Letters

Discovery of Aminofurazan-azabenzimidazoles as Inhibitors of Rho-Kinase with High Kinase Selectivity and Antihypertensive Activity

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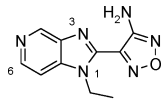
Abstract: The discovery, proposed binding mode, and optimization of a novel class of Rho-kinase inhibitors are presented. Appropriate substitution on the 6-position of the azabenzimidazole core provided subnanomolar enzyme potency in vitro while dramatically improving selectivity over a panel of other kinases. Pharmacokinetic data was obtained for the most potent and selective examples and one (**6n**) has been shown to lower blood pressure in a rat model of hypertension.

Despite many available treatments, hypertension remains a prevalent problem. In fact, some 30% of hypertensive patients are unable to reach their blood pressure goals. Thus, a new anti-hypertensive treatment, which acts on a broader patient population, would be an important addition to existing treatments.

A number of vasoconstrictive agents, including angiotensin II, endothelin-1, and urotensin-II, exert their effect through RhoA and the downstream kinase Rho-kinase (ROCK1).[1] Because of its central role in the control of smooth muscle contraction, inhibition of ROCK1 could lead to a more broadly efficacious anti-hypertensive agent.[2] ROCK1 inhibitors have been shown to relax vascular smooth muscle and lower blood pressure in several animal models of hypertension.[3] Therefore, we began an effort to identify potent ROCK1 inhibitors with pharmacokinetic profiles consistent with once daily oral dosing.

To manage the inherent risk involved with developing a kinase inhibitor for a chronic indication, a high premium was placed on identifying ROCK1 inhibitors that were not only potent, but also highly selective over other protein kinases (initial goal: >100-fold). An internal screening effort led to the discovery of the aminofurazan (oxadiazole), azabenzimidazole **1**, a potent inhibitor of ROCK1 (IC_{50} = 19 nM).[4] Although **1** had good kinase selectivity (IC_{50} >10 000 nM, >500-fold, over a range of 30 diverse protein kinases), several kinases were inhibited with submicromolar activity (Table 1). Inhibition of RSK1 and p70S6K[5] was nearly equipotent with ROCK1 inhibition; in addition, **1** has been reported to be a very potent (3 nM) inhibitor of MSK1.[6] Modest selectivity (ranging from 10- to 30-fold) was also observed against CDK2 and GSK3β.[7]



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Two general syntheses of these analogs are outlined in Scheme 1. The original route, which provided compounds **6a**−**d** and **6f**, starts with oxidation of 4-chloro-3-nitropyridine (**1**) to provide 4-chloro-3-nitro-2-pyridone.[8] This intermediate was then treated with $POCl_3$ to provide 2,4-dichloro-5-nitropyridine (**3**). The 4-chloride was then preferentially displaced by ethylamine at room temperature, followed by displacement of the 6-chloride. Although the second displacement could be achieved by anion formation with NaH, followed by heating in DMF, a milder and more generally useful method involved heating the chloride and nucleophile in the presence of K_2CO_3 in CH_3CN. Hydrogenation of the nitro group, followed by amide coupling with cyanoacetic acid and cyclo-dehydration provided the cyanomethyl-azabenzimidazoles **5**. Transformation of the cyanomethyl group to the aminofurazan was then accomplished by preparation of the α-cyano oxime with sodium nitrite and aqueous acid, followed by a two-step sequence involving addition of hydroxylamine and closure to **6a**−**e** by heating in Et_3N/THF. Deprotection of **6e** with sulfuric acid provided the free aniline **6f**; amide coupling to **6f** then provided inhibitors **6g**−**n**.

A more efficient method for preparing analogs at the 6-position (**6o**−**p)** was then developed utilizing CuI-promoted couplings[9] to the late-stage C6-bromide **10**, Scheme 2. This bromide was prepared similarly to the analogs above starting from 2,4-dibromo-5-nitropyridine (**8**).

During the course of initial SAR studies around the azabenzimidazole core, we quickly found that substitution on the 6-position was tolerated and could provide improved kinase selectivity. Addition of a methoxy group on the 6-position (**6a**) led to a 4-fold improvement in potency against ROCK1 and an increase in selectivity with respect to most other kinases (Table 1). The incorporation of an unsubstituted phenoxy group provided compound **6b**, although less potent than **6a**, and increased selectivity over CDK2 and GSK3β.

Substitution on the phenoxy substituent revealed that the 3-acetamide **6c** provided a >10-fold boost in ROCK1 potency

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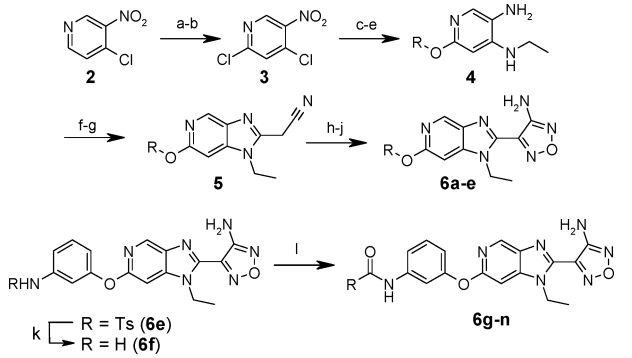
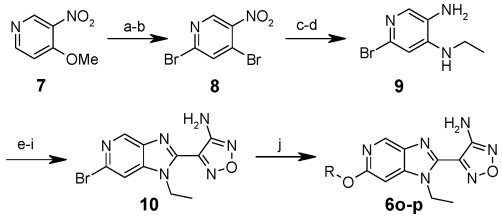
Scheme 1[a]



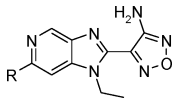
[a] Reagents and conditions: (a) KO*t*-Bu, *t*-BuOOH, THF/NH_3, −33 °C to rt; (b) $POCl_3$, tol, Δ; (c) $EtNH_2$, THF, rt; (d) ArOH, NaH, DMF, Δ or ArOH, K_2CO_3, CH_3CN, Δ; (e) Pd/C, H_2; (f) cyanoacetic acid, EDCI, Et_3N, CH_2Cl_2; (g) HOAc, Δ; (h) $NaNO_2$, HCl, MeOH; (i) NH_2OH, Et_3N, THF, Δ; (j) Et_3N, THF, Δ; (k) H_2SO_4, Δ; (l) ROCl, py.

Scheme 2[a]



[a] Reagents and conditions: (a) KO*t*-Bu, *t*-BuOOH, THF/NH_3, −33 °C to rt; (b) $POBr_3$, CH_3CN, Δ; (c) $EtNH_2$, THF, rt; (d) Fe, HOAc, Δ; (e) cyanoacetic acid, EDCI, Et_3N, CH_2Cl_2; (f) HOAc, Δ; (g) $NaNO_2$, HCl, MeOH; (h) NH_2OH, Et_3N, THF, Δ; (i) Et_3N, THF, Δ; (j) ArOH, CuI, 1,10-phenatholine, Cs_2CO_3, tol, Δ.

Table 1. Kinase Selectivity Data of 6-Substituted Aminofurazanyl-azabenzimidazole ROCK1 Inhibitors



cmpd	R	ROCK1 IC_{50}[a] (nM)	fold selectivity RSK1	p70S6K	MSK1	CDK2	GSK3β
1	H	19	0.63	3.1	0.18	22	53
6a	methoxy	4.4	18	15	2.3	55	250
6b	phenoxy	30	10	18	1.5	>130	>500
6c	3-acetamido-phenoxy	1.8	18	14	2.8	>1000	>1000
6d	4-acetamido-phenoxy	90	12	9.2	9.7	110	>160
6o	3-acetyl-phenoxy	13	12	6.2	12	460	>1000
6p	3-methyl-sulfonyl-phenoxy	33	2.7	0.7	3.9	nd	150
6f	3-amino-phenoxy	18	8.9	13	9.4	320	300

[a] ROCK1 IC_{50} values have been evaluated for standard error. Kinase data reported is the mean of 2 or more determinations; all kinase assays generated data within 2-fold of the mean.

while retaining >1000-fold selectivity over CDK2 and GSK3β. In addition, selectivity over RSK1, p70S6K, and MSK1 was comparable to that of **6b**. Moving the acetamide group to the 4-position of the phenyl ring (**6d**) or replacing it with acetyl (**6o**), methanesulfonyl (**6p**), or amino (**6f**) led to varying degrees of selectivity, but no compound attained the level of potency and selectivity provided by **6c**.

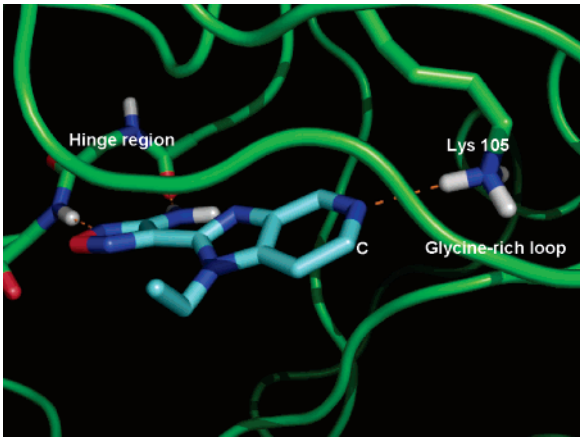


Figure 1. Inhibitor **1** docked into a homology model of ROCK1. Interactions with the hinge region and conserved Lys105 residue are highlighted.[11]

Having achieved high selectivity over CDK2 and GSK3β, we turned our attention to improving selectivity over the remaining kinases and optimization of the pharmacokinetic parameters of these compounds.

As **6c** was both potent and selective, it was chosen as the starting point in this phase of the investigation. Extending the acetamide with large (and substituted) alkyl groups provided no advantages over the parent acetamide **6c** (data not shown). In contrast, the benzoyl-substituted compound **6g**, though not as potent or selective as the acetamide **6c**, did provide a framework for further optimization (Table 2). Addition of a tertiary amine (dimethylamino and morpholino, **6h** and **6i**) provided a small increase in ROCK1 potency (∼1 nM) but also dramatically improved selectivity over RSK1. Activity at MSK1 was also attenuated, though selectivity over p70S6K was largely unchanged.

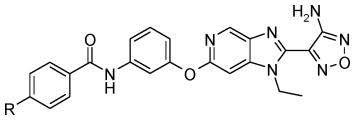
Changing from a pendent aromatic to aliphatic amine, exemplified by analogs **6j**−**l**, provided some improvement in the kinase profile with RSK1 selectivity improved to >100-fold. Selectivity against MSK1 was also increased in these analogs, most notably **6j** and **6k**. Increasing the length of the spacer to the amine group in the form of an ethoxy group led to **6m** and **6n**. Both retained excellent RSK1 selectivity and good to excellent MSK1 selectivity, although p70S6K selectivity was somewhat reduced relative to compound **6j**.

Docking studies[10,11] using a homology model of ROCK1 suggested that the aminofurazan head group of **1** makes two key hydrogen bonds with the hinge region of the ATP-binding site and that the 5-nitrogen of the azabenzimidazole makes contact with the catalytic Lys105, Figure 1. Based on the model shown, substituents off the C6 position of the azabenzimidazole core can orient toward the glycine-rich loop. It is possible that sequence differences in the glycine-rich loop lead to the selectivity observed between these kinases. Unfortunately, the known mobility of this loop in kinases has made the identification of specific interactions responsible for the selectivity gains made at this position difficult using homology models.

Functional activities of these ROCK1 inhibitors were investigated by measuring relaxation of phenylephrine contracted rat aortic rings. Inhibitor **6n** proved to be the most potent inhibitor in this assay, with an IC_{50} of 35 nM, less than 20-fold shifted relative to its IC_{50} in the ROCK1 biochemical assay. Other examples (**6g**−**m**) in Table 2 show a generally larger shift, but still demonstrate potent tissue relaxation.

The pharmacokinetic parameters of selected analogs were evaluated (Table 2). In every case the morpholine-substituted

Table 2. Kinase Selectivity and Rat Pharmacokinetic Data of Benzoyl-Substituted ROCK1 Inhibitors



			fold selectivity				rat PK parameters[c]					
cmpd	R	ROCK1 IC_{50}[a] (nM)	RSK1	p70S6K	MSK1	Aortic IC_{50}[b] (nM)	dose iv/po (mg/kg)	C_{max} iv/po (ng/mL)	$T_{1/2}$[d] (h)	CL[d] (mL/min/kg)	Vd_{ss}[d] (L/kg)	Oral F[e] (%)
6g	H	4.0	3.8	3.0	2.2	106 ± 18	1.2/3.7	11 700 ± 1700/ 6940 ± 1490	9.6 ± 0.9	0.18 ± 0.02	0.16 ± 0.02	35 ± 5
6h	dimethyl-amino	2.0	45	7.0	13	106 ± 35	0.85/2.0	18 600 ± 900/ 3620 ± 2630	8.2, 8.6[f]	0.14, 0.14[f]	0.09, 0.09[f]	9, 11[f]
6i	morpholino	≤1	≥60	≥10	≥26	59 ± 15	2.1/2.1	1500 ± 300/ 890 ± 115	0.9 ± 0.2	8.6 ± 2.5	0.57 ± 0.09	23 ± 6
6j	aminomethyl	≤1	≥70	≥17	≥70	107 ± 53	1.0/2.2	9900 ± 2500/ 255 ± 112	1.4 ± 0.3	2.3 ± 0.75	0.11 ± 0.03	4 ± 2
6k	dimethylamino-methyl	≤1	≥80	≥3	≥52	74 ± 7	1.0/2.2	630 ± 100/ 160 ± 16	1.9 ± 0.4	30 ± 5.1	2.4 ± 0.4	47 ± 9
6l	morpholino-methyl	≤1	≥120	≥10	≥35	102 ± 13	1.1/2.2	1100 ± 120/ 684 ± 145	1.4 ± 0.1	10.7 ± 1.9	1.1 ± 0.1	64 ± 11
6m	dimethylamino-ethoxy	≤1	≥80	≥3	≥65	79 ± 19	2.4/3.3	410 ± 70/ 410 ± 15	2.1 ± 0.7	121 ± 17	8.9 ± 2.2	40 ± 19
6n	morpholino-ethoxy	1.8	83	2.9	29	35 ± 7	1.6/3.3	1270 ± 70/ 800 ± 327	2.2 ± 0.6	7.6 ± 1.0	1.3 ± 0.2	69, 78[f]

