Supplementary information for

Preclinical Characterization of 22-(4'-Pyridinecarbonyl) Jorunnamycin A Against Lung Cancer Cell Invasion and Angiogenesis via AKT/mTOR Signaling

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Number of tables: 3

Supplementary Methods

Pharmacokinetic Parameters Prediction. The online web server known as "pkCSM-tools" (http://biosig.unimelb.edu.au/pkcsm/) was utilized for predictions of pharmacokinetic features such as absorption, distribution, metabolism, excretion, and toxicity.

Supplementary Figures and Tables

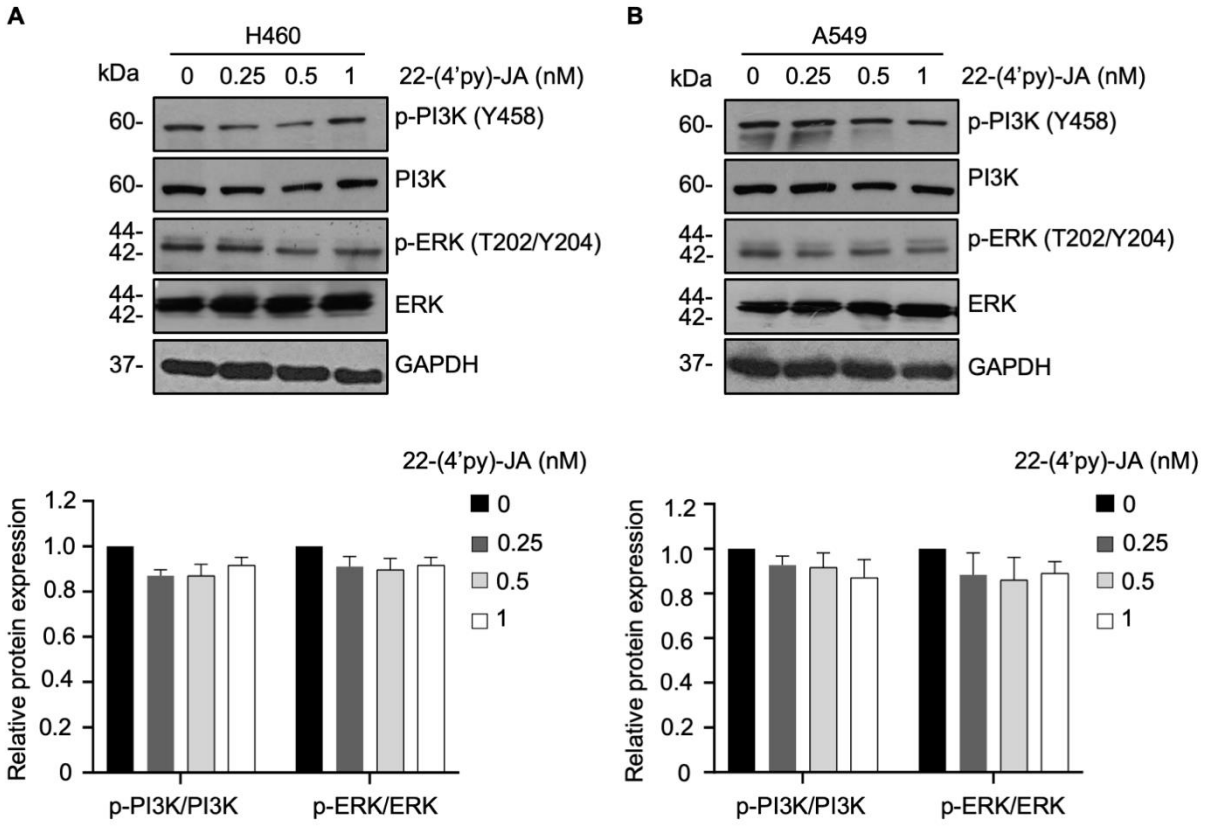


Figure S1. The effect of 22-(4'py)-JA on PI3K and ERK signaling. (A) H460 and (B) A549 cells were treated with non-toxic concentration of 22-(4'py)-JA for 24 h. The protein expression of p-PI3K, PI3K, p-ERK1/2, and ERK1/2 were investigated by western blot analysis and quantified relatively to those of GAPDH. Plots are mean ± SEM. **p* < 0.05 vs. control group (n=3).

