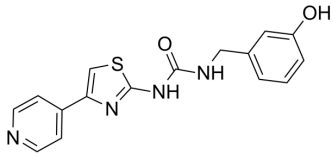
RKI-1447

Cat. No.:	HY-15755
CAS No.:	1342278-01-6
Molecular Formula:	$C_{16}H_{14}N_4O_2S$
Molecular Weight:	326.37
Target:	ROCK
Pathway:	Cell Cycle/DNA Damage; Cytoskeleton; Stem Cell/Wnt; TGF-beta/Smad
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 50 mg/mL (153.20 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	3.0640 mL	15.3200 mL	30.6401 mL
5 mM	0.6128 mL	3.0640 mL	6.1280 mL
10 mM	0.3064 mL	1.5320 mL	3.0640 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (7.66 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.66 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

RKI-1447 is a potent small molecule inhibitor of ROCK1 and ROCK2 with IC_{50} values of 14.5 nM and 6.2 nM, respectively.

IC_{50} & Target

ROCK2	ROCK1
6.2 nM (IC_{50})	14.5 nM (IC_{50})

In Vitro

RKI-1447 is a Type I kinase inhibitor that binds the ATP binding site through interactions with the hinge region and the DFG motif. RKI-1447 suppresses phosphorylation of the ROCK substrates mLC-2 and MYPT-1 in human cancer cells, but has no effect on the phosphorylation levels of the AKT, MEK and S6 kinase at concentrations as high as 10 μM. RKI-1447 is also highly selective at inhibiting ROCK-mediated cytoskeleton re-organization. RKI-1447 inhibits migration, invasion and

	anchorage-independent tumor growth of breast cancer cells[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	RKI-1447 is highly effective at inhibiting the outgrowth of mammary tumors in a transgenic mouse model. RKI-1447 inhibits mammary tumor growth by 87% and on average the mammary tumors from RKI-1447 treated mice are 7.7 fold smaller compared to those tumors from mice treated with the vehicle control[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Compounds are tested on three separate days with 8 point dilutions performed in duplicate to determine average IC_{50} values. The assay conditions are optimized to 15 µL of kinase reaction volume with 5 ng of enzyme in 50 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, and 0.01% Brij-35. The reaction is incubated for 1 h at room temperature in the presence of 1.5 µM of peptide substrate with 12.5 µM of ATP (for ROCK1) or 2 µM of substrate with 50 µM of ATP (for ROCK2). The reaction is then stopped and the ratio of phosphorylated to unphosphorylated peptides is determined by selective cleavage of only the unphosphorylated peptide. The ratio of the signals at 445 nm and 520 nm is measured. IC_{50} values are determined using fitted curves with GraphPad Prism 5 software[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	NIH 3T3 cells are plated at 8000 cells/well in 8-chamber slides in serum free media for 24 hours, and treated with vehicle, 1 µM RKI-1447 or 1 µM RKI-1313 for 1 hour. The cells are then stimulated with complete media, 10 µM LPA, 200 ng/mL bradykinin or 30 ng/mL PDGF for 30 mins. After stimulation, the cells are fixed in 4% paraformaldehyde and stained with Texas-Red Phalloidin. Mounting medium containing DAPI is then added and at least 100 cells per well are observed[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Mice: MMTV/neu transgenic mice are treated i.p. daily for 14 days with either Vehicle [20%-2-hydroxypropyl-beta-cyclodextrin (HPCD)] or 200 mpk RKI-1447 dissolved in freshly prepared HPCD. The percent change in volume is calculated on the basis of the tumor volume on the last day of treatment (Vf) relative to that on the day of initiation of treatment (V0). The average percent change in tumor volume is then calculated for each treatment group[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Int J Mol Sci. 2023 Jul 28, 24(15), 12079.
- Virology. 2024 Dec:600:110233.
- Med Sci Monit. 2020 Feb 6;26:e919220.

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REFERENCES

[1]. Patel RA, et al. RKI-1447 is a potent inhibitor of the Rho-associated ROCK kinases with anti-invasive and antitumor activities in breast cancer. Cancer Res. 2012 Oct 1;72(19):5025-34.

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 F, Monmouth Junction, NJ 08852, USA