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Discovery and optimization of indoles and 7-azaindoles as Rho kinase (ROCK) inhibitors (part-I)

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ABSTRACT

Rho kinase (ROCK) inhibitors are potential therapeutic agents to treat disorders such as hypertension, multiple sclerosis, cancers, and glaucoma. Here, we disclose the synthesis, optimization, biological evaluation of potent indole and 7-azaindole based ROCK inhibitors that have high potency on ROCK (IC_{50} = 1 nM) with 740-fold selectivity over PKA (**47**). Moreover, **47** showed very good DMPK properties making it a good candidate for further development. Finally, docking studies with a homology model of ROCK-II were performed to rationalize the binding mode of these compounds and showed the compounds bound in both orientations to take advantage to H-bonds with Lys-121 of ROCK-II.

Rho kinase (ROCK) is a serine/threonine kinase of significant interest in drug discovery owing to its fundamental role in vital signal transduction pathways central to many essential cellular activities, including contraction, adhesion and migration. Once activated by the GTP-bound G-protein Rho, ROCK phosphorylates multiple substrates including myosin light chain (MLC), which ultimately results in cellular contraction.[1] The inhibition of the ROCK pathway might provide a means of therapy for diseases such as hypertension,[2,3] glaucoma,[4,5] multiple sclerosis,[6] stroke,[7] asthma,[8] erectile dysfunction,[9] central nervous system disorders,[10] and tumor metastasis,[11] among other conditions. Most known ROCK inhibitors are non-selective for the two isoforms (ROCK-I and ROCK-II), which have approximately 92% sequence homology in the kinase domain.[12] The isoquinoline ROCK inhibitor fasudil, used in Japan to treat cerebral vasospasm,[13] is an ATP-competitive inhibitor of modest potency, especially in cell-based assays.[14] Recently, several different families of ROCK inhibitors have been published by our group[15–21] along with IOP-lowering effects of two series in glaucomatous rats.[20,21] Herein, we discuss structure–activity relationships of new ROCK inhibitors based on the indole and azaindole scaffolds (Fig. 1).

In previous Letters, we have disclosed benzimidazole **1**[18] and benzothiazole **2**[19] as potent ROCK inhibitors (Fig. 1). We wished to expand our exploration to the corresponding indole and azaindole-based inhibitors shown in general structure **3.** This new scaffold would allow for substitution patterns not accessible in the earlier ring systems, permitting chemoselective substitutions at the N1, C2, and C3 ring positions of the indole/azaindole nucleus. We expected that indoles with C3 amides might have especially different properties from the benzothiazole 2-amides, since the amide carbonyl would be positioned differently to interact with the side chain amino group of Lys-121 in the ATP-binding site of ROCK-II. Our previous work[15–21] led us to believe that there may be four potential interactions of scaffold **3** within the ATP binding pocket that are relevant to ROCK potency and selectivity, shown in Figure 1 for indole/azaindole scaffold **3**. These include (1) Hydrogen bonds between the backbone NH of Met-172 in ROCK-II and a nitrogen-containing H-bond acceptor in the inhibitor (R^1). This interaction may also be complemented by a second hydrogen bond with the backbone carbonyl of Glu-170; (2) Hydrogen bond between an acceptor of the inhibitor to Lys-121, accomplished by the benzimidazole N3 of inhibitor **1** and by the amide carbonyl of benzothiazole **2**; (3) Hydrophobic interactions between the glycine-rich P-loop and an electron rich aromatic ring of the inhibitor, accomplished by the chroman ring of benzimidazole **1** and the

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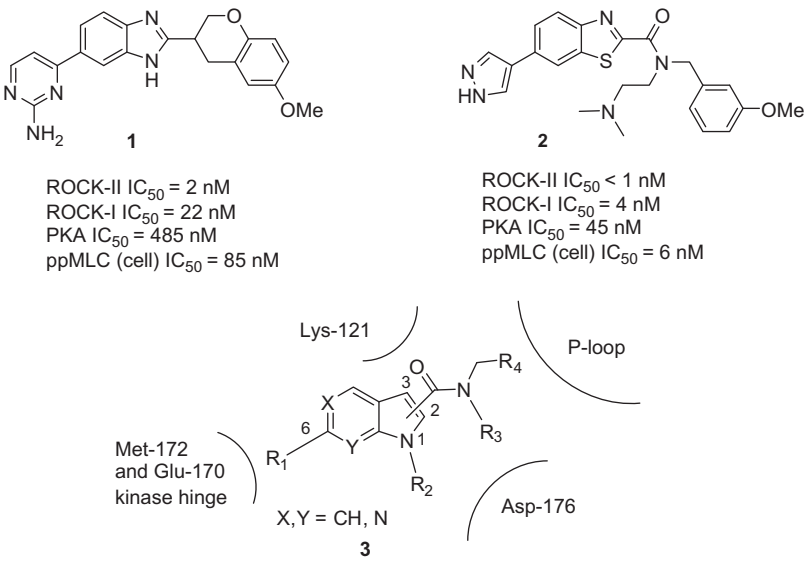


Figure 1. Benzimidazole and benzothiazole ROCK inhibitors **1**–**2** and proposed interactions of indole/azaindole **3** within the ATP binding pocket of ROCK-II.

