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Novel Class of LIM-Kinase 2 Inhibitors for the Treatment of Ocular Hypertension and Associated Glaucoma

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Abstract: The discovery of a pyrrolopyrimidine class of LIM-kinase 2 (LIMK2) inhibitors is reported. These LIMK2 inhibitors show good potency in enzymatic and cellular assays and good selectivity against ROCK. After topical dosing to the eye in a steroid induced mouse model of ocular hypertension, the compounds reduce intraocular pressure to baseline levels. The compounds also increase outflow facility in a pig eye perfusion assay. These results suggest LIMK2 may be an effective target for treating ocular hypertension and associated glaucoma.

Open-angle glaucoma is a progressive neurodegenerative disease of the inner retina and optic nerve head. One significant risk factor in glaucoma is increased intraocular pressure (IOP[a]). High IOP can contribute to optic nerve head damage, resulting in vision loss and blindness. The IOP is regulated by the fluid dynamics of aqueous humor in the anterior chamber. Aqueous humor is produced by the ciliary body and flows through the anterior chamber before exiting primarily through the trabecular meshwork.[1] In ocular hypertensive patients, conventional outflow facility through the trabecular meshwork has decreased because of structural changes in the tissue.[2]

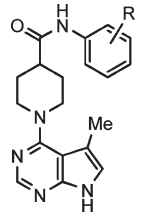
It is estimated that 2.2 million people in the U.S. suffer from open-angle glaucoma.[3] All approved therapies to treat glaucoma are directed at lowering IOP. Carbonic anhydrase inhibitors, β-blockers, and α-2 adrenergic receptor agonists decrease aqueous humor production,[4−6] and prostaglandin analogues increase uveoscleral outflow facility through structures other than the trabecular meshwork.[7] Even with the multiple therapies available, many patients experience significant side effects or fail to adequately control IOP, resulting in disease progression and the need for invasive surgery to control IOP.[8,9] It is reported that 50−75% of patients are unable to control their glaucoma with topical medical treatment.[10] Consequently, new and more effective therapies are needed.

Increasing conventional outflow facility through the trabecular meshwork is a promising approach to treat open-angle glaucoma, as this mechanism would directly reverse the causes of ocular hypertension. One approach to increasing outflow facility is to relax the trabecular meshwork cells through disruption of the actin cytoskeleton. To this end, several ROCK inhibitors have been developed that show some promise in reducing IOP in the clinic.[11]However, the reported compounds are associated with significant side effects, especially conjunctival hyperemia, which may limit their usefulness.

LIM-kinases (LIMK1 and LIMK2) are downstream from ROCK in the pathways that regulate the polymerization of actin filaments. LIM kinases phosphorylate and deactivate cofilin, a protein that depolymerizes actin filaments.[12,13] Inhibition of LIMK in the trabecular meshwork could promote depolymerization of the actin filaments, resulting in relaxation of the tissue, increased outflow facility, and reduced IOP. Similar to reported ROCK knockout mice,[14−16] LIMK2 knockout mice had lower IOP when compared to their wild type littermates.[17] As the LIM-kinases are downstream of ROCK in the signaling pathway, inhibition of these targets could be associated with fewer side effects.

Herein we report the discovery of a series of novel compounds that inhibit LIMK2[18] and their use in lowering IOP in animal models. Our objective was to develop potent inhibitors of LIMK2 with selectivity against ROCK that could be incorporated into an aqueous formulation for topical application to the eye. Compounds were assayed in a primary screen for in vitro inhibition of cofilin phosphorylation by LIMK2 and further evaluated for inhibition of cofilin phosphorylation in pig trabecular meshwork cells using Western blot analysis. Several highly potent compounds were identified in

Table 1. Aryl Analogues of Compound **1**



Compd	R	LIMK2 IC_{50} (nM)
1	H	800
5a	2-Me	11500
5b	3-Me	650
5c	4-Me	1300
5d	3-OMe	520
5e	4-OMe	1500
5f	3-OPh	64
5g	3-Br	300

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[a] Abbreviations: IOP, intraocular pressure; ROCK, Rho kinase; LIMK, LIM-kinase; HPMC, hydroxypropylmethylcelluose; SEM, standard error of the mean; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

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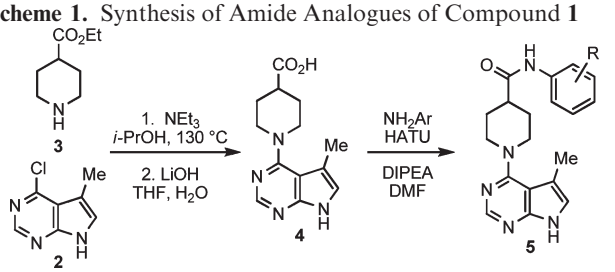
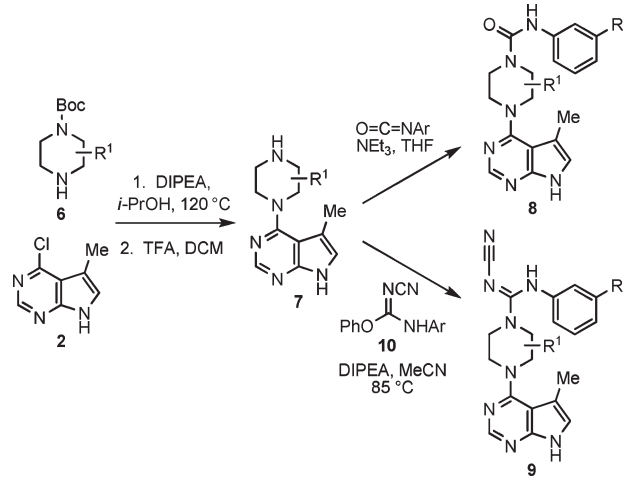
Scheme 1. Synthesis of Amide Analogues of Compound **1**



Scheme 2. Synthesis of Urea and Cyanoguanidine Analogues of Compound **1**



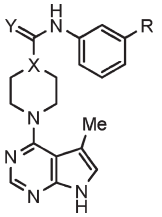
the cell based screen. A formulation screen was conducted to identify compounds suitable for topical instillation in a steroid-induced mouse model of ocular hypertension.[19]

Through high-throughput screening and elaboration of the resulting hits, we identified an active chemotype typified by pyrrolopyrimidine compound **1** (Table 1). This compound was an attractive lead due to having submicromolar activity and a modular structure amenable to rapid determination of SAR. Analogues of this compound were made to optimize potency and improve properties for topical dosing.

Amide analogues of **1** were synthesized as described in Scheme 1. Nucleophilic substitution of chloropyrrolopyrimidine **2**[20] with ethyl isonipecotate **3**, followed by saponification of the ester, gave acid **4**. Coupling of acid **4** with anilines using HATU or other coupling reagents afforded amides **5**. In addition to amides, urea and cyanoguanidine analogues of **1** were prepared (Scheme 2). Pyrrolopyrimidine **2** was reacted with substituted Boc-piperazines **6**, followed by Boc deprotection, to give amines **7**. Amines **7** could be converted to ureas **8** by reaction with isocyanates, purchased commercially or prepared from anilines and triphosgene. Alternatively, amines **7** could be converted to cyanoguanidines **9** by reaction with phenyl carbamimidates **10**, prepared from anilines and diphenyl-*N*-cyanocarbonimidate.

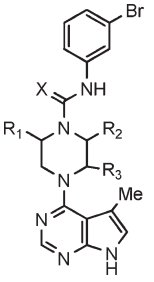
SAR around the aryl ring of **1** was quickly established as illustrated by **5a**−**g** (Table 1). Meta substitution with a variety of substituents (**5b**, **5d**, **5f**, and **5g**) gave improved potency compared with the unsubstituted parent, while para substitution (**5c** and **5e**) reduced activity and ortho substitution (**5a**) almost completely eliminated it. This trend was consistent throughout the work on the project. Especially potent in the meta position were aryl ethers. For example, phenyl ether **5f** had an IC_{50} of 64 nM. However, these aryl ethers had very

