Supplemental Material

Supp. Material 1. General Chemical Methods for Benzamidobenzoic Acid Compounds

Supplemental Figures

Supp. Fig. 1. Characterization of cyst-like structures after growth of amoeba under different culture conditions. (A.) Trophozoites were cultured in standard NCM (Glc), NCM with no glucose but supplemented with 9 mM mannose (Man), NCM without glucose (-Glc), or in NCM with 50 mM 3-bromopyruvate (3BP). Images are before (left) and after (right) addition of 0.25% SDS. (B.) Trophozoite recovered from cysts generated by culture in NCM with no glucose supplemented by mannose. Scale bar = 20 μm.

Supplemental Data Files

Supp. Data 1. Alignment of HKs and Glcks. HKs and Glcks with resolved structures were aligned using T-Coffee (version 11.00.d625267 (2016-01-11 15:25:41 – Revision d625267 – Build 507), which uses structural information to inform the alignment. The yellow highlights denote residues that interact with glucose in the binding pocket based on PDB site. The first 4 characters are the PDB ID. HsGCK, human glucokinase; KlHK; *K. lactis* hexokinase, ScHK P1, yeast hexokinase P1, TcGlcK, *T. cruzi* glucokinase, NfGlcK, *N. fowleri* glucokinase. The "*" indicated in the consensus line (cons) are fully conserved residues, the ":" indicates highly similar residue conservation, and "." indicates weak conservation.

Supplemental Material 1

Enzymatic and structural characterization of the *Naegleria fowleri* glucokinase

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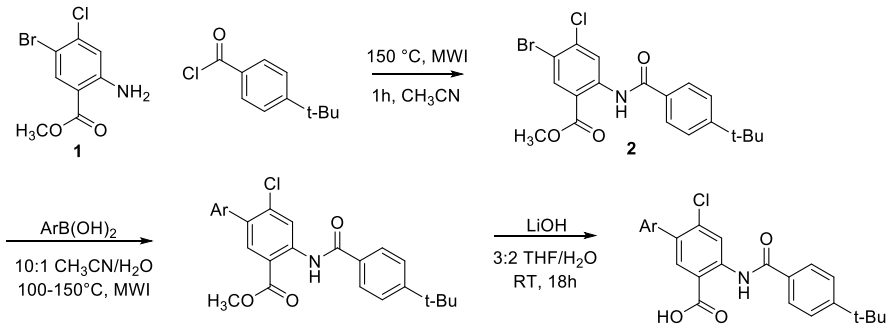
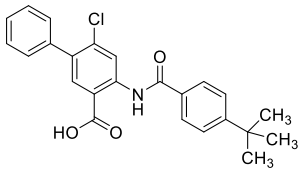
General Chemical Methods for Benzamidobenzoic Acid Compounds (Golden group, University of Wisconsin-Madison)

Compounds not described below were purchased from commercial vendors. Purity of all final compounds was confirmed by HPLC/MS analysis and determined to be ≥ 95%. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-Inova 400 MHz NMR Spectrometer (operating at 400 and 101 MHz, respectively) or a Varian Unity-Inova 500 MHz NMR Spectrometer (operating at 500 and 126 MHz, respectively) or Bruker Ascend 400 MHz Spectrometer (operating at 400 and 101 MHz, respectively) in $CDCl_3$ (residual internal standard $CHCl_3$ = δ 7.26), DMSO-d_6 (residual internal standard CD_3SOCD_2H = δ 2.50), CD_3OD (residual internal standard CD_3OH = δ 3.31), or acetone-d_6 (residual internal standard CD_3COCD_2H = δ 2.05). The chemical shifts (δ) reported are given in parts per million (ppm), and the coupling constants (J) are in hertz (Hz). The spin multiplicities are reported as s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, p = pentuplet, dd = doublet of doublet, dt = doublet of triplet, td = triplet of doublet, tt = triplet of triplet, and m= multiplet. Flash chromatography separations were carried out using a Teledyne Isco CombiFlash Rf 200 purification system with silica gel columns. The LC-MS analysis was performed on an Agilent 1290 Infinity II HPLC system with 1290 Infinity II Diode Array Detector and an Agilent 6120 Quadrupole LC-MS system. The analytical chromatography method utilized the following parameters: Poroshell 120 EC-C18, 1.9 µm column, UV detection wavelength = 254 nm, Flow rate = 1.0 mL/min, Gradient = 5-100% LC-MS grade Methanol over 4 min; The organic mobile phase and aqueous mobile phase contained 0.1% LC-MS grade formic acid. The mass spectrometer utilized the following parameters: an Agilent multimode source that simultaneously acquires ESI+/APCI+; Final compounds were determined to be ≥ 95% purity by UV-LCMS at 254 nm.

The syntheses and characterization of several benzamidobenzoic acid compounds listed herein have been previously described. Specifically, SID144241589 (ML205, entry 5, Table 1) is described in a probe report on the NIH bookshelf.[1] SID162211071, SID162211077, SID144241590, SID162211078, SID162211083, SID162211084, SID162211075, SID162211076, SID162211086, and SID162211072 (entries 6-11, 14-15, and 18-19, Table 1) have been previously reported.[2] Characterization of resynthesized compounds matched previous reports. In some cases, compounds were prepared by modified methods, which are described below. New compounds are also described.

Scheme 1. General synthetic approach to benzamidobenzoic acids





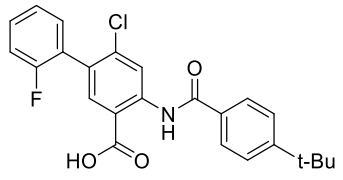
SID144241590

4-(4-*(t*-butyl)benzamido)-6-chloro-[1,1'-biphenyl]-3-carboxylic acid, SID144241590, entry 8, Table 1. Prepared according to Scheme 1.

Step 1: To a G30 microwave vial was added CH_3CN (10.0 mL), methyl 2-amino-4-chloro-5-bromobenzoate (2.0 g, 7.6 mmol, 1 equiv.), and 4-*t*-butylbenzoyl chloride (1.6 mL, 8.3 mmol, 1.1 equiv.). The reaction was sealed and heated at 150 °C by microwave irradiation for 1h with stirring and then allowed to cool to rt. The resulting precipitate was filtered to collect the desired product **2** as an oatmeal-colored solid (1.6 g, 3.8 mmol, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=11.94 (br s, 1H), 9.20 (s, 1H), 8.29 (s, 1H), 7.95 (d, *J*=8.8 Hz, 2H), 7.55 (d, *J*=8.8 Hz, 2H), 3.98 (s, 3H), 1.36 (s, 9H).

Step 2: To a G4 microwave vial was added **2** (53.3 mg, 0.125 mmol, 1 equiv.), phenylboronic acid (18.3 mg, 0.15 mmol, 1.2 equiv.), bis(triphenylphosphine)palladium dichloride (6.3 mg, 0.01 mmol, 0.07 equiv.), and Na_2CO_3 (18.0 mg, 0.17 mmol, 1.4 equiv.) before addition of a 10:1 (v/v) CH_3CN/H_2O solution (1.0 mL). The mixture was heated at 100 °C by microwave irradiation for 30 min with stirring and then allowed to cool to rt. The reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with H_2O (3 x 10 mL) and brine (10 mL). The separated organic layer was then dried over $MgSO_4$, filtered, and concentrated to give a yellow oil. The yellow oil was adsorbed to silica and purified by 4 g silica column MPLC (0-25% CH_2Cl_2/hexanes linear gradient over 15 min). The fractions eluting from 10.5-14.5 min were combined and concentrated to give the desired product methyl 4-(4-(tert-butyl)benzamido)-6-chloro-[1,1'-biphenyl]-3-carboxylate as a white solid (15.9 mg, 0.04 mmol, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=12.02 (br s, 1H), 9.19 (d, *J*=0.3 Hz, 1H), 9.07 (d, *J*=0.4 Hz, 1H), 8.00 (d, *J*=8.8 Hz, 2H), 7.75 (d, *J*=8.8 Hz, 2H), 7.46-7.37 (m, 5H), 3.96 (s, 3H), 1.37 (s, 9H).

Step 3: To a 20 mL screwtop vial was added THF (0.4 mL) and methyl 4-(4-(tert-butyl)benzamido)-6-chloro-[1,1'-biphenyl]-3-carboxylate (15.9 mg, 0.04 mmol, 1 equiv.), the suspension was stirred until a solution was achieved. To this was added a 1.3 M aqueous solution of LiOH (0.2 mL, 7 equiv.), and the resulting mixture was stirred at rt for 18h. The reaction mixture was acidified with 1 M HCl (aq.) to pH 2, this was filtered to collect **SID144241590** (7.5 mg, 0.02 mmol, 49% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ=11.82 (br s, 1H), 9.22 (s, 1H), 8.15 (s, 1H), 7.97 (d, *J*=8.5 Hz, 2H), 7.55 (d, *J*=8.4 Hz, 2H), 7.49-7.39 (m, 5H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ=165.9, 156.2, 142.1, 140.5, 138.2, 135.2, 134.1, 131.6, 129.6, 128.4, 128.1, 127.4, 126.1, 121.8, 112.5, 110.1, 35.2, 31.3. HPLC t_R = 6.84 min; purity >98%.

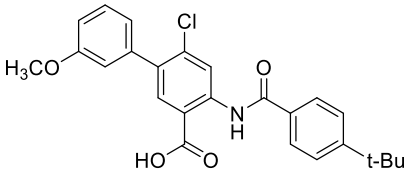


SID162211083

4-(4-(*t*-butyl)benzamido)-6-chloro-2'-fluoro-[1,1'-biphenyl]-3-carboxylic acid. SID144241590, entry 10, Table 1. Prepared according to Scheme 1.

To a G10 microwave vial was added aryl bromide **2** (100 mg, 0.235 mmol, 1 equiv.), 2-fluorophenylboronic acid (39.6 mg, 0.283 mmol, 1.2 equiv.), bis(triphenylphosphine)palladium dichloride (16.5 mg, 0.024 mmol, 0.10 equiv.) and Na_2CO_3 (37.4 mg, 0.353 mmol, 1.5 equiv.) before addition of a 10:1 (v/v) CH_3CN/H_2O solution (2.0 mL). The mixture was heated at 150 °C by microwave irradiation for 20 min with stirring and then allowed to cool to rt. The mixture was diluted with EtOAc (10 mL) and washed with H_2O (2x10 mL) and brine (2x10 mL). The separated organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give a brown oil. The oil was adsorbed to silica and purified via 4 g silica column MPLC (0-5% EtOAc/hexanes linear gradient over 15 min) to give methyl 4-(4-(*tert*-butyl)benzamido)-6-chloro-2'-fluoro-[1,1'-biphenyl]-3-carboxylate as a white solid (72.4 mg, 0.165 mmol, 58% yield). Characterization matched previous literature report.[2]

Methyl 4-(4-(*tert*-butyl)benzamido)-6-chloro-2'-fluoro-[1,1'-biphenyl]-3-carboxylate was subjected to the same conditions as described in *Step 3* (above) to afford **SID144241590** as a white solid (44.9 mg, 0.105 mmol, 64% yield). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ=12.33 (s, 1H), 9.23 (s, 1H), 8.16 (s, 1H), 8.00 (d, *J*=8.5 Hz, 2H), 7.65 (d, *J*= 8.5 Hz, 2H), 7.53 (tdd, *J*=7.5 Hz, 5.2 Hz, 1.8 Hz, 1H), 7.46 (td, *J*=7.6 Hz, 1.8 Hz, 1H), 7.34 (t, *J*=1.75 Hz, 1H), 7.28 (t, *J*=9.2 Hz, 1H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $(CD_3)_2CO$) δ= 166.0 (d, *J*=8.1 Hz), 160.6 (d, *J*=245.8 Hz), 156.8, 143.6 (d *J*=14.8 Hz), 140.3, 135.1, 132.7, 132.6, 132.6, 131. 5 (d *J*=8.2 Hz), 129.7, 128.1, 126.8, 126.2, 125.3, (d, *J*=3.6 Hz), 121.1 (d, *J*=5.3 Hz), 116.6, 116.3, 35.6, 31.4. HPLC RT = 6.85 min; purity >98%.



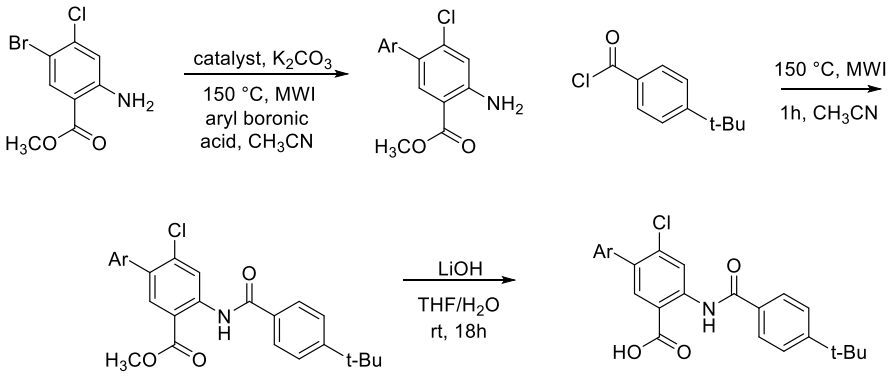
SID162211075

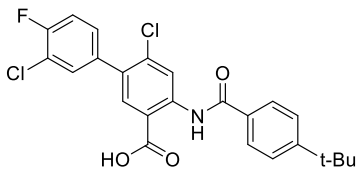
4-(4-(*t*-butyl)benzamido)-6-chloro-3'-methoxy-[1,1'-biphenyl]-3-carboxylic acid. SID162211075, entry 14, Table 1. Prepared according to Scheme 1.

To a G10 microwave vial was added aryl bromide **2** (100 mg, 0.235 mmol, 1 equiv.), 3-methoxyphenylboronic acid (43.0 mg, 0.283 mmol, 1.2 equiv.), bis(triphenylphosphine)palladium dichloride (16.5 mg, 0.024 mmol, 0.10 equiv.) and Na_2CO_3 (37.4 mg, 0.353 mmol, 1.5 equiv.) before addition of a 10:1 (v/v) CH_3CN/H_2O solution (2.0 mL). The mixture was heated at 150 °C by microwave irradiation for 30 min with stirring and then allowed to cool to rt. The mixture was diluted with EtOAc (10 mL) and washed with H_2O (2x10 mL) and brine (2x10 mL). The separated organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give a brown oil. The oil was adsorbed to silica and purified via 4 g silica column MPLC (0-5% EtOAc/hexanes linear gradient over 15 min) to give methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-3'-methoxy-[1,1'-biphenyl]-3-carboxylate as a white solid (83.1 mg, 0.184 mmol, 78% yield). Characterization matched previous literature report.[2]

Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-3'-methoxy-[1,1'-biphenyl]-3-carboxylate was subjected to the same conditions as described in *Step 3* (above) to afford **SID162211075** as a white solid (41.7 mg, 0.095 mmol, 52% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ=9.03 (s, 1H), 8.08 (s, 1H), 7.95 (d, *J*=8.5 Hz, 2H), 7.59 (d, *J*=8.6 Hz, 2H), 7.35 (m, 1H), 6.98 (m, 3H), 3.84 (s, 3H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, Methanol-d4) δ=171.0, 167.4, 160.9, 157.4, 142.5, 140.9 139.3, 136.2, 134.9, 132.7, 130.4, 128.3, 127.0, 122.8, 122.1, 116.2, 114.5, 55.8, 49.6, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 35.9, 31.5. HPLC RT = 6.93 min. HPLC Purity >98%.

Scheme 2. Alternative procedure for synthesis of benzamidobenzoic acids





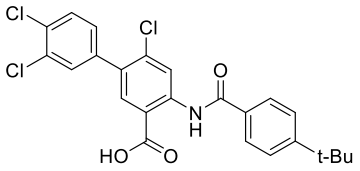
SID162211073

4-(4-(*t*-butyl)benzamido)-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylic acid. SID162211073, entry 12, Table 1. Prepared according to Scheme 2.

Step 1. **Methyl 4-amino-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate.** To a microwave vial was added 3-chloro-4-fluorophenyl)boronic acid (0.078 g, 0.45 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate **2** (0.098 g, 0.37 mmol), 1,1'-bis(di-*t*-butylphosphino)ferrocene palladium dichloride, (5.0 mg, 7.4 µmol) and K_2CO_3 (0.077 g, 0.56 mmol). The vial was evacuated with argon 3 times, and then degassed CH_3CN (1.5 mL) and water (1.5 mL) were added. The reaction stirred at 110 °C for 60 minutes in the microwave. After cooling to rt, the reaction was diluted with EtOAc (10 mL) and washed with saturated $NaHCO_3$ (12 mL). The separated organic extracts were dried with $MgSO_4$, filtered and concentrated. The crude product was purified by reverse-phase MPLC (10 - 100% CH_3CN:H_2O) to provide pure methyl 4-amino-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.038 g, 0.12 mmol, 32% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.43 (dd, J_1 = 7.0 Hz, J_2 = 2.2 Hz, 1H), 7.29 – 7.24 (m, 1H), 7.16 (t, J = 8.6 Hz, 1H), 6.81 (s, 1H), 3.87 (s, 3H).

Step 2. **Methyl 4-(4-(*t*-butyl)benzamido)-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate.** To a microwave vial was added methyl 4-amino-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.038 g, 0.12 mmol), acetonitrile (2 mL) and 4-(*t*-butyl)benzoyl chloride (0.026 g, 0.024 mL, 0.13 mmol). The reaction was stirred at 150 °C in the microwave for 60 min. After cooling to rt, the reaction was diluted with saturated $NaHCO_3$ (10 mL) and extracted with EtOAc (2 x 10 mL). The separated organic layers were combined and dried with $MgSO_4$, filtered and adsorbed to silica Purification by MPLC (0 - 15% EtOAc:hexanes) afforded methyl 4-(4-(*t*-butyl)benzamido)-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.037 g, 0.078 mmol, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.03 (s, 1H), 9.20 (s, 1H), 8.02 (s, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 7.49 (dd, J_1 = 7.0 Hz, J_2 = 2.2 Hz, 1H), 7.35 – 7.30 (m, 1H), 7.22 (t, J = 8.7 Hz, 1H), 3.98 (s, 3H), 1.37 (s, 9H).

