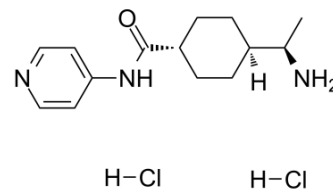


Y-27632 dihydrochloride

Cat. No.:	HY-10583		
CAS No.:	129830-38-2		
Molecular Formula:	C ₁₄ H ₂₃ Cl ₂ N ₃ O		
Molecular Weight:	320.26		
Target:	ROCK; Apoptosis		
Pathway:	Cell Cycle/DNA Damage; Cytoskeleton; Stem Cell/Wnt; TGF-beta/Smad; Apoptosis		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

H₂O : ≥ 124 mg/mL (387.19 mM)

DMSO : ≥ 32 mg/mL (99.92 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent		Mass		
	Concentration		1 mg	5 mg	10 mg
	1 mM		3.1225 mL	15.6123 mL	31.2246 mL
	5 mM		0.6245 mL	3.1225 mL	6.2449 mL
	10 mM		0.3122 mL	1.5612 mL	3.1225 mL

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

BIOLOGICAL ACTIVITY

Description

Y-27632 dihydrochloride is an orally active, ATP-competitive inhibitor of **ROCK-I** and **ROCK-II**, with K_is of 220 and 300 nM, respectively. Y-27632 dihydrochloride induces **apoptosis**. Y-27632 dihydrochloride primes human induced pluripotent stem cells (iPSCs) to selectively differentiate towards mesendodermal lineage via epithelial-mesenchymal transition-like modulation^{[1][2][3][4]}.

IC₅₀ & Target

ROCK-I 220 nM (Ki)	ROCK-II 300 nM (Ki)	PKN 3.1 μM (Ki)	Citron kinase 5.3 μM (Ki)
PKCα 73 μM (Ki)	PKA 25 μM (Ki)		

In Vitro	<p>Y-27632 inhibits the ROCK family of kinases 100 times more potently than other kinases including protein kinase C, cAMP-dependent kinase and myosin light chain kinase. Y-27632 prolongs the lag time and delays the appearance of BrdU-labeled cells in a concentration-dependent manner, delays of about 1 and 4 h are noticed in the Swiss 3T3 cells treated with 10 and 100 μM Y-27632, respectively^[1].</p> <p>Y-27632 promotes neuronal differentiation of adipose tissue-derived stem cells (ADSCs). Compared to 1.0 and 2.5 μM Y-27632 induced groups, percentages of neuroal-like cells achieved a peak in the 5.0 μM Y-27632 induced group^[2].</p>
In Vivo	<p>Y-27632 (5 and 10 mg/kg) significantly prolongs the onset time of myoclonic jerks when compare with saline group. Y-27632 (5 and 10 mg/kg) significantly prolongs the onset time of clonic convulsions when compare with saline group^[3].</p> <p>Treatment with Dimethylnitrosamine (DMN) causes a significant decrease in rat body and liver weight (DMN-S group) compared with control animals (S-S group). Oral Y27632 (30 mg/kg) essentially prevents this DMN-induced rat body and liver weight loss (DMN-Y group)^[4].</p>

PROTOCOL

Kinase Assay ^[1]

Recombinant ROCK-I, ROCK-II, PKN, or citron kinase is expressed in HeLa cells as Myc-tagged proteins by transfection using Lipofectamine, and is precipitated from the cell lysates by the use of 9E10 monoclonal anti-Myc antibody coupled to G protein-Sepharose. Recovered immunocomplexes are incubated with various concentrations of [³²P]ATP and 10 mg of histone type 2 as substrates in the absence or presence of various concentrations of either Y-27632 or Y-30141 at 30°C for 30 min in a total volume of 30 μ L of the kinase buffer containing 50 mM HEPES-NaOH, pH 7.4, 10 mM MgCl₂, 5 mM MnCl₂, 0.02% Brij 35, and 2 mM dithiothreitol. PKCa is incubated with 5 μ M [³²P]ATP and 200 μ g/mL histone type 2 as substrates in the absence or presence of various concentrations of either Y-27632 or Y-30141 at 30°C for 10 min in a kinase buffer containing 50 mM Tris-HCl, pH 7.5, 0.5 mM CaCl₂, 5 mM magnesium acetate, 25 μ g/mL phosphatidyl serine, 50 ng/mL 12-O-tetradecanoylphorbol-13-acetate and 0.001% leupeptin in a total volume of 30 μ L. Incubation is terminated by the addition of 10 μ L of 43 Laemmli sample buffer. After boiling for 5 min, the mixture is subjected to SDS-polyacrylamide gel electrophoresis on a 16% gel. The gel is stained with Coomassie Brilliant Blue, and then dried. The bands corresponding to histone type 2 are excised, and the radioactivity is measured^[1].

