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2,3-Diaminopyrazines as rho kinase inhibitors

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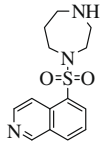
ABSTRACT

Inhibition of rho kinase (ROCK) has been recognized as an important target for a number of diseases, including glaucoma. Herein we report SAR development around two hits from a kinase library that led to the discovery of the ROCK inhibitor compound **38**. In vitro and in vivo analysis of this compound, including its effects in a monkey model of glaucoma will be discussed.

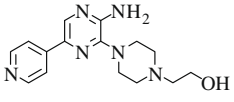
Rho kinase (Rho-associated coiled–coil-containing protein kinase or ROCK) is a serine/threonine protein kinase that is a member of the AGC family.[1] There are two known isoforms (ROCK 1 and ROCK 2) which have high sequence homology particularly in the kinase domain (92%).[2] Activation of ROCK leads to phosphorylation of various target proteins, with myosin light chain (MLC) being one of ROCK's main substrates.[3] Inhibition of ROCK has been proposed as a mechanism for treating a number of diseases including renal disease,[4] hypertension,[5] cancer,[6] ischemia[7] and glaucoma.[8] The latter represented the focus of our program, as there was strong evidence to suggest that ROCK inhibitors had the potential to lower intraocular pressure (IOP) by increasing uveoscleral and trabecular (conventional) outflow.[9] Furthermore, phase I clinical data has shown significant dose-dependant IOP lowering effects in humans using a ROCK inhibitor.[10] To date, the only marketed rho kinase inhibitor (Fasudil, **1**) is prescribed in Japan for the treatment of cerebral vasospasm after hemorrhage in the subarachnoid space leading to ischemia (Fig. 1).



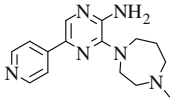
1

Figure 1. Structure of Fasudil.

Screening of a focused kinase library uncovered two aminopyrazine hits (**2** and **3**) that were of particular interest to us (Fig. 2). Although both had fairly weak ROCK activity, docking of compound **3** into a ROCK 1 homology model suggested there was scope for picking up additional binding interactions to improve the potency.



2
ROCK 1 IC_{50} = 4600 nM
ROCK 2 IC_{50} = 2500 nM



3
ROCK 1 IC_{50} = 4800 nM
ROCK 2 IC_{50} = 3100 nM

Figure 2. Hits obtained from focused kinase library.

It was decided to focus on compound **3** as the initial hit, using a structure-based approach towards improving the potency. An in-house developed homology model (Fig. 3) suggested that the 4-pyridyl substituent bound to the hinge region of the kinase. This was somewhat validated by looking at the activity of other compounds in the focused library, where non nitrogen-containing aryl replacements for pyridine, as well as other pyridine isomers resulted in a loss of activity. It was our belief that there was an opportunity to enhance the binding efficiency in the hinge region via modified pyridines, or heterocycles that had the potential to pick up additional binding interactions. Furthermore, there

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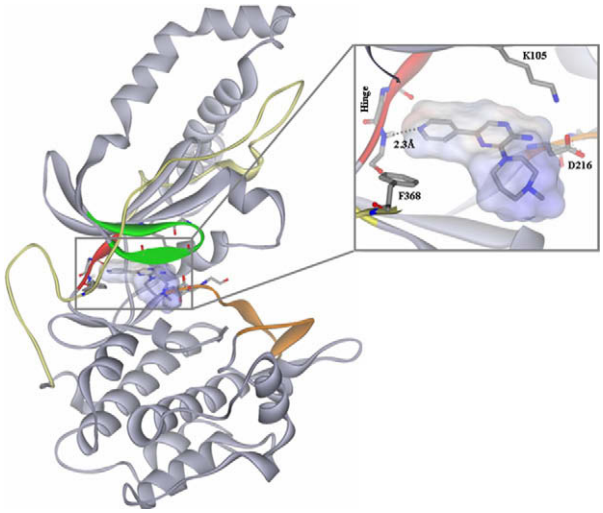


