Discovery of a Type III Inhibitor of LIM Kinase 2 that Binds in a DFG-out Conformation

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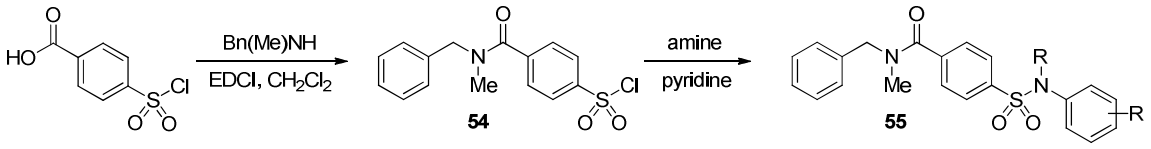
Contents:

Chemical Methods. All reactions were conducted under a static atmosphere of argon or nitrogen and stirred magnetically unless otherwise noted. Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. Flash column chromatography was carried out using pre-packed silica gel columns from Biotage or ISCO, or by slurry preparation using EMD silica gel 60 (particle size 0.040-0.063 mm). ^{1}H and ^{13}C NMR spectra were collected on Bruker ARX300, DRX400 or DPX400, or Varian Mercury 400 MHz NMR spectrometers. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane in δ-units, and coupling constants (*J*-values) are given in hertz (Hz). Data are reported in the following format: chemical shift, multiplicity, coupling constants, and assignment. Reactions were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F_{254}) and were visualized with UV light. Analytical HPLC spectra were collected on Shimadzu HPLC systems equipped with a UV detector measuring absorbance at 220 and 254 nm. Mass spectra were obtained on Waters ZQ or ZMD LCMS systems equipped with an auto-sampler, and ELSD detector, a UV detector measuring absorbance at 220 and 254 nm, and a mass detector. High resolution mass spectra were obtained on a Waters LCT Premier XE Micromass® MS Technologies instrument equipped with an auto-sampler. Elemental analysis was conducted by Robertson Microlit Laboratory, Madison, NJ.

1. Experimental Methods

1.1 Synthesis and characterization of substrates

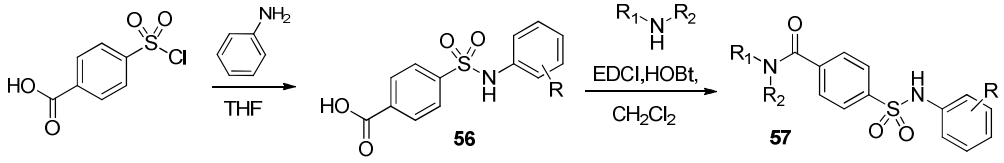
1.1.1 General Method A for Preparation of Sulfonamides



Preparation of 4-(benzyl(methyl)carbamoyl)benzene-1-sulfonyl chloride (54): 4-(Chlorosulfonyl) benzoic acid (1.0 equiv., 4.54 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.8 equiv.) were stirred in CH_2Cl_2 (0.5 M) for 2 hours. Substituted anilines (1 equiv.) were added to the solution at room temperature over 30 minutes and stirred for 3 hours. The crude reaction was diluted with CH_2Cl_2 and washed once with H_2O and dried over $MgSO_4$. Solvent was removed in vacuo to give product which was carried directly onto the next step.

Preparation of 4-(N-arylsulfamoyl)benzamides (55): Pyridine (0.5 equiv.) was added to a stirring solution of 4-(benzyl(methyl)carbamoyl)benzene-1-sulfonyl chloride (**54**, 1.2 equiv.) and the corresponding amine (1.0 equiv.) in CH_2Cl_2 (3 mL) and the reaction was stirred overnight. The crude reaction was concentrated in vacuo and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to give the desired benzamides **55**.

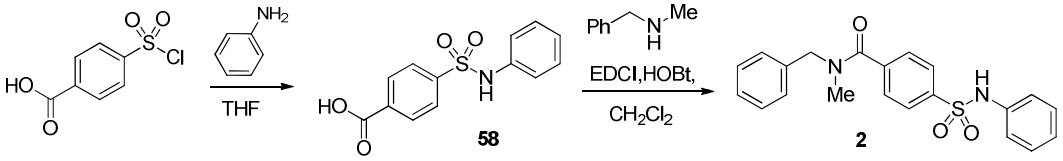
1.1.2 General Method B for Preparation of Sulfonamides



Preparation of 4-(*N*-arylsulfamoyl)benzoic acid (56): Substituted aniline (10 equiv) was added dropwise to a stirring solution of 4-(chlorosulfonyl)benzoic acid (1 equiv.) in THF (0.1M) at room temperature and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 and washed three times with 1*N* HCl, once with brine, and dried over $MgSO_4$. The organic layer was concentrated under vacuum and crystallized from a hot methanol/H_2O mixture to give benzoic acid **#.**

Preparation of 4-(*N*-arylsulfamoyl)benzamides (57): Benzoic acid # (1 equiv., 0.18 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.2 equiv.), and *N*-hydroxybenzotriazole (1 equiv.) are stirred in CH_2Cl_2 for 1 hour. The amine (1.5 equiv.) was added to the solution at room temperature and stirred overnight. The crude reaction was concentrated under vacuum and directly purified via silica gel column chromatography.

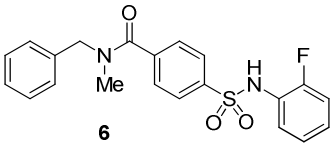
1.1.3 Procedure and characterization sulfonamide 2.



Preparation of 4-(*N*-phenylsulfamoyl)benzoic acid (58): Aniline (8.26 mL, 90.6 mmol) was added dropwise to a stirring solution of 4-(chlorosulfonyl)benzoic acid (2.0 g, 9.06 mmol) in THF (100 mL) at room temperature and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed three time with 1*N* HCl, once with brine, and dried over $MgSO_4$. The organic layer was concentrated under vacuum and crystallized from a hot methanol/H_2O mixture to give 4-(*N*-phenylsulfamoyl)benzoic acid (2.2 g, 7.93 mmol, 88% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.42 - 2.48 (m, 5 H) 3.33 (br. s., 79 H) 6.96 - 7.06 (m, 5 H) 7.15 - 7.21 (m, 3 H) 7.77 - 7.82 (m, 3 H) 7.98 - 8.03 (m, 3 H) 10.38 (s, 2 H); MS (ES+) $[M+H]^+ = 276$.

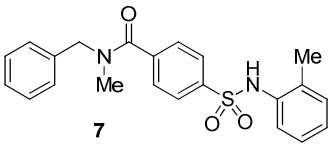
Preparation of *N*-benzyl-*N*-methyl-4-(N-phenylsulfamoyl)benzamide (2): 4-(*N*-Phenylsulfamoyl) benzoic acid (**58**, 0.05 g, 0.18 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.041 g, 0.216 mmol), and hydroxybenzotriazole (0.024 g, 0.18 mmol) are stirred in CH_2Cl_2 (5 mL) for 1 hour. *N*-Methylbenzyl amine (35 uL, 0.27 mmol) was added to the solution at room temperature and stirred overnight. The crude reaction was concentrated under vacuum and directly purified via silica gel chromatography. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.82, 3.04 (br. s., 3 H) 4.42, 4.75 (br. s., 2 H) 7.01 - 7.15 (m, 4 H) 7.08 (d, *J*=16.10 Hz, 2 H) 7.16 - 7.27 (m, 2 H) 7.29 - 7.44 (m, 4 H) 7.48 - 7.63 (m, 2 H) 7.75 - 7.97 (m, 2 H); MS (ES+) [M+H]+ = 380.

1.1.4 Synthesis and characterization sulfonamides 6-18 in table 1.



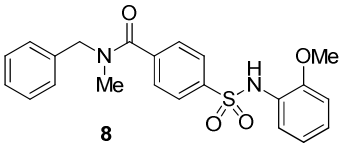
6

Preparation of *N*-benzyl-4-(*N*-(2-flurorophenyl)sulfamoyl)-*N*-methylbenzamide (6). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.84, 3.05 (s, 3 H) 4.44, 4.76 (s, 2 H) 6.81 - 7.03 (m, 1 H) 7.06 - 7.23 (m, 3 H) 7.28 - 7.40 (m, 4 H) 7.42 - 7.50 (m, 1 H) 7.51 - 7.64 (m, 2 H) 7.73 - 7.97 (m, 2 H); MS (ES+) [M+H]+ = 399.



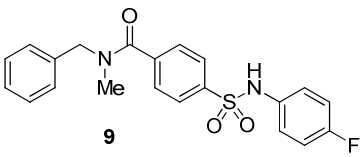
7

Preparation of *N*-benzyl-*N*-methyl-4-(*N*-(*o*-tolyl)sulfamoyl)benzamide (7). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 1.95, 2.04 (s, 3 H) 2.86, 3.06 (s, 3 H) 4.48, 4.78 (s, 2 H) 6.95 - 7.21 (m, 5 H) 7.27 - 7.45 (m, 4 H) 7.53 - 7.62 (m, 2 H) 7.68 - 7.85 (m, 2 H); MS (ES+) [M+H]+ = 395.



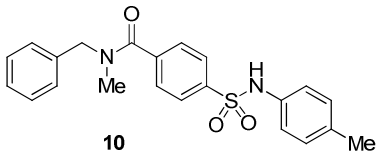
8

Preparation of *N*-benzyl-4-(*N*-(2-methoxyphenyl)sulfamoyl)-*N*-methylbenzamide (8). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.83, 3.03 (s, 3 H) 3.36, 3.53 (s, 3 H) 4.44, 4.76 (s, 2 H) 6.67 - 6.95 (m, 2 H) 7.05 - 7.22 (m, 2 H) 7.28 - 7.47 (m, 5 H) 7.49 - 7.59 (m, 2 H) 7.67 - 7.85 (m, 2 H); MS (ES+) [M+H]+ = 411/



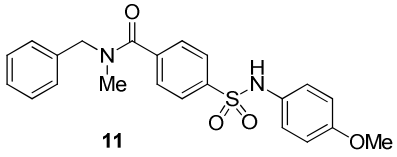
9

Preparation of *N*-benzyl-4-(*N*-(4-fluorophenyl)sulfamoyl)-*N*-methylbenzamide (9). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.77, 2.98 (s, 3 H) 4.37, 4.69 (s, 2 H) 6.81 - 7.11 (m, 5 H) 7.19 - 7.38 (m, 4 H) 7.44 - 7.66 (m, 2 H) 7.66 - 8.00 (m, 2 H). MS (ES+) [M+H]+ = 399.



