Supporting Information

Discovery of Highly Potent and Selective Urea-based ROCK Inhibitors and Their Effects on Intraocular Pressure in Rats

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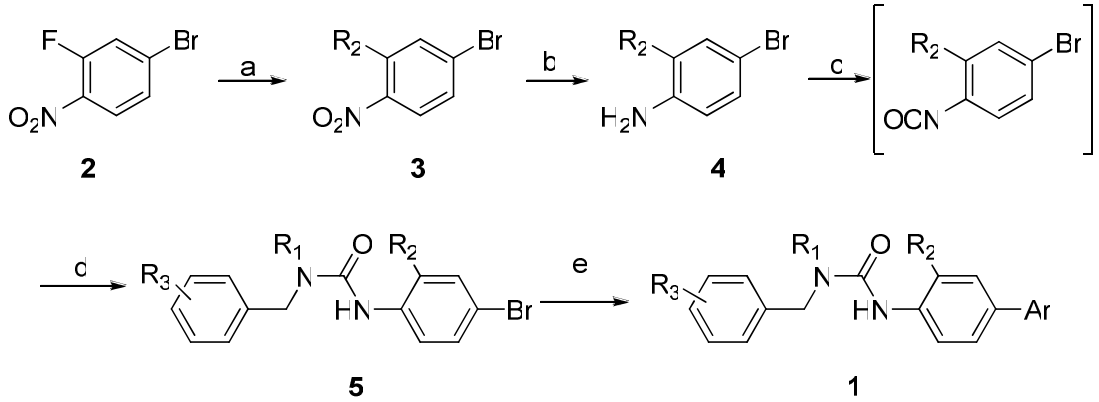
1. Synthetic Procedures and Characterization

Commercially available reagents and anhydrous solvents were used without further purification unless otherwise specified. Thin layer chromatography (TLC) analyses were performed with precolated silica gel 60 F254 to monitor the reaction. The mass spectra were recorded by LC/MS with Finnigan LCQ Advantage MAX spectrometer of Thermo Electron® to monitor the reaction and identify the target compounds. Flash chromatography was performed on prepacked columns of silica gel (230-400 Mesh, 40-63 μm) by CombiFlash® with EtOAc/hexane or MeOH/CH_2Cl_2 as the eluents to purify the intermediates. Preparative HPLC was performed on SunFire C_{18} OBD 10μm (30x250mm) with 50%MeOH /CH_3CN as solvent A, and H_2O+0.1% TFA as solvent B to purify the final targeted compounds. Analytic HPLC was performed on agilent technologies 1200 series with CH_3CN (Solvent B) / H_2O+0.9%CH_3CN+0.1% TFA (Solvent A) as eluent to identify the purity of the targeted compounds (gradient from 0%B to 100%B in 10 min). NMR spectra were recorded with a Bruker® 400 MHz spectrometer at ambient temperature with the residual solvent peaks as internal standards. The line positions of multiplets were given in ppm (δ) and the coupling constants (*J*) were given in Hertz. The high-resolution mass spectra (HRMS, electrospray ionization) experiments were performed with a Thermo Finnigan orbitrap mass analyzer. Data were acquired in the positive ion mode at a resolving power of 100000 at m/z 400. Calibration was performed with an external calibration mixture immediately prior to analysis.

1.1 Synthetic procedure



Scheme 1. Reagents and conditions: (a) Amine or alcohol nucleophile, Cs_2CO_3, DMF, rt; (b) $SnCl_2{\cdot}2H_2O$, EtOAc; (c) Triphosgene, $NaHCO_3$, CH_2Cl_2, 0 °C; (d) Benzylamine derivatives, CH_2Cl_2, 0 °C; (e) Boronic acid pinacol ester, $Ph(PPh_3)_4$, K_2CO_3, Dioxane, H_2O, 95 °C or (i) Bispinacolatodiboron, $PdCl_2(dppf)$, KOAc, Dioxane, reflux, (ii) Ar-Cl, $Ph(PPh_3)_4$, K_2CO_3, Dioxane, H_2O, 95 °C.

General synthetic procedures:

To a mixture of 4-bromo-2-fluoro-nitrobenzene (1 mmol), Cs_2CO_3 (3 mmol) in DMF (5 mL) was added an amine or an alcohol nucleophile (1.05 mmol). After the complete conversion of starting material as detected by TLC, the mixture was quenched by water (2 mL) and extracted with EtOAc (5 mL × 3). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified through silica gel to give **3**.

To a solution of **3** (1 mmol) in EtOAc (5 mL) was added tin chloride dehydrate (3 mmol). After stirring in room temperature until the complete disappearance of **3** as detected by TLC and LC/MS, the mixture was quenched by water (2 mL) and extracted with EtOAC (5 mL × 3). The combined organic extracts were washed with saturated brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified through silica gel to give aniline **4**.

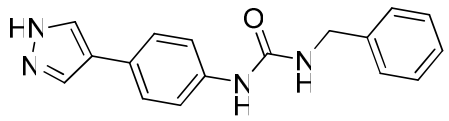
Triphosgen (0.33 mmol) was added in portions to a mixture of **4** (1 mmol), saturated $NaHCO_3$ (1.2 mL) in dichloromethane (2 mL) at 0 °C to produce the isocyanate intermediate.

To a solution of 3-substituted aldehyde (10 mmol) in methanol (20 mL) was added a primary amine (10 mmol). After stirring at room temperature for 15 min, the solution was cooled to 0 °C prior to the addition of sodium borohydride (5 mmol). The resulting solution was stirred at room temperature for 1 h. After the addition of water (3 mL), methanol was removed under reduced pressure and the resulting aqueous phase was extracted with EtOAc. The combined extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the secondary benzylamine derivative. Then secondary benylamine or commercial available primary benylamine (0.2 mmol) was added to a

mixture of the isocyanate intermediate or a commercial available isocyanate (0.2 mmol) in dicholoromethane at 0 °C. After stirring at room temperature for 0.5-12 h, the mixture was extracted with dicholoromethane, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give the crude bromide urea **5** which was used in the next step without further purification.

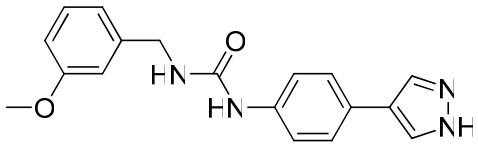
Crude **5** (0.2 mmol) and the boronic acid pinacol ester (0.3 equiv) were dissolved in degassed 5:1 dioxane/H_2O. $Pd(PPh_3)_4$ (0.02 mmol) and 2M solution of K_2CO_3 (0.6 mmol) were then added sequentially under Argon and the mixture was heated at 95 ^oC for 2h. After cooling to room temperature, the mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated under reduced pressure to give crude **1a-1j**, and **1n-1z**. These crude products were subjected to preparative HPLC to give **1a-1j**, and **1n-1z** as white solid.

