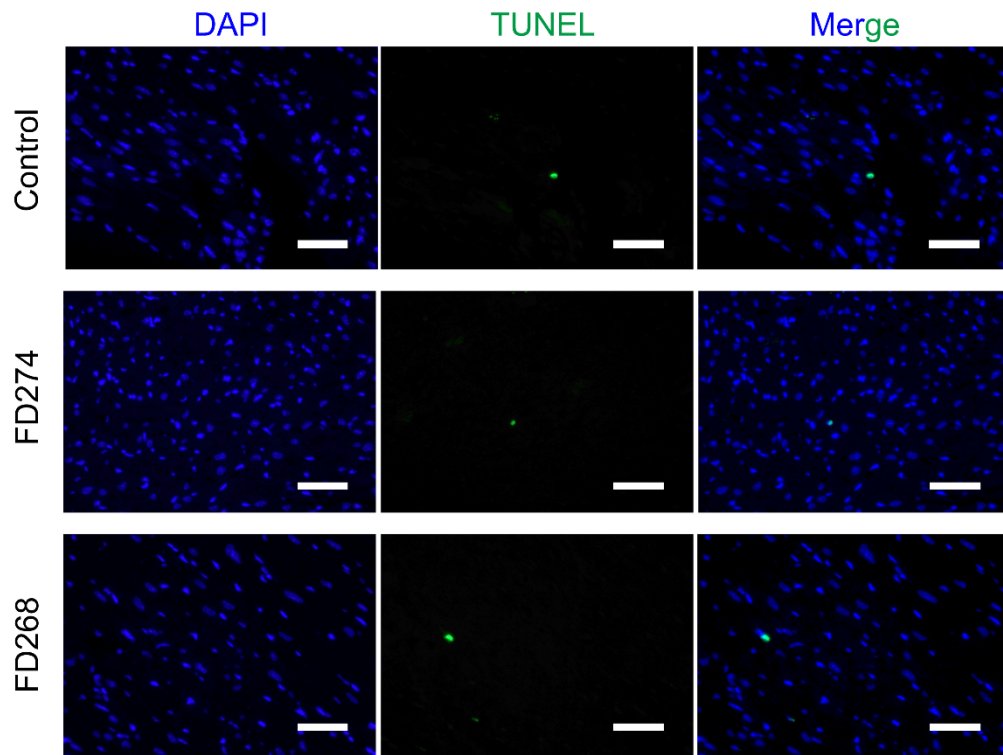


Figure S3 H&E staining of the liver, spleen, lung, and kidney sections of mice on the fourth day after respectively treated with vehicle (control), FD274, and FD268. Scale bar = 50 μ m.