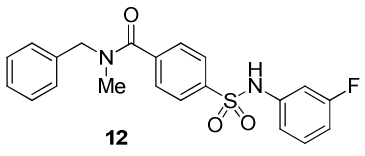
10

Preparation of *N*-benzyl-*N*-methyl-4-(*N*-(*p*-tolyl)sulfamoyl)benzamide (10). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.13, 2.18 (s, 3 H) 2.76, 2.97 (s, 2 3) 4.35, 4.69 (s, 2 H) 6.80 - 7.11 (m, 5 H) 7.18 - 7.35 (m, 4 H) 7.40 - 7.55 (m, 2 H) 7.64 - 7.84 (m, 2 H); MS (ES+) [M+H]+ = 395.



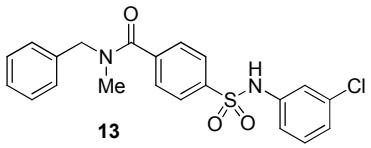
11

Preparation of *N*-benzyl-4-(*N*-(4-methoxyphenyl)sulfamoyl)-*N*-methylbenzamide (11). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.77, 2.98 (s, 3 H) 3.62, 3.66 (s, 3 H) 4.37, 4.69 (s, 2 H) 6.61 - 6.75 (m, 2 H) 6.81 - 6.96 (m, 2 H) 7.02 - 7.13 (m, 1 H) 7.21 - 7.36 (m, 4 H) 7.48 (dd, *J*=13.34, 8.05 Hz, 2 H) 7.60 - 7.77 (m, 2 H); MS (ES+) [M+H]+ = 411.



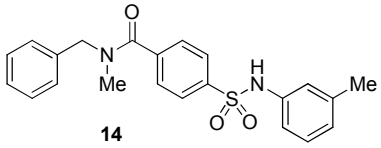
12

Preparation of *N*-benzyl-4-(*N*-(3-flurorophenyl)sulfamoyl)-*N*-methylbenzamide (12). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.83, 3.05 (s, 3 H) 4.43, 4.76 (s, 2 H) 6.71 - 7.00 (m, 3 H) 7.06 - 7.25 (m, 2 H) 7.28 - 7.42 (m, 4 H) 7.52 - 7.65 (m, 2 H) 7.80 - 7.97 (m, 2 H); MS (ES+) [M+H]+ = 399.



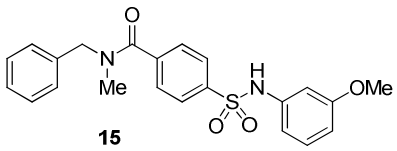
13

Preparation of *N*-benzyl-4-(*N*-(3-chlorophenyl)sulfamoyl)-*N*-methylbenzamide (13). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.76, 2.98 (s, 3 H) 4.36, 4.69 (s, 2 H) 6.92 - 7.19 (m, 5 H) 7.21 - 7.36 (m, 4 H) 7.46 - 7.59 (m, 2 H) 7.69 - 7.88 (m, 2 H); MS (ES+) [M+H]+ = 415.



14

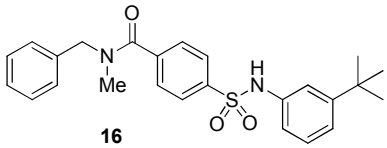
Preparation of *N*-benzyl-*N*-methyl-4-(*N*-(*m*-tolyl)sulfamoyl)benzamide (14). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.11, 2.17 (s, 3 H) 2.75, 2.96 (s, 3 H) 4.35, 4.68 (s, 2 H) 6.75 - 6.89 (m, 3 H) 6.91 - 7.09 (m, 2 H) 7.18 - 7.35 (m, 4 H) 7.42 - 7.56 (m, 2 H) 7.66 - 7.88 (m, 2 H); MS (ES+) [M+H]+ = 395.



15

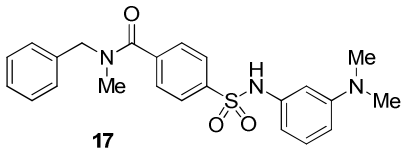
Preparation of *N*-benzyl-4-(*N*-(3-methoxyphenyl)sulfamoyl)-*N*-methylbenzamide (15). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers*

observed) δ ppm 2.82, 3.03 (s, 3 H) 3.65, 3.71 (s, 3 H) 4.41, 4.75 (s, 2 H) 6.53 - 6.73 (m, 3 H) 7.01 - 7.18 (m, 2 H) 7.26 - 7.39 (m, 4 H) 7.49 - 7.65 (m, 2 H) 7.77 - 7.96 (m, 2 H); MS (ES+) [M+H]+ = 411.



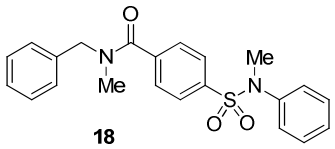
16

Preparation of *N*-benzyl-4-(*N*-(3-(*tert*-butyl)phenyl)sulfamoyl)-*N*-methylbenzamide (16). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 1.16, 1.23 (s, 9 H) 2.82, 3.02 (s, 3 H) 4.41, 4.75 (s, 2 H) 6.87 - 6.98 (m, 1 H) 7.03 - 7.20 (m, 4 H) 7.26 - 7.42 (m, 4 H) 7.50 - 7.62 (m, 2 H) 7.73 - 7.89 (m, 2 H); MS (ES+) [M+H]+ = 437.



17

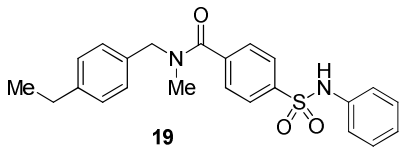
Preparation of *N*-benzyl-4-(*N*-(3-(dimethylamino)phenyl)sulfamoyl)-*N*-methylbenzamide (17). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.77, 2.85 (s, 6 H) 2.82, 3.03 (s, 3 H) 4.41, 4.75 (s, 2 H) 6.36 - 6.51 (m, 3 H) 6.91 - 7.05 (m, 1 H) 7.11 (d, *J*=7.06 Hz, 1 H) 7.30 - 7.44 (m, 4 H) 7.48 - 7.61 (m, 2 H) 7.74 - 7.95 (m, 2 H); MS (ES+) [M+H]+ = 424.



18

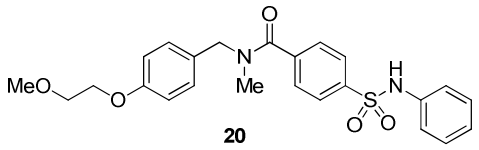
Preparation of *N*-benzyl-*N*-methyl-4-(*N*-methyl-*N*-phenylsulfamoyl)benzamide (18). Title compound was synthesized according to general method A. ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.88, 3.07 (s, 3 H) 3.20, 3.23 (s, 3 H) 4.50, 4.78 (s, 2 H) 7.02 - 7.22 (m, 3 H) 7.22 - 7.43 (m, 7 H) 7.53 - 7.71 (m, 4 H); MS (ES+) [M+H]+ = 395.

1.1.5 Synthesis and characterization sulfonamides 19-30 in table 2.



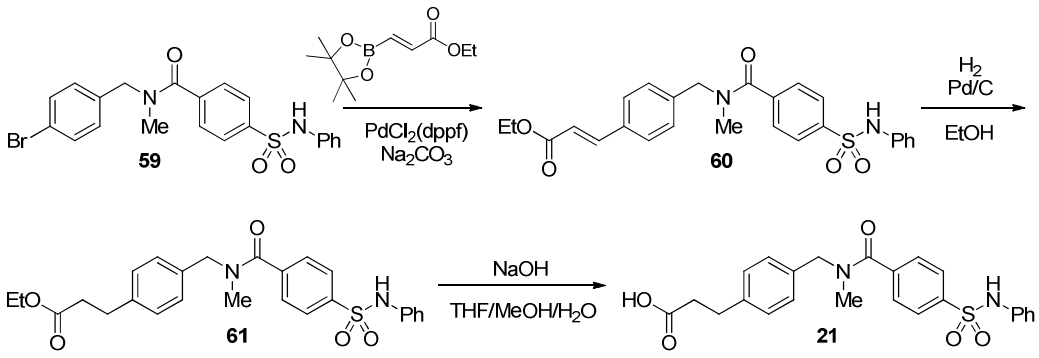
19

Preparation of *N*-(4-ethylbenzyl)-*N*-ethyl-4-(*N*-phenylsulfamoyl)benzamide (19). Prepared by general method B to give product (52 mg, 73% yield) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.25 (t, *J*=7.61 Hz, 3 H) 2.67 (q, *J*=7.64 Hz, 2 H) 2.79, 3.06 (s, 3 H) 4.37, 4.71 (s, 2 H) 6.53, 6.56 (br. s., 1 H) 6.99 - 7.10 (m, 3 H) 7.12 - 7.28 (m, 6 H) 7.49 - 7.53 (m, 2 H) 7.76, 7.80 (d, *J*=7.94 Hz, *J*=7.94 Hz 2 H); MS (ES+) $[M+H]^+$ = 409.



20

Preparation of *N*-(4-(2-methoxyethoxy)benzyl)-*N*-methyl-4-(*N*-phenylsulfamoyl)benzamide (20). Prepared by general method B to give product (60 mg, 64% yield). ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 2.70, 2.97 (s, 1 H) 3.41 (s, 3 H) 3.67 - 3.77 (m, 2 H) 4.07 (br. s., 2 H) 4.26, 4.61 (s, 2 H) 6.56 (br. s., 1 H) 6.81 - 7.12 (m, 6 H) 7.19 (m, 2 H) 7.42 (d, *J*=8.38 Hz, 2 H) 7.71 (dd, *J*=12.35, 8.16 Hz, 2 H); MS (ES+) $[M+H]^+$ = 455.



