Product Data Sheet

Product Name: H-1152
Cat. No.: GC36207

Chemical Properties

Cas. No.	451462-58-1		
SMILES	CC1=CN=CC2=C1C(S(=O)(N3[C@@H](C)CNCCC3)=O)=CC=C2		
Formula	$C_{16}H_{21}N_3O_2S$	M.Wt	319.42
Solubility	DMF: 15 mg/ml,DMSO: 12.5 mg/ml,Ethanol: 20 mg/ml,PBS (pH 7.2): 10 mg/ml	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	H-1152		

Protocol

Kinase experiment:

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 MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 40 µM S6-peptide, various concentrations of [γ-32P]ATP and purified Rho-kinase. The reactions are started by the addition of [γ-32P]ATP and carried out at 30°C for 5 min. The Michaelis-Menten equation is used to calculate the Michaelis constant (Km) and maximal velocity (Vmax) of Rho-kinase. Data are further analyzed with secondary plot to calculate the inhibitory constant (Ki)[2].

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Cell experiment:

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

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.

Background

Product Data Sheet

Background

Rho-associated kinase (ROCK), activated by GTP-linked Rho, phosphorylates targets that are involved in cytoskeletal remodeling, smooth muscle contraction, and neuronal development. H-1152 is a potent, specific, ATP-competitive, and cell permeable inhibitor of ROCK (K_i = 1.6 nM).[1,2] It is a more potent inhibitor of ROCK than either Y-27632 (K_i = 140 nM) or HA-1077 (K_i = 330 nM).[2] H-1152 poorly inhibits PKA, PKC, and myosin light chain kinase (K_i = 0.63, 9.27, and 10.1 μM, respectively).[2] It has been used to examine the role of ROCK in such diverse processes as stress fiber assembly,[3] vasoconstriction,[4] as well as spontaneously tonic smooth muscle[5] and neurite extension.[6]

1.Sasaki, Y., Suzuki, M., and Hidaka, H.The novel and specific Rho-kinase inhibitor (S)-(+)-2-methyl-1-[(4-methyl-5-isoquinoline)sulfonyl]-homopiperazine as a probing molecule for Rho-kinase-involved pathwayPharmacol. Ther.93225-232(2002) 2.Ikenoya, M., Hidaka, H., Hosoya, T., 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 inhibitorJ. Neurochem.819-16(2002) 3.Davies, S.L., Gibbons, C.E., Vizard, T., et al.Ca2+-sensing receptor induces Rho kinase-mediated actin stress fiber assembly and altered cell morphology, but not in response to aromatic amino acidsAm. J. Physiol. Cell Physiol.290C1543-C1551(2006) 4.Johnson, R.P., El-Yazbi, A.F., Takeya, K., et al.Ca2+ sensitization via phosphorylation of myosin phosphatase targeting subunit at threonine-855 by Rho kinase contributes to the arterial myogenic responseJ. Physiol.587(11)2537-2553(2009) 5.Rattan, S., and Patel, C.A.Selectivity of Rho kinase (ROCK) inhibitors in the spontaneously tonic smooth muscleAm. J. Physiol. Gastrointest. Liver Physiol.294(3)G687-G693(2008) 6.Fuentes, E.O., Leemhuis, J., Stark, G.B., et al.Rho kinase inhibitors Y27632 and H1152 augment neurite extension in the presence of cultured Schwann cellsJ. Brachial Plex. Peripher. Nerve Inj.3(19)(2008)

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA