ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

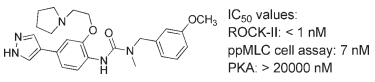
Discovery of Potent and Selective Urea-Based ROCK Inhibitors and Their Effects on Intraocular Pressure in Rats

Yan Yin,[†] Michael D. Cameron,[†] Li Lin,[†] Susan Khan,[†] Thomas Schröter,[†] Wayne Grant,[†] Jennifer Pocas,[†] Yen Ting Chen,[†] Stephan Schürer,[‡] Alok Pachori,[†] Philip LoGrasso,*[,†] and Yangbo Feng*[,†]

[†]Translational Research Institute and Department of Molecular Therapeutics, 130 Scripps Way, #2A1, Jupiter, Florida 33458, and [‡]Department of Pharmacology and Center for Computational Science, University of Miami, Miami, Florida 33136

ABSTRACT A series of urea-based Rho kinase (ROCK) inhibitors were designed and evaluated. The discovered compounds had excellent enzyme and cellular potency, high kinase selectivity, high aqueous solubility, good porcine corneal penetration, and appropriate DMPK profiles for topical applications as antiglaucoma therapeutics.

KEYWORDS Glaucoma, ROCK, IOP, kinase inhibitor, urea, pyrazole



Glaucoma is a sight-threatening optic neuropathy disease, and it is estimated that over 60 million people suffer from this disease worldwide.[1] Primary open-angle glaucoma, its most common form, is associated with blockage of aqueous humor outflow, which results in elevated intraocular pressure (IOP), leading to optic nerve damage, progressive vision loss, and irreversible blindness. Extensive efforts have been made to develop IOP-lowering drugs that balance the rate of aqueous humor produced by ciliary processes and the rate of aqueous outflow.[2−4] However, current medical treatments are unable to efficiently control IOP or lead to unacceptable side effects in many patients. Therefore, the discovery of more effective and noninvasive antiglaucoma medications is still needed.

Rho kinase (ROCK), a serine/threonine kinase, is a downstream effector of RhoA. Two isoforms of ROCK were identified as ROCK-I (or ROCKβ) and ROCK-II (ROCKα),[5] and both were shown to be expressed in human trabecular meshwork (TM) and ciliary muscle (CM) cells.[6] Upon activation by GTP-bound RhoA, ROCK phosphorylates the myosin light chain (MLC) and myosin phosphatase, leading to smooth muscle contraction.[7] Recently, ROCK has attracted numerous and diverse interest, especially as a new antiglaucoma target.[8−11] Some ATP-competitive small molecule ROCK inhibitors including Y-27632,[8] Y-39983,[9] SNJ-1656,[10] and H-1152P[11] were reported to relax the TM cells and increase the aqueous outflow facility and decrease IOP in animal models or clinical trials. SR3677 (Figure 1),[12] a potent ROCK-II inhibitor developed by our group for the treatment of glaucoma, exhibited excellent enzyme and cell-based MLC bisphosphorylation[13,14] (ppMLC) potency and high selectivity. Pharmacology studies showed that SR3677 inhibited TM MLC phosphorylation and significantly increased ex vivo aqueous humor outflow in porcine eye.[12] The hydrophobic interaction between the benzodioxane moiety of SR3677 and the hydrophobic pocket under the P-loop of

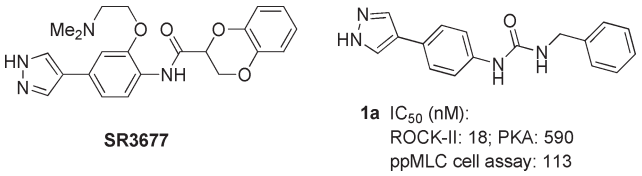


Figure 1. ROCK-II inhibitors.

