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The development of benzimidazoles as selective rho kinase inhibitors

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ABSTRACT

Rho Kinase (ROCK) is a serine/threonine kinase whose inhibition could prove beneficial in numerous therapeutic areas. We have developed a promising class of ATP-competitive inhibitors based upon a benzimidazole scaffold, which show excellent potency toward ROCK (IC_{50} <10 nM). This report details the optimization of selectivity for ROCK over other related kinases such as Protein kinase A (PKA).

Rho Kinase (ROCK) is one of the over 500 known constituents in the human kinome. This serine/threonine kinase is ubiquitously expressed throughout vascular tissue and plays an important role in essential signal transduction pathways.[1] Upon activation by RhoA, ROCK mediates several fundamental cellular processes such as contraction, migration, adhesion, and cytoskeleton regulation. As such, the inhibition of ROCK has been hypothesized to be of potential therapeutic utility for a range of diseases including stroke,[2] hypertension,[3] multiple sclerosis,[4] asthma,[5] erectile dysfunction,[6] glaucoma,[7] and central nervous system disorders.[8] Currently, the only clinically approved ROCK inhibitor is fasudil, which is used in Japan for the treatment of cerebral vasospasm.[9]

Many groups, including ours, have pursued small molecule inhibitors of ROCK based upon several different scaffolds including indazoles,[10] isoquinolines,[11] aminofurazans,[12] chromanamides,[13] and benzimidazoles.[14] Though structurally distinct, these series all act as ATP-competitive ROCK inhibitors. Close homology of the ATP binding pockets within the human kinome makes kinase selectivity a major issue in the development of small molecule inhibitors. We previously reported that benzimidazole-based ROCK inhibitors (Fig. 1) have excellent potency in both biochemical (IC_{50} <10 nM) and cell-based (IC_{50} <100 nM) assays.[14] Molecular modeling studies and preliminary SAR indicated that the high potency was largely due to three key interactions within the ROCK[15] binding pocket: (i) H-bonding between the pyridyl nitrogen and M172 of the hinge region, (ii) water-mediated H-bonding of an imidazole nitrogen and L121, and (iii) hydrophobic interactions between the aryl moiety and the P-loop. To evaluate their selectivity for ROCK over other members of the kinome, the potency against the closely related protein kinase A (PKA) was also determined.[16,17] Unfortunately, the initial compounds synthesized proved to be relatively potent (IC_{50} <500 nM) inhibitors of PKA also. We hoped to improve upon the selectivity for ROCK over PKA, which could also provide greater selectivity over less closely related kinases.

In a related aniline-based scaffold (Fig. 2), the addition of a polar side chain (e.g., *N*,*N*-dimethylaminoethoxy in **2**) dramatically improved selectivity for ROCK over PKA (472-fold vs 63-fold for the unsubstituted analog).[13] This improved selectivity was attributed to the interaction of the polar side chain with an aspartic acid residue (D176) in the ROCK binding pocket that is not present in PKA.

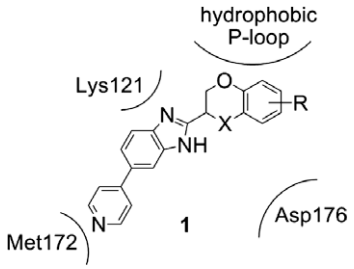

Figure 1. Previously disclosed ROCK inhibitor **1** (X = CH_2, O).

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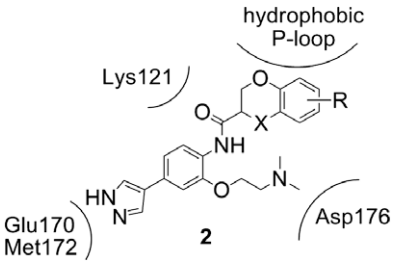


Figure 2. Key modeling interactions of inhibitor **2** with the ATP binding pocket of ROCK (X = CH_2, O).

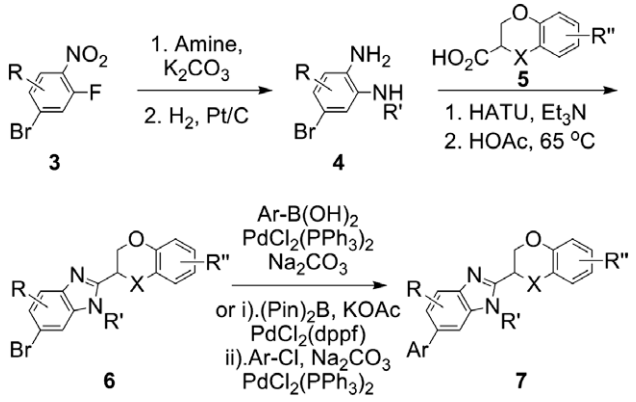
Modeling suggests that the other key interactions in the aniline-amide series are similar to those in the benzimidazole series.[13] Incorporating an interaction analogous to the amine-D176 H-bond into the benzimidazole scaffold might similarly improve the selectivity against PKA. This Letter details our ultimately successful efforts to identify low nanomolar inhibitors of ROCK with affinity for PKA in the micromolar range.

The general synthetic route for the benzimidazoles is shown in Scheme 1. Commercially available 4-bromo-2-fluoronitrobenzenes[18] **3** were converted into phenylenediamines **4** via nucleophilic aromatic substitution followed by chemoselective reduction of the nitro group. Peptide coupling with carboxylic acids **5** followed by an acid catalyzed cyclodehydration formed the benzimidazole heterocycles **6**. Palladium catalyzed cross-coupling reactions were then utilized to install the heteroaromatic hinge binding moiety, resulting in the ROCK inhibitors **7**.[19]

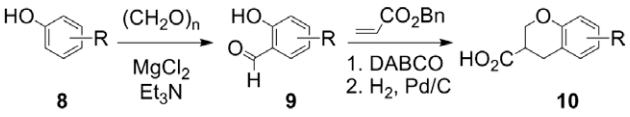
Chroman acids **10** which were not commercially available were synthesized as shown in Scheme 2. Salicylaldehydes **9** were formed via an orthoformylation reaction from the corresponding phenol **8**. They were then heated with benzyl acrylate in the presence of DABCO to give the corresponding chromene derivatives. Reduction of both the olefin and the benzyl ester occurred upon exposure to standard hydrogenation conditions, thus giving the chromans **10**.[20]

A series of 6-carboxamide-substituted chromans were synthesized as shown in Scheme 3.[21] Access to the methyl ester **11** was achieved through the standard route (Schemes 1 and 2). Saponification of the ester, followed by HATU-mediated amide formation gave the desired analogs **12**.

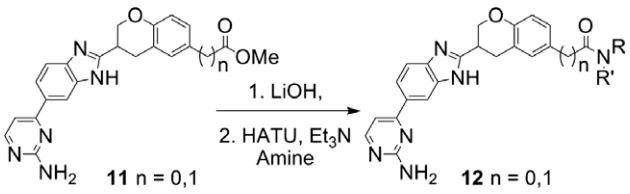
Drawing from our experience in the aniline-amide scaffold (i.e., **2**), it was felt that the addition of a side chain on the benzimidazole core might lead to an improvement in selectivity for ROCK over PKA. To test this hypothesis, alkyl or alkoxy groups were systematically incorporated onto the benzimidazole core of either benzodioxane **13** or chroman **18** (Table 1). As previously noted,[14] the



Scheme 1. General synthesis of benzimidazoles **7**.

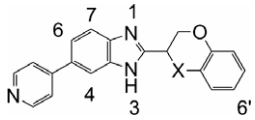


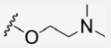
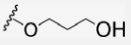
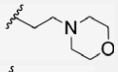
Scheme 2. General synthesis of chromans **10**.



Scheme 3. General synthesis of amides **12**.

Table 1
Benzimidazole substituent effects and PKA selectivity[a]

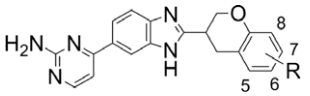


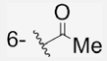
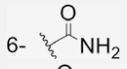
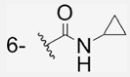
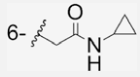
#	Substitution	X	ROCK (nM)	PKA (nM)	PKA/ ROCK
13	None	O	7.5	355	47
14	7-Me	O	240	1380	6
15	6-CF3	O	2970	3080	1
16	6-CF3	O	>20,000	>20,000	1
17	6-Me, 7-Me	O	>20,000	>20,000	1
18	None	CH_2	1.7	252	148
19	6'-OMe, 7- 	CH_2	440	>20,000	45
20	6'-OMe, 7- 	CH_2	183	1590	9
21	4-OMe	CH_2	4.1	187	46
22	3-Me	O	15	1960	131
23	1-Me	O	1780	3130	2
24	3- 	O	24	850	35
25	3- 	O	190	1000	5
26	3- 	O	680	8810	13
27	3- 	O	130	6120	47
28	3- 	O	11	310	28
29	3- 	O	27	509	19

[a] IC_{50} values are means of two or more experiments with errors within 143% of the mean.