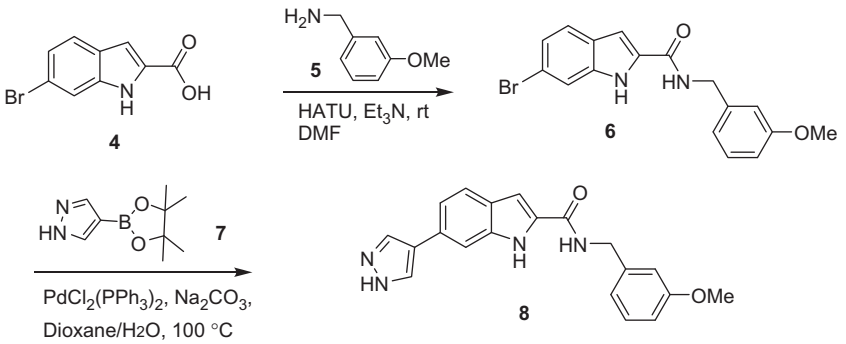
methoxybenzyl group of benzothiazole **2**; and (4) Polar interactions between the inhibitor and Asp-176 in ROCK-II, accomplished by the N-2-dimethylaminoethyl group of benzothiazole inhibitor **2**. The first three of these interactions can confer high ROCK affinity. The fourth interaction, accessed by indole/azaindole inhibitors through a side chain (R^2 or R^3 in structure **3**) will also perturb many pharmaceutical properties of these ROCK inhibitors such as potency, selectivity, cell permeation, brain penetration, aqueous solubility, and in vitro and in vivo pharmacokinetic properties.

Since the indole scaffold **3** lacks the nitrogen atom in benzimidazole **1**, which is responsible for a key interaction (with Lys-121 in ROCK-II), we believed that a C2 or C3 carbonyl group would be essential for this scaffold to obtain high ROCK affinity. Given that indole 2-carboxamides would possibly be isosteric with benzothiazole 2-carboxamides such as inhibitor **2**, we designed an expeditious synthesis of indole **8**, as shown in Scheme 1. Briefly, the sequence began with the commercially available acid **4**. HATU-mediated amide coupling and a Suzuki heteroarylation reaction allowed for the assembly of indole **8**, which was a potent ROCK inhibitor with good inhibitory activity in both enzyme assays and cell-based myosin light chain bis-phosphorylation assays (ppMLC)[22] (ROCK-II IC_{50} = 2 nM, ppMLC IC_{50} = 28 nM).[23] Especially noteworthy was the much reduced activity against protein kinase A (PKA, IC_{50} = 6700 nM, >3000-fold selectivity), a closely homologous kinase chosen to indicate the selectivity potential against other members of the human kinome,[24] compared to the benzimidazole and benzothiazole based ROCK inhibitors **1** and **2**.

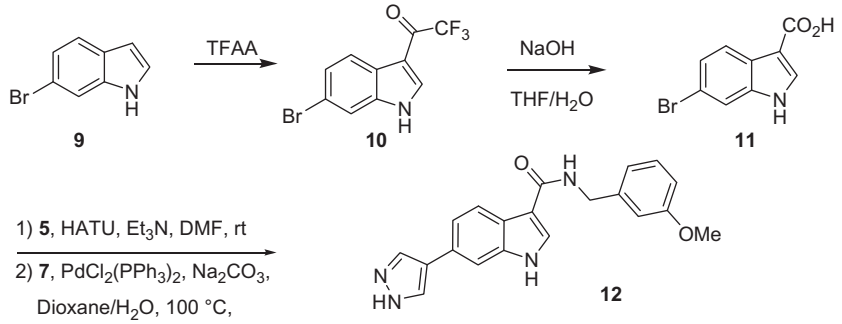
In parallel, we pursued the synthesis of an analogous indole 3-carboxamide **12**. Placing the amide carbonyl group at the indole C3 position was not as established in our ROCK inhibitor studies. As shown in Scheme 2, acylation and hydrolysis of 6-bromoindole **9** gave acid **11**, which was then converted into pyrazole **12** by the same two-step sequence used in the synthesis of indole 2-carboxamide ROCK inhibitor **8**. Remarkably, the unsubstituted indole 3-carboxamide **12** was also an extremely potent ROCK inhibitor in both biochemical and cell-based assays (ROCK-II IC_{50} <1 nM, ppMLC IC_{50} = 4 nM).

