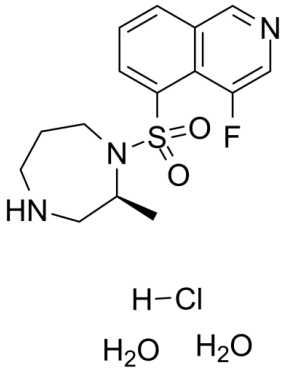
Data Sheet

Product Name:	Ripasudil
Cat. No.:	CS-3401
CAS No.:	887375-67-9
Molecular Formula:	C15H23ClFN3O4S
Molecular Weight:	395.88
Target:	ROCK
Pathway:	Cell Cycle/DNA Damage; Stem Cell/Wnt; TGF-beta/Smad
Solubility:	H2O : ≥ 50 mg/mL (126.30 mM)



BIOLOGICAL ACTIVITY:

Ripasudil (K-115) is a specific inhibitor of **ROCK**, with **IC_{50}**s of 19 and 51 nM for **ROCK2** and **ROCK1**, respectively. IC50 & Target: IC50: 19 nM (ROCK2), 51 nM (ROCK1)[1] **In Vitro:** Ripasudil (K-115) is a potent inhibitor of ROCK, with IC_{50}s of 19 and 51 nM for ROCK2 and ROCK1, respectively. Ripasudil also shows less potent inhibitory activities against CaMKIIα, PKACα and PKC, with IC_{50}s of 370 nM, 2.1 μM and 27 μM, respectively[1]. Ripasudil (K-115; 1, 10 μM) induces cytoskeletal changes, including retraction and cell rounding and reduced actin bundles of cultured trabecular meshwork (TM) cells. Ripasudil (5 μM) sifnificantly reduces transendothelial electrical resistance (TEER), and increases FITC-dextran permeability in Schlemm's canal endothelial (SCE) cell monolayers[2]. **In Vivo:** Ripasudil (K-115) reduces intraocular pressure (IOP) in a concentration-dependent manner at concentrations between 0.1% and 0.4% in monkey eyes and 0.0625% to 0.5% in rabbit eyes, respectively[1]. Ripasudil (K-115; 1 mg/kg, p.o. daily) shows a neuroprotective effect on retinal ganglion cells (RGCs) after nerve crush (NC). Ripasudil also inhibits the oxidative stress induced by axonal injury in mice. Ripasudil suppresses the time-dependent production of ROS in RGCs after NC injury[3].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]**ROCK 1 (0.75 ng/mL)** and **ROCK 2 (0.5 ng/mL)** are incubated with **various concentrations of Ripasudil**, Y-27632, or HA-1077 at 25°C for 90 min in 50 mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM EGTA, 30 mM Long S6 Kinase Substrate peptide, and 1 mM ATP in a total volume of 40 mL. PKACa, PKC, and CaMKIIa are also incubated with various concentrations of Ripasudil, Y-27632, or HA-1077. **PKACa (0.0625 ng/mL)** is incubated at 25°C for 30 min in 40 mM Tris-HCl buffer (pH 7.5) containing 20 mM $MgCl_2$, 1 mg/ mL BSA, 5 mM Kemptide peptide substrate, and 1 mM ATP in a total volume of 40 mL. **PKC (0.025 ng/mL)** is incubated at 25°C for 80 min in 20 mM Tris-HCl buffer (pH 7.5) containing 20 mM $MgCl_2$, 0.4 mM $CaCl_2$, 0.1 mg/mL BSA, 0.25 mM EGTA, 25 ng/mL phosphatidylserine, 2.5 ng/mL diacylglycerol, 0.0075% Triton-X-100, 25 mM DTT, 10 mM Neurogranin (28-43) peptide substrate, and 1 mM ATP in a total volume of 40 mL. **CaMKIIa (0.025 ng/mL)** is incubated at 25°C for 90 min in 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM $MgCl_2$, 2 mM $CaCl_2$, 0.04 mg/mL BSA, 16 mg/mL purified calmodulin from bovine testis, 500 mM DTT, 50 mM Autocamitide 2, and 1 mM ATP in a total volume of 40 mL. After incubation, 40 mL of KinaseGlo Luminescent Kinase Assay solution is added, and allowed to remain at 25°C for 10 min, and Relative Light Units (RLU) are measured using a luminometer. The RLU without test compound is set as 100% (Control value), and that without enzyme and compound is set as 0% (Normal value). The reaction rate (% of control) is then calculated from the RLU with addition of each concentration of test compounds, and the 50% inhibitory concentrations (IC_{50}) are determined by logistic regression analysis using SAS[1]. **Cell Assay:** Ripasudil is dissolved in PBS.[2]**Trabecular meshwork (TM) cells** are plated on 6 well plates at a density of **1×10^4 cells per well** in DMEM containing 10% FBS. Following overnight culture, when cells have reached semiconfluence, **1 or 10 μM of Ripasudil**, 10 μM of Y-27632, or 10 μM of fasudil are added to culture wells. **PBS is used as a control vehicle**. After 60 min, drug solutions are removed and replaced with DMEM containing 10% FBS. Cells are observed by phase-contrast microscopy and photographed 60 min after drug application and 2 h after drug removal. For immunohistochemistry, TM cells are plated on gelatin-coated 8 well chamber slides at a