Table 2. Ureas and Cyanoguanidines



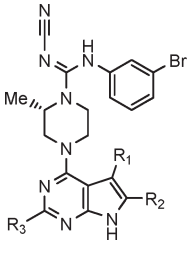
Compd	X	Y	R	LIMK2 IC_{50} (nM)
5b	CH	O	Me	650
5d	CH	O	OMe	520
5g	CH	O	Br	300
11a	N	O	Me	92
11b	N	O	OMe	140
11c	N	O	Br	38
12	N	NCN	Br	11

Table 3. Piperazine Substitution



Compd	X	R_1	R_2	R_3	LIMK2 IC_{50} (nM)
11c	O	H	H	H	38
12	NCN	H	H	H	11
13	O	(*S*)-Me	H	H	3.9
14	NCN	(*S*)-Me	H	H	0.9
15	O	(*R*)-Me	H	H	37
16	NCN	(*S*)-*i*-Pr	H	H	4.2
17	NCN	Me (trans)	H	Me	3.9
18	O	Me (cis)	Me	H	133
19	O	$-CH_2CH_2-$	$= R_1$	H	16
20	NCN	$-CH_2-$	H	$= R_1$	76

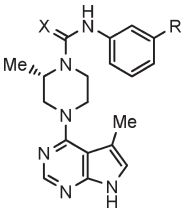
Table 4. Pyrrolopyrimidine Substitution



Compd	R_1	R_2	R_3	LIMK2 IC_{50} (nM)
14	Me	H	H	0.9
21a[a]	H	H	H	8.1
21b	Cl	H	H	1.2
21c	H	Me	H	4.2
21d	H	H	Cl	> 1000

[a] Racemate.

Table 5. Aryl Analogues with Cell-Based Activity



Compd	X	R	LIMK2 IC_{50} (nM)	Cellular % inh at 10 nM[a]
14	NCN	Br	0.9	50
22a	NSO_2Me	Br	6.8	48
22b	NCN	Cl	1.6	49
22c	O	Cl	2.3	0
22d	NCN	F	1.8	39
22e	NCN	Br, 4-F	1.5	63
22f	O	Br, 4-F	3.2	51
22g	NCN	CF_3	1.9	12
22h	NCN	CN	1.2	18
22i	NCN	*t*-Bu	1.1	10
22j	O	$OCONMe_2$	1.2	61
22k	NCN	CONH-*i*-Pr	3.5	0
22l	O	CONH-*i*-Pr	5.5	0
22m	NCN	$CONH(CH_2)_2OH$	0.8	9
22n	O	$CONH(CH_2)_2OH$	3.0	0
22o	O	$CONHCH(CH_2OH)_2$	3.0	27
22p	NCN	$CONH(CH_2)_2NMe_2$	3.4	0
22q	NCN	SO_2NH-*i*-Pr	1.7	0
22r	NCN	$SO_2NH(CH_2)_2OH$	2.9	22

[a] Inhibition of cofilin phosphorylation in pig trabecular meshwork cells as determined by Western blot analysis. Compounds tested in triplicate at 100, 10, and 1 nM. For clarity, only results at 10 nM are shown.

poor solubility which limited their use in aqueous topical formulations. Another substitution at the meta position of the aryl ring that showed good potency was bromine (**5g**). Analogues of aryl bromide **5g** had sufficient solubility to formulate for in vivo assays and were readily accessible, facilitating further SAR development.

We next turned our attention to the piperidine ring. Replacement of the piperidine with piperazine to give ureas **11a**−**c** (Table 2) improved potency 4- to 8-fold over the parent analogues **5b**, **5d**, and **5g**. For bromide **11c**, modification of the urea to give cyanoguanidine **12** gave an additional 3-fold boost in potency.

The piperazine ring allowed us to easily introduce further substitution in an effort to improve potency (Table 3). The best results were achieved by introducing a group in the S configuration at R_1. Thus, an (*S*)-methyl group at the R_1 position gave a 10-fold increase in potency in the urea (**13**) and cyanoguanidine series (**14**). The enantiomeric (*R*)-methyl group at R_1 (**15**) did not boost potency. Larger groups at R_1, such as (*S*)-*i*-Pr (**16**), were also fairly well tolerated, as were *trans*-dimethyl substitution at R_1 and R_3 (**17**) and bridging between R_1 and R_2 (**19**). However, *cis*-dimethyl substitution at R_1 and R_2 (**18**) and bridging between R_1 and R_3 (**20**) resulted in reduced potency.