chroman variants were consistently more potent than benzodioxanes in the ROCK assays, but these analogs showed similar potency in the PKA assay.[22] Incorporation of a substituent at the 6- or 7-position of the benzimidazole reduced affinity for ROCK (cf. **14**–**17**). The lack of tolerance for even small groups on these positions likely indicates that this portion of the inhibitor makes close contact with the enzyme; this would be unexpected if the 6- or 7-positions projected toward the solvent exposed D176 residue. To allow for a more direct comparison, two of the polar side chains from the aniline series that were most effective in boosting selectivity over PKA were also tested (e.g., **19**, **20**). Incorporation of these groups led to significantly less affinity (>100-fold) for both ROCK and PKA. It should be noted that these compounds also have a 6′-methoxy group on the chroman, a modification that generally boosts ROCK potency and selectivity over PKA (discussed later in this Letter, see Table 2), suggesting that without the 6′-methoxy the PKA

Table 2
Exploration of chroman substituent effects on PKA selectivity[a]



#	Substitution	ROCK (nM)	PKA (nM)	PKA/ ROCK
30	6-OMe	1.7	485	285
31	5-OMe	25	310	12
32	7-OMe	95	435	4.6
33	8-OMe	256	232	0.9
34	none	8.0	195	24
35	6-O/Pr	316	4320	14
36	6-F	4.7	147	31
37	6-Br	10	82	8.2
38	6-CN	43	713	17
39		40	923	23
40		2.7	691	256
41		4.0	803	200
42		1.2	497	414
43		0.8	78	98

[a] IC_{50} values are means of two or more experiments with errors within 139% of the mean.

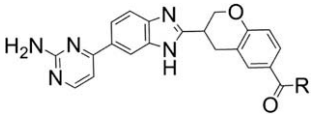
selectivity of analogs **19** and **20** would likely be even lower. The 4-position of the benzimidazole core was next examined. Derivative **21** showed that a methoxy group was well tolerated, however the selectivity against PKA was diminished relative to **18** (~3-fold decrease). Larger alkoxy groups might improve the selectivity, though similar interactions might be achieved through more synthetically-accessible N-3 substituted analogs. Overall no improvement in the desired selectivity was found by adding substituents on any of the three benzimidazole carbon atoms.

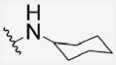
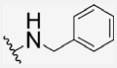
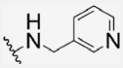
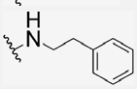
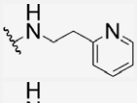
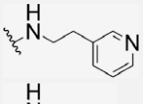
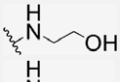
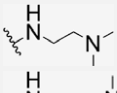
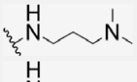
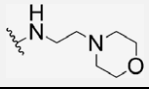
Substitutions on the two nitrogen atoms of the benzimidazole core were next explored. A methyl group at N-1 was poorly tolerated,[23] while the N-3 methyl derivative was well tolerated, exhibiting only a small decrease in affinity for ROCK and a larger decrease in affinity for PKA.[24] Many N-3 substituted analogs were subsequently synthesized in search of further improvement in the selectivity over PKA; only a representative sample of those tested is shown in Table 1. As can be seen, while several substituents were tolerated by ROCK, in no case was superior selectivity over PKA achieved. The tolerance of fairly large groups (**29**, for example) indicates that groups on N-3 were likely projected into the vacant, solvent exposed portion of the binding pocket near D176 in ROCK, however the lack of improved selectivity implies that no important interaction with D176 was occurring. Thus addition of a polar side chain to any position on the benzimidazole core, to date, has failed to yield the same dramatic increase in PKA selectivity that was seen in the previous aniline series. For example, the morpholinoethyl group and related amines confers added PKA selectivity with no loss of ROCK affinity in the aniline series, while some potency is lost with benzimidiazoles (see compound **27**). This disparity suggests that the precise orientations and conformational preferences of the benzimidazole and aniline-based ROCK inhibitors are significantly different in the ATP binding pocket, despite their superficial similarities.[13b]

Having been unable to improve the selectivity for ROCK over PKA through modifications of the benzimidazole core, attention was turned to the chroman to achieve the same objective (Table 2). These explorations were primarily carried out with the aminopyrimidine group replacing the pyridine moiety due to the lower levels of inhibition of cytochrome P_{450} enzymes observed in inhibitors bearing this group.[25] An initial, limited screen of substituted chromans led to the identification of 6-methoxychroman **30** as a promising lead, with near 300-fold selectivity for PKA. The alternate methoxy regioisomers (cf. **31**–**33**) were found to be both less potent against ROCK and less selective over PKA. These findings support our modeling-based hypothesis that the chroman ring is in close contact with the enzyme at the 7- and 8-positions.[14] The 5-position, however, appears to be solvent exposed and therefore shows greater tolerance toward substitution.

