Supplementary Material (ESI) for Medicinal Chemistry Communications

Asymmetric synthesis of potent chroman-based Rho kinase (ROCK-II) inhibitors

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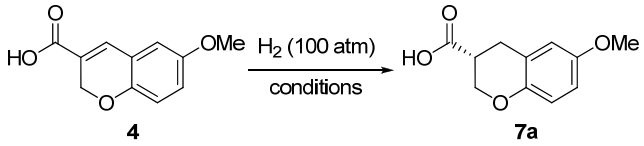
Electronic Supplementary Information

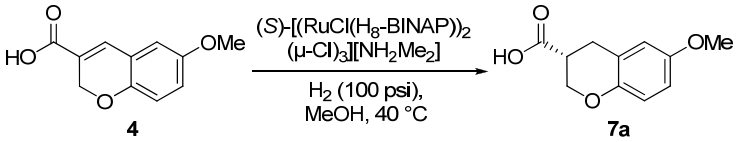
Table SI-1. Examination of reaction conditions for the asymmetric hydrogenation of chromene 4.



Entry	Catalyst and reagent	Temp (˚C)	Solvent	Time (h)	Conv (%)	ee (%)[a]
1	(*S*)-BINAP·$RuCl_2$ (0.4 mol %) HCO_2Na (10 eq)	70	MeOH:H_2O (2:1)	8	100	78
2	(*R*,*R*)-Ph-BPE-$Rh(cod)_2BF_4$ (6 mol %)	rt	MeOH:H_2O (3:1)	14	70	67
3	(*S*,*S*)-Mandyphos SL-M004-1 (1 mol %) $Rh(NBD)_2BF_4$ (1 mol %)	rt	MeOH	2.25	100	85
4	(*S*)-[($RuCl(H_8$-BINAP))$_2$(μ-$Cl)_3$][NH_2Me_2] (0.5 mol %) HCO_2Na (10 eq)	70	MeOH:H_2O (2:1)	3	100	89
5	(*S*)-Segphos-Ph·$RuCl_2$·1/2Me_2NH_4Cl (0.5 mol %) HCO_2Na (10 eq)	70	MeOH:H_2O (2:1)	4.33	100	82
6	(*S*)-Synphos·$Ru(BF_4)_2$ (0.5 mol %) HCO_2Na (6 eq)	50	MeOH	12	85	71
7	(*S*)-C_3Tunephos (0.5 mol %) $Ru(cod)(Me\text{-}allyl)_2$ (0.5 mol %) HCO_2Na (3 eq)	50	MeOH	8	55	82
8	Walphos (W001-1) (0.5 mol %) $[Rh(nbd)_2]BF_4$ (0.5 mol %)	40	MeOH	20	100	30

[a] % ee determined by chiral HPLC.

Table SI-2. Optimization of reaction conditions for the asymmetric hydrogenation of chromene 4 using H_8-BINAP.



entry	S/C[a] (mol%)	Formate salt	Time (h)	Conv. (%)	ee (%)[b]
1	400:1 (0.25%)	HCO_2Na (3 eq)	2	96	89
2	400:1 (0.25%)	HCO_2Na (1 eq)	2	95	87.5
3	400:1 (0.25%)	HCO_2Cs (3 eq)	2	100	89
4	400:1 (0.25%)	HCO_2Cs (0.2 eq)	2	97	88
5	600:1 (0.17%)	HCO_2K (3 eq)	20	94	90
6	600:1 (0.17%)	HCO_2Li (3 eq)	4	99	89.5
7	600:1 (0.17%)	HCO_2Cs (3 eq)	11	100	90
8	1000:1 (0.1%)	HCO_2Cs (3.5 eq)	14	100	89
9	1500:1 (0.07%)	HCO_2Cs (3.5 eq)	18	93	89

[a] Molar ratio of acid **4** to catalyst.
[b] % ee determined by chiral HPLC.

General Methods

All reaction solvents were of reagent grade and used as received. Unless indicated otherwise, all reactions were conducted under an atmosphere of argon using oven-dried (140 °C) glassware. Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 (C^{13}) NMR spectra were recorded on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. The proton signal for residual non-deuterated solvent (δ 7.26 for $CHCl_3$ and δ 2.50 for DMSO) was used as an internal reference for ^{1}H NMR spectra. For C^{13} NMR spectra, chemical shifts are reported relative to the δ 77.0 ppm resonance of $CDCl_3$ or the δ 39.5 ppm resonance of DMSO. Coupling constants are reported in Hz. Mass spectra were recorded on a Finnigan LCQ Advantage MAX spectrometer (Thermo Electron Corp.). Optical rotations were measured using a quartz cell with 0.5 mL capacity and a 10 cm path length.

Analytical HPLC analyses were performed with a ZORBAX Eclipse XDB-C18 column (4.6 mm × 150 mm) and UV detection in the range of 215−280 nm. Chiral analytical HPLC analyses were performed with a Chiralpak AD-RH column (4.6 mm × 150 mm) and UV detection in the range of 215−280 nm. The solvents used on the mobile phase were water with 0.1% TFA and acetonitrile. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} glass plates pre-coated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light or by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid). Flash column chromatography was generally performed on pre-packed columns of silica gel (230−400 mesh, 40−63 μm) by CombiFlash.

6-Methoxy-2*H*-chromene-3-carbonitrile (3).

To a solution of 2-hydroxy-5-methoxybenzaldehyde (26.6 g, 175 mmol) in acrylonitrile (20 mL) was added 1,4-diazabicyclo[2,2,2]octane (DABCO, 9.0 g). An additional portion of acrylonitrile (40 mL) was then added, and the mixture was refluxed under argon for 5 h. The reaction mixture was cooled, diluted with ether (0.5 L), and washed with 10% aqueous NaOH, 0.5 M aqueous H_2SO_4, and water. The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. The solid residue was re-crystallized from methanol (300 mL) to afford chromene **3** (19.2 g) as a light-yellow solid. Two additional crops were obtained by concentrating the supernatant liquid and additional re-crystallization for a combined yield of 24.1 g (74%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (br s, 1 H), 6.90 (m, 3 H), 4.80 (d, J = 1.3 Hz, 2 H), 3.71 (s, 3 H).

6-Methoxy-2*H*-chromene-3-carboxylic acid (4).