In an alternative route, crude bromide **5** (0.2 mmol) and the 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (0.4 mmol) were dissolved in degassed dioxane (2 mL) in a sealed tube. $PdCl_2$(dppf) (0.04 mmol) and KOAc (0.6 mmol) were added sequentially. The mixture was heated at 80 ^oC for 2h. After cooling to room temperature, the mixture was diluted with water and extracted with ethyl acetate. The organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the crude boronic acid ester intermediate. Then the crude boronic acid ester intermediate (0.2 mmol) and an aromatic chloride (0.3 mmol) were dissolved in degassed dioxane/H_2O (2 mL, 5:1 by volume) in a sealed tube. $Pd(PPh_3)_4$ (0.02 mmol) and 2M solution of K_2CO_3 (0.6 mmol) were added sequentially. The mixture was heated at 95 ^oC for 2h. After cooling to room temperature, the mixture was diluted with water and extracted with ethyl acetate. The organic layers were combined, dried over sodium sulfate and concentrated *in vacuo*. The remaining residue was purified by preparative HPLC to give **1k-1m** as white solids.

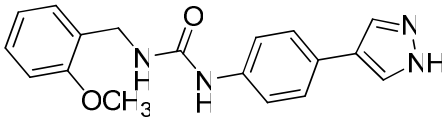


1-(4-(1H-pyrazol-4-yl)phenyl)-3-benzylurea (1a): Prepared using the **General synthetic procedures**. 75% yield in two steps**.** ^1H NMR (DMSO-d_6, 400 MHz) δ 12.81

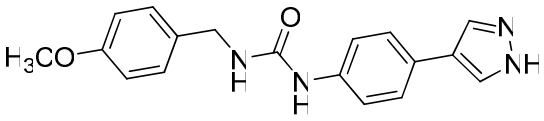
(br, s, 1H), 8.52 (s, 1H), 7.93 (s, 2H), 7.45 (dd, *J*=6.8, 2.0 Hz, 2H), 7.38 (dd, *J*=6.8, 2.0 Hz, 2H), 7.36-7.30 (m, 4H), 7.26-7.22 (m, 1H), 6.58 (t, *J*=5.6 Hz, 1H), 4.30 (d, *J*=5.6 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.67, 155.19, 150.36, 140.35, 138.40, 128.27, 127.08, 126.67, 125.95, 125.41, 121.10, 118.07, 42.71; LC/MS ($M+H^+$): 293.12; HRMS (ESI-Orbitrap) Calcd for $C_{17}H_{17}N_4O$: 293.1402 [$M+H^+$], Found 293.1393.



1-(4-(1H-pyrazol-4-yl)phenyl)-3-(3-methoxybenzyl)urea (1b): 86% yield in two steps**.** ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.52 (s, 1H), 7.94 (s, 2H), 7.45 (d, *J*=8.8 Hz, 2H), 7.37 (d, *J*=8.8 Hz, 2H), 7.25 (m, 1H), 6.87 (m, 2H), 6.80 (m, 1H), 6.57 (t, *J*=5.6 Hz, 1H), 4.27 (d, *J*=5.6 Hz, 2H), 3.74 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.30, 155.20, 142.00, 138.43, 137.56, 130.15, 129.34, 125.91, 125.42, 121.13, 119.22, 118.06, 112.76, 111.99, 54.95, 42.67; LC/MS ($M+H^+$): 323.14; HRMS (ESI-Orbitrap) Calcd for $C_{18}H_{19}N_4O_2$ [$M+H^+$]: 323.1508, Found 323.1496.

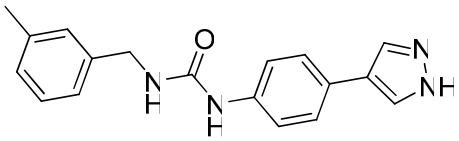


1-(4-(1H-Pyrazol-4-yl)phenyl)-3-(2-methoxybenzyl)urea (1c): 79% yield in two steps**.** ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.55 (s, 1H), 7.94 (s, 2H), 7.44 (d, *J*=8.4 Hz, 2H), 7.36 (d, *J*=8.4 Hz, 2H), 7.27-7.22 (m, 2H), 7.00-6.98 (m, 1H), 6.94-6.90 (m, 1H), 6.41 (t, *J*=5.6 Hz, 1H), 4.25 (d, *J*=5.6 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.79, 156.77, 155.12, 138.50, 130.16, 128.10, 127.98, 127.62, 125.80, 125.44, 121.16, 120.13, 117.93, 110.45, 55.29, 38.17; LC/MS ($M+H^+$) 323.15; HRMS (ESI-Orbitrap) Calcd for $C_{18}H_{19}N_4O_2$ [$M+H^+$]: 323.1508, Found 323.1497.

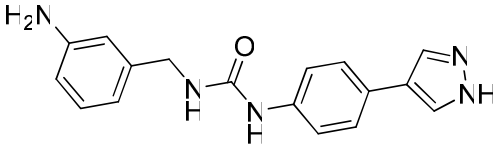


1-(4-(1H-pyrazol-4-yl)phenyl)-3-(4-methoxybenzyl)urea (1d): 68% yield in two steps**.** ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.47 (s, 1H), 7.94 (s, 2H), 7.44 (d, *J*=8.0 Hz, 2H), 7.37 (d, *J*=8.0 Hz, 2H), 7.22 (d, *J*=8.0 Hz, 2H), 6.89 (d, *J*=8.0 Hz, 2H), 6.49 (t, *J*=4.8 Hz, 1H), 4.22 (d, *J*=4.8 Hz, 2H), 2.73 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.15,

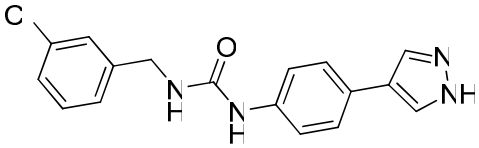
157.94, 155.12, 138.45, 134.18, 132.22, 128.46, 125.87, 125.41, 121.13, 118.02, 113.68, 55.03, 42.19; LC/MS ($M+H^+$) 323.14; HRMS (ESI-Orbitrap) Calcd for $C_{18}H_{19}N_4O_2$ [$M+H^+$]: 323.1508, Found 323.1497.



1-(4-(1H-pyrazol-4-yl)phenyl)-3-(3-methylbenzyl)urea (1e): 80% yield in two steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.51 (s, 1H), 7.94 (s, 2H), 7.44 (d, *J*=8.8 Hz, 2H), 7.37 (d, *J*=8.8 Hz, 2H), 7.24-7.20 (m, 1H), 7.11-7.05 (m, 3H), 6.55 (t, *J*=5.6 Hz, 1H), 4.26 (d, *J*=5.6 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.49, 155.16, 140.23, 138.51, 137.32, 130.14, 128.18, 127.70, 127.31, 125.76, 125.44, 124.21, 121.20, 118.03, 42.68, 20.99; LC/MS ($M+H^+$): 307.16; HRMS (ESI-Orbitrap) Calcd for $C_{18}H_{19}N_4O$ [$M+H^+$]: 307.1559, Found 307.1548.

