Bioorganic & Medicinal Chemistry Letters 18 (2008) 6390–6393

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Benzimidazole- and benzoxazole-based inhibitors of Rho kinase

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ARTICLE INFO

Article history:
Received 8 September 2008
Revised 16 October 2008
Accepted 17 October 2008
Available online 25 October 2008

Keywords:
Rock-II
Rho kinase
Glaucoma
Hypertension
Benzimidazole
Benzoxazole
Chroman
Aminopyrimidine
Pyrazole

ABSTRACT

Inhibitors of Rho kinase have been developed based on two distinct scaffolds, benzimidazoles, and benzoxazoles. SAR studies and efforts to optimize the initial lead compounds are described. Novel selective inhibitors of ROCK-II with excellent potency in both enzyme and cell-based assays were obtained. These inhibitors possess good microsomal stability, low cytochrome P-450 inhibitions and good oral bioavailability.

Rho kinase (ROCK) is a serine/threonine kinase that is an important regulator in smooth cell contraction. Two isoforms of Rho kinase are known, ROCK-I (or ROKβ) and ROCK-II (or ROKα), which share 92% sequence identity in the ATP binding pocket.[1] ROCK is activated via the binding of the GTP-bound G protein Rho. Once activated, ROCK phosphorylates numerous substrates affecting cellular contraction, cytoskeleton regulation, and microtubule regulation. One example of these effects is the phosphorylation of myosin light chain (MLC) which, when phosphorylated, leads to cell contraction. ROCK is closely related to other members of the AGC family of kinases such as CDC42-binding kinase (MRCK), protein kinase A (PKA) and protein kinase B (Akt).[2]

There are many potential therapeutic applications for an inhibitor of ROCK, including hypertension,[3] inflammation,[4] ischemic stroke,[5] cancer,[6] multiple sclerosis,[7] erectile dysfunction,[8] and glaucoma.[9] So far, this potential has only been realized by one clinically approved inhibitor (fasudil, marketed in Japan for cerebral vasospasm[10]). Our goal was to identify and develop potent and selective inhibitors of ROCK with acceptable pharmacokinetic and safety profiles. Our initial progress toward this goal has been previously reported.[11,12] Additionally, there are several other groups working toward the development of novel Rho kinase inhibitors.[13]

A focused medicinal chemistry effort based on literature ROCK inhibitors and leads from an in-house HTS campaign[14] led to amide **1** (Fig. 1) as an early lead compound[12] with high affinity for ROCK-II in enzyme assays[15] (IC_{50} < 2 nM), good selectivity over the related kinase PKA[16] (IC_{50} = 127 nM), and high potency in a cell-based myosin light chain *bis*-phosphorylation (ppMLC) assay[17] (IC_{50} = 25 nM). While these initial data were promising, we sought to address the potential lability of the central amide bond, which upon hydrolysis would lead to primary anilines, a group often associated with potential toxicity. One strategy for amide replacement involved the synthesis of the benzoxazole and benzimidazole ring-fused analogs **2** and **3**.

We found that both benzoxazole **2** and benzimidazole **3** maintain many of the desirable properties of amide **1**. In particular,

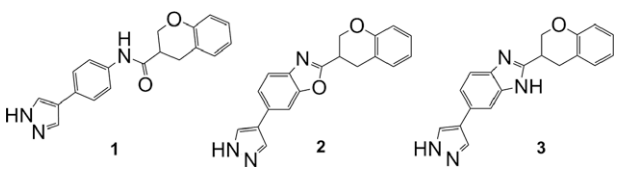

Figure 1. Structures of lead compounds **1**–**3**.

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doi:10.1016/j.bmcl.2008.10.095

benzoxazole **2** has high affinity for ROCK-II (IC_{50} = 21 nM) with moderate PKA selectivity (IC_{50} = 1206 nM). The potency in the cell-based ppMLC assay was, however, reduced (IC_{50} = 330 nM). Benzimidazole **3** has high ROCK-II affinity (IC_{50} = 27 nM) and cell-based potency (IC_{50} = 86 nM), but with reduced selectivity for PKA (IC_{50} = 168 nM). With these promising initial results, we sought to optimize both scaffolds as novel ROCK inhibitors.