A mixture of chromene **3** (24.0 g, 128 mmol) in 10% aqueous NaOH (400 mL) was refluxed for 5 h. To this mixture was added charcoal (1 g) and the resulting mixture was re-heated to reflux briefly, then cooled to rt and filtered. The filtrate was acidified to pH 1 with concentrated HCl, and cooled in an ice bath. The resulting precipitate was collected by filtration, washed with water, and dried under vacuum. The crude product was re-crystallized from acetonitrile (1.2 L)

to yield acid **4** (23.8 g, 90%) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (br s, 1 H), 7.42 (s, 1 H), 7.00 (d, J = 2.9 Hz, 1 H), 6.85 (dd, J = 8.7 Hz, 2.9 Hz, 1 H), 6.79 (d, J = 8.8 Hz, 1 H), 4.83 (d, J = 1.4 Hz, 2 H), 3.70 (s, 3 H).

(2*R*)-*N*-[6-Methoxy-2*H*-chromene-3-carbonyl]bornane-10,2-sultam (5a).

To a suspension of acid **4** (12.6 g, 60.9 mmol) in anhydrous methylene chloride (100 mL) was added oxalyl chloride (7 mL), followed by *ca.* 4 drops of DMF. The mixture was stirred under a gas outlet tube filled with Drierite overnight. The reaction mixture was concentrated to dryness, and dried under vacuum to obtain the corresponding acid chloride as a yellow solid (13.7 g, 100%), which was taken to the next reaction without further purification.

NaH (60% in mineral oil, 1.63 g, 40.6 mmol) and anhydrous toluene (250 mL) were sequentially added to (2*R*)-bornane-10,2-sultam (6.24 g, 26.9 mmol). The suspension was sonicated for 5 min and stirred at room temperature under argon for 3 h. The mixture was cooled in an ice bath, treated with the acid chloride (6.97 g, 31.0 mmol), and stirred at rt for 2 h under argon. The reaction was quenched with silica gel (50 g) and treated with hexanes (100 mL). After stirring for 10 min, the mixture was purified by flash column chromatography (10- 30% ethyl acetate in hexanes) to afford sultam **5a** (11.1 g, 95%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (br s, 1H), 6.80 (m, 2 H), 6.73 (d, J = 2.4 Hz, 1 H), 5.03 (dd, J = 13.8, 1.1 Hz, 1 H), 4.81 (dd, J = 13.8, 1.1 Hz, 1 H), 4.12 (dd, J = 7.7, 4.6 Hz, 1 H), 3.77 (s, 3 H), 3.54 (d, J = 13.6 Hz, 1 H), 3.43 (d, J = 13.7 Hz, 1 H), 2.05 (m, 1 H), 1.93 (m, 4 H), 1.43 (m, 2 H), 1.30 (s, 3 H), 1.02 (s, 3 H); MS (ES+) *m/z* for $C_{21}H_{26}NO_5S$ $[M+H]^+$ calcd 404, found 404.

(2*S*)-*N*-[6-methoxy-2*H*-chromene-3-carbonyl]bornane-10,2-sultam (5b).

Compound **5b** was synthesized from acid **4** using the similar procedure used to prepare compound **5a** (92%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (br s, 1H), 6.80 (m, 2 H), 6.73 (d, J = 2.4 Hz, 1 H), 5.03 (dd, J = 13.6, 1.2 Hz, 1 H), 4.81 (dd, J = 14.0, 1.2 Hz, 1 H), 4.12 (dd, J = 7.6, 4.8 Hz, 1 H), 3.77 (s, 3 H), 3.54 (d, J = 13.6 Hz, 1 H), 3.43 (d, J = 13.6 Hz, 1 H), 2.05 (m, 1 H), 1.93 (m, 4 H), 1.43 (m, 2 H), 1.30 (s, 3 H), 1.02 (s, 3 H); MS (ES+) *m/z* for $C_{21}H_{26}NO_5S$ $[M+H]^+$ calcd 404, found 404.

(2*R*)-*N*-[(3*R*)-6-methoxychroman-3-carbonyl]bornane-10,2-sultam (6a).

To a solution of chromene **5a** 2.020 g (5.00 mmol) in anhydrous THF (50 mL) under Ar at -50 ˚C was added L-Selectride (1 M in THF, 6.5 mL) dropwise over a 5 min period. The reaction mixture was stirred vigorously at -50 ˚C for 45 min and quenched at -55 ˚C by dropwise addition of an aqueous 2 M sulfuric acid solution (25 mL). The cooling bath was removed and the reaction mixture was stirred at ambient temperature in an open flask for 2 h. The mixture was partitioned between ether (120 mL) and water (80 mL). After separation, the organic phase was washed with water and saturated sodium bicarbonate. The aqueous phases were combined and extracted with ether. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (0-30% ethyl acetate in

hexanes) to afford chromane **6a** (1.42 g, 98:2 dr determined by ^{1}H-NMR). Further re-crystallization in cyclohexane (60 mL) gave the diastereomerically pure chromane 1.21 g (60%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, J = 8.9 Hz, 1 H), 6.69 (dd, J = 8.9, 3.0 Hz, 1 H), 6.59 (d, J = 2.9 Hz, 1 H), 4.44 (ddd, J = 10.7, 3.3, 2.0 Hz, 1 H), 4.04 (t, J = 10.3 Hz, 1 H), 3.92 (t, J = 6.3 Hz, 1 H), 3.74 (s, 3 H), 3.58 (m, 1 H), 3.54 (d, J = 13.9 Hz, 1 H), 3.47 (d, J = 13.9 Hz, 1 H), 3.03 (m, 2 H), 2.08 (m, 2 H), 1.90 (m, 2 H), 1.41 (m, 2 H), 1.20 (s, 3 H), 0.99 (s, 3 H); MS (ES+) *m/z* for $C_{21}H_{28}NO_5S$ $[M+H]^+$ calcd 406, found 406.

(2*S*)-*N*-[(3*S*)-6-methoxychroman-3-carbonyl]bornane-10,2-sultam (6b).

Compound **6b** was synthesized from chromene **5b** using the similar procedure used to prepare compound **6a** (70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, J = 8.8 Hz, 1 H), 6.69 (dd, J = 8.8, 2.8 Hz, 1 H), 6.59 (d, J = 2.8 Hz, 1 H), 4.44 (ddd, J = 10.8, 3.2, 2.0 Hz, 1 H), 4.04 (t, J = 10.4 Hz, 1 H), 3.92 (t, J = 6.4 Hz, 1 H), 3.74 (s, 3 H), 3.58 (m, 1 H), 3.54 (d, J = 14.0 Hz, 1 H), 3.47 (d, J = 14.0 Hz, 1 H), 3.03 (m, 2 H), 2.08 (m, 2 H), 1.90 (m, 2 H), 1.41 (m, 2 H), 1.20 (s, 3 H), 0.99 (s, 3 H); MS (ES+) *m/z* for $C_{21}H_{28}NO_5S$ $[M+H]^+$ calcd 406, found 406.

