产品名称：(ALPHAS)-ALPHA-[[[5-(3-甲基-1H-吲唑-5-基)-3-吡啶基]氧]甲基]苯乙胺
产品别名：A-674563

生物活性：

Description	A-674563 is a potent and selective Akt1 inhibitor with a K_i of 11 nM.					
IC_{50} & Target	Akt1	PKA	PKCγ	PKCδ	SRC	CDK2
	11 nM (Ki)	16 nM (Ki)	1.2 μM (Ki)	360 nM (Ki)	13 μM (Ki)	46 nM (Ki)
	ERK2	GSK3β	CK2	Chk1	RSK2	MAPK-AP2
	260 nM (Ki)	110 nM (Ki)	5.4 μM (Ki)	2.6 μM (Ki)	580 nM (Ki)	1.1 μM (Ki)
In Vitro	A-674563 slows proliferation of tumor cells with an EC50 of 0.4 μM[1]. A563 (0-10 μM) significantly decreases GSK3 and MDM2 phosphorylation in STS cells. A563 shows inhibitory effect on all STS cell lines, with IC50 values at 48 hours ranging from 0.22±0.034 μM (SW684) to 0.35 ±0.06 μM (SKLMS1). A563 induces G2 cell cycle arrest and apoptosis in STS cells. A563 (1 μM/12 hr) upregulates the expression of GADD45A independent of p53[2]. A-674563 (10-1000 nM) is anti-proliferative and cytotoxic in cultured human melanoma cells, induces melanoma cell apoptotic death, inhibited by caspase inhibitors, and inhibits melanoma cells via Akt-dependent and -independent mechanisms[3]. A-674563 is cytotoxic and anti-proliferative when added to U937 and AmL progenitor cells, activates caspase-3/9 and apoptosis in U937 and AmL progenitor cells, and manipulates other signalings in AmL cells whiling blocking Akt[4].					
In Vivo	A-674563 (40 mg/kg/d, p.o.) shows no significant monotherapy activity, but the efficacy of the combination therapy (A-674563+paclitaxel) is significantly improved in the PC-3 prostate cancer xenograft model. A-674563 (20, 100 mg/kg) increases plasma insulin in an oral glucose tolerance test[1]. A563 (20 mg/kg/bid; p.o.) exhibits slow tumor growth and a significant difference in tumor volume without significant weight loss of mice. A563-treated tumors express increased levels of GADD45α and decreased levels of PCNA (a nuclear marker for proliferation). Additionally, TUNEL assay staining levels (marker for apoptosis) increase in the A563-treated specimens[2]. A-674563 (25, 100 mg/kg, lavage daily) potently inhibits A375 xenograft growth in mice[3]. A-674563 (15, 40 mg/kg) injection inhibits U937 xenograft in vivo growth, and improves mice survival[4].					

In Vitro:

DMSO : 83.33 mg/mL (**232.48 mM;** Need ultrasonic)

H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	2.7899 mL	13.9493 mL	27.8987 mL
	5 mM	0.5580 mL	2.7899 mL	5.5797 mL
	10 mM	0.2790 mL	1.3949 mL	2.7899 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

Solvent&Solubility	——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶 1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: ≥ 2.08 mg/mL (5.80 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (5.80 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 20.8 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。
References	[1]. Luo Y, et al. Potent and selective inhibitors of Akt kinases slow the progress of tumors in vivo. Mol Cancer Ther, 2005, 4(6), 977-986. [2]. Xu L, et al. Concurrent targeting Akt and sphingosine kinase 1 by A-674563 in acute myeloid leukemia cells. Biochem Biophys Res Commun. 2016 Apr 15;472(4):662-8. [3]. Zhu QS, et al. Soft tissue sarcoma cells are highly sensitive to AKT blockade: a role for p53-independent up-regulation of GADD45 alpha. Cancer Res, 2008, 68(8), 2895-2903. [4]. Wang A, et al. Dual inhibition of AKT/FLT3-ITD by A674563 overcomes FLT3 ligand-induced drug resistance in FLT3-ITD positive AML. Oncotarget. 2016 May 17;7(20):29131-42. [5]. Zou Y, et al. Pre-clinical assessment of A-674563 as an anti-melanoma agent. Biochem Biophys Res Commun. 2016 Aug 12;477(1):1-8.

实验参考：

Cell Assay	The cells on 96-well plates are gently washed with 200 μL of PBS. Alamar Blue reagent is diluted 1:10 in normal growth media. The diluted Alamar Blue reagent (100 μL) is added to each well on the 96-well plates and incubated until the reaction is complete. Analysis is done using an fmax Fluorescence Microplate Reader, set at the excitation wavelength of 544 nm and emission wavelength of 595 nm. Data are analyzed using SOFTmax PRO software. [1]
Animal Administration	Immunocompromised male scid mice are at 6 to 8 weeks of age. The 1×10^6 3T3-Akt1 or 2×10^6 MiaPaCa-2 and PC-3 cells in 50% Matrigel are inoculated s.c. into the flank. For early treatment studies, mice are randomLy assigned to treatment groups and therapy is initiated the day after inoculation. Ten animals are assigned to each group, including controls. For established tumor studies, tumors are allowed to reach a designated size and mice are assigned to treatment groups of equal tumor size (n=10 mice per group). Tumor size is evaluated by twice weekly measurements with digital calipers. Tumor volume is estimated using the formula: $V=L\times W^2/2$. A-443654 is given s.c. in a vehicle of 0.2% HPMC. A-674563 is given orally in a vehicle of 5% dextrose. Gemcitabine and paclitaxel are added to the assay. [1]
References	[1]. Luo Y, et al. Potent and selective inhibitors of Akt kinases slow the progress of tumors in vivo. Mol Cancer Ther, 2005, 4(6), 977-986. [2]. Xu L, et al. Concurrent targeting Akt and sphingosine kinase 1 by A-674563 in acute myeloid leukemia cells. Biochem Biophys Res Commun. 2016 Apr 15;472(4):662-8. [3]. Zhu QS, et al. Soft tissue sarcoma cells are highly sensitive to AKT blockade: a role for p53-independent up-regulation of GADD45 alpha. Cancer Res, 2008, 68(8), 2895-2903. [4]. Wang A, et al. Dual inhibition of AKT/FLT3-ITD by A674563 overcomes FLT3 ligand-induced drug resistance in FLT3-ITD positive AML. Oncotarget. 2016 May 17;7(20):29131-42.

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