Preparation of *N*-(4-bromo-benzyl)-*N*-methyl-4-phenylsulfamoyl-benzamide (59). Starting with 4-phenylsulfamoyl benzoic acid (**58**, 200 mg, 0.72 mmol) and following general method B, the title compound was isolated as a yellowish solid (167 mg, 50% yield). ^{1}H NMR (400 MHz, Chloroform-*d,*

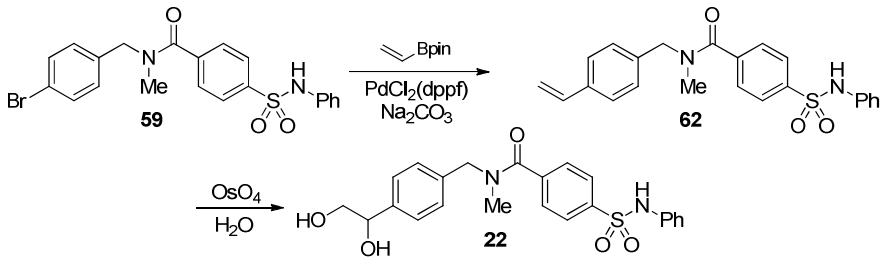
rotamers observed) δ ppm 2.81, 3.06 (s, 3 H) 4.36, 4.70 (s, 2 H) 6.98 - 7.16 (m, 5 H) 7.22 - 7.28 (m, 3 H) 7.47 - 7.52 (m, 4 H) 7.69-7.87 (m, 2 H); MS (ES+) [M+H] = 459.

Preparation of (*E*)-3-(4-{[methyl-(4-phenylsulfamoyl-benzoyl)-amino]-methyl}-phenyl)-acrylic acid ethyl ester (60). *N*-(4-Bromo-benzyl)-*N*-methyl-4-phenylsulfamoylbenzamide (**59**, 71 mg, 0.16 mmol), (*E)*-3-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-acrylic acid ethyl ester (42 mg, 0.19 mmol), $PdCl_2$(dppf) (8 mg, 6 mol%) and Na_2CO_3 (36 mg, 0.34 mmol) were combined in THF/H_2O (4:1, 2.5 mL) and heated in the microwave at 120 ^oC for 10 minutes. The mixture was filtered and concentrated. Purification by flash chromatography (40% EtOAc/hexanes) afforded the desired compound (15 mg, 17% yield). ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.36 (t, *J* = 7.20 Hz, 3 H), 2.83, 3.08 (s, 3 H), 4.28 (q, J = 7.20 Hz, 2 H), 4.43, 4.76 (s, 2 H), 6.45, (d, *J* = 16 Hz, 1 H), 6.70 (br. s., 1 H), 7.02 - 7.17 (m, 5 H) 7.25 - 7.38 (m, 4 H) 7.51 - 7.55 (m, 2 H) 7.67-7.83 (m, 3 H); MS (ES+) [M+H] = 479.

Preparation of 3-(4-{[methyl-(4-phenylsulfamoylbenzoyl)-amino]-methyl}-phenyl)-propionic acid ethyl ester (61). (*E*)-3-(4-{[Methyl-(4-phenylsulfamoyl-benzoyl)-amino]-methyl}-phenyl)-acrylic acid ethyl ester (**60**, 15 mg, 0.032 mmol) was dissolved in EtOH (2 mL) and Pd/C (10wt%, 50% wet, ~1 mg, 5 mol%) was added. The mixture was placed under hydrogen at atmospheric pressure and stirred at room temperature for 45 minutes. The mixture was filtered through Celite, and concentrated and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to afford the desired compound (13 mg, 85% yield). ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.26 (t, *J* = 7.20 Hz, 3 H) 2.63 (t, *J* = 7.6 Hz, 2 H) 2.79, 3.06 (s, 3 H) 3.03 (t, *J* = 7.60 Hz, 2 H) 4.14 (q, *J* = 7.20 Hz, 2 H) 4.37, 4.71 (s, 2 H) 6.88 (br. s., 1 H) 7.02 - 7.16 (m, 5 H) 7.20 - 7.37 (m, 4 H) 7.48 - 7.50 (m, 2 H) 7.75-7.81 (m, 2 H); MS (ES+) [M+H] = 481.

Preparation of 3-(4-((*N*-methyl-4-(*N*-phenylsulfamoyl)benzamido)methyl)phenyl)propanoic acid (21). 3-(4-{[Methyl-(4-phenylsulfamoyl-benzoyl)-amino]-methyl}-phenyl)-propionic acid ethyl ester (**61**, 12 mg, 0.025 mmol) was dissolved in THF/MeOH (1:1 v:v, 2 mL) and 1M NaOH (0.5 mL) added. The mixture was stirred at room temperature for 40 minutes, concentrated and purified by prep HPLC

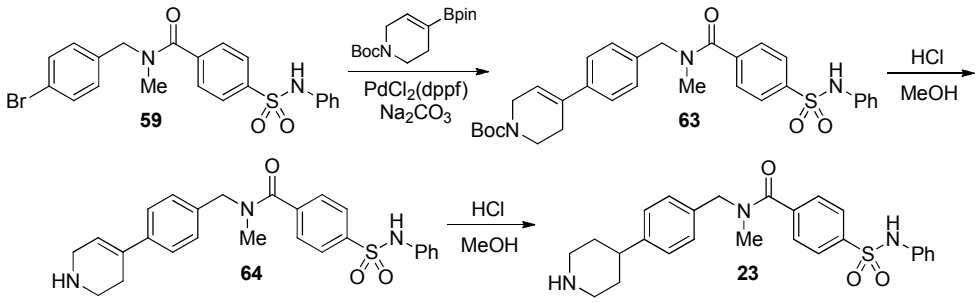
(30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to afford the desired compound (3 mg, 26% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.47 - 2.59 (m, 2 H) 2.80, 3.03 (s, 3 H) 2.87 - 2.97 (m, 2 H) 4.37, 4.71 (s, 2 H) 6.95 - 7.13 (m, 4 H) 7.15 - 7.34 (m, 5 H) 7.54 (dd, *J*=17.43, 8.08 Hz, 2 H) 7.75 - 7.90 (m, 2 H); MS (ES+) $[M+H]^+ = 453$.



Preparation of *N*-methyl-4-phenylsulfamoyl-*N*-(4-vinyl-benzyl)-benzamide (62). *N*-(4-Bromo-benzyl)-*N*-methyl-4-phenylsulfamoyl-benzamide (**59**, 35 mg, 0.076 mmol), 4,4,5,5-tetramethyl-2-vinyl[1,3,2]dioxaborolane (14 mg, 0.091 mmol), $PdCl_2$(dppf) (4 mg, 6 mol%) and Na_2CO_3 (17 mg, 0.16 mmol) were combined in THF/H_2O (4:1 v:V, 2 mL) and heated in the microwave at 120 ^oC for 10 minutes. The mixture was filtered and concentrated. Purification by flash chromatography (20-40% EtOAc/hexanes) afforded the desired compound (12 mg, 39% yield). ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 2.80, 3.08 (s, 3 H) 4.40, 4.74 (s, 2 H) 5.28 (d, *J* = 10.0 Hz, 1 H) 5.78 (d, *J* = 17.6 Hz, 1 H) 6.75 (m, 1 H) 6.95 - 7.16 (m, 5 H) 7.24 - 7.35 (m, 3 H) 7.41 - 7.51 (m, 4 H) 7.70-7.81 (m, 2 H); MS (ES+) [M+H] = 407.

Preparation of *N*-(4-(1,2-dihydroxyethyl)benzyl)-*N*-methyl-4-(*N*-phenylsulfamoyl)benzamide (22). *N*-Methyl-4-phenylsulfamoyl-*N*-(4-vinylbenzyl)benzamide (**62**, 11 mg, 0.028 mmol) was dissolved in pyridine (1 mL) and OsO_4 (4 wt% solution in H_2O, 14 mg, 0.056 mmol) was added. The mixture was stirred at room temperature overnight. 10% aqueous $NaHSO_3$ (0.5 mL) was added and the mixture stirred for 2 hours. The mixture was filtered, concentrated and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to give product (5 mg, 41% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 2.82, 3.04 (s, 3

H) 3.63 (m, 2 H) 4.41 (s, 1 H) 4.66 - 4.75 (m, 2 H) 7.02 - 7.16 (m, 4 H) 7.16 - 7.27 (m, 2 H) 7.30 - 7.45 (m, 3 H) 7.49 - 7.62 (m, 2 H) 7.75 - 7.93 (m, 2 H); MS (ES+) $[M+H]^+ = 441$.

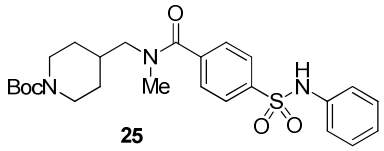


Preparation of 4-(4-{[methyl-(4-phenylsulfamoyl-benzoyl)-amino]-methyl}-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylic acid *tert*-butyl ester (63). *N*-(4-Bromo-benzyl)-*N*-methyl-4-phenylsulfamoylbenzamide (**59**, 60 mg, 0.13 mmol), 4-(4,4,5,5-tetramethyl-l[1,3,2]dioxaborolan-2-yl)-3,6-dihydro-2H-pyridine-1-carboxylic acid *tert*-butyl ester (49 mg, 0.16mmol), $PdCl_2$(dppf) (6 mg, 6 mol%) and Na_2CO_3 (29 mg, 0.27 mmol) were combined in THF/H_2O (4:1, 2.5 mL) and heated in the microwave at 120 ^oC for 10 minutes. The mixture was filtered and concentrated. Purification by flash chromatography (40% EtOAc/hexanes) afforded the desired compound (20 mg, 27% yield). ^1H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.26, 1.48 (s, 9 H) 2.54 (m, 2 H) 2.81, 3.07 (s, 3 H) 3.66 (m, 2 H) 4.13 (m, 2 H) 4.40, 4.74 (s, 2 H) 6.07, (m, 1 H) 6.73 (m, 1 H) 7.02 - 7.17 (m, 5 H) 7.25 - 7.38 (m, 4 H) 7.49 - 7.54 (m, 2 H) 7.75-7.82 (m, 2 H); MS (ES+) [M+H] = 562.

