Product Data Sheet

Product Name: A-674563
Cat. No.: GC11875

Chemical Properties

Cas. No.	552325-73-2		
Chemical Name	(2S)-1-[5-(3-methyl-2H-indazol-5-yl)pyridin-3-yl]oxy-3-phenylpropan-2-amine		
SMILES	CC1=C2C=C(C=CC2=NN1)C3=CC(=CN=C3)OCC(CC4=CC=CC=C4)N		
Formula	$C_{22}H_{22}N_4O$	M.Wt	358.44
Solubility	≥ 4.9mg/mL in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Caution: Product has not been fully validated for medical applications. For research use only.
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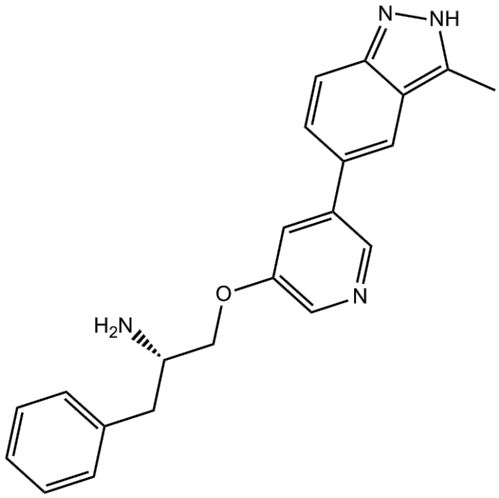
Structure



Protocol

Cell experiment [1]:	
Cell lines	A549 cells, A427 cells, NCI-H23 cells, NCI-H358 cells, NCI-H1975 cells, NCI-H1650 cells (human non-small cell lung cancer cell lines)
Preparation Method	A549 cells, A427 cells, NCI-H23 cells, NCI-H358 cells, NCI-H1975 cells and NCI-H1650 cells were maintained in RPMI 1640 medium supplemented with 10% FBS at 37°C, 5% CO_2. Cells were treated with A-674563 at increasing concentrations for 72 hours for viability assay, at IC_{50} concentrations for 24 hours for BrdU flow cytometry cell cycle analysis, and for 30 minutes, 1 hour and 2 hours for Western blot analysis of AKT, CDK2 and related proteins.
Reaction Conditions	0.03-0.70μM; 1h-72h

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Applications	A-674563 reduced NSCLC cell survival and proliferation and lowered IC_{50} values across all six cell lines. A-674563 decreased G0/G1 phase proportion and increased S phase proportion in A549, A427, NCI-H23 and NCI-H358 cells. A-674563 increased p-AKT(Ser473), p-AKT(Thr308) and p-AKT-1 expression, and decreased p-CDK2 expression in A549 and A427 cells.
Animal experiment [2]:	
Animal models	C57BL/6J mice
Preparation Method	C57BL/6J mice were intratracheally administered lenti-CX3CR1 for 144 hours, then intravenously injected with A-674563 (100mg/kg) 1 hour before exposure to room air or hyperoxia (95-100% oxygen) for 24h, 48h, or 72h. Mice were sacrificed after exposure for analysis.
Dosage form	100mg/kg; i.v.; single injection
Applications	A-674563 decreased LC3-II/LC3-I ratio, CD11b expression and CX3CR1 expression in lung tissues, increased p62 expression, reduced LC3 co-localization within CD11b positive cells and CX3CR1 co-localization with CD11b, and alleviated pulmonary fibrosis.

References:[1] Chorner PM, Moorehead RA. A-674563, a putative AKT1 inhibitor that also suppresses CDK2 activity, inhibits human NSCLC cell growth more effectively than the pan-AKT inhibitor, MK-2206. PLoS ONE. 2018 Feb 22;13(2):e0193344.[2] Chen Y, Zhang H, Li F, et al. Inhibition of CX3C receptor 1-mediated autophagy in macrophages alleviates pulmonary fibrosis in hyperoxic lung injury. Life Sci. 2020;259:118286.

Background

A-674563 is an orally active Akt inhibitor (K_i=11nM). A-674563 binds to the Akt ATP site and reduces phosphorylation of downstream targets including GSK3 and FOXO to inhibit tumor cell proliferation

and survival. A-674563 maintains chondrocyte marker expression by inhibiting Sox9 degradation. A-674563 can be used for research on tumors and cartilage degeneration[1-4].