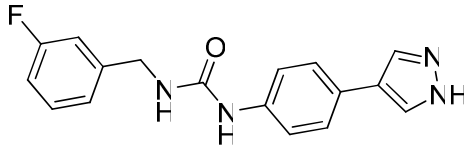


1-(4-(1H-pyrazol-4-yl)phenyl)-3-(3-aminobenzyl)urea (1f): 1-(4-(1H-pyrazol-4-yl)phenyl)-3-(3-nitrobenzyl)urea was prepared using the **General synthetic procedures**. Then the nitro group was reduced with $TiCl_2 \cdot 2H_2O$ (3 equiv) in EtOAc to give **1f** in 35% overall yield (three steps)**.** ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.64 (s, 1H), 7.94 (s, 2H), 7.46 (d, *J*=8.8 Hz, 2H), 7.38 (d, *J*=8.8 Hz, 2H), 7.12-7.08 (m, 1H), 6.75-6.69 (m, 3H), 6.68 (t, *J*=5.6 Hz, 1H), 4.29 (d, *J*=5.6 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.05, 157.74, 155.18, 151.30, 141.59, 138.44, 129.10, 125.91, 125.40, 121.10, 120.40, 118.01, 115.84, 115.76, 42.65; LC/MS ($M+H^+$) 308.04; HRMS (ESI-Orbitrap) Calcd for $C_{17}H_{18}N_5O$ [$M+H^+$]: 308.1511, Found 308.1501.

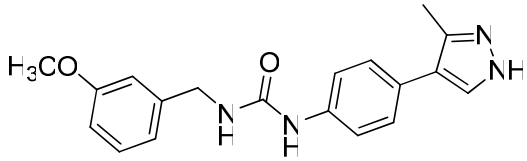


1-(4-(1H-pyrazol-4-yl)phenyl)-3-(3-chlorobenzyl)urea (1g): 75% yield in two steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.59 (s, 1H), 7.94 (s, 2H), 7.47-7.45 (m, 2H), 7.39-7.35 (m, 4H), 7.31-7.26 (m, 2H), 6.67 (t, *J*=5.6 Hz, 1H), 4.30 (d, *J*=5.6 Hz, 2H); ^{13}C NMR

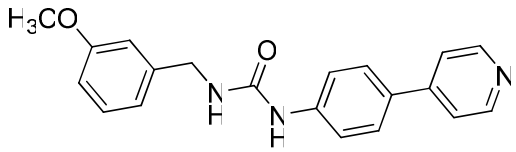
(DMSO-d_6, 100 MHz) δ 155.20, 143.23, 138.33, 132.93, 130.14, 126.80, 126.55, 126.02, 125.72, 125.42, 121.11, 118.15, 113.89, 111.56, 42.18; LC/MS ($M+H^+$): 327.16; HRMS (ESI-Orbitrap) Calcd for $C_{17}H_{16}ClN_4O$ [$M+H^+$]: 327.1013, Found 327.1002.



1-(4-(1H-pyrazol-4-yl)phenyl)-3-(3-fluorobenzyl)urea (1h): 69% yield in two steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.60 (s, 1H), 7.96 (s, 2H), 7.48-7.46 (m, 2H), 7.40-7.36 (m, 3H), 7.16-7.04 (m, 3H), 6.67 (t, *J*=5.6 Hz, 1H), 4.32 (t, *J*=5.6 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.43, 161.01, 155.20, 143.61, 138.37, 130.14, 125.95, 125.42, 123.00, 122.97, 121.13, 118.14, 113.73, 113.44, 42.23; LC/MS ($M+H^+$) 311.09; HRMS (ESI-Orbitrap) Calcd for $C_{17}H_{16}FN_4O$ [$M+H^+$]: 311.1308, Found 311.1297.

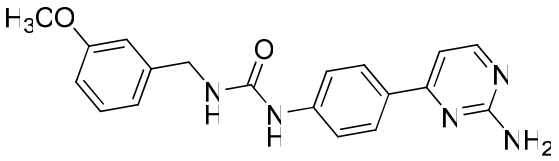


1-(3-Methoxybenzyl)-3-(4-(3-methyl-1H-pyrazol-4-yl)phenyl)urea (1i): 79% yield in two steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.55 (s, 1H), 7.70 (s, 1H), 7.41 (dd, *J*=6.8, 2.0 Hz, 2H), 7.30 (dd, *J*=6.8, 2.0 Hz, 2H), 7.27-7.23 (m, 1H), 6.89-6.87 (m, 2H), 6.82-6.80 (m, 1H), 6.58 (t, *J*=5.6 Hz, 1H), 4.27 (d, *J*=5.6 Hz, 2H), 3.74 (s, 3H); 2.33 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.30, 158.57, 155.23, 141.99, 138.46, 133.26, 129.34, 127.06, 126.17, 119.21, 118.64, 117.99, 112.75, 111.99, 54.94, 42.66, 11.38; LC/MS ($M+H^+$) 337.08; HRMS (ESI-Orbitrap) Calcd for $C_{19}H_{21}N_4O_2$ [$M+H^+$]: 337.1665, Found 337.1652.

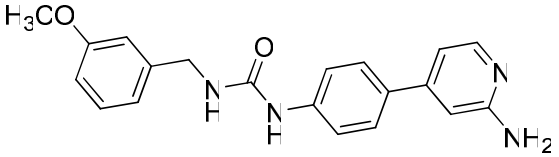


1-(3-Methoxybenzyl)-3-(4-(pyridin-4-yl)phenyl)urea (1j): 80% yield in two steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.06 (s, 1H), 8.31-8.30 (m, 1H), 8.10-8.08 (m, 2H), 7.60-7.58 (m, 4H), 7.33-7.31 (m, 1H), 7.27-7.23 (m, 1H), 6.89-6.80 (m, 4H), 4.29 (d, *J*=6.0 Hz, 2H), 3.74 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.31, 154.89, 153.27, 144.01, 143.52, 141.72, 129.36, 128.57, 126.43, 121.74, 119.22, 117.87, 112.77, 112.05,

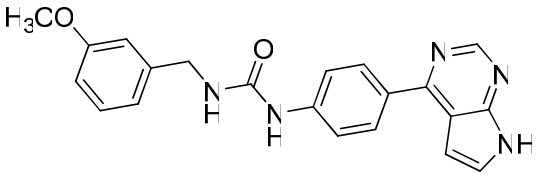
54.94, 42.66; LC/MS ($M+H^+$) 334.09; HRMS (ESI-Orbitrap) Calcd for $C_{20}H_{20}N_3O_2$ [$M+H^+$]: 334.1556, Found 334.1544.



1-(4-(2-Aminopyrimidin-4-yl)phenyl)-3-(3-methoxybenzyl)urea (1k): 75% yield in three steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.04 (s, 1H), 8.76 (d, *J*=6.8 Hz, 2H), 8.1 (d, *J*=6.8 Hz, 2H), 7.93-7.91 (m, 2H), 7.64-7.62 (m, 2H), 7.28-7.23 (m, 1H), 6.90-6.88 (m, 2H), 6.86-6.81 (m, 2H), 4.29 (d, *J*=5.6 Hz, 2H), 3.74 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.42, 159.31, 158.23, 154.77, 150.88, 144.79, 141.68, 129.37, 128.85, 127.21, 119.24, 117.26, 112.81, 112.05, 104.95, 54.96, 42.68; LC/MS ($M+H^+$) 350.05; HRMS (ESI-Orbitrap) Calcd for $C_{19}H_{20}N_5O_2$ [$M+H^+$]: 350.1617, Found 350.1605.