(*R*)-6-methoxychroman-3-carboxylic acid (7a).

Chromane **6a** (1.21 g, 2.98 mmol) was dissolved in THF (120 mL) and the solution was cooled to 0 ˚C. To this solution was added water (31 mL) and hydrogen peroxide (50% aqueous solution, 13 mL), followed by 1 M aqueous LiOH (4.4 mL). The reaction mixture was stirred at 0 ˚C for 20 min, then quenched with 2 M aqueous H_2SO_4 (1.3 mL), warmed to ambient temperature, and partitioned between ether (300 mL) and water (150 mL). The organic phase was washed with additional water (200 mL), and 1 M aqueous Na_2SO_3 (200 mL). The aqueous phases were combined and re-extracted with ether (300 mL). The combined organic phases were extracted twice with 25% concentrated ammonium hydroxide in water (2 × 100 mL) and once with water. The combined aqueous extracts were acidified with 6 M aqueous HCl (140 mL) at 0 ˚C and then extracted with methylene chloride (3 × 150 mL). The combined methylene chloride layers were washed with water (100 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was re-crystallized from cyclohexane (60 mL), washed with hexanes, and dried under vacuum to yield acid **7a** (580 mg, 93%, (*R*)-enantiomer, >99% ee) as a colorless solid: >99% ee by HPLC analysis (Chiralpak AD-RH column eluted with a shallow water-acetonitrile gradient (17% acetonitrile for 1 min, then 17-20% acetonitrile over 10 min) and 0.1 % TFA at 0.80 mL/min, column temperature at 75 ˚C, and detection at 215 nm), t_R = 9.60 min; $[\alpha]_D^{27}$ = -2.51 (c = 0.796, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (d, J = 8.9 Hz, 1 H), 6.69 (dd, J = 8.9, 2.9 Hz, 1 H), 6.62 (d, J = 2.9 Hz, 1 H), 4.37 (m, 1 H), 4.15 (m, 1 H), 3.75 (s, 3 H), 3.06 (m, 3 H)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3, 38.4, 55.7, 66.2, 113.8, 114.0, 117.4, 120.5, 148.0, 153.7, 177.9; MS (ES-) *m/z* for $C_{11}H_{11}O_4$ $[M-H]^+$ calcd 207, found 207.

(*S*)-6-methoxychroman-3-carboxylic acid (7b).

Acid **7b** was synthesized from chromene **6b** using the similar procedure used to prepare compound **7a** (94%): >99% ee by HPLC analysis, t_R = 9.25 min; $[\alpha]_D^{27}$ = +2.04 (*c* = 0.981, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, *J* = 8.8 Hz, 1 H), 6.69 (dd, *J* = 8.8, 2.8 Hz, 1 H), 6.63 (d, *J* = 2.8 Hz, 1 H), 4.39 (m, 1 H), 4.16 (m, 1 H), 3.75 (s, 3 H), 3.06 (m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3, 38.4, 55.7, 66.2, 113.8, 114.0, 117.4, 120.5, 148.0, 153.7, 177.9; MS (ES-) *m/z* for $C_{11}H_{11}O_4$ $[M-H]^+$ calcd 207, found 207.

(*S*)-6-methoxychroman-3-carboxylic acid (7b) via asymmetric hydrogenation.

Chromene **4** (6.19 g, 30 mmol) and (*R*)-[$(RuCl(H_8\text{-BINAP}))_2(\mu\text{-}Cl)_3$][$NH_2Me_2$] (Strem, 26mg, 0.030 mmol Ru) was loaded into a 300 mL-sized Ace glass pressure hydrogenation flask with a Teflon screw-cap and a stir bar. The gas inlet was equipped with a rubber septa and the solid mix was flushed with a gentle stream of argon (15 min). A solution of cesium formate (21.35 g, 120 mmol) in methanol (120 mL) was degassed, purged with argon, and the solution was transferred into the hydrogenation flask via thick cannula under argon. The hydrogenation flask was connected to the Parr hydrogenator gas inlet and placed on ambient bath on a stir plate behind a blast shield and the argon was replaced with hydrogen (5-times vacuum/hydrogen purge). The heating was turned on and the mixture was stirred at 40 ˚C under 100 psi (=7 atm) of hydrogen for 20 h. (*Note*: 100 psi is more than what is recommended for the Parr shaker equipment – a protective blast shield and a carefully-inspected hydrogenation flask was used without any incident. However, a steel autoclave would have been a safer alternative. There was almost no conversion when the hydrogenation was attempted under H_2 balloon.) The resulting colorless homogeneous mixture was cooled to ambient temperature, carefully vented, transferred into a 1 L round-bottom flask and evaporated to dryness. The residue was diluted with water (200 mL) and methylene chloride (200 mL), acidified with 6 M aqueous HCl (30 mL), and separated. The aqueous phase was re-extracted with additional dichloromethane (200 mL). The organic extracts were washed with water (200 mL), dried over $MgSO_4$, and evaporated to provide acid **7b** (6.30 g, 89% ee) as a colorless solid.

The obtained solid was dissolved in acetonitrile (1.75 L) and the solution was warmed to close to reflux. (*S*,*S*)-chloramphenicol base (6.36 g, 30 mmol) was added in one portion and the mixture was stirred at gentle reflux until complete dissolution (*ca.* 15 min). The mixture was then allowed to crystallize at ambient temperature overnight (16 h). The precipitate was collected by filtration, washed with MeCN, and dried to provide the chloramphenicol base salt of compound **7b** as a pale yellow solid (11.01 g, 87.5% yield, 99% ee). (*Note*: After a second re-crystallization of the chloramphenicol base salt, the enantiomeric purity is further improved to >99.6%. However, the recrystallization procedure requires a large volume of acetonitrile, and 99% ee was acceptable for our purposes.) This salt was treated with ether (250 mL) and water (200 mL), acidified with 6 M aqueous HCl (10 mL) stirred for 10 min, and the layers were separated. The aqueous phase was re-extracted with ether (250 mL). The organic extracts were washed with

water 250mL, combined, dried over $MgSO_4$, and concentrated to dryness to obtain chromane acid **7b** (5.46 g, 87%, 99% ee) as a colorless crystalline solid.

(*S*)-6-methoxychroman-3-carboxylic acid (7a) via asymmetric hydrogenation.

Acid **7a** was synthesized from chromene **4** using the similar hydrogenation procedure used to prepare compound **7a**, except (*S*)-[(RuCl(H_8-BINAP))$_2$(μ-Cl)$_3$][NH_2Me_2] was used as the catalyst, and (*R*,*R*)-chloramphenicol base was used as the chiral resolving agent (86%): >99% ee by HPLC analysis.

tert-Butyl 4-(4-nitro-3-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-1H-pyrazole-1-carboxylate (9a).