The SAR around the pyrrolopyrimidine is summarized in Table 4. The methyl group at R_1 (**14**) was responsible for a significant increase in potency compared to the unsubstituted compound **21a**. The methyl group could be replaced with a chloride or other small group as in **21b** with little loss of potency. Substitution at R_2 (**21c**) was tolerated, while halogen substitution at R_3 (**21d**) was not.

Table 6. Profile of Selected Compounds

	IC_{50} (nM)				
Compd	LIMK 2	LIMK 1	ROCK 1	ROCK 2	Δ IOP ± SEM[a] (mmHg)
14	0.9	0.5	260	2700	3.7 ± 0.4
22e	1.5	3.7	310	2200	
22f	3.2	6.6	51	280	3.6 ± 0.6
22j	1.2	3.2	490	1100	3.6 ± 0.6

[a] Reduction in intraocular pressure at 1 h postdose in a dexamethsone induced ocular hypertensive mouse following topical instillation of 5 μL of 1 mg/mL xanthan gum based suspension of compound, compared to vehicle dosed mice (**14**: $n = 30$, vehicle $n = 33$; **22f**: $n = 13$, vehicle $n = 14$; 22j: $n = 18$, vehicle $n = 17$).

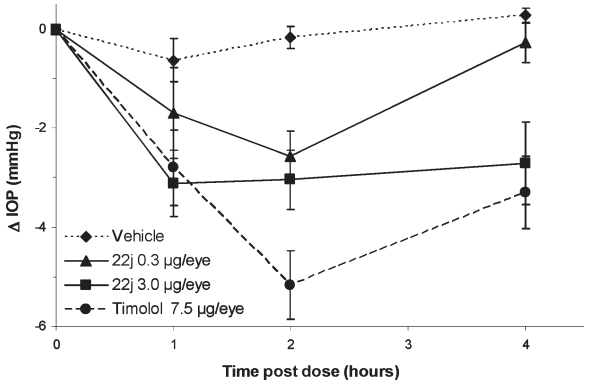


Figure 1. Change in IOP in a dexamethsone induced ocular hypertensive mouse following topical instillation of 3 μL of a 1 mg/mL or 0.1 mg/mL HPMC based solution of **22j** or of a 2.5 mg/mL solution of timolol (vehicle, $n = 10$; 0.3 μg of **22j**, $n = 10$; 3 μg of **22j**, $n = 9$; timolol, $n = 10$).

With potency established by **13** and **14**, multiple aryl analogues of ureas, cyanoguanidines, and sulfonylguanidines were prepared with the goal of optimizing selectivity, solubility, and activity in cells (Table 5). We discovered that a wide range of groups were tolerated at the meta position of the aryl ring, including halides, alkyl groups, carbamates, amides, and sulfonamides (**22a**−**r**). The compounds were further differentiated in the cell based assay. Compounds were tested at three concentrations (100, 10, and 1 nM) with the goal of identifying compounds exhibiting > 50% inhibition of cofilin phosphorylation in pig trabecular meshwork cells at 10 nM. Four compounds (**14**, **22e**, **22f**, and **22j**) were identified that met these criteria.

The four compounds active in the cellular assay were also tested for in vitro inhibition of LIMK1, ROCK1, and ROCK2 (Table 6). The compounds generally showed comparable activity between LIMK1 and LIMK2, and **14**, **22e**, and **22j** showed > 100-fold selectivity for LIMK2 against ROCK1 and > 900-fold selectivity against ROCK2. Compound **22f** was less selective, only 16-fold selective against ROCK1 and 88-fold against ROCK2.

