产品名称：Y-39983 HCl
产品别名：Y-33075 dihydrochloride

生物活性：

Description	Y-33075 dihydrochloride is a selective ROCK inhibitor with an IC50 of 3.6 nM.		
IC_{50} & Target	ROCK	PKC	CaMKII
	3.6 nM (IC_{50})	420 nM (IC_{50})	810 nM (IC_{50})
In Vitro	Y-33075 (Y-39983) is a potent ROCK inhibitor, with an IC_{50} of 3.6 nM. Y-33075 also inhibits PKC and CaMKII more potently than Y-27632, and the IC_{50}s of Y-27632 and Y-33075 for PKC are 9.0 μM and 0.42 μM, respectively, whereas the IC_{50}s of Y-27632 and Y-33075 for CaMKII are 26 μM and 0.81 μM, respectively. The IC_{50}s of Y-27632 and Y-33075 for PKC is 82 and 117 times those for ROCK, respectively, whereas the IC_{50}s of Y-27632 and Y-33075 for CaMKII is 236 and 225 times those for ROCK, respectively[1]. Y-33075 (Y-39983, 10 μM) extends neurites in the retinal ganglion cells (RGCs) compared with those in RGCs treated without Y-39983[2]. Y-33075 (Y-39983, 1 μM) inhibits the contraction of rabbit ciliary artery segments evoked by histamine in Ca^{2+}-free solutions. Y-33075 (10 μM) shows no effect on the $[Ca^{2+}]i$ increase with the high-potassium (high-K) solution[3].		
In Vivo	In rabbits, Y-39983 (≥ 0.01%) significantly lowers intraocular pressure (IOP) at 2 hours after topical administration. In monkeys, Y-39983 (0.05%)-treated eyes show significant reduction of IOP between 2 and 7 hours after topical administration[1]. Y-39983 (100 μM) increases the regenerating axons of retinal ganglion cells (RGCs) in the eyes of the rats[2].		
Solvent&Solubility	***In Vitro:***		
	DMSO : 100 mg/mL (**283.09 mM**; Need ultrasonic)		
	H_2O : 50 mg/mL (**141.54 mM**; Need ultrasonic)		

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.8309 mL	14.1543 mL	28.3086 mL
	5 mM	0.5662 mL	2.8309 mL	5.6617 mL
	10 mM	0.2831 mL	1.4154 mL	2.8309 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；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

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 (7.08 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.08 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 (7.08 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.08 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 (7.08 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.08 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]. Hideki Tokushige, et al. Effects of Topical Administration of Y-39983, a Selective Rho-Associated Protein Kinase Inhibitor, on Ocular Tissues in Rabbits and Monkeys Invest. Ophthalmol. Vis. Sci. July 2007 vol. 48no. 7 3216-3222 [2]. Tokushige H, et al. Effects of Y-39983, a selective Rho-associated protein kinase inhibitor, on blood flow in optic nerve head in rabbits and axonal regeneration of retinal ganglion cells in rats. Curr Eye Res. 2011 Oct;36(10):964-70. [3]. Watabe H, et al. Effects of Rho-associated protein kinase inhibitors Y-27632 and Y-39983 on isolated rabbit ciliary arteries.Jpn J Ophthalmol. 2011 Jul;55(4):411-7. Epub 2011 Jun 11.

实验参考：

Cell Assay	In brief, retinal cell suspensions are obtained from dissected retinas of Wistar rats by papain treatment. Retinal ganglion cells (RGCs) are purified by the panning method using anti-rat CD11 antibody for removal of microglia cells and anti-rat Thy-1 antibody for isolation of ganglion cells. The purified RGCs (5000 cells/plate) are seeded into 24-well plates coated by 50 μg/mL of poly-l-lysine and 2 μg/mL of merosin, and are cultured in serum-free neurobasal medium supplemented with 2% B27 supplement, 50 ng/mL BDNF, 50 ng/mL CNTF, 5 μM forskolin, and 1 mM glutamine under a 95% air-5% CO_2 atmosphere at 37°C. After completion of 24-hour cultivation, RGCs are cultured in medium with or without 10 μM Y-33075 as the final concentration for 24 hours and morphologically observed by phase-contrast microscopy. The concentration used is determined based on the effect of Y-33075 on trabecular meshwork contraction in vitro. Since this study is conducted in order to confirm whether Y-33075 has a potential of effect on axonal regeneration of RGCs, the effect is unquantitateively evaluated[2].
	In brief, SD rats is anesthetized with an intraperitoneal injection of sodium pentobarbital (0.4 mg/kg body weight), and the optic nerve of one eye is transected 4 to 6 mm posterior to the eyeball, taking care to avoid injury to the ophthalmic artery. The anterior branch of the sciatic nerve is excised and sutured autologously to the optic nerve stump with nylon sutures. The other end of the graft is sutured to the temporalis muscle. A small piece (3 mm × 3 mm) of gelatin sponge soaked with 10 μM Y-33075 or saline as a control is implanted in the space behind the optic stump after optic nerve transection in intact animals. Five μL of 0.12 mM or 1.2 mM Y-33075 solution or saline is administered into the vitreous body to final concentrations of 10 μM or 100 μM, respectively. The concentrations of Y-33075 used is determined as 10 μM that is effective in the in vitro study on axonal regeneration of RGCs, and also as 100 μM in order to confirm the dose response of