To a solution of 2-(pyrrolidin-1-yl)ethanol (0.120 g, 1.04 mmol) in THF (15 mL) at 0 °C was added NaH (60% oil dispersion, 0.054 g, 1.35 mmol). After stirring for 15 min, 4-bromo-2-fluoro-1-nitrobenzene (**8**) (0.22 g, 1.00 mmol) was added and the resulting mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed by rotary evaporation and the residue was partitioned between saturated aqueous $NaHCO_3$ (25 mL) and ethyl acetate (25 mL). The aqueous layer was further extracted with ethyl acetate (2 × 25 mL) and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated to dryness. Without further purification, the residue was dissolved in a 3:2 mixture of ethanol:toluene (10 mL). 1*H*-pyrazoleboronic acid pinacol ester (0.291 g, 1.50 mmol), K_2CO_3 (2 M aqueous solution, 1.5 mL, 3.00 mmol), and tetrakis(triphenylphosphine)palladium (0) (0.060 mg, 0.052 mmol) were added, and the reaction mixture was degassed, purged with argon, and heated to 140 °C for 1 h by microwave irradiation. After cooling to room temperature, the solvent was removed, partitioned between ethyl acetate (10 mL) and saturated aqueous $NaHCO_3$, and separated. The aqueous layer was extracted with two additional portions of ethyl acetate (10 mL). The organic layers were combined, dried, and concentrated under reduced pressure. The residue was then treated with dioxane (5 mL), a catalytic amount of DMAP (*ca.* 5 mg), and Boc_2O (0.262 g, 1.2 mmol), and the resulting solution was stirred overnight at rt. The solvent was removed and purified by flash column chromatography (0.5-10% MeOH in CH_2Cl_2) to yield pyrazole **9a** (65%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, J = 0.8 Hz, 1 H), 8.03 (d, J = 0.8 Hz, 1 H), 7.96 (d, J = 8.4 Hz, 1 H),7.30 (d, J = 1.6 Hz, 1 H), 7.23 (dd, J = 8.4, 1.6 Hz, 1 H), 4.66 (t, J = 4.4 Hz, 1 H), 3.41 (m, 2 H), 3.25 (m, 4 H), 2.08 (m, 4 H), 1.93 (m, 2 H), 1.70 (s, 9 H); MS (ES+) *m/z* for $C_{20}H_{27}N_4O_5$ $[M+H]^+$ calcd 403, found 403.

(*R*)-tert-Butyl 4-(3-(1-methylpyrrolidin-3-yloxy)-4-nitrophenyl)-1H-pyrazole-1-carboxylate (9b).

Compound **9b** was synthesized from nitrobenzene **8** using the similar procedure used to prepare compound **9a** (61%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 0.8 Hz, 1 H), 7.98 (d, J = 0.8 Hz, 1 H), 7.90 (d, J = 8.4 Hz, 1 H), 7.15 (dd, J = 8.4, 1.6 Hz, 1 H), 7.06 (d, J = 1.6 Hz, 1 H), 5.00 (m, 1 H), 3.09 (dd, J = 10.4, 6.0 Hz, 1 H), 2.78 (m, 2 H), 2.67-2.62 (m, 1 H), 2.43 (s, 3 H),

2.42-2.34 (m, 1 H), 2.15-2.08 (m, 2 H), 1.69 (s, 9 H); MS (ES+) *m/z* for $C_{19}H_{25}N_4O_5$ $[M+H]^+$ calcd 389, found 389.

(*S*)-tert-Butyl 4-(3-(1-methylpyrrolidin-3-yloxy)-4-nitrophenyl)-1H-pyrazole-1-carboxylate (9c).

Compound **9c** was synthesized from nitrobenzene **8** using the similar procedure used to prepare compound **9a** (72%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* = 0.8 Hz, 1 H), 7.98 (d, *J* = 0.8 Hz, 1 H), 7.90 (d, *J* = 8.4 Hz, 1 H), 7.15 (dd, *J* = 8.4, 1.6 Hz, 1 H), 7.06 (d, *J* = 1.6 Hz, 1 H), 5.00 (m, 1 H), 3.09 (dd, *J* = 10.4, 6.0 Hz, 1 H), 2.78 (m, 2 H), 2.67-2.62 (m, 1 H), 2.43 (s, 3 H), 2.42-2.34 (m, 1 H), 2.15-2.08 (m, 2 H), 1.69 (s, 9 H); MS (ES+) *m/z* for $C_{19}H_{25}N_4O_5$ $[M+H]^+$ calcd 389, found 389.

tert-Butyl 4-(3-(3-hydroxypropoxy)-4-nitrophenyl)-1H-pyrazole-1-carboxylate (9d).

Compound **9d** was synthesized from nitrobenzene **8** using the similar procedure used to prepare compound **9a** (74%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, *J* = 0.6 Hz, 1 H), 8.48 (d, *J* = 0.6 Hz, 1 H), 7.92 (d, *J* = 8.5 Hz, 1 H), 7.70 (d, *J* = 1.5 Hz, 1 H), 7.51 (dd, *J* = 8.4, 1.6 Hz, 1 H), 4.60 (t, *J* = 5.1 Hz, 1 H), 4.33 (t, *J* = 6.1 Hz, 2 H), 3.59 (q, *J* = 5.3 Hz, 2 H), 1.90 (quint, *J* = 6.1 Hz, 2 H), 1.62 (s, 9H); MS (ES+) *m/z* for $C_{17}H_{22}N_3O_6$ $[M+H]^+$ calcd 364, found 364.

tert-Butyl 4-(3-(2-(dimethylamino)ethoxy)-4-nitrophenyl)-1H-pyrazole-1-carboxylate (9e).

Compound **9e** was synthesized from nitrobenzene **8** using the similar procedure used to prepare compound **9a** (72%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 0.8 Hz, 1 H), 8.05 (d, *J* = 0.8 Hz, 1 H), 7.98 (d, *J* = 7.31 (d, *J* = 1.6 Hz, 1 H), 7.26 (dd, *J* = 8.4, 1.6 Hz, 1 H), 4.79 (t, *J* = 4.4 Hz, 1 H), 3.50 (t, *J* = 4.4 Hz, 1 H), 2.97 (s, 6 H), 1.70 (s, 9 H); MS (ES+) *m/z* for $C_{18}H_{25}N_4O_5$ $[M+H]^+$ calcd 377, found 377.

tert-Butyl 4-(4-amino-3-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-1H-pyrazole-1-carboxylate (10a).