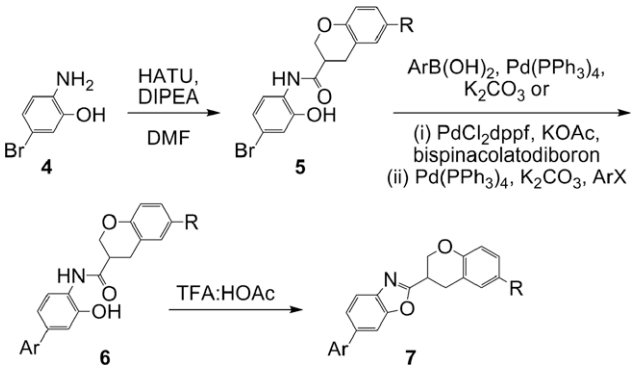
Analogs **7** of benzoxazole **2** were readily synthesized by a short sequence (Scheme 1) that began with amide coupling of commercially available aniline **4** and a chroman carboxylic acid to yield amide **5.** The nitrogen heterocycle was then incorporated through standard palladium coupling chemistry, giving intermediate **6**. Closure of the benzoxazole ring was lastly accomplished under acidic conditions, giving the desired product **7**.

Similarly, a short sequence (Scheme 2) involving sequential amide coupling, dehydration, and Suzuki coupling with an appropriate aryl boronic acid (or alternatively a two-step palladium catalyzed borylation/Suzuki coupling sequence with an aryl halide) gave the targeted benzimidazoles **11**.

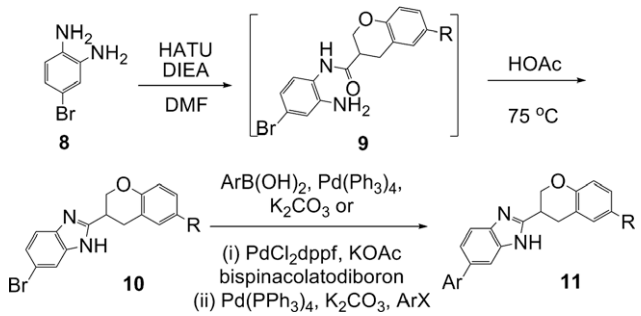
The necessary chromanylcarboxylic acids were synthesized as previously described,[12] or according to Scheme 3. The isopropoxy chroman **13** was derived from the corresponding methoxy substrate via BBr_3 mediated demethylation followed by alkylation. The carboxamide **15** was synthesized from the methyl ester via a two-step hydrolysis/peptide coupling sequence.

We studied the effect of various nitrogen-containing heterocycles upon ROCK-II potency and selectivity. Representative analogs are shown in Table 1. Addition of a methyl group on the 3-position of the pyrazole modestly improved the ROCK-II potency, PKA selectivity and stability to human liver microsomes, though potency in the cell-based assays (ppMLC) was reduced. More promising was compound **18**. The pyridine substitution in this compound improved the enzyme potency, significantly raised PKA selectivity (158-fold vs 6-fold), and improved cell-based potency (IC_{50} < 6 nM). Unfortunately, **18** was found to be a potent inhibitor of several cytochrome p450 (CYP) metabolizing enzymes, as were lead compounds **2** and **3**.[18] The CYP inhibition was effectively reduced by using 2-aminopyrimidine as the heterocycle. Thus benzimidazole **19** and benzoxazole **20** had minimal inhibition of the CYP enzymes studied, with high stability to human liver microsomes, and with high potency in ROCK-II enzyme and cell-based assays.

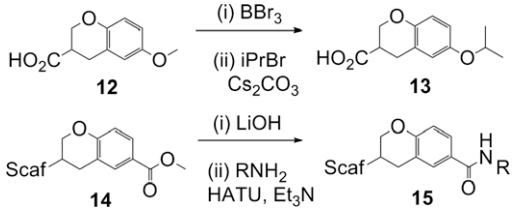
Next we explored the effect of various substituents on the chroman ring, hoping that improved interactions within the ROCK-II binding pocket would lead to improvements in ROCK-II affinity and PKA selectivity. To this end, several 6-substituted chromans were incorporated into the scaffolds as shown in Table 2.[19] The addition of a methoxy or an amide group improved both potency and PKA selectivity. Compound **21** had high affinity for ROCK-II (2 nM) with enhanced selectivity versus PKA (240-fold). Benzoxazole **22** was similarly potent and significantly more selective (ROCK-II IC_{50} = 2 nM, ~950-fold selectivity for PKA). Larger alkoxy groups were not well-tolerated (e.g., isopropoxy derivatives **23** and **24**). Fluoro substitution (e.g., **25** and **26**), a methyl group (**27**) or a



Scheme 1. Synthesis of benzoxazole **7**.

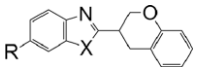


Scheme 2. Synthesis of benzimidazole **11**.



Scheme 3. Synthesis of chroman derivatives.