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

Cell Assay ^[1]

HeLa cells are plated at a density of 3×10^4 cells per 3.5-cm dish. The cells are cultured in DMEM containing 10% FBS in the presence of 10 mM Thymidine for 16 h. After the cells are washed with DMEM containing 10% FBS, they are cultured for an additional 8 h, and then 40 ng/mL of Nocodazole is added. After 11.5 h of the Nocodazole treatment, various concentrations of Y-27632 (0-300 μ M), Y-30141, or vehicle is added and the cells are incubated for another 30 min^[1].

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

Animal Administration ^{[3][4]}

Mice^[3]

Male, inbred Swiss albino mice (2-3 months old) weighing 25-30 g are used. Mice are injected with a sub-convulsive dose of PTZ (35 mg/kg, i.p.) (on Mondays, Wednesdays and Fridays) of each week for a total of 11 injections. After each PTZ injection, mice are observed for 30 min and the occurrence of convulsive activity is recorded. After 30 min, the mice are then injected with either Fasudil (25 mg/kg, i.p.) or Y-27632 (5 mg/kg, i.p.) and returned to their home cages until the next injection. Control mice for Fasudil and Y-27632 receives saline.

Rats^[4]

Male Wistar Kind A rats (200-250 g) are used. DMN (1 g/mL) is diluted ten times with saline (final concentration 1%) and 10 mg/kg per day of DMN is injected intraperitoneally (i.p.) on the first 3 days of each week for 4 weeks. Y27632 is given orally once per day at a dose of 30 mg/kg for 4 weeks starting on the day of the first injection of DMN. The dose of 30 mg/kg corrects hypertension in several rat models without toxicity. Twenty rats are randomized into four experimental groups (n=5 in each group) as follows: (1) S-S (injection of saline i.p. and oral administration of saline);

(2) S-Y (injection of saline i.p. and oral administration of Y27632); (3) DMN-S (DMN i.p. and oral administration of saline); (4) DMN-Y (DMN i.p. and oral administration of Y27632). The rats are weighed every week. They are sacrificed at the end of the fourth week and the liver is excised. In addition, a blood sample is taken immediately before the rats are sacrificed.

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CUSTOMER VALIDATION

- Cell Stem Cell. 2020 Jul 14;S1934-5909(20)30288-5.
- Cell Stem Cell. 2020 Jul 2;27(1):125-136.e7.
- Cell Stem Cell. 2020 Jun 4;26(6):845-861.e12.
- Adv Sci. 2020 Jun 17;7(15):1903583.
- Biomaterials. 2020 Aug 5.

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- [2]. Xue ZW, et al. Rho-associated coiled kinase inhibitor Y-27632 promotes neuronal-like differentiation of adult human adipose tissue-derived stem cells. Chin Med J (Engl). 2012 Sep;125(18):3332-5.
- [3]. Inan S, et al. Antiepileptic effects of two Rho-kinase inhibitors, Y-27632 and fasudil, in mice. Br J Pharmacol. 2008 Sep;155(1):44-51.
- [4]. Tada S, et al. A selective ROCK inhibitor, Y27632, prevents dimethylnitrosamine-induced hepatic fibrosis in rats. J Hepatol. 2001 Apr;34(4):529-36.
- [5]. Maldonado M, et al. ROCK inhibitor primes human induced pluripotent stem cells to selectively differentiate towards mesodermal lineage via epithelial-mesenchymal transition-like modulation. Stem Cell Res. 2016 Sep;17(2):222-227.

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

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