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In vivo optimization of 2,3-diaminopyrazine Rho Kinase inhibitors for the treatment of glaucoma

Hwang-Hsing Chen, Abdelmoulah Namil, Bryon Severns, Jennifer Ward, Curtis Kelly, Colene Drace, Marsha A. McLaughlin, Shenouda Yacoub, Byron Li, Raj Patil, Naj Sharif, Mark R. Hellberg, Andrew Rusinko, Iok-Hou Pang, Keith D. Combrink *

Alcon Laboratories Inc, A Division of Novartis, 6201 South Freeway, Fort Worth, TX 76134, USA

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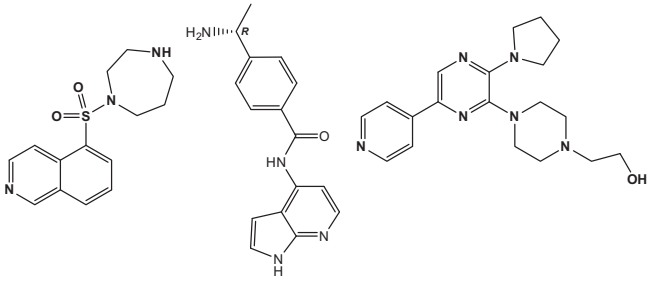
ABSTRACT

A series of 2,3,6-pyrazine Rho Kinase inhibitors were optimized for in vivo activity for topical ocular dosing. Modifications of the 2-(piperazin-1-yl)pyrazine derivatives produced compounds with improved solubility and physicochemical properties. Modifications of the 6-pyrazine substituent led to improvements in in vitro potency. Compound **9** had the best in vitro and in vivo potency of EC_{50} = 260 nM with a 30% reduction of IOP in a non-human primate model at a dose of 0.33%.

ROCK, Rho associated protein kinase is from the ACG family of serine/threonine kinases.[1] ROCK exists in two isoforms ROCK I and ROCK II that are 65% homologous in amino acid sequence and 92% homology in their kinase domain.[1] ROCK phosphorylates Lim Kinase 1 (LIMK1) and Lim Kinase 2 (LIMK2) at conserved threonines in the activation loop increasing LIMK activity.[1] ROCK also directly phosphorylates myosin light chain (MLC), and the myosin-binding subunit (MYPT1) of the myosin phosphatase to inhibit catalytic activity.[1] Rho Kinase activation leads to a number of actin-myosin mediated processes such as cell motility, smooth muscle contraction and phagocytosis.[1] Inhibition of ROCK has been explored as a therapeutic target for hypertension,[2–6] regeneration of nerve fibers,[7] oncology,[8] inflammatory disorders,[9] asthma[10] and glaucoma.[11–14] Fasudil, **1**, a Rho Kinase inhibitor, is used in Japan for the treatment of cerebral vasospasms after a subarachnoid hemorrhage.[15]



1, Fasudil **2**, Y-39983 **3**

Primary open angle glaucoma (POAG) is the most common form of glaucoma with elevated intraocular pressure as a risk factor for progression to visual loss.[16,17] The increase in intraocular pressure (IOP) is thought to be due to increased outflow resistance due to changes in the trabecular meshwork (TM). It has been shown that inhibition of Rho Kinase alters the response of TM cells to migration, adhesion and cell shape.[18] SNJ-1656 (**2**, Y-39983), a Rho Kinase inhibitor was recently shown to reduce intraocular pressure 22% in humans after a topical ocular instillation of a 0.1% solution.[19] AR-12286, a Rho Kinase inhibitor, has also been reported

* Corresponding author. Tel.: +1 817 253 3038.
E-mail addresses: kdcom1@yahoo.com, keith.combrink@gmail.com (K.D. Combrink).

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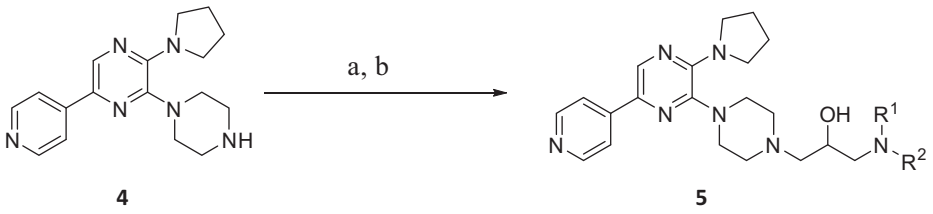
to reduce IOP in humans by 20% after a topical ocular dose of 0.25%.[20] In both cases, the IOP reduction was observed for about 6 h and IOP returned to normal by 24 h after the initial dose.

At Alcon, a previous screening effort identified a series of novel 2,3-diamino pyrazines that were further optimized to give derivative **3**.[21] Compound **3** lowered IOP by 33–37% in a non-human primate model at concentrations of 1% and two consecutive doses of 2%.[21] Due to the high concentrations required to give the desired IOP reduction a more potent analog was needed. Compound **2**, Y-39983 was slightly more potent in our cell based assay and indicated that improved cellular potency was possible. We also thought that improving the aqueous humor (AH) concentrations may also provide an improvement in in vivo potency.

