Technical Data

Molecular Weight (MW)			320.26
Formula			$C_{14}H_{21}N_3O.2HCl$
CAS No.			129830-38-2
Storage	3 years -20℃powder		
	6 months-80℃in solvent		
Synonyms	N/A		
Solubility (25°C) *	*In vitro*	DMSO	64 mg/mL warmed (199.83 mM)
		Water	14 mg/mL (43.71 mM)
		Ethanol	<1 mg/mL (<1 mM)
	In vivo	Saline	30 mg/mL

* <1 mg/ml means slightly soluble or insoluble.
* Please note that Selleck tests the solubility of all compounds in-house, and the actual solubility may differ slightly from published values. This is normal and is due to slight batch-to-batch variations.

Chemical Name	(1R,4r)-4-((R)-1-aminoethyl)-N-(pyridin-4-yl)cyclohexanecarboxamide dihydrochloride

Preparing Stock Solutions

	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
50 mM	0.0624 mL	0.3122 mL	0.6245 mL

Biological Activity

Description	Y-27632 2HCl is a selective **ROCK1 (p160ROCK)** inhibitor with **K_i** of 140 nM in a cell-free assay, exhibits >200-fold selectivity over other kinases, including PKC, cAMP-dependent protein kinase, MLCK and PAK.					
Targets	ROCK1	ROCK2				
IC50	140 nM (K_i) [1]	300 nM (K_i) [6]				
In vitro	Y-27632 2HCl inhibits ROCK-II while displaying little activity against PKC, cAMP-dependent protein kinase and myosin light-chain kinase (MLCK) with K_i of 26 μM, 25 μM and > 250 μM, respectively, as well as PKA activated by another Rho-family GTPase member, Cdc42. Y-27632 2HCl inhibits smooth-muscle contraction induces by various agonists including phenylephrine, histamine, acetylcholine, serotonin, endothelin, and thromboxane with IC50 of 0.3-1 μM, by selectively inhibiting Ca^{2+} sensitization. Y-27632 2HCl suppresses Rho-induced, p160ROCK-mediated formation of stress fibres in cultured cells. [1] Y-27632 2HCl					

	treatment blocks both Rho-mediated activation of actomyosin and LPA-stimulated invasive activity of MM1 cells in a concentration-dependent manner. [2] Y-27632 2HCl treatment is not only sufficient to initiate formation of exuberant axonal processes but also facilitates axonal maturation during the very early stages of axonogenesis, while largely sparing axon elongation. [3] In human embryonic stem (hES) cells, Y-27632 2HCl treatment at 10 μM markedly diminishes dissociation-induced apoptosis even in serum-free suspension (SFEB) culture, increases cloning efficiency (from ~1% to ~27%), facilitates subcloning after gene transfer, and enables SFEB-cultured hES cells to survive and differentiate into $Bf1^+$ cortical and basal telencephalic progenitors. [4]
In vivo	Oral administration of Y-27632 2HCl at 30 mg/kg significantly decreases the blood pressure in a dose-dependent manner in spontaneous hypertensive rats, renal hypertensive rats, as well as deoxycorticosterone acetate (DOCA)-salt hypertensive rats. [1] When Y-27632 2HCl is continuously administered at a rate of 0.55 μL per hour by implanted pumps for 11 days tumor cell invasion (MM1 cells expressing Val14-RhoA in rats) is significantly delayed. [2] By inhibiting ROCK, Y-27632 2HCl treatment attenuates hypoxia-induced angiogenesis and vascular remodeling in the pulmonary circulation. [5] Pretreatment with Y-27632 has a protective effect against tumor formation in albino mice with Ehrlich ascites carcinoma. [7]
Features	

Protocol (Only for Reference)

Kinase Assay: [1]

Phosphorylation reactions	The p160ROCK is expressed in COS cells as tagged full-length proteins, and immunoprecipitated by the use of anti-tag antibodies. The p160ROCK (30 ng) is incubated with 40 μM [γ-^{32}P]ATP (3.3 Ci/mmol) and with 3 μg of either histone (HF2A), dephosphorylated casein or MBP in the presence of various concentrations of Y-27632 2HCl at 30 °C in a total volume of 31 μL. A 7 μL aliquot is taken at 0, 5, 10, and 20 minutes, mixed with an equal volume of 2 × Laemmli sample buffer, and applied to SDS-PAGE. The gel is stained with Commassie Blue, dried and subjected to analysis by a Bioimage Analyzer BAS2000. The Y-27632 2HCl concentration required to inhibit p160ROCK activity by 50% (IC50 value) is obtained. K_i value is calculated according to the equation, $K_i = IC50/(1 + S/K_m)$, where S and K_m represent concentrations of and K_m value for ATP.