Preparation of *N*-methyl-4-phenylsulfamoyl-*N*-[4-(1,2.3.6-tetrahydro-pyridin-4-yl)-benzyl]-benzamide (64). 4-(4-{[Methyl-(4-phenylsulfamoyl-benzoyl)-amino]-methyl}-phenyl)-3,6-dihydro-2H-pyridine-1-carboxylic acid *tert*-butyl ester (**63**, 20 mg, 0.036mmol) was dissolved in MeOH (2 mL) and HCl (4M in dioxane, 4 uL, 0.1 mmol) was added. The mixture was stirred at room temperature overnight. The mixture was concentrated and taken on to the next step without purification. MS (ES+) [M+H] = 462

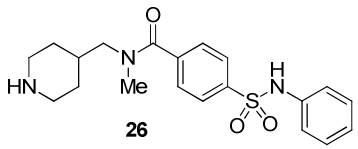
Preparation of *N*-Methyl-4-phenylsulfamoyl-*N*-(4-piperidin-4-yl-benzyl)-benzamide (23). The crude residue was dissolved in EtOH (1 mL) and Pd/C (50% wet, 2 mg, 10 wt%) was added. The mixture was placed under hydrogen at atmospheric pressure and stirred at room temperature for 30

minutes. The mixture was filtered through Celite, concentrated and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to give product (4 mg, 40% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 1.84 - 1.98 (m, 2 H) 2.05 - 2.12 (m, 2 H) 2.83, 3.03 (s, 3 H) 2.92 (br. s., 1 H) 3.08 - 3.23 (m, 2 H) 3.45 - 3.59 (m, 2 H) 4.41, 4.73 (s, 2 H) 6.97 - 7.14 (m, 4 H) 7.15 - 7.39 (m, 5 H) 7.46 - 7.61 (m, 2 H) 7.72 - 7.95 (m, 2 H); MS (ES+) $[M+H]^+$ = 464.



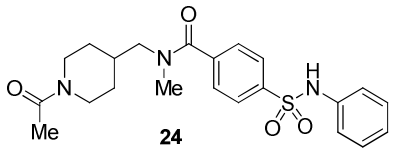
25

Preparation of t*ert*-butyl 4-((*N*-methyl-4-(*N*-phenylsulfamoyl)benzamido)methyl)piperidine-1-carboxylate (25). Prepared by general method B. ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 0.64 - 0.81 (m, 1 H) 1.19 - 1.27 (m, 1 H) 1.38, 1.41 (s, 9 H) 1.57 - 1.98 (m, 2 H) 2.49 - 2.75 (m, 2 H) 2.84 (s, 2 H) 2.95 - 3.02 (m, 1 H) 3.02 (s, 1 H) 3.32 - 3.41 (m, 1 H) 3.97 - 4.16 (m, 2 H) 6.48 - 6.56 (m, 1 H) 6.97 - 7.04 (m, 1 H) 6.97 - 7.04 (m, 2 H) 7.06 - 7.13 (m, 1 H) 7.15 - 7.21 (m, 2 H) 7.31 - 7.44 (m, 2 H) 7.65 - 7.79 (m, 2 H); MS (ES+) $[M+H]^+$ = 488.



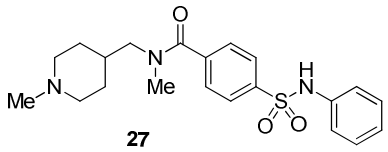
26

Preparation of *N*-methyl-4-(*N*-phenylsulfamoyl)-*N*-(piperidin-4-ylmethyl)benzamide (26). A solution of 4M HCl in dioxane (4 mL) was added to a stirring solution of *tert*-butyl 4-((*N*methyl-4-(*N*-phenylsulfamoyl)benzamido)methyl)piperidine-1-carboxylate (**25**, 139 mg, 0.285 mmol) in diethyl ether (20 mL) and stirred at room temperature for 3 days. The resulting solid was filtered and washed with diethyl ether to give product as the HCl salt (73 mg, 65% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 1.00 - 1.22 (m, 1 H) 1.43 - 1.87 (m, 2 H) 1.93 - 2.06 (m, 2 H) 2.09 - 2.29 (m, 1 H) 2.91 - 3.23 (m, 5 H) 3.40 - 3.57 (m, 3 H) 7.03 - 7.14 (m, 3 H) 7.17 - 7.31 (m, 2 H) 7.46 - 7.62 (m, 2 H) 7.86 (d, *J*=8.08 Hz, 2 H); MS (ES+) $[M+H]^+$ = 388.



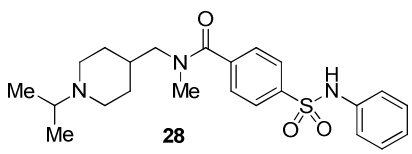
24

Preparation of *N*-((1-acetylpiperidin-4-yl)methyl)-*N*-methyl-4-(N-phenylsulfamoyl)benzamide (24). Acetic anhydride (0.25 mL) was added to a stirring solution of *N*-methyl-4-(*N*-phenylsulfamoyl)-*N*-(piperidin-4-ylmethyl)benzamide (**26**, 50 mg, 0.117 mmol) in pyridine (0.5 mL) at room temperature, and stirred overnight. The crude reaction was diluted with EtOAc and washed twice with H_2O, twice with 1N HCl, once with brine, and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was triturated with diethyl ether and filtered to give product. (19 mg, % yield). ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 0.62 - 0.86 (m, 1 H) 1.25 - 1.38 (m, 1 H) 1.57 (m, 1 H) 1.71 - 1.97 (m, 2 H) 2.49 - 2.75 (m, 1 H) 2.92, 3.10 (s, 3 H) 2.98 - 3.07 (m, 1 H) 3.11 - 3.20 (m, 1 H) 3.40 - 3.55 (m, 2 H) 3.40 - 3.55 (m, 2 H) 3.77 - 4.00 (m, 1 H) 4.32 - 4.52 (m, 1 H) 4.56 (s, 3 H) 7.02 - 7.15 (m, 3 H) 7.17 - 7.29 (m, 2 H) 7.43 - 7.59 (m, 2 H) 7.77 - 7.89 (m, 2 H); MS (ES+) $[M+H]^+$ = 430.



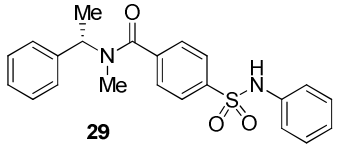
27

Preparation of *N*-methyl-*N*-((1-methylpiperidin-4-yl)methyl)-4-(N-phenylsulfamoyl)benzamide (27). Title compound synthesized according to general method B and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to give product (11 mg, 15% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 1.46 - 1.74 (m, 2 H) 1.80 - 2.13 (m, 3 H) 2.65 - 3.15 (m, 8 H) 3.37 - 3.50 (m, 3 H) 3.64 - 3.71 (m, 1 H) 6.93 - 7.07 (m, 3 H) 7.11 - 7.25 (m, 2 H) 7.41 - 7.54 (m, 2 H) 7.78 (d, *J*=8.38 Hz, 2 H); MS (ES+) $[M+H]^+$ = 402.



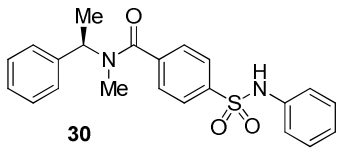
28

Preparation of *N*-((1-isopropylpiperidin-4-yl)methyl)-*N*-methyl-4-(N-phenylsulfamoyl)benzamide (28). Title compound synthesized according to general method B and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to give product (22 mg, 31% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *rotamers observed*) δ ppm 1.06 - 1.25, 1.53-1.70 (m, 2 H) 1.30, 1.37 (d, *J*=6.62 Hz, *J*=6.62 Hz 6 H) 1.77 - 2.27 (m, 3 H) 2.87 - 3.28 (m, 5 H) 3.44 - 3.60 (m, 4 H) 7.02 - 7.14 (m, 3 H) 7.17 - 7.27 (m, 2 H) 7.47 - 7.59 (m, 2 H) 7.85 (d, *J*=8.38 Hz, 2 H) ; MS (ES+) $[M+H]^+$ = 430.



29

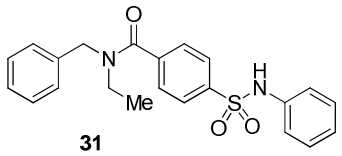
Preparation of (*S*)-*N*-methyl-*N*-(-1-phenethyl)-4-(*N*-phenylsulfamoyl)benzamide (29). Title compound synthesized according to general method B to give product (51 mg, 75% yield) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.53 - 1.64 (m, 3 H) 2.53, 2.85 (br. s., 3 H) 4.78 - 4.90, 6.10-6.20 (m, 1 H) 6.98 (s, 1 H) 7.04 - 7.09 (m, 2 H) 7.10 - 7.20 (m, 2 H) 7.21 - 7.27 (m, 2 H) 7.29 - 7.42 (m, 4 H) 7.49 (br. s., 2 H) 7.75 - 7.85 (m, 2 H); MS (ES+) $[M+H]^+$ = 395.



30

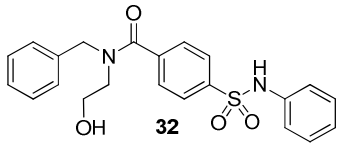
Preparation of (*R*)-*N*-methyl-*N*-(-1-phenethyl)-4-(*N*-phenylsulfamoyl)benzamide (30). Title compound synthesized according to general method B to give product (63 mg, 89% yield) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.60 (m, *J*=12.60 Hz, 3 H) 2.53, 2.85 (br. s., 3 H) 4.78 - 4.90, 6.15 (m, 1 H) 6.89 (s, 1 H) 7.04 - 7.10 (m, 2 H) 7.11 - 7.21 (m, 2 H) 7.21 - 7.27 (m, 2 H) 7.30 - 7.42 (m, 4 H) 7.44 - 7.59 (m, 2 H) 7.75 - 7.83 (m, 2 H); MS (ES+) $[M+H]^+$ = 395.

