BIOLOGICAL ACTIVITY:
SLx-2119 is a selective inhibitor of ROCK2 with IC$_{50}$ of 105 nM, more than 200 fold selectivity over ROCK1 (IC$_{50}$=24 μM).

In Vitro: SLx-2119 (40 μM) induces significant down-regulations of Tsp-1 and CTGF mRNA levels in PASMC. The microarray hybridized with aRNA from HMVEC treated with SLx-2119, shows a 5-times higher background than the other arrays.[1]

In Vivo: KD025 (100, 200 or 300 mg/kg, i.p.) dose-dependently reduces infarct volume after transient middle cerebral artery occlusion. KD025 is at least as efficacious in aged, diabetic or female mice, as in normal adult males.[2]

PROTOCOL (Extracted from published papers and Only for reference)
Kinase Assay: [1]Cell-free enzyme assays are performed to determine the selective inhibition of ROCK1 and ROCK2 by SLx-2119. Reactions are performed on non-binding surface microplates. Four mU of human ROCK1 and ROCK2 are used to phosphorylate 30 μM of the synthetic ROCK peptide substrate S6 Long, prepared at American Peptide with the addition of 10 μM ATP, containing $^{32}$P-ATP in the presence of 10 mM Mg$^{2+}$, 50 mM Tris, pH 7.5, 0.1 mM EGTA and 1 mM DTT at room temperature. One unit is the amount of kinase needed to catalyze the transfer of 1 nmol phosphate/min to the peptide. The reactions are allowed to proceed for 45 minutes and then stopped with 3% phosphoric acid to a final concentration of 1%. The reactions are captured on phospho cellulose filtration microplates and washed with 75 mM phosphoric acid and methanol using a vacuum manifold. Phosphorylation is measured on a Perkin-Elmer MicroBeta 1450. Cell Assay: SLx-2119 is dissolved in DMSO to obtain a stock solution of 20 mM. Western blots are used to determine whether HMVEC, NHDF and PASMC express ROCK1 and ROCK2. The cells are incubated for 24 hours in 3 mL culture media containing SLx-2119. All cells are collected at passage 3 and lysed on ice in 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5% tritonX-100, 10% glycerol, 10 mM NaF and a protease inhibitor cocktail. Protein concentration is determined using a BCA protein assay reagent. Cell lysates (35 μg) are separated on 7.5% or 12.5% SDS-PAGE polyacrylamide gels and transferred to PVDF membrane filters. Membranes are blocked in 5% non-fat milk in TBS containing 0.1% Tween 20. Blots are probed with antibodies to ROCK1, ROCK2 or actin and washed well before incubation with HRP-conjugated secondary antibodies and visualization with an enhanced chemiluminescence (ECL) kit. Animal Administration: SLx-2119 is dissolved 0.4% methylcellulose.[2] Young adult (C57BL/6, 2-3 months old, male 22-30 g, female 16-23 g), aged (C57BL/6, 12 months old, 33-52 g) are used in all experiments. Vehicle (0.4% methylcellulose) or KD025 (100, 200 or 300 mg/kg) is administered every 12 h via orogastric gavage. The dosing paradigm is chosen based on the pharmacokinetic profile after oral administration in mice. Atorvastatin (4 mg/mL) is dissolved in phosphate-buffered saline (pH 7.4) containing 45% 3-hydroxypropyl-B-cyclodextrin and 10% ethanol, and administered at a dose of 20 mg/kg per day as a single daily intraperitoneal injection for 2 weeks.

References:

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: 609-228-6898    Fax: 609-228-5909    E-mail: tech@MedChemExpress.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA