Discovery and Preliminary SAR of a New Class of *Rho* Kinase Inhibitors for the Treatment of Eye Diseases

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Abstract

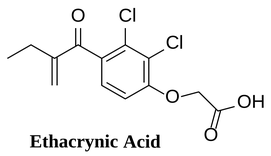
Inhibition of Rho-associated protein kinases (ROCKs) in the trabecular outflow pathway of the eye is a new approach to lower intraocular pressure (IOP) in patients with glaucoma. Aerie Pharmaceuticals has filed an NDA for netarsudil, the lead compound from a series of a*lpha*-aryl-*beta*-amino isoquinolyl amide ROCK inhibitors (*K*i = 0.2-10.3 nM). This series also demonstrates activity at other kinases and transporters. In contrast to amino acid-based kinase inhibitors, herein is presented a novel class of cyclopropyl-based kinase inhibitors that have advantages in stability. While all of the features of this class are not yet clear, they have demonstrated potent *in vivo* IOP lowering ability.

Introduction

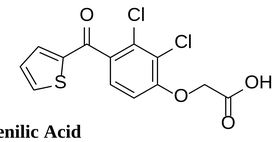
One of the most common causes of blindness worldwide is glaucoma, a group of diseases characterized by progressive optic nerve damage. Elevated IOP is a major risk factor for glaucoma and lowering IOP is currently the only approved treatment. Over the last several years, Aerie has developed new ROCK inhibitors as a way to directly treat the diseased tissue of the trabecular meshwork and lower IOP in patients with glaucoma.

The Rho Kinases are serine/threonine protein kinases that exist as 2 isoforms, ROCK1 and ROCK2, which are widely expressed in many tissues including the trabecular meshwork. ROCK promotes the assembly of actin stress fibers and focal adhesions and regulates cell contraction and motility. Inhibition of ROCK results in increased aqueous humor outflow through the trabecular meshwork with concomitant reduction of IOP in animal models, including rat and monkey.[1] SNJ-1656 (also known as Y-39983) was the first ROCK inhibitor to demonstrate an IOP-lowering effect in human subjects.[2]

Separately, research has been undertaken to investigate the IOP-lowering ability of ethacrynic acid and its analogues, such as tienilic acid.[3]



Ethacrynic Acid

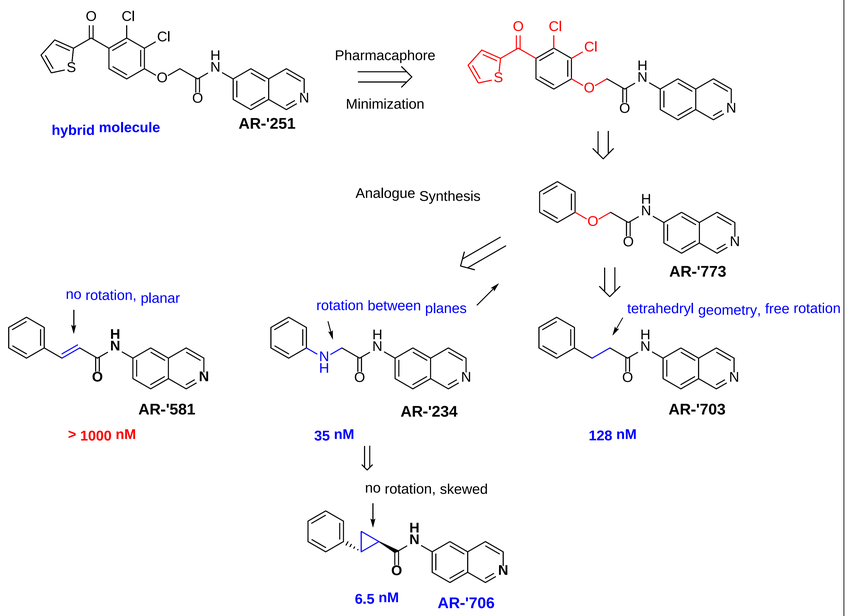


Tienilic Acid

To investigate the IOP lowering activity of this type of structure, hybrid molecules consisting of ethacrynic and tienilic acid isoquinolyl amides were synthesized. These compounds were screened for protein kinase inhibitory activity, including ROCK inhibition, and for cytotoxicity. To investigate the role that the *alpha, beta* unsaturated ester played in the activity of these molecules, in particular sulfhydryl activity, select molecules were either reduced to their corresponding alkanes, or cyclopropanated. Select compounds were tested for ocular hypotensive activity and tolerability in normotensive Dutch Belted rabbits and Formosan Rock monkeys. Representative compounds were subsequently screened for inhibitory activity against a panel of kinase proteins to assess whether a second target other than Rho kinase might contribute to their IOP-lowering activity.