Animal Study: [1] [7]

Animal Models	Male Wistar rats with spontaneous or induced hypertension; Swiss albino mice with Ehrlich ascites carcinoma
Formulation	Dissolved in DMSO, and diluted in saline (Rat); 0.9% NaCl (Mice)
Dosages	30 mg/kg/day (Rat); 0-10 mg/kg (mice)
Administration	Orally (Rat); i.p. (Mice)

Conversion of different model animals based on BSA (Value based on data from FDA Draft Guidelines)

Species	Mouse	Rat	Rabbit	Guinea pig	Hamster	Dog
Weight (kg)	0.02	0.15	1.8	0.4	0.08	10
Body Surface Area (m^2)	0.007	0.025	0.15	0.05	0.02	0.5
K_m factor	3	6	12	8	5	20

Animal A (mg/kg) = Animal B (mg/kg) multiplied by Animal B K_m

$$\overline{\text{Animal A } K_m}$$

For example, to modify the dose of resveratrol used for a mouse (22.4 mg/kg) to a dose based on the BSA for a rat, multiply 22.4 mg/kg by the K_m factor for a mouse and then divide by the K_m factor for a rat. This calculation results in a rat equivalent dose for resveratrol of 11.2 mg/kg.

$$\text{Rat dose (mg/kg)} = \text{mouse dose (22.4 mg/kg)} \times \frac{\text{mouse } K_m(3)}{\text{rat } K_m(6)} = 11.2 \text{ mg/kg}$$

1

References

[1] Uehata M, et al. Nature, 1997, 389(6654), 990-994.

[2] Itoh K, et al. Nat Med, 1999, 5(2), 221-225.

[3] Bito H, et al. Neuron, 2000, 26(2), 431-441.

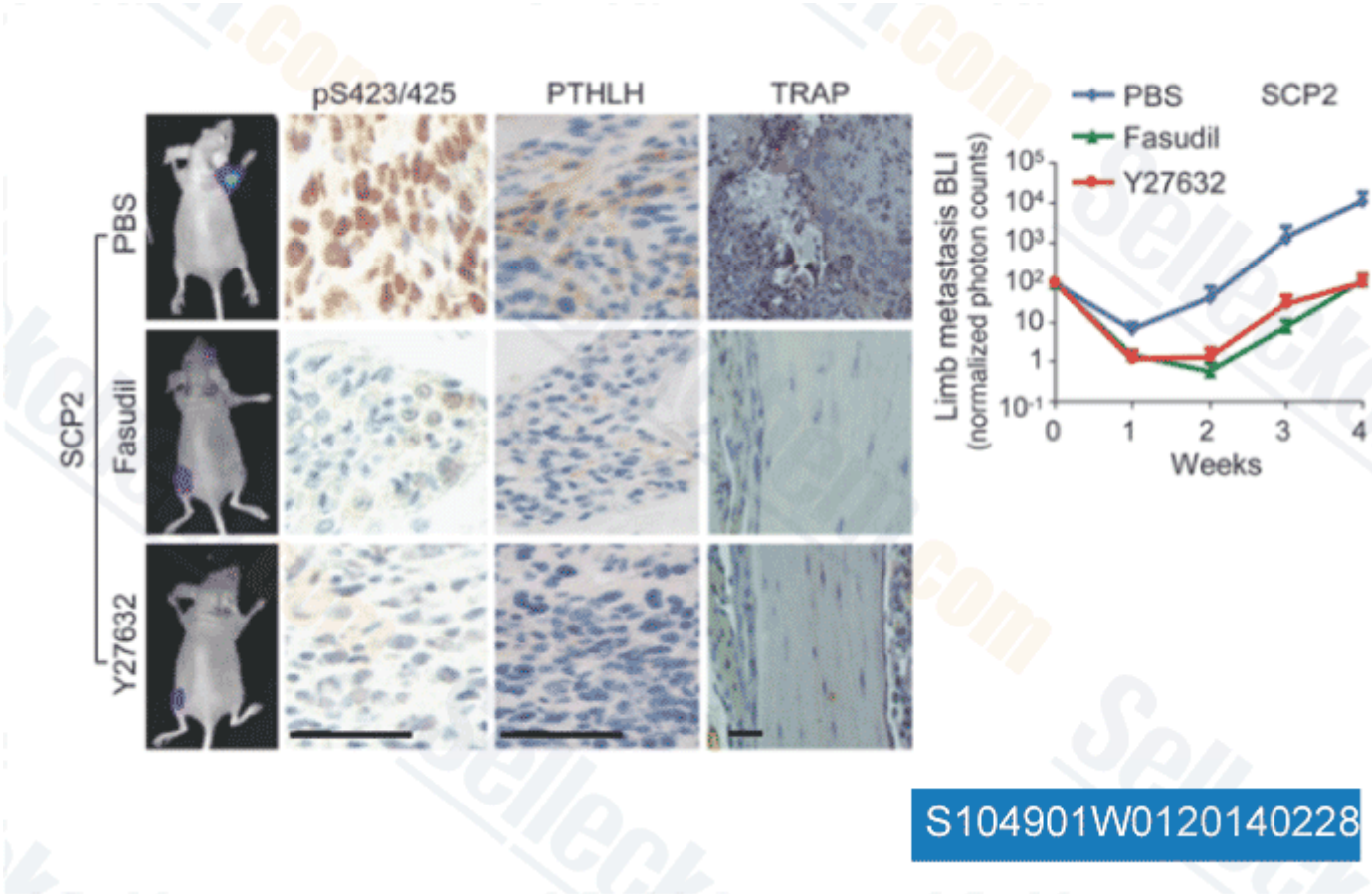
[4] Watanabe K, et al. Nat Biotechnol, 2007, 25(6), 681-686.