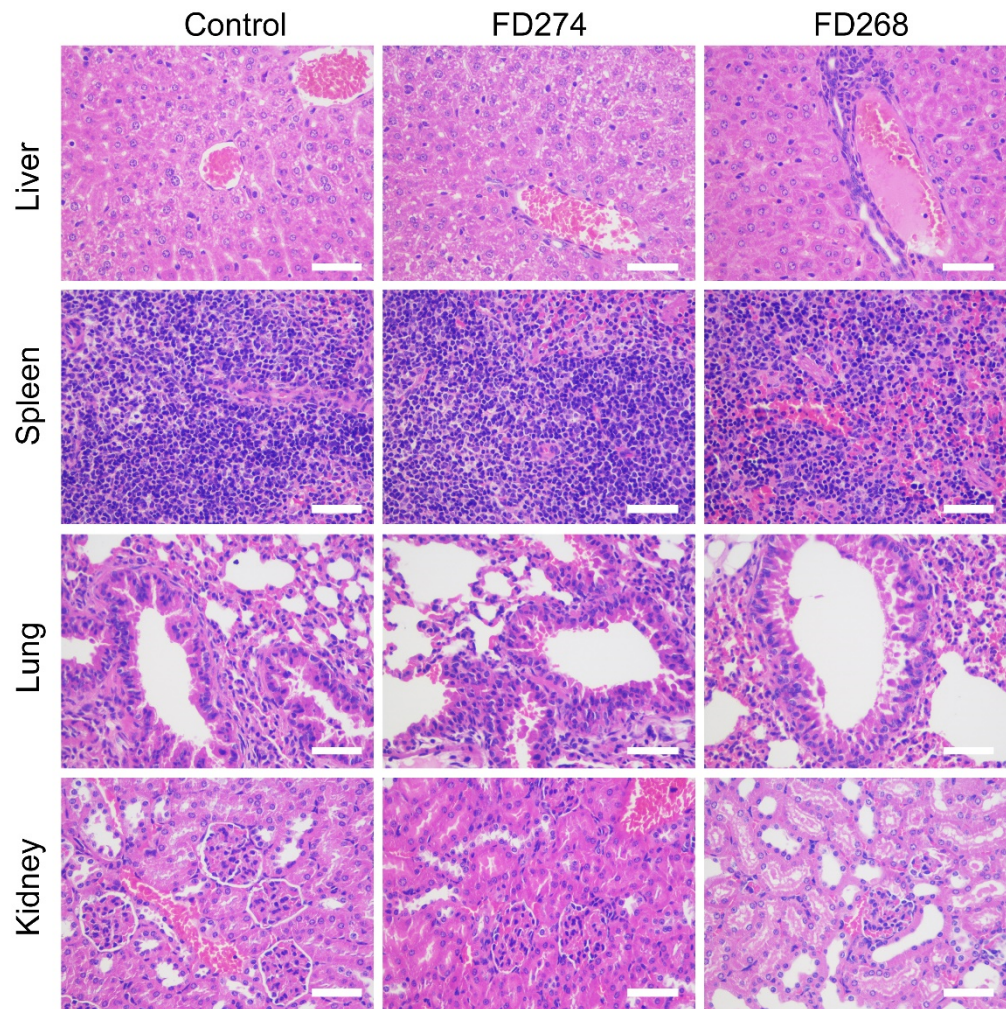


Figure S4 Representative CLSM images of H9C2 cells stained by DCFH-DA after respectively treated with various concentrations of FD274 (**a**) and FD268 (**b**), scale bar = 100 μ m.

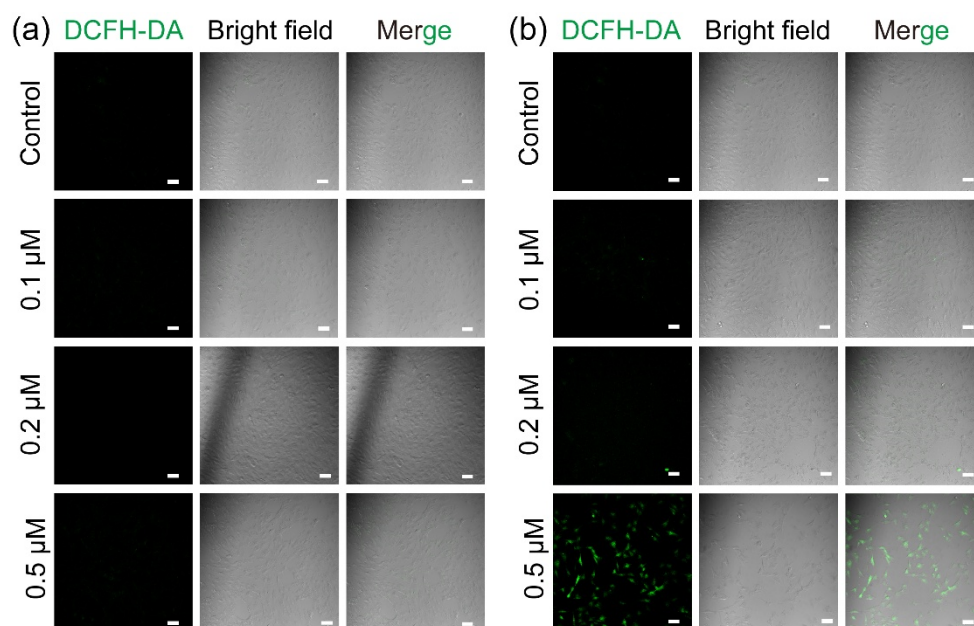


Figure S5 The apoptosis level of FD274 group (a) and FD268 group (b) was measured as the ratio of Annexin V-positive cells and both Annexin V- and PI-positive cells (n = 3). *** $p < 0.001$

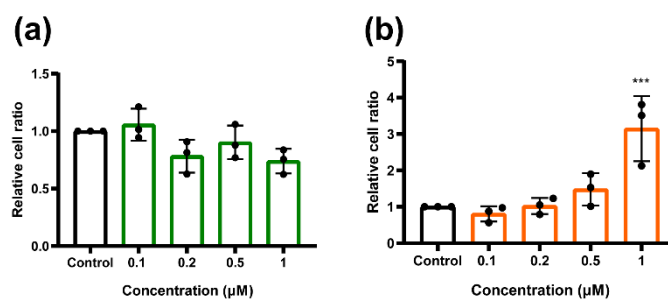


Figure S6 The normalized isobologram for the combination of FD274 and Gilteritinib against HL-60 **(a)** and MV-4-11 **(b)** generated by CompuSyn software.

