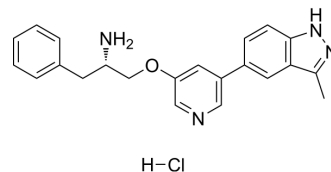


A-674563 hydrochloride

Cat. No.:	HY-13254A
CAS No.:	2070009-66-2
Molecular Formula:	C ₂₂ H ₂₃ ClN ₄ O
Molecular Weight:	394.9
Target:	Akt
Pathway:	PI3K/Akt/mTOR
Storage:	4°C, stored under nitrogen * In solvent : -80°C, 6 months; -20°C, 1 month (stored under nitrogen)



SOLVENT & SOLUBILITY

In Vitro

H₂O : 100 mg/mL (253.23 mM; Need ultrasonic)
DMSO : 50 mg/mL (126.61 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg
			1 mM	2.5323 mL	12.6614 mL
	5 mM	0.5065 mL	2.5323 mL	5.0646 mL	
	10 mM	0.2532 mL	1.2661 mL	2.5323 mL	

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

In Vivo

- Add each solvent one by one: PBS
Solubility: 100 mg/mL (253.23 mM); Clear solution; Need ultrasonic and warming and heat to 60°C
- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
Solubility: ≥ 2.08 mg/mL (5.27 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
Solubility: ≥ 2.08 mg/mL (5.27 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
Solubility: ≥ 2.08 mg/mL (5.27 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

A-674563 hydrochloride is a potent and selective Akt1 inhibitor with K_i of 11 nM.

IC₅₀ & Target

Akt1 11 nM (Ki)	PKA 16 nM (Ki)	CDK2 46 nM (Ki)	GSK3β 110 nM (Ki)
ERK2	PKCδ	RSK2	MAPK-AP2

	260 nM (Ki)	360 nM (Ki)	580 nM (Ki)	1.1 μM (Ki)
	PKCγ 1.2 μM (Ki)	Chk1 2.6 μM (Ki)	CK2 5.4 μM (Ki)	SRC 13 μM (Ki)
In Vitro	<p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>			
In Vivo	<p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>			

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. 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.

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Animal Administration ^[1]

Immunocompromised male scid mice are at 6 to 8 weeks of age. 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. Gemcitabine and paclitaxel are added to the assay.

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

CUSTOMER VALIDATION

- Aging. 2020 Nov 17;12(22):23114-23128.
- Cell Signal. 2023 Jun 9;109:110753.
- J Proteome Res. 2018 Oct 5;17(10):3360-3369.
- Oncotarget. 2016 May 17;7(20):29131-42.

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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, 4(6), 977-986.
- [2]. 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.
- [3]. 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.
- [4]. 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.
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Caution: Product has not been fully validated for medical applications. For research use only.

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA