Development of Dihydropyridone Indazole Amides as Selective Rho-Kinase Inhibitors

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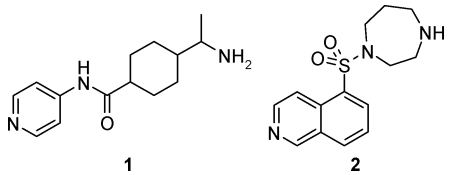
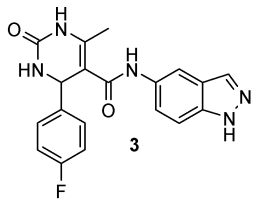
Abstract: Rho kinase (ROCK1) mediates vascular smooth muscle contraction and is a potential target for the treatment of hypertension and related disorders. Indazole amide **3** was identified as a potent and selective ROCK1 inhibitor but possessed poor oral bioavailability. Optimization of this lead resulted in the discovery of a series of dihydropyridones, exemplified by **13**, with improved pharmacokinetic parameters relative to the initial lead. Indazole substitution played a critical role in decreasing clearance and improving oral bioavailability.

Rho-associated kinase (ROCK1)[1] is a serine/threonine kinase that has been implicated in a variety of cellular processes including vascular smooth muscle contraction, stress-fiber formation, cell migration, and gene expression.[2] In vascular smooth muscle contraction, ROCK1 plays a central role in the calcium-sensitization pathway.[3] Activation of ROCK1 indirectly regulates the phosphorylation state of myosin light chain, leading to increased vascular smooth muscle contraction. Inhibition of this pathway represents a promising strategy for the treatment of a variety of cardiovascular diseases, including hypertension.[4] Several ROCK1 inhibitors have been reported in the literature,[5] including **1** (Y-27632)[3] and **2** (Fasudil).[6] These compounds have been used extensively to elucidate the role of Rho-kinase in vivo. Importantly, studies have shown that both compounds lower blood pressure in preclinical models of hypertension.[3,6] Given these encouraging observations, we sought to develop a potent, selective ROCK1 inhibitor for use as an antihypertensive agent.



From the outset, achieving kinase selectivity was a high priority. Over 500 protein kinases with a range of biological functions have been identified to date, and undesired kinase activity could negatively impact the long-term safety and tolerability of a ROCK1 inhibitor.[7] To assess potential kinase selectivity issues, compounds were screened against a panel of 33 kinases including rat RSK1[8] and p70S6K. RSK1 and p70S6K were of particular concern: both are involved in cell proliferation and their active sites share a high degree of sequence homology with ROCK1.[9]



ROCK1 IC_{50} = 14 nM
Rat aorta IC_{50} = 760 nM
RSK1 IC_{50} = 3100 nM
p70S6K IC_{50} = 2850 nM

Figure 1. Activity and kinase selectivity of the initial dihydropyrimidinone lead. Data represent the average value for two or more measurements. Standard error is typically within 2-fold of the reported mean.

Screening identified dihydropyrimidinyl indazole amide **3** as promising lead. Not only was **3** a potent ROCK1 inhibitor, but it also possessed exciting selectivity (>30-fold) against a panel of 33 kinases, including RSK1 and p70S6K (Figure 1).

Compound **3** was further evaluated in the rat aortic ring dilation assay to establish functional activity.[10] Although compound **3** displayed cellular activity, a significant shift (>50-fold) was observed relative to in vitro potency. In addition, the pharmacokinetic profile of **3** was characterized by low oral bioavailability and high clearance (Oral F = not quantifiable, Cl = 50 mL/min/kg) in rats. Based on these observations, the primary goal for lead optimization was to identify modifications that would improve the pharmacokinetic profile for this chemical series.

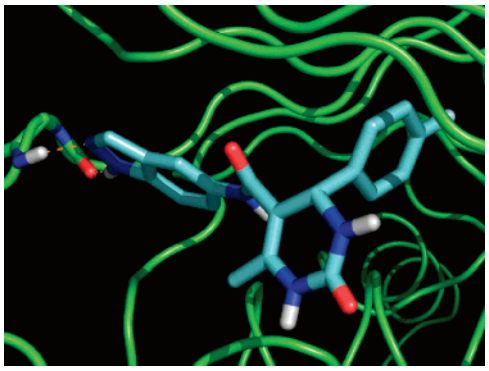


Figure 2. Structure of **3** docked in the active site of the ROCK1 homology model. Key hydrogen bonds to the protein backbone are highlighted in green.

