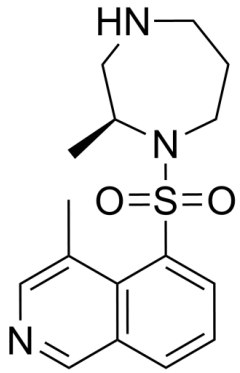
Data Sheet

Product Name:	H-1152
Cat. No.:	CS-1536
CAS No.:	451462-58-1
Molecular Formula:	$C_{16}H_{21}N_3O_2S$
Molecular Weight:	319.42
Target:	ROCK
Pathway:	Cell Cycle/DNA Damage; Cytoskeleton; Stem Cell/Wnt; TGF-beta/Smad
Solubility:	10 mM in DMSO



BIOLOGICAL ACTIVITY:

H-1152 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**. IC50 & Target: IC50: 12 nM (ROCK2)[1]
Ki: 1.6 nM (ROCK)[2] ***In Vitro:*** H-1152 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].

PROTOCOL (Extracted from published papers and Only for reference)

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]. **Cell Assay:** H-1152 is diluted in water and added in an additional 10 μL to cultures.[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].

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.

CAIndexNames:

Isoquinoline, 5-[[(2S)-hexahydro-2-methyl-1H-1,4-diazepin-1-yl]sulfonyl]-4-methyl-

SMILES:

CC1=CN=CC2=C1C(S(=O)(N3[C@@H](C)CNCCC3)=O)=CC=C2

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