

Product Data Sheet

Product Name: AR-13324 mesylate

Cat. No.: GC19034

Chemical Properties

Cas. No. 1422144-42-0

SMILES CC1=CC(C)=C(C(OCC2=CC=C([C@@H](CN)C(NC3=CC(C=CN=C4)=C4C=C3)=O)C=C2)=O)C=C1.CS(=O)(O)=O.CS(=O)(O)=O

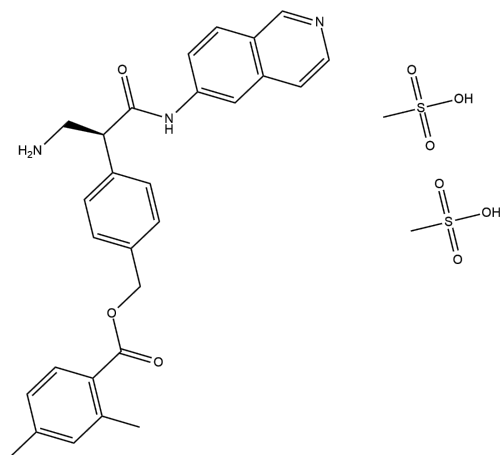
Formula $C_{30}H_{35}N_3O_9S_2$ M.Wt 645.74

Solubility DMSO : 100 mg/mL (154.86 mM; Need ultrasonic) Storage Store at -20°C

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C 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



Caution: Product has not been fully validated for medical applications. For research use only.

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Protocol

Cell experiment [1]:	
Cell lines	Primary porcine trabecular meshwork cells and primary human trabecular meshwork cells
Preparation Method	Primary porcine trabecular meshwork cells were plated on fibronectin-coated glass-bottom 96-well plates, exposed to AR-13324 mesylate, fixed, stained with Alexa Fluor 488-phalloidin and Hoechst, and analyzed for actin-stress-fiber length. Serum-starved primary human trabecular meshwork cells received vehicle, transforming growth factor- β 2, AR-13324 mesylate, or both treatments before staining for α -smooth muscle actin, fibroblast-specific protein 1, and collagen 1A.
Reaction Conditions	Primary porcine trabecular meshwork cells: 0, 0.015, 0.138, and 1.2 μ M; 6h. Primary human trabecular meshwork cells: 500nM; 24h (with an 8ng/mL transforming growth factor- β 2 co-treatment group)
Applications	AR-13324 mesylate dose-dependently disrupted actin stress fibers in primary porcine trabecular meshwork cells with an IC ₅₀ of 79nM. At 500nM, AR-13324 mesylate suppressed transforming growth factor- β 2-induced increases in fibrotic markers in primary human trabecular meshwork cells.
Animal experiment [2]:	
Animal models	Seven adult female normotensive cynomolgus monkeys weighing 3-5kg
Preparation Method	AR-13324 mesylate was instilled into one eye and an equal volume of vehicle into the contralateral eye. Outflow facility was measured before dosing and 6h afterward; aqueous-humor flow and intraocular pressure were measured across baseline and dosing days.
Dosage form	0.04%; 50 μ L/eye, administered as 25 μ L $\times$ 2; single topical ocular dose; 6h

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Applications	AR-13324 mesylate increased outflow facility by 53%, reduced intraocular pressure by 25% from baseline, and decreased aqueous-humor flow by 23% from baseline.
References: [1] Lin CW, Sherman B, Moore LA, Laethem CL, Lu DW, Pattabiraman PP, et al. Discovery and Preclinical Development of Netarsudil, a Novel Ocular Hypotensive Agent for the Treatment of Glaucoma. J Ocul Pharmacol Ther. 2018;34(1-2):40-51. doi:10.1089/jop.2017.0023. [2] Wang RF, Williamson JE, Kopczynski C, Serle JB. Effect of 0.04% AR-13324, a ROCK, and norepinephrine transporter inhibitor, on aqueous humor dynamics in normotensive monkey eyes. J Glaucoma. 2015;24(1):51-54. doi:10.1097/IJG.0b013e3182952213. [4] Kiel JW, Kopczynski CC. Effect of AR-13324 on episcleral venous pr	

Background

AR-13324 mesylate is a potent inhibitor of Rho-associated protein kinases ROCK1 and ROCK2, with K_i values of 1nM for both isoforms^[1]. AR-13324 mesylate inhibits the Rho/ROCK pathway and the norepinephrine transporter, relaxes trabecular meshwork cells, disrupts actin stress fibers and focal adhesions, and thereby promotes conventional aqueous-humor outflow^[1]. AR-13324 mesylate is used in research on trabecular meshwork function, aqueous-humor dynamics, ocular hypertension, and glaucoma-related animal models^[1].