Nitrobenzene **9a** (0.264 g, 0.655 mmol) was dissolved in a mixture of ethyl acetate (1 mL) and methanol (5 mL). To this solution was added 10% palladium on carbon (0.04 g) and the reaction was stirred under a hydrogen atmosphere using a balloon filled with hydrogen gas for 20 h at room temperature. After removal of the catalyst by filtration through a pad of CELITE™, the filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography (2-18% methanol in CH_2Cl_2 with 0.1% NH_4OH) to afford 0.211 g (86%) of aniline **10a** as a light purple oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, *J* = 0.8 Hz, 1 H), 8.17 (d, *J* = 0.8 Hz, 1 H), 7.20 (d, *J* = 1.6 Hz, 1 H), 7.07 (dd, *J* = 8.4, 1.6 Hz, 1 H), 6.65 (d, *J* = 8.0 Hz, 1 H), 4.88 (br s, 2 H), 4.13 (t, *J* = 6.0 Hz, 2 H), 4.13 (t, *J* = 6.0 Hz, 2 H), 2.86 (br m, 2 H), 2.60 (br m, 4 H), 1.71 (br m, 4 H),1.60 (s, 9 H); MS (ES+) *m/z* for $C_{20}H_{29}N_4O_3$ $[M+H]^+$ calcd 373, found 373.

(*R*)-tert-butyl 4-(4-amino-3-(1-methylpyrrolidin-3-yloxy)phenyl)-1H-pyrazole-1-carboxylate (10b).

Aniline **10b** was synthesized from nitrobenzene **9b** using the similar procedure used to prepare compound **10a** (87%): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.50 (d, *J* = 0.4 Hz, 1 H), 8.16 (d, *J* = 0.8 Hz, 1 H), 7.04 (m, 2 H), 6.64 (d, *J* = 8.4 Hz, 1 H), 4.94 m, 1 H), 4.80 (br s, 2 H), 2.82 (dd, *J* = 10.4, 6.0 Hz, 1 H), 2.68 (m, 1 H), 2.60 (d, *J* = 10.4 Hz, 1 H), 2.39 (m, 1 H), 2.32 (m, 1 H), 2.27 (s, 3 H), 1.84 (m, 1 H), 1.60 (s, 9 H); MS (ES+) *m/z* for $C_{19}H_{27}N_4O_3$ $[M+H]^+$ calcd 359, found 359.

(*S*)-tert-butyl 4-(4-amino-3-(1-methylpyrrolidin-3-yloxy)phenyl)-1H-pyrazole-1-carboxylate (10c).

Aniline **10c** was synthesized from nitrobenzene **9c** using the similar procedure used to prepare compound **10a** (72%): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.50 (d, *J* = 0.8 Hz, 1 H), 8.16 (d, *J* = 0.8 Hz, 1 H), 7.04 (m, 2 H), 6.64 (d, *J* = 8.4 Hz, 1 H), 4.94 m, 1 H), 4.80 (br s, 2 H), 2.82 (dd, *J* = 10.4, 6.0 Hz, 1 H), 2.68 (m, 1 H), 2.60 (d, *J* = 10.4 Hz, 1 H), 2.39 (m, 1 H), 2.32 (m, 1 H), 2.27 (s, 3 H), 1.84 (m, 1 H), 1.60 (s, 9 H); MS (ES+) *m/z* for $C_{19}H_{27}N_4O_3$ $[M+H]^+$ calcd 359, found 359.

tert-Butyl 4-(4-amino-3-(3-hydroxypropoxy)phenyl)-1H-pyrazole-1-carboxylate (10d).

Aniline **10d** was synthesized from nitrobenzene **9d** using the similar procedure used to prepare compound **10a** (75%): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.49 (s, 1 H), 8.16 (s, 1 H), 7.15 (d, *J* = 1.6 Hz, 1 H), 7.05 (dd, *J* = 8.0, 1.7 Hz, 1 H), 6.63 (d, *J* = 8.0 Hz, 1 H), 4.80 (br s, 2 H), 4.53 (t, *J* = 5.1 Hz, 1 H), 4.09 (t, *J* = 6.2 Hz, 2 H), 3.61 (q, *J* = 5.7 Hz, 2 H), 1.90 (quint, *J* = 6.2 Hz, 2 H), 1.60 (s, 9 H); MS (ES+) *m/z* for $C_{17}H_{24}N_3O_4$ $[M+H]^+$ calcd 334, found 334.

tert-Butyl 4-(4-amino-3-(2-(dimethylamino)ethoxy)phenyl)-1H-pyrazole-1-carboxylate (10e).

Aniline **10e** was synthesized from nitrobenzene **9e** using the similar procedure used to prepare compound **10a** (86%): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.50 (d, *J* = 0.8 Hz, 1 H), 8.17 (d, *J* = 0.8 Hz, 1 H), 7.19 (d, *J* = 1.6 Hz, 1 H), 7.06 (dd, *J* = 8.4, 1.6 Hz, 1 H), 6.64 (d, *J* = 8.0 Hz, 1 H), 4.84 (br s, 2 H), 4.09 (t, *J* = 6.0 Hz, 2 H), 4.09 (t, *J* = 6.2 Hz, 2 H), 2.65 (t, *J* = 6.0 Hz, 2 H), 2.25 (s, 6 H), 1.60 (s, 9 H); MS (ES+) *m/z* for $C_{18}H_{27}N_4O_3$ $[M+H]^+$ calcd 347, found 347.

(*S*)-N-(4-(1H-pyrazol-4-yl)-2-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-6-methoxychroman-3-carboxamide ((*S*)-11a).

To a solution of aniline **10a** (40 mg, 0.107 mmol) in CH_2Cl_2 (5 mL) was added acid **7b** (23 mg, 0.111 mmol), N-hydroxyazabenzotriazole (HOAt, 16 mg, 0.118 mmol), and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 27 mg, 0.141 mmol). The resulting