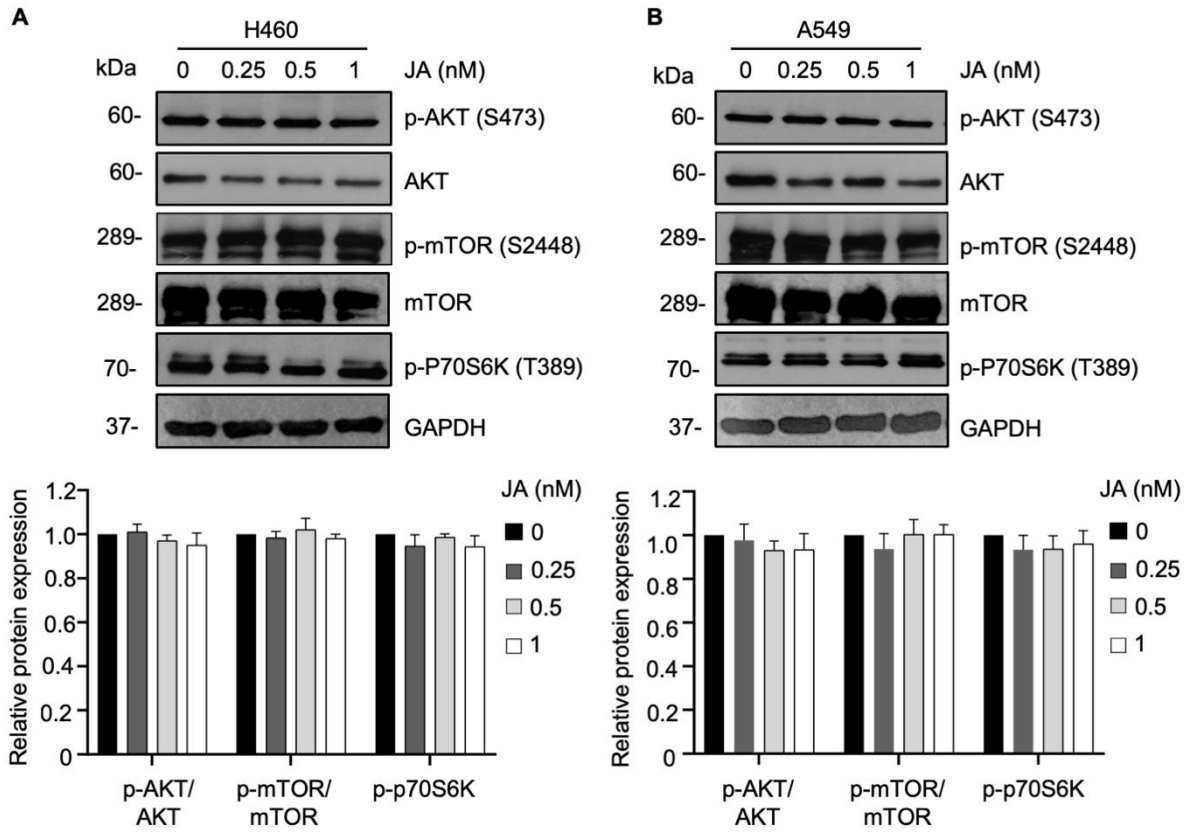


Figure S2. The effect of JA on AKT/mTOR/p70S6K signaling. (A) H460 and (B) A549 cells were treated with JA (0-1 nM) for 24h. The protein expression of p-AKT, AKT, p-mTOR, mTOR, and p-p70S6K were investigated by western blot analysis and quantified relatively to those of GAPDH. Plots are mean ± SEM. *$p < 0.05$ vs. control group (n=3).