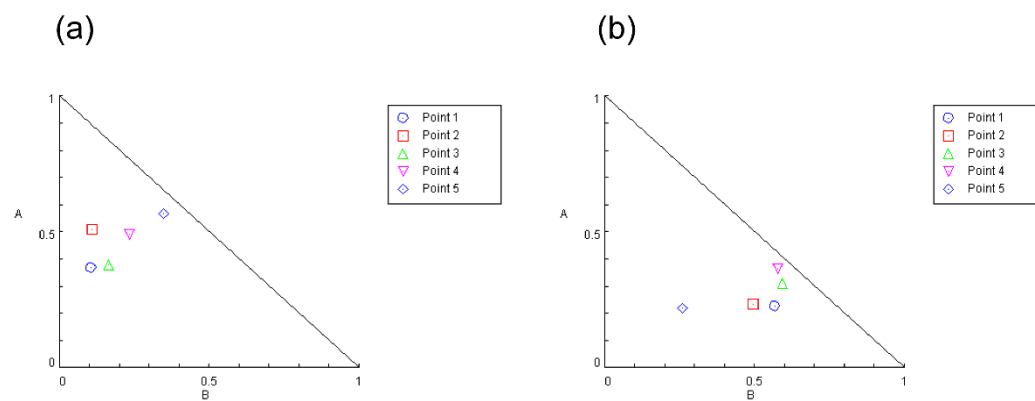


Figure S7 The apoptosis level of HL-60 (a) and MV-4-11 (b) was measured as the ratio of Annexin V-positive cells (early apoptosis) and both Annexin V- and PI-positive cells (late apoptosis) (n = 3).c-d: The normalized isobologram for the combination of FD274 and Gilteritinib against HL-60 (c) and MV-4-11 (d) generated by CompuSyn software. ****** $p < 0.01$ vs control group, ******** $p < 0.0001$ vs control group; **###** $p < 0.001$ vs FD274 alone, **####** $p < 0.0001$ vs FD274 alone; **\$** $p < 0.05$ vs Gilteritinib alone, **\$\$\$** $p < 0.001$ vs Gilteritinib alone, **\$\$\$\$** $p < 0.0001$ vs Gilteritinib alone