1.1.6 Synthesis and characterization sulfonamides 31-36 in table 3.



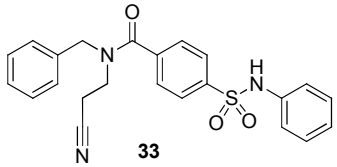
31

Preparation of *N*-benzyl-*N*-ethyl-4-(*N*-phenylsulfamoyl)benzamide (31). Title compound synthesized according to general method B to give product (41 mg, 57% yield) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 0.98, 1.16 (t, *J*=6.84 Hz, *J*=6.50 Hz, 3 H) 3.04, 3.49 (q, *J*=6.76 Hz, *J*=6.54 Hz, 2 H) 4.33, 4.70(s., 2 H) 6.77, 6.88 (br. s., 1 H) 6.93 - 7.12 (m, 4 H) 7.13 - 7.20 (m, 2 H) 7.23 - 7.48 (m, 6 H) 7.66, 7.73 (d, *J*=7.94 Hz, *J*=7.72 2 H); MS (ES+) $[M+H]^+$ = 395.



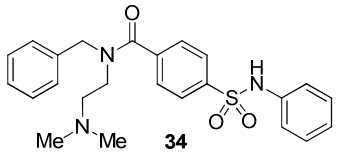
32

Preparation of *N*-benzyl-*N*-(2-hydroxyethyl)-4-(*N*-phenylsulfamoyl)benzamide (32). Title compound synthesized according to general method B to give product (18 mg, 12% yield). ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 3.22, 3.65 (m., 2 H) 3.56, 3.74-3.86 (m, 2 H) 4.44, 4.78 (s, 2 H) 6.61 - 6.76 (m, 1 H) 6.96 - 7.13 (m, 4 H) 7.18 (t, *J*=7.50 Hz, 2 H) 7.23 - 7.37 (m, 4 H) 7.45 (d, *J*=7.94 Hz, 2 H) 7.68 (d, *J*=8.16 Hz, 2 H); MS (ES+) $[M+H]^+$ = 411.



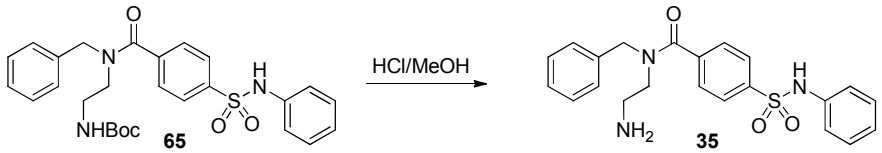
33

Preparation of *N*-benzyl-*N*-(2-cyanoethyl)-4-(*N*-phenylsulfamoyl)benzamide (33). Title compound synthesized according to general method B to give product (63 mg, 90% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *no rotamers observed*) δ ppm 2.56 (t, *J*=6.57 Hz, 1 H) 2.80 (t, *J*=6.32 Hz, 2 H) 2.97 (t, *J*=6.69 Hz, 1 H) 3.71 (t, *J*=6.19 Hz, 2 H) 7.00 - 7.19 (m, 4 H) 7.20 - 7.26 (m, 2 H) 7.32 - 7.43 (m, 5 H) 7.49 - 7.61 (m, 2 H) 7.78 (d, *J*=8.08 Hz, 2 H); MS (ES+) $[M+H]^+$ = 420



34

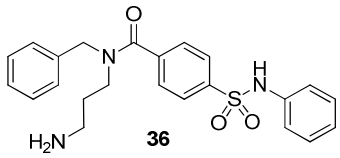
Preparation of *N*-benzyl-*N*-(2-(dimethylamino)ethyl)-4-(*N*-phenylsulfamoyl)benzamide (34). Title compound synthesized according to general method B to give product (25 mg, 32% yield) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.88 (br. s., 3 H) 2.20 (br. s., 4 H) 2.50 (br. s., 1 H) 3.09 (br. s., 1 H) 3.53 (br. s., 1 H) 4.41 (br. s., 1 H) 4.72 (br. s., 1 H) 6.94 - 7.11 (m, 3 H) 7.13 - 7.22 (m, 4 H) 7.23 - 7.33 (m, 4 H) 7.43 (br. s., 2 H) 7.70 (m, *J*=15.00 Hz, 2 H); MS (ES+) $[M+H]^+$ = 438.



65 35

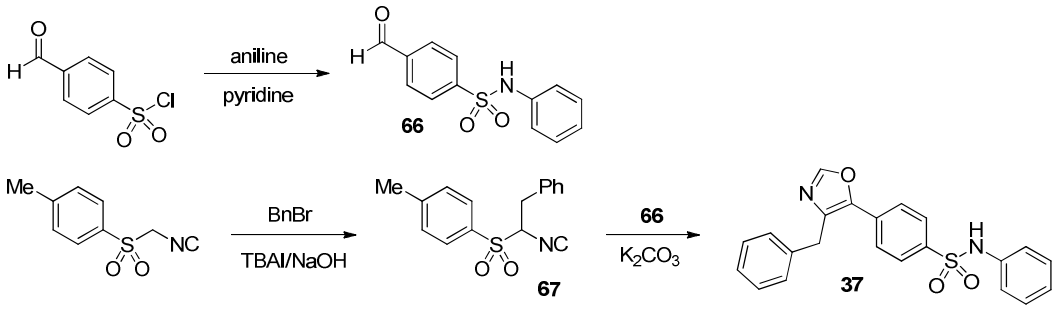
Preparation of *tert*-butyl (2-(*N*-benzyl-4-(*N*-phenylsulfamoyl)benzamido)ethyl)carbamate (65). Title compound synthesized according to general method B to give product (54 mg, 59% yield). ^{1}H NMR (400 MHz, Chloroform-*d, rotamers observed*) δ ppm 1.46 (s, 9 H) 3.09 - 3.25, 3.35-3.46 (m, 2 H) 3.35 - 3.46, 3.58-3.67 (m, 2 H) 4.47, 4.82 (s, 2 H) 6.50 - 6.67 (m, 1 H) 6.97 - 7.17 (m, 4 H) 7.19 - 7.28 (m, 2 H) 7.29 - 7.43 (m, 4 H) 7.49 (d, *J*=8.08 Hz, 2 H) 7.67 - 7.91 (m, 2 H); MS (ES+) $[M+H]^+$ = 510.

Preparation of *N*-benzyl-*N*-(2-aminoethyl)-4-(*N*-phenylsulfamoyl)benzamide (35). 4M HCl in dioxane (0.5 mL) was added to a stirring solution of *tert*-butyl (2-(*N*-benzyl-4-(*N*-phenylsulfamoyl) benzamido)ethyl)carbamate (44 mg, 0.086 mmol) in methanol at room temperature and stirred overnight. The crude reaction was concentrated *in vacuo* and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to give product (32 mg, 84% yield). ^{1}H NMR (400 MHz, Methanol-d_4, *no rotamers observed*) δ ppm 1.61 - 1.74 (m, 2 H) 2.23 - 2.44 (m, 2 H) 3.03 (br. s., 2 H) 5.52 - 5.64 (m, 3 H) 5.64 - 5.75 (m, 4 H) 5.86 - 5.97 (m, 3 H) 6.13 (d, *J*=8.34 Hz, 2 H) 6.33 (d, *J*=7.83 Hz, 2 H); MS (ES+) $[M+H]^+$ = 410.



Preparation of *N*-(3-aminopropyl)-*N*-benzyl-4-(*N*-phenylsulfamoyl)benzamide (36). Under a nitrogen atmosphere, *N*-benzyl-*N*-(2-cyanoethyl)-4-(*N*-phenylsulfamoyl)benzamide (**33**, 60 mg, 0.143 mmol) and a slurry of Raney Nickel (1 mL) were added to a solution of 4M ammonia in methanol (20 mL) and hydrogenated at 50 psi overnight. The slurry was filtered over a celite pad and washed with methanol. The solution was concentrated *in vacuo* and the crude mixture was purified by silica gel chromatography ($MeOH/CH_2Cl_2$ eluent) to give product (0.010 g, 0.021 mmol, 17% yield). ^1H NMR (400 MHz, Methanol-d_4, *no rotamers observed*) δ ppm 1.95 (quin, *J*=6.95 Hz, 2 H) 2.97 (t, *J*=7.07 Hz, 2 H) 3.62 (t, *J*=6.82 Hz, 2 H) 4.47 (s, 2 H) 6.98 - 7.24 (m, 7 H) 7.28 - 7.43 (m, 4 H) 7.54 (d, *J*=8.08 Hz, 2 H) 7.80 (d, *J*=8.34 Hz, 1 H); MS (ES+) $[M+H]^+ = 424$.

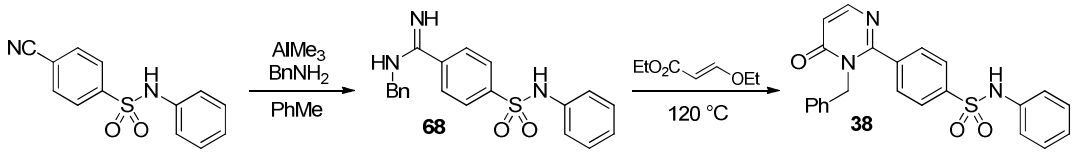
1.1.7 Synthesis and characterization of heterocycle analogs 37-41 in table 4.



Preparation of 4-formyl-N-phenylbenzenesulfonamide (66). 4-Formylbenzene-1-sulfonyl chloride (500 mg, 2.44 mmol), aniline (0.25 mL, 2.69 mmol), and pyridine (0.22 mL, 2.69 mmol) were taken up into 10 mL DCM and stirred at room temperature for 2.5 hours. The reaction was concentrated in vacuo and purified using silica gel chromatograpy to produce aldehyde **9** as a yellow foam (402 mg, 63% yield). ^1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 6.70 (d, *J*=14.40 Hz, 1 H) 7.03 - 7.14 (m, 2 H) 7.15 - 7.26 (m, 2 H) 7.42 (d, *J*=8.08 Hz, 1 H) 7.69 - 8.08 (m, 4 H) 10.08 (s, 1 H). MS (ES+) $[M+NH_4]^+ = 279$.