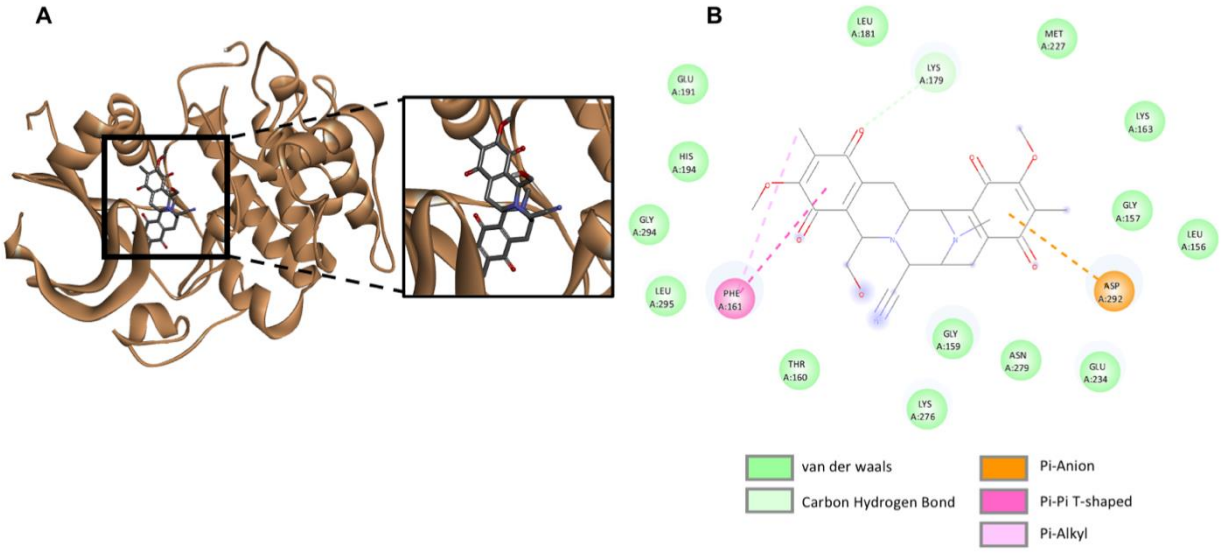
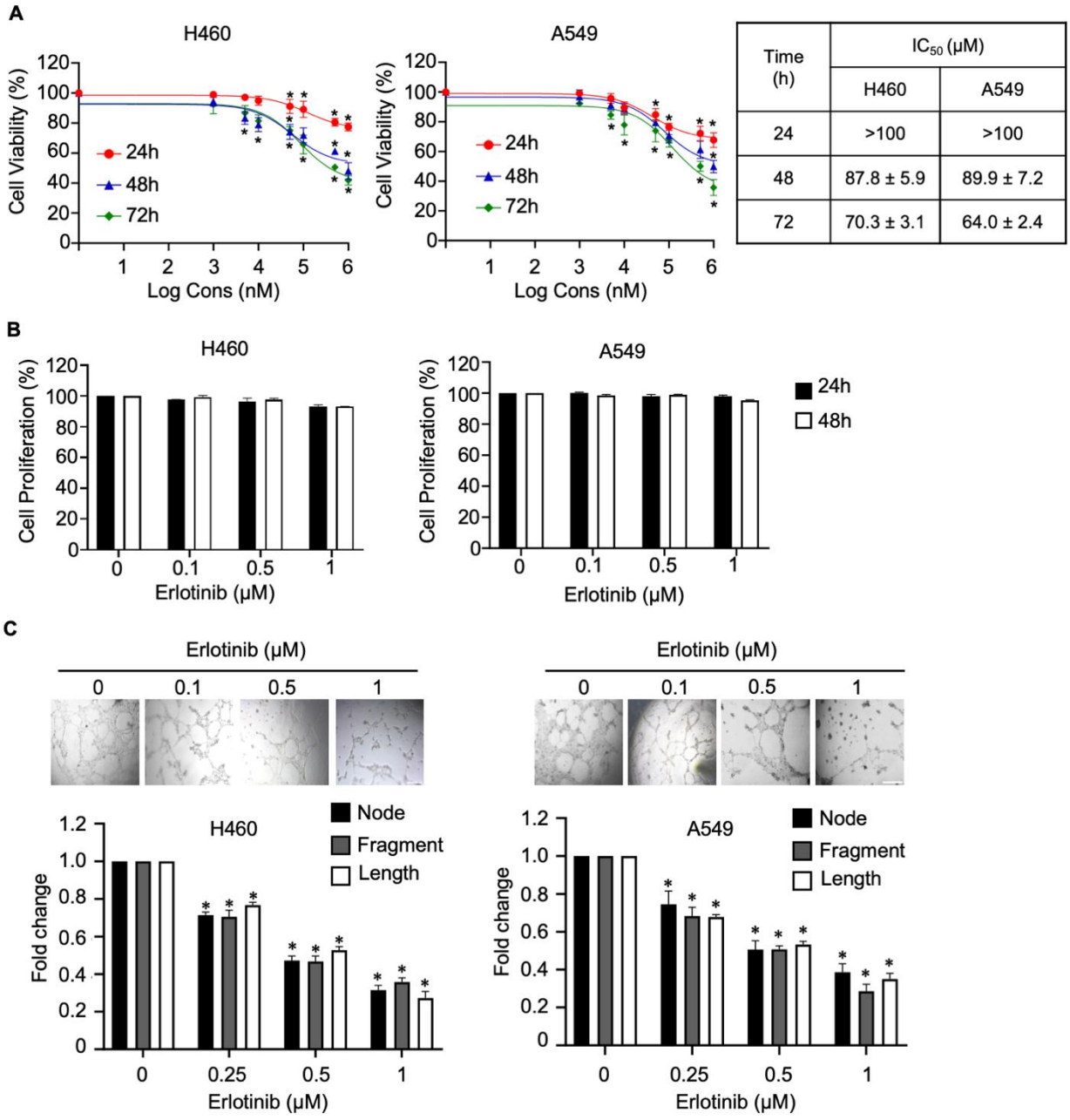


Figure S3. Molecular docking and dynamic interaction of JA and AKT1. Interactions between JA and AKT1 (PDB ID: 3MVH) are shown in (A) 3D and (B) 2D images. Molecular docking was performed using PyRx Autodock Vina, and schematization of the interaction between JA and AKT1 was obtained using the BIOVIA Discovery Studio Visualizer.



