Potent, Selective and Orally Bioavailable Dihydropyrimidine Inhibitors of Rho Kinase (ROCK1) as Potential Therapeutic Agents for Cardiovascular Diseases

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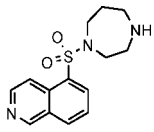
Abstract: Recent studies using known Rho-associated kinase isoform 1 (ROCK1) inhibitors along with cellular and molecular biology data have revealed a pivotal role of this enzyme in many aspects of cardiovascular function. Here we report a series of ROCK1 inhibitors which were originally derived from a dihydropyrimidinone core **1**. Our efforts focused on the optimization of dihydropyrimidine **2**, which resulted in the identification of a series of dihydropyrimidines with improved pharmacokinetics and P450 properties.

Rho-associated kinase isoform 1 (ROCK1[a])[1] is an enzyme involved in diverse cellular signaling functions such as smooth muscle contraction, cytoskeleton rearrangement, cell migration, and proliferation. Recent studies using ROCK1 inhibitors such as **30** (Fausidil)[2,3] and **31** (Y-27632)[3] along with cellular and molecular biology data have revealed a pivotal role of this serine-threonine kinase in many aspects of cardiovascular function. ROCK1 is a potential therapeutic target in the treatment of cardiovascular diseases such as hypertension.[4]



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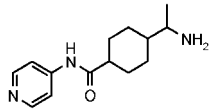

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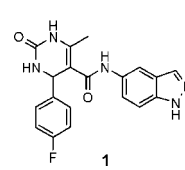
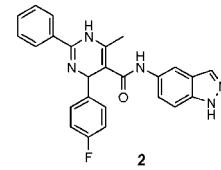
Recently, we and others described the discovery of 5-substituted indazoles as ROCK1 inhibitors, including a new class of dihydropyrimidinone derived amides (**1**) (Table 1).[5] After extensive lead optimization, the dihydropyrimidinone series was found to have limited oral bioavailability and cellular activity.

Table 1. Activity, Kinase Selectivity, and Pharmacokinetics of the Initial Dihydropyrimidinone and Dihydropyrimidine Leads



1



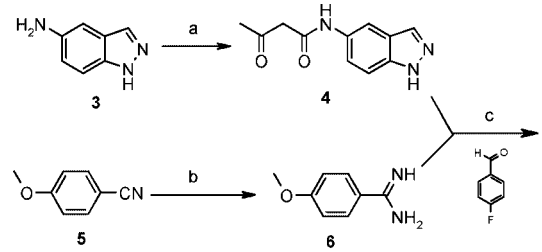
2

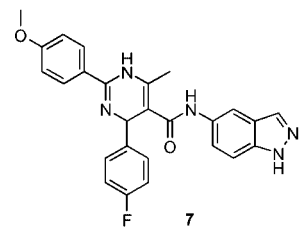
	IC_{50}[9] (nM)	
ROCK1	14	105
RSK1	3100	5300
p70S6K	2850	4500
rat aorta	760	1300
	pharmacokinetics (rat)[10]	
dose[11] (mg/kg)	1.3	1.8
$T_{1/2}$ (h)	0.7 ± 0.2	1.0 ± 0.7
CL (mL/min/kg)	49 ± 5	15 ± 2.8
V_{dss} (L/kg)	2.2 ± 0.2	0.8 ± 0.2
oral F (%)	not detectable	16 ± 7.5

During the course of the chemistry effort, an alternative scaffold containing a dihydropyrimidine core (**2**) was identified. In comparison to the dihydropyrimidinone **1**, the dihydropyrimidine **2** exhibited improved pharmacokinetics (PK) and diminished ROCK1 activity. In addition, compound **2** had significant P450 liabilities (2C9, 0.64 μM; 2D6, 0.09 μM; 3A4, 0.35 μM). Overall, compound **2** maintained good selectivity (>30 fold) against a panel of 33 kinases, including RSK1[6] and p70S6K[6] (Table 1) and had reasonable activity in the phenylepherine induced rat aortic tissue functional assay. Our initial goal was to survey substitution around the dihydropyrimidine core and to determine if the improved PK was general to the series and if the undesired P450 inhibition could be overcome.

