Supporting Information

Optimization of a Novel Series of TRPV4 Antagonists with *In vivo* Activity in a Model of Pulmonary Edema

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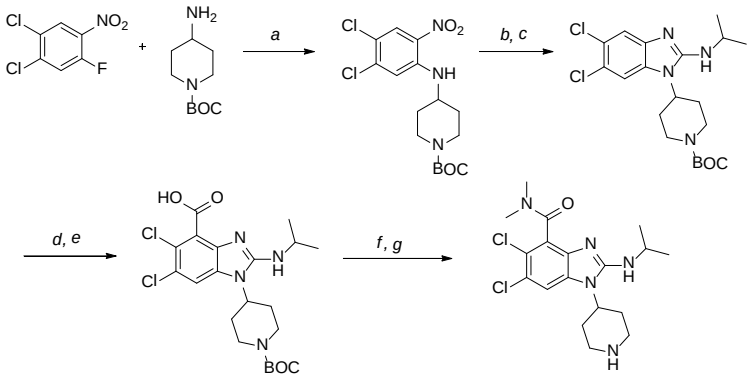
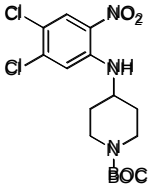
Experimental Procedures:

General information: Commercially available reagents were used without additional purification. All reactions were carried out using anhydrous solvents purchased from Sigma-Aldrich. The reagent, 2-(4-bromophenyl)-2-methylpropanenitrile, 98% was purchased from Combi-Blocks Inc. (cat# CS-9085). The reagent, 4-(4-bromophenyl)oxane-4-carbonitrile, was purchased from Ukraine OrgSynthesis Ltd. (cat# BBV-223169). All other reagents were purchased from Sigma-Aldrich unless otherwise noted. Reactions were monitored using an Agilent Technologies 1100 series 6110 quadrupole LCMS system with ultraviolet detection at 254 nm and 220 nm wavelengths in electrospray ionization mode (eV). A 3.0 x 50 mm Sunfire C18 column (5 um) was used with a 10 – 100% solvent gradient over 2.5 min with a 1 mL/min flow rate. An acetonitrile/water solvent system modified with 0.1% TFA was used unless otherwise noted. Normal phase purification was performed on a Combiflash Rf chromatography system by Teledyne Isco using RediSep normal phase silica gel columns and ultraviolet detection at 254 nm. Reverse phase chromatography was conducted on a Waters 2525 binary gradient HPLC system with a Waters 2996 photodiode array detector. Sample collection used UV detection at 220 nm. All samples were purified with gradient elution utilizing acetonitrile and water solvents with both solvent systems containing 0.1 % TFA. All NMR spectra were recorded on Bruker AVANCE spectrometers operating at either 400 or 500 MHz for ^{1}H and 126 MHz for ^{13}C. Chemical shifts were referenced to the corresponding solvent as referenced. HRMS-ESI data was recorded on a Waters QTOF Premiere running in W optics with positive ion electrospray. Elemental analysis was performed by Quantitative Technologies Inc. (info@QTIonline.com, 908-534-4445).

Scheme 1: Synthesis of 1-(4-piperidinyl)-benzimidazole precursor[a]

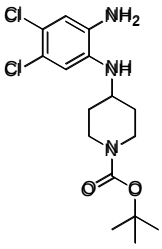


[a](a) Na_2CO_3, MeCN, 25 °C (b) Fe, NH_4Cl, $EtOH/H_2O$, 70 °C (c) isopropylisothiocyanate, pyridine, 70 °C, then EDC (d) *sec*-BuLi, THF, -78 °C, then $ClCO_2Et$ (e) KOH, $THF/EtOH/H_2O$, 70 °C (f) Me_2NH, T3P, $(^iPr)_2NEt$, DCM, 0 °C (g) HCl in Et_2O, MeOH, 25 °C



tert-butyl 4-((4,5-dichloro-2-nitrophenyl)amino)piperidine-1-carboxylate (8)

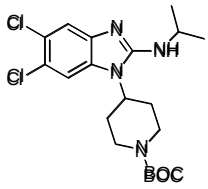
3,4-dichloro-6-fluorobenzonitrile (9.83 mL, 74.9 mmol) was dissolved in acetonitrile (250 mL) and treated with sodium carbonate (23.8 g, 225 mmol). The 1-*N*-BOC-4-aminopiperidine (TCI) (15.0 g, 74.9 mmol) was added dropwise. The solution quickly turned orange-yellow. The reaction was allowed to stir for 3 days at ambient temperature. The reaction mixture was filtered through celite, and the filter cake was washed with EtOAc and DCM leaving the sodium carbonate. The combined washings were concentrated to provide the title compound as an orange solid (74 %). This material was carried on to the next step without further purification. 1H NMR (400 MHz, CHLOROFORM-*d*) δ 8.30 (s, 1H), 8.02 (d, *J* = 7.03 Hz, 1H), 6.98 (s, 1H), 4.03 (m, 2H), 3.61 (m, 1H), 3.06 (m, 2H), 2.04 (m, 2H), 1.59 (m, 2H), 1.48 (s, 9H); LCMS 426.1 $(M+23)^+$; T_R = 3.51 min.



tert-butyl 4-((2-amino-4,5-dichlorophenyl)amino)piperidine-1-carboxylate

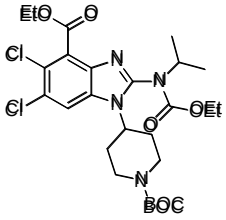
tert-butyl 4-((4,5-dichloro-2-nitrophenyl)amino)piperidine-1-carboxylate (21.5 g, 55.1 mmol) was suspended in ethanol (103 mL) and Water (34.4 mL). The suspension was treated with ammonium chloride (1.47 g, 27.5 mmol), iron powder (325 mesh) (14.5 g, 260 mmol), and then heated to 70 °C. After 1.5 h the reaction was allowed to cool slowly to ambient temperature for the night. Heating was resumed the next day for 4h. The reaction was cooled and filtered through celite. The filtrate was concentrated and taken up in EtOAc. The organics were washed with 1*N* NaOH (1 x 10 mL) and saturated aqueous $NaHCO_3$ solution (1 x 10 mL). The organic layer was then concentrated onto silica gel for

purification on a 120 g silica gel cartridge (85 mL/min elution rate; 30 – 40 % EtOAc/hexanes, 30 min gradient) to provide the title compound as a white crystalline solid (31 %). LCMS 360 $(M+H)^+$; T_R = 2.93 min.



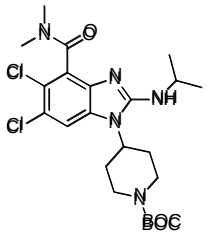
***tert*-butyl 4-(5,6-dichloro-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)piperidine -1-carboxylate (9)**

tert-butyl 4-((2-amino-4,5-dichlorophenyl)amino)piperidine-1-carboxylate (6.10 g, 16.9 mmol) was dissolved in pyridine (33.9 mL) and treated with isopropylisothiocyanate (1.82 mL, 16.9 mmol) at 80 °C. After 2 h, the reaction was cooled to ambient temperature and treated with EDC (3.90 g, 20.3 mmol). The reaction was allowed to stir at room temperature for 18 h. The reaction mixture was concentrated, and the resulting crude product was dissolved in EtOAc. The organic solution was washed with saturated aqueous $CuSO_4$ solution (2 x 20 mL) and brine (1 x 10 mL). The organics were dried over $MgSO_4$, filtered, and concentrated onto silica gel for purification on a 330 g silica gel cartridge (200 mL/min elution rate; 30 % EtOAc/hexanes, 15 min; 30 – 80 % EtOAc/hexanes, 15 min) to provide the title compound (78 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.51 (s, 1H), 7.24 (s, 1H), 4.36 (m, 2H), 4.22 (dq, *J* = 6.59, 13.22 Hz, 1H), 2.86 (t, *J* = 12.59 Hz, 2H), 2.24 (qd, *J* = 4.53, 12.59 Hz, 2H), 1.87 (d, *J* = 12.34 Hz, 2H), 1.53 (s, 9H), 1.34 (d, 6H); LCMS 427.1 $(M+H)^+$, T_R = 2.44 min.



ethyl 5,6-dichloro-1-(1-{[(1,1-dimethylethyl)oxy]carbonyl}-4-piperidinyl)-2-[[(ethyloxy)carbonyl](1-methylethyl)amino]-1H-benzimidazole-4-carboxylate

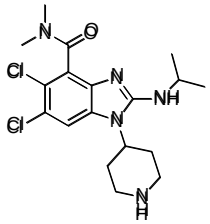
The *tert*-butyl 4-(5,6-dichloro-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate (1.00 g, 2.34 mmol) was dissolved in tetrahydrofuran (11.7 mL) and cooled to -78 °C. *Sec*-butyllithium (5.85 mL, 8.19 mmol) was added slowly, and the reaction was allowed to stir at -78 °C for 1 h. Ethyl chloroformate (0.779 mL, 8.19 mmol) was added, and the reaction was then allowed to warm slowly to ambient temperature. The reaction was quenched with saturated aqueous NH_4Cl solution (1 x 10 mL). The layers were separated, and the organic layer was dried over $MgSO_4$, filtered, and concentrated to an off-white foam. This crude material was carried on to the next step without further purification. LCMS 571.2 $(M+H)^+$, T_R = 3.49 min.



***tert*-butyl 4-(5,6-dichloro-4-(dimethylcarbamoyl)-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate**

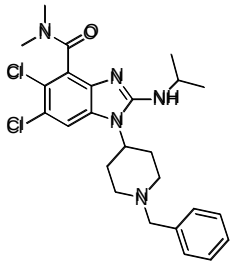
ethyl 5,6-dichloro-1-(1-{[(1,1-dimethylethyl)oxy]carbonyl}-4-piperidinyl)-2-[[(ethyloxy)carbonyl](1-methyl ethyl)amino]-1H-benzimidazole-4-carboxylate (1.40 g, 2.45

mmol) was dissolved in THF (6 mL), EtOH (2 mL), and water (2 mL). Potassium hydroxide (0.69 g, 12.3 mmol) was added, and the reaction was heated to 60 °C for 2 days. The reaction was cooled to room temperature and concentrated to a yellow foam. The foam was dissolved in DCM (20 mL) and washed with saturated aqueous NH_4Cl solution (10 mL). The organic layer was passed through a hydrophobic frit and concentrated. The crude acid **10** was dissolved in DCM (11.5 mL), and the resulting solution was cooled to 0 °C. $(^{i}Pr)_2NEt$ (0.127 mL, 0.69 mmol) and Me_2NH (3.45 mL, 6.90 mmol) were added to the reaction mixture followed by dropwise addition of T3P (2.87 mL, 4.83 mmol). The resulting reaction was allowed to warm slowly to room temperature and stir for 3 days. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (1 x 10 mL). The layers were separated using a hydrophobic frit, and the organics were concentrated onto silica gel for purification on a 40 g silica gel column (40 mL/min elution rate; 60 % EtOAc/hexanes, 3 min; 60 – 100 % EtOAc/hexanes, 20 min gradient) to provide the title compound as a white solid (33 %, 3 steps). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.17 (s, 1H), 4.89 (d, *J* = 5.77 Hz, 1H), 4.23 (m, 2H), 4.12 (m, 2H), 3.17 (s, 3H), 2.85 (s, 3H), 2.84 (m, 1H), 2.03 - 2.15 (m, 2H), 1.77 (m, 2H), 1.51 (s, 9H), 1.12 (d, *J* = 6.27 Hz, 3H), 1.05 (d, *J* = 6.27 Hz, 3H); LCMS 498.2 $(M+H)^+$, T_R = 2.40 min.



5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide

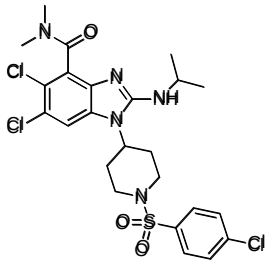
Tert-butyl 4-(5,6-dichloro-4-(dimethylcarbamoyl)-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate (0.562 g, 1.13 mmol) was suspended in THF (5 mL) and only partially dissolved. DCM (5 mL) was added to the slurry to fully dissolve the material. HCl (0.85 mL, 3.38 mmol) was added to the homogeneous solution at 25 °C and allowed to stir for 10 min. Stirring was stopped, and the solution was allowed to stand for 18 h. A white precipitate formed. The solid was collected in a medium frit, washed with Et_2O, and then dried *in vacuo* to provide the title compound as a white solid (100 %) which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (br. s., 1H), 8.99 (br. s., 1H), 8.21 (s, 1H), 5.24 (m, 1H), 4.21 (m, 1H), 3.44 - 3.53 (m, 2H), 3.07 (s, 3H), 2.97 - 3.11 (m, 2H), 2.79 (s, 3H), 2.64 - 2.76 (m, 2H), 1.91 - 2.04 (m, 2H), 1.29 - 1.35 (m, 6H); LCMS 398.1 $(M+H)^+$, T_R = 1.54 min.



1-(1-benzylpiperidin-4-yl)-5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (4)

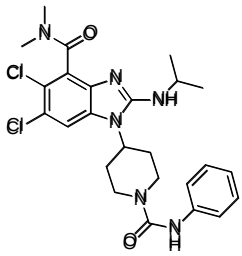
5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide·HCl (0.032 g, 0.074 mmol) was dissolved in dry MeCN (1.0 mL) and methanol (1.0 mL) and treated with benzyl bromide (20.0 µL, 0.168 mmol) and K_2CO_3 (0.031 g, 0.222

mmol). The reaction was allowed to stir at 25 °C for 1 h. The reaction was concentrated, dissolved in DMSO (1.0 mL), and purified by reverse phase-HPLC (Sunfire Prep C18 column, 30 x 150 mm; 16 min run with at-column dilution; 10 – 50 % MeCN/water/0.1 % TFA gradient elution; 220 nm detection) to provide the title compound (83 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.97 (s, 1H), 7.37 - 7.48 (m, 5H), 5.20 (m, 1H), 4.31 (s, 2H), 4.06 (m, 1H), 3.55 – 3.69 (m, 2H), 3.23 – 3.36 (m, 2H), 3.14 (s, 3H), 2.89 - 2.95 (m, 2H), 2.92 (s, 3H), 1.99 - 2.18 (m, 2H), 1.29 (d, *J* = 6.27 Hz, 3H), 1.19 (d, *J* = 6.27 Hz, 3H); LCMS 488.2 $(M+H)^+$, T_R = 1.90 min.