Preparation of 1-((1-isocyano-2-phenylethyl)sulfonyl)-4-methylbenzene (67). 1-((Isocyanomethyl) sulfonyl)-4-methylbenzene (TosMIC, 195 mg, 1.0 mmol) dissolved in 4 mL DCM was added to tetrabutylammonium iodide (0.13 mL, 1.1 mmol) in 2.9 mL of 6N NaOH. The reaction was stirred at room temperature overnight. The reaction was quenched with water and extracted with DCM. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude material (361 mg) was taken on without further purification. MS (ES+) $[M+NH_4]^+ = 303$.

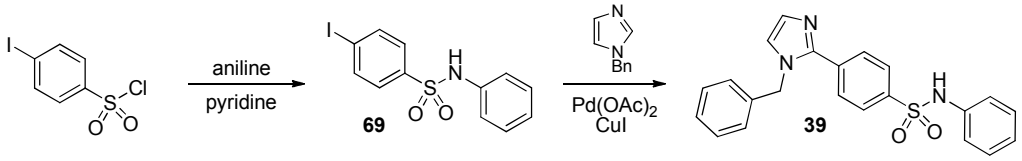
Preparation of 4-(4-benzyloxazol-5-yl)-N-phenylbenzenesulfonamide (37). 1-((1-Isocyano-2-phenylethyl)sulfonyl)-4-methylbenzene (**67**, 285 mg, 1.0 mmol), 4-formyl-N-phenyl benzenesulfonamide (**66**, 200 mg, 0.80 mmol), and K_2CO_3 (50 mg, 0.36 mmol) were taken up into 4 mL MeOH. The reaction was stirred at 60 °C. overnight. The reaction was cooled to room temperature and diluted with 2 mL H_2O and 1 mL ACN and filtered. The solute was concentrated and purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to afford the title compound **11** (101 mg, 32% yield). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 4.17 (s, 2 H) 6.59 - 6.66 (m, 1 H) 7.09 (dd, *J*=8.59, 1.26 Hz, 2 H) 7.16 (s, 1 H) 7.25 (s, 4 H) 7.28 - 7.36 (m, 3 H) 7.67 (d, *J*=8.84 Hz, 2 H) 7.77 - 7.85 (m, 2 H) 7.94 (s, 1 H); MS (ES+) $[M+H]^+ = 391$.



Preparation of *N*-benzyl-4-(*N*-phenylsulfamoyl)benzimidamide (68). A 10 mL round-bottomed flask was charged with benzyl amine (21 uL, 0.194 mmol) and the system was purgued with nitrogen and cooled to 0 °C. Trimethylaluminum (2M solution in toluene, 0.15 mL, 0.291 mmol) was added slowly via synringe. The reaction was warmed to room temperature. 4-cyano-*N*-phenylbenzenesulfonamide[1] (100 mg, 0.387 mmol, dissolved in 3 mL toluene) was added to the reaction. The reaction was heated to 80 °C for 4 days, upon which the reaction had stalled at approximately 25%% conversion. The reaction was cooled to room temperature, filtered, and the

filtrate was concentrated under vacuum. The crude residue was used in the next step without further purification. MS (ES+) $[M+H]^+ = 366$.

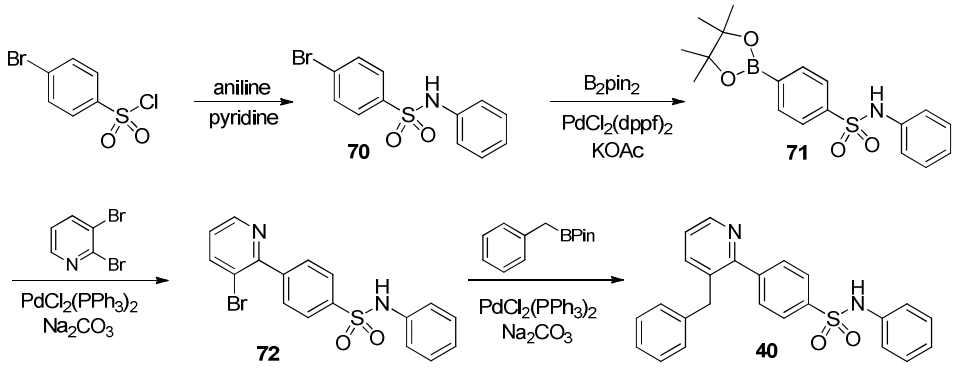
Preparation of 4-(1-benzyl-6-oxo-1,6-dihydropyrimidin-2-yl)-*N*-phenylbenzenesulfonamide (38). Ethyl (*E*)-3-ethoxyacrylate (0.5 mL) was added to the crude residue from the previous step and heated to 80 °C for 18 hours. The reaction was monitored by LCMS, which showed full consumption of the amidine #. The reaction was quenched with saturated NH_4Cl (aq.) and extracted twice with ethyl acetate/THF (1:1 v:v). The organic layers were washed with brine and dried over MgSO4. The product and excess ethyl ethoxyacrylate had similar retention times by HPLC and TLC under a variety of mobile phases. The crude reaction was purified by prep HPLC (30 x 100mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min). A singular clean product fraction was isolated to provide 1 mg of the desired product in 95% purity. ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 5.03 (s, 2H) 6.36 (d, *J*=7.6 Hz, 1 H) 6.83 (d, *J*=7.1 Hz, 2 H) 7.06 - 7.12 (m, 3 H) 7.19 - 7.29 (m, 5 H) 7.51 (m, 2H), 7.81 (m, 2H) 8.01 (d, *J*=7.6 Hz, 1 H). MS (ES+) $[M+H]^+ = 418$.



Preparation of 4-iodo-N-phenylbenzenesulfonamide (69). 4-Iodobenzene-1-sulfonyl chloride (5.1 g, 16.9 mmol), aniline (1.7 mL, 18.6 mmol), and pyridine (1.5 mL, 18.6 mmol) were taken up into 25 mL DCM and stirred at room temperature for 1 hour. The reaction was diluted with 150 mL of ethyl acetate and the organic layer was washed sequentially with 1N HCl, water, saturated $NaHCO_3$, and brine. The organic layer was dried over sodium sulfate, filtered, and the solvent was removed in vacuo to provide aryl iodide **1** (5.7 g, 94% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.35 (s, 1H), 7.94 (d, 2H), 7.48 (d, 2H), 7.25 (m, 2H), 7.06 (m, 3H). MS (ES+) $[M+H]^+ = 360$.

Preparation of 4-(1-benzyl-1H-imidazol-2-yl)-N-phenylbenzenesulfonamide (39). 1-Benzyl-1H-imidazole (79 mg, 0.50 mmol), 4-iodo-N-phenylbenzenesulfonamide (**69**, 360 mg, 1.0 mmol), CuI (190 mg, 1.0 mmol), and $Pd(OAc)_2$ (6 mg, 5 mol%) were taken up into 3 mL DMF and purged with nitrogen

for 5 minutes. The reaction was sealed and heated at 140° C for two days. The reaction was quenched with 100 mL sat. NH_4Cl and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered, and the solvent was removed in vacuo. The crude residue was purified by silica gel chromatography to provide imidazole **2** (20 mg, 10% yield). ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 5.24 - 5.37 (m, 2 H) 6.92 - 7.02 (m, 2 H) 7.03 - 7.16 (m, 4 H) 7.17 - 7.38 (m, 6 H) 7.55 - 7.68 (m, 2 H) 7.80 (d, *J*=8.59 Hz, 2 H). MS (ES+) $[M+H]^+$ = 390.

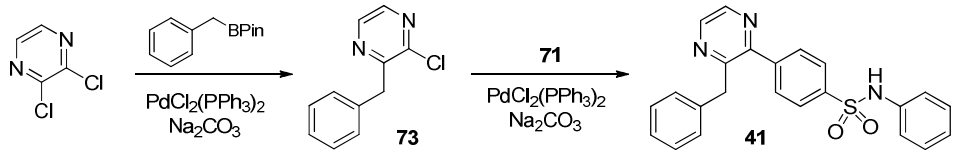


Preparation of 4-bromo-N-phenylbenzenesulfonamide (70). 4-Bromobenzene-1-sulfonyl chloride (1.77 g, 6.9 mmol), aniline (0.70 mL, 7.6 mmol), and pyridine (1.5 mL, 18.6 mmol) were taken up into 25 mL DCM and stirred at room temperature for one hour. The reaction was diluted with ethyl acetate and the organic layer was washed sequentially with 1N HCl, water, saturated $NaHCO_3$, and brine. The organic layer was dried over sodium sulfate, filtered, and the solvent was removed in vacuo to provide aryl bromide **3 (**2.1 g, 98% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 6.95 - 7.14 (m, 3 H) 7.15 - 7.37 (m, 2 H) 7.62 - 7.70 (m, 2 H) 7.71 - 7.88 (m, 2 H) 10.36 (s, 1 H). MS (ES+) $[M+NH_4]^+$ = 329.

Preparation of N-phenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (71). To a solution of 4-bromo-N-phenylbenzenesulfonamide (**70**, 200 mg, 0.64 mmol) in 3 mL of 1,4-dioxane was added dipinacolborane (180 mg, 0.70 mmol), $PdCl_2(dppf)_2$ (52 mg, 10 mol%), and potassium acetate (188 mg, 1.92 mmol). The reaction was stirred overnight at room temperature. The reaction was filtered and the solvent was removed in vacuo. This crude material was used in the next step without purification. MS (ES+) $[M+NH_4]^+$ = 377.