Having established that both indole 2- and 3-carboxamides are suitable starting points for ROCK inhibitor design, we then targeted the synthesis and evaluation of their azaindole analogs. It was expected that an additional basic nitrogen atom present in the scaffold would enhance aqueous solubility and perhaps confer more favorable drug properties. The prototype 5-azaindole 2-carboxamide analog was prepared based on the route of Scheme 3. It began with the iodination of commercially available amine **13**. Heating iodide **14** with pyruvic acid, DABCO and $Pd(OAc)_2$ gave the cyclization[25] product 5-azaindole-2-carboxylic acid **15**.[25] HATU amide coupling followed by a Suzuki heteroarylation yielded the desired amide **16**. Surprisingly, compound **16** was only a modestly potent ROCK inhibitor (ROCK-II IC_{50} = 190 nM).

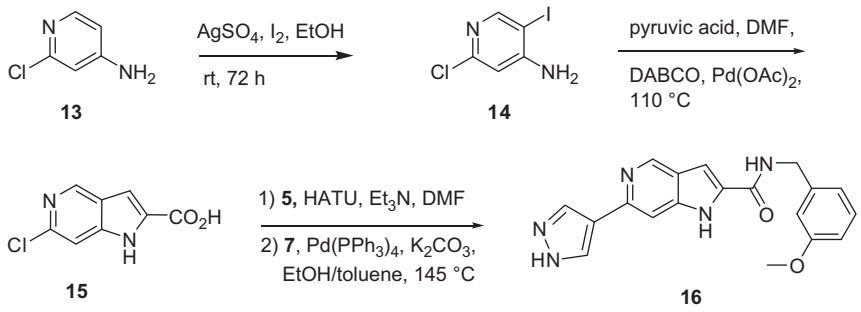
The effect of an added nitrogen atom in the scaffold was further investigated, however, with the 7-azaindole regioisomer, which was accessed as shown in Scheme 4. Pivaloylation of amine **17**, followed by directed halogenation,[26] and acidic pivaloyl group re-



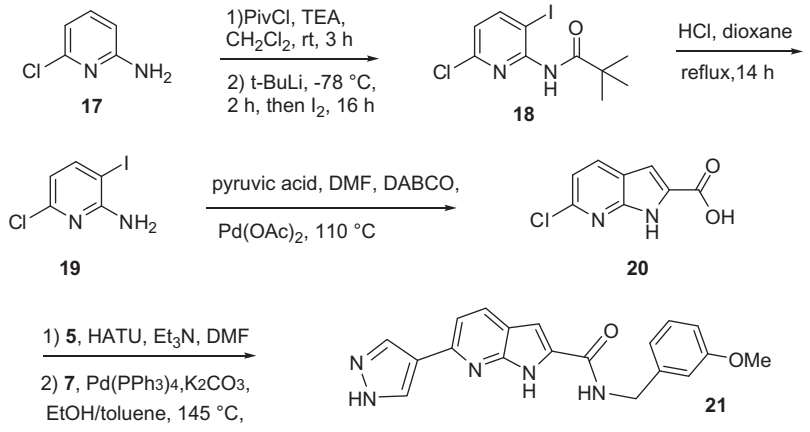
Scheme 1. Synthesis of indole 2-carboxamide ROCK inhibitor **8**.



Scheme 2. Synthesis of indole 3-carboxamide ROCK inhibitor **12**.



Scheme 3. Synthesis of 5-azaindole 2-carboxamide ROCK inhibitor **16**.

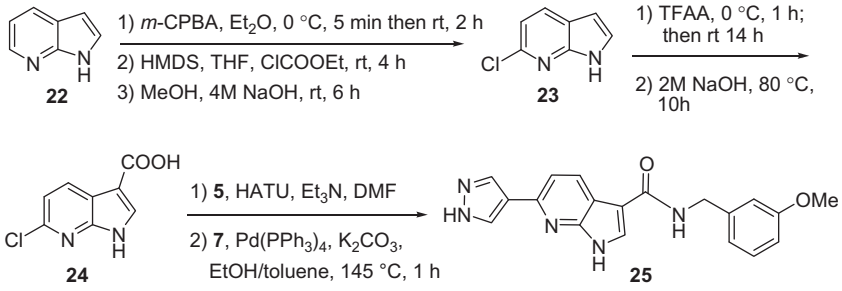


Scheme 4. Synthesis of 7-azaindole 2-carboxamide ROCK inhibitor **21**.