[5] Hyvelin JM, et al. Circ Res, 2005, 97(2), 185-191.

[6] Ishizaki T, et al. Mol Pharmacol. 2000, 57(5), 976-983.

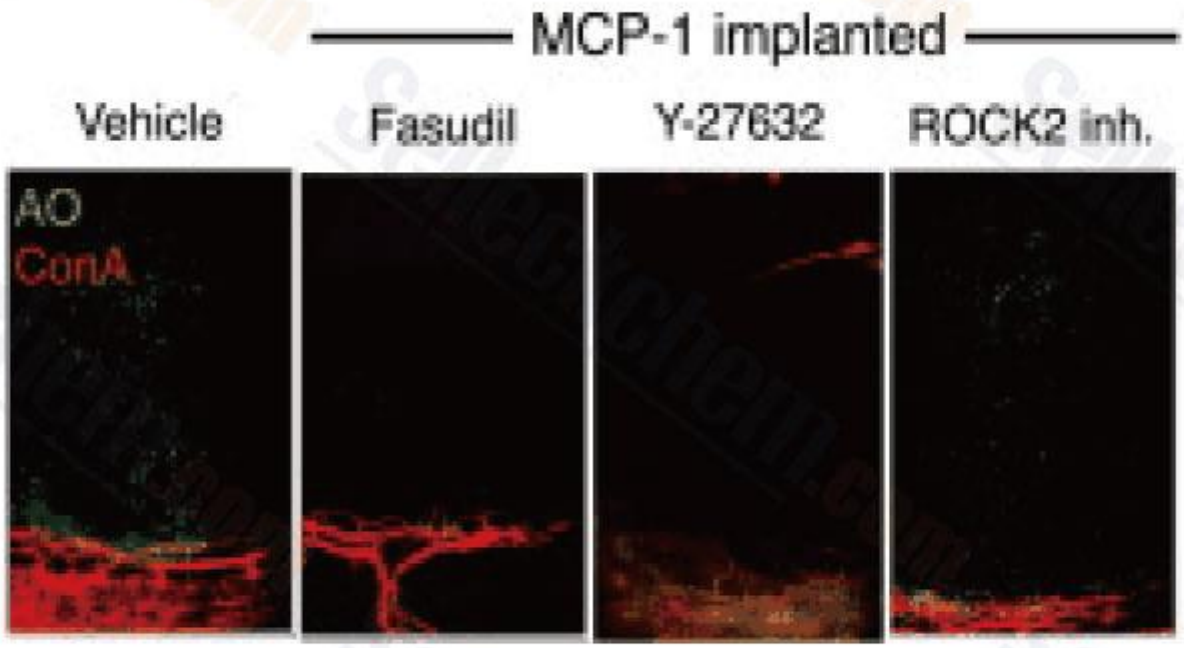
[7] Isler D, et al. Pharmacol Rep. 2014, 66(1), 114-120.

Customer Product Validation



Data from [**J Clin Invest**, 2014, 124(4), 1646-59]

Y-27632 2HCl purchased from **Selleck**



S1049Y0120150625

Data from [Data independently produced by **Cell Rep**, 2015, 10(7), 1173-86]

Y-27632 2HCl purchased from **Selleck**

ROCK-Mediated Regulation of Inflammatory Leukocyte Infiltration during CNV. Ex vivo imaging of impact of ROCK inhibitors on MCP-1-mediated leukocyte transmigration. AO(+) leukocytes and Con A(+) angiogenic vessels in MCP-1-implanted corneas, 2 hr after AO injection, 24 hr after pellet implantation with vehicle, fasudil, Y-27632, or ROCK2 inhibitor treatment. This shows quantification of the number of AO(+) leukocytes in areas of MCP-1-implanted corneas 2 hr after AO injection, 24 hr after pellet implantation.

Y-27632 2HCl has been referenced in 42 publications.