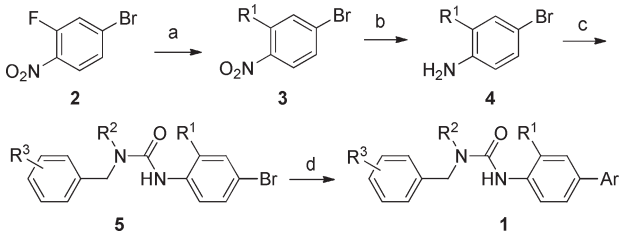
ROCK-II appears to be one of the key elements for its high potency.[12] Its high selectivity was proposed to be mainly due to the H-bond between the protonated tertiary amine of the dimethylaminoethoxy moiety and the carboxylate side chain of Asp176 of ROCK-II.[12]

To develop diversified chemotypes as novel ROCK-II inhibitors for the treatment of glaucoma, a urea-based ROCK inhibitor **1a** (Figure 1) was recently identified in our group. In this compound, an achiral benzyl amine moiety was used to replace the chiral 3-substituted benzodioxane group in SR3677. We anticipated that the benzyl group would pick up the hydrophobic interactions between the benzodioxane group and the hydrophobic pocket under the P-loop of ROCK since the distance between the carbonyl group and the terminal phenyl ring of **1a** is similar to that in SR3677 (three bonds). As shown in Figure 1, **1a** exhibited good enzyme and cellular potency (IC_{50} = 18 and 113 nM, respectively). However, the corresponding tetrahydroisoquinoline (instead of benzylamine)-based analogue of **1a** was a modest ROCK inhibitor (IC_{50} = 280 nM) despite a resemblance to the benzodioxane group. Herein, we report the optimization of **1a** to obtain potent and selective ROCK

Received Date: February 18, 2010
Accepted Date: March 27, 2010
Published on Web Date: April 05, 2010

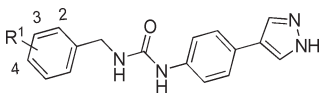
DOI: 10.1021/ml1000382 | *ACS Med. Chem. Lett.* **2010**, *1*, 175–179

Scheme 1[a]



[a] Reagents and conditions: (a) Amine or alcohol nucleophile, Cs_2CO_3, DMF. (b) $SnCl_2 \cdot H_2O$, EtOAc. (c) (i) Triphosgene, $NaHCO_3$, CH_2Cl_2, 0 °C; (ii) benzylamine derivatives, CH_2Cl_2, 0 °C. (d) Boronic acid pinacol ester, $Ph(PPh_3)_4$, K_2CO_3, dioxane, H_2O, 95 °C or (i) *bis*-pinacolato-diboron, $PdCl_2$(dppf), KOAc, dioxane, reflux; (ii) Ar−Cl, $Ph(PPh_3)_4$, dioxane, K_2CO_3, H_2O, 95 °C.

Table 1. SAR of Substitutions on the Benzyl Amine Phenyl Ring



		IC$_{50}$[a] (nM)		
compd	R^1	ROCK-II	ppMLC	PKA
1b	2-OCH_3	26	>2700	654
1c	3-OCH_3	2	4	882
1d	4-OCH_3	2750	ND[b]	ND[b]
1e	3-CH_3	33	>2700	2630
1f	3-NH_2	3	360	405
1g	3-Cl	17	41	454
1h	3-F	3	20	322

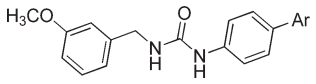
[a] IC_{50} values were means of two or more experiments with errors within 40 % of the mean. [b] Not determined.

inhibitors with appropriate properties to be used as potential antiglaucoma therapeutics.

A short route to synthesize **1a** and its analogues is shown in Scheme 1. Starting from 2-fluoro-4-bromonitrobenzene (**2**), a nucleophilic aromatic substitution reaction was applied to introduce a substitution to give 4-bromonitrobenzene **3**. Compound **3** was then reduced to aniline **4** using $SnCl_2 \cdot 2H_2O$. Urea **5** was obtained by adding a benzylamine derivative (the secondary amine was prepared by a reductive amination reaction using a substituted benzaldehyde and a primary amine and using $NaCNBH_3$ as the reducing agent in methanol at room temperature) to a mixture of **4**, triphosgene, and saturated $NaHCO_3$ in dichloromethane at 0 °C. Finally, the targeted inhibitor **1** was prepared through a Suzuki reaction or through a two-step borylation/Suzuki coupling reaction.