moval gave iodide **19**. Cyclization gave the key azaindole carboxylic acid **20**. The two-step coupling and Suzuki process under conditions analogous to those in Scheme 3 gave the desired 7-azaindole 2-carboxamide **21**. In contrast to the 5-azaindole analog **16**, this 7-azaindole 2-carboxamide, like its indole analog **8**, was found to be a potent ROCK inhibitor (ROCK-II IC_{50} = 2 nM), suggesting that further optimization efforts in the 7-azaindole scaffold were also warranted.

Given the encouraging result with 7-azaindole-2-carboxamide inhibitor **21**, we then sought its analogous 3-carboxamide regioisomer. The synthesis (Scheme 5) began with oxidation of commercially available 7-azaindole (**22**) to the corresponding *N*-oxide.[27] A known two-step procedure (chlorination plus acylation, then hydrolysis)[28] gave 6-chloro-7-azaindole **23**. Carboxylation, HATU-mediated amine coupling, and a Suzuki heteroarylation then gave the desired 7-azaindole 3-carboxamide **25**, which likes its indole analog **12** was found to be a potent ROCK inhibitor (ROCK-II IC_{50} = 2 nM) with excellent selectivity against PKA (IC_{50} = 8100 nM, >4000-fold).

The C6 pyrazole group of these inhibitors is anticipated to engage in key hydrogen bonding interactions to the kinase hinge (Fig. 1), and is essential for activity in several ROCK inhibitors.[19] Analogs with other heteroaryl groups were prepared to explore the tolerance of other hinge-binding moieties (Table 1). These compounds were accessed by coupling appropriate indole/azaindole 2-carboxylic acid with *m*-methoxy benzyl amine followed by heteroarylation under Suzuki conditions. The most potent 2-carboxamide indole has a pyrazole as the hinge-binding group. While aminopyrimidine **27** also had good affinity for ROCK, pyridine **26** and aminopyridine **28** were less potent inhibitors. 7-Azaindoles were found to bind to ROCK with high affinity and they resembled their indole analogs in preferring pyrazole-type groups (**21**, **31**, and **32**). However, the PKA selectivity was much reduced (**21** vs **8**). In comparison, 5-azaindoles (**16**, **29**, and **30**) exhibited



Scheme 5. Synthesis of 7-azaindole 3-carboxamide ROCK inhibitor **25**.

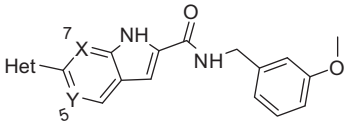
weak activity in this study and hence, their SAR was not studied further.

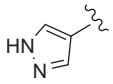
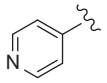
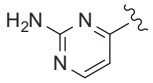
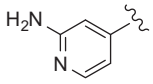
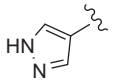
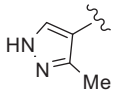
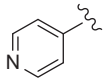
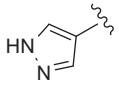
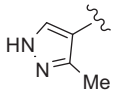
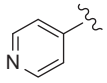
A similar SAR study with indole- and 7-azaindole-3-carboxamides (Table 2) also revealed that pyrazole is the most effective hinge binding group. Interestingly, several incongruities between the two series also became apparent. First, in contrast to the 2-carboxamides, the pyridine group was well tolerated in both indole and 7-azaindole3-carboxamide series (**33**–**34**). Second, 7-azaindole 3-carboxamides (**25**, **34**) showed an enhanced PKA selectivity as compared to both the 7-azaindole 2-carboxamide (**21** and **32** in Table 1) and the indole 3-carboxamide (**12**, **33**) counterparts.

