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产品名称：H1152 Dihydrochloride
产品别名：H-1152 dihydrochloride

生物活性：

Description

H-1152 dihydrochloride is a membrane-permeable and selective ROCK inhibitor, with a K_i value of 1.6 nM, and an IC_{50} value of 12 nM for ROCK2.

IC_{50} & Target

ROCKII	CaMKII	PKG	AuroraA	PKA
12 nM (IC_{50})	0.18 μM (IC_{50})	0.36 μM (IC_{50})	0.745 μM (IC_{50})	3.03 μM (IC_{50})
Src	PKC	Abl	MKK4	MLCK
3.06 μM (IC_{50})	5.68 μM (IC_{50})	7.77 μM (IC_{50})	16.9 μM (IC_{50})	28.3 μM (IC_{50})
EGFR	GSK3α	AMPK	P38α	
50 μM (IC_{50})	60.7 μM (IC_{50})	100 μM (IC_{50})	100 μM (IC_{50})	

In Vitro

H-1152 dihydrochloride is an inhibitor of Rho-kinase, with an IC_{50} of 12 nM for ROCK2. H-1152 (H-1152P) also shows less inhibitory activities against CaMKII, PKG, AuroraA, PKA, Src, PKC, MLCK, Abl, EGFR, MKK4, GSK3α, AMPK, and P38α, with IC_{50}s of 0.180, 0.360, 0.745, 3.03, 3.06, 5.68, 28.3, 7.77, 50.0, 16.9, 60.7, 100, and 100 μM, respectively[1]. H-1152 potently inhibits Rho kinase, with a K_i of 1.6 nM, and slightly suppresses PKA, PKC and MLCK, with K_i0.63, 9.27, and 10.1 μM, respectively. H-1152 (0.1-10 μM) highly inhibits MARCKS phosphorylation, with an IC s of$_{50}$ value of 2.5 μM in LPA-treated cells, but shows no such obvious effects in PDBu-treated cells[2]. H-1152 (0.5-10 μM) cuases no decreased neuronal survival. H-1152 (1, 5 or 10 μM) also exerts no alterations in the ratios of different neuronal morphologies. Furthermore, H-1152 (10 μM) increases neurite length in both BMP4 and LIF cultures[3].

Solvent&Solubility

In Vitro:

DMSO : ≥ 32 mg/mL (81.56 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.5488 mL	12.7440 mL	25.4881 mL
5 mM	0.5098 mL	2.5488 mL	5.0976 mL
10 mM	0.2549 mL	1.2744 mL	2.5488 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→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (5.30 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (5.30 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 20.8 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。 2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.08 mg/mL (5.30 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (5.30 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 20.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Tamura M, et al. Development of specific Rho-kinase inhibitors and their clinical application. Biochim Biophys Acta. 2005 Dec 30;1754(1-2):245-52. Epub 2005 Sep 12. [2]. Ikenoya M, et al. Inhibition of rho-kinase-induced myristoylated alanine-rich C kinase substrate (MARCKS) phosphorylation in human neuronal cells by H-1152, a novel and specific Rho-kinase inhibitor. J Neurochem. 2002 Apr;81(1):9-16. [3]. Lie M, et al. Accelerated neurite growth from spiral ganglion neurons exposed to the Rho kinase inhibitor H-1152. Neuroscience. 2010 Aug 25;169(2):855-62.
实验参考：	
Cell Assay	Briefly, cells are routinely plated on poly-d-lysine/laminin coated 96 well plates or in 16 well glass culture slides. Control medium contained Dulbecco's modified Eagles medium/Hams F12(1:1) (DMEM/F12), 2 mM l-glutamine, N2 mix (1:100 dilution), 0.63 mL of 45% glucose for each 100 mL of DMEM/F12, neurotrophin 3 (NT3; final concentration, 8 ng/mL), BDNF (final concentration 8 ng/mL), and 10% fetal bovine serum heat inactivated before use. LIF cultures contain control medium+LIF (50 ng/mL). BMP4 cultures contain control medium+bone morphogenetic protein 4 (BMP4; 25 ng/mL). Total volume of culture is 110 μL. ROCK inhibitor H-1152 is diluted in water and added in an additional 10 μL to cultures 24 h after plating. Water is added to controls. Eighteen hours after the addition of inhibitor, cultures are fixed in 4% paraformaldehyde (1 h at room temperature for peroxidase-linked labeling and 20 min at room temperature for fluorescence labeling). For ArrayScan/Cellomics automated analysis: Cells are plated in a total volume of 50 μL on 384 well plastic plates previously coated with poly-d-lysine/laminin, and cultured in the same medium[3].
Kinase Assay	Inhibitors (including H-1152) are added at the indicated concentrations to 50 μL of the assay mixture 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 40 μM S6-peptide, various concentrations of [γ-^{32}P]ATP and purified Rho-kinase. The reactions are started by the addition of [γ-^{32}P]ATP and carried out at 30°C for 5 min. The Michaelis-Menten equation is used to calculate the Michaelis constant (K_m) and maximal velocity (V_{max}) of Rho-kinase. Data are further analyzed with secondary plot to calculate the inhibitory constant (K_i)[2].
	[1]. Tamura M, et al. Development of specific Rho-kinase inhibitors and their clinical application. Biochim Biophys Acta. 2005 Dec 30;1754(1-2):245-52. Epub 2005 Sep 12. [2]. Ikenoya M, et al. Inhibition of rho-kinase-induced myristoylated alanine-rich C kinase substrate

References	(MARCKS) phosphorylation in human neuronal cells by H-1152, a novel and specific Rho-kinase inhibitor. J Neurochem. 2002 Apr;81(1):9-16. [3]. Lie M, et al. Accelerated neurite growth from spiral ganglion neurons exposed to the Rho kinase inhibitor H-1152. Neuroscience. 2010 Aug 25;169(2):855-62.