5,6-dichloro-1-(1-((4-chlorophenyl)sulfonyl)piperidin-4-yl)-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (5)

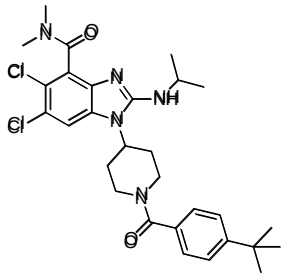
5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide·HCl (0.040 g, 0.092 mmol) was suspended in DCM (1.26 mL) and treated with triethylamine (0.042 mL, 0.301 mmol) at 0 °C. The resulting solution was then treated with 4-chlorophenylsulfonyl chloride (0.021 g, 0.100 mmol). The reaction was allowed to warm to ambient temperature and stir for 18 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (2 mL) and diluted with DCM (10 mL). The layers were shaken and separated, and the organics were concentrated. The crude product was dissolved in DMF (1 mL) and purified by reverse phase-HPLC (Sunfire Prep C18 column, 30 x 150 mm; 16 min run with at-column dilution; 20 – 60 % MeCN/water/0.1 % TFA gradient elution; 220 nm detection) to provide the title compound. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.71 (d, *J* = 8.78 Hz, 2H), 7.57 (d, *J* = 9.54 Hz, 2H), 7.53 (s, 1H), 4.62 - 4.75 (m, 1H), 3.84 - 4.05 (m, 3H), 3.14 (s, 3H), 2.95 (s, 3H), 2.47 - 2.58 (m, 2H), 2.32 - 2.46 (m, 2H), 1.92 - 2.10 (m, 2H), 1.22 (d, *J* = 6.27 Hz, 3H), 1.13 (d, 3H); LCMS 572.1 $(M+H)^+$, T_R = 2.49 min.



5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(1-(phenylcarbamoyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide (6)

5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide·HCl (0.020 g, 0.046 mmol) was suspended in DCM (500 µl) and treated with phenyl isocyanate (5.03 µL, 0.046 mmol). Approx. 0.5 mL of saturated aqueous $NaHCO_3$ solution was added, and the biphasic mixture formed a solution. This was stirred vigorously for 3 days. The layers were separated, and the organic phase was concentrated and dissolved in DMSO (1 mL). The DMSO solution was subjected to reverse phase-HPLC (Sunfire Prep C18 column, 30 x 150 mm; 16 min run with at-column dilution; 20 – 60 % MeCN/water/0.1 % TFA gradient elution; 220 nm detection) to provide the title compound (45 %). ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.87 (s, 1H), 7.40 (d, *J* = 8.53 Hz, 2H), 7.30 (t, *J* = 7.53 Hz, 2H), 7.05 (t, *J* = 7.53 Hz, 1H),

4.68 (m, *J* = 8.03 Hz, 1H), 4.39 - 4.50 (m, 2H), 3.19 (s, 3H), 3.02 - 3.14 (m, 2H), 2.94 (s, 3H), 2.36 - 2.49 (m, 2H), 1.96 - 2.05 (m, 2H), 1.42 (d, *J* = 6.5 Hz, 3H), 1.41 (d, *J* = 6.53 Hz, 3H); LCMS 517.1 $(M+H)^+$, T_R = 2.20 min.

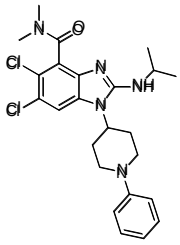


1-(1-(4-(*tert*-butyl)benzoyl)piperidin-4-yl)-5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (7)

5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(piperidin-4-yl)-1*H*-benzo[*d*] imidazole-4-carboxamide·HCl (0.075 g, 0.173 mmol) was suspended in DCM (1.5 mL) and treated with triethylamine (89 µl, 0.637 mmol) and 4-tert-butylbenzoyl chloride (0.031 g, 0.159 mmol). The reaction was allowed to stir at room temperature for 2 h and then diluted with DCM and washed with water (1 x 10 mL). The DCM layer was passed through a hydrophobic frit and concentrated. The crude product was dissolved in DMSO (1 mL) and subjected to reverse phase-HPLC (Sunfire Prep C18 column, 30 x 150 mm; 16 min run with at-column dilution; 20 – 60 % MeCN/water/0.1 % TFA gradient elution; 220 nm detection) to provide the title compound (71 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 9.10 (br.s., 1H), 7.46 (d, *J* = 8.28 Hz, 2H), 7.45 (s, 1H), 7.40 (d, *J* = 8.28 Hz, 2H), 5.02 (m, 1H), 4.06 (m, 1H), 3.00 – 3.49 (m, 4H), 3.11 (s, 3H), 2.92 (s, 3H), 1.96 - 2.09 (m, 2H), 1.82 - 1.95 (m, 2H), 1.33 (s, 9H), 1.24 (d, *J* = 6.27 Hz, 3H), 1.21 (d, *J* = 6.27 Hz, 3H); LCMS 558.3 $(M+H)^+$, T_R = 1.91 min.

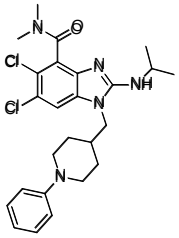
General procedure for the synthesis of arylpiperidines 1, 2, and 12-20 via the Pd-catalyzed amination of arylbromides:

5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide·HCl (0.140 g, 0.323 mmol) was dissolved in dry 1,4-Dioxane (1.61 mL) in a 20 mL scintillation vial and treated with palladium(II) acetate (7.32 mg, 0.032 mmol), di-*tert*-butylbiphenylphosphine (19.2 mg, 0.064 mmol), bromobenzene (0.034 mL, 0.322 mmol), and cesium carbonate (0.315 g, 0.966 mmol). The suspension was purged with nitrogen, capped, and heated to 100 °C. After 18 h, the reaction was cooled to ambient temperature and filtered through celite. The solid residue was washed with THF, and the combined organics were concentrated. The crude product was dissolved in DMSO (1 mL) and purified by reverse phase-HPLC (Sunfire Prep C18 column, 30 x 150 mm; 16 min run with at-column dilution; 20 – 60 % MeCN/water/0.1 % TFA gradient elution; 220 nm detection).



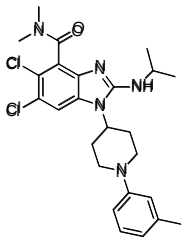
5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(1-phenylpiperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide (1)

The title compound was prepared and purified by the general procedure outlined above to provide the title compound (44 %). 1H NMR (500 MHz, DMSO-*d*6) δ 7.49 (s, 1H), 7.22 - 7.31 (m, *J* = 8.30 Hz, 2H), 7.08 (d, *J* = 8.30 Hz, 2H), 6.87 (t, *J* = 7.32 Hz, 1H), 4.55 (m, 1H), 4.06 (m, 1H), 3.90 (m, *J* = 12.21 Hz, 2H), 3.06 (s, 3H), 2.87 - 2.97 (m, 2H), 2.76 (s, 3H), 2.37 - 2.47 (m, 2H), 1.82 - 1.94 (m, 2H), 1.27 (d, *J* = 2.93 Hz, 3H), 1.26 (d, *J* = 2.44 Hz, 3H); LCMS 474.2 $(M+H)^+$, T_R = 1.58 min.



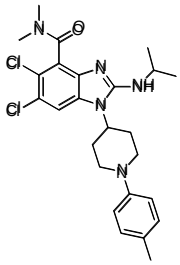
5,6-Dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-((1-phenylpiperidin-4-yl)methyl)-1*H*-benzo[*d*]imidazole-4-carboxamide (2)

Compound **2** was synthesized from 3,4-dichloro-6-fluorobenzonitrile and *tert*-butyl 4-(aminomethyl)piperidine-1-carboxylate using the same procedures followed for the synthesis of compound **1**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 (s, 1H), 7.25 (m, 2H), 7.05 (m, 2H), 6.87 (m, 1H), 4.11 (dd, *J* = 5.37, 14.16 Hz, 1H), 4.06 (d, *J* = 7.32 Hz, 2H), 3.64 - 3.74 (m, 2H), 3.07 (s, 3H), 2.77 (s, 3H), 2.69 - 2.76 (m, 2H), 2.00 (m, 1H), 1.59 - 1.68 (m, 2H), 1.40 – 1.50 (m, 2H), 1.27 (d, *J* = 6.1 Hz, 3H), 1.26 (d, *J* = 6.1 Hz, 3H); LCMS 488.2 $(M+H)^+$, T_R = 1.31 min.