Having determined that the 6-position was optimal for substituents, several derivatives were synthesized in the search for an improved selectivity for ROCK over PKA. Increasing the size of the alkoxy substituent led only to a large decrease in the ROCK potency (e.g. **35**). This lack of tolerance for larger alkoxy groups is interesting given the high potency maintained with several other larger substituents (vide infra, Table 3). Relatively small groups such as F and Br were tolerated, but unsurprisingly did not improve upon the selectivity of **30**. Nitrile or sulfonamide incorporation resulted in PKA affinities near 1 μM, however the accompanying increase in the ROCK IC_{50} values negated any benefit. Greater success was ulti-

Table 3
Relative PKA selectivity of chroman-6-carboxamides[a]



#	R	ROCK (nM)	PKA (nM)	PKA/ROCK
44		129	1730	13
45		1.1	20	18
46		<1	78	78
47		0.78	82	105
48		1.7	700	412
49		0.43	305	709
50		0.25	442	1768
51		2.8	370	132
52		1.5	328	219
53		1.2	272	227
54		1.2	1530	1275

[a] IC_{50} values are means of two or more experiments with errors within 92% of the mean.

Table 4
Stability to human liver microsomes (HLM), in vivo pharmacokinetic data (Rat) and cell-based potencies (ppMLC) for chosen ROCK inhibitors[a]

#	$t_{1/2}$ HLM (min)	Cl (mU min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	AUC po (μM/h)	C_{max} Po (μM)	Oral F%	ppMLC (nM)
30	28	17	0.9	2.3	1.9	0.90	35	85
42	79	29	1.4	2.0	0.01	0.009	<1	210
50	1.5	58	5.9	3.6	0.08	0.005	6	250
53	100	27	4.6	3.0	0.01	0.003	<1	2700
54	15	19	0.5	0.7	0.0	0.0	0	1200

[a] Data was generated from three determinations. Dosed at 1.0 mg/kg (iv) Hid or 2.0 mg/kg (po).

mately achieved with 6-keto or 6-carboxamide derivatives. For instance the 6-acetylchroman **40** showed low nanomolar affinity for ROCK and a PKA selectivity ratio of 256. Primary amide **41** was similar in potency and selectivity to acetylchroman **40**. Secondary amide **42** had increased ROCK and PKA potency with a selectivity ratio of over 400. The importance of the carbonyl location was next evaluated through the testing of homologue **43**. This derivative exhibited sub-nanomolar ROCK potency, but also has very high affinity for PKA. These results led us to focus our efforts on an in-depth exploration of the carboxamide-substituted chromans similar to **42**.

A diverse collection of amides was synthesized and assayed against both ROCK and PKA; a representative sample is shown in Table 3. Tertiary amides such as **44** were uniformly found to be much less potent in both assays. Larger cycloalkyl amides such as **45** possessed excellent ROCK potency, but the PKA potency was also greatly increased compared to the smaller cyclopropyl group of **42**. Similarly, benzyl amide **46** and its pyridyl analog **47** both were potent ROCK inhibitors but had eroded selectivity versus PKA. Significant selectivity was found by further extending the aryl moiety, for example, **48**, raising the PKA IC_{50} to near micromolar levels while maintaining low nanomolar ROCK potency. The corresponding pyridines showed a further 5–7-fold increase in ROCK potency, with a smaller 2–3-fold increase in PKA potency leading to excellent selectivity ratios. It is unclear whether or not the pyridyl nitrogen is making a specific binding site interaction which leads to the improved selectivity. To further explore the potential for hydrogen bonding interactions within the enzyme's binding pocket, polar groups were incorporated and assayed. Smaller polar chains (e.g., **51**–**53**) were found to result in similar ROCK and PKA affinities to the cyclopropylamide **42**. The larger morpholino group in **54,** however, showed a remarkable 1300-fold preference for ROCK over PKA. As a general trend, the larger 2-ethylamine derivatives possessed more favorable selectivity profiles, with large distal functionality being optimal (e.g. **54**).