Preparation of dihydropyrimidines such as **7** was carried out in a two-step process utilizing the modified Biginelli cyclization as the key ring forming reaction.[7] Condensation of 5-aminoindazole with diketene afforded ketoamide **4**, which underwent

Scheme 1. Synthesis of Dihydropyrimidines[a]





7

ROCK1 IC_{50}[9] 46 nM

Pharmacokinetics (rat)[10]

Dose[11]	1.2 mg/kg
$T_{1/2}$	169 ± 21 min
Cl	55 ± 5.4 mL/min/kg
Vdss	5.10 ± 0.9 L/kg
Oral F	35 ± 6.3 %

[a] (a) Diketene, CH_3CN, 84%; (b) $Al(CH_3)_3$, NH_4Cl, toluene, 95%; KOAc (2 equiv), DMSO, 100 °C, 3 h, 32%.

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Department of Vascular Biology.
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[a] Abbreviations: ATP, adenosine triphosphate; ROCK1, Rho-associated kinase isoform 1; RSK1, ribosomal S6 kinase 1; SAR, structure activity relationship; SHR, spontaneously hypertensive rat.

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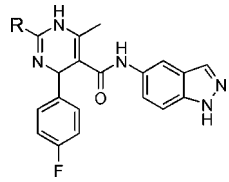
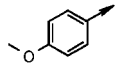
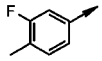
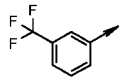
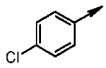
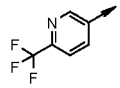
Table 2. SAR of 2-Phenyl Substituents



R	ROCK1 IC_{50} (nM)[9]	R	ROCK1 IC_{50} (nM)[9]
 **7**	46	 **12**	8
 **8**	101	 **13**	105
 **9**	104	 **14**	288
 **10**	129	 **15**	180
 **11**	129	 **16**	75

Biginelli condensation in the presence of an appropriately substituted amidine[8] **6** and an aldehyde to afford **7** (Scheme 1). Optimal conditions were determined to be excess KOAc in DMSO at 100 °C for ~3 h. The overall yields were modest but sufficient to complete the preparation of a large number of compounds to determine initial SAR.

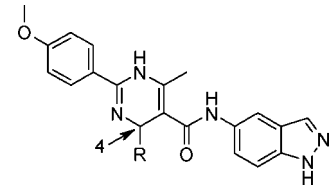
Our initial array focused on simple substitution on the aryl ring introduced from the benzamidine **6** (Table 2). In general, a wide variety of substitutions were tolerated. The 4-MeO compound **7** had the most promising rat PK (oral bioavailability of 35% vs 0% for cmpd **12**) and was selected for further exploration.

SAR around the 4-position was well established and appeared to parallel that of the dihydropyrimidinone and dihydropyridone series reported recently.[5] From this small array (Table 3), the 2-F,4-Cl substitution found in compound **21** was optimal for activity against ROCK1.

Compound **21** had >100-fold selectivity over selected kinases and good oral bioavailability at 58% (Table 4). In addition, it had encouraging functional activity in the rat aortic contraction assay (IC_{50} = 200 nM). Unfortunately, there was significant P450 inhibition against CYP2D6 (Table 5). This undesired CYP2D6 activity appeared to be general for the 4-MeO series and proved to be insurmountable.

Concurrently, the crystal structure of the complex of compound **12** with ROCK1 was solved. As expected, the indazole binds with the ROCK1 hinge region by forming two key hydrogen bonding interactions (Figure 1). In addition, there is a potential interaction between the 4-pyridine nitrogen and Lys200. This interaction may be responsible for the increased binding affinity associated with compounds containing hydrogen bond acceptors in the para position (e.g., MeO in compound **7**).

Table 3. Screening of a Series of Substituted Aldehydes



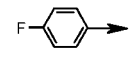
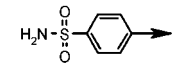
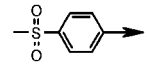
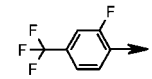
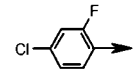
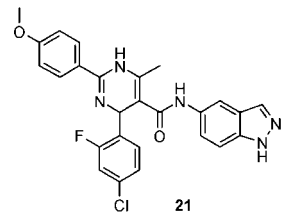
R	ROCK1 IC_{50} (nM)[9]	R	ROCK1 IC_{50} (nM)[9]
 **7**	46	 **19**	68
 **17**	150	 **20**	26
Me **18**	863	 **21**	5

Table 4. Activity, Kinase Selectivity, and Pharmacokinetics of Compound **21**



	IC_{50} (nM)[9]
ROCK1	5
RSK1	510
p70S6K	2100
Rat Aorta	200
Pharmacokinetics (rat)[10]	
Dose[11]	1.1 (mg/kg)
$T_{1/2}$	68 ± 26 min
Cl	108 ± 21 mL/min/kg
Vdss	6.46 ± 2.5 L/kg
Oral F	58 ± 25 %

Because of the P450 issues associated with the 4-MeO series (compound **21**), we then focused our efforts on substitution of pyridyl ring found in compound **12**. In comparison with compound **21** (Table 5), the ROCK1 enzyme activity was maintained and CYP2D6 inhibition was substantially dimin-

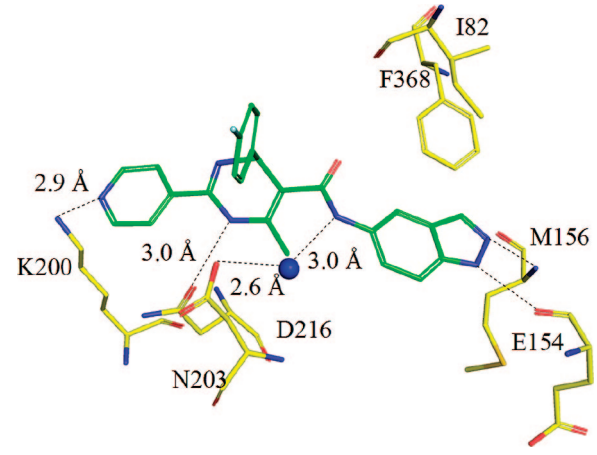
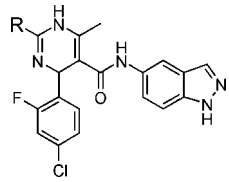


Figure 1. Crystal structure of compound **12** bound to the ATP site of ROCK1. An unexpected hydrogen bond interaction was observed with the unsubstituted 4-pyridyl nitrogen and Lys200. The blue sphere represents a water molecule.

Table 5. Optimizing P450 of Compound **21**



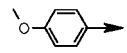
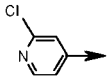
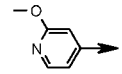
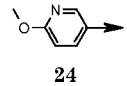
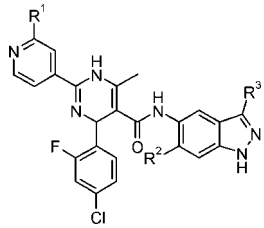
R	ROCK1 IC_{50} (nM)[9]	IC_{50} (uM)[9] CYP2D6	IC_{50} (uM)[9] CYP3A4	Oral F (%)
 **21**	5	0.20	2.7	58
 **22**	4	5.7	5.5	7
 **23**	2	3.7	4.3	21
 **24**	3	1.2	2.5	27

Table 6. Substituted Indazole Analogues



compd	R^1	R^2	R^3	ROCK1 IC_{50} (nM)[9]
22	Cl	H	H	4
25	Cl	F	H	6
26	Cl	H	Me	15
23	MeO	H	H	2
27	MeO	F	H	6
28	EtO	F	H	11
29	Me	F	H	10

ished. However, the oral bioavailability was decreased. Interestingly, ortho substitution on the pyridine does not affect the activity, suggesting the hydrogen bond interaction with Lys200 may be weak.

On the basis of the related dihydropyridone series,[5] it was determined that C6 substitution on the indazole was crucial for improving oral bioavailability. With this in mind, a small series of dihydropyrimidines with a variety of substituted pyridines and incorporation of the key C6 fluoroindazole substitution was prepared.

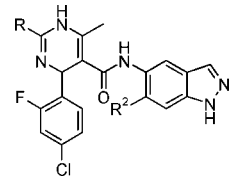
Indazole substitution resulted in only minimal loss of binding activity when compared to unsubstituted compound **22** (Table 6). Two compounds, **25** and **27**, were then advanced to P450 (Table 7) and rat PK studies. Both compounds had dramatically improved oral bioavailability (49% and 53%), and the chloro analogue **25** showed an improvement in half-life in rats (Table 8) and was selected for in vivo efficacy studies.

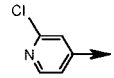
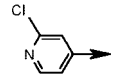
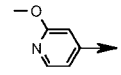
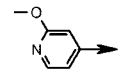
Compound **25** (Rat aorta IC_{50} = 256 nM) was profiled in a spontaneously hypertensive rat (SHR) model of hypertension.

