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产品名称：Y-27632 二盐酸盐
产品别名：Y-27632

生物活性：

Description

Y-27632 is an ATP-competitive inhibitor of ROCK-I and ROCK-II, with Ki of 220 nM and 300 nM for ROCK-I and ROCK-II, respectively, which primes human induced pluripotent stem cells (hIPSCs) to selectively differentiate towards mesendodermal lineage via epithelial-mesenchymal transition-like modulation.

IC_{50} & Target

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

In Vitro

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]. 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].

In Vivo

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]. 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].

Solvent&Solubility

In Vitro:

DMSO : 50 mg/mL (**202.15 mM;** Need ultrasonic)

H_2O : 5 mg/mL (**20.22 mM;** ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	4.0430 mL	20.2151 mL	40.4302 mL
5 mM	0.8086 mL	4.0430 mL	8.0860 mL
10 mM	0.4043 mL	2.0215 mL	4.0430 mL

*请根据产品在不同溶剂中的溶解度，选择合适的溶剂配制储备液；该产品在溶液状态不稳定，建议您现用现配，即刻使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (10.11 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (10.11 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；

	向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (10.11 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (10.11 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (10.11 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (10.11 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Ishizaki T, et al. Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. Mol Pharmacol. 2000 May;57(5):976-83. [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 towardsmesendodermal lineage via epithelial-mesenchymal transition-like modulation. Stem Cell Res. 2016 Sep;17(2):222-227.

实验参考：

Cell Assay	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 μM), Y-30141, or vehicle is added and the cells are incubated for another 30 min[1].
Animal Administration	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.
Kinase Assay	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 [^{32}P]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 µL of the kinase buffer containing 50 mM HEPES-NaOH, pH 7.4, 10 mM $MgCl_2$, 5 mM $MnCl_2$, 0.02% Briji 35, and 2 mM dithiothreitol. PKCa is incubated with 5 µM [^{32}P]ATP and 200 µg/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 $CaCl_2$, 5 mM magnesium acetate, 25 µg/mL phosphatidyl serine, 50 ng/mL 12-O-tetradecanoylphorbol-13-acetate and 0.001% leupeptin in a total volume of 30 µL. Incubation is terminated by the addition of 10 µL 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].
References	[1]. Ishizaki T, et al. Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. Mol Pharmacol. 2000 May;57(5):976-83. [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 towardsmesendodermal lineage via epithelial-mesenchymal transition-like modulation. Stem Cell Res. 2016 Sep;17(2):222-227.