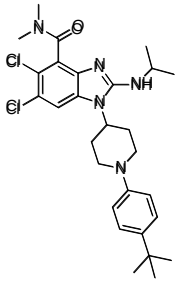
5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(1-(*meta*-tolyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide (12)

The title compound was prepared and purified by the general procedure outlined above to provide the title compound (20 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 (s, 1H), 7.18 (t, *J* = 7.81 Hz, 1H), 6.93 (s, 1H), 6.89 (d, *J* = 7.81 Hz, 1H), 6.72 (d, *J* = 7.32 Hz, 1H), 4.51 - 4.61 (m, 1H), 4.08 (dd, *J* = 6.35, 12.70 Hz, 1H), 3.88 (d, *J* = 12.70 Hz, 2H), 3.06 (s, 3H), 2.94 (dd, *J* = 7.81, 18.56 Hz, 1H), 2.76 (s, 3H), 2.38 – 2.48 (m, 2H), 2.29 (s, 3H), 1.83 - 1.95 (m, 2H), 1.27 (d, *J* = 1.95 Hz, 3H), 1.26 (d, *J* = 2.44 Hz, 3H); LCMS 488.2 $(M+H)^+$, T_R = 1.62 min.



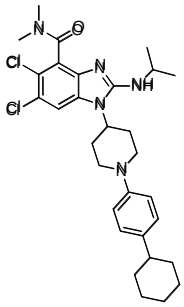
5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(1-(*para*-tolyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazole-4-carboxamide (13)

The title compound was prepared and purified by the general procedure outlined above to provide the title compound (46 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61 (s, 1H), 7.17(d, *J* = 7.32 Hz, 2H), 7.12 (d, *J* = 7.32 Hz, 2H), 4.61 (m, 1H), 4.09 (dd, *J* = 5.86, 12.21 Hz, 1H), 3.80 – 3.86 (m, 2H), 3.06 (s, 3H), 3.00 – 3.10 (m, 2H), 2.77 (s, 3H), 2.27 (s, 3H), 2.46 – 2.58 (m, 2H), 1.88 - 2.01 (m, 2H), 1.28 (d, *J* = 6.35 Hz, 3H), 1.26 (d, *J* = 6.35 Hz, 3H); LCMS 488.3 $(M+H)^+$, T_R = 1.51 min.



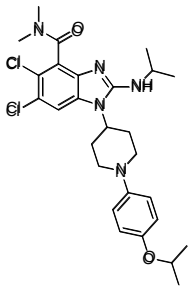
1-(1-(4-(*tert*-butyl)phenyl)piperidin-4-yl)-5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (14)

The title compound was prepared and purified by the general procedure outlined above to provide the title compound (33 %). 1H NMR (500 MHz, DMSO-*d*6) δ 7.47 (s, 1H), 7.34 (d, *J* = 8.30 Hz, 2H), 7.07 (d, *J* = 7.81 Hz, 2H), 4.51 - 4.60 (m, 1H), 4.07 (dd, *J* = 6.84, 13.67 Hz, 1H), 3.81 - 3.88 (m, 2H), 3.06 (s, 3H), 2.92 - 3.03 (m, 2H), 2.76 (s, 3H), 2.39 - 2.49 (m, 2H), 1.83 - 1.95 (m, 2H), 1.27 (s, 9H), 1.26 (d, *J* = 6.84 Hz, 6H); LCMS 488.3 $(M+H)^+$, T_R = 1.51 min.



5,6-dichloro-1-(1-(4-cyclohexylphenyl)piperidin-4-yl)-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (15)

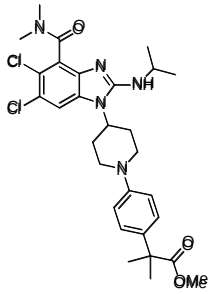
The title compound was prepared and purified by the general procedure outlined above to provide the title compound (28 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.97 (s, 1H), 7.55 (d, *J* = 8.78 Hz, 2H), 7.35 (d, *J* = 8.53 Hz, 2H), 3.71 - 4.02 (m, 5H), 3.20 - 3.34 (m, 2H), 3.16 (s, 3H), 2.96 (s, 3H), 2.55 (br. s., 1H), 2.22 (br. s., 2H), 1.86 (d, *J* = 7.53 Hz, 4H), 1.73 - 1.81 (m, 1H), 1.38 - 1.45 (m, 3H), 1.36 (d, *J* = 6.53 Hz, 3H), 1.25 (d, *J* = 6.53 Hz, 3H); LCMS 556.3 $(M+H)^+$, T_R = 1.94 min.



5,6-dichloro-1-(1-(4-isopropoxyphenyl)piperidin-4-yl)-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (16)

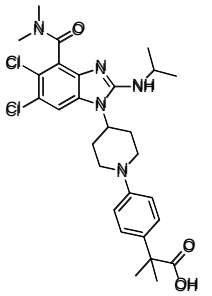
The title compound was prepared and purified by the general procedure outlined above to provide the title compound (20 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.34 (s, 1H), 6.94 (d, *J* = 9.03 Hz, 2H), 6.86 (d, *J* = 9.03 Hz, 2H), 4.54 (d, *J* = 7.03 Hz, 1H), 4.48 (m, *J* = 6.02 Hz, 1H), 4.07

(m, *J* = 7.03 Hz, 1H), 3.98 (m, *J* = 3.51, 8.03, 12.17 Hz, 1H), 3.58 - 3.68 (m, 2H), 3.21 (s, 3H), 2.89 (s, 3H), 2.72 - 2.83 (m, 2H), 2.33 - 2.51 (m, *J* = 5.02, 13.05, 25.60 Hz, 2H), 1.86 - 1.95 (m, 1H), 1.79 (d, *J* = 11.04 Hz, 1H), 1.33 (d, *J* = 6.02 Hz, 6H), 1.23 (d, *J* = 6.27 Hz, 3H), 1.19 (d, *J* = 6.53 Hz, 3H); LCMS 533.3 $(M+H)^+$, T_R = 1.54 min.



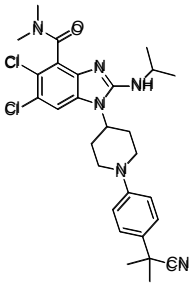
methyl 2-(4-(4-(5,6-dichloro-4-(dimethylcarbamoyl)-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)piperidin-1-yl)phenyl)-2-methylpropanoate (17)

The title compound was prepared and purified by the general procedure outlined above to provide the title compound (17 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 9.01 (br. s., 2H), 7.97 (s, 1H), 7.58 (d, *J* = 8.78 Hz, 2H), 7.51 (d, *J* = 8.78 Hz, 2H), 5.34 (m, 1H), 3.75 - 4.03 (m, 5H), 3.68 (s, 3H), 3.21 - 3.31 (m, 2H), 3.18 (s, 3H), 2.99 (s, 3H), 2.21 - 2.34 (m, 2H), 1.59 (s, 6H), 1.35 (d, *J* = 6.02 Hz, 3H), 1.22 (d, *J* = 6.02 Hz, 3H); LCMS 574.3 $(M+H)^+$, T_R = 2.39 min.