Compounds **14**, **22f**, and **22j** were selected for in vivo experiments and were formulated as suspensions using a xanthan gum based vehicle. These were then assayed in the dexamethasone induced ocular hypertensive mouse model.[19] A single 5 μL drop of 1 mg/mL formulation of each compound was administered to one eye of an ocular hypertensive mouse, and IOP was measured after 1 h. At this 5 μg dose, **14**,

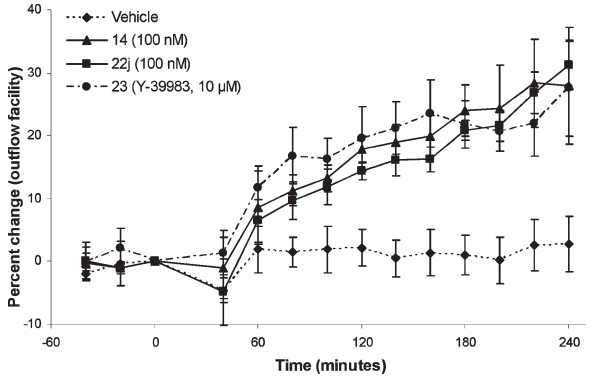


Figure 2. Change in outflow facility in a perfused pig eye upon infusion of 100 nM **14**, 100 nM **22j**, or 10 μM **23** (vehicle, $n = 6$; **14**, $n = 6$; **22j**, $n = 5$; **23**, $n = 7$).

22f, and **22j** lowered IOP by 3.6−3.7 mmHg relative to vehicle dosed control animals (Table 6). This reduction in IOP effectively represents a return to the baseline IOP measured in normotensive animals.[21] Systemic exposure on topical dosing was below the limits of quantitation for **22j** (< 18 nM).

Compound **22j** was reformulated as 1 and 0.1 mg/mL solutions using a cosolvent/HPMC based vehicle and compared to a marketed formulation of the β-blocker timolol in the ocular hypertensive mouse model at 1, 2, and 4 h postdose (Figure 1). With this formulation, **22j** showed a dose dependent response with maximal efficacy at 1−2 h (3 μg dose, ΔIOP = −3.1 mmHg). While **22j** did not have the same maximal effect as timolol (ΔIOP = −5.2 mmHg) at 2 h, the effect at other time points and the duration of action were similar.

To investigate the mechanism of action of the LIMK2 inhibitors, **14** and **22j** were also examined in a pig eye perfusion assay (Figure 2). In this experiment, a solution of the compound is infused at a constant pressure into a mounted anterior chamber of an enucleated pig eye. With fixed pressure, changes in inflow volume are directly attributable to changes in outflow facility. Consistent with the proposed mechanism of action for LIMK2 inhibitors, **14** and **22j** increased outflow facility by about 30% at 100 nM. This result compared favorably with the effects of clinical ROCK inhibitor Y-39983 (**23**)[11] which increased outflow facility by 28% at 10 μM.

In conclusion, we report the discovery of a novel class of LIMK2 inhibitors and their optimization for topical ocular dosing. Treatment of ocular hypertensive mice results in a significant reduction in IOP, and the compounds increase outflow facility in pig eye perfusion assays. These results suggest that LIMK2 may be a valid target for the treatment of glaucoma. Further elaboration and development of LIMK2 inhibitors for the treatment of glaucoma will be reported later.