In the design of inhibitors **8**, **12**, **21**, **25** and their analogs shown in Tables 1 and 2, we relied upon our previous studies of other scaffolds[19] to choose a *meta*-methoxy benzyl group to interact with the glycine rich P-loop region of the ATP binding pocket. In order to explore the tolerance of ROCK for indoles and 7-azaindoles with other amide groups, commercially available amines were used in the penultimate step to access diverse amides of indole-3-carboxylic acids and 7-azaindole-2-carboxylic acids followed by a Suzuki coupling for installation of the hinge binding group. It was especially of interest to vary the amide groups in the 3-carboxamides, since the C3 regiochemistry could alter the established preferences seen with benzothiazole 2-carboxamides (such as inhibitor **2**).[19] As shown in Table 3, affinity for ROCK was the highest for indole **12**, which contains a *m*-methoxy benzyl group. Less inhibition was observed for methoxy regioisomers **35** and **36**, and for fluorinated analogs **37** and **38** (compounds **35** and **38** still gave high ROCK potency, though). An (*R*)-benzylic methyl group (compound **39**) was superior to the (*S*)-benzylic methyl group (**40**), and nonaromatic and arylethyl moieties (**41**–**43**) were less optimal. It is also interesting to note that a 2,5-dimethoxy substituted benzyl amide was not tolerated (**44**) although the individual methoxy substitution at each position gave potent ROCK inhibitors (compounds **12** and **35**). In light of these results, we preferred the mono *m*-methoxy benzyl substituent in all further studies involving inhibitors belonging to the indole and azaindole scaffolds. This trend in favor of a *meta*-methoxy group and the (*R*)-benzylic methyl substituent is also present in 7-azaindole 2-carboxamides (Table 3, compounds **21**, **45**–**48**). However, PKA selectivity was slightly reduced in the 7-azaindole series.

The excellent potency shown in both the 2-carboxamides and 3-carboxamidesof the indole as well as the 7-azaindole series is somewhat surprising considering the key interactions proposed are the hydrogen bonds in the hinge binding area and the interaction between the amide carbonyl group and the side chain amino group of residue Lys-121 in ROCK-II. To understand these observations, we used previously described methods[29] to perform docking studies of several indole and azaindole inhibitors in a ROCK-II homology model. These studies led us to propose that two distinctly different flipped orientations of the indole nucleus, as shown in Figure 2, are important to consider. In indole 2-carboxamides such as azaindole **21** (compound shown in green in

Table 1
Exploration of the C6 hinge-binding group of indole, 5-azaindole, and 7-azaindole 2-carboxamide scaffolds

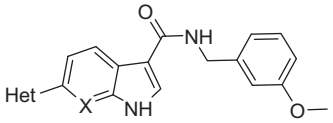


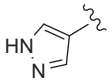
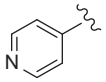
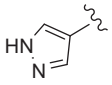
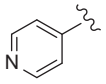
Compd	X	Y	Het	IC_{50}[a] (nM)		
				ROCK-II	ROCK-I	PKA
8	CH	CH		2	19	6700
26	CH	CH		160	1900	>20000
27	CH	CH		36	480	11000
28	CH	CH		1200	2100	>20000
16	CH	N		190	870	9400
29	CH	N		530	1200	>20000
30	CH	N		420	1100	>20000
21	N	CH		2	12	140
31	N	CH		5	19	230
32	N	CH		220	720	17000

[a] Average of two or more measurements with standard derivations ⩽30%.

Fig. 2), the indole NH is projected away from Asp-176 of ROCK-II. In the other orientation (as in azaindole **25** shown in pink), predominant for most if not all 3-carboxamides, the indole NH is instead projected towards Asp-176. Importantly, both orientations enable H-bonding interactions between the Lys-121 side chain amino group of the protein and the indole carbonyl oxygen as well as interactions with the hinge binding group and the P-loop recognition elements, lowering the overall energy of binding.

Table 2
Exploration of the C6 hinge-binding group in the indole- and 7-azaindole-3 carboxamide scaffolds



Compd	X	Het	IC_{50}[a] (nM)		
			ROCK-II	ROCK-I	PKA
12	CH		<1	<1	510
33	CH		2	11	730
25	N		2	11	8100
34	N		7	48	10000

[a] Average of two or more measurements with standard derivations ⩽30%.

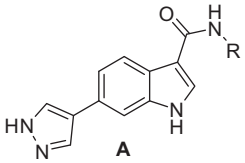
The ability of the nitrogens in the pyrazole hinge-binding group to serve as a hydrogen bond acceptor/donor group, interacting with Met-172 and Glu-170 through tautomerization or transposition via single bond rotation, is possibly another key factor giving rise to high potency in both regioisomeric series. Additionally, this hypothesis also can be used to address the weak ROCK affinity of the 2-carboxamides containing a 4-pyridyl group at C6, a hinge-binding group lacking an NH donor and in which the location of the H-bond accepting atom is fixed. This binding motif is consistent with all SAR results observed in our indole and azaindole series, especially when the indole NH or the amide NH is substituted (see part-II).