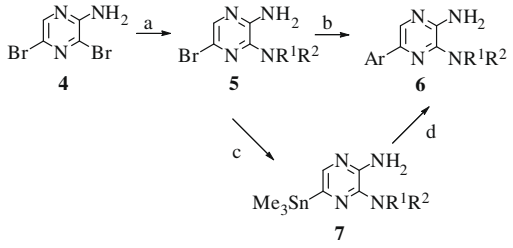
Figure 3. Docking model of **3** in the catalytic domain of ROCK 1. The activation loop (orange), glycine rich loop (green), hinge (red) and the tail (yellow) are indicated in the enzyme structure (PDB code: 2ETO).

appeared to be a pocket accessible from the 2-amino substituent that offered the potential of accessing previously unexplored space. Finally, different amino groups to replace the 3-substituted 4-methylhomopiperidine provided an opportunity to improve binding to the aspartic acid residue (D216) within the Asp-Phe-Gly (DFG) motif.

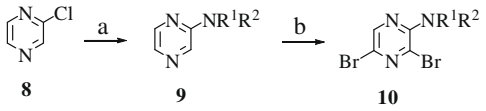
A synthetic protocol was devised to allow for diversity of analogs and rapid SAR development. 5-Aryl analogs could be easily prepared from 2-amino-3,5-dibromopyrazine as shown in Scheme 1. Regioselective SnAr displacement at the 3-position[11] provided diaminopyrazines **5** which could be elaborated to the final compounds **7** via a Suzuki coupling reaction.[12] Alternatively, the stannanes **7** could be formed[13] and coupled with a suitable aryl halide under Stille coupling conditions.[12]

For 2-substituted analogs additional steps had to be introduced (Scheme 2). 2-Chloropyrazine was functionalized with a suitable amine and dibrominated[14] to form the aminodibromopyrazines **10**. This was then progressed as in Scheme 1.

Table 1 summarizes the SAR of modifying the hinge-binding pyridine ring. Simple substituents that affected the electronic

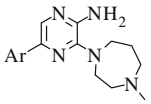


Scheme 1. Reagents and conditions: (a) HNR^1R^2, sealed tube, DMSO, 130 °C; (b) $ArB(OH)_2/ArB(OR)_2$, $Pd(dppf)Cl_2$, Na_2CO_3, DMF, H_2O, 100 °C or $ArB(OH)_2/ArB(OR)_2$, $Pd(dppf)Cl_2$, K_2CO_3, DMSO, 100 °C; (c) $(SnMe_3)_2$, $Pd(PPh_3)_4$, toluene, reflux; (d) ArBr, $Pd(PPh_3)_4$, toluene, reflux.



Scheme 2. Reagents and conditions: (a) HNR^1R^2, sealed tube, DMSO, 130 °C; (b) *N*-bromosuccinimide, DMSO, 0 °C to room temperature.

Table 1
Effect of modifications of Ar group on ROCK 1 and ROCK 2 affinity



Compound	Ar	ROCK 1[15] IC_{50}[a] (nM)	ROCK 2[15] IC_{50}[a] (nM)
3	4-Pyridine	4600	2500
11	4-(2-Methyl)pyridine	>100,000	>100,000
12	4-(3-Methyl)pyridine	120,000	58,000
13	4-(2-Amino)pyridine	25,000	53,000
14	3-Pyrazole	37,000	2900
15	4-(7-Aza)indole	1500	530
16	4-Indazole	9600	2500
17	5-Indazole	2300	950
18	6-Indazole	>10,000	>10,000

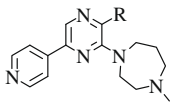
[a] IC_{50} values represent means of at least three determinations for each compound reported in the table. Standard deviation generally <50%.

nature of the pyridine or added steric bulk (methyl, amino; compounds **11–13**) were detrimental to potency. However, pyrazole or bicyclic pyridine replacements where two nitrogens were available for binding (compounds **14–18**) generally maintained the potency (particularly against ROCK 2), although the substitution pattern was important. The most effective substituent was 4-(7-aza)indole **15**, which led to a significant improvement in both ROCK 1 and ROCK 2 potency.