mixture was stirred at 0 °C then warmed to room temperature over 11 h. The residue was dissolved in ethyl acetate (20 mL) and washed twice with saturated aqueous $NaHCO_3$ (5 mL) and once with brine (5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was dissolved in a 33% trifluoroacetic acid (TFA) solution in CH_2Cl_2 (1.5 mL) and stirred for 2 h. The solvent was removed and the excess TFA was removed by repeated evaporation with toluene *in vacuo*. The residue was partitioned with CH_2Cl_2 (10 mL) and a saturated solution of aqueous Na_2HCO_3 (10 mL), and the aqueous layer was extracted with additional CH_2Cl_2. The organic layers were combined, dried over sodium sulfate, filtered, and concentrated. Purification of the crude product by flash column chromatography (2-15% methanol in CH_2Cl_2 with 0.1% NH_4OH) provided 28.6 mg (58%) of chroman (*S*)-**11a** as a colorless solid: $[\alpha]_D^{27}$ = -45.20 (*c* = 0.469, CF_3CH_2OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (br s, 1 H), 9.37 (s, 1 H), 8.18 (br s, 1 H), 7.92 (br s, 1 H), 7.84 (d, *J* = 8.4 Hz, 1 H), 7.32 (d, *J* = 2.0 Hz, 1 H), 7.18 (dd, *J* = 8.4, 2.0 Hz, 1 H), 6.73-6.67 (m, 3 H), 4.37 (m, 1 H), 4.20 (t, *J* = 6.0 Hz, 2 H), 3.94 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.02 (dd, *J* = 16.0, 10.4 Hz, 1 H), 2.91 (dd, *J* = 15.6, 4.8 Hz, 1 H), 2.81 (t, *J* = 6.0 Hz, 2 H), 2.57 (m, 4 H), 1.69 (m, 4 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.1, 27.9, 53.9, 54.2, 55.3, 67.1, 68.4, 110.6, 113.3, 114.1, 116.7, 117.5, 121.0, 121.7, 122.6, 125.4, 125.8, 129.8, 136.2, 147.6, 149.5, 153.0, 170.2; MS (ES+) *m/z* for $C_{26}H_{31}N_4O_4$ $[M+H]^+$ calcd 463, found 463.

(*R*)-N-(4-(1H-pyrazol-4-yl)-2-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-6-methoxychroman-3-carboxamide ((*R*)-11a).

Amide **(*R*)-11a** was synthesized from aniline **10a** and acid **7a** using the similar procedure used to prepare compound **(*S*)-11a** (56%): $[\alpha]_D^{27}$ = +45.39 (*c* = 0.445, CF_3CH_2OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (br s, 1 H), 9.36 (s, 1 H), 8.18 (br s, 1 H), 7.92 (br s, 1 H), 7.84 (d, *J* = 8.4 Hz, 1 H), 7.32 (d, *J* = 1.6 Hz, 1 H), 7.18 (dd, *J* = 8.4, 2.0 Hz, 1 H), 6.73-6.69 (m, 3 H), 4.37 (m, 1 H), 4.20 (t, *J* = 6.0 Hz, 2 H), 3.94 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.02 (dd, *J* = 15.6, 10.4 Hz, 1 H), 2.91 (dd, *J* = 15.6, 4.0 Hz, 1 H), 2.81 (t, *J* = 6.0 Hz, 2 H), 2.57 (m, 4 H), 1.69 (m, 4 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.1, 27.9, 53.9, 54.2, 55.3, 67.1, 68.4, 110.6, 113.3, 114.1, 116.7, 117.5, 121.0, 121.7, 122.6, 125.4, 125.8, 129.8, 136.2, 147.6, 149.5, 153.0, 170.2; MS (ES+) *m/z* for $C_{26}H_{31}N_4O_4$ $[M+H]^+$ calcd 463, found 463.

(*S*)-6-methoxy-N-(2-((*R*)-1-methylpyrrolidin-3-yloxy)-4-(1H-pyrazol-4-yl)phenyl)chroman-3-carboxamide ((*S*)-11b).

Amide **(*S*)-11b** was synthesized from aniline **10b** and acid **7b** using the similar procedure used to prepare compound **(*S*)-11a** (49%): $[\alpha]_D^{27}$ = +0.244 (*c* = 0.409, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (br s, 1 H), 9.24 (s, 1 H), 8.18 (br s, 1 H), 7.91 (br s, 1 H), 7.87 (d, *J* = 8.0 Hz, 1 H), 7.15 (m, 2 H), 6.73-6.67 (m, 3 H), 4.97 (m, 1 H), 4.37 (m, 1 H), 3.96 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.20-3.16 (m, 1 H), 3.02 (dd, *J* = 16.0, 9.6 Hz, 1 H), 2.92 (dd, *J* = 16.0, 5.2 Hz, 1 H), 2.81 (dd, *J* = 10.4, 6.0 Hz, 1 H), 2.74 (m, 1 H), 2.68 (dd, J = 10.0, 3.2 Hz, 1 H), 2.43-2.36 (m,

1 H), 2.33 (m, 1 H), 2.29 (s, 3 H), 1.95 (m, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.8, 31.9, 41.6, 54.2, 54.9, 55.3, 67.1, 77.7, 110.4, 113.3, 114.1, 116.7, 117.2, 120.9, 121.8, 122.8, 125.2, 125.8, 129.7, 136.2, 147.6, 148.2, 153.0, 170.3; MS (ES+) *m/z* for $C_{25}H_{29}N_4O_4$ $[M+H]^+$ calcd 449, found 449.

(*R*)-6-methoxy-N-(2-((*R*)-1-methylpyrrolidin-3-yloxy)-4-(1H-pyrazol-4-yl)phenyl)chroman-3-carboxamide ((*R*)-11b).

Amide **(*R*)-11b** was synthesized from aniline **10b** and acid **7a** using the similar procedure used to prepare compound **(*S*)-11a** (45%): $[\alpha]_D^{27}$ = -9.82 (*c* = 0.387, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (br s, 1 H), 9.24 (s, 1 H), 8.18 (br s, 1 H), 7.93 (br s, 1 H), 7.87 (d, *J* = 8.0 Hz, 1 H), 7.15 (m, 2 H), 6.73-6.67 (m, 3 H), 4.97 (m, 1 H), 4.37 (m, 1 H), 3.95 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.19-3.14 (m, 1 H), 3.02 (dd, *J* = 16.4, 9.6 Hz, 1 H), 2.92 (dd, *J* = 16.4, 6.0 Hz, 1 H), 2.80 (dd, *J* = 10.4, 6.0 Hz, 1 H), 2.73 (m, 1 H), 2.68 (dd, J = 10.0, 2.8 Hz, 1 H), 2.43-2.36 (m, 1 H), 2.33 (m, 1 H), 2.29 (s, 3 H), 1.94 (m, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.9, 32.2, 41.7, 54.5, 55.3, 61.4, 67.1, 78.2, 110.6, 113.3, 114.1, 116.7, 117.2, 120.9, 121.8, 122.7, 125.4, 125.9, 129.7, 136.2, 147.6, 148.4, 153.0, 170.3; MS (ES+) *m/z* for $C_{25}H_{29}N_4O_4$ $[M+H]^+$ calcd 449, found 449.

