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产品名称：A-443654
产品别名：A-443654

生物活性：

Description	A-443654 is a pan-Akt inhibitor and has equal potency against Akt1, Akt2, or Akt3 within cells (K_i=160 pM).						
IC_{50} & Target	Akt1	Akt2	Akt3	PKA	RSK2	PKCγ	CDK2
	160 pM (Ki)	160 pM (Ki)	160 pM (Ki)	6.3 nM (Ki)	11 nM (Ki)	24 nM (Ki)	24 nM (Ki)
	PKCδ	GSK3β	ERK2	cKIT	Chk1	CK2	SRC
	33 nM (Ki)	41 nM (Ki)	340 nM (Ki)	1.2 μM (Ki)	2.3 μM (Ki)	2.4 μM (Ki)	2.6 μM (Ki)
	KDR	MAPK-AP2	Flt1				
	3.1 μM (Ki)	3.3 μM (Ki)	3.6 μM (Ki)				
In Vitro	A-443654 exhibits a Ki of 160 pM, a 30,000-fold improvement in potency versus the initial lead molecule. A-443654 is 40-fold selective for Akt over PKA. A-443654 inhibits Akt1, Akt2, or Akt3 equally within cells. A-443654 reduces the P-GSK3 in a dose-responsive manner in all three cell lines. A-443654 inhibits the proliferation of tumor cells with EC50 of 0.1 μM[1]. A-443654-induced morphological changes occur very rapidly (within 2 to 4 h) in both 10A and 10CA1a cells, with 10CA1a cells more sensitive to A-443654 than the 10A cells. A-443654 alone at 2 μM causes the 10CA1a cells, but not the 10A cells, to detach from the plate after 12 h, whereas 1 μM of A-443654 causes 10CA1a cells to detach from the plate after 12 h. FACScan Analysis of rapamycin and A-443654 effects on DNA content in 10A and 10CA1a cells. In contrast, A-443654 at 2 and 5 μM decreases Bcl-2 levels by 30 to 40% in the 10CA1a cells at 8h. The combination of rapamycin with 2 or 5 μM A-443654, however, markedly decreases Bcl-2 protein levels by appr 40 to 50% in the 10A cells and by appr 70% in the 10CA1a cells, respectively[2]. A-443654 demonstrates the greatest selective effect on the mutant cells compared to the WT cells with greater than 3.5 fold relative growth inhibition of the mutant cells[3].						
In Vivo	A-443654 (7.5 mg/kg/d, s.c.) inhibits tumor growth in the 3T3-Akt1 flank tumor model. A-443654 (50 mg/kg, s.c.) induces apoptosis in 3T3-Akt1 flank tumors. A-443654 (30 mg/kg, s.c.) leads to increased levels of phosphorylated Akt1 in MiaPaCa-2 tumors[1].						

In Vitro:

DMSO : ≥ 100 mg/mL (251.59 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	2.5159 mL	12.5796 mL	25.1591 mL
	5 mM	0.5032 mL	2.5159 mL	5.0318 mL
	10 mM	0.2516 mL	1.2580 mL	2.5159 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:

Solvent&Solubility	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： ——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: ≥ 2.5 mg/mL (6.29 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.29 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 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。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.29 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.29 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Luo Y, 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. [2]. Zheng J, et al. Rapamycin sensitizes Akt inhibition in malignant human breast epithelial cells. Cancer Lett. 2010 Oct 1;296(1):74-87. [3]. Gallia GL, et al. Inhibition of Akt inhibits growth of glioblastoma and glioblastoma stem-like cells. Mol Cancer Ther. 2009 Feb;8(2):386-93. [4]. Zhao Y, et al. Estrogen receptor alpha and beta regulate actin polymerization and spatial memory through an SRC-1/mTORC2-dependent pathway in the hippocampus of female mice. J Steroid Biochem Mol Biol. 2017 Nov;174:96-113.

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 as per manufacturer's instructions. Analysis is done using an fmaxFluorescence Microplate Reader, set at the excitation wavelength of 544 nm and emission wavelength of 595 nm. Data are analyzed using SOFTmax PRO software provided by the manufacturer. [1]
	Immunocompromised male scid mice are 6 to 8 weeks of age. The 3T3-Akt1 cell line is developed and characterized in our laboratory. The 1×10^6 3T3-Akt1 or 2×10^6 MiaPaCa-2 and PC-3 cells in

Animal Administration	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. [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 Jun;4(6):977-86. [2]. Zheng J, et al. Rapamycin sensitizes Akt inhibition in malignant human breast epithelial cells. Cancer Lett. 2010 Oct 1;296(1):74-87. [3]. Gallia GL, et al. Inhibition of Akt inhibits growth of glioblastoma and glioblastoma stem-like cells. Mol Cancer Ther. 2009 Feb;8(2):386-93. [4]. Zhao Y, et al. Estrogen receptor alpha and beta regulate actin polymerization and spatial memory through an SRC-1/mTORC2-dependent pathway in the hippocampus of female mice. J Steroid Biochem Mol Biol. 2017 Nov;174:96-113.