Animal Administration	Y-33075. Six weeks after surgery, rats is anesthetized with an intraperitoneal injection of sodium pentobarbital (0.4 mg/kg body weight), and 4-Di-10ASP is embedded in the transplanted sciatic nerve to retrogradely label RGCs with axonal regeneration into the sciatic nerve. Three days after dye embedding, rats is euthanized and the eyes is enucleated for preparation of retinal flat-mounts. The posterior eyecup is then separated from the vitreous body and postfixed with 4% paraformaldehyde solution in phosphate buffer for around 1 hour at room temperature. Fluorescence micrographs of the labeled cells is imported using a fluorescence microscope connected to a computer. Labeled cells is counted using image analysis software. As a normal group, the subsequent procedure for retrograde labeling with 4-Di-10ASP is performed without grafting sciatic nerve and administering the test drug. Statistical analysis is performed using logarithmically transformed values due to differences in variance among the groups. The statistical significance of differences between the normal and saline groups and the saline and Y-33075 groups is examined by t-test (onesided) and William's test (one-sided). Findings of $p < 0.05$ is considered significant[2].
Kinase Assay	Recombinant ROCK (ROK α/ROCK II), purified protein kinase C (PKC: mixture of α, β, γ isoforms), and recombinant calmodulin-dependent protein kinase II (CaMK II) are used in the assay. ROCK (0.2 U/mL) is incubated with 1 μM [γ-^{32}P] ATP and 10 μg/mL histone as substrates in the absence or presence of various concentrations of Y-27632, Y-33075, or staurosporine at room temperature for 20 minutes in 20 mM MOPS (3-(N-morpholino)propanesulfonic acid) buffer (pH 7.2) containing 0.1 mg/mL bovine serum albumin (BSA), 5 mM dithiothreitol [DTT], 10 mM β-glycerophosphate, 50 μM Na_3VO_4, and 10 mM $MgCl_2$ in a total volume of 100 μL. PKC (10 ng/mL) is incubated with 1 μM [γ-^{32}P] ATP and 20 μM PKC substrate in the absence or presence of various concentrations of Y-27632, Y-33075, or staurosporine at room temperature for 30 minutes in 20 mM MOPS buffer (pH 7.5) containing 0.1 mg/mL BSA, 10 mM DTT, 10 mM β-glycerophosphate, 50 μM Na_3VO_4, 2 mM $CaCl_2$, 20 μg/mL phosphatidyl-l-serine, and 10 mM $MgCl_2$ in a total volume of 100 μL. CaMK II (125 U/mL) is incubated with 1 μM [γ-^{32}P] ATP, 10 μM calmodulin, and 20 μM CaMK II substrate, in the absence or presence of various concentrations of Y-27632, Y-33075, or staurosporine at room temperature for 30 minutes in 20 mM MOPS buffer (pH 7.5) containing 0.2 mg/mL BSA, 0.5 mM DTT, 0.1 mM β-glycerophosphate, 50 μM Na_3VO_4, 1 mM $CaCl_2$, and 5 mM $MgCl_2$ in a total volume of 100 μL. Incubation is terminated by the addition of 100 μL of 0.7% phosphoric acid. A 160 μL portion of the mixture is transferred to Multiscreen-PH plate. A positively charged phosphocellulose filter absorbs the substrate that binds ^{32}P. The filter is washed with 300 μL of 0.5% phosphoric acid and then twice with purified water and then dried. The radioactivity of the dried filter is measured with a liquid scintillation counter. Results are presented as 50% inhibitory concentrations and 95% confidence intervals (CIs)[1].
References	[1]. Hideki Tokushige, et al. Effects of Topical Administration of Y-39983, a Selective Rho-Associated Protein Kinase Inhibitor, on Ocular Tissues in Rabbits and Monkeys Invest. Ophthalmol. Vis. Sci. July 2007 vol. 48no. 7 3216-3222 [2]. Tokushige H, et al. Effects of Y-39983, a selective Rho-associated protein kinase inhibitor, on blood flow in optic nerve head in rabbits and axonal regeneration of retinal ganglion cells in rats. Curr Eye Res. 2011 Oct;36(10):964-70. [3]. Watabe H, et al. Effects of Rho-associated protein kinase inhibitors Y-27632 and Y-39983 on isolated rabbit ciliary arteries.Jpn J Ophthalmol. 2011 Jul;55(4):411-7. Epub 2011 Jun 11.