(*S*)-6-methoxy-N-(2-((*S*)-1-methylpyrrolidin-3-yloxy)-4-(1H-pyrazol-4-yl)phenyl)chroman-3-carboxamide ((*S*)-11c).

Amide **(*S*)-11c** was synthesized from aniline **10c** and acid **7b** using the similar procedure used to prepare compound **(*S*)-11a** (50%): $[\alpha]_D^{27}$ = +10.26 (*c* = 0.312, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (br s, 1 H), 9.24 (s, 1 H), 8.18 (br s, 1 H), 7.91 (br s, 1 H), 7.87 (d, *J* = 8.0 Hz, 1 H), 7.15 (m, 2 H), 6.73-6.67 (m, 3 H), 4.97 (m, 1 H), 4.37 (m, 1 H), 3.95 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.21-3.14 (m, 1 H), 3.02 (dd, *J* = 16.4, 9.6 Hz, 1 H), 2.92 (dd, *J* = 16.4, 5.6 Hz, 1 H), 2.80 (dd, *J* = 10.4, 6.4 Hz, 1 H), 2.73 (m, 1 H), 2.68 (dd, J = 10.0, 2.8 Hz, 1 H), 2.43-2.37 (m, 1 H), 2.33 (m, 1 H), 2.29 (s, 3 H), 1.93 (m, 1 H); MS (ES+) *m/z* for $C_{25}H_{29}N_4O_4$ $[M+H]^+$ calcd 449, found 449.

(*R*)-6-methoxy-N-(2-((*S*)-1-methylpyrrolidin-3-yloxy)-4-(1H-pyrazol-4-yl)phenyl)chroman-3-carboxamide ((*R*)-11c).

Amide **(*R*)-11c** was synthesized from aniline **10c** and acid **7a** using the similar procedure used to prepare compound **(*S*)-11a** (47%): $[\alpha]_D^{27}$ = -0.248 (*c* = 0.404, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (br s, 1 H), 9.24 (s, 1 H), 8.18 (br s, 1 H), 7.91 (br s, 1 H), 7.87 (d, *J* = 8.0 Hz, 1 H), 7.15 (m, 2 H), 6.74-6.67 (m, 3 H), 4.97 (m, 1 H), 4.37 (m, 1 H), 3.96 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.21-3.15 (m, 1 H), 3.03 (dd, *J* = 16.0, 9.6 Hz, 1 H), 2.92 (dd, *J* = 16.4, 5.6 Hz, 1 H), 2.81 (dd, *J* = 10.4, 6.0 Hz, 1 H), 2.74 (m, 1 H), 2.68 (dd, J = 10.0, 3.2 Hz, 1 H), 2.43-2.37 (m, 1 H), 2.33 (m, 1 H), 2.29 (s, 3 H), 1.94 (m, 1 H); MS (ES+) *m/z* for $C_{25}H_{29}N_4O_4$ $[M+H]^+$ calcd 449, found 449.

(*S*)-N-(2-(3-hydroxypropoxy)-4-(1H-pyrazol-4-yl)phenyl)-6-methoxychroman-3-carboxamide ((*S*)-11d).

Amide **(*S*)-11d** was synthesized from aniline **10d** and acid **7b** using the similar procedure used to prepare compound **(*S*)-11a** (51%): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.89 (br s, 1 H), 9.23 (s, 1 H), 8.18 (br s, 1 H), 7.91 (br s, 1 H), 7.84 (d, *J* = 8.4 Hz, 1 H), 7.27 (d, *J* = 1.6 Hz, 1 H), 7.14 (dd, *J* = 8.4, 1.6 Hz, 1 H), 6.73-6.67 (m, 3 H), 4.61 (t, *J* = 5.2 Hz, 1 H), 4.36 (m, 1 H), 4.16 (t, *J* = 6.0 Hz, 2 H), 3.95 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.64 (q, *J* = 6.0 Hz, 2 H), 3.19-3.13 (m, 1 H), 3.02 (dd, *J* = 16.4, 10.0 Hz, 1 H), 2.91 (dd, *J* = 16.4, 5.6 Hz, 1 H), 1.93 (quint, *J* = 6.0 Hz, 2 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 27.8, 32.0, 55.3, 57.7, 65.9, 67.1, 109.3, 113.3, 114.1, 116.7, 116.9, 121.0, 121.8, 122.6, 125.1, 125.3, 129.8, 136.2, 147.6, 149.8, 153.0, 170.3; MS (ES+) *m/z* for $C_{23}H_{26}N_3O_5$ $[M+H]^+$ calcd 424, found 424.

(*R*)-N-(2-(3-hydroxypropoxy)-4-(1H-pyrazol-4-yl)phenyl)-6-methoxychroman-3-carboxamide ((*R*)-11d).

Amide **(*R*)-11d** was synthesized from aniline **10d** and acid **7a** using the similar procedure used to prepare compound **(*S*)-11a** (54%): ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.88 (br s, 1 H), 9.23 (s, 1 H), 8.18 (br s, 1 H), 7.91 (br s, 1 H), 7.84 (d, *J* = 8.4 Hz, 1 H), 7.27 (d, *J* = 1.6 Hz, 1 H), 7.14 (dd, *J* = 8.4, 1.6 Hz, 1 H), 6.73-6.67 (m, 3 H), 4.61 (t, *J* = 4.8 Hz, 1 H), 4.36 (m, 1 H), 4.16 (t, *J* = 6.0 Hz, 2 H), 3.95 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.64 (q, *J* = 6.0 Hz, 2 H), 3.18-3.13 (m, 1 H), 3.02 (dd, *J* = 16.4, 10.0 Hz, 1 H), 2.91 (dd, *J* = 16.8, 6.0 Hz, 1 H), 1.93 (quint, *J* = 6.0 Hz, 2 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 27.8, 32.0, 55.3, 57.7, 65.9, 67.1, 109.3, 113.3, 114.1, 116.7, 116.9, 121.0, 121.8, 122.6, 125.1, 125.3, 129.8, 136.2, 147.6, 149.8, 153.0, 170.3; MS (ES+) *m/z* for $C_{23}H_{26}N_3O_5$ $[M+H]^+$ calcd 424, found 424.

(*S*)-N-(2-(2-(dimethylamino)ethoxy)-4-(1H-pyrazol-4-yl)phenyl)-6-methoxychroman-3-carboxamide ((*S*)-1).