The structure−activity relationship (SAR) of substitutions on the benzyl ring was first investigated. As shown in Table 1, mapping the 2-, 3-, and 4-positions (compounds **1b**−**d**) demonstrated that a methoxy group at the 2- (**1b**) and 3-positions (**1c**) was well-tolerated, while substitution at the 4-position was not. The 3-position substitution was optimal with **1c** having improved enzyme and cell potency

Table 2. SAR of the Hinge-Binding Moiety



		IC$_{50}$[a] (nM)		
compd	Ar	ROCK-II	ppMLC	PKA
1i	3-methyl-1*H*-pyrazol-4-yl	2	90	816
1j	pyridin-4-yl	5	348	2304
1k	2-aminopyrimidine-4-yl	13	397	1031
1l	2-aminopyridin-4-yl	44	>2700	5142
1m	7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl	188	>2700	744

[a] IC_{50} values were means of two or more experiments with errors within 40 % of the mean.

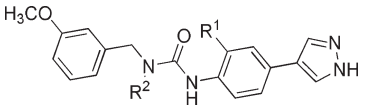
(Table 1) and higher selectivity against PKA as compared to **1a** (440- and 33-fold for **1c** and **1a**, respectively). Substitution by groups other than methoxy at the 3-position was also studied. All substituents investigated (NH_2, Me, Cl, and F) could be tolerated in the enzyme assays. However, the 3-Me (**1e**) and 3-NH_2 (**1f**) analogues showed reduced potency in cell assays. One potential explanation for the low cell potency of **1b** (as compared to **1c**) was likely due to its lower aqueous solubility caused by intramolecular H-bonding between the methoxy group and the benzyl NH group (6-membered ring). This intramolecular H-bond was not capable in the 3-position isomer (**1c**) and hence the better aqueous solubility.[15] Another possible explanation for decreased cell potency could be due to different albumin binding between **1b** and **1c**. The 3-Cl (**1g**) and 3-F (**1h**) analogues had good cell potency, but their selectivity against PKA (27- and 107-fold, respectively) was still lower than that of **1c**. Therefore, the 3-methoxyl-benzyl amine moiety was used in subsequent SAR studies.

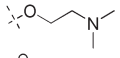
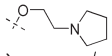
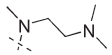
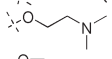
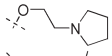
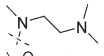
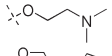
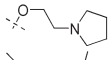
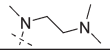
Several heterocycles were applied as the hinge-binding moiety to replace the 4-pyrazole in **1c**. As shown in Table 2, the addition of a methyl group to the 3-position of the pyrazole ring resulted in a compound (**1i**) with similar ROCK-II potency and PKA selectivity but with lower cellular potency (IC_{50} = 90 vs 4 nM in **1c**). Replacement of the pyrazole by 6-membered heterocycles, including pyridine, 2-aminopyrimidine, and 2-aminopyridine (**1j**−**l**), also gave potent ROCK inhibitors, but again, the cellular potency (IC_{50} = 348, 397, and > 2700 nM, respectively) of these compounds was much worse than that of **1c**. Application of the pyrrolopyrimidine (**1m**) group decreased not only the enzymatic potency (IC_{50} = 188 nM) but also the selectivity against PKA. As a result, compound **1c** was still the lead for further optimizations.

SAR studies on the central phenyl ring and on the urea bond were performed to improve the pharmaceutical properties of these urea-based ROCK inhibitors (Table 3). Thus, both electron-donating groups (dimethyl amino **1n** and methoxy **1o**) and electron-withdrawing groups (chloro **1p** and fluoro **1q**) were introduced to the central phenyl ring at the position ortho to the urea group. Notably, all resulting compounds **1n**−**q** exhibited excellent ROCK-II potency