Step 3. **4-(4-(*t*-butyl)benzamido)-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylic acid.** To a flask was added methyl 4-(4-(*t*-butyl)benzamido)-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.037 g, 0.078 mmol), THF (1 mL), LiOH (0.013 g, 0.55 mmol) and water (1 mL). The reaction stirred for 18h at rt and was then acidified to pH 2 - 3 with aqueous 1.0 M HCl and extracted with CH_2Cl_2 (3 x 10 mL). The organic layers were combined and then dried with $MgSO_4$, filtered and adsorbed to silica then purified by column chromatography (0 - 5% MeOH:CH_2Cl_2) to afford 4-(4-(*t*-butyl)benzamido)-3',6-dichloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylic acid **SID162211073** (0.030 g, 0.065 mmol, 84% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 8.98 (s. 1H), 8.03 (s, 1H), 8.5 Hz, 2H), 7.73 (dd, J_1 = 7.2 Hz, J_2 = 2.2 Hz, 1H), 7.64 (d, J = 8.5 Hz, 2H), 7.57 – 7.48 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.1, 164.9, 157.0 (d, J = 248.0 Hz), 155.6, 141.4, 136.8, 135.2 (d, J = 3.9 Hz), 133.7, 131.7, 131.4, 131.2, 130.3 (d, J = 7.7 Hz), 127.0, 126.0, 120.3, 119.4 (d, J = 17.8 Hz), 116.9 (d, J = 21.0 Hz), 115.7, 34.8, 30.9. LCMS RT: 4.528 min. LCMS purity 93.4%. HRMS (ESI) *m/z* calcd for $C_{24}H_{20}Cl_2FNO_3$ $[M+H]^+$ 460.0876, found 460.0877.



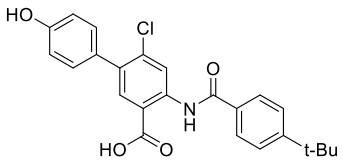
SID162211080

4-(4-(*t*-butyl)benzamido)-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylic acid. SID162211080, entry 13, Table 1. Prepared according to Scheme 2.

Methyl 4-amino-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 1. (3,4-dichlorophenyl)boronic acid (0.084 g, 0.44 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate (0.097 g, 0.37 mmol), 1,1'-bis(di-*t*-butylphosphino)ferrocene palladium dichloride, (5.0 mg, 7.3 µmol) and K_2CO_3 (0.076 g, 0.55 mmol), degassed acetonitrile (1.5 mL) and water (1.5 mL). The crude product was purified by reverse-phase MPLC (10 - 100% CH_3CN:H_2O) to provide methyl 4-amino-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylate (0.047 g, 0.14 mmol, 39% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.46 (d, J = 8.2 Hz, 2H), 7.25 (dd, J_1 = 8.3 Hz, J_2 = 2.1 Hz, 1H), 6.80 (s, 1H), 3.87 (s, 3H).

Methyl 4-(4-(*t*-butyl)benzamido)-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 2. methyl 4-amino-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylate (0.047 g, 0.14 mmol), acetonitrile (2 mL) and 4-(*t*-butyl)benzoyl chloride (0.031 g, 0.028 mL, 0.16 mmol). Purified by MPLC (0 - 15% EtOAc: hexanes) to produce methyl 4-(4-(*t*-butyl)benzamido)-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylate (0.049 g, 0.10 mmol, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.03 (br s, 1H), 9.21 (s, 1H), 8.03 (s, 1H), 7.99 (d, J = 8.5 Hz, 2H), 7.59 – 7.50 (m, 4H), 7.32 (dd, J_1 = 8.3 Hz, J_2 = 2.1 Hz, 1H), 3.98 (s, 3H), 1.37 (s, 9H).

4-(4-(*t*-butyl)benzamido)-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylic acid. Prepared as described above, Scheme 2, Step 3. Methyl 4-(4-(*t*-butyl)benzamido)-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylate (0.044 g, 0.089 mmol), THF (1 mL), LiOH (0.015 g, 0.62 mmol) and water (1 mL). Purified by MPLC (0 - 5% MeOH:CH_2Cl_2) to afford 4-(4-(*t*-butyl)benzamido)-3',4',6-trichloro-[1,1'-biphenyl]-3-carboxylic acid **SID162211080** (0.038 g, 0.080 mmol, 90% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (br s, 1H), 8.98 (s, 1H), 8.04 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.79 – 7.75 (m , 2H), 7.64 (d, J = 8.5 Hz, 2H), 7.51 – 7.48 (m, 1H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.0, 164.9, 155.6, 141.6, 138.1, 136.4, 133.6, 131.4, 131.21, 131.18, 131.0, 130.9, 130.5, 129.8, 127.0, 125.9, 120.3, 34.8, 30.9. LCMS RT: 2.769 min. LCMS purity 100%. HRMS (ESI) *m/z* calcd for $C_{24}H_{20}Cl_3NO_3$ $[M+H]^+$ 476.0581, found 476.0582.



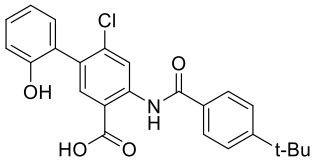
SID162211079

4-(4-(*t*-butyl)benzamido)-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid, SID162211079, entry 16, Table 1. Prepared according to Scheme 2.

Methyl 4-amino-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 1. (4-hydroxyphenyl) boronic acid (0.063 g, 0.46 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate (0.10 g, 0.382 mmol), 1,1'-bis(di-*t*-butylphosphino)ferrocene palladium dichloride, (5.2 mg, 7.6 μmol) and K_2CO_3 (0.079 g, 0.57 mmol), degassed acetonitrile (1.5 mL) and water (1.5 mL). The crude product was purified by reverse-phase MPLC (10 - 100% CH_3CN:water) to provide methyl 4-amino-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.022 g, 0.079 mmol, 21% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.79 (s, 1H), 5.76 (br s, 2H), 4.83 (br s, 1H), 3.86 (s, 3H).

Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 2. methyl 4-amino-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.026 g, 0.094 mmol), acetonitrile (4 mL) and 4-(*t*-butyl)benzoyl chloride (0.020 g, 0.019 mL, 0.10 mmol). Purified by MPLC (0 - 25% EtOAc:hexanes) to afford methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.036 g, 0.082 mmol, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.00 (s, 1H), 9.16 (s, 1H), 8.04 (s, 1H), 8.00 (d, *J* = 8.3 Hz, 2H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.3 Hz, 2H), 6.91 (d, *J* = 8.2 Hz, 2H), 3.96 (s, 3H), 1.37 (s, 9H).

4-(4-(*t*-butyl)benzamido)-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid. Prepared as described above, Scheme 2, Step 3. methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.026 g, 0.059 mmol), THF (1 mL), LiOH (0.011 g, 0.48 mmol) and water (1 mL). Purified by MPLC (0 - 5% MeOH:CH_2Cl_2) to afford 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid **SID162211079** (0.009 g, 0.021 mmol, 36% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H), 7.99 (s, 1H), 7.94 (d, *J* = 8.5 Hz, 2H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 1.33 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.9, 164.6, 157.0, 155.1, 140.4, 134.9, 133.6, 133.5, 131.7, 130.4, 128.7, 127.0, 125.8, 119.8, 118.1, 115.1, 34.8, 30.9. LCMS RT: 3.758 min. LCMS purity 100%. HRMS (ESI) *m/z* calcd for $C_{24}H_{22}ClNO_4$ $[M+H]^+$ 424.1309, found 424.1310.



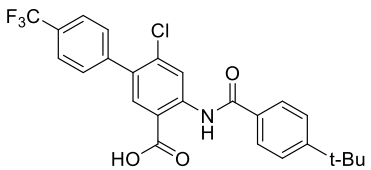
SID162211085

4-(4-(*t*-butyl)benzamido)-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid, SID162211085, entry 17, Table 1. Prepared according to Scheme 2.

Methyl 4-amino-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 1. (2-hydroxyphenyl) boronic acid (0.066 g, 0.48 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate (0.11 g, 0.40 mmol), 1,1'-bis(di-*t*-butylphosphino)ferrocene palladium dichloride, (5.4 mg, 7.9 µmol), K_2CO_3 (0.082 g, 0.59 mmol), degassed acetonitrile (1.5 mL) and water (1.5 mL). The crude product was purified by reverse-phase MPLC (10 - 100% MeCN:water) to provide pure methyl 4-amino-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.045 g, 0.16 mmol, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.43 – 7.32 (m, 4H), 6.82 (s, 1H), 3.86 (s, 3H).

Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 2. methyl 4-amino-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.045 g, 0.16 mmol), acetonitrile (2 mL) and 4-(*t*-butyl)benzoyl chloride (0.035 g, 0.032 mL, 0.18 mmol). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.063 g, 0.14 mmol, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (br s, 1H), 9.15 (s, 1H), 8.06 (s, 1H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.53 – 7.47 (m, 1H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.38 – 7.32 (m, 3H), 3.90 (s, 3H), 1.35 (s, 9H).

4-(4-(*t*-butyl)benzamido)-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid. Prepared as described above, Scheme 2, Step 3. Methyl 4-(4-*(t*-butyl)benzamido)-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylate (0.063 g, 0.14 mmol), THF (1 mL), LiOH (0.024 g, 1.0 mmol) and water (1 mL). Concentrated after workup to produce 4-(4-(*t*-butyl)benzamido)-6-chloro-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid **SID162211085** (0.033 g, 0.078 mmol, 54% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.37 (br s, 1H), 9.50 (br s, 1H), 8.87 (s, 1H), 7.99 (br s, 1H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.24 – 7.19 (m, 1H), 7.10 (dd, J_1 = 7.5 Hz, J_2 = 1.7 Hz, 1H), 6.94 (dd, J_1 = 8.2 Hz, J_2 = 1.2 Hz, 1H), 6.86 (td, J_1 = 7.4 Hz, J_2 = 1.1 Hz, 1H), 1.33 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.0, 164.5, 154.9, 154.7, 140.7, 134.5, 132.0, 131.0, 130.9, 129.0, 127.1, 125.8, 125.7, 118.8, 118.7, 115.5, 34.7, 30.9. LCMS RT: 3.796 min. LCMS purity 97.8%. HRMS (ESI) *m/z* calcd for $C_{24}H_{22}ClNO_4$ $[M+H]^+$ 424.1309, found 424.1310.



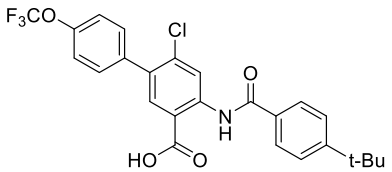
SID162211070

4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylic acid. SID162211070, entry 20, Table 1. Prepared according to Scheme 2.

Methyl 4-amino-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 1. (4-(trifluoromethyl)phenyl)boronic acid (0.046 g, 0.24 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate (0.053 g, 0.20 mmol), 1,1'-bis(di-*t*-butylphosphino)ferrocene palladium dichloride (6.5 mg, 10 µmol), K_2CO_3 (0.042 g, 0.30 mmol), degassed acetonitrile (1.5 mL) and water (1.5 mL). The crude product was purified by reverse-phase MPLC (10 - 100% CH_3CN:water) to afford methyl 4-amino-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylate (0.033 g, 0.100 mmol, 50% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.65 (d, *J* = 7.9 Hz, 2H), 7.52 (d, *J* = 7.9 Hz, 2H), 6.82 (s, 1H), 5.88 (br s, 2H), 3.87 (s, 3H).