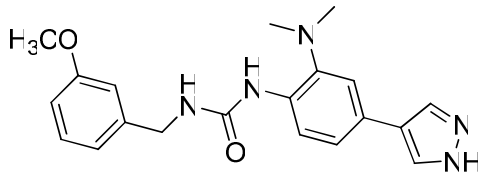


1-(4-(2-Aminopyridin-4-yl)phenyl)-3-(3-methoxybenzyl)urea (1l): 45% yield in three steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.99 (s, 1H), 7.96-7.94 (m, 1H), 7.77 (s, 2H), 7.70 (dd, *J*=6.8, 1.6 Hz, 2H), 7.60 (dd, *J*=6.8, 1.6 Hz, 2H), 7.27-7.23 (m, 1H), 7.19-7.17 (m, 1H), 7.12-7.10 (m, 1H), 6.89-6.87 (m, 2H), 6.83-6.80 (m, 2H), 4.29 (d, *J*=6 Hz, 2H), 3.74 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.30, 154.91, 154.43, 153.22, 143.39, 141.75, 136.67, 129.37, 127.79, 127.10, 119.23, 117.81, 112.78, 112.03, 109.85, 107.20, 54.95, 42.66; LC/MS ($M+H^+$) 349.10; HRMS (ESI-Orbitrap) Calcd for $C_{20}H_{21}N_4O_2$ [$M+H^+$]: 349.1665, Found 349.1653.

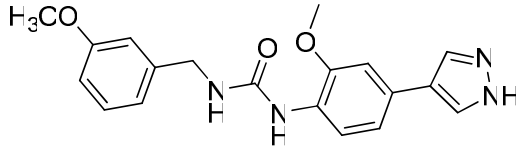


1-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)phenyl)-3-(3-methoxybenzyl)urea (1m): 61% yield in three steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.48 (br, s, 1H), 8.97 (s, 1H), 8.84 (s, 1H), 8.10 (d, *J*=8.8 Hz, 2H), 7.72 (s, 1H), 7.66 (d, *J*=8.8 Hz, 2H), 7.28-7.24 (m, 1H), 6.99 (s, 1H), 6.91-6.89 (m, 2H), 6.84-6.81 (m, 1H), 6.77 (t, *J*=5.6 Hz, 1H), 4.31 (d,

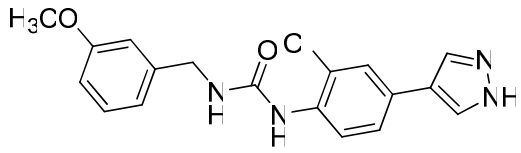
J=5.6 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.32, 158.04, 154.90, 153.18, 152.20, 148.26, 143.53, 141.73, 129.84, 129.38, 129.06, 119.25, 117.54, 113.67, 112.81, 112.06, 101.43, 54.96, 42.70; LC/MS ($M+H^+$) 374.06; HRMS (ESI-Orbitrap) Calcd for $C_{21}H_{20}N_5O_2$ [$M+H^+$]: 374.1617, Found 374.1604.



1-(2-(Dimethylamino)-4-(1H-pyrazol-4-yl)phenyl)-3-(3-methoxybenzyl)urea (**1n**): 47% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.20 (s, 1H), 8.13 (s, 2H), 8.01 (s, 1H), 7.48-7.44 (m, 2H), 7.32 (s, 1H), 7.28-7.24 (m, 1H), 6.90-6.88 (m, 2H), 6.82 (t, *J*=5.2 Hz, 1H), 4.29 (d, *J*=5.2 Hz, 2H), 3.74 (s, 3H), 2.76 (s, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.45, 157.73, 157.37, 155.34, 140.63, 138.99, 130.16, 129.82, 128.51, 122.40, 119.73, 118.37, 116.09, 113.25, 111.90, 111.24, 54.05, 44.00, 42.09; LC/MS ($M+H^+$) 366.03; HRMS (ESI-Orbitrap) Calcd for $C_{20}H_{24}N_5O_2$ [$M+H^+$]:366.1930, Found 366.1921 .

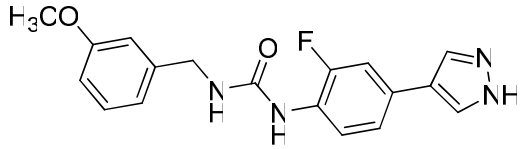


1-(2-Methoxy-4-(1H-pyrazol-4-yl)phenyl)-3-(3-methoxybenzyl)urea (**1o**): 55% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.05-8.03 (m, 1H), 7.99-7.98 (m, 3H), 7.27-7.23 (m, 2H), 7.18-7.17 (m, 1H), 7.10-7.07 (m, 1H), 6.88-6.86 (m, 2H), 6.83-6.80 (m, 1H), 4.36-4.27 (m, 2H), 3.89 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.32, 158.10, 156.23, 155.14, 147.71, 141.82, 129.39, 127.40, 126.11, 121.42, 119.28, 118.28, 117.20, 112.82, 112.03, 107.80, 55.79, 54.96, 42.68; LC/MS ($M+H^+$) 353.01.

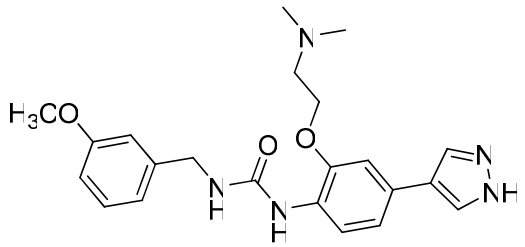


1-(2-Chloro-4-(1H-pyrazol-4-yl)phenyl)-3-(3-methoxybenzyl)urea (**1p**): 73% yield in two steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.91 (s, br, 1H), 8.18 (s, 1H), 8.11-8.07 (m, 2H), 7.90 (s, 1H), 7.67-7.66 (m, 1H), 7.50-7.48 (m, 1H), 7.39 (t, *J*=5.6 Hz, 1H), 7.29-

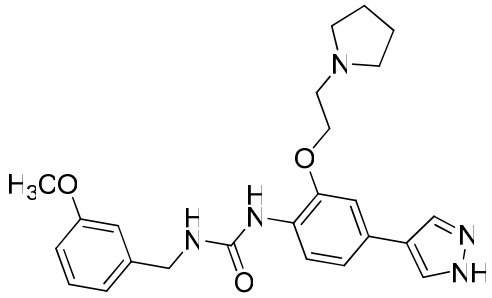
7.25 (m, 1H), 6.90-6.88 (m, 2H), 6.84-6.82 (m, 1H), 4.29 (d, *J*=5.6 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.34, 154.80, 152.95, 141.47, 136.03, 134.27, 129.45, 127.77, 125.23, 124.10, 121.88, 121.24, 119.79, 119.35, 112.89, 112.16, 54.98, 42.78; LC/MS ($M+H^+$) 357.05.