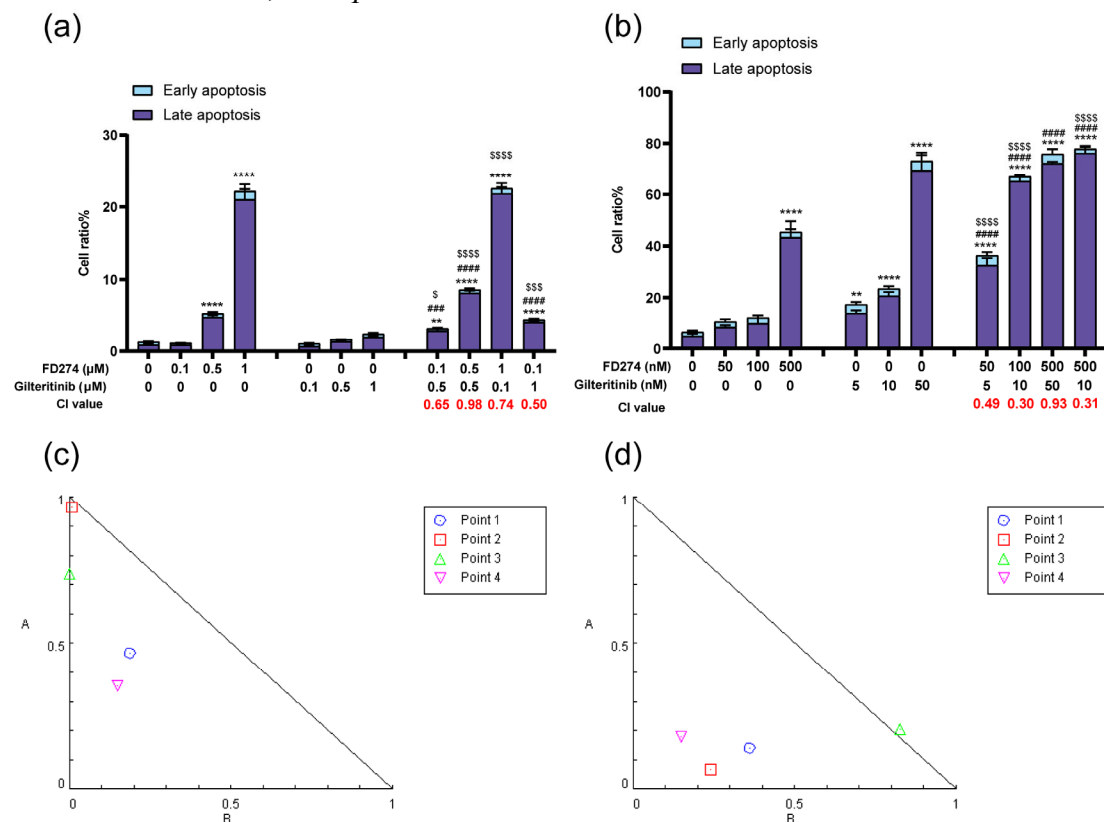


Figure S8 The expression levels of AKT, p-AKT, p-mTOR, p70S6K, p- p70S6K, 4E-BP1, and p-4E-BP1 in MV-4-11 cells after respectively treated with FD274, Gilteritinib, and the combination of these two compounds were determined by western blot.

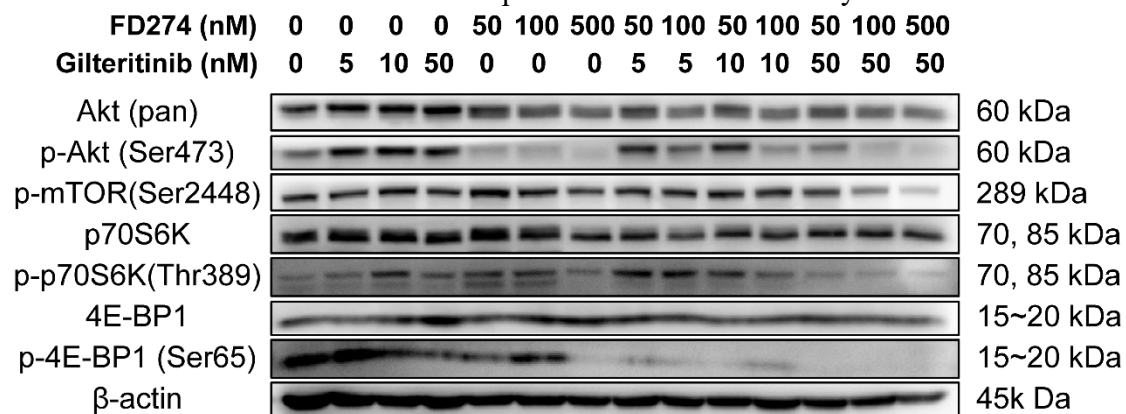
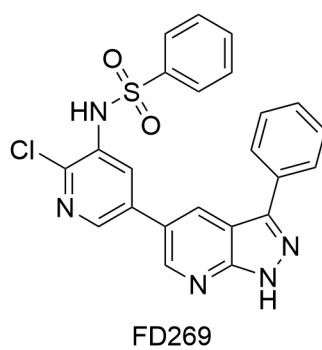


Figure S9. The chemical structure of FD269.



References

- [1] Yang, C.; Chen, Y.; Wu, T.; Gao, Y.; Liu, X.; Yang, Y.; Ling, Y.; Jia, Y.; Deng, M.; Wang, J.; Zhou, Y., Discovery of N-(2-chloro-5-(3-(pyridin-4-yl)-1H-pyrazolo[3,4-b]pyridin-5-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide (FD274) as a highly potent PI3K/mTOR dual inhibitor for the treatment of acute myeloid leukemia. *Eur. J. Med. Chem.* **2023**, *258*, 115543.