Amide **(*S*)-1** was synthesized from aniline **10e** and acid **7b** using the similar procedure used to prepare compound **(*S*)-11a** (61%): $[\alpha]_D^{27}$ = +9.76 (*c* = 0.840, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.89 (br s, 1 H), 9.50 (s, 1 H), 8.18 (br s, 1 H), 7.92 (br s, 1 H), 7.87 (d, *J* = 8.4 Hz, 1 H), 7.33 (d, *J* = 1.6 Hz, 1 H), 7.19 (dd, *J* = 8.4, 1.6 Hz, 1 H), 6.74-6.67 (m, 3 H), 4.37 (m, 1 H), 4.18 (t, *J* = 6.0 Hz, 2 H), 3.95 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.03 (dd, *J* = 15.6, 9.6 Hz, 1 H), 2.92 (dd, *J* = 16.0, 4.4 Hz, 1 H), 2.65 (t, *J* = 6.0 Hz, 2 H), 2.26 (s, 6 H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 27.8, 45.4, 55.3, 57.6, 67.1, 67.7, 111.2, 113.3, 114.1, 116.7, 117.7, 120.9, 121.8, 122.5, 125.3, 126.1, 129.7, 136.2, 147.6, 149.4, 153.0, 170.1; MS (ES+) *m/z* for $C_{24}H_{29}N_4O_4$ $[M+H]^+$ calcd 437, found 437.

(*R*)-N-(2-(2-(dimethylamino)ethoxy)-4-(1H-pyrazol-4-yl)phenyl)-6-methoxychroman-3-carboxamide ((*R*)-1).

Amide **(*R*)-1** was synthesized from aniline **10e** and acid **7a** using the similar procedure used to prepare compound **(*S*)-11a** (66%): $[\alpha]_D^{27}$ = -9.59 (*c* = 0.709, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (br s, 1 H), 9.50 (s, 1 H), 8.18 (br s, 1 H), 7.92 (br s, 1 H), 7.87 (d, *J* = 8.4 Hz, 1 H), 7.33 (d, *J* = 1.6 Hz, 1 H), 7.19 (dd, *J* = 8.4, 1.6 Hz, 1 H), 6.74-6.67 (m, 3 H), 4.37 (m, 1 H), 4.18 (t, *J* = 6.0 Hz, 2 H), 3.95 (t, *J* = 10.0 Hz, 1 H), 3.69 (s, 3 H), 3.03 (dd, *J* = 15.6, 9.6 Hz, 1 H), 2.92 (dd, *J* = 16.0, 4.4 Hz, 1 H), 2.65 (t, *J* = 6.0 Hz, 2 H), 2.26 (s, 6 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.8, 45.4, 55.3, 57.6, 67.1, 67.7, 111.2, 113.3, 114.1, 116.7, 117.7, 120.9, 121.8, 122.5, 125.3, 126.1, 129.7, 136.2, 147.6, 149.4, 153.0, 170.1; MS (ES+) *m/z* for $C_{24}H_{29}N_4O_4$ $[M+H]^+$ calcd 437, found 437.

Enzyme assays

All experiments were performed in Greiner FIA black 384-well low volume plates (#784076). PKA and MRCKα were purchased from Upstate (now part of Millipore), and ROCK-II (amino acid 1-543) was cloned and purified as described previously.[1] Enzyme inhibitors were dispensed in a 90% DMSO/10% water mixture using a 384 head offline Pintool system (GNF Systems).

ROCK-II

Assays were performed using the HTRF KinEASE STK S2 (STK substrate2-biotin) kit (Cisbio #62ST2PEC). A 5 µL mixture of 1 µM STK2 substrate and 20 µM ATP in STK-buffer was added to the wells using a BioRAPTR FRD™ Workstation (Aurora Discovery). Inhibitor (20 nL) at various concentrations was then dispensed. Reaction was started by addition of 0.5 nM ROCK-II in STK-buffer (5 µL). After 4 h at rt, the reaction was stopped by addition of 10 µL mixture of 1× STK antibody-Cryptate in detection buffer and 62.5 nM Sa-XL in detection buffer. After 1 h at rt, the plates were read on the Viewlux in HTRF mode (PerkinElmer Life Sciences).

PKA assay

A 5 µL mixture of a 60 µM kemptide and 20 µM ATP in Kinase buffer (50 mM Hepes pH 7.3, 10 mM $MgCl_2$, 0.1% BSA, 2 mM DTT) was added to the wells using a BioRAPTR FRD™ Workstation (Aurora Discovery). 20 nL of inhibitor at various concentrations was then dispensed. Reaction was started by the addition of 0.5 nM PKA (Upstate #14-440) in Kinase buffer (5 µL of kinase buffer alone for high control wells). After 70 min at rt, the reaction was stopped by addition of 10 µL Kinase-Glo reagent (Promega) and the plate was read after 10 min incubation time at rt on the Viewlux in luminescence mode (PerkinElmer Life Sciences).

MRCK assay

A 5 µL mixture of 40 µM S6-peptide (LCB-AKRRRLSSLRA-NH_2) and 10 µM ATP in Kinase buffer (50 mM Hepes pH 7.3, 10 mM MgCl2, 0.1 % BSA, 2 mM DTT) was added to the wells using a BioRAPTR FRD™ Workstation (Aurora Discovery). 20 nL of inhibitor at various

concentrations was then dispensed. Reaction was started by the addition of 12 nM MRCK (Upstate #14-691) in Kinase buffer (5 μL of kinase buffer alone for high control wells). After 75 min at rt, the reaction was stopped by addition of 10 μL Kinase-Glo reagent and the plate was read after 10 min incubation time at rt on the Viewlux in luminescence mode.

ppMLC assay

ROCK inhibitor activity was determined in the ppMLC assay as previously described.[2] Briefly, A7r5 cells (ATCC) were plated at 5000 cells/well in a 96-well Packard View Plate (Perkin-Elmer) in DMEM + 10% FBS. Following attachment, cells were serum starved for 4 h and treated with inhibitor in 0.25% DMSO final concentration for 1 h at 37 °C. Cells were then treated with 10 μM LPA for 10 min, fixed with 4% paraformaldehyde for 20 min, washed in 0.1 M glycine and permeabilized in 0.2% Triton X for 20 min. Cells were then washed once in PBS and blocked in LI-COR blocking buffer (LI-COR Biosciences) for 1 h at 25 °C. Cells were probed for phosphorylated myosin light chain 20 using 55 ng/mL primary rabbit antibody (Cell Signaling Technology, #3674) and incubated overnight at 4 °C. Following three washes, cells were probed with goat-anti-rabbit IR800 antibody (LI-COR Biosciences 926-32211) (2 μg/mL in LI-COR block + 0.025% Tween-20) for 1 h at 25 °C. Nuclei were stained with TO-PRO-3 iodide (642/661, Invitrogen) (1:4000) for 30 min, washed twice in PBS/0.05% Tween-20, and read with an Odyssey infrared imaging system (LI-COR Biosciences).

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