We next turned our attention to the 2-amino substituent. Initial attempts at replacing the primary amino group were disappointing. The monomethyl, dimethyl and diethyl analogs **19–21** provided no real improvement in potency whilst introduction of polar functionality via an ethyl linkage (compounds **22–24**) generally resulted in a drop in potency. However, introduction of a saturated heterocyclic ring system markedly improved the potency against ROCK 2 with the most potent compound **25** showing a 10-fold improvement in potency over the initial hit (Table 2). Somewhat surprisingly, heterocyclic analogs **25** and **26** showed a significant level of selectivity for ROCK 2 over ROCK 1 (18-fold and 32-fold, respectively) suggesting the pocket accessible from the 2-amino position differs between the two isoforms. Additionally, the lack of selectivity seen for the diethyl analog **21** suggests a level of conformational constraint is necessary within this pocket to improve the ROCK 2 activity.

Finally, the 3-position was targeted for SAR development. The most potent 2-amino substituent (pyrrolidine) was retained as

Table 2
Effect of modifications of 2′-R group on ROCK 1 and ROCK 2 affinity



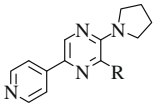
Compound	R	ROCK 1 IC_{50}[a] (nM)	ROCK 2 IC_{50}[a] (nM)
3	$-NH_2$	4600	2500
19	–NHMe	6900	2400
20	$-NMe_2$	6500	1700
21	$-NEt_2$	2600	6700
22	$-NH(CH_2)_2OH$	42,000	3000
23	$-NH(CH_2)_2OMe$	10,000	52,000
24	$-NMe(CH_2)_2OMe$	48,000	45000
25	1-Pyrrolidine	6000	330
26	1-Piperidine	18,000	560
27	1-Morpholine	68,000	6300

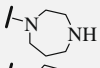
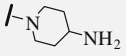
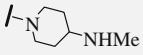
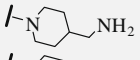
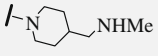
[a] IC_{50} values represent means of at least three determinations for each compound reported in the table. Standard deviation generally < 50%.

was the 4-pyridyl substituent in the 5-position (due to synthetic accessibility). Removal of the *N*-methyl substituent from the homopiperazine analog (compound **28**) produced a highly significant improvement in potency against both ROCK 1 and ROCK 2. The NH-piperazine **29** resulted in similar potency as did the 4-aminopiperidine **30** (although the N-methylated analog **31** was not as well tolerated). Further homologation of the latter two compounds led to **32** and **33** with the best combined activity (Table 3).

In order to further elaborate on the developing SAR, some additional compounds were synthesized to take advantage of the increased potency. The initial hit **2** was revisited, as was the 4-(7-aza)-indole replacement for the 4-pyridyl hinge binder (Table 4). The homopiperazine analog of scaffold A (compound **34**) produced a modest improvement in potency. However, the piperazine analog **35** caused a highly significant improvement in potency (particularly against ROCK 1) producing the best combined activity seen in this series. The 4-aminopiperidine analog **36** did not, however, have the beneficial effect seen in the 4-pyridyl series. Modifications to the hit compound **2** (scaffold B) also showed improvements. Introduction of substituted 2-amino groups had a markedly positive effect, particularly with the pyrrolidine **38**.