DOI: 10.1021/ml1000382 | *ACS Med. Chem. Lett.* **2010**, *1*, 175–179

Table 3. SAR of Side Chains on the Central Phenyl Ring



cmpd	R^1	R^2	IC_{50} [a] (nM)		
			ROCK-II	ppMLC	PKA
1n	-$N(CH_3)_2$	H	<1	nd[b]	38
1o	-OCH_3	H	<1	4	163
1p	-Cl	H	<1	4	313
1q	-F	H	<1	28	1821
1r		H	<1	<4	3900
1s		H	<1	<4	2727
1t		H	<1	6	3573
1u		CH_3	<1	<4	5225
1v		CH_3	<1	7	>20000
1w		CH_3	8	170	4618
1x		C_2H_5	3	4	>20000
1y		C_2H_5	<1	16	18090
1z		C_2H_5	6	62	5004

[a] IC_{50} values were means of two or more experiments with errors within 40% of the mean. [b] Not determined.

(1 nM or below) and high cellular activity. However, these compounds were also potent inhibitors for PKA with the exception of **1q** (IC_{50} = 1821 nM). The introduction of a large tertiary amine-containing side chain was applied to reduce PKA inhibition and to enhance the aqueous solubility. As shown in Table 3, compounds **1r**−**t** exhibited not only excellent ROCK inhibition (IC_{50} values below 1 nM) and cell-based potency (<4, <4, and 6 nM, respectively) but also very low PKA inhibitions (IC_{50} = 3900, 2727, and 3573 nM, respectively). Alkylation of the urea NH of **1r**−**t**, which decreases the H-bonding numbers of the inhibitor, was also investigated. Both methylation (inhibitors **1u**−**w**) and ethylation (**1x**−**z**) were applied on the free NH of the benzyl amine group. These compounds exhibited excellent enzyme and cellular potency and reduced PKA activity with the exception of compounds **1w** and **1z**, where R^1 is *N*,*N*,*N*′-trimethylethyldiamine. The biochemical potency of all of the inhibitors in Table 3 exceeds that of all well-known ROCK inhibitors such as Y-27632, Fasudil, or H-1152P. Indeed, **1v** and **1y** are >110-fold more potent than Y-27632 in biochemical assays and >100-fold more potent in cell assays.[16] Similarly, Fasudil and H-1152P are both weak inhibitors in cell-based assays with IC_{50} values in the micromolar range.[16]

The selectivity of inhibitors **1x**−**z** against a few other kinases was evaluated (see Table S1 in the Supporting Information). These compounds were generally inactive against JNK3 and p38, in addition to PKA, and only moderately active against MRCKα. Screening against ROCK-I demonstrated that these compounds were pan-ROCK inhibitors, although the ROCK-I activity was lower. Inhibition to four selected cytochrome P450 isoforms, 1A2, 2C9, 2D6, and 3A4, was also studied, and results showed that inhibition of P450 was not a concern for these compounds with the highest inhibition being ~80% at 10 μM for CYP2D6 on compounds **1x** and **1z** (Table S1 of the Supporting Information).

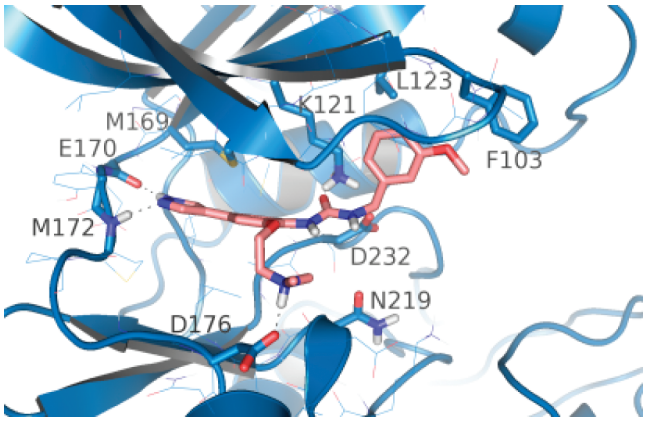


Figure 2. Compound **1r** docked to the ROCK-II ATP-binding pocket.