The drug metabolism and pharmacokinetic (DMPK) properties of several of the more selective benzimidazole-based inhibitors were determined as shown in Table 4. The majority of synthesized inhibitors showed good stability toward human liver microsomes (HLM); the notable exceptions were the arylethylamide derivatives such as **50**. While the modestly PKA-selective methoxychroman **30** showed good systemic pharmacokinetic properties, the much more selective amides had very poor oral bioavailability which limits their usefulness as orally-administered agents. These inhibitors could prove useful in topical formulations for local administration; in such instances the poor oral bioavailability might be beneficial in reducing potential side effects resulting from inadvertent systemic exposure. The cell-based activity[26] of these inhibitors was also determined and found to be significantly lower than the in vitro binding assay. An increase in the cell-based potency might be necessary to further evaluate these compounds for topically treated indications such as glaucoma.[27]

In summary, benzimidazole-based ROCK inhibitors with high potency have been optimized to improve selectivity over the closely related kinase PKA.[17] The enhanced selectivity was achieved through the substitution of the chroman ring with various carboxamide groups. These derivatives maintained low- or sub-nanomolar potency against ROCK with approximately micromolar activity against PKA. Poor oral bioavailability precludes the use of these inhibitors by oral delivery, however they might be well-suited for topical applications such as for the treatment of glaucoma. The in vivo evaluation of benzimidazole ROCK inhibitors as anti-glaucoma agents is the subject of ongoing investigations to be reported in future publications.

Acknowledgments

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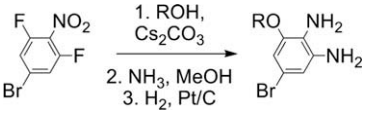
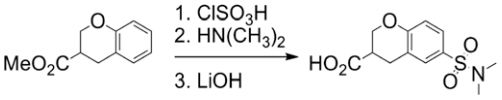
15. In this Letter, only the affinity for the ROCK-II isoform is reported. ROCK-I affinity was monitored as well, but isoform selectivity in these series did not exceed 10-fold.

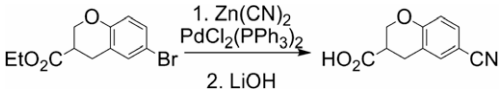
16. ROCK and PKA assays were performed as described in: Schröter, T.; Minond, D.; Weiser, A.; Dao, C.; Habel, J.; Spicer, T.; Chase, P.; Baillargeon, P.; Scampavia, L.; Schürer, S. C.; Chung, C.; Mader, C.; Southern, M.; Tsinoremas, N.; LoGrasso, P.; Hodder, P. *J. Biomol. Screen.* **2008**, *13*, 17.
17. In many instances the compounds reported herein were also evaluated versus a selected panel of other kinases (e.g., MRCKα, AKT, p38, JNK1, JNK3, etc.). PKA inhibition was the most frequently seen unwanted activity in early leads and so was used as a surrogate marker for potential broad kinase selectivity. In the amide series (see Fig. 2 and Ref. 13) enhanced selectivity for PKA was found to generally correlate with selectivity for many other kinases of concern.
18. 2-Amino-4-bromo-3-methoxy-1-nitrobenzene is not commercially available and was synthesized according to: Mallory, F.; Wood, C.; Hurwitz, B. *J. Org. Chem.* **1964**, *29*, 2605.
19. (a) Compounds **19** and **20** were made similarly to Scheme 1, however the starting material 4-bromo 2-6-difluoronitrobenzene was used as shown below.



(b) Compound **23** was made similarly to Scheme 1, however 4-bromo-1-fluoro-2-nitrobenzene was used as the starting material.
20. The 6-sulfonamidechroman was synthesized as below:



The 6-cyanochroman was synthesized as follows:



21. The 6-acetylchroman **40** was also synthesized from this route. The Weinreb amide was made as shown, followed by methyl ketone formation via treatment with MeMgBr in THF.
22. The corresponding 2-substituted chromans and tetrahydronaphthalenes were also briefly examined and shown to be less potent and selective compared to the 3-substituted chromans.
23. Larger substituents at N-1 were similarly inactive, data not shown. For clarity in this discussion, the pyridyl-containing site is consistently termed C-5 rather than renumbering all sites after defining the unsubstituted N as N-1.
24. This promising compound was unfortunately also found to be a strong inhibitor of the cytochrome P_{450} enzymes.
25. 4-Pyridyl-containing inhibitors in this series commonly inhibit one or more of CYP_{450} isoforms 2C9, 2D6, 3A4, and 1A2. Aminopyrimidine analogs of these same compounds are known to lack CYP_{450} affinity (cf. Table 1, Ref. 14).
26. Schröter, T.; Griffin, E.; Weiser, A.; Feng, Y.; LoGrasso, P. *Biochem. Biophys. Res. Commun.* **2008**, *374*, 356.
27. Our earlier work (Ref. 14) showed that fluorine-containing analogs in this series show significant (even 10-fold) improvement in potency in cell-based assays. There is also a general trend for enhanced cell-based potency among analogs with low total polar surface area (tPSA).