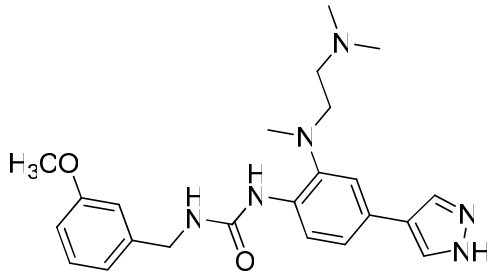
1-(2-Fluoro-4-(1H-pyrazol-4-yl)phenyl)-3-(3-methoxybenzyl)urea (**1q**): 70% yield in two steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.34-8.33 (m, 1H), 8.08-8.02 (m, 3H), 7.48-7.44 (m, 1H), 7.35-7.32 (m, 1H), 7.28-7.24 (m, 1H), 7.01 (t, *J*=5.6 Hz, 1H), 6.89-6.87 (m, 2H), 6.84-6.81 (m, 1H), 4.29 (d, *J*=5.6 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.33, 154.90, 153.30, 150.92, 141.63, 130.62, 129.42, 127.10, 125.85, 120.85, 120.22, 119.23, 112.77, 112.10, 111.54, 111.35, 54.95, 42.70; LC/MS ($M+H^+$) 341.03.



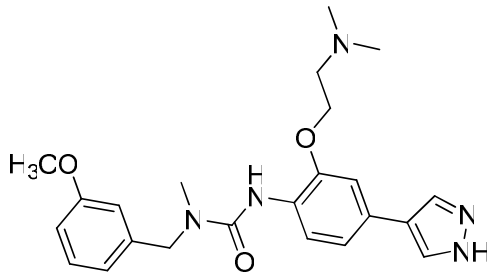
1-(2-(2-(Dimethylamino)ethoxy)-4-(1H-pyrazol-4-yl)phenyl)-3-(3-methoxybenzyl)urea (**1r**): 39% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.59 (s, br, 1H), 8.00-7.95 (m, 3H), 7.87 (s, 1H), 7.28-7.24 (m, 2H), 7.17-7.13 (m, 2H), 6.90-6.88 (m, 2H), 6.82 (t, *J*=5.6 Hz, 1H), 4.44 (t, *J*=5.2 Hz, 2H), 4.29 (t, *J*=5.6 Hz, 2H), 3.75 (s, 3H), 3.54 (t, *J*=5.2 Hz, 2H), 2.90 (s, 3H), 2.89 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.31, 158.90, 158.56, 155.24, 146.50, 141.87, 129.37, 127.33, 126.61, 121.19, 119.52, 119.34, 117.99, 112.90, 112.02, 109.06, 62.54, 55.49, 54.92, 42.70, 42.60; LC/MS ($M+H^+$) 410.03; HRMS (ESI-Orbitrap) Calcd for $C_{22}H_{28}N_5O_3$ [$M+H^+$]: 410.2192, Found 412.2191



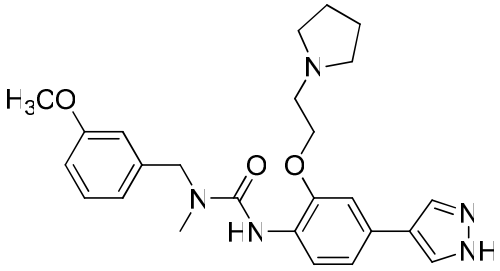
1-(4-(1H-pyrazol-4-yl)-2-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-3-(3-methoxybenzyl)urea (**1s**): 45% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.69 (s, br, 1H), 8.01 (s, 2H), 7.92-7.90 (m, 2H), 7.29-7.25 (m, 2H), 7.18-7.15 (m, 2H), 6.90-6.88 (m, 2H), 6.83 (t, *J*=6.0 Hz, 1H), 4.44-4.41 (m, 2H), 4.28 (d, *J*=6.0 Hz, 2H), 3.75 (s, 3H), 3.62-3.61 (m, 4H), 3.18-3.13 (m, 2H), 2.05-2.03 (m, 2H), 1.89-1.86 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.33, 158.69, 158.32, 155.30, 146.77, 141.84, 129.39, 127.31, 126.84, 121.18, 119.95, 119.28, 118.08, 112.89, 112.00, 109.29, 63.81, 54.93, 53.65, 52.85, 42.68, 22.48; LC/MS ($M+H^+$) 436.09; HRMS (ESI-Orbitrap) Calcd for $C_{24}H_{30}N_5O_3$ [$M+H^+$]:436.2349, Found 436.2335.



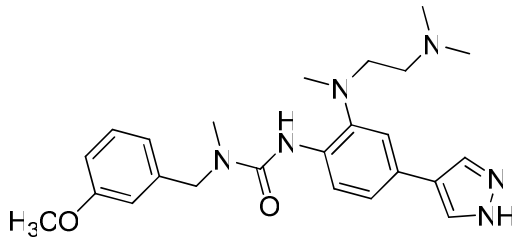
1-(2-((2-(Dimethylamino)ethyl)(methyl)amino)-4-(1H-pyrazol-4-yl)phenyl)-3-(3-methoxybenzyl)urea (**1t**): 45% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.36 (s, br, 1H), 8.13 (s, 1H), 7.99-7.97 (m, 3H), 7.44-7.43 (m, 1H), 7.32-7.25 (m, 3H), 6.91-6.89 (m, 2H), 6.84 (t, *J*=5.6 Hz, 1H), 4.29 (d, *J*=5.6 Hz, 2H), 3.75 (s, 3H), 3.25 (t, *J*=5.2 Hz, 2H), 3.22 (t, *J*=5.2 Hz, 2H), 2.76 (s, 3H), 2.75 (s, 3H), 2.60 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.32, 158.88, 158.48, 155.39, 141.90, 141.03, 133.34, 129.37, 126.89, 121.90, 121.08, 119.61, 119.22, 118.49, 112.78, 111.99, 54.93, 53.85, 49.59, 42.80, 42.69, 42.22; LC/MS ($M+H^+$) 423.03; HRMS (ESI-Orbitrap) Calcd for $C_{23}H_{31}N_6O_2$ [$M+H^+$]: 423.2508, Found 423.2497.



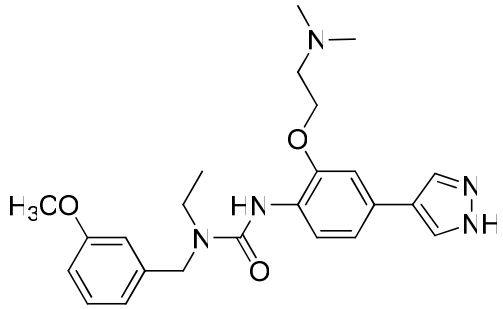
3-(2-(2-(Dimethylamino)ethoxy)-4-(1H-pyrazol-4-yl)phenyl)-1-(3-methoxybenzyl)-1-methylurea (**1u**): 37% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.66 (s, br, 1H), 8.05 (s, 2H), 7.84 (s, 1H), 7.58-7.56 (m, 1H), 7.32-7.28 (m, 2H), 7.21-7.19 (m, 1H), 6.88-6.84 (m, 3H), 4.54 (s, 2H), 4.48 (t, *J*=4.8 Hz, 2H), 3.75 (s, 3H), 3.49 (t, *J*=4.8 Hz, 2H), 2.96 (s, 3H), 2.84 (s, 3H), 2.83 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.44, 158.47, 158.12, 155.90, 149.07, 139.85, 129.66, 129.09, 126.30, 123.51, 121.00, 119.27, 117.76, 113.10, 112.14, 109.20, 62.12, 55.59, 54.98, 51.25, 42.54, 34.35; LC/MS ($M+H^+$) 423.99; HRMS (ESI-Orbitrap) Calcd for $C_{23}H_{30}N_5O_3$ [$M+H^+$]:424.2349, Found 424.2340.