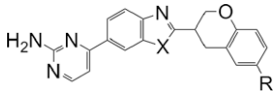
Table 1
Varying the nitrogen heterocycle of benzimidazoles and benzoxazoles.



Compound	R	X	Enzyme assays IC_{50}[a] (nM)		Cell assay ppMLC IC_{50}[a] (nM)	$t_{1/2}$[b] (min) HLM
			ROCK-II	PKA		
2		O	21	1206	330	40
3		N	27	168	86	20
16		N	9	122	nd[c]	30
17		O	9	778	533	70
18		N	2	317	<6	29
19		N	8	198	66	44
20		O	27	1336	nd	57

[a] Values are means of 2 or more experiments with errors within 15% of the mean.
[b] 2 mg/mL human liver microsomes were used in stability studies.
[c] nd, not determined.

Table 2
Evaluation of 6-substituted chromans.



Compound	R	X	Enzyme assays IC_{50}[a] (nM)		Cell assay ppMLC IC_{50}[a] (nM)	$t_{1/2}$[b] (min) HLM
			ROCK-II	PKA		
21	–OMe	N	2	602	99	28
22	–OMe	O	2	1911	46	34
23	–OiPr	N	296	6382	nd	nd
24	–OiPr	O	294	nd	nd	nd
25	–F	N	6	270	nd	45
26	–F	O	15	2912	28	91
27	–Me	O	78	6389	nd	25
28	$-CO_2Me$	N	6	10,190	271	18
29	$-CO_2Me$	O	24	nd	nd	nd
30	-CONH-◁	N	<1	669	212	79
31	–CONHBn	N	<1	75	23	25

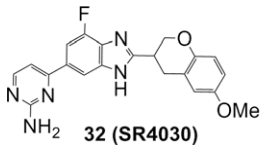
nd, not determined.
[a] Values are means of 2 or more experiments with errors within 80% of the mean.
[b] 2 mg/mL human liver microsomes were used in stability studies.

carbomethoxy group (**28**, **29**) were all tolerated, though with somewhat reduced affinity for ROCK-II. Replacement of the ester with amides not only improved microsomal stability, as expected, but also resulted in higher potency (benzimidazole **30, 31**). Interestingly, even larger C-6 amide substituents were tolerated (e.g., benzimidazole **31**) whereas larger alkoxy groups (e.g., benzimidazole **23**) were not. This survey of 6-substituted chroman derivatives led us to conclude that methoxy and carboxamide substituents are particularly helpful in enhancing affinity for ROCK-II.

A limited SAR study on the effects of substituents on the benzimidazole ring system (not shown) revealed that in general only small groups were well-tolerated. Benzimidazole **32**, with a fluorine atom incorporated (Fig. 2),[20] had excellent affinity for ROCK-II, selectivity against PKA, and high potency in the cell-based ppMLC assay (IC_{50} < 6 nM). Additionally, this compound was screened against several related kinases, and proved to have excellent selectivity against all kinases tested. Screening against ROCK-I for compound **32** and those listed in Tables 1 and 2 is undergoing in our labs. Preliminary results (data not shown) demonstrated that these compounds were typically pan-ROCK inhibitors.

The most promising derivatives in both the benzimidazole and benzoxazole scaffolds were evaluated for their in vivo pharmacokinetic properties (Table 3). All of the compounds shown had both high oral bioavailability and systemic exposure. The high bioavailability of benzoxazole **22** (F = 63%) was especially noteworthy.

To identify likely binding modes of our inhibitors, we docked benzimidazole **21** and benzoxazole **22** into a model of ROCK-II as previously described.[12,21] The initial docking results failed to show any direct interaction between the enzyme and the benzimidazole (or benzoxazole) nitrogen, an interaction anticipated based upon the binding mode of related ROCK-II inhibitors.[12,21] Inclusion of a



32 (SR4030)

ROCK-II: < 1 nM
ppMLC: < 6 nM

PKA: 397 nM
Akt1: 5000 nM
MRCK: 1095 nM
p38: > 20000 nM
JNK1: 14100 nM
JNK3: 16800 nM

Figure 2. Structure and IC_{50} values of fluorinated derivative **32**.