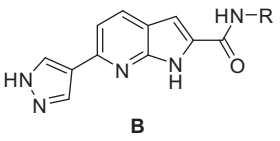
The in vitro and in vivo pharmacokinetic properties were also used to evaluate these novel ROCK inhibitors. The most important observations from the in vitro data (Table 4) are that the human microsomal stability is greatly reduced by moving the carboxamide from the C2 to C3 position (**8** vs **12**, **21** vs **25**), and that the 7-azaindole scaffold is much cleaner than the indole scaffold in terms of CYP-450 enzyme inhibitions (**21/47** vs **8**, **25** vs **12**). Replacement of the pyrazole by an aminopyrimidine group significantly lowered the CYPs inhibitions but it also reduced the microsomal stability (**27** vs **8**). On the other hand, replacement of the pyrazole by a pyridine moiety slightly increased the CYPs inhibitions and decreased the microsomal stability (**33** vs **12**). Interestingly, the 3A4 enzyme inhibition is much lower in the 2-methoxy benzyl amide (**35**) than in the 3-methoxy benzyl amide (**12**). It is also important to note that a methyl substitution at the benzylic position helped to reduce CYPs inhibitions (**39** vs **12**) in the indole 3-carboxamide scaffold while a similar substitution in the 7-azaindole 3-carboxamide scaffold did not change much the microsomal stability and the CYPs inhibitions (**47** vs **21**).

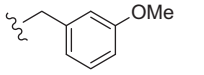
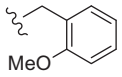
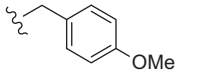
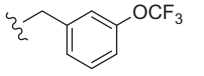
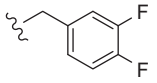
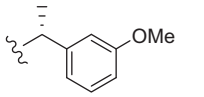
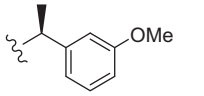
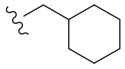
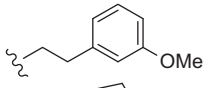
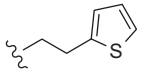
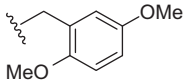
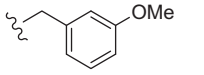
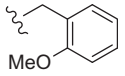
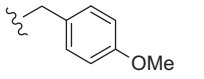
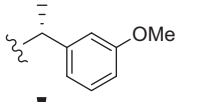
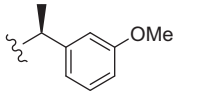
The in vivo pharmacokinetic data shown in Table 5 demonstrated that all these selected indole and 7-azaindole based ROCK inhibitors had low clearance (Cl), low volume of distribution (V_{ss}), and high AUC in iv dosing. However, only compounds **8**, **21**, and **47** exhibited a good half-life ($t_{1/2}$), a fair oral bioavailability (%*F*), and a high C_{max} and AUC in oral (po) dosing. These in vivo pharmacological data correlated well with the corresponding microsomal stability: high microsomal stability generally gave high oral absorbance.

In conclusion, we have synthesized a series of 2- and 3-carboxamide indoles and azaindoles and evaluated their biological activity against the Rho kinase. These compounds possess high ROCK-II potency and moderate to excellent PKA selectivity. The overall best compounds for oral dosing, considering target activity, selectivity, CYP 450 inhibitions, and pharmacokinetic properties, are the 7-azaindole 2-carboxamides **21** (also coded as SR**7355**) and **47** (also

Table 3
Exploration of the benzyl amide group in the indole and 7-azaindole-2 carboxamide scaffold


A


B

Compd	R	Structure	IC_{50}[a] (nM)		
			ROCK-II	ROCK-I	PKA
12		A	<1	<1	510
35		A	9	23	970
36		A	750	1900	>20000
37		A	220	760	4000
38		A	8	34	360
39		A	<1	2	930
40		A	130	500	16000
41		A	76	170	2800
42		A	170	240	1900
43		A	3	18	500
44		A	740	1340	13100
21		B	2	12	140
45		B	5	130	150
46		B	180	500	>20000
47		B	1	6	740
48		B	290	560	11000

[a] Average of two or more measurements with standard derivations ⩽30%.

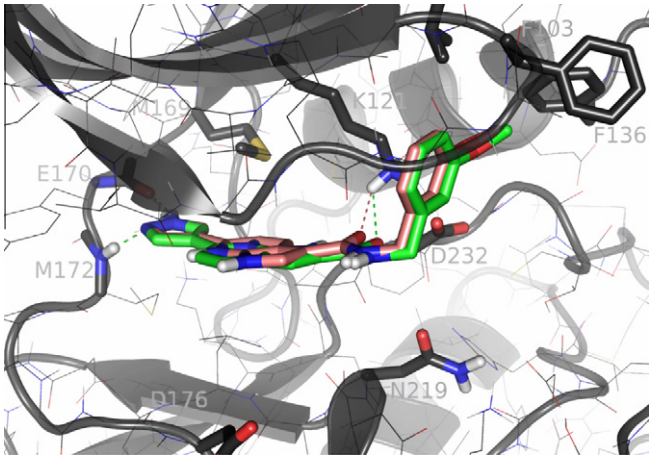


Figure 2. Proposed two alternate binding modes of 7-azaindole 2- and 3-carboxamides (compound **21** and **25**, respectively).