The substituted alcohol Rho Kinase inhibitors were prepared by addition of piperazine **4** to an appropriately substituted epoxide (Scheme 1). The intermediate epoxide was then opened by the addition of an amine to give the desired amino alcohols. The acetamides were prepared by alkylation chemistry followed by removal of the Boc protecting group, followed by treatment of the newly liberated amine with the corresponding acid chloride (Scheme 2). The indazole derivatives **8**–**10** were prepared in a similar manner as shown below (Scheme 3).

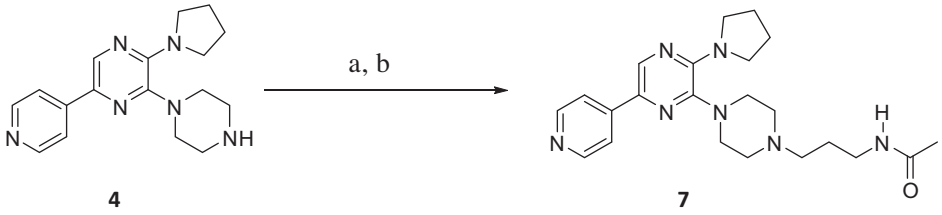
Compounds were evaluated in vitro using a commercial kinase assay kit using truncated Rho Kinase (ROCK) enzyme.[23] Rho Kinase inhibitors induce a change in cell shape in GTM3, a transformed human trabecular meshwork cell line, with cells appearing to round-up on the edges (see Fig. 1). The dose dependent and reversible response can be quantified using a cell impedance assay.[12] Several impedance assays at different doses are run to generate a dose response and an IC_{50} is calculated based on the dose response curves (Fig. 2). For pharmacokinetic studies, aqueous humor levels were measured 30 min after a single topical ocular dose in Dutch Belted rabbits.[24] In vivo studies included a guinea pig hyperemia assessment. After a single drop (10 μl) of a dose of 10–300 μg, percent incidence of hyperemia is assessed to find a no effect dose or the lowest dose that produces a hundred percent incidence of hyperemia.[25] Measurement of IOP reduction in non-human primates is determined following topical ocular application. Concentrations are increased until efficacy is established or the concentration reaches a dose that has been deemed the maximum safe dose (MDT maximum dose tested) established in a preliminary rabbit single drop study.[25]

In the pyridine series, substitution on the terminal piperazine nitrogen with alcohol substituents was generally well tolerated with all of the compounds within 2-fold of Y-39983 potency in the ROCK II enzyme assay. (Table 1, entries **3**, **5** and **6**). Activity in the cell impedance assay was 4-fold weaker for the alcohol substituent (entry **3**) and 10-fold weaker for the amino alcohol and alcohol acetamide derivatives (entries **5** and **6**) compared to **2**, Y-39983. The pyridine derivatives had generally good aqueous solubility but relatively poor activity in the cell impedance assay. With the exception of compound 5, the alcohol substituents (entries **3**, **5** and **6**) had equivalent or better AH levels but this improvement was offset by a loss of cell based potency. In the alcohol derivatives, hyperemia as judged in a guinea pig model was reduced (entries **3**, **5** and **6**) as was IOP efficacy. Amide derivative **7** had AH levels and



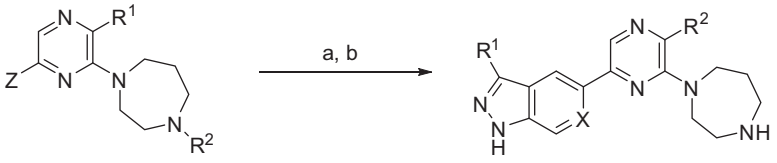
4 5

Scheme 1. Synthesis of alcohol derivatives. Reagents and conditions: (a) Compound **4**, 2-Bromo-methyloxirane, acetonitrile, microwave 140 °C, (b) NHR^1R^2, MeOH, sealed tube, 70 °C, overnight.



4 7

Scheme 2. Synthesis of acetamide derivatives. Reagents and conditions: (a), **4**, acetonitrile, (3-bromopropyl)-carbamic acid tert-butyl ester 70 °C, overnight, (b), 4.0 M HCl in dioxane, (c), CH_3COCl, DCM,TEA, RT.



8-10

Scheme 3. Synthesis of indazole derivatives. Reagents and conditions: (a), Z = Me_3Sn– 100 °C, 5-bromo-6-azaindazole,overnight, toluene, $Pd(PPh_3)_4$; Z = Cl, 1H-indazole-5-boronic acid pinacol ester, $Pd(dppf)Cl_2$, 100 °C, (b) rt 4.0 M HCl in dioxane.