Methyl 4-(4-(t-butyl)benzamido)-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 2. methyl 4-amino-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylate (0.033 g, 0.10 mmol), acetonitrile (2 mL) and 4-(*t*-butyl)benzoyl chloride (0.022 g, 0.020 mL, 0.11 mmol). Purified by reverse-phase MPLC (10 - 100% CH_3CN:water) to afford methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylate (0.024 g, 0.048 mmol, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.05 (s, 1H), 9.22 (s, 1H), 8.06 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.71 (d, *J* = 8.1 Hz, 2H), 7.59 – 7.55 (m, 4H), 3.97 (s, 3H), 1.37 (s, 9H).

4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylic acid. Prepared as described above, Scheme 2, Step 3. Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylate (0.024 g, 0.048 mmol), THF (1 mL), LiOH (8.1 mg, 0.34 mmol) and water (1 mL). Purified by MPLC (0 - 5% MeOH:CH_2Cl_2) to produce 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-carboxylic acid **SID162211070** (0.012 g, 0.025 mmol, 52% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H), 9.01 (s, 1H), 8.06 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.87 (d, *J* = 7.8 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 2H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.1, 164.9, 155.6, 141.6, 141.5, 136.7, 133.6, 132.5, 131.1, 130.2, 128.4 (q, *J* = 25.3 Hz), 127.0, 125.9, 125.3 (q, *J* = 2.8 Hz), 124.3 (q, *J* = 216.4 Hz), 120.4, 115.8, 34.8, 30.9. LCMS RT: 4.405 min. LCMS purity 100%. HRMS (ESI) *m/z* calcd for $C_{25}H_{21}ClF_3NO_3$ $[M+H]^+$ 476.1234, found 476.1235.



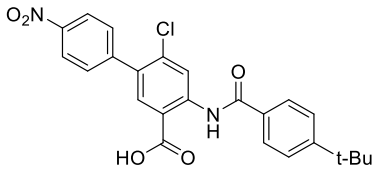
SID162211074

4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-3-carboxylic acid, SID162211074, entry 21, Table 1. Prepared according to Scheme 2.

Methyl 4-amino-6-chloro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 1. (4-(trifluoromethoxy)phenyl)boronic acid (0.097 g, 0.47 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate (0.10 g, 0.39 mmol), 1,1'-bis (di-*t*-butylphosphino) ferrocene palladium dichloride, (5.4 mg, 7.9 µmol), K_2CO_3 (0.082 g, 0.59 mmol), degassed acetonitrile (1.5 mL) and water (1.5 mL). The crude product was purified by reverse-phase MPLC (10 - 100% CH_3CN:water) to afford methyl 4-amino-6-chloro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-3-carboxylate (0.053 g, 0.15 mmol, 39% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.43 (d, *J* = 8.7 Hz, 2H), 7.26 – 7.23 (m, 2H), 6.84 (s, 1H), 3.87 (s, 3H).

Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 2. methyl 4-amino-6-chloro-4'-(trifluoromethoxy) -[1,1'-biphenyl]-3-carboxylate (0.053 g, 0.15 mmol), acetonitrile (2 mL) and 4-(*t*-butyl) benzoyl chloride (0.033 g, 0.031 mL, 0.17 mmol). Purified by MPLC (0 - 15% EtOAc:hexanes) to afford methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'(trifluoromethoxy)- [1,1'-biphenyl]-3-carboxylate (0.049 g, 0.097 mmol, 63% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 12.03 (s, 1H), 9.20 (s, 1H), 8.05 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 3.97 (s, 3H), 1.37 (s, 9H).

4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-3-carboxylic acid. Prepared as described above, Scheme 2, Step 3. Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'(trifluoromethoxy)- [1,1'-biphenyl]-3-carboxylate (0.049 g, 0.097 mmol), THF (1 mL), LiOH (0.016 g, 0.68 mmol) and water (1 mL). Purified by MPLC (0 - 5% MeOH:CH_2Cl_2) to afford 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-3-carboxylic acid **SID162211074** (0.036 g, 0.073 mmol, 76% yield). ^1H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 8.99 (s, 1H), 8.04 (s, 1H), 7.91 (d, *J* = 8.5 Hz, 2H), 7.66 – 7.61 (m, 4H), 7.49 (d, *J* = 8.5 Hz, 2H), 1.34 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.1, 164.9, 155.6, 148.0, 141.3, 136.8, 136.7, 133.6, 132.5, 131.4, 131.2, 127.0, 126.0, 120.9, 120.4, 120.1 (q, *J* = 256.6 Hz), 115.8, 34.8, 30.9. LCMS RT: 4.519 min. LCMS purity 100%. HRMS (ESI) *m/z* calcd for $C_{25}H_{21}ClF_3NO_4$ $[M+H]^+$ 492.1183, found 492.1184.



SID162211081

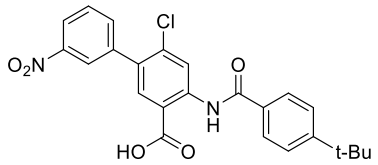
4-(4-(*t*-butyl)benzamido)-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylic acid, SID162211081, entry 22, Table 1. Prepared according to Scheme 2.

Methyl 4-amino-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 1. (4-nitrophenyl)boronic acid (0.15 g, 0.87 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate (0.19 g, 0.72 mmol), 1,1'-bis(di-t-butylphosphino)ferrocene palladium dichloride, (9.8 mg, 0.014 mmol), K_2CO_3 (0.150 g, 1.08 mmol), degassed acetonitrile (1.5 mL) and water (1.5 mL). The crude product was purified by reverse-phase MPLC (10 - 100% CH_3CN:water) to provide pure methyl 4-amino-6-chloro-4'-

nitro-[1,1'-biphenyl]-3-carboxylate (0.15 g, 0.50 mmol, 68% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 8.9 Hz, 2H), 7.87 (s, 1H), 7.59 (d, *J* = 8.9 Hz, 2H), 6.84 (s, 1H), 3.88 (s, 3H).

Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 2. methyl 4-amino-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylate (0.15 g, 0.50 mmol), acetonitrile (3 mL) and 4-(*t*-butyl)benzoyl chloride (0.11 g, 0.099 mL, 0.55 mmol). Purified by MPLC (0 - 15% EtOAc:hexanes) to afford methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylate (0.20 g, 0.43 mmol, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.07 (br s, 1H), 9.25 (s, 1H), 8.32 (d, *J* = 8.8 Hz, 2H), 8.08 (s, 1H), 8.01 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.6 Hz, 2H), 3.99 (s, 3H), 1.38 (s, 9H).

4-(4-(*t*-butyl)benzamido)-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylic acid. Prepared as described above, Scheme 2, Step 3. Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylate (0.027 g, 0.058 mmol), THF (1 mL), LiOH (9.7 mg, 0.41 mmol) and water (1 mL) Purified by MPLC (0 - 5% MeOH:CH_2Cl_2) to afford 4-(4-(*t*-butyl)benzamido)-6-chloro-4'-nitro-[1,1'-biphenyl]-3-carboxylic acid **SID162211081** (0.016 g, 0.035 mmol, 61% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H), 9.02 (s, 1H), 8.34 (d, *J* = 8.8 Hz, 2H), 8.08 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 2H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.0, 164.9, 155.6, 147.0, 144.1, 141.8, 136.4, 133.6, 131.8, 131.1, 130.8, 127.0, 125.9, 123.5, 120.5, 116.2, 34.8, 30.9. LCMS RT: 4.226 min. LCMS purity 100%. HRMS (ESI) *m/z* calcd for $C_{24}H_{21}ClN_2O_5$ $[M+H]^+$ 453.1211, found 453.1212.



SID162211082

4-(4-(*t*-butyl)benzamido)-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylic acid, SID162211082, entry 23, Table 1. Prepared according to Scheme 2.

Methyl 4-amino-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 1. (3-nitrophenyl)boronic acid (0.15 g, 0.89 mmol), methyl 2-amino-5-bromo-4-chlorobenzoate (0.20 g, 0.74 mmol), 1,1'-bis (di-*t*-butylphosphino)ferrocene palladium dichloride, (10 mg, 0.015 mmol), K_2CO_3 (0.15 g, 1.08 mmol), degassed acetonitrile (1.5 mL) and water (1.5 mL). The crude product was purified by reverse-phase MPLC (10 - 100% CH_3CN:water) to provide pure methyl 4-amino-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate (0.17 g, 0.55 mmol, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (t, *J* = 2.0 Hz, 1H), 8.22 – 8.18 (m, 1H), 7.87 (s, 1H), 7.75 – 7.45 (m, 1H), 7.57 (t, *J* = 8.2 Hz, 1H), 6.85 (s, 1H), 3.88 (s, 3H).

Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate. Prepared as described above, Scheme 2, Step 2. methyl 4-amino-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate (0.16 g, 0.52 mmol), acetonitrile (5 mL) and 4-(*t*-butyl)benzoyl chloride (0.11 g, 0.10 mL, 0.57 mmol). Purified by MPLC (0 - 15% EtOAc:hexanes) to afford methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-3'-nitro-[1,1'-

biphenyl]-3-carboxylate (0.22 g, 0.46 mmol, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.07 (s, 1H), 9.25 (s, 1H), 8.33 (t, *J* = 2.0 Hz, 1H), 8.29 – 8.25 (m, 1H), 8.09 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.84 – 7.81 (m, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 3.99 (s, 3H), 1.38 (s, 9H).

4-(4-(*t*-butyl)benzamido)-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylic acid. Prepared as described above, Scheme 2, Step 3. Methyl 4-(4-(*t*-butyl)benzamido)-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate (0.026 g, 0.055 mmol), THF (1 mL), LiOH (9.2 mg, 0.38 mmol) and water (1 mL). Purified by reverse phase MPLC (10 - 100% CH_3CN:water) to afford 4-(4-(*t*-butyl)benzamido)-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylic acid **SID162211082** (0.015 g, 0.033 mmol, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (br s, 1H), 9.01 (s, 1H), 8.33 – 8.29 (m, 2H), 8.11 (s, 1H), 8.00 – 7.96 (m, 1H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.83 – 7.78 (m, 1H), 7.64 (d, *J* = 8.5 Hz, 2H), 1.34 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.9, 164.9, 155.6, 147.7, 141.7, 139.1, 136.11, 136.07, 133.8, 131.4, 131.3, 130.1, 127.1, 125.9, 124.0, 122.9, 120.3, 34.8, 30.9. LCMS RT: 4.134 min. LCMS purity 95.2%. HRMS (ESI) *m/z* calcd for $C_{24}H_{21}ClN_2O_5$ $[M+H]^+$ 453.1211, found 453.1212.

References

[1]Sharlow, E.; Golden, J.E.; Dodson, H.; Morris, M.; Hesser, M.; Lyda, T.; Leimgruber, S.; Schroeder, C.E.; Flaherty, D.P.; Weiner, W.S.; Simpson, D.; Lazo, J.S.; Aubé, J.; **Morris, J.C.** in *Probe Reports from the NIH Molecular Libraries Program* [Internet] (Ed.:National Center for Biotechnology Information (US)), NCBI, US National Library of Medicine, Bethesda, MD, **2010**, http://www.ncbi.nlm.nih.gov/books/NBK63599/.