Inhibitor **1r** was docked into the catalytic domain of human ROCK-II by methods described previously.[12] The obtained binding motif of the enzyme−ligand complex with the highest docking score was shown in Figure 2, and the docking pose explained very well the observed SAR. There were four H-bonds between the enzyme and the ligand: two between the pyrazole headgroup and the Glu170 and Met172 in the hinge-binding region, one between the urea carbonyl group and the side-chain N−H of Lys121 in the phosphate binding site, and the last H-bond between the protonated tertiary amine of the dimethylaminoethoxy moiety of the ligand and the side chain carboxylate of Asp176 in the ribose site. In addition to hydrogen-bonding interactions, another important contributor to the high potency and selectivity of these inhibitors was the hydrophobic interaction between the benzyl amine phenyl moiety of ligand and the hydrophobic pocket under the P-loop of the enzyme, which was composed of the side chains of Phe103, Leu123, and Phe136.

High aqueous solubility and high corneal penetration are critical for these compounds to be used in glaucoma therapy by topical administration.[17] Thus, these properties of compounds **1r** and **1x**−**z** were evaluated. Results showed that these compounds had excellent aqueous solubility at pH 5.5 (1844 μg/mL for **1x**, 3147 μg/mL for **1y**, and 2847 μg/mL for **1z**), and the porcine cornea penetration is similar to that of the IOP-reducing drug, timolol.[18]

A large fraction of the administrated dose from ophthalmic drops is absorbed via the tear duct and enters systemic circulation via the nasolacrimal duct. Systemic exposure of ROCK inhibitors has the potential for cardiovascular or other systemic side effects.[16] ROCK inhibitors developed as anti-glaucoma therapeutics are expected to be rapidly metabolized upon entering general circulation to relieve these safety concerns. The rat PK properties of compounds **1r**, **1u**, and **1x**−**z** (see Table S2 in the Supporting Information) demonstrated

DOI: 10.1021/ml1000382 | ACS Med. Chem. Lett. 2010, 1, 175–179

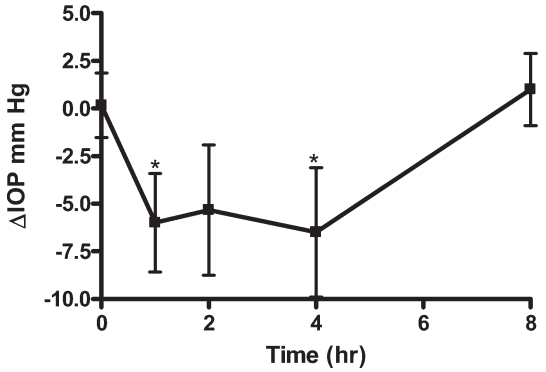


Figure 3. Rat IOP effects of **1y** (IOP decreases relative to vehicle). Decreases at 1 and 4 h were statistically significant with $p < 0.05$.

that these ROCK inhibitors have high clearance (Cl), low oral C_{max}, and very low bioavailability (F), which we believe are desirable for topical antiglaucoma applications.

To test the effect of these novel urea-based ROCK inhibitors on lowering the IOP, compound **1y** was applied to rat eyes. As shown in Figure 3, statistically significant decreases in IOP were detected when 40 μg (2 × 20 μL drops of a 0.1 % solution) of **1y** was applied to the eyes of Brown Norway rats (n = 7/group) housed under constant low light conditions. Initial IOP was 29 mmHg. This elevated IOP model has been suggested to be akin to the magnitude changes seen in glaucoma patients,[19,20] making it a reasonable experimental model reflective of clinical conditions and therefore helpful in compound development. The maximal IOP decrease was ~7 mmHg relative to that of vehicle from 1 to 4 h, returning to baseline at 8 h as compared to the vehicle. These results demonstrated that these urea-based ROCK inhibitors are good candidates for further IOP studies. One challenge will be to improve upon the duration of action for this class of compounds, which will be future optimization focus driven by ocular stability assays and insights of the inhibitors' physicochemical properties.

