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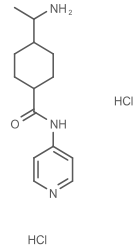
| **Product Name** | : | Y-27632 dihydrochloride |
| **Catalog Number** | : | T1725 |
| **CAS Number** | : | 129830-38-2 |
| **Molecular Formula** | : | C14H21N3O·2HCl |
| **Molecular Weight** | : | 320.26 |




**Description**: Y-27632 is a selective inhibitor of ROCKs including p160ROCK (Ki: 140 nM) and ROCK2 (IC50: 800 nM).
**Storage**: 2 years -80°C in solvent; 3 years -20°C powder;

**Solubility**

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| DMSO | 199.8 mM |
| Water | 43.7 mM |

( < 1 mg/ml refers to the product slightly soluble or insoluble )

**Receptor (IC50)**

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|---|---|
| ROCK1 (p160ROCK) | 140 nM (Ki, cell free) |
| ROCK2 | 300 nM (Ki, cell free) |

### In vitro Activity

The inhibited potency of Y-27632 against the ROCK family is 100 times 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]. The application of a selective Rho-associated kinase (ROCK) inhibitor, Y-27632, to hES cells markedly diminishes dissociation-induced apoptosis, increases cloning efficiency (from approximately 1% to approximately 27%) and facilitates subcloning after gene transfer. Furthermore, dissociated hES cells treated with Y-27632 are protected from apoptosis even in serum-free suspension (SFEB) culture and form floating aggregates [2]. 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 neuronal-like cells achieved a peak in the 5.0 μM Y-27632 induced group [3]. Y-27632 selectively inhibits smooth-muscle contraction by inhibiting Ca2+ sensitization. We identified the Y-27632 target as a Rho-associated protein kinase, p160ROCK. Y-27632 consistently suppresses Rho-induced [4].

### In vivo Activity

Y-27632 significantly decreased the blood pressure in a dose-dependent manner in spontaneously hypertensive rats: a fall of 50 mm Hg was still observed 7 h after administration of 30 mg/kg of Y-27632. The same dose of this compound also caused a significant and persistent fall in blood pressure in renal hypertensive rats, as well as in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. On the other hand, administration of the same dose of Y-27632 caused only a slight and transient fall in blood pressure in control Wistar rats [4]. Y-27632 (5-10 mg/kg) and fasudil 5-25 (mg/kg) diminished onset of myoclonic jerks, clonic convulsions and tonic hindlimb extensions in mice given pentylenetetrazole [5].

### Kinase Assay

Recombinant ROCK1/2, 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 μL of the kinase buffer containing 50 mM HEPES-NaOH, pH 7.4, 10 mM MgCl2, 5 mM MnCl2, 0.02% Briji 35, and 2 mM dithiothreitol. PKCa is incubated with 5 μM [32P]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 phosphatidylserine, 50 ng/mL 12-O-tetradecanoyl phorbol-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**

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) or vehicle is added and the cells are incubated for another 30 min [1].

Cell line:

**Animal Experiment**

Animal Model: Wistar rats with spontaneous or induced hypertension; Swiss albino mice with Ehrlich ascites carcinoma

Reference

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. Watanabe K, et al. A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol. 2007 Jun;25(6):681-6.
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. Uehata M, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature. 1997 Oct 30;389(6654):990-4.
5. 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.