H-1152 dihydrochloride

Cat. No.: HY-15720A

CAS No.: 871543-07-6

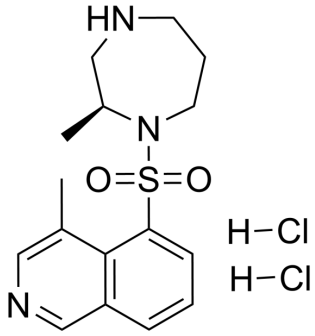
Molecular Formula: $C_{16}H_{23}Cl_2N_3O_2S$

Molecular Weight: 392.34

Target: ROCK

Pathway: Cell Cycle/DNA Damage; Cytoskeleton; Stem Cell/Wnt; TGF-beta/Smad

Storage: 4°C, sealed storage, away from moisture
* In solvent : -80°C, 2 years; -20°C, 1 year (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

In Vitro

H_2O : 35.71 mg/mL (91.02 mM; Need ultrasonic)
DMSO : 10 mg/mL (25.49 mM; ultrasonic and warming and heat to 60°C)

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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: PBS
   Solubility: 50 mg/mL (127.44 mM); Clear solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (5.30 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (5.30 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (5.30 mM); Clear solution

BIOLOGICAL ACTIVITY

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
12 nM (IC_{50})	0.18 μM (IC_{50})	0.36 μM (IC_{50})	0.745 μM (IC_{50})

	PKA 3.03 μM (IC_{50})	Src 3.06 μM (IC_{50})	PKC 5.68 μM (IC_{50})	Abl 7.77 μM (IC_{50})
	MKK4 16.9 μM (IC_{50})	MLCK 28.3 μM (IC_{50})	EGFR 50 μM (IC_{50})	GSK3α 60.7 μM (IC_{50})
	AMPK 100 μM (IC_{50})	P38α 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_is of?0.63, 9.27, and 10.1 μM, respectively. H-1152 (0.1-10 μM) highly inhibits MARCKS phosphorylation, with an IC_{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].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [2]

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].

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Cell Assay [3]

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].

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CUSTOMER VALIDATION

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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.

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