Table 7. P450 Inhibition of Compounds **25** and **27**

compd	P450 IC_{50} (μM)[9] CYP2C9	CYP2D6	CYP3A4
25	2.5	5.2	2.5
27	8.4	2.1	5.3

Table 8. Comparison of Indazole and 6-F-Indazole Analogues[10]



R	R^2	ROCK1 IC_{50} (nM)[9]	$T_{1/2}$ (min)	CL (mL/min/kg)	Vdss (L/kg)	% F
 **22**	H	4	62 ± 15	36 ± 15	1.6 ± 0.33	7 ± 2.7
 **25**	F	7	105 ± 32	36 ± 4.8	2.0 ± 0.24	49 ± 10
 **23**	H	2	34 ± 3.2	88 ± 2.4	2.0 ± 0.2	21 ± 9.7
 **27**	F	6	46 ± 9.7	46 ± 7.4	2.5 ± 0.41	53 ± 12

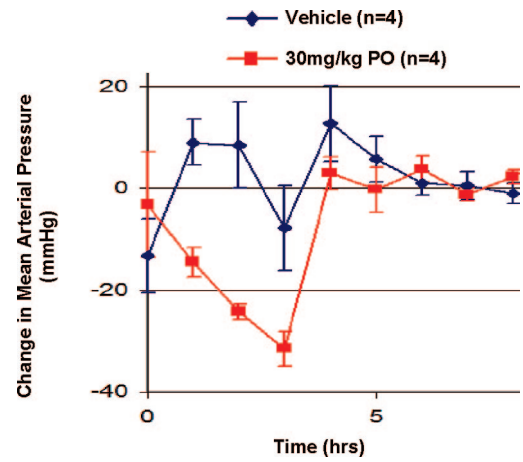


Figure 2. Effect of **25** on mean arterial pressure in conscious SHR. Compound was administered by single dose oral gavage. Reported data represent the average decrease in mean arterial pressure for five treated animals.

At 30 mg/kg (po), compound **25** induced a 25 mmHg (t = 3 h) drop in blood pressure, thus demonstrating potent in vivo activity for the series (Figure 2).

Conclusion

A series of potent and selective dihydropyrimidine inhibitors of ROCK1 have been described. From the initial lead **2**, significant improvements were made in the overall profile for this series of ROCK1 inhibitors. Incorporation of substitution next to the 4-pyridyl nitrogen provided compounds with

improved P450 profiles. In addition, indazole substitution provided very encouraging DMPK profiles. Final optimization led to compound **25**, which has promising oral bioavailability (49% in rat and 19% in monkey), good half-life (1.8 h in rat and 2.2 h in monkey), maintained good selectivity against a panel of 31 kinases (>100 fold), as well as RSK1[6] and p70S6K[6] (RSK1 IC_{50} = 398 nM, p70S6K IC_{50} = 1000 nM), and a dramatically improved P450 profile (>2.2 μM at all isozymes tested). Finally, oral administration of compound **25** gave a robust 25 mmHg reduction mean arterial pressure in spontaneously hypertensive rats at a single dose of 30 mg/kg.

Note Added after ASAP Publication. This paper published ASAP on October 9, 2008 with an incorrect presentation of compounds **30** and **31**. The correct version was published on October 15, 2008.

Supporting Information Available: Synthetic procedures and characterization data for all compounds. Procedures for ROCK assays and acute SHR studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Riento, K.; Ridley, A. J. ROCKs: Multifunctional kinases in cell behavior. *Nat. Rev. Mol. Cell Biol.* **2003***4*446−456. Two isoforms of Rho-kinase (ROCK1 or ROKβ and ROCK2 or ROKα) are known and they share >90% homology in the kinase domain and their relative function in vivo is not well understood to date. We have not observed differences in SAR between the two isoforms in the current chemical series (data not shown).

(2) Asano, T.; Ikegaki, I.; Satoh, S.; Suzuki, Y.; Shibuya, M.; Takayasu, M.; Hidaka, H. Mechanism of action of a novel antivasospasm drug, HA1077. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 1033–1040.

(3) Uehata, M.; Ishizaki, T.; Satoh, H.; Ono, T.; Kawahara, T.; Morishita, T.; Tamakawa, H.; Yamagami, K.; Innui, J.; Maekawa, M.; Narumiya, S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* **1997**, *389*, 990–994.