Preparation of 4-(3-bromopyridin-2-yl)-N-phenylbenzenesulfonamide (72). 2,3-Dibromopyridine (118 mg, 0.50 mmol) was taken up into 4 mL ACN/H_2O (1:1 v:v). To this solution was added *N*-phenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (**71**, 230 mg, 0.64 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 10 mol%), and Na_2CO_3 (212 mg, 2.0 mmol). The reaction was microwaved at 150° C for 10 minutes. The completed reaction was filtered and the solvent removed in vacuo. The material was purified using silica gel column chromatography to yield bromopyridine **5** (65 mg, 34% yield). ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 7.03 - 7.18 (m, 3 H) 7.18 - 7.28 (m, 2 H) 7.38 (dd, *J*=8.08, 4.80 Hz, 1 H) 7.68 - 7.77 (m, 2 H) 7.83 - 7.92 (m, 2 H) 8.21 (dd, *J*=8.08, 1.26 Hz, 1 H) 8.54 (s, 1 H) 8.59 (dd, *J*=4.55, 1.26 Hz, 1 H). MS (ES+) $[M+H]^+$ = 389.

Preparation of 4-(3-benzylpyridin-2-yl)-N-phenylbenzenesulfonamide (40). 4-(3-Bromopyridin-2-yl)-N-phenylbenzenesulfonamide (**72**, 65 mg, 0.17 mmol) was dissolved in 4 mL ACN/H_2O (1:1 v:v). To this solution was added 2-benzyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**5**, 0.045 mL, 0.20 mmol), $PdCl_2(PPh_3)_2$ (12 mg, 10 mol%), and Na_2CO_3 (71 mg, 0.68 mmol). The reaction was microwaved at 150° C for 10 minutes. The reaction was filtered and the solvent removed in vacuo. The material was purified by prep HPLC (30 x 250mm C18 column, 5–95% acetonitrile:water (10 mM ammonium acetate), 15 min, 45 mL/min) to afford the desired product (**6**, 6 mg, 9% yield). ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 3.95 (s, 2 H) 6.81 (dd, J=6.32, 2.78 Hz, 2 H) 6.99 - 7.30 (m, 9 H) 7.38 - 7.52 (m, 3 H) 7.74 - 7.92 (m, 3 H) 8.46 (dd, J=4.80, 1.52 Hz, 1 H). MS (ES+) $[M+H]^+$ = 401.



Preparation of 2-benzyl-3-chloropyrazine (73). 2,3-Dichloropyridine (0.053 mL, 0.50 mmol) was dissolved in 4 mL ACN/H_2O (1:1 v:v). 2-Benzyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.11 mL, 0.50 mmol), $PdCl_2(PPh_3)_2$ (35 mg,10 mol%), and Na_2CO_3 (212 mg, 2.0 mmol) were added. The reaction was microwaved at 150° C for 10 minutes. The reaction was filtered and the solvent removed

in vacuo. The material was used without further purification (102 mg, 100% yield). MS (ES+) $[M+H]^+$ = 205.

Preparation of 4-(3-benzylpyrazin-2-yl)-N-phenylbenzenesulfonamide (41). 2-Benzyl-3-chloro-pyrazine (**73**, 102 mg, 0.50 mmol) was taken up into 4 mL ACN/H_2O (1:1 v:v). To this was added *N*-phenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (**71**, 180 mg, 0.50 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 10 mol%), and Na_2CO_3 (212 mg, 2.0 mmol). The reaction was microwaved at 150° C for 10 minutes. The reaction was filtered and the solvent removed in vacuo. The material was purified using silica gel chromatography to provide the title compound **8** as a white solid (40 mg, 20% yield). ^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 6.80 (dd, *J*=6.82, 2.53 Hz, 2 H) 7.03 - 7.20 (m, 6 H) 7.21 - 7.31 (m, 2 H) 7.45 - 7.54 (m, 2 H) 7.79 - 7.88 (m, 2 H) 8.56 (d, *J*=2.53 Hz, 1 H) 8.63 (d, *J*=2.53 Hz, 1 H). MS (ES+) $[M+H]^+$ = 402.

2. In Vitro Methods

2.1 Expression and Purification of LIMK2

Full length human LIMK2 was expressed using the BAC-to-BAC® Baculovirus Expression System (Invitrogen). Recombinant baculovirus was made according to the manufacturer's directions as set forth in the instruction manual. Briefly, the plasmids (pFactBac1 or pFastBacHT) carrying the LIMK2 inserts were transformed into MAX efficiency DH10Bac competent *E. coli* to generate a recombinant bacmid. The DH10Bac *E. coli* host strain contains a baculovirus shuttle vector (bacmid) with a mini-attTn7 target site and a helper plasmid, and allows generation of a recombinant bacmid following transposition between the mini-Tn7 element on the pFastBac vector and the min-attTn7 target site on the bacmid. The transposition reaction occurs in the presence of transposition proteins supplied by the helper plasmid. Cells were plated and the white colonies picked for bacmid isolation as described in the instruction manual.

The isolated bacmid DNA was transfected into SF9 cells to generate a recombinant baculovirus, and virus was collected five days after transfection. Virus was amplified in T75 flasks at a multiplicity of infection (MOI) of 0.2. The amplified virus was used to infect SF9 cells at a MOI 5 for protein expression.

For small scale purification of the LIMK2 constructs, a 50 ml culture of Sf9 cells infected with the recombinant baculovirus was used. The cells were harvested by centrifugation for 5 minutes at 500 x g. The cells were then resuspended in lysis buffer (5 volumes per gram of cells). A typical lysis buffer contains the following: 50 mM HEPES (pH 8.0), 300 mM KCl, 10% glycerol, 1% NP-40, 15mM imidazole, 1mM benzamidine, and Roche complete protease inhibitors (1 tablet per 50 ml of cell lysate). The cellular suspension was lysed by one passage through a Microfluidics Microfluidizer M-110Y at a liquid pressure of 14,000 to 20,000 psi followed by centrifugation of the lysate at 60,000 x g for 15 minutes at 4 °C.

The supernatant was then loaded directly onto a chromatography matrix containing Cobalt ion covalently attached to nitrilotriacetic acid NTA . The chromatography matrix was equilibrated in the

same buffer as the protein loading solution. The ion charged resin typically has a binding capacity equivalent to 5 to 10 mg histidine-tagged protein per ml of packed resin. The amount of extract that can be loaded onto the column depends on the amount of soluble histidine-tagged protein in the extract. The column was then washed in a stepwise fashion, first with: 50 mM HEPES (pH 8.0), 300 mM KCl, 10% glycerol, 1% NP-40, 15 mM imidazole, 1 mM benzamidine; second, with 20 mM HEPES (pH 8.0), 500 mM KCl, 10% glycerol, and 20 mM imidazole; third, with 20 mM HEPES (pH 8.0), 100 mM KCl, 10% glycerol, and 20 mM imidazole; followed by elution with 250 mM imidazole in the same buffer. The LIMK2 protein solution was then analyzed by SDS-PAGE and Western blot using commercial antibodies directed to both the carboxyl terminus and internal catalytic domains of the protein. For storage purposes the protein was dialyzed into 50 mM Tris (pH 7.5), 150 mM NaCl, 0.1% BME, 0.03% Brij-35, and 50% glycerol.

Large scale LIMK2 purification was done in a Wave Bioreactor (Wave Biotech) with 10L culture volumes. 10L of cell culture at 2-3 x 10^6 viable cells/mL were infected at an MOI=5 pfu/cell and harvested at 48 hours post infection. Yields were typically 0.5-1 mg/ L of insect cell culture. Purity was ≥ 80% based on SDS-PAGE followed by quantitative densitometry of Coomassie stained protein bands.

2.2 Expression and Purification of Biotinylated Cofilin

Four liters of sterile fermenter media (containing 80 g peptone, 60 g yeast extract, 24 g Na_2HPO_4, 12 g KH_2PO_4, 4 g $(NH_4)_2SO_4$), pH 7.0, was poured into New Brunswick Bioflow 110 fermenter and autoclaved for 45 minutes. The following sterile solutions were then added to the fermenter media: 2 mL of 1M $MgCl_2$ + 5mM $CaCl_2$ mixture, 12 mL of 40% glucose, and 0.5 mL of 10% antifoam solution. The vessel containing fermenter media was inoculated with 40ml overnight culture expressing N-terminal his-tagged human cofilin. The pH, DO2, and thermo probes were plugged in and the cooling water to the vessel and condenser was connected along with the acid, base, and feeding pumps. The temperature was set to 37 °C, agitation to 400 rpm, air flow rate at 3 dL/min, DO2 control at 40% (cascade to agitation) and pH 7.0. When the OD500 reached 6.0, the temperature was reduced to 25 °C,

and protein expression was induced by feeding 10% lactose and 1 mM biotin at maximal flow rate to a final concentration of 0.5% lactose and 50 μM biotin. Cells from the fermentor were pellet by centrifugation and pellets were resuspended in lysis buffer (20 mM Tris pH 7.5, 500 mM NaCl, 0.5 mM DTT, 10mM $MgCl_2$, 20mM imidazole, 10% glycerol, 1 mM benzamidine, 50 μg/mL DNase I, 1X Sigma protease inhibitor cocktail -EDTA) using 5 mL per gram of cell pellet. Cells were completely lysed after 2 passes through a microfluidizer. Lysate was cleared by centrifugation at 21,000 rpm for 30 minutes in a JA 25.5 rotor.

Protein was batch purified from lysate as follows. 3 mL of pre-equilibrated Ni-NTA resin (Qiagen) was used per each liter of culture. The lysate and resin mixture was poured into a shake flask (without aeration ridges to prevent foaming) and shook at ~200 rpm at 4 °C for 1-2 hours. Resin was pelleted by a spin at 1000 rpm for 10 minutes and washed with 20 column volumes lysis buffer followed by another spin. Resin/protein mixture was then washed with 20 column volumes wash buffer (20 mM Tris pH 7.5, 300 mM NaCl, 0.5mM DTT, 10% glycerol, 20 mM imidazole*)* followed by another spin. Resin was then packed into an Waters AP-2 column and connect to an FPLC. The column was washed at 2 mL/min with wash buffer until baseline reached. Protein was eluted with a gradient to 100% elution buffer (20 mM Tris pH 7.5, 300 mM NaCl, 0.5 mM DTT, 10% glycerol, 250 mM imidazole) and 1 mL fractions were collected. Peak fractions were analyzed by SDS-PAGE and Coomassie staining. Desired fractions were pooled and dialyzed overnight at 4 °C in storage buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 40% glycerol). Protein concentration was measured by the Bradford method and protein then stored at –20 °C.