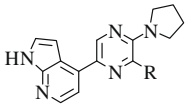
Table 3
Effect of modifications of 3′-R group on ROCK 1 and ROCK 2 affinity



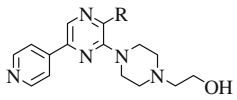
Compound	R	ROCK 1 IC_{50}[a] (nM)	ROCK 2 IC_{50}[a] (nM)
28		320	39
29		610	62
30		290	62
31		1800	250
32		130	28
33		150	49

[a] IC_{50} values represent means of at least three determinations for each compound reported in the table. Standard deviation generally < 50%.

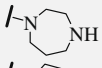
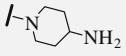
Table 4
Effect of modifications of 3′-R group on ROCK 1 and ROCK 2 affinity



A



B

Compound	Scaffold	R	ROCK 1 IC_{50}[a] (nM)	ROCK 2 IC_{50}[a] (nM)
34	A		170	21
35	A		34	17
36	A		300	89
37	B	NMe_2	1400	300
38	B	1-Pyrrolidine	300	78

[a] IC_{50} values represent means of at least three determinations for each compound reported in the table. Standard deviation generally <50%.

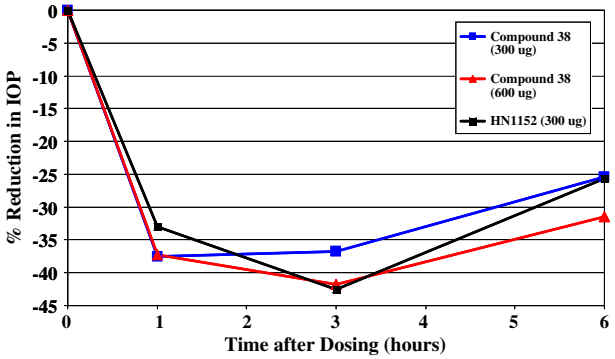


Figure 4. IOP lowering effects of compound **38** and HN1152 in the lasered monkey over a 6 h timeframe (n = 8–9). Baseline IOP was between 36.0 and 39.0 mmHg. Compounds were administered as a single 30 μL dose of a 1% solution (300 μg) or two 30 μL doses separated by at least 5 min (600 μg).

The next step in the process was to evaluate compounds of interest in a rabbit safety model of ocular dosing.[16] A number of compounds were tested for their effects on eye irritation, with compound **38** showing the best profile. Only mild hyperemia (reddening of the eye) was observed with no discharge or swelling apparent. This profile was very similar to one of the known rho kinase inhibitors, HN1152[17] (ROCK 1 IC_{50} = 55 nM, ROCK 2 IC_{50} = 34 nM) which had an excellent IOP lowering profile. Compound **38** was then tested in a further battery of in vitro assays where it was shown to have an acceptable profile (>2 μM inhibitory activity against a panel of seven cytochrome P450's,[18] 100% remaining after 1 h in human liver microsomes[19]). Additionally, compound **38** was subjected to a kinase selectivity panel where it showed high levels of selectivity. Only 20 of 213 kinases tested returned activity of greater than 50% inhibition at 10 μM. This compared favorably with HN1152 which showed activity of greater than 50% inhibition at 10 μM against 91 of the 213 kinases.

Based on the favorable in vitro data and the safety profile of compound **38**, it was decided to measure its efficacy in a model for intraocular pressure lowering (lasered cymologous monkey).[16] Compound **38** was topically dosed at 300 μg and 600 μg in a study where HN1152 was used as a positive control (Fig. 4).

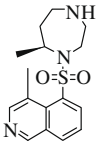
Compound **38** showed a robust, highly efficacious effect in the monkey, reducing the intraocular pressure by an average of 33% at the 300 μg dose and 37% at the 600 μg dose. This compared very well with HN1152 which produced an average reduction of 34%. It was interesting to note that at the highest dose, compound **38** had a sustained effect on the intraocular pressure that was maintained after 6 h, whereas HN1152 returned to levels matching that of the lower dose of compound **38**. Furthermore, the magnitude of the IOP lowering compares well with published data for other compounds such as Y-39983 (14.3% maximum reduction in IOP for a 0.05% solution).[20]

In conclusion, SAR development around two hits from a focused kinase library led to a number of ROCK inhibitors that had sub 500 nM activity against ROCK 1 and sub 100 nM activity against ROCK 2. One of these compounds (compound **38**) had a suitable profile for in vivo determination of its effects on lowering intraocular pressure. The compound was found to be highly effective, showing improved efficacy over HN1152 at the highest dose.