To provide insight into its binding mode, compound **3** was docked into the active site of our ROCK1 homology model (Figure 2).[11] The lowest energy structure oriented the indazole toward the hinge region of the kinase, making two key hydrogen bonds with the protein backbone. This binding mode is consistent with previously described kinase inhibitors bearing an indazole ring.[4] Indeed, methylation of compound **3** at N1 of the indazole resulted in a dramatic loss of activity (ROCK1 IC_{50}

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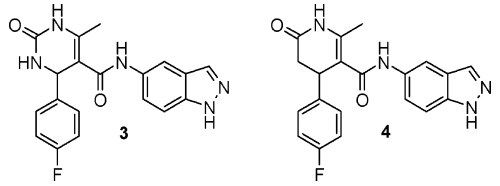
Table 1. Profiles of Initial Screening Hit and Pyridone Analog



cmpd	**3**	**4**
	IC_{50}[a] (nM)	
ROCK1	14	51
rat aorta	760	200
RSK1	3100	1410
p70S6K	2850	1100
	rat PK parameters[b]	
dose[c] (mg/kg)	1.3	1.6
C_{max}[c] (ng/mL)	567 ± 112	668 ± 121
$T_{1/2}$ (h)	0.7 ± 0.2	1.0 ± 0.5
Cl (mL/min/kg)	49 ± 5	47 ± 10
Vdss (L/kg)	2.2 ± 0.2	2.1 ± 0.3
F (%)	not quantifiable	14 ± 5

[a] Data represent the average value for two or more measurements. Standard error is typically within 2-fold of the reported mean. [b] PK data were determined from an iv/po crossover study. Pharmacokinetic parameters represent average values from three tested animals. [c] Values are reported for the iv leg of the study.

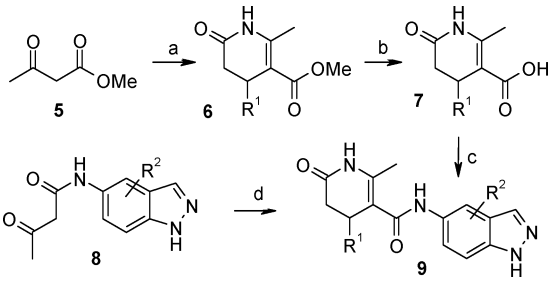
> 2500 nM). In the model, the aryl group occupies a hydrophobic region beneath the glycine-rich loop. However, no significant interactions between the dihydropyrimidinone core and the protein were identified, suggesting that this heterocycle may serve primarily as a scaffold to appropriately orient the indazole and the aryl group for optimal binding.

Because our binding hypothesis suggested that modification of the dihydropyrimidinone core could be tolerated, our initial strategy focused on identifying core replacements with improved cellular activity and oral bioavailability. Mindful of previous work that drew a correlation between hydrogen bond count and oral bioavailability in rats,[12] compounds with a decreased hydrogen bond count in the central ring were targeted. Indeed, simple modification of the core to a pyridone **4** was well tolerated (Table 1) and selectivity against the kinase panel was maintained. Importantly, rat pharmacokinetic studies demonstrated that **4** had enhanced oral bioavailability (14%) relative to **3**. Furthermore, these modifications decreased the shift between the in vitro enzyme assay and the rat aortic ring dilation assay from greater than 50-fold for compound **3** to 4-fold for compound **4**. Therefore, optimization of **4** became the new focus of our lead optimization efforts.

Dihydropyridone analogs were rapidly synthesized by two routes outlined in Scheme 1. Methylacetoacetate was condensed with Meldrum's acid, an aryl aldehyde, and ammonium acetate to afford the substituted dihydropyridone **6**.[13] Saponification of the ester provided acid **7**, which smoothly underwent coupling with an indazole to provide indazole amide **9**. Alternatively, ketoamide **8** could be treated with Meldrum's acid, an aryl aldehyde, and ammonium acetate to provide indazole amide **9** in a single step.