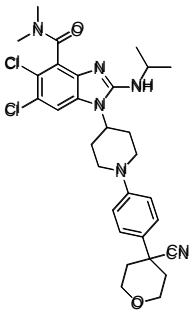
2-(4-(4-(5,6-dichloro-4-(dimethylcarbamoyl)-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)piperidin-1-yl)phenyl)-2-methylpropanoic acid (18)

Methyl 2-(4-(4-(5,6-dichloro-4-(dimethylcarbamoyl)-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)piperidin-1-yl)phenyl)-2-methylpropanoate (**17**) (0.074 g, 0.129 mmol) was dissolved in THF (0.52 mL), methanol (0.52 mL), and water (0.26 mL) and treated with KOH (0.036 g, 0.644 mmol). The solution was heated to 50 °C for 18 h. The reaction was then cooled to ambient temperature and quenched with saturated aqueous NH_4Cl solution (1 mL). The organics were diluted with EtOAc, and the layers were separated. The aqueous layer was washed with EtOAc (2 x 5 mL). The combined organics were dried over $MgSO_4$, filtered, and concentrated. The crude product was dissolved in DMSO (1 mL) and submitted to reverse phase HPLC purification (Sunfire Prep C18 column, 30 x 150 mm; 16 min run with at-column dilution; 20 – 60 % MeCN/water/0.1 % TFA gradient elution; 220 nm detection) to provide the title compound (14 %). ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.93 (s, 1H), 7.47 (d, *J* = 9.03 Hz, 2H), 7.29 (d, *J* = 8.78 Hz, 2H), 4.75 – 4.85 (m, 2H), 4.03 - 4.13 (m, *J* = 6.43, 6.43 Hz, 1H), 3.89 - 3.97 (m, 2H), 3.23 - 3.29 (m, 1H), 3.20 (s, 3H), 2.94 (s, 3H), 2.67 - 2.83 (m, 2H), 2.11 - 2.20 (m, 2H), 1.57 (s, 6H), 1.43 (d, *J* = 6.40 Hz, 3H), 1.41 (d, *J* = 6.40 Hz, 3H); LCMS 560.2 $(M+H)^+$, T_R = 2.30 min.



5,6-dichloro-1-(1-(4-(2-cyanopropan-2-yl)phenyl)piperidin-4-yl)-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (19)

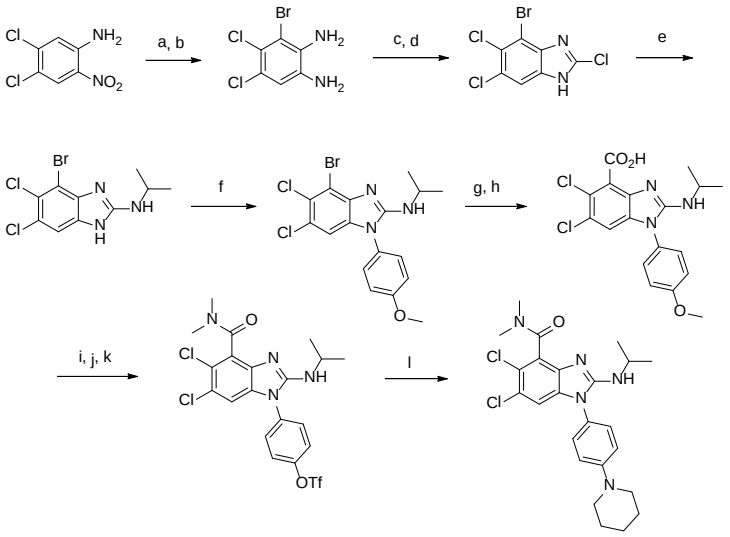
The title compound was prepared and purified by the general procedure outlined above to provide the title compound (48 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.80 (s, 1H), 7.57 (d, *J* = 9.03 Hz, 2H), 7.50 (d, *J* = 8.78 Hz, 2H), 5.28 (m, 1H), 4.01 (m, 1H), 3.74 - 3.85 (m, 2H), 3.57 - 3.71 (m, 2H), 3.16 (s, 3H), 2.96 (s, 3H), 2.92 – 3.30 (m, 2H), 2.10 - 2.22 (m, 2H), 1.74 (s, 6H), 1.34 (d, *J* = 6.27 Hz, 3H), 1.24 (d, *J* = 6.27 Hz, 3H); ^{13}C NMR (126 MHz, METHANOL-d_4) δ 165.6, 151.8, 151.2, 135.2, 130.8, 128.7, 128.5, 127.3, 126.0, 124.5, 123.2, 118.7, 115.0, 54.8, 50.7, 38.1, 35.0, 29.4, 28.8, 22.7; LCMS 541.3 $(M+H)^{+}$, T_R = 1.75 min; HRMS 541.2249 ($C_{28}H_{35}N_6OCl_2$); see appendix for attached NMR spectra.



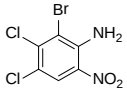
5,6-dichloro-1-(1-(4-(4-cyanotetrahydro-2*H*-pyran-4-yl)phenyl)piperidin-4-yl)-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (20)

The title compound was prepared and purified by the general procedure outlined above to provide the title compound (7 %). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 9.11 (br. s., 1H), 7.65 (s, 1H), 7.51 (d, *J* = 9.03 Hz, 2H), 7.35 (d, *J* = 8.78 Hz, 2H), 5.12 (m, 1H), 4.01 - 4.16 (m, 3H), 3.85 – 3.95 (m, 2H), 3.76 - 3.85 (m, 2H), 3.36 - 3.51 (m, 2H), 3.05 – 3.20 (m, 2H), 2.93 (s, 3H), 2.65 – 2.85 (m, 2H), 2.67 (s, 3H), 2.00 - 2.18 (m, 4H), 1.29 (d, *J* = 6.27 Hz, 3H), 1.23 (d, *J* = 6.27 Hz, 3H); LCMS 583.2 $(M+H)^{+}$, T_R = 1.70 min.

Synthesis of 5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(4-piperidin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole-4-carboxamide (3)[b]

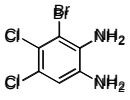


[b](a) Br_2, $(ClCH_2)_2$, 97 °C, (b) $SnCl_2$, 70 °C, (c) CDI, MeCN, 80 °C, (d) $POCl_3$, 120 °C, (e) $^{i}PrNH_2$, 130 °C, (f) 4-MeO-PhB$(OH)_2$, $Cu(OAc)_2$, TEA, (g) CuCN, DMF, 180 °C, (h) KOH, $(CH_2OH)_2$, 180 °C, (i) Me_2NH, T3P, DIEA, (j) BBr_3, DCM, -78 °C, (k) Tf_2O, TEA, DCM, 0 °C, (l) piperidine, Cs_2CO_3, $Pd(OAc)_2$, (2-biphenyl)di-*tert*-butylphosphine, dioxane, 100 °C



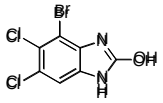
2-bromo-3,4-dichloro-6-nitroaniline

4,5-dichloro-2-nitroaniline (10.0 g, 48.3 mmol) and bromine (3.0 mL, 58.2 mmol) in 1,2-dichloroethane (120 mL) was stirred at 97 °C (with a condenser) for 18 h. After cooling to ambient temperature, the reaction was washed with $Na_2S_2O_3$ (2 x 10 mL). The organics were dried over Na_2SO_4, filtered, and concentrated to give the desired product (99%) as a bright yellow solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 6.82 (br. s., 2 H) 8.34 (s, 1 H).



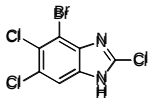
3-bromo-4,5-dichlorobenzene-1,2-diamine

A suspension of 2-bromo-3,4-dichloro-6-nitroaniline (8.00 g, 28.0 mmol) and tin(II) chloride (22.0 g, 116 mmol) in ethanol (300 mL) was stirred at 70 °C for 4 h. The reaction was cooled to ambient temperature and poured into ice water. Saturated aqueous $NaHCO_3$ was added, and the reaction mixture was extracted into EtOAc. The organic layer was washed with brine (1 x 10 mL), dried over Na_2SO_4, filtered, and concentrated to give the desired product (100%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.10 (s, 2H) 5.30 (s, 2H) 6.71 (s, 1H); LCMS 256.8 $(M+H)^+$, T_R = 2.63 min.