Table 4
In vitro DMPK properties for selected compounds

Compd	$t_{1/2}$ (min)	CYP-450 Enzyme inhibition at 10 μM (%)			
	HLM	1A2	2C9	2D6	3A4
8	80	42	87	81	87
12	25	87	97	51	72
21	53	4	38	32	22
25	15	5	86	12	46
27	17	12	14	8	14
33	10	67	99	75	80
35	21	89	91	34	37
39	24	39	90	33	56
47	59	13	60	41	23

Table 5
In vivo (rat) pharmacokinetic data[a] for selected inhibitors

Cmpd	Cl (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	AUC iv (μM h)	AUC po (μM h)	C_{max} po (μM)	Oral *F* (%)
8	3.2	0.46	2.0	15.3	8.7	1.5	28
12	4.9	0.26	1.1	10.0	1.0	0.5	5
21	2.1	0.27	2.0	22.4	14.4	1.8	32
25	7.6	0.52	1.0	6.4	0.3	0.1	3
39	5.8	0.37	1.1	8.1	1.4	0.3	9
47	6.6	0.94	2.0	7.6	5.0	0.6	33

[a] Data were the average from three determinations with standard derivations ⩽30%. Dosed at 1.0 mg/kg (iv) or 2.0 mg/kg (po).

coded as SR**8363**). Further SAR studies for indole and 7-azaindole based ROCK compounds bearing substitutions on the indole NH and/or the amide NH positions, or the indole C3 position are addressed in the following Letter (part-II).