density of 1 × 10^4 cells per well in DMEM containing 10% FBS. After overnight culture, when cells reach semiconfluence, cell are incubated in **Ripasudil at 1 or 10 μM**, Y-27632 at 10 μM, or fasudil at 10 μM for 60 min. PBS is used as a control vehicle. Drug solutions are removed and replaced with DMEM containing 10% FBS after 2 h. Cells are fixed with 4% paraformaldehyde in PBS for 15 min then washed with cytoskeletal buffer (10 mM MES, 150 mM NaCl, 5 mM EGTA, 5 mM $MgCl_2$, 5 mM glucose, pH 6.1) and serum buffer (10% FBS in PBS). Cells are permeabilized with 0.5% Triton X-100 in PBS for 12 min at room temperature and blocked with serum buffer for at least 2 h at 4°C. Filamentous actin (F-actin) is labeled with 0.05 mg/mL Phalloidin-TRITC for 1 h at room temperature. After washing with PBS, cells are mounted with commercial mounting medium containing DAPI and observed using a fluorescence microscope. The exposure to take images for F-actin and DAPI are 0.1 and 0.05 sec, respectively[2]. **Animal Administration:** Ripasudil is dissolved in vehicle with ophthalmic formulation.[1]Rabbits[1]
In the **rabbit** experiments, 50 mL of vehicle or **Ripasudil at concentrations of 0.0625%, 0.125%, 0.25, or 0.5%** is instilled into one **eye**. Intraocular pressure (IOP) is measured in both eyes before and 0.5, 1, 2, 3, 4, and 5 h after instillation. The contralateral eye is not treated. Animals are administered all concentrations of Ripasudil assigned using the Latin square method with intervals of at least 2 d.
Monkeys[1]
In the **monkey** experiments, 20 mL of **Ripasudil at concentrations of 0.1%, 0.2%, or 0.4%**, and latanoprost at a concentration of 0.005% are instilled into one **eye**. IOP is measured in both eyes before and 1, 2, 4, 6, and 8 h after instillation. The contralateral eye is not treated. Animals are arranged to receive all formulations with intervals of at least 1 week using the Latin square method. The IOPs are compared with the results for the instillation side at pre-dose and at each time point after instillation of Ripasudil, and are compared with both eyes at each time point.

References:

[1]. Isobe T, et al. Effects of K-115, a rho-kinase inhibitor, on aqueous humor dynamics in rabbits. Curr Eye Res. 2014 Aug;39(8):813-22.

[2]. Kaneko Y, et al. Effects of K-115 (Ripasudil), a novel ROCK inhibitor, on trabecular meshwork and Schlemm's canal endothelial cells. Sci Rep. 2016 Jan 19;6:19640.

[3]. Yamamoto K, et al. The novel Rho kinase (ROCK) inhibitor K-115: a new candidate drug for neuroprotective treatment in glaucoma. Invest Ophthalmol Vis Sci. 2014 Oct 2;55(11):7126-36.

CAIndexNames:

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

SMILES:

FC1=CN=CC2=C1C(S(N3CCCNC[C@@H]3C)(=O)=O)=CC=C2.[H]Cl.O.O

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