Time (h)	IC_{50} (μM)	
	H460	A549
24	>100	>100
48	87.8 ± 5.9	89.9 ± 7.2
72	70.3 ± 3.1	64.0 ± 2.4

Figure S4. The anti-cancer activities of erlotinib on NSCLC cells. (A) H460 and A549 were treated with Erlotinib (0-100 μM) for 24, 48, and 72 h. Cell viability was analyzed by the MTT assay and represented as a percentage of cell viability. (B) H460 and A549 cells were treated with non-toxic concentrations of Erlotinib (0-1μM) for 24 and 48 h. Cell proliferation was evaluated by the MTT assay. (C) HUVECs were cultured in the medium obtained from H460 and A549 cells

treated with or without Erlotinib. After 24 h, tube formation was imaged, and its parameters were quantified using ImageJ software. Plots present the fold change of node number, fragment number, and fragment length. Scale bar, 100 μm. The data are presented as the mean ± SEM ($n = 3$). *$p < 0.05$ vs untreated control cells. All data were analyzed by using Analysis of variance (ANOVA) followed by the Tukey post-hoc test.

Table S1. The interactions between AKT1 (PDB:3MVH) and 22-(4'py)-JA or JA.

Compound	Binding interaction	Amino acids
22-(4'py)-JA	Hydrogen	LEU156, GLY162, LYS179, GLU234
	Hydrophobic	LEU156, ALA177, MET281
	Van der Walls	GLY157, LYS158, GLY159, PHE161, LYS163, ILE165, LEU181, LYS276, GLU278, ASN279, ASP292, GLY294, LEU295, THR312, PHE438
	Electrostatic	ASP274
JA	Hydrogen	LYS179
	Hydrophobic	PHE161
	Van der Walls	LEU156, GLY157, GLY159, THR160, LYS163, LEU181, GLU191, HIS194, MET227, GLU234, LYS276, ASN279, LEU295
	Electrostatic	ASP292

Table S2. Pharmacokinetic parameters of 22-(4'py)-JA and JA obtained from pkCSM-tools

Parameter	Model	Unit	22-(4'py)-JA	JA	Accepted Value
Absorption	Water solubility	Log mol/L	-3.558	-3.562	-
	Intestinal absorption	% absorbed	71.223	60.436	>30%
	Skin permeability	Log Kp	-2.741	-2.767	>-2.5
Distribution	BBB permeability	Log BB	-1.397	-1.053	<0.3
	CNS permeability	Log PS	-3.203	-3.542	>-2
Metabolism	CYP2D6 substrate	Yes/No	No	No	-
	CYP3A4 substrate	Yes/No	Yes	Yes	-
Excretion	Total clearance	Log mL/min/kg	0.675	0.789	-
	Renal OCT2 substrate	Yes/No	No	No	No
Toxicity	AMES toxicity	Yes/No	No	No	No
	Maximum tolerated dose (human)	Log mg/kg/day	-0.922	-0.322	<0.477
	hERG I inhibitor	Yes/No	No	No	No
	hERG II inhibitor	Yes/No	No	No	No
	Hepatotoxicity	Yes/No	Yes	Yes	No
	Skin sensitization	Yes/No	No	No	No
	Oral rat chronic toxicity	Log mg/kg bw/day	1.094	2.25	-

Table S3. List of premiers used for qRT-PCR.

Genes	Primer sequences	Tm (°C)
GAPDH	F: ACATCGCTCAGACACCATG	61.00
	R: TGTAGTTGAGGTCAATGAAGGG	61.00
MMP-2	F: GAAGTATGGGAACGCCGATGG	61.14
	R:TTGTCGCGGTCGTAGTCCTCA	63.49
MMP-9	F: CCTGGAGACCTGAGAACCAATC	61.18
	R: CCACCCGAGTGTAACCATAGC	58.00
VEGF	F:TGCTTCTGAGTTGCCCAGGA	61.42
	R:TGGTTTCAATGGTGTGAGGACATAG	61.04
HIF1-α	F:TTTGCTGAAGACACAGAAGCAAAGA	61.61
	R:GGAAGTCATCATCCATTGGGATA	59.34