In vitro, primary porcine trabecular meshwork cells treated with AR-13324 mesylate (0, 0.015, 0.138, and 1.2 μ M; 6h) showed dose-dependent disruption of actin stress fibers, with an IC_{50} of 79nM^[1]. In primary human trabecular meshwork cells, AR-13324 mesylate (500nM; 24h) suppressed increases in α -smooth muscle actin, fibroblast-specific protein 1, and collagen 1A induced by transforming growth factor- β 2 (8ng/mL; 24h), supporting an antifibrotic cellular response^[1]. In bioengineered extracellular-matrix hydrogels containing primary human trabecular meshwork cells, transforming growth factor- β 2 (2.5ng/mL; 5 days) was used to induce a contractile phenotype, after which AR-13324 mesylate (0.0001, 0.001, 0.01, 0.1, and 1.0 μ M; 5 days) reversed hydrogel contraction dose-

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dependently; activity was significant from 0.01 μ M and the EC₅₀ was 35.9 nM^[2].

In vivo, normotensive Dutch Belted rabbits received AR-13324 mesylate (0.005%, 0.01%, 0.02%, and 0.04%; 30 μ L/eye; topical ocular instillation once daily for 3 days), producing dose-dependent maximal intraocular-pressure reductions of 2.5 $\pm$ 0.2, 4.6 $\pm$ 0.2, 5.0 $\pm$ 0.6, and 8.1 $\pm$ 0.7 mmHg at 4 or 8 h after the third dose^[1]. In seven normotensive female cynomolgus monkeys, a single topical ocular dose of AR-13324 mesylate (0.04%; 50 μ L/eye, administered as 25 μ L $\times$ 2; 6 h) increased outflow facility by 53%, reduced intraocular pressure by 25% from baseline, and decreased aqueous-humor flow by 23% from baseline^[3]. In male Dutch Belted rabbits, AR-13324 mesylate (0.04%; topical ocular instillation once daily for 3 days; assessment 3 h after the final dose) reduced intraocular pressure by 39% and episcleral venous pressure by 35%, without changing arterial pressure, carotid blood flow, or heart rate^[4]. In living C57BL/6 and CD1 mice, a single topical ocular dose of AR-13324 mesylate (0.04%; 10 μ L/eye; 45 min) widened the trabecular meshwork and increased the cross-sectional area of Schlemm's canal and outflow vessels^[5]. In normotensive Labrador retrievers, AR-13324 mesylate (0.02%; topical ocular instillation once or twice daily for 5 days) produced no significant pressure reduction with once-daily dosing, whereas twice-daily dosing yielded a small mean reduction of 0.6 mmHg across the dosing period^[6]. In normal and ADAMTS10-open-angle-glaucoma Beagles, AR-13324 mesylate (0.02%; topical ocular instillation once daily during week 2 and twice daily during week 3) produced marginal pressure reductions that were not statistically or biologically relevant and increased conjunctival hyperemia^[7].

References:

- [1] Lin CW, Sherman B, Moore LA, Laethem CL, Lu DW, Pattabiraman PP, et al. Discovery and Preclinical Development of Netarsudil, a Novel Ocular Hypotensive Agent for the Treatment of Glaucoma. *J Ocul Pharmacol Ther.* 2018;34(1-2):40-51. doi:10.1089/jop.2017.0023.
- [2] Bagué T, Singh A, Ghosh R, Yoo H, Kelly C, deLong MA, et al. Effects of Netarsudil-Family Rho Kinase Inhibitors on Human Trabecular Meshwork Cell Contractility and Actin Remodeling Using a Bioengineered ECM Hydrogel. *Front Ophthalmol.* 2022;2:948397. doi:10.3389/fopht.2022.948397.
- [3] Wang RF, Williamson JE, Kopczynski C, Serle JB. Effect of 0.04% AR-13324, a ROCK, and norepinephrine transporter inhibitor, on aqueous humor dynamics in normotensive monkey eyes. *J Glaucoma.* 2015;24(1):51-54. doi:10.1097/IJG.0b013e3182952213.

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- [4] Kiel JW, Kopczynski CC. Effect of AR-13324 on episcleral venous pressure in Dutch belted rabbits. J Ocul Pharmacol Ther. 2015;31(3):146-151. doi:10.1089/jop.2014.0146.
- [5] Li G, Mukherjee D, Navarro I, Ashpole NE, Sherwood JM, Chang J, et al. Visualization of conventional outflow tissue responses to netarsudil in living mouse eyes. Eur J Pharmacol. 2016;787:20-31. doi:10.1016/j.ejphar.2016.04.002.
- [6] Yang VY, Miller PE, Keys DA, La Croix NC. Effects of 0.02% netarsudil ophthalmic solution on intraocular pressure of normotensive dogs. Vet Ophthalmol. 2021;24(Suppl 1):87-95. doi:10.1111/vop.12736.
- [7] Leary KA, Lin KT, Steibel JP, Harman CD, Komáromy AM. Safety and efficacy of topically administered netarsudil in normal and glaucomatous dogs with ADAMTS10-open-angle glaucoma. Vet Ophthalmol. 2021;24(Suppl 1):75-86. doi:10.1111/vop.12734.

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