(4) (a) Amano, M.; Fukata, Y.; Kaibuchi, K. Regulation and functions of Rho-associated kinase. *Exp. Cell Res.* **2000**, *261*, 44–51. (b) Hu, E.; Lee, D. Rho kinase as a potential therapeutic target for cardiovascular diseases: opportunities and challenges. *Expert Opin. Ther. Targets* **2005**, *9*, 715–736. (c) Hu, E.; Lee, D. Rho kinase inhibitors as potential therapeutic agents for cardiovascular diseases. *Curr. Opin. Invest. Drugs* **2003**, *4*, 1065–1075. (d) Hirooka, Y.; Shimokawa, H. Therapeutic potential of Rho-kinase inhibitors in cardiovascular diseases. *Am. J. Cardiovasc. Drugs* **2005**, *5*, 31–39. (e) Lee, D. L.; Webb, R. C.; Jin, L. Hypertension and RhoA/Rhokinase signaling in the vasculature: highlights from the recent literature. *Hypertension* **2004**, *44*, 796–799. (f) Rikitake, Y.; Liao, J. K. ROCKs as therapeutic targets in cardiovascular diseases. *Expert Rev. Cardiovasc. Ther.* **2005**, *3*, 441–451. (g) Teixeira, C. B.; Webb, R. C. Targeting the vascular RhoA-Rho-kinase signaling pathway in hypertension. *Drug Discovery Today: Ther. Strat.* **2005**, *2*, 193–199.

(5) (a) Goodman, K. B.; Cui, H.; Dowdell, S. E.; Gaitanopoulos, D. E.; Ivy, R. L.; Sehon, C. A.; Stavenger, R. A.; Wang, G. Z.; Viet, A. Q.; Xu, W.; Ye, G.; Semus, S. F.; Evans, C.; Fries, H. E.; Jolivette, L. J.; Kirkpatrick, R. B.; Dul, E.; Khandekar, S.; Yi, T.; Jung, D. K.; Wright, L. L.; Smith, G. K.; Behm, D. J.; Doe, C. P.; Bentley, R.; Hu, E.; Lee, D. Development of Dihydropyridone Indazole Amides as Selective Rho-Kinase Inhibitors. *J. Med. Chem.* **2007**, *50* (1), 6–9. (b) Feng, Y.; Cameron, M. D.; Frackowiak, B.; Griffin, E.; Lin, L.; Ruiz, C.; Schroeter, T.; LoGrasso, P. Structure−activity relationships, and drug metabolism and pharmacokinetic properties for indazole piperazine and indazole piperidine inhibitors of ROCK-II. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2355–2360.

(6) (a) Inhibition of RSK1 or p70S6K has been implicated in a number of cellular functions. Although the specific consequence of inhibition is not clearly understood, knowing the cross-activity of this series, we sought to remove the activities against these targets. For a leading reference on RSK1, see: Hauge, C.; Frödin, M. RSK and MSK in MAP kinase signalling. *J. Cell Sci.* **2006**, *119*, 3021–3033. (b) RAJIV6For a leading reference on p70S6K, see: Berven, L. A.; Crouch, M. F. Cellular function of p70S6K: A role in regulating cell motility. *Immunol. Cell Biol.* **2000**, *78*, 447–451.

(7) (a) Biginelli, P. Aldehyde-Urea derivatives of Aceto- and Oxaloacetic Acids. *Gazz. Chim. Ital.* **1893**, *23*, 360–413. (b) Beck, G.; Kesseller, K.; Baader, E.; Bartman, W.; Berrgmann, A.; Granzer, B.; Jendralla, H.; Kerekjarto, B. V.; Krause, R.; Paulas, E.; Schubert, W.; Wess, G. Synthesis and biological activity of new HMG-CoA reductase inhibitors. 1. Lactones of pyridine- and pyrimidine-substituted 3,5-dihydroxy-6-heptenoic (-heptanoic) acids. *J. Med. Chem.* **1990**, *33* (1), 52–60.

(8) Garigipati, R. S. An efficient conversion of nitriles to amidines. *Tetrahedron. Lett.* **1990**, *31* (14), 1969–1972.

(9) Data represent the average value for two or more measurements. Standard error is typically within 2-fold of the reported mean.

(10) PK data were determined from an iv/po crossover study. Pharmacokinetic parameters represent average values from three tested animals.

(11) Values are reported for the iv leg of the study.

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