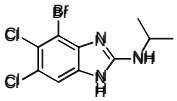
4-bromo-5,6-dichloro-1*H*-benzo[*d*]imidazol-2-ol

A solution of 3-bromo-4,5-dichloro-1,2-benzenediamine (7.17 g, 28.0 mmol) and CDI (13.6 g, 84.0 mmol) in MeCN (100 mL) was stirred at 80 °C for 3 h. After this time, a yellow solid precipitated out of the reaction mixture. The reaction was allowed to cool to room temperature, and the yellow solid was collected by filtration. The solid was washed with water, MeCN, and air dried to give the desired product (92%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 11.21 (s, 1H), 7.16 (s, 1H); LCMS 282.9 $(M+H)^+$, T_R = 2.45 min.



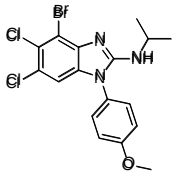
4-bromo-2,5,6-trichloro-1*H*-benzo[*d*]imidazole

A suspension of 4-bromo-5,6-dichloro-1*H*-benzo[*d*]imidazol-2-ol (7.36 g, 26.1 mmol) in phosphorus oxychloride (30 mL, 322 mmol) was heated to 120 °C for 3 days. The reaction was concentrated to a solid and then suspended in ice water. The resulting reaction mixture was neutralized with K_2CO_3 powder until pH~9. A resulting solid was collected by filtration, washed with water, and air dried to give the desired product (98%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 7.82 (s, 1H); LCMS 300.8 $(M+H)^+$, T_R = 2.80 min.



4-bromo-5,6-dichloro-*N*-isopropyl-1*H*-benzo[*d*]imidazol-2-amine

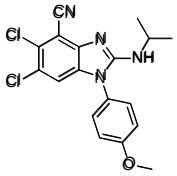
A suspension of 4-bromo-2,5,6-trichloro-1H-benzimidazole (7.65 g, 25.5 mmol) and isopropylamine (22 mL, 258 mmol) in 1,4-Dioxane (5 mL) was placed in a sealed tube and heated to 130 °C for 4 days. After cooling to ambient temperature, the reaction was concentrated and purified on a 120 g silica gel column (0 - 50 % EtOAc/hexanes gradient elution; 20 min; 50 % EtOAc/hexanes, 5 min) to give the desired product (34%) as a beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (br. s., 1H), 7.29 (s, 1H), 7.14 (d, J = 8.28 Hz, 1H), 3.83 - 4.01 (m, 1H), 1.20 (d, J = 6.53 Hz, 6H); LCMS 300.8 $(M+H)^+$, T_R = 2.80 min.



4-bromo-5,6-dichloro-*N*-isopropyl-1-(4-methoxyphenyl)-1*H*-benzo[*d*]imidazol-2-amine

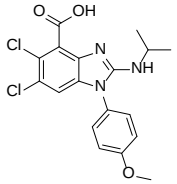
A suspension of 4-bromo-5,6-dichloro-N-(1-methylethyl)-1H-benzimidazol-2-amine (1.70 g, 5.26 mmol), copper(II) acetate (3.82 g, 21.1 mmol), [4-(methyloxy)phenyl]boronic acid (1.60 g, 10.5 mmol), and triethylamine (2.93 mL, 21.1 mmol) in 1,2-dichloroethane (17 mL) was stirred at room temperature for 2 days. Water was added, and the reaction was filtered through a pad of Celite. The filtrate was extracted with DCM, dried over Na_2SO_4, filtered, and concentrated. The residue was purified on a 40 g silica gel column (0 – 50 % EtOAc/hexanes gradient elution, 20 min; 50 % EtOAc/hexanes, 5 min) to give the desired

product (43%). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.29 (d, *J* = 8.78 Hz, 2H), 7.12 (d, *J* = 8.78 Hz, 2H), 6.88 (s, 1H), 4.24 (m, 1H), 3.92 (s, 3H), 1.25 (d, *J* = 6.27 Hz, 6H); LCMS 430.0, 431.9 $(M+H)^+$, T_R = 2.75 min.



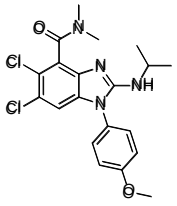
5,6-dichloro-2-(isopropylamino)-1-(4-methoxyphenyl)-1*H*-benzo[*d*]imidazole-4-carbonitrile

4-bromo-5,6-dichloro-N-(1-methylethyl)-1-[4-(methyloxy)phenyl]-1*H*-benzimidazol-2-amine (1.05 g, 2.44 mmol) and copper(I) cyanide (1.55 g, 17.3 mmol) in DMF (5 mL) was heated to 180 °C in a microwave reactor for 1 h. The reaction was cooled to ambient temperature, quenched with brine, and stirred for 15 min. The reaction mixture was washed with EtOAc (3 x 10 mL), and the combined organic extracts were washed with brine (1 x 10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified on a 40 g silica gel column (0 – 25 % EtOAc/hexanes gradient elution, 15 min; 25 % EtOAc/hexanes, 5 min; 25 – 50 % EtOAc/hexanes gradient elution, 15 min) to give the desired product (74%) as an off-white solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.28 (d, *J* = 9.03 Hz, 2H), 7.13 (d, *J* = 9.03 Hz, 2H), 7.01 (s, 1H), 4.36 (m, 1H), 3.92 (s, 3H), 1.27 (d, *J* = 6.27 Hz, 6H); LCMS 375.0 $(M+H)^+$, T_R = 3.20 min.



5,6-dichloro-2-(isopropylamino)-1-(4-methoxyphenyl)-1*H*-benzo[*d*]imidazole-4-carboxylic acid

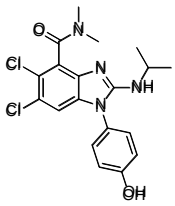
A suspension of 5,6-dichloro-2-[(1-methylethyl)amino]-1-[4-(methyloxy)phenyl]-1*H*-benzimidazole-4-carbonitrile (0.679 g, 1.81 mmol) and potassium hydroxide (0.609 g, 10.9 mmol) in ethylene glycol (4 mL) and water (0.2 mL) was subjected to microwave heating at 180 °C for 1 h, followed by heating at 185 °C for 1 h. The reaction was cooled to room temperature and acidified with 6 N HCl until pH ~5. The resulting solid precipitate was filtered, washed with H_2O, and air dried to give the desired product (0.673 g, 80 % yield, 85 % pure) as a beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38 (d, *J* = 8.78 Hz, 2H), 7.17 (d, *J* = 8.78 Hz, 2H), 6.86 (s, 1H), 6.53 (d, *J* = 8.03 Hz, 1H), 4.10 (m, *J* = 6.53, 13.30, 19.83 Hz, 1H), 3.38 (s, 3H), 1.16 (d, *J* = 6.53 Hz 6H); LCMS 394.0 $(M+H)^+$, T_R = 2.36 min.



5,6-dichloro-2-(isopropylamino)-1-(4-methoxyphenyl)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide

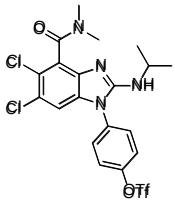
T3P (4.00 mL, 6.72 mmol) was added to a 0 °C solution of 5,6-dichloro-2-[(1-methylethyl)amino]-1-[4-(methyloxy)phenyl]-1*H*-benzimidazole-4-carboxylic acid (0.673 g, 1.71 mmol), dimethylamine hydrochloride (0.696 g, 8.54 mmol) and diisopropylethylamine (5.00 mL, 28.6 mmol) in dichloromethane (5.0 mL). After stirring for 30 min at 0 °C, the reaction was allowed to warm to ambient

temperature overnight. The reaction was partitioned between EtOAc and water, and the organic layer was dried over $MgSO_4$, filtered, and concentrated to give the crude product (0.630 g, 88 %) as a beige solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.42 (d, *J* = 8.78 Hz, 2H), 7.18 (d, *J* = 9.03 Hz, 2H), 6.95 (s, 1H), 3.95 (s, 3H), 4.00 – 3.90 (m, 1H), 3.23 (s, 3H), 3.02 (s, 3H), 1.21 (dd, *J* = 6.53, 2.01 Hz, 6H); LCMS 421.1 $(M+H)^+$, T_R = 2.29 min.



