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Chroman-3-amides as potent Rho kinase inhibitors

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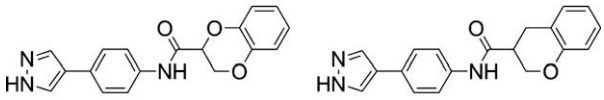
ABSTRACT

Inhibition of Rho kinase (ROCK) is an attractive strategy for the treatment of diseases such as hypertension, glaucoma, and cancer. Here we report chroman-3-amides as highly potent ROCK inhibitors with sufficient kinase selectivity, excellent cell activity, good microsomal stability, and desirable pharmacokinetic properties for study as potential therapeutic agents.

ROCK-I and ROCK-II are highly homologous isoforms[1] of Rho kinase (ROCK), a downstream effector of the small GTPase Rho. These serine-threonine kinases have numerous cellular functions,[2] including regulation of the formation of stress fibers and focal adhesions via the phosphorylation of myosin light chain (MLC).[3] Inhibition of ROCK promotes effects such as relaxation of vascular smooth muscle fibers,[4] alteration of intercellular junctions in the trabecular meshwork of the eye,[5] and promotion of neuronal outgrowth.[6] ROCK inhibitors have been proposed for treating hypertension,[4] ischemic stroke,[7] cancer,[8] erectile dysfunction,[9] glaucoma,[10] multiple sclerosis,[11] and spinal cord injury.[12] Fasudil, the only marketed ROCK inhibitor, is used in Japan for cerebral vasospasm. In recent years, a variety of other ROCK inhibitors have been reported,[13] including aminofurazans,[14] indazoles,[15] and isoquinolines.[16]

We began a program to identify potent and selective ROCK inhibitors with pharmacokinetic (PK) profiles favorable for therapy. The analysis of known ROCK ligands, paired with the study of hits from a high-throughput screen, led us to identify benzodioxane **1** as a promising lead (Fig. 1). This compound was a potent inhibitor of ROCK-II (IC_{50} = 2 nM) with fair selectivity (~100-fold) over the highly homologous protein kinase A (PKA)[17] and was potent in a cell-based myosin light chain *bis*-phosphorylation assay (ppMLC)[18] (IC_{50} = 72 nM). In PK studies, benzodioxane **1** had modest stability in vitro upon exposure to the human liver microsome



1 **2**

Figure 1. Structures of benzodioxane **1** and chroman **2**.

(HLM, $t_{1/2}$ = 20 min) and had essentially no oral bioavailability in rats (F < 1%).

We reasoned that the instability of benzodioxane **1** toward liver microsomes might correlate with electron deficiency of the amide. Chroman **2**, in which the oxygen atom α- to the amide in compound **1** was replaced with a methylene group was prepared and indeed had improved human microsomal stability ($t_{1/2}$ = 65 min) with similar affinity for ROCK-II (IC_{50} < 2 nM) and selectivity over PKA (Table 1). Moreover, compound **2** showed enhanced potency in the cell-based ppMLC assay (IC_{50} = 25 nM) and slightly better oral bioavailability (F = 6%).

The straightforward synthesis of compound **2** and its close analogs is outlined in Scheme 1. Coupling of acid **3** with bromoaniline **4** followed by Suzuki heteroarylation with 1*H*-4-pyrazoleboronic acid pinacol ester under microwave irradiation gave compounds with the general structure **5**.

Dual routes to the preparation of the substituted chroman-3-carboxylic acid are shown in Scheme 2. Ethoxycarbonylation of 4-chromanone **6** gave β-ketoester **7**.[19] Cationic hydrogenation of the ketone to chroman **8**, followed by saponification afforded acid **9**. Alternatively, substituted salicylaldehyde **10** and benzyl acrylate

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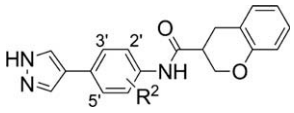
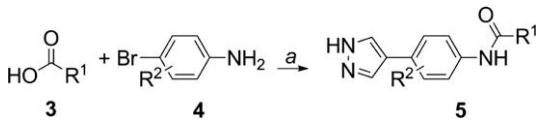
Table 1
Kinase inhibition and stability of chroman **2** and analogs.