Pyridone derivatives with varying aryl groups (R^1) were prepared and evaluated for ROCK1 inhibition. Close analogs of **4** incorporating a variety of aryl substituents had similar activity and selectivity (Table 2). Replacement of fluorine with trifluoromethyl resulted in a 10-fold increase in ROCK1 potency and maintained the desired selectivity profile. However, these modifications did not significantly improve oral bioavailability.

Scheme 1. Synthesis of Dihydropyridones[a]



[a] Reagents: (a) Meldrum's acid, R^1CHO, NH_4OAc, HOAc; (b) NaOH, MeOH, H_2O; (c) 5-aminoindazole, EDC, Et_3N, DMF; (d) Meldrum's acid, R^1CHO, NH_4OAc, AcOH.

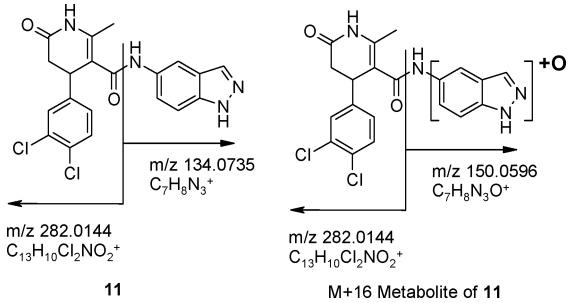


Figure 3. LC/MS/MS analysis of parent **11** and its M+16 metabolite generated by incubation with rat hepatocytes.

We then investigated whether metabolism could be limiting oral bioavailability. Compound **11** was incubated with rat hepatocytes, and incubates were subsequently analyzed by LC/MS/MS for the presence of metabolites (Figure 3). Three chromatographically resolved M+16 metabolites were observed. Subsequent fragmentation of one of these metabolites identified a fragment (*m/z* 150.0596), which suggested that oxidation was occurring on the indazole. Concurrently, the dihydropyridone fragment was recovered unchanged (*m/z* 282.0144), supporting the conclusion that oxidation had occurred on the indazole. Based on these observations, we directed our chemical strategy toward compounds with diverse indazole substitution patterns in an attempt to block sites of metabolism and potentially improve oral bioavailability and half-life by reducing clearance.

A variety of indazole substitution patterns were explored (Table 3). Substitution at C3 and C6 had little effect on ROCK1 inhibition (**13** and **15**) or kinase selectivity. In contrast, addition of a fluorine substituent at C4 dramatically reduced potency (**14**). Interestingly, while substitution at C7 maintained ROCK1 potency, selectivity against key kinases suffered (**16**). In contrast to its modest effects on ROCK1 activity, indazole substitution

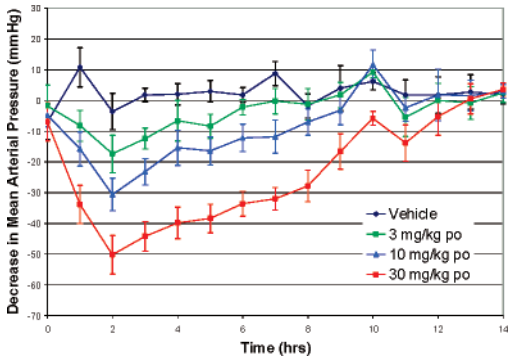
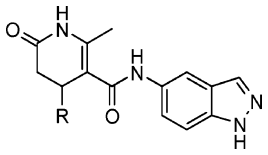


Figure 4. Effect of **15** on mean arterial pressure in spontaneously hypertensive rats. Compound was administered by single dose oral gavage. Reported data represent the average decrease in mean arterial pressure for five treated animals.

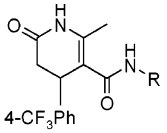
Table 2. Profile of Dihydropyridones with Varying Aryl Rings



		kinase IC_{50}[a] (nM)				rat PK parameters[b]					
compd	R	ROCK1	RSK1	p70S6K	rat aorta[a] IC_{50} (nM)	dose[c] (mg/kg)	C_{max}[c] (ng/mL)	$T_{1/2}$ (h)	Cl (mL/min/kg)	Vdss (L/kg)	Oral F (%)
4	4-FPh	51	1410	1150	200	1.6	668 ± 121	1.0 ± 0.5	47 ± 10	2.1 ± 0.3	14 ± 5
10	4-CF_3Ph	5	740	nd	39	0.9	315 ± 43	0.7 ± 0.1	51 ± 4	2.3 ± 0.2	22 ± 4
11	3,4-diClPh	8	620	560	nd	0.9	334 ± 29	0.8 ± 0.01	57 ± 1	2.4 ± 0.3	1 ± 1
12	2-naphthyl	9	1100	1550	76	1.7	700 ± 35	0.9 ± 0.1	45 ± 8	1.8 ± 0.3	13 ± 2