[a] Kinase data reported is the mean of 2 or more determinations; all kinase assays generated data within 2-fold of the mean. [b] Values are an average of 3−4 determinations. [c] PK parameters are presented as means ± standard deviation where $n = 3$ or as individual values where $n < 3$ due to technical difficulties. For full experimental details see the Supporting Information. [d] Dose administered as an iv infusion over 30 min. [e] Dose administered as a solution by oral gavage. [f] Data for only two animals is given due to technical difficulties in the experiment.

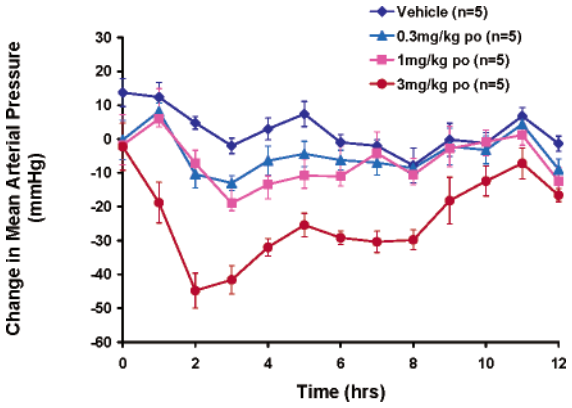


Figure 2. Effect of **6n** on mean arterial pressure in conscious SHR.

analogs possess higher bioavailability than their dimethylamine counterparts. With exceptions (notably the low bioavailability of **6h** and **6j**), their pharmacokinetic profiles were suitable for in vivo pharmacological evaluation in an animal hypertension model.

From these studies, compound **6n** possessed the best overall profile (kinase selectivity, half-life, bioavailability, and potency in the rat aortic contraction assay). The blood pressure lowering effect of compound **6n** was evaluated in spontaneously hypertensive rats (SHR). Oral dosing (0.3−3.0 mg/kg)[12] of **6n** led to a robust and dose-dependent decrease in blood pressure as shown in Figure 2. In comparison, the published ROCK1 inhibitor Fasudil (**11**)[13] provided only a ∼15 mmHg drop in mean arterial pressure at 30 mg/kg (data not shown).

In summary, we have demonstrated that the aminofurazanyl-azabenzimidazole structure is a useful template for the development of ROCK1 inhibitors. ROCK1 potency and kinase selectivity were dramatically improved by addition of a substituted phenoxy group at the 6-position of the azabenzimidazole, and several analogs have promising rat PK profiles. In addition, **6n** was shown to dramatically lower blood pressure in a rat model of hypertension. Further studies on the pharmacology of these inhibitors and SAR around the core template will be reported in due course.

Supporting Information Available: Experimental details and characterization data for all compounds and details for in vitro and in vivo assays. This information is available free of charge via the Internet at http://pubs.acs.org.

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(12) Plasma levels were obtained for a 3 mg/kg dose of **6n** in a satellite group of spontaneously hypertensive rats ($n = 3$). $T = 0.5$ h, 280 ± 23 ng/mL; $T = 1$ h, 330 ± 15 ng/mL; $T = 2$ h, 290 ± 24 ng/mL; $T = 4$ h, 150 ± 40 ng/mL; $T = 8$ h, 88 ± 20 ng/mL.

(13) Fasudil (**11**) is 5-(hexahydro-1*H*-1,4-diazepin-1-ylsulfonyl)-quinoline.

JM060873P