References and notes

1. Breitenlechner, C.; Gabel, M.; Engh, R.; Bossemeyer, D. *Oncol. Res.* **2004**, *14*, 267.
2. (a) Amino, M.; Fukata, Y.; Kaibuchi, K. *Exp. Cell Res.* **2000**, *261*, 44; (b) Riento, K.; Ridley, A. J. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 446.
3. Somlyo, A. P.; Somlyo, A. V. *J. Physiol.* **2000**, *522*, 177.
4. Sharpe, C. C.; Hendry, B. M. *J. Am. Soc. Nephrol.* **2003**, *14*, 261.

5. Doggrell, S. A. *Exp. Opin. Invest. Drugs* **2005**, *14*, 1157.
6. Aznar, S.; Pilar, F.-V.; Espina, C.; Lacal, J. C. *Cancer Lett.* **2004**, *206*, 181.
7. Hu, E.; Lee, D. *Exp. Opin. Ther. Targets* **2005**, *9*, 715.
8. Novak, G. D. *Exp. Opin. Emerg. Drugs* **2003**, *8*, 251.
9. Rao, P. V.; Deng, P. F.; Kumar, J.; Epstein, D. L. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 1029.
10. Tanihara, H.; Inatani, M.; Honjo, M.; Tokushige, H.; Azuma, J.; Araie, M. *Arch. Ophthalmol.* **2008**, *126*, 309.
11. Vitse, O.; Laurent, F.; Pocock, T. M.; Benezech, V.; Zamik, L.; Elliott, K. R.; Subra, G.; Portet, K.; Bompart, J.; Chapat, J. P.; Sunall, R. C.; Michel, A.; Bonnet, P. A. *Bioorg. Med. Chem.* **1999**, *7*, 1059.
12. Miyaura, N.; Suzuki, A. *J. Chem. Soc., Chem. Commun.* **1979**, 866.
13. Milstein, D.; Stille, J. K. *J. Am. Chem. Soc.* **1978**, *100*, 3636.
14. Jiang, B.; Cai-guang, Y.; Wen-Nan, X.; Wang, J. *Bioorg. Med. Chem.* **2001**, *9*, 1149.
15. ROCK 1 and ROCK 2 binding data was obtained using human recombinant rho kinase. Substrate and ATP concentrations used were 200 nM and 10 μM, respectively, while the enzyme concentration was 3.96×10^{-3}, units per well. Enzyme reaction was allowed to proceed for 60 min at 23 °C (well volume of 20 μL) then 60 μL of the binding solution (IMAP kit provided by Molecular Devices, Sunnyvale, CA) added to each well, and incubated for a further 30 min at 23 °C. Reaction mixtures were then analyzed by fluorescence polarization.
16. Hellberg, M. R.; Sallee, V. L.; McLaughlin, M. A.; Sharif, N. A.; Desantis, L.; Dean, T. R.; Zinke, P. W. *J. Ocul. Pharmacol. Ther.* **2001**, *5*, 421.
17. HN1152 (also known as H-1152 or dimethyl-fasudil) has the structure shown below.



18. The seven cytochrome P450's tested were 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4.
19. The percentage of parent compound remaining after 1 h was determined by incubation with human liver microsomes for 60 min. The reported result is a mean of two separate analyses performed in duplicate.
20. Tokushie, H.; Inatani, M.; Nemoto, S.; Sakai, H.; Katayama, K.; Uehata, M.; Tanihata, H. *Invest. Ophthalmol. Vis. Sci.* **2007**, *48*, 3216.