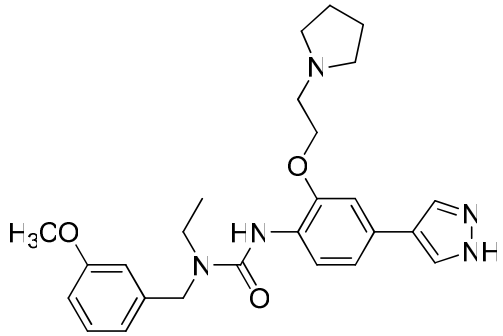
3-(4-(1H-pyrazol-4-yl)-2-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-1-(3-methoxybenzyl)-1-methylurea (**1v**): 41% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.67 (s, br, 1H), 8.06 (s, 2H), 7.97 (s, 1H), 7.48-7.46 (m, 1H), 7.33-7.32 (m, 2H), 7.22-7.19 (m, 1H), 6.89-6.81 (m, 3H), 4.54 (s, 2H), 4.49-4.47 (m, 2H), 3.76 (s, 3H), 3.54-3.53 (m, 6H), 3.09-3.06 (m, 2H), 2.96 (s, 3H), 2.00-1.98 (m, 2H), 1.85-1.78 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.48, 159.38, 157.77, 156.12, 149.56, 139.79, 129.72, 129.65, 126.09, 124.33, 121.08, 119.07, 117.76, 112.93, 112.16, 109.29, 63.19, 55.01, 53.57, 52.75, 51.28, 34.45, 22.46; LC/MS ($M+H^+$) 450.10; HRMS (ESI-Orbitrap) Calcd for $C_{25}H_{32}N_5O_3$ [$M+H^+$]:450.2505, Found 450.2498.



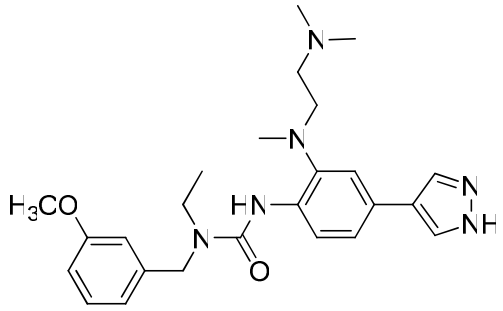
3-(2-((2-(Dimethylamino)ethyl)(methyl)amino)-4-(1H-pyrazol-4-yl)phenyl)-1-(3-methoxybenzyl)-1-methylurea (**1w**): 48% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.40 (s, br, 1H), 8.30 (s, 1H), 8.06-8.05 (m, 2H), 7.44-7.42 (m, 2H), 7.34-7.31 (m, 2H), 6.89-6.83 (m, 3H), 4.58 (s, 2H), 3.76 (s, 3H), 3.37 (t, *J*=6.0 Hz, 2H), 3.08 (t. *J*=6.0 Hz, 2H), 3.01 (s, 3H), 2.66 (s, 3H), 2.65 (s, 3H), 2.61 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.52, 158.44, 158.10, 156.34, 143.69, 139.74, 132.00, 129.76, 124.65, 121.18, 120.91, 118.89, 117.55, 115.03, 112.77, 112.16, 55.01, 53.80, 51.35, 47.46, 43.88, 42.09, 34.76; LC/MS ($M+H^+$) 437.01; HRMS (ESI-Orbitrap) Calcd for $C_{24}H_{33}N_6O_2$ [$M+H^+$]:437.2665, Found 437.2656.



3-(2-(2-(Dimethylamino)ethoxy)-4-(1H-pyrazol-4-yl)phenyl)-1-ethyl-1-(3-methoxybenzyl)urea (**1x**): 48% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.72 (s, br, 1H), 8.05 (s, 2H), 7.75 (s, 1H), 7.57-7.55 (m, 1H), 7.33-7.29 (m, 2H), 7.21-7.19 (m, 1H), 6.90-6.86 (m, 3H), 4.56 (s, 2H), 4.47 (t, *J*=4.8 Hz, 2H), 3.76 (s, 3H), 3.46 (t, *J*=4.8 Hz, 2H), 3.37 (q, *J*=6.8 Hz, 2H), 2.83 (s, 3H), 2.82 (s, 3H), 1.12 (t, *J*=6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.40, 158.44, 158.10, 155.30, 149.07, 140.31, 129.63, 129.07, 126.30, 123.71, 121.01, 119.19, 117.75, 113.02, 112.08, 109.17, 62.12, 55.59, 54.98, 48.89, 42.57, 41.22, 13.25; LC/MS ($M+H^+$) 438.01; HRMS (ESI-Orbitrap) Calcd for $C_{24}H_{32}N_5O_3$ [$M+H^+$]:438.2505, Found 438.2496.



3-(4-(1H-pyrazol-4-yl)-2-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-1-ethyl-1-(3-methoxybenzyl)urea (**1y**) : 46% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.76 (s, br, 1H), 8.06 (s, 2H), 7.85 (s, 1H), 7.50-7.48 (m, 1H), 7.34-7.30 (m, 2H), 7.22-7.20 (m, 1H), 6.89-6.86 (m, 3H), 4.56 (s, 2H), 4.46 (m, 2H), 3.76 (s, 3H), 3.40-3.35 (m, 6H), 3.09-3.07 (m, 2H), 1.99-1.98 (m, 2H), 1.84-1.80 (m, 2H), 1.15-1.11 (m, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.44, 158.21, 157.88, 155.47, 149.48, 140.24, 129.67, 129.54, 126.10, 124.38, 120.94, 119.01, 117.76, 112.86, 112.10, 109.25, 63.20, 55.01, 53.60, 52.77, 48.87, 41.29, 22.47, 13.25; LC/MS ($M+H^+$) 464.10; HRMS (ESI-Orbitrap) Calcd for $C_{26}H_{34}N_5O_3$ [$M+H^+$]:464.2662, Found 464.2655.