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References and notes

1. Riento, K.; Ridley, A. *J. Nat. Rev. Mol. Cell. Biol.* **2003**, *4*, 446.
2. Uehata, M.; Ishizaki, T.; Sato, H.; Ono, T.; Kawahara, T.; Morishita, T.; Tamakawa, H.; Yamagami, K.; Inui, J.; Maekawa, M.; Narumiya, S. *Nature* **1997**, *389*, 990.
3. Doe, C.; Bentley, R.; Behm, D. J.; Lafferty, R.; Stavenger, R.; Jung, D.; Bamford, M.; Panchal, T.; Grygielko, E.; Wright, L. L.; Smith, G. K.; Chen, Z.; Webb, C.; Khandekar, S.; Yi, T.; Kirkpatrick, R.; Dul, E.; Jolivette, L.; Marino, J. P., Jr.; Willette, R.; Lee, D.; Hu, E. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 89.
4. Tanihara, H.; Inatani, M.; Honjo, M.; Tokushige, H.; Azuma, J.; Araie, M. *Arch. Ophthalmol.* **2008**, *3*, 309.
5. Waki, M.; Yoshida, Y.; Oka, T.; Azuma, M. *Curr. Eye Res.* **2001**, *22*, 470.
6. Rudick, R.; Mi, S.; Sandrock, A. *Expert Opin. Biol. Ther.* **2008**, *8*, 1561.
7. Shin, H.; Salomone, S.; Ayata, C. *Expert Opin. Ther. Targets* **2008**, *12*, 1547.
8. Oka, M.; Fagan, K.; Jones, P.; McMurtry, I. *Br. J. Pharmacol.* **2008**, *155*, 444.
9. Bivalacqua, T. J.; Champion, H. C.; Usta, M. F.; Cellek, S.; Chitaley, K.; Webb, R. C.; Lewis, R. L.; Mills, T. M.; Hellstrom, W. J. G.; Kadowitz, P. *J. Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9121.
10. Kubo, T.; Yamaguchi, A.; Iwata, N.; Yamashita, T. *Ther. Clin. Risk Manag.* **2008**, *4*, 605.
11. Micuda, S.; Rosel, D.; Ryska, A.; Brabek, J. *Curr. Cancer Drug Targets* **2010**, *10*, 127.
12. Nakagawa, O.; Fujisawa, K.; Ishizaki, T.; Saito, Y.; Nakao, K.; Narumiya, S. *FEBS Lett.* **1996**, *392*, 189.
13. Shibuya, M.; Suzuki, Y.; Sugita, K.; Saito, I.; Sasaki, T.; Takakura, K.; Nagata, I.; Kikuchi, H.; Takemae, T.; Hidaka, H., et al *J. Neurosurg.* **1992**, *76*, 571.
14. Hydroxyfasudil, the active metabolite of fasudil, is generally effective in cell-based assays at or above 1 μM; se:e Nakamura, K.; Nishimura, J.; Hirano, K.; Ibayashi, S.; Fujishima, M.; Kanaide, H. *J. Cereb. Blood Flow Metab.* **2001**, *21*, 876.
15. Feng, Y.; Cameron, M.; Frackowiak, B.; Griffin, E.; Lin, L.; Ruiz, C.; Schröter, T.; LoGrasso, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2355.
16. Chen, Y. T.; Bannister, T.; Weiser, A.; Griffin, E.; Lin, L.; Ruiz, C.; Cameron, M.; Schürer, S.; Duckett, D.; Schröter, T.; LoGrasso, P.; Feng, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6406.
17. Sessions, E. H.; Yin, Y.; Bannister, T. B.; Weiser, A.; Griffin, E.; Pocas, J.; Cameron, M.; Ruiz, C.; Lin, L.; Schürer, S.; Schröter, T.; LoGrasso, P.; Feng, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6390.
18. Sessions, E. H.; Smolinksi, M.; Wang, B.; Frackowiak, B.; Chowdhury, S.; Yin, Y.; Chen, Y. T.; Ruiz, C.; Lin, L.; Pocas, J.; Schröter, T.; Cameron, M.; LoGrasso, P.; Feng, Y.; Bannister, T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1939.
19. Yin, Y.; Lin, L.; Ruiz, C.; Cameron, M.; Pocas, J.; Grant, W.; Schröter, T.; Chen, W.; Duckett, D.; Schürer, S.; LoGrasso, P.; Feng, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6686.
20. Fang, X.; Yin, Y.; Chen, Y. T.; Yao, L.; Wang, B.; Cameron, M. D.; Lin, L.; Khan, S.; Ruiz, C.; Schröter, T.; Grant, W.; Weiser, A.; Pocas, J.; Pachori, A.; Schürer, S.; Lo Grasso, P.; Feng, Y. *J. Med. Chem.* **2010**, *53*, 5727.
21. Yin, Y.; Cameron, M. D.; Lin, L.; Khan, S.; Schröter, T.; Grant, W.; Pocas, J.; Chen, Y. T.; Schürer, S.; Pachori, A.; LoGrasso, P.; Feng, Y. *ACS Med. Chem. Lett.* **2010**, *1*, 175.
22. (a) Schröter, T.; Minond, D.; Weiser, A.; Dao, C.; Habel, J.; Spicer, T.; Chase, P.; Baillargeon, P.; Scampavia, L.; Schürer, S. C.; Chung, C.; Mader, C.; Southern, M.; Tsinoremas, N.; LoGrasso, P.; Hodder, P. *J. Biomol. Screen.* **2008**, *13*, 17; (b) Schröter, T.; Griffin, E.; Weiser, A.; Feng, Y.; LoGrasso, P. *Biochem. Biophys. Res. Commun.* **2008**, *374*, 356.
23. For benzothiazoles (e.g., inhibitor 2 in Fig. 1) the amide substituent is important for cell-based activity and added ROCK-II potency. The benzothiazole analog of indole 8 has the following binding profile: ROCK-II IC_{50} = 18 nM, ROCK-I IC_{50} = 76 nM, PKA IC_{50} = 5300 nM, ppMLC IC_{50} = 2700 nM.
24. In many instances the compounds reported herein were also evaluated versus a selected panel of other kinases (e.g., JNK3). No off-target activity <5 μM was seen. Development compounds will be profiled versus an extended panel of >300 kinases.
25. Fyfe, M. C. T.; Thomas, G. H.; Gardner, L. S.; Bradley, S. E.; Gattrell, W.; Rasamison, C. M.; Shah, V. K. WO 2006067532, 2006.
26. Krulle, T. M.; Rowley, R. J.; Thomas, G. H. WO 2006059164, 2006.
27. Berdini, V.; Boyle, R.G.; Saxty, G.; Walker, D. W.; Woodhead, S. J.; Wyatt, P. G.; Caldwell, J.; Collins, I.; Da Fonseca, T. F. WO 2006046024, 2006.
28. Minakata, S.; Komatsu, M.; Ohshiro, Y. *Synthesis* **1992**, *7*, 661.
29. Feng, Y.; Yin, Y.; Weiser, A.; Griffin, E.; Cameron, M. D.; Lin, L.; Ruiz, C.; Schürer, S. C.; Inoue, T.; Rao, P. V.; Schröter, T.; LoGrasso, P. *J. Med. Chem.* **2008**, *51*, 6642.