[a] Data represent the average value for two or more measurements. Standard error is typically within 2-fold of the reported mean. [b] PK data were determined from an iv/po crossover study. Pharmacokinetic parameters represent average values from three tested animals. [c] Values are reported for the iv leg of the study.

Table 3. Effect of Indazole Substitution on Kinase Selectivity and Rat DMPK Parameters



		Kinase IC_{50} (nM)[a]			Rat DMPK Parameters[b]						
compd	R	ROCK1	RSK	p70S6K	rat aorta[a] IC_{50} (nM)	dose[c] (mg/kg)	Cmax[c] (ng/mL)	$T_{1/2}$ (h)	Cl (mL/min/kg)	Vdss (L/kg)	Oral F(%)
10		5	740	nd	39	0.9	315 ± 43	0.7 ± 0.1	51 ± 4	2.3 ± 0.2	22 ± 10
13		9	1950	4790	130	1.3	672 ± 190	2.0 ± 0.1	24 ± 5	2.8 ± 0.4	18 ± 2
14		2500	>10,000	>10,000	nd	nd	nd	nd	nd	nd	nd
15		14	780	1940	190	1.8	1565 ± 367	1.0 ± 0.25	25 ± 6	1.4 ± 0.3	61 ± 9
16		8	90	590	nd	0.9	424 ± 85	1.2 ± 0.2	81 ± 4	3.9 ± 0.3	29 ± 13

[a] Data represent the average value for two or more measurements. Standard error is typically within 2-fold of the reported mean. [b] PK data were determined from an iv/po crossover study. Pharmacokinetic parameters represent average values from three tested animals. [c] Values are reported for the iv leg of the study.

at C3, C6, and C7 had a pronounced effect on the rat PK profile. While substitution at C3 and C7 had only modest impact on oral bioavailability (**13** and **17**), incorporation of fluorine at C6 dramatically increased oral bioavailability (**15**). This mode of substitution provided a general handle for improving pharmacokinetics in this series. Importantly, the improved profile of **15** provided an ideal tool compound to explore the effects of indazole-derived ROCK1 inhibitors in vivo.

Spontaneously hypertensive rats (SHRs) were treated with compound **15** to establish its effect on mean arterial pressure (Figure 4). Indeed, treatment of animals with **15** resulted in a dose-dependent decrease in mean arterial pressure.[14] A maximum decrease of 50 mmHg was observed approximately 2 h after oral administration at 30 mg/kg. These data represent a significant increase in efficacy compared to **2** (∼15 mmHg decrease at 30 mg/kg, data not shown).

In conclusion, we have described a series of potent and selective inhibitors of Rho-associated kinase. Optimization of the initial dihydropyrimidinone lead **3** resulted in a series of dihydropyridones exemplified by **4** with improved cellular activity and oral bioavailability. Evaluation of indazole substitution led to the identification of 6-fluoroindazole **15**, a compound with 61% oral bioavailability. Compound **15** dramatically reduced mean arterial pressure in spontaneously hypertensive rats after oral administration. Further studies around the indazole-dihydropyridones will be reported in due course.

Supporting Information Available: Synthetic procedures and characterization data for all compounds. Background information on the ROCK homology model. In vitro incubation and analytical methodologies for metabolite ID experiments. Procedures for enzyme assays and acute SHR studies. This information is available free of charge via the Internet at http://pubs.acs.org.

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(1) Two isoforms of Rho-kinase (ROCK1 or ROKβ and ROCK2 or ROKα) are known, and they share >90% homology in the kinase domain. In this chemical series, similar SAR was observed for both isoforms (data not shown).

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time post dose (h)	plasma concn (ng/mL)
0.5	193
1	192
2	190
4	110
8	74
24	2

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