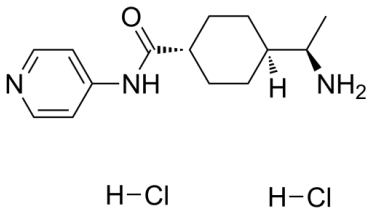
Data Sheet

Product Name:	Y-27632 (dihydrochloride)
Cat. No.:	CS-0878
CAS No.:	129830-38-2
Molecular Formula:	$C_{14}H_{23}Cl_2N_3O$
Molecular Weight:	320.26
Target:	Organoid; ROCK
Pathway:	Cell Cycle/DNA Damage; Cytoskeleton; Stem Cell/Wnt; TGF-beta/Smad
Solubility:	DMSO : 33.33 mg/mL (104.07 mM; Need ultrasonic); H2O : 100 mg/mL (312.25 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

Y-27632 dihydrochloride is an orally active and ATP-competitive **ROCK** (**Rho-kinase**) inhibitor (ROCK-I K_i=220 nM; ROCK-II K_i=300 nM). Y-27632 dihydrochloride shows antiepileptic effects[1][2][3][4]. IC50 & Target:Ki: 220/300 nM (ROCK-I/II)[1] ***In Vitro:*** Y-27632 (1-5 μM; 0-60 min) promotes neuronal differentiation of adipose tissue-derived stem cells (ADSCs) [3].

Y-27632 (1-5 μM; 0-60 min) induces the expression of NSE, MAP-2 and nestin in ADSCs [3]. ***In Vivo:*** Y-27632 (oral gavage; 30 mg/kg; once daily; 4 w) prevents dimethylnitrosamine-induced hepatic fibrosis in rats[1].

Y-27632 (oral gavage; 5-10 mg/kg; once) shows antiepileptic effects in epilepsy induced by PTZ and MES[2].

PROTOCOL (Extracted from published papers and Only for reference)

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 [^{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 CaCl2, 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]. **Cell Assay:** Y-27632 is dissolved in water and stored[1].[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 μM), Y-30141, or vehicle is added and the cells are incubated for another 30 min[1]. **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.

References:

[1]. Tada S, et al. A selective ROCK inhibitor, Y27632, prevents dimethylnitrosamine-induced hepatic fibrosis in rats. J Hepatol. 2001 Apr;34(4):529-36.

[2]. 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.

[3]. 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.

[4]. Ishizaki T, et al. Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. Mol Pharmacol. 2000 May;57(5):976-83.

CAIndexNames:

Cyclohexanecarboxamide, 4-[(1R)-1-aminoethyl]-N-4-pyridinyl-, hydrochloride (1:2), trans-

SMILES:

O=C([C@@H]1CC[C@]([C@H](N)C)([H])CC1)NC2=CC=NC=C2.[H]Cl.[H]Cl

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