

Product Data Sheet

Product Name: A-443654

Cat. No.: GC12502

Chemical Properties

Cas. No. 552325-16-3

Chemical Name (2S)-1-(1H-indol-3-yl)-3-[5-(3-methyl-2H-indazol-5-yl)pyridin-3-yl]oxypropan-2-amine

SMILES CC1=C2C=C(C=CC2=NN1)C3=CC(=CN=C3)OCC(CC4=CNC5=CC=CC=C54)N

Formula $C_{24}H_{23}N_5O$ M.Wt 397.48

Solubility ≥ 19.85 mg/mL in DMSO, ≥ 44.3 mg/mL in EtOH 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.

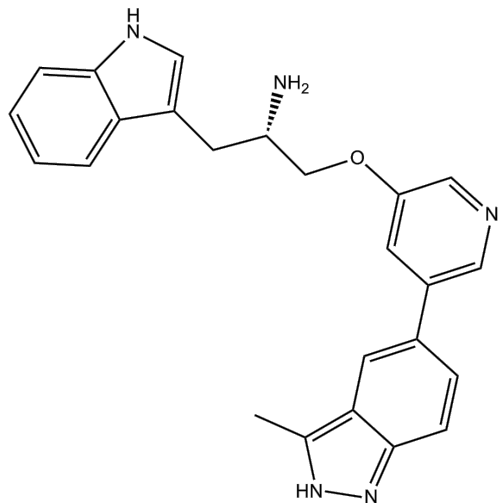
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

Cell experiment: The cells on 96-well plates are gently washed with 200 μ L of PBS. Alamar Blue reagent is diluted 1:10 in normal growth media. The diluted Alamar Blue reagent (100 μ L) 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.

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Animal experiment: 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 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.

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.

Background

A-443654 is a potent and selective inhibitor of Akt with K_i value of 160 pM [1]. The Akt kinases play important roles in cellular signal transduction and take participate in the regulation of cell transformation and tumor progression. The activities of them are usually elevated in human malignancies. A-443654 is a pan inhibitor of Akt1, 2 and 3. It binds to the ATP-binding site of Akt and inhibits the kinase activity reversibly. A-443654 shows selectivity against Akt. The inhibition ability of it for Akt is much higher than that for other kinases. It is found that A-443654 can suppress the phosphorylation of the downstream proteins of Akt while increased the Ser473 and Thr308

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phosphorylation of Akt [1].

In murine FL5.12 cells stably transfected with human Akt1, 2 or 3, A-443654 inhibited the phosphorylation of GSK3 dose-dependently. A-443654 at a concentration of 0.6 μ M inhibited Akt and induced G2/M accumulation in H1299 cells. In MiaPaCa-2 cells, treatment of A-443654 for 48 h resulted in a suppression of tumor proliferation with EC50 value of 100 nM. In chronic lymphocytic leukemia cells, A-443654 also inhibited the activity of Akt and induced apoptosis with EC50 value of 0.63 μ M. Besides that, A-443654 was found to cause decreased phosphorylation of GSK3, FOXO3, TSC2 and mTOR in MiaPaCa-2 cells. It is also reported that A-443654 was effective in blocking the phosphorylation of 4EBP-1 and S6 protein in both T47D and LNCaP cells [1, 2, 3 and 4]. A-443654 is not oral available. In mice model with human MiaPaCa-2 pancreatic cell xenograft, the administration of A-443654 at a dose of 7.5mg/kg/d significantly inhibited tumor growth. A-443654 also inhibited tumor growth in 3T3 murine fibroblast model expressing active Akt. When using the combination of A-443654 and rapamycin in mice model with MiaPaCa-2 pancreatic cancer xenograft, the administration showed more efficacy than each monotherapy [1].

References:

- 1.Luo Y, Shoemaker A R, Liu X, et al. Potent and selective inhibitors of Akt kinases slow the progress of tumors in vivo. *Molecular cancer therapeutics*, 2005, 4(6): 977-986.
- 2.Liu X, Shi Y, Woods K W, et al. Akt inhibitor a-443654 interferes with mitotic progression by regulating aurora a kinase expression. *Neoplasia*, 2008, 10(8): 828-837.
- 3.Merce de Frias M, Iglesias-Serret D, Cosials A M, et al. Akt inhibitors induce apoptosis in chronic lymphocytic leukemia cells. *haematologica*, 2009, 94(12): 1698-1707.
- 4.Cherrin C, Haskell K, Howell B, et al. An allosteric Akt inhibitor effectively blocks Akt signaling and tumor growth with only transient effects on glucose and insulin levels in vivo. *Cancer biology & therapy*, 2010, 9(7): 493-503.

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