In conclusion, we have developed a series of potent and selective urea-based novel ROCK-II inhibitors. These inhibitors exhibited low inhibitions over PKA, MRCKα, JNK3, p38, and selected cytochrome P450 isoforms, had good aqueous solubility and corneal penetration, and possessed high systemic clearance and low oral bioavailability. All of these properties are desirable for these ROCK inhibitors to be used in the topical administration as antiglaucoma agents. Significant IOP-lowering effects on rat eyes were observed for compound **1y**, further demonstrating the effectiveness of these urea-based ROCK inhibitors as potential antiglaucoma therapeutics. Future work will be directed at improving the duration of action for these compounds so that IOP lowering could be accomplished by aq.d dosing regimen. Once these properties are achieved, ocular toxicology studies that examine conjunctival hyperemia will be investigated in appropriate species as other ROCK inhibitors have shown hyperemia in rabbits and monkeys after long-term dosing.[9]

SUPPORTING INFORMATION AVAILABLE Details for synthetic procedures, biological studies, and analytical data for compounds **1a**−**1z**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *To whom correspondence should be addressed. (Y.F.) Tel: 561-228-2201. E-mail: yfeng@scripps.edu. (P.L.) Tel: 561-228-2230. E-mail: lograsso@scripps.edu.

ACKNOWLEDGMENT We thank Professors Patrick Griffin and William R. Roush for their support. We are grateful to Dr. Derek Duckett and Weimin Chen for p38 and JNK assays and to Dr. Michael Chalmers for HRMS. Experimental help from Claudia Ruiz and Shannon Clapp are also greatly appreciated.

REFERENCES

(1) Quigley, H. A.; Broman, A. T. The number of people with glaucoma worldwide in 2010 and 2020. *Br. J. Ophthalmol.* **2006,** *90*, 262–267.
(2) Coakes, R. L.; Brubaker, R. F. The mechanism of timolol in lowering intraocular pressure in the normal eye. *Arch. Ophthalmol.* **1978,** *96*, 2045–2048.
(3) Toris, C. B.; Gleason, M. L.; Camras, C. B.; Yablonski, M. E. Effects of brimonidine on aqueous humor dynamics in human eyes. *Arch. Ophthalmol.* **1995,** *113*, 1514–1517.
(4) Lippa, E. A.; Carlson, L. E.; Ehinger, B.; Eriksson, L. O.; Finnstrom, K.; Holmin, C.; Nilsson, S. E.; Nyman, K.; Raitta, C.; Ringvold, A. Dose response and duration of action of dorzolamide, a topical carbonic anhydrase inhibitor. *Arch. Ophthalmol.* **1992,** *110*, 495–499.
(5) Nakagawa, O.; Fujisawa, K.; Ishizaki, T.; Saito, Y.; Nakao, K.; Narumiya, S. ROCK-I and ROCK-II, two isoforms of Rho-associated coil-coil forming protein serine/threonine kinase in mice. *FEBS Lett.* **1996,** *392*, 189–193.
(6) Nakajima, E.; Nakajima, T.; Minagawa, Y.; Shearer, T. R.; Azuma, M. Contribution of ROCK in contraction of trabecular meshwork: Proposed mechanism for regulating aqueous outflow in monkey and human eyes. *J. Pharm. Sci.* **2005,** *47*, 701–708.
(7) Uehata, M.; Ishizaki, T.; Satoh, H.; Ono, T.; Kawahara, T.; Morishita, T.; Tamakawa, H.; Yamagami, K.; Inui, J.; Maekawa, M.; Narumiya, S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* **1997,** *389*, 990–994.
(8) Waki, M.; Yoshida, Y.; Oka, T.; Azuma, M. Reduction of intraocular pressure by topical administration of an inhibitor of the Rho-associated protein kinase. *Curr. Eye Res.* **2001,** *22*, 470–474.
(9) Tokusbige, H.; Inatani, M.; Nemoto, S.; Sakaki, H.; Katayama, K.; Uebata, M.; Tanibara, H. Effects of topical administration of Y-39983, a selective Rho-associated protein kinase inhibitor, on ocular tissues in rabbits and monkeys. *Invest. Ophthalmol. Vis. Sci.* **2007,** *48*, 3216–3222.
(10) Tanihara, H.; Inatani, M.; Honjo, M.; Tokushige, H.; Azuma, J.; Araie, M. Intraocular pressure-lowering effects and safety of topical administration of a selective ROCK inhibitor, SNJ-1656, in healthy volunteers. *Arch. Ophthalmol.* **2008,** *126*, 309–315.
(11) Nishio, M.; Fukunaga, T.; Sugimoto, M.; Ikesugi, K.; Sumi, K.; Hidaka, H.; Uji, Y. The effect of the H-1152p, a potent Rho-associated coiled coil-formed protein kinase inhibitor, in rabbit normal and ocular hypertensive eyes. *Curr. Eye Res.* **2009,** *34*, 282–286.