Table 3
In vivo (rat) pharmacokinetic data for inhibitors **21**, **22**, and **32.**[a]

Compound	Cl (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	AUC po (μM h)	C_{max} po (μM)	Oral F (%)
21	17 ± 2.6	0.89 ± 0.11	2.3 ± 0.2	1.9 ± 0.7	0.90 ± 0.58	35 ± 13
22	8.4 ± 0.3	1.6 ± 0.1	2.6 ± 0.2	6.6 ± 1.3	0.97 ± 0.20	63 ± 12
32	12 ± 4.4	0.63 ± 0.20	1.4 ± 0.1	2.2 ± 0.3	0.74 ± 0.03	29 ± 4.4

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

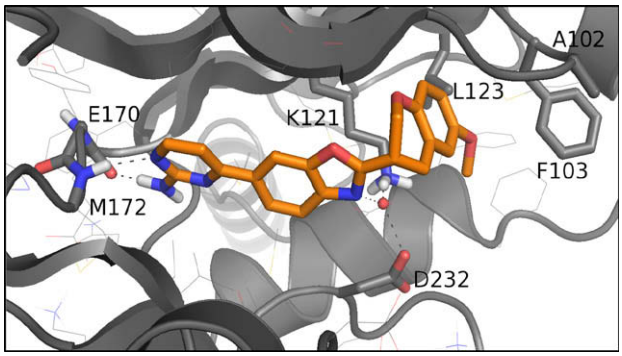


Figure 3. Optimized conformation of **22** docked into the ROCK-II structure.

water molecule, which is frequently observed in the phosphate binding region in ROCK crystal structures,[22,23] could act as a bridge between the benzimidazole nitrogen and the enzyme. Thus, we optimized the enzyme–ligand complex with a water molecule by molecular dynamics and minimization and created a docking model from the minimized complex. Into this enzyme structure **21** and **22** were docked and the best conformation obtained for **22** is shown in Figure 3 (**21** has essentially the same conformation). Figure 3 shows the *S* enantiomer of **22** and our modeling indicated that the opposite enantiomer showed a similar binding mode.[21]

In this model, the 2-aminopyrimidine moiety forms a typical two hydrogen bond pattern with the Glu170 and Met172 residues of the hinge region. An important hydrophobic interaction is formed by the chroman moiety with the hydrophobic binding pocket under the P-loop which is characterized by Phe103, Ala102, Leu123, and the carbon chain of Lys121. The benzoxazole or benzimidazole groups potentially interact with the Lys121 and Asp232 side chains via a water bridge. Docking experiments starting from an alternate ROCK-II structure suggested that an alternate orientation of the central benzimidazole/benzoxazole ring may also be possible, however, the essential hydrogen bonding with the hinge region and the hydrophobic interactions of the chroman are maintained in that structure as well.

In conclusion, a potent series of benzimidazole- and benzoxazole-based ROCK inhibitors have been developed from the initial amide lead **1**. Optimization of the nitrogen heterocycle led to compounds with improved microsomal stability and reduced CYP inhibition, while substitution of the chroman ring produced compounds with improved potency and selectivity against PKA. The benzoxazole series have good ROCK-II potency and selectivity against PKA with excellent pharmacokinetic properties. The benzimidazole series possess excellent biochemical and cell-based potency, with ~100-fold selectivity over PKA and many also have good pharmacokinetic properties. Future efforts to optimize these compounds will focus on the enantioselective synthesis and evaluation of both enantiomers, the development of isoform selective ROCK (I and II) inhibitors and the achievement of pharmacokinetic properties tailored to specific therapeutic applications. These studies will be reported in due course.

Acknowledgments

We thank Prof. Patrick Griffin and Prof. William Roush for their support and Dr. Derek Duckett and Ms. Weimin Chen for the counter screening against p38 and JNK kinases.

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15. Assays were performed using the STK2 kinase system from Cisbio. 5 μL mixture of a 1-μM STK2 substrate and ATP (20 μM) in STK-buffer was added to the wells using a BioRAPTR FRD™ Workstation (Aurora Discovery). 20 nL of test compounds was dispensed. Reaction was started by addition 0.5 nM ROCK-II in STK-buffer. After 4 h at rt the reaction was stopped by addition of 10 μL of 1× antibody and 62.5 nM Sa-XL in detection buffer. After 1 h at rt the plates were read on the Viewlux in HTRF mode.
16. PKA was chosen as a routine preliminary kinase counterscreen because it is among the kinases most closely related to ROCK, along with MRCK and Akt. Promising compounds will be more thoroughly profiled, particularly against a much larger panel of kinases and also assessed for affinity to other relevant receptors, enzymes, and ion channels.
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19. Preliminary SAR, not shown, indicated that substitution at the 6-position is preferred.
20. The synthesis of **32** follows the Scheme 2 procedures, starting with the fluorinated diamine, which is available by SnCl2 reduction of the nitro group of commercially available 2-amino-4-bromo-6-fluoro-nitrobenzene.
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