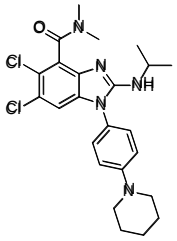
5,6-dichloro-1-(4-hydroxyphenyl)-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide

Boron tribromide (0.500 mL, 5.29 mmol) was added to a -78 °C solution of 5,6-dichloro-2-(isopropylamino)-1-(4-methoxyphenyl)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (0.548 g, 1.30 mmol) in dichloromethane (10.0 mL). The resulting reaction mixture was stirred at -78 °C for 1 h and then allowed to warm slowly to ambient temperature overnight. The reaction was cooled to 0 °C and quenched with water (2 mL) and saturated aqueous sodium bicarbonate solution (2 mL). A gray precipitate was collected in a frit, washed with water, and air dried to provide the desired product (0.500 g, 94 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 7.24 (d, *J* = 8.78 Hz, 2H), 6.97 (d, *J* = 8.78 Hz, 2H), 6.83 (s, 1H), 6.35 (d, *J* = 8.28 Hz, 1H), 4.06 (m, *J* = 6.53, 13.05, 21.33 Hz, 1H), 3.06 (s, 3H), 2.80 (s, 3H), 1.15 (dd, *J* = 6.53, 5.02 Hz, 6H); LCMS 407.1 $(M+H)^+$, T_R = 2.09 min.



4-(5,6-dichloro-4-(dimethylcarbamoyl)-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)phenyl trifluoromethanesulfonate

Trifluoromethanesulfonic anhydride (0.101 mL, 0.600 mmol) was added to a 0 °C solution of 5,6-dichloro-1-(4-hydroxyphenyl)-2-(isopropylamino)-*N,N*-dimethyl-1*H*-benzo[*d*]imidazole-4-carboxamide (0.122 g, 0.300 mmol) and triethylamine (0.167 mL, 1.20 mmol) in dichloromethane (3.0 mL). After stirring at room temperature for 2 h, water was added (3 mL). The layers were separated, and the aqueous layer was washed with dichloromethane (2 x 10 mL). The combined organics were dried over sodium sulfate, filtered, and concentrated. The crude product was purified on a 12 g silica gel column (0 – 50 % EtOAc/hexanes gradient elution, 15 min; 50 % EtOAc/hexanes, 3 min; 50 – 100 % EtOAc/hexanes gradient elution, 18 min; 100 % EtOAc, 8 min) to provide the desired product (0.100 g, 64 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, *J* = 9.03 Hz, 2H), 7.72 (d, *J* = 9.29 Hz, 2H), 6.99 (s, 1H), 6.72 (d, *J* = 8.03 Hz, 1H), 4.08 (m, *J* = 6.53, 13.05, 20.33 Hz, 1H), 3.07 (s, 3H), 2.81 (s, 3H), 1.18 (d, *J* = 5.02 Hz, 6H); LCMS 539.0 $(M+H)^+$, T_R = 2.62 min.



5,6-dichloro-2-(isopropylamino)-*N,N*-dimethyl-1-(4-(piperidin-1-yl)phenyl)-1*H*-benzo[*d*]imidazole-4-carboxamide

A suspension containing 4-(5,6-dichloro-4-(dimethylcarbamoyl)-2-(isopropylamino)-1*H*-benzo[*d*]imidazol-1-yl)phenyl trifluoromethanesulfonate (0.100 g, 0.185 mmol), piperidine (0.037 mL, 0.371 mmol), cesium carbonate (0.060 g, 0.185 mmol), (2-biphenyl)di-*tert*-butylphosphine (0.011 g, 0.037 mmol), and palladium (II) acetate (0.010 g, 0.045 mmol) in 1,4-dioxane (1.0 mL) was stirred at 100 °C for 18 h. The reaction was filtered through celite and purified by reverse phase-HPLC (Sunfire C18 OBD column; 20 - 60 % MeCN/water (0.1 % TFA) gradient; 16 min run ^{w}/ at-column dilution; flow rate = 45 mL/min) and concentrated. The crude product was purified a second time by reverse phase-HPLC (Sunfire C18 OBD column; 50 – 100 % MeCN/water (0.1 % NH_4OH) gradient; 16 min run ^{w}/at-column dilution; flow rate = 45 mL/min) to provide the desired product (0.003 g, 2.9 %) as a white solid. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ 7.19 (d, *J* = 8.28 Hz, 2H), 7.10 (d, *J* = 6.27 Hz, 2H), 6.93 (s, 1H), 4.33 (m, 1H), 3.34 - 3.32 (m, 4H), 3.27 (s, 3H), 2.99 (s, 3H), 1.73 - 1.83 (m, 4H), 1.61 - 1.71 (m, 2H), 1.22 (dd, *J* = 6.53, 8.28 Hz, 6H); LCMS 474.1 $(M+H)^{+}$, T_R = 2.28 min.

Assay Procedures:

TRPV4 FLIPR Assay: HEK MSRII cells were thawed and suspended in the cell plating medium (DMEM/F12 1:1 with L-glutamine with 15 mM HEPES @ pH 7.3, 10 % FBS, 1 % Penicillin-Streptomycin solution, 1 % L-glutamine) at 15 K cells/50 uL. TRPV4 BacMam virus was added to the cells at a final concentration of 1 % and gently mixed. Cells were then plated at 15 K cells/well, allowed to stand at room temperature for 1 h and then incubated at 37 °C with 5 % CO_2 for 24 to 72 h in a tissue culture incubator. Media is then removed and cells were dye loaded with 2 uM Fura-4, 0.5 mM Brilliant Black (Molecular Devices Corporation R8033-BLA4) and 2.5 uM Probenecid (Sigma P8761). Plates are then run on a FLIPR Tetra 384. Blockers are added 10 min prior to TRPV4 activation with GSK1016790A. Data analysis was conducted following a 11 point blocker concentration curve using the Activity Base XE curve fitting module.

cardiac ion channel selectivity assays: Full curve IC_{50} data was generated for hERG on a PatchXpress 7000A parallel patch clamp system (Molecular Devices) using HEK293 cells stably expressing hERG (incubated at 37 °C in 5 % CO_2). A five-point serial dilution of test compound is performed from DMSO stock solution. Full curve IC_{50} data was generated for the K v1.5 channel on a PatchXpress 7000A parallel patch clamp system (Molecular Devices) using CHO cells stably expressing the human K v1.5 channel. Full curve IC_{50} data was generated for the Ca v1.2 (L-type) channel by measuring fluorescence due to calcium immobilization in HEK293 cells on a FLIPR Tetra 384 (Molecular

Devices) with a Calcium 4 assay kit (Molecular Devices Corporation R8141). HEK cells were stably expressed with human recombinant auxiliary calcium channel subunits: $\alpha 2\delta 1$ and $\beta 2a$ and treated with a BacMam encoding the pore forming $\alpha 1C$ subunit on the day prior to the assay. HEK cells were prepared by diluting to 300K/mL in DMEM/F12 containing 10 % FBS. Full curve IC_{50} data was generated for the Na v1.5 channel on an IonWorks Quattro 384 (Molecular Devices) using HEK293 cells stably expressing the human Na v1.5 channel.