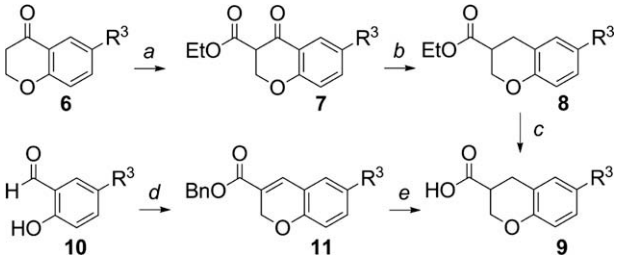


Compound	R^2	Enzyme assays IC_{50}[a] (nM)		ppMLC cell assay IC_{50}[a] (nM)	$t_{1/2}$[b] (min) HLM
		ROCK-II	PKA		
2	H	2	127	25	65
17	3′-OMe	4	290	7	8
18	2′-OMe	3	109	6	14
19	2′-F	4	87	80	40
20	3′,5′-F	4	82	15	37
21	2′-OMe,5′-F	5	117	6	25
22	2′-F,5′-OMe	17	531	8	13
23	2′-Dimethyl-aminoethoxy	3	1416	6	69
24	3′-Dimethyl-aminoethoxy	640	>20,000	nd[c]	nd[c]

[a] Values are means of two or more experiments. The error in these values is within ±30% of the mean.
[b] 2 mg/mL human liver microsomes were used in stability studies.
[c] Not determined.



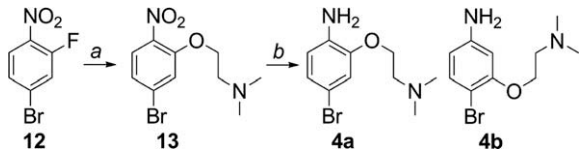
Scheme 1. Reagents and conditions: (i) HATU, *N*-methylmorpholine, DMF, rt, 14 h; (ii) 1*H*-4-pyrazoleboronic acid pinacol ester, $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O, 1 h, 140 °C, μW.



Scheme 2. (a) Reagents and conditions: (i) LiHMDS, THF, −78 °C, 30 min; (ii) ethylchloro-formate, THF, −78 °C, 1 h; (b) Et_3SiH, TFA, rt, 14 h; (c) LiOH, methanol, THF, H_2O, rt, 2 h; (d) benzyl acrylate, DABCO, 0.5 h, 160 °C, μW; (e) H_2, 10% Pd/C, methanol, rt, 14 h.

condensed to give chromene **11**, which upon hydrogenolysis also provided acid **9**.

We explored the effects of structural variation in the central ring using commercially available 4-bromoaryl amines and also dimethylaminoethoxy analogs **4a** and **4b.** Amine **4a** was prepared from 4-bromo-2-fluoro-1-nitrobenzene (**12**) via nucleophilic substitution followed by stannous chloride reduction (Scheme 3). 3-Dimethylaminoethoxy analog **4b** was prepared by a literature



Scheme 3. Reagents and conditions: (a) NaH, 2-(dimethylamino)ethanol, THF, rt, 7 h, 92%; (b) $SnCl_2$, ethanol, 70 °C, 1.5 h, 71%.

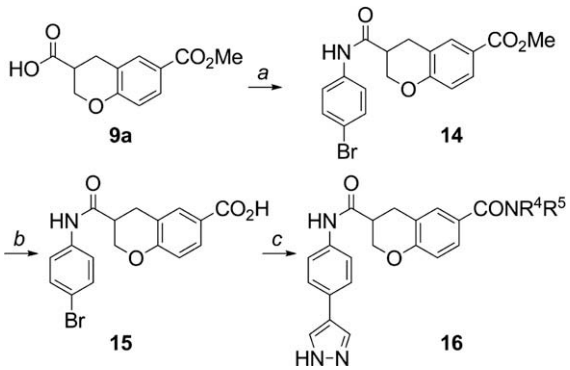
procedure.[20] Coupling of functionalized acid **9** and the substituted anilines and Suzuki coupling was accomplished by the two-step sequence illustrated in Scheme 1 to give the desired inhibitors.

Inhibitors containing an amide group at C6 of the chroman ring were prepared as described in Scheme 4. In this route, acid **9a** was coupled to bromoaniline (**4**, R^2 = H) to give bromide **14**, which was saponified to acid **15**. Coupling of acid **15** with available amines and microwave-assisted Suzuki heteroarylation gave chroman amides with the general structure **16**.

Table 1 summarizes the effects of substitution in the central phenyl ring upon affinity for ROCK-II and PKA, activity in the cell-based ppMLC assay, and stability in vitro to a preparation of human liver microsomes (HLM).[21] Compounds with R^2 = methoxy in the 2′ or 3′ position (inhibitors **17** and **18**) retained ROCK-II potency relative to **2** with enhanced cell-based activity (3- to 4-fold) but were less stable to HLM. Several fluorinated analogs (compounds **19**–**22**) have similar potency and cell-based activity yet have neither enhanced PKA selectivity nor increased stability. Most encouraging, however, was the finding that inhibitor **23**, with a 2-dimethylaminoethoxy substituent, maintained the potency and stability of the lead with a marked improvement in PKA selectivity (PKA/ROCK-II ratio = 470, vs 60 for inhibitor **2**) and with slightly enhanced activity (fourfold) in the cell-based ppMLC assay. Regioisomer **24** was curiously a far less effective ROCK-II inhibitor.

