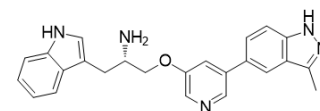


A-443654

Cat. No.:	HY-10425		
CAS No.:	552325-16-3		
Molecular Formula:	C ₂₄ H ₂₃ N ₅ O		
Molecular Weight:	397.47		
Target:	Akt		
Pathway:	PI3K/Akt/mTOR		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 100 mg/mL (251.59 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration \ Mass	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	

Please refer to the solubility information to select the appropriate solvent.

In Vivo

- Add each solvent one by one: **10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline**
Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution
- Add each solvent one by one: **10% DMSO >> 90% (20% SBE-β-CD in saline)**
Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution
- Add each solvent one by one: **10% DMSO >> 90% corn oil**
Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

A-443654 is a potent Akt1/2/3 inhibitor, with a K_i of 160 pM for Akt1.

IC₅₀ & Target

K_i: 160 pM (Akt1)

In Vitro

A-443654 exhibits a K_i 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. FACSscan 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].

PROTOCOL

Cell Assay ^[1]

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.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration ^[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 1×10⁶ 3T3-Akt1 or 2×10⁶ 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×W²/2. A-443654 is given s.c. in a vehicle of 0.2% HPMC. A-674563 is given orally in a vehicle of 5% dextrose.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Steroid Biochem Mol Biol. 2017 Nov;174:96-113.
- CNS Neurosci Ther. 2019 Feb 3.
- CNS Neurosci Ther. 2018 Jun;24(6):495-507.

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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.

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

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