3-(2-((2-(Dimethylamino)ethyl)(methyl)amino)-4-(1H-pyrazol-4-yl)phenyl)-1-ethyl-1-(3-methoxybenzyl)urea (**1z**): 44% yield in four steps. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.42 (s, br, 1H), 8.19 (s, 1H), 8.05 (s, 2H), 7.50-7.48 (m, 1H), 7.43-7.42 (m, 1H), 7.34-7.30 (m, 2H), 6.89-6.86 (m, 3H), 4.58 (s, 2H), 3.76 (s, 3H), 3.47 (q, *J*=6.8 Hz, 2H), 3.41 (t, *J*=6.4 Hz, 2H), 3.33 (t, *J*=6.4 Hz, 2H), 3.03 (s, 3H), 3.02 (s, 3H), 2.54 (s, 3H), 1.15 (t, *J*=6.8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.49, 158.36, 158.03, 155.57, 143.22, 140.14, 132.20, 129.73, 129.42, 121.31, 120.91, 118.89, 117.66, 114.96, 112.73, 112.16, 55.03, 53.78, 49.09, 47.56, 44.05, 42.13, 41.61, 13.39; LC/MS ($M+H^+$) 451.06; HRMS (ESI-Orbitrap) Calcd for $C_{25}H_{35}N_6O_2$ [$M+H^+$]:451.2821, Found 451.2805.

1.2 Purity of compounds 1a-1z.

cmpd	RT[a] min.	Purity,% 215 nm	Purity,% 230 nm	Purity,% 254 nm	Purity,% 280 nm	Purity,% 310 nm
1a	6.7	100	100	100	100	nd[b]
1b	6.5	100	100	100	100	100
1c	6.9	100	100	100	100	100
1d	6.6	100	100	100	100	100
1e	7.2	100	100	100	100	100
1f	4.8	100	100	100	100	nd[b]
1g	7.3	100	100	100	100	100
1h	6.8	100	100	100	100	100
1i	6.6	100	100	100	100	nd[b]
1j	6.3	100	100	100	100	100
1k	6.4	100	100	100	100	100
1l	6.5	100	100	100	100	100
1m	6.4	100	100	100	100	100
1n	6.1	100	100	100	100	100
1o	7.1	97	100	100	100	100
1p	7.4	100	100	100	100	100
1q	7.1	100	100	100	100	100
1r	6.1	98	95	100	100	nd[b]
1s	6.1	100	100	100	100	100
1t	6.2	100	100	100	100	100
1u	6.3	100	100	100	100	100
1v	6.6	100	100	100	100	100
1w	6.6	100	100	100	100	100
1x	6.6	100	100	100	100	100
1y	6.9	97	100	100	100	100
1z	6.9	100	100	100	100	100

[a] Retation time; [b]Not determined.

2. Biological tests

Biochemical Screening

All experiments were performed in Greiner FIA black 384-well low volume plate. All active enzymes but ROCK-II 1-543 which was cloned and purified as described in ref.8a were purchased from Upstate. Test compounds were dispensed in 90% DMSO/10% water using a 384 head oppline Pintool system (GNF Systems).

2.1 ROCK-I/II biochemical assays

Assays were performed using the STK2 kinase system from Cisbio. A 5 µL mixture of a 1µM STK2 substrate and ATP (ROCK-I: 4 µM, ROCK-II: 20 µM) in STK-buffer was added to the wells using a BioRAPTR FRD^{TM} Workstation (Aurora Discovery). 20 nL of test compounds was dispensed. Reaction was started by the addition of 5 µL of 2.5 nM ROCK-I (Upstate #14-601) or 0.5 nM ROCK-II in STK-buffer. After 4h at RT the reaction was stopped by addition of 10 µL of 1× antibody and 62.5 nM Sa-XL in detection buffer. After 1h at RT the plates were read on the Viewlux in HTRF mode.

2.2 PKA biochemical assay

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 FRDTM Workstation (Aurora Discovery). 20 nL of test compounds was dispensed. Reaction was started by addition of 5 µL of 0.5 nM PKA (Upstate #14-440) in Kinase buffer (5 µL of kinase buffer for high wells). After 70 min at RT the reaction was stopped by addition of 10 µL Kinase-Glo reagent and plate was read after 10 min incubation time at RT on the Viewlux in luminescene mode.

2.3 MRCKα biochemical assay

K_m for ATP and S6-peptide (LCD-AKRRRLSSLRA-NH_2) were determined by titrating various concentrations of ATP versus a constant concentration of peptide and vice versa using radioactive filter binding assays. S6-peptide, MRCKα and ATP were mixed and reaction was started by addition of ATP and 1 µCi of ^{33}P-ATP/data point. After

indication time point aliquots were removed and reaction was stopped by 1 volume of 100 nM EDTA and 15 mm PPi. Reaction was transferred to Millipore HTS PH plates and incubated for 5 min at rt, washed 5 times with 100 mM phosphoric acid (1.15%). 50 uL/well scintillation cocktail was added and incorporation of radioactive phosphate was measured. For determination of IC_{50}s the determined biochemical parameters were used in a Kinase-Glo assay. As a final condition 5 µl mixture of a 40 µM S6-peptide (LCB-AKRRRRLSSLRA-NH_2) and 10 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^{TM} Workstation (Aurora Discovery). 20 nL of test compounds was dispensed. Reaction was stated by addition of 5 µL of 12 nM MRCK (Upstate #14-691) in Kinase buffer (5 µL of kinase buffer for high wells). After 75 min at rt the reaction was stopped by addition of 10 µL Kinase-Glo reagent and plate was read after 10 min incubation time at rt on the Viewlux in luminescence mode.

2.4 JNK3 and p38 biochemical assays:

Enzyme inhibition studies were performed in 384-well polystyrene HTRF plates (Grainier). For JNK3 incubations were performed for 15 min at ambient temperature (~22 °C) with 0.2 µM biotinylated Flag-ATF2, 1 µM ATP, 0.3 nM activated JNK3α1 (with a control in the absence of kinase to determine the basal signal). For p38 incubations were performed for 30 min at ambient temperature (~22 °C) with, 0.4 µM biotinylated Flag-ATF2, 10 µM ATP, 0.125 nM activated p38 (with a control in the absence of kinase to determine the basal signal). The reactions were carried out in 10 µL volumes containing the final buffer concentrations; 50 mM Hepes pH 7.0, 2.5 mM $MgCl_2$, 0.1 mg/mL bovine serum albumin, 1mM DL-dithiothreitol, 0.01% Triton X-100 and 5% DMSO (all from Sigma-Aldrich). A 10 point titration of all compounds was carried out in 3-fold dilutions from 2 µM – 10 pM. After the allotted time the kinase reaction was terminated by the addition 10 µL of quenching solution [50 mM Hepes, pH 7.0 with 14 mM EDTA, 0.01% Triton X-100, 200 Mm KF (all from Sigma-Aldrich)]. The detection reagents, streptavidin-xl;-APC (400 nM) and europium cryptate-labeled rabbit polyclonal anti-phospho-ATF2 (0.43 ng/well) were purchased from CisBio. The HTRF signal was

detected using a viewlux plate reader (PerkinElmer) 1h post-quenching. IC_{50} values were determined by fitting the data to the equation for a four-parameter logistic.