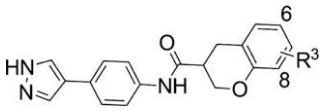
We next investigated the effects of substituent R^3 on the chroman ring (Table 2). Compound **25**, with a C8 methoxy group, had less affinity for ROCK-II than chroman **2**, whereas 6-methoxy substituted analog **26** retained ROCK-II potency with enhanced selectivity (140-fold) over PKA, although microsomal stability was reduced. Other small groups at the C6 position were well-tolerated with respect to ROCK-II inhibition, with compounds **27** and **31** displaying high potency in the cell-based ppMLC assay. Replacing the metabolically labile ester with an amide led to compound **33**, a potent ROCK-II inhibitor with over 200-fold selectivity for PKA. In brief, chromans containing 6-methoxy, 6-fluoro, and 6-primary amide substitutions (inhibitors **26**, **27**, and **33**, respectively) possessed the best balance of properties.

We sought to determine if the effects of substituents R^2 and R^3 in tuning ROCK-II inhibition, selectivity, stability, and especially potency in the cell-based assay could be combined. Hence, we prepared a series of chromans with the 2′-methoxy, 3′-methoxy, or 2′-dimethylaminoethoxy group and these compounds were more effective in the cell-based assay relative to their unsubstituted counterparts (Table 3). The PKA selectivity trend was mixed for the 2′- and 3′-methoxy compounds. However, 2′-dimethylaminoethoxy analogs **38** and **47** were remarkably selective (>10,000-fold) for ROCK-II over PKA.



Scheme 4. Reagents and conditions: (a) 4-bromoaniline, HATU, *N*-methylmorpholine, DMF, rt, 14 h; (b) LiOH, methanol, THF, rt, 2 h, 90%; (c) (i) NHR^1R^2, HATU, *N*-methylmorpholine, DMF, rt, 14 h; (ii) 1*H*-4-pyrazoleboronic acid pinacol ester, $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O, 1 h, 140 °C, μW.

Table 2
Kinase inhibition and stability of substituted chromans.



Compound	R^3	Enzyme assays IC_{50}[a] (nM)		ppMLC cell assay IC_{50}[a] (nM)	$t_{1/2}$[b] (min) HLM
		ROCK –II	PKA		
25	8-OMe	285	3734	nd[c]	nd
26	6-OMe	3	437	38	29
27	6-F	4	270	71	105
28	6-Cl	5	>20,000	3487	138
29	6-Me	10	2000	4000	34
30	6-CO_2H	30	16850	751	nd
31	6-CO_2Me	10	>20,000	22	20
32	6-$CONMe_2$	86	1644	500	70
33	6-$CONH(CH_2)_2NMe_2$	<2	206	47	35
34	6-$CON(Me)(CH_2)_2NMe_2$	93	>20,000	1500	51
35	6-$CON(CH_2CH_2)_2NMe$	78	3062	nd[c]	41

[a] Average of two or more measurements. The error in these values is within ±30% of the average.
[b] 2 mg/mL human liver microsomes were used in stability studies.
[c] Not determined.

Compounds **23**, **38**, and **41** were selected for further selectivity screening and were essentially inactive against several other kinases except for MRCKα (Table 4),[22] the selectivity for which was moderate (~100-fold). Pharmacokinetic evaluation in rats showed that these compounds had improved oral bioavailability (27–35%) relative to lead compounds **1** and **2** (Table 5). Inhibitor **38** displayed an especially intriguing pharmacokinetic profile, with high systemic exposure upon oral delivery.

Inhibitor **38** was docked into the catalytic domain of ROCK-II (gray, modeled from PDB entry 2ETR, chain A) by methods described previously[22a] and the obtained binding mode is illustrated in Figure 2. This picture shows the *S* enantiomer of **38** and our docking suggested that the opposite enantiomer had a similar binding motif.[22a] Compound **38** (orange) forms four hydrogen bonds with ROCK-II: two between the pyrazole group and residues Glu170 and Met172 in the kinase hinge region, a third between the chroman amide carbonyl and Lys121 in the phosphate binding region, and a fourth between the dimethylaminoethoxy moiety and Asp176. Co-crystal structures of ROCK with ligands Fasudil and Y-27632 have been previously reported.[23] The docking pose shown in Figure 2 suggests that our ROCK-II inhibitors have combined the ligand–enzyme interactions from both Fasudil and Y-27632.