Pharmacokinetic Studies:

Pharmacokinetic studies were conducted on a single day in male Sprague-Dawley rats using a non-crossover design with two animals per route of administration (i.v. and p.o.). Studies were conducted in a cassette fashion with up to 5 compounds per cassette. At least three days prior to the start of the study, rats received surgically implanted femoral vein, femoral artery, and gastric catheters for *i.v.* infusion of the test molecules, blood sampling, and oral dose administration, respectively. Dose solutions were filtered prior to administration with the actual dose administered quantified. All PK parameters were calculated based on actual dosage administered to each animal. For *i.v.* infusion, a dose volume of 4 mL/kg in 5 % DMSO, 20 % Cavitron (pH 4) was administered for a target dose of 1 mg/kg; i.v. infusions were carried out over 30 minutes. Oral administration via gastric bolus used a 16 mL/kg dose volume in 5 % DMSO, 6 % Cavitron (pH 4) for a target dose of 2 mg/kg. Blood samples (110 uL) were collected into sodium heparinized tubes at predetermined time points up to 24 hours following compound administration. Blood samples were centrifuged to obtain plasma, and 30 uL plasma aliquots of each sample were either analyzed immediately or were stored at -80°C until analyzed. Proteins were precipitated by the addition of 120 uL of acetonitrile containing an appropriate internal standard. Samples were centrifuged to remove precipitate, and aliquots of the resulting supernatant were analyzed for test compound concentrations by HPLC-MS/MS. Analytes were quantified by comparison to a standard curve prepared in identical matrix using Analyst 1.5.1 software. Pharmacokinetic parameters were calculated via noncompartmental analysis with linear-log trapezoidal calculation of area under the curve using Phoenix Winnonlin 6.1.0 software. Oral bioavailability was calculated in a non-crossover fashion for each oral study animal by the ratio of oral dose-normalized area under the curve (0-∞) to the mean i.v. dose-normalized area under the curve (0-∞).

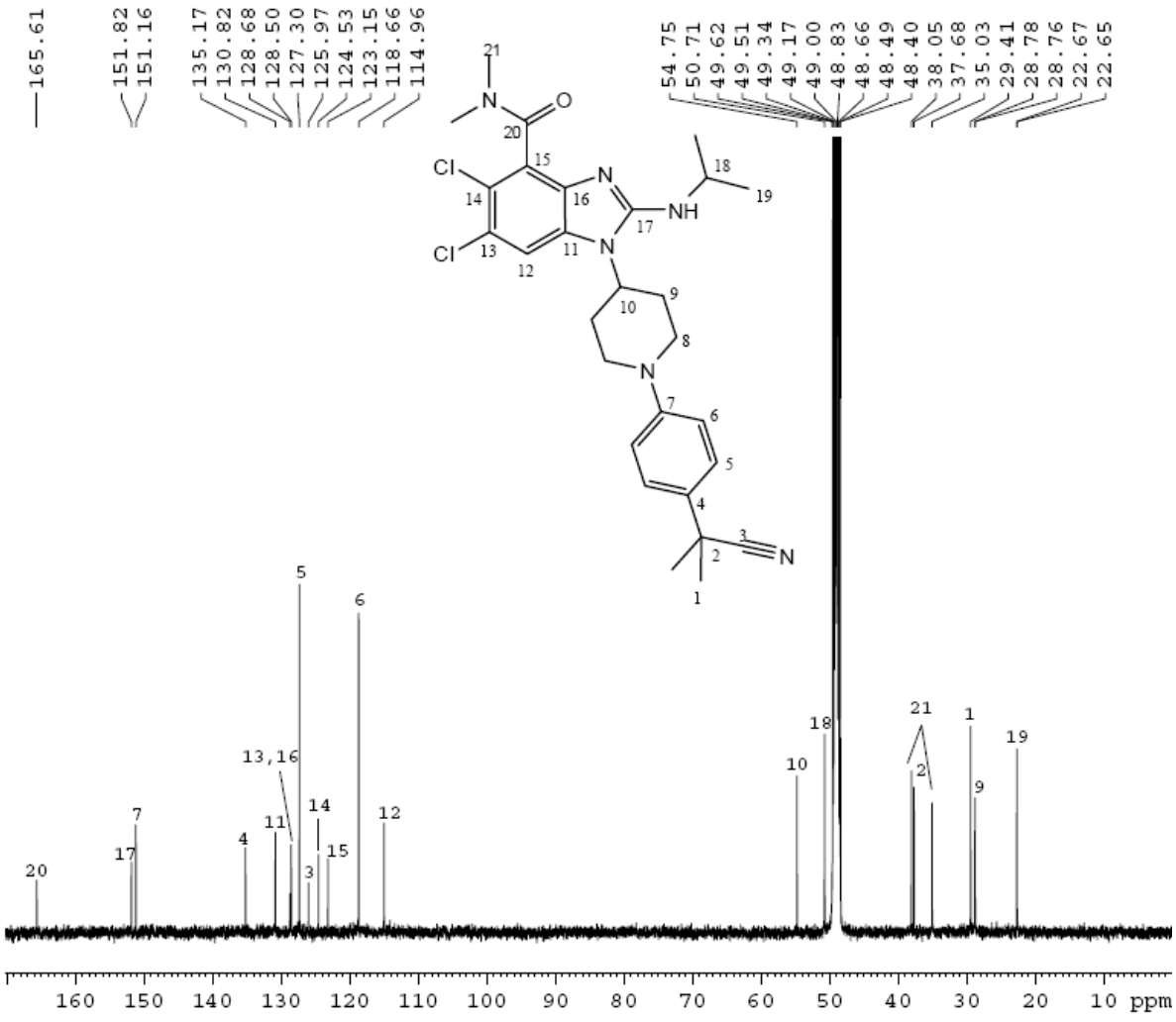
Pharmacodynamic Study:

Male Sprague-Dawley rats with jugular and carotid catheters (Charles River – Raleigh) were anesthetized with 2-4% isoflurane, weighed and catheters removed from the nap of the neck. A switch to urethane anesthetic was performed by slowly dosing 1.2 g/kg *i.v.* urethane (0.5g/mL stock in saline) in the absence of isoflurane. The carotid IA catheter was connected via a 3 way connector to a Harvard infusion pump and a pressure tranducer. Saline infusion (10 μL/min) was maintained during the study to keep the IA line patent. Control hemodynamics were recorded for ~30 min (HR, MAP) prior to dosing the TRPV4 antagonist, **19**/vehicle via the *i.v.* line. 10 min

later the GSK1016790A dose response was started with doses separated by 5 min periods (0.0003, 0.001, 0.003, 0.01, 0.03, 0.1. 0.2 mg/kg *i.v.*). At lower doses the drop in mean arterial pressure in response to GSK1016790A was transient and therefore changes in MAP were measured as the peak drop in mean arterial pressure, assessed as a 1 min average centered at the peak drop during the 5 min time frame. At the end of the study blood was collected from the IA line for analysis of test compound concentrations into lithium heparinized tubes. Lithium tubes were spun for 3 min at 10,000 rpm and plasma was drawn into separate tubes for storage at -80 C. Sample analysis was performed as described above for pharmacokinetic studies. Both lungs were harvested and weighed and a lung/body weight ratio was calculated. Compound **19** was dosed in 2 % DMSO, PEG-200. TRPV4 agonist, GSK1016790A, was dosed in 1 % DMSO, 29 % Cavitron.

Appendix

^{13}C-NMR of compound **19** in *d*-MeOD at 126 MHz



^{1}H-NMR of compound **19** in *d*-MeOD at 500 MHz