In vitro, A549, A427, NCI-H23, NCI-H358, NCI-H1975 and NCI-H1650 cells were treated with 0.03-0.70μM A-674563 for 1-72 hours. A-674563 reduced cell viability and proliferation, decreased G0/G1 phase cell proportion and increased S phase cell proportion[5]. A375, WM-115 and SK-Mel-2 cells were treated with 10-1000nM A-674563 for 30-72 hours. A-674563 inhibited cell proliferation and colony formation, increased dead cell number, early and late apoptotic cell proportion, and histone-DNA apoptotic signal, blocked Akt(Thr-308) and S6K1 phosphorylation[6]. U937 cells were treated with 10-500nM A-674563 for 12-72 hours. A-674563 dose-dependently inhibited cell viability and proliferation, induced cell death and reduced colony formation, activated caspase-3 and caspase-9 and promoted apoptosis[7].

In vivo, CX3CR1-overexpressing C57BL/6J mice received a single tail vein injection of 100mg/kg A-674563. One hour after the injection, the mice were exposed to hyperoxia for 24-72 hours. A-674563 decreased LC3-II/LC3-I ratio and CD11b expression, increased p62 expression, reduced LC3 co-localization within CD11b positive cells and CX3CR1 co-localization with CD11b, alleviated pulmonary fibrosis[8]. C57BL/6 mice received a single intravenous injection of 100mg/kg A-674563 1 hour before ischemia. The mice were then exposed to 67% H_2 and 33% O_2 for 4 hours of reperfusion. A-674563 increased infarct size percentage, elevated serum creatine kinase, creatine kinase MB isoenzyme and cardiac troponin I levels, decreased p-Akt1 expression[9]. Immunocompromised male SCID mice bearing 3T3-Akt1, MiaPaCa-2 and PC-3 xenograft tumors received oral 40mg/kg A-674563 for 20 days. A-674563 slowed tumor growth in xenograft tumors model, enhanced paclitaxel antitumor efficacy in the PC-3 model, caused body weight loss and increased plasma insulin[10].

References:
[1] Kobayashi T, Fujita K, Kamatani T, et al. A-674563 increases chondrocyte marker expression in cultured chondrocytes by inhibiting Sox9 degradation. Biochem Biophys Res Commun. 2018 Jan 1;495(1):1468-1475.
[2] Hirai T, Wang W, Murono N, et al. Potential role of Akt in the regulation of fibroblast growth factor 21 by berberine. J Nat Med. 2024 Jan;78(1):169-179.
[3] Goßmann M, Linder P, Thomas U, et al. Integration of mechanical conditioning into a high

throughput contractility assay for cardiac safety assessment. J Pharmacol Toxicol Methods. 2020 Sep;105:106892.
[4] Chen O, Cao Z, Li H, et al. High-concentration hydrogen protects mouse heart against ischemia/reperfusion injury through activation of thePI3K/Akt1 pathway. Sci Rep. 2017 Nov 1;7(1):14871.
[5] Chorner PM, Moorehead RA, et al. A-674563, a putative AKT1 inhibitor that also suppresses CDK2 activity, inhibits human NSCLC cell growth more effectively than the pan-AKT inhibitor, MK-2206. PLoS ONE. 2018 Feb 22;13(2):e0193344.
[6] Zou Y, Fan G, Wang X. Pre-clinical assessment of A-674563 as an anti-melanoma agent. Biochem Biophys Res Commun. 2016;473(4):1243-1250.
[7] Xu L, Zhang Y, Gao M, et al. Concurrent targeting Akt and sphingosine kinase 1 by A-674563 in acute myeloid leukemia cells. Biochem Biophys Res Commun. 2016 Apr 8;472(4):662-668.
[8] Chen Y, Zhang H, Li F, et al. Inhibition of CX3C receptor 1-mediated autophagy in macrophages alleviates pulmonary fibrosis in hyperoxic lung injury. Life Sci. 2020;259:118286.
[9] Chen O, Cao Z, Li H, et al. High-concentration hydrogen protects mouse heart against ischemia/reperfusion injury through activation of the PI3K/Akt1 pathway. Sci Rep. 2017 Nov 1;7:14871.
[10] Luo Y, Shoemaker AR, Liu X, et al. Potent and selective inhibitors of Akt kinases slow the progress of tumors in vivo. Mol Cancer Ther. 2005 Jun;4(6):977-86.