2.3 In Vitro LIMK2 Inhibition Assay

An *in vitro* assay used to identify LIMK2 inhibitors was developed. The analytical readout was the incorporation of ^{33}P from ATP into biotinylated-cofilin substrate immobilized on streptavidin coated

flash plates (Perkin Elmer Biosciences). Plates were counted on a scintillation counter equipped with a plate reader (TopCount, Packard Bioscience, Meriden, CT). 384 well streptavidin FlashPlates from Perkin Elmer (Cat# SMP410A001PK) were used.

Rock1 purified at Lexicon using a similar procedure described above for LIMK2, was used to activate LIMK2. Specifically, a 20 μL mixture of 0.6 μM ROCK1, 5 nM LIMK2, and cold ATP (from 5-500 μM) was preincubated in kinase assay buffer (30 mM HEPES, pH 8.0; mM $MgCl_2$; 5 mM DTT; 0.1% Pluronic F-68) at room temperature for 30 minutes. Compounds were then acoustically dispensed using a Labcyte® Echo™ 550 (Labcyte Inc., Sunnyvale, CA) compound reformatter and 12-point dose responses were performed in four independent dilutions. The LIMK2 assay was then initiated upon addition of ^{33}P-ATP, 0.5 μM biotinylated-cofilin (Vmax is reached at 0.5 μM), and 0.5 μM Rock1 inhibitor[2] in a final reaction volume of 50 μL. For kinetic studies, the final compound concentrations varied from 0 to 100 nM. The reaction was incubated at room temperature for 60 minutes, washed 3 times with 75 μL of stop/wash buffer (1X stop/was buffer contains 50 mM EDTA and 20 mM Tris (pH 7.4)), and then the plates were read on the scintillation counter. Separate experiments not shown indicate that none of our compounds inhibit ROCK1, that even 2 nM ROCK1 does not phosphorylate cofilin, and LIMK2 does not phosphorylate cofilin unless first activated by ROCK1.[3]

Calculating IC50 Values

The IC_{50} of a compound with regard to a given target is determined by fitting the relevant data, using the Levenburg Marquardt algorithm, to the equation:

$$y = A + ((B-A)/(1+((C/x)^D)))$$

wherein A is the minimum y value; B is the maximum y value; C is the IC_{50}; and D is the slope. The calculation of the IC_{50} is performed using XLFit4 software (ID Business Solutions Inc., Bridgewater, NJ 08807) for Microsoft Excel (the above equation is model 205 of that software).

3. Crystallographic information

3.1 Expression and purification of LIMK2

Crystallography was done by Proteros Biostructures Gmbh, Martinsried, Germany. Different plasmid constructs comprising the kinase domain of LIMK2 were fused to a GST- or a His_6-tag in a pFastBac vector to allow efficient expression in insect cells. Recombinant baculoviruses were generated and amplified according to manufacturer's protocols. For expression of recombinant LIMK2 by a titerless infection protocol, 5 L of Sf9 cells were infected with 150 mL of high titer virus stock (HTVS) and grown for 64 hours on a BioWave 50 SPS (WaveBiotech). After expression, cells were harvested by centrifugation and stored frozen (-80 °C) until purification.

All purification steps were performed at 4 °C on chromatography stations and columns obtained from GE Healthcare (ÄKTA system). Between purification steps, the protein was kept on ice or in a cold room/fridge at 4 °C unless noted otherwise. Before the protein was pooled, samples were analyzed on pre-cast SDS gels (10%) obtained from Invitrogen.

The initial purification step for LIMK2 from insect cell culture lysates was performed by NiNTA affinity chromatography using a 20 mL His prep 16/10 FF column (GE Healthcare). The cell lysate was loaded onto the column at 1 mL/min. The column was washed with 100 mL of starting buffer and eluted with 50 ml of NiNTA elution buffer. LIMK 2 containing elution fractions were pooled and dialysed against 2 L NiNTA starting buffer in the presence of TEV protease in order to remove the HIS tag and uncleaved fusion protein by subsequent reverse NiNTA chromatography. The flow fractions of the reverse NiNTA affinity chromatography were pooled and dialysed two times against 2 L TRIS buffer in order to remove imidazole and salt before the protein was applied to an anion exchange Mono Q 5/50 GL column (GE Healthcare). Bound protein was washed until the absorption reached a base line and eluted by a 2 step gradient until 1 M NaCl. The protein eluted at 150 mM NaCl. LIMK2 containing elution fractions were pooled and concentrated. The elution fractions from the anion exchange chromatography were concentrated and finally purified by size exclusion chromatography using a Superdex75 26/60 column (GE Healthcare). LIMK 2 kinase domain

eluted as a monomeric peak. The amount of finally purified protein is 0.5 – 1.5 mg/L insect cell expression culture. This procedure yielded homogenous protein with a purity greater 95 % as judged from coomassie stained SDS-PAGE.

The purified protein was used in crystallization trials employing both, a standard screen with approximately 1200 different conditions, as well as crystallization conditions identified using literature data. Conditions initially obtained have been optimized using standard strategies, systematically varying parameters critically influencing crystallization, such as temperature, protein concentration, drop ratio, and others. These conditions were also refined by systematically varying pH or precipitant concentrations.

3.2 Data collection and processing

A cryo-protocol was established using PROTEROS Standard Protocols. Crystals have been flash-frozen and measured at a temperature of 100 K.

The X-ray diffraction data have been collected from complex crystals of human LIMK2 with the ligand **22** at the SWISS LIGHT SOURCE (SLS, Villigen, Switzerland) using cryogenic conditions.

The crystals belong to space group P 21. Data were processed using the programs XDS and XSCALE.

Table 1. Data collection and processing statistics for sulfonamide **22** (MM7-4)

Ligand	**MM7-4**
X-ray source	PXI/X06SA (SLS)[1]
Wavelength [Å]	1.0000
Detector	PILATUS 6M
Temperature [K]	100
Space group	P 2_1
Cell: a; b; c; [Å]	51.57; 77.90; 86.39
α; β; γ; [°]	90.0; 100.8; 90.0
Resolution [Å]	2.60 (2.99-2.77)[2]
Unique reflections	20329 (3495)[2]
Multiplicity	3.9 (4.1)[2]
Completeness [%]	97.6 (99.9)[2]
R_{sym} [%] [3]	5.3 (40.8)[2]
R_{meas} [%] [4]	6.1 (46.8)[2]
Mean(I)/sd [5]	17.16 (3.67)[2]

[1] SWISS LIGHT SOURCE (SLS, Villigen, Switzerland)
[2] values in parenthesis refer to the resolution bin with R_{sym} = 40.8 %.

[3] $$R_{sym} = \frac{\sum_h \sum_i^{n_h} |\widehat{I}_h - I_{h,i}|}{\sum_h \sum_i^{n_h} I_{h,i}} \text{ with } \widehat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$$

where $I_{h,i}$ is the intensity value of the ith measurement of h

[4] $$R_{meas} = \frac{\sum_h \sqrt{\frac{n_h}{n_h - 1}} \sum_i^{n_h} |\widehat{I}_h - I_{h,i}|}{\sum_h \sum_i^{n_h} I_{h,i}} \text{ with } \widehat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$$

where $I_{h,i}$ is the intensity value of the ith measurement of h

[5] calculated from independent reflections

3.3 Structure Modeling and Refinement

The phase information necessary to determine and analyze the structure was obtained by molecular replacement. A previously solved structure of human LIMK2 was used as a search model.

Subsequent model building and refinement was performed according to standard protocols with the software packages CCP4 and COOT. For the calculation of the free R-factor, a measure to cross-validate the correctness of the final model, about 4.5 % of measured reflections were excluded from the refinement procedure (see Table 2).

TLS refinement (using REFMAC5, CCP4) has been carried out, which resulted in lower R-factors and higher quality of the electron density map.

The ligand parameterization was carried out with CHEMSKETCH. LIBCHECK (CCP4) was used for generation of the corresponding library files.

The water model was built with the "Find waters"-algorithm of COOT by putting water molecules in peaks of the F_o-F_c map contoured at 3.0 σ followed by refinement with REFMAC5 and checking all waters with the validation tool of COOT. The criteria for the list of suspicious waters were: B-factor greater 80 $Å^2$, $2F_o$-F_c map less than 1.2 σ, distance to closest contact less than 2.3 Å or more than 3.5 Å. The suspicious water molecules and those in the active site (distance to inhibitor less than 10 Å) were checked manually. The occupancy of side chains, which were in negative peaks in the F_o-F_c map (contoured at -3.0 σ), were set to zero and subsequently to 0.5 if a positive peak occurred after the next refinement cycle.

The Ramachandran Plot of the final model shows 86.7% of all residues in the most favored region, 12.5% in the additionally allowed region, and 0.6% in the generously allowed region. The residue ARG450(B) is found in the disallowed region of the Ramachandran plot (Table 2). This is either confirmed by the electron density map or could not be modeled in another sensible conformation. Statistics of the final structure and the refinement process are listed in Table 2.

Table 2. Refinement statistics for sulfonamide **22** (**MM7-4**)

Ligand	**MM7-4**
Resolution [Å]	84.82-2.60
Number of reflections (working / test)	19409 / 919
R_{cryst} [%]	22.0
R_{free}[%] [2]	27.4
Total number of atoms:	
Protein	4417
Water	30
Ligand	62
Deviation from ideal geometry: [3]	
Bond lengths [Å]	0.006
Bond angles [°]	0.93
Bonded B's [Å^2] [4]	1.3
Ramachandran plot: [5]	
Most favoured regions [%]	86.7
Additional allowed regions [%]	12.5
Generously allowed regions [%]	0.6
Disallowed regions [%]	0.2

[1] Values as defined in REFMAC5, without sigma cut-off
[2] Test-set contains 4.5 % of measured reflections
[3] Root mean square deviations from geometric target values
[4] Calculated with MOLEMAN
[5] Calculated with PROCHECK

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