Supporting Information Available: Experimental details and analytical data of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Weinreb, R. N.; Toris, C. B.; Gabelt, B. T.; Lindsey, J. D.; Kaufman, P. L. Effects of prostaglandins on the aqueous humor outflow pathways. *Surv. Ophthalmol.* **2002**, *47* (Suppl. 1), S53–S64.
(2) Toris, C. B.; Koepsell, S. A.; Yablonski, M. E.; Camras, C. B. Aqueous humor dynamics in ocular hypertensive patients. *J. Glaucoma* **2002**, *11*, 253–258.
(3) Friedman, D. S.; Wolfs, R. C.; O'Colmain, B. J.; Klein, B. E.; Taylor, H. R.; West, S.; Leske, M. C.; Mitchell, P.; Congdon, N.; Kempen, J. Prevalence of open-angle glaucoma among adults in the United States. *Arch. Ophthalmol.* **2004**, *122*, 532–538.
(4) Coakes, R. L.; Brubaker, R. F. The mechanism of timolol in lowering intraocular pressure in the normal eye. *Arch. Ophthalmol.* **1978**, *96*, 2045–2048.
(5) 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.
(6) Lippa, E. A.; Carlson, L. E.; Ehinger, B.; et al. Dose response and duration of action of dorzolamide, a topical carbonic anhydrase inhibitor. *Arch. Ophthalmol.* **1992**, *110*, 495–499.
(7) Gabelt, B. T.; Kaufman, P. L. Prostaglandin $F_{2\alpha}$ increases uveoscleral outflow in the cynomolgus monkey. *Exp. Eye Res.* **1989**, *49*, 389–402.
(8) Schwartz, K.; Budenz, D. Current management of glaucoma. *Curr. Opin. Ophthalmol.* **2004**, *15*, 119–126.
(9) Reyes, E.; Izquierdo, N. J.; Blasini, M. Adverse drugs reactions associated with glaucoma medications. *Bol.—Asoc. Med. P. R.* **1997**, *89*, 51–55.
(10) Lewis, T. (Principal Author). Optometric Clinical Practice Guideline, Care of Patient with Open Angle Glaucoma: Reference Guide for Clinicians. Updated Aug 17, **2002**. http://www.aoa.org/documents/CPG-9.pdf (accessed Sep 28, **2009**).
(11) 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.
(12) Arber, S.; Barbayannis, F. A.; Hanser, H.; Schneider, C.; Stanyon, C. A.; Bernard, O.; Caroni, P. Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. *Nature* **1998**, *393*, 805–809.
(13) Yang, N.; Higuchi, O.; Ohashi, K.; Nagata, K.; Wada, A.; Kangawa, K.; Nishida, E.; Mizuno, K. Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* **1998**, *393*, 809–812.
(14) Lindsey, J. D.; Weinreb, R. N. Elevated intraocular pressure and transgenic applications in the mouse. *J. Glaucoma* **2005**, *14*, 318–320.
(15) John, S. W.; Anderson, M. G.; Smith, R. S. Mouse genetics: a tool to help unlock the mechanisms of glaucoma. *J. Glaucoma* **1999**, *8*, 400–412.
(16) Whitlock, N. A.; Harrison, B.; Mixon, T.; Yu, X.-Q.; Wilson, A.; Gerhardt, B.; Eberhart, D. E.; Abuin, A.; Rice, D. S. Decreased intraocular pressure in mice following either pharmacological or genetic inhibition of ROCK. *J. Ocul. Pharmacol. Ther.* **2009**, *25*, 187–194.
(17) Whitlock, A; Rice, D. S.; et al. et al. Manuscript in preparation.
(18) We are aware of only one other report of LIM kinase inhibitors in the literature: Ross-Macdonald, P.; de Silva, H.; Guo, Q.; Xiao, H.; Hung, C.-Y.; Penhallow, B.; Markwalder, J.; He, L.; Attar, R. M.; Lin, T.; Seitz, S.; Tilford, C.; Wardwell-Swanson, J.; Jackson, D. Identification of a nonkinase target mediating cytotoxicity of novel kinase inhibitors. *Mol. Cancer Ther.* **2008**, *7*, 3490–3498.
(19) Whitlock, A.; McKnight, B.; Rice, D. S. Increased Intraocular Pressure in Mice Treated with Dexamethasone. *Abstracts of Papers*, 2008 Annual Meeting of the Association for Research in Vision and Ophthalmology, Ft. Lauderdale, FL; Association for Research in Vision and Ophthalmology: Rockville, MD, 2008; Abstract 2100/A211.
(20) West, R. A. 4-Hydroxypyrrolo[2,3-*d*]pyrimidine: Mannich reaction. *J. Org. Chem.* **1961**, *26*, 4959–4961.
(21) The average IOP for normotensive mice in our experiments was 13.9 ± 0.9 mmHg. The average IOP for the dexamethasone induced ocular hypertensive mice was 17.7 ± 1.1 mmHg, representing a 3.8 mmHg increase from baseline.