2.5 ppMLC cell-based assays (In Cell Western – ppMLC phosphorylation with A7r5 cells):

1) A7r5 cells are maintained in 20% FBS supplemented DMEM at 5% CO2, 37C in a humid environment. Only splitting 1:2 every 3 days.
2) A7r5 cells are plated in clear-bottomed Packard View plates at 5,000 cells/well and allowed to attach overnight.
3) Next day, serum starve in 0.05% FBS DMEM x 4hours.
4) Cells are treated with compound x 1hour (13333nM – 18nM final concentration) from 384 well plate containing 2mM-0.9uM compound or 20% DMSO for controls.
5) Cells are treated with 10uM LPA x 10 min.
6) Stain for PP-MLC (Cell Signalling #), followed by gαrIR 800 (LICOR #) and To Pro 3-iodide (Invitrogen #)
    - Fix cells in 4% paraformaldehyde in PBS x 25min at room temperature
    - Gently remove; wash 1x with 0.1M glycine
    - Permeate with 0.2% Triton-X x 20min
    - Wash 1x PBS
    - Block with Licor Blocking Buffer (1:1 in PBS) x1.5 hr rocking RT
    - Incubate with primary antibody o/n at 4C rocking: PPMLC (1:200) in Licor Blocking buffer
    - Next day, wash 2x PBST, 1X Licor-T
    - wash 1x in licor blocking buffer + 0.05% Tween 20
    - Incubate with secondary antibody x 1hr rocking at RT: gαrIR 800 (1:500) in Licor blocking buffer + 0.05% Tween (may want to 1:1 Licor buffer/Licor-T buffer)
    - Wash 2x PBST, 1X Licor-T
    - incubate with To Pro (1:4000) x 30 min RT (no rocking) in Licor buffer
    - Wash 2x PBS
7) Remove PBS completely before reading plate on Odyssey LICOR Infrared Scanner

8) Read both the 800 and 700 channels, taking the RAW data.
9) Determine %Phosphorylation normalized to nuclei fluorescence.

3. Pharmacokinetics

Pharmacokinetics studies were conducted in Sprague Dawley rats. The compound was formulated in a generic formulation at 1 mg/mL (e.g. 10:10:80, DMSO:Tween 80:water, v:v:v) and dosed at 1 mg/kg intravenous into the femoral vein or 2 mg/kg by oral gavage. Blood was obtained at t = 5 min, 15 min, 30 min, 1h, 2h, 4h, 6h, and 8h. Blood was collected into EDTA containing tubes and plasma was generated by standard centrifugation methods. All procedures and handling were according to standard operating procedures approved by IACUC at Scripps Florida.

In order to assess in vivo pharmacokinetic parameters an LC-MS/MS bioanalytical method was developed where 25 µL of plasma was treated with 125 µL of acetonitrile containing an internal standard in a Millipore Multscreen Slovinter 0.45 micron low binding PTFE hydrophilic filter plate (#MSRLN0450) and allowed to shake at room temperature for five minute. The plate was then centrifuged for 5 minutes at 4000 rpm in a tabletop centrifuge and the filtrate was collected in a polypropylene capture plate. The filtrate (10 µL) is injected using an Agilent 1200 HPLC equipped with a Thermo Betasil C18 HPLC column 5 µ (50 × 2.1 mm) #70105-052130. Mobile Phase A was water with 0.1% formic acid. Mobile phase B was acetonitrile with 0.1% formic acid. Flow rate was 375 µL/min using a gradient of 90% A/10% B from 0 - 0.5 min, ramped to 5%A95%B at 2 min, held at 5%A95%B until 3 min, ramped to 90% A/10% B at 4 min, and held at 90% A10% B until 7 min.

An API Sciex 4000 equipped with a turbo ion spray source was used for all analytical measurement. MRM methods were developed in position ion mode. Peak areas of the analyte ion were measured against the peak areas of the internal standard. Data was fit using WinNonLin using an IV bolus model.

3.1 Cytochrome P450s inhibition

P450s inhibition for four major isoforms are evaluated using a cocktail inhibition assay where the metabolism of specific marker substrate (CYP1A2 phenaceten demethylation

to acetaminophen); CYP2C9, tolbutamide hydroxylation to hydrocytolbutamide; CYP2D6, bufuralol hydroxylation to 4'-Hydroxybufuralol; and CYP3A4, midazolam hydroxylation to 1'-Hydroxymidazolam) in the presence or absence of 10 μM probe compound is evaluated. The concentration of each marker substrate is approximately its Km. Conditions were similar to those described by Tesino and Patonay (Testino and Patonay, 2003) except 2C19 was not evaluated as we found that stock solution of the 2C19 probe substrate, omeprazole, had poor stability. Specific inhibitors for each isoform are included in each run to validate the system.

3.2 Solubility

The solubility of compounds was tested at pH 5.5 which is near the lower end of the acceptable pH range for ophthalmic dosing. Compounds were inverted for 24 hours in test tubes containing 1-2 mg of compound with 1 mL of pH 5.5 potassium phosphate buffer. The samples were centrifuged and analyzed by HPLC (Agilent 1100 with diode-array detector). Peak area will be compared to a standard of known concentration.

3.3 Porcine corneal penetration

Porcine corneal penetration was estimated using freshly isolated porcine corneas. A Teflon three chamber dialysis chamber (Harvard apparatus) was used where drug in phosphate buffer, pH 7.4, was added to the center chamber. Instead of using dialysis membranes, porcine cornea was used to separate to three chambers. Permeability was calculated by determining the diffusion of drug from the center chamber into the two end chambers over time.

3.4 IOP in rat model

An elevated rat IOP model was used to evaluate the IOP lowering effects of compounds. Initial IOP was 29 mm Hg. The IOP was measured in Brown Norway rats that were housed in constant low-light conditions to reduce circadian IOP changes. Measurements were made using a rebound tonometer with n = 7 in the vehicle and drug treated groups. The reported IOP at each time point is the average of five readings. Compounds were formulated in 50 mM citrate buffer, pH 5.5 w:v.

Counterscreen data for selected compounds:

Table S1. Selectivity over other kinases and cytochrome P450 isoforms

cmpd	% inh. at 10 μM	IC_{50} (nM)[a]			
	1A2/2C9/2D6/3A4	MRCKα	JNK3	p38	ROCK-I
1x	13/73/77/59	nd[b]	>20000	nd[b]	26
1y	3/54/69/61	1198	>20000	>20000	15
1z	0/49/80/52	1892	>20000	>20000	55

[a] IC_{50} values were means of 2 or more experiments with errors within 40% of the mean.
[b] Not determined.

Rat pharmacological data for selected compounds:

Table S2. Rat pharmacokinetics of selected compounds[a]

cmpd	Cl[b] (mL/min/kg)	Vd[b] (L/kg)	$t_{1/2}$[b] (h)	AUC[b] (μM*h)	C_{max}[c] (nM)	F[c] (%)
1r	94	4.8	1.2	0.4	23	6
1u	13	1.2	1.3	3.1	<10	0
1x	27	1.6	1.1	1.4	39	2
1y	60	3.6	0.7	0.6	<10	0
1z	34	2.4	1.2	1.1	45	2

[a] Data reported were the mean of 3 determinations and the standard error is within 30% of the mean.
[b] iv, 1mg/kg. [c] po, 2 mg/kg.