[1]Rao, P.V.; Deng, P.-F.; Kumar, J.; Epstein, D.L. *Invest. Ophthalmol. Vis. Sci.* **2001**, 42, 1029.
[2]Tanihara, H.; Inatani, M.; Honjo. M.; Tokushige, H.; Azuma, J.; Araie, M. *Arch. Ophthalmol.* **2008**, 1*26*, 309
[3]Epstein, D.L.; Freddo, T.F., Basset-Chu, S.; Chung, M.; Karageuzian, L. Influence of Ethacrynic Acid on Outflow Facility in the Monkey and Calf Eye iovs.arvojournals.org/data/ Journals /IOVS/933133/2067.pdf
[4] Sturdivant J.M., Royalty, S. M.; Lin, C-W.; Moore, L.A.; Yingling, J. D.; Laethem, C.L.; Sherman, B.; Heintzelman, G.R.; Kopczynski, C.C.; deLong, M.A. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2475.

SAR Results



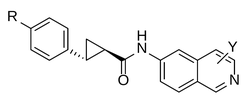


Table 1. SAR Data

Compound	R	Y	ROCK1[a] *Ki* nM	ROCK2[a] *Ki* nM	Other Kinases *Ki* nM	PTM[c] IC_{50} nM
AR-'251	n.a.	H	**55 nM**	**20 nM**	> 10,000 nM JAK3	1335 nM
AR-'773	n.a.	H	**105 nM**	**95 nM**	6700 nM JAK3	> 10,000 nM
AR-'703	n.a.	H	**128 nM**	**99 nM**	2900 nM JAK3	> 10,000 nM
AR-'581	n.a.	H	**n.t.**	**1250 nM**		> 10,000 nM
AR-'234	n.a.	H	**35 nM**	**21 nM**		7300 nM
AR-'706	H	H	**6.5 nM**	**6.3 nM**	70 nM JAK3	805 nM
AR-'193	Carboxypiperidyl amido	H	**0.5 nM**	**0.5 nM**	70 nM IKKb	55 nM
AR-'122	Piperidyl-sulfamido	Cl	**10 nM**	**10 nM**	15 nM JAK2	900 nM
AR-'215	Methyl-pyridylamido	F	**0.2 nM**	**0.1 nM**	80 nM JAK3	30 nM

n.a. not applicable, [a] ROCK enzyme inhibition assay, [b,c], Porcine (PTM) trabecular meshwork cell assays[4] ; data represent average of at least duplicate runs.
JAK: Janus kinase; IKKb: Inhibitor of NF*kappa*B kinase subunit *beta*

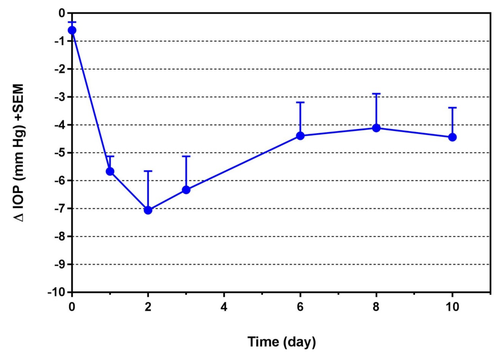
Results

Having found nM activity in the hybrid compound AR-'251, an examination of the minimum pharmacophore ensued. Stripping off all functionality, including the 'sulfhydryl-reactive portion" that had burdened ethacrynic acid-type molecules with irritation, revealed compounds that still had about 100 nM inhibitory activity at ROCK (AR-'773 & AR-'703). Removing the ability of the molecule to freely rotate by inserting an alkene (AR-'581) caused a dramatic loss of activity, while the switch from the lone-pair-containing O to the heteroatom N (AR-'234) caused a three-fold increase in potency. Making the linker rigid, but skewed by the insertion of a cyclopropyl moiety, caused a dramatic swing in activity to single-digit nM at both ROCK1 and ROCK2 (AR-'706), and introduced nM inhibitory activity at JAK3.

By adding back non-sulfhydryl-reactive groups to the core, sub-nanomolar activity at both ROCK isoforms was achieved. In addition, the changes introduced other, potentially desirable kinase inhibition such as inhibition of JAK2, JAK3 and IKKb at nanomolar levels.

In vivo testing demonstrated that compounds possessing a cyclopropyl acid amide had a distinct pharmacologic profile from compounds containing an amino acid skeleton. In rabbit studies, topical treatment with a cyclopropyl derivative produced mean IOP reductions of only 1-2 mm Hg ($p<0.01$) at 2-4 hours after dosing. Irritation scores were typically only trace (+0.5) hyperemia (Draize scale 0-3) lasting 4-8 hours. The addition of a distal nitrogen atom produced a series of compounds with maximal efficacy in the PTM assay and improved tolerability. Testing of these compounds in *in vitro* stability assays revealed that they were resistant to racemization and other forms of *in vivo* degradation. Particularly promising were the IOP reductions achieved with intracameral (IC) injection of a sustained release formulation of AR-'215 (Figure 1).

Figure 1. Rabbit ΔIOP following IC Injection (n=3)



Conclusions

Beginning with ethacrynic acid and its analogues, compounds were developed that contained cyclopropyl isoquinolylamides. The cyclopropyl compounds were potent ROCK inhibitors, and produced significant reductions in IOP in normotensive animal models. In addition, some compounds have activity against interesting anti-inflammatory targets. Removal of sulfhydryl activity preserved efficacy while improving tolerability in normotensive rabbits and monkeys. This class of Rho kinase inhibitors is undergoing further study.