DOI: 10.1021/ml1000382 | *ACS Med. Chem. Lett.* **2010,** *1*, 175–179

(12) Feng, Y.; Yin, Y.; Weiser, A.; Griffin, E.; Cameron, M.; Lin, L.; Ruiz, C.; Schürer, S. C.; Inoue, T.; Rao, V.; Schröter, T.; LoGrasso, P. Discovery of Substituted 4-(Pyrazol-4-yl)-phenylbenzodioxane-2-carboxamides as Potent and Highly Selective Rho Kinase (ROCK-II) Inhibitors. *J. Med. Chem.* **2008,** *51*, 6642–6645.

(13) Schröter, T.; Minond, D.; Weiser, A.; Dao, C.; Habel, J.; Spicer, T.; Chase, P.; Baillargeon, P.; Scampavia, L.; Schürer, S.; Chung, C.; Mader, C.; Southern, M.; Tsinoremas, N.; LoGrasso, P.; Hodder, P. Comparison of miniaturized time-resolved fluorescence resonance energy transfer and enzyme-coupled luciferase high-throughput screening assays to discovery inhibitors of Rho-kinase II (ROCK-II). *J. Biomol. Screening* **2008,** *13*, 17–28.

(14) Schröter, T.; Griffin, E.; Weiser, A.; Feng, Y.; LoGrasso, P. Detection of myosin light chain phosphorylation-A cell-based assay for screening Rho-kinase inhibitors. *Biochem. Biophys. Res. Commun.* **2008,** *374*, 356–360.

(15) As indicated in the Supporting Information, in enzyme assays, compounds were diluted directly from 90% DMSO/H_2O media into the assay plates, while in ppMLC cell assays, compounds were diluted from 20% DMSO/H_2O or aqueous buffer media into the assay plates. Therefore, there was no solubility issue for compound **1b** in the enzyme assays, but it could be a problem in the cell assay. For details, see refs 13 and 14.

(16) Ikenoya, M.; Hidaka, H.; Hosoya, T.; Suzuki, M.; Yamamoto, N.; Sasaki, Y. 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,** *81*, 9–16.

(17) Shirasaki, Y. Molecular design for enhancement of ocular penetration. *J. Pharm. Sci.* **2007,** *97*, 2462–2496.

(18) Corneal permeability was tested in a three-chamber dialysis system with porcine cornea acting as the partitions between the chambers. The cornea was orientated so that the exterior surface was facing the central compound compartment. All three chambers contained pH 7.4 buffer, and the central chamber also contained 100 μM compound. Compound levels were determined in the two terminal chambers at 4 h. Values are the means of the two chambers. The glaucoma drug timolol was used as a reference. Concentrations in the buffer chambers at the 4 h time point were 1.6 μM for timolol, 1.1 μM for **1x**, 1.5 μM for **1y**, and 1.0 μM for **1z**.

(19) Pang, I. H.; Clark, A. F. Rodent models for glaucoma retinopathy and optic neuropathy. *J. Glaucoma* **2007,** *16*, 483–505.

(20) Pang, I. H.; Wang, W. H.; Clark, A. F. Acute effects of glaucoma medications on rat intraocular pressure. *Exp. Eye Res.* **2005,** *80*, 207–214.

DOI: 10.1021/ml1000382 | *ACS Med. Chem. Lett.* **2010,** *1*, 175–179