In this model the chroman group also interacts in a hydrophobic binding region composed of Phe103 of the flexible P loop, Leu123, the carbon chain of Lys121, and Phe136.[23b] The 6-methoxy group on the chroman may maximize this interaction and explain the high ROCK-II affinity seen in this series. Exceptional PKA selectivity may be due to three factors: (i) increased steric hindrance for binding to PKA due to Thr184 versus Ala231 in ROCK-II (ii) poor interaction of the dimethylaminoethoxy moiety with Glu128 in PKA, in contrast to its favorable interaction with the corresponding Asp176 in ROCK-II, and (iii) optimized interaction of the chroman ring with the hydrophobic binding region and the flexible and distinct P loop of ROCK-II.

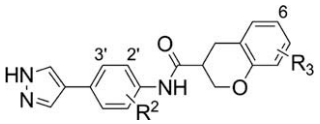
In summary, we have synthesized a series of chroman-3-amides with remarkable inhibition of ROCK-II and other favorable properties for further study. Assessment of the in vivo efficacy of these inhibitors in therapeutically relevant animal models, SAR studies in the pyrrole region, the enantioselective synthesis and evaluation of both enantiomers, and a detailed analysis of factors affecting affinity for the enzyme isoforms, ROCK-I and ROCK-II, are underway and will be reported in due course.

Table 4
Kinase inhibition of selected chromans.

Compound	Enzyme assay IC_{50}[a] (nM)					
	ROCK-II	PKA	AKT1	MRCKα	JNK3	p38
23	3	1416	>20,000	210	>20,000	>20,000
38	<2	>20,000	>20,000	150	>20,000	>20,000
41	<2	7316	>20,000	79	>20,000	>20,000

[a] Average of two or more measurements with error in these values within ±60% of the average.

Table 3
Kinase inhibition and stability of substituted chromans.



Compound	R^3	R^2	Enzyme assay IC_{50}[a] (nM)		ppMLC cell assay IC_{50}[a] (nM)	$t_{1/2}$[b] (min) HLM
			ROCK-II	PKA		
36	6-OMe	3′-OMe	3	1348	<6	6
37	6-OMe	2′-OMe	3	327	<6	12
38						
(SR3850)	6-OMe	2′-Dimethyl-aminoethoxy	<2	>20,000	<6	46
39	6-F	3′-OMe	7	1518	<6	28
40	6-F	2′-OMe	3	705	<6	23
41	6-F	2′-Dimethyl-aminoethoxy	<2	7316	<6	56
42	6-Cl	3′-OMe	2	955	38	36
43	6-Cl	2′-OMe	3	1259	66	36
44	6-Cl	2′-Dimethyl-aminoethoxy	5	2390	< 6	62
45	6-Me	3′-OMe	12	1628	52	6
46	6-Me	2′-OMe	4	1764	35	13
47	6-Me	2′-Dimethyl-aminoethoxy	<2	>20,000	16	62

[a] Average of two or more measurements with error in these values within ±30% of the average.
[b] 2 mg/mL human liver microsomes were used in stability studies.

Table 5
Rat ADME data for selected ROCK-II inhibitors[a].

	Compound		
	23	**38**	**41**
Dose iv/po (mg/kg)	1.0/2.0	1.0/2.0	1.0/2.0
Cl (mL/min/kg)	67 ± 11	23 ± 2	61 ± 11
Vss (L/kg)	4.6 ± 0.9	1.7 ± 0.1	5.9 ± 0.9
$t_{1/2}$ (h)	0.9 ± 0.4	1.0 ± 0.1	1.5 ± 0.2
Cmax po (μM)	0.17 ± 0.04	0.32 ± 0.07	0.10 ± 0.01
Oral F (%)	33 ± 11	35 ± 2	27 ± 5

[a] Data was generated from three determinations.

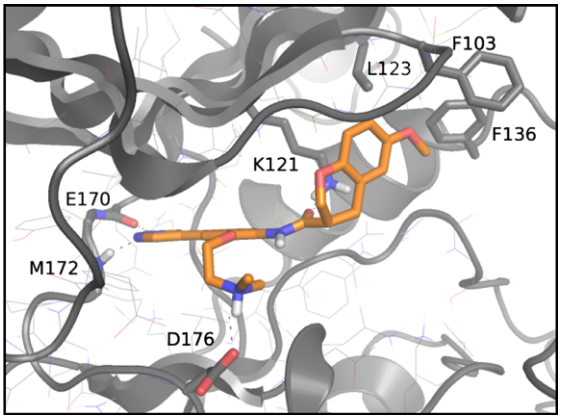


Figure 2. Docking of compound **38** into the ROCK-II kinase domain.

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