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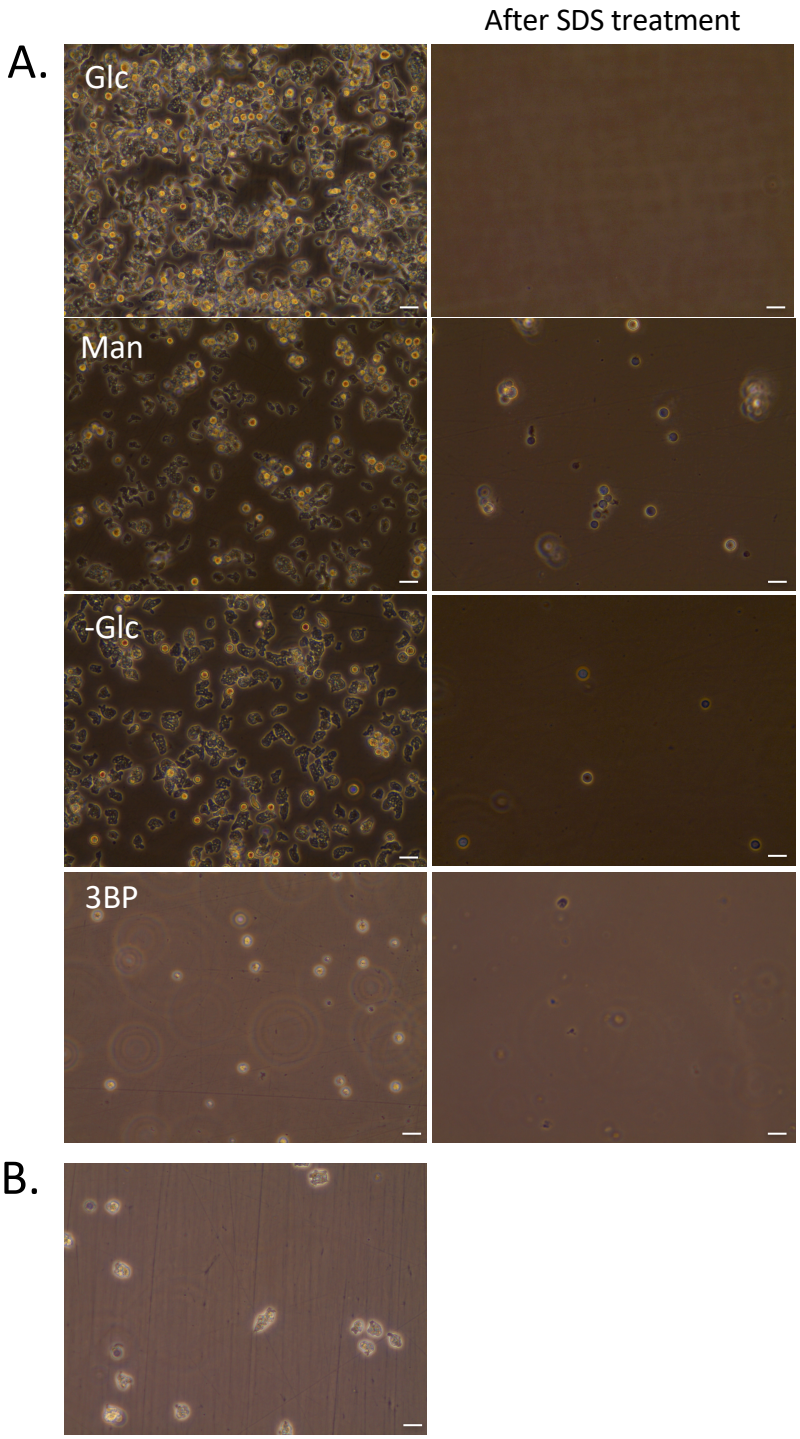
After SDS treatment
A.
Glc
Man
-Glc
3BP
B.

Supplemental Data 1

```
3VEY_HsGCK         1 MALT--------------------LVEQILAEFQLQEEDLKKVMRRMQKEMDRGLRLETHEEASV   45
3O8M_KlHK          1 MVRLGPKKPPARKGSMADVPANLMEQIHGLETLFTVSSEKMRSIVKHFISELDKGLSKK---GGNI   63
3B8A_ScHK_P1       1 MVHLGPKKPQARKGSMADVPKELMDEIHQLEDMFTVDSETLRKVVKHFIDELNKGLTKK---GGNI   63
2Q2R_TcGlcK        1 APAGSH-----------------------------------MNIKELSLHELCEELKTP---AWNA   28
6DA0_NfGlcK        1 MAHHHHHHMPERMISS----------SSRSSMSSSTSSEEPLVIDEDFINQVEKHYRKS---LKNI   53

cons               1                                          : .    :: .          .    66


3VEY_HsGCK        46 KMLPTYVRSTPEGSEVGDFLSLDLGGTNFRVMLVKVGEGEEGQWSVKTKHQMYSIPEDAMTGT-AE  110
3O8M_KlHK         64 PMIPGWVVEYPTGKETGDFLALDLGGTNLRVVLVKLGGNHDF---DT-TQNKYRLPDHLRTGT-SE  124
3B8A_ScHK_P1      64 PMIPGWVMEFPTGKESGNYLAIDLGGTNLRVVLVKLSGNHTF---DT-TQSKYKLPHDMRTTKHQE  125
2Q2R_TcGlcK       29 ----------------PLTFVGDVGGTSARMGFVREGKNDSV---HA-CVTRYSMKRKDI------   68
6DA0_NfGlcK       54 PF--------------QYIVGVDVGATNTRIAIQFIINE-DQ---DD-EVFMTKFPCNTS------   94

cons              67                    .  *:*.*. *: :                    :  .          132


3VEY_HsGCK       111 MLFDYISECISDFLDKHQ--MKH-KKLPLGFTFSFPVRHEDIDKGILLNW-TKGFKASGAEGNNVV  172
3O8M_KlHK        125 QLWSFIAKCLKEFVDEWYPDGVS-EPLPLGFTFSYPASQKKINSGVLQRW-TKGFDIEGVEGHDVV  188
3B8A_ScHK_P1     126 ELWSFIADSLKDFMVEQE-LLNTKDTLPLGFTFSYPASQNKINEGILQRW-TKGFDIPNVEGHDVV  189
2Q2R_TcGlcK       69 ---TEIIEFFNEIIELMPASVMK-RVKAGVINVPGPVTG--GAVGG----PFNNLK--------G-  115
6DA0_NfGlcK       95 ---THLANYLAAYGKAMVKAVGK-GSAAGSIALAGPVTG--DKVRI---TNYKEHD--------Q-  142

cons             133      : . :                 .  : .. *.              :  .           198


3VEY_HsGCK       173 GLLRDAIKRRGDFEMDVVAMVNDTVATMISCYYED-------------------------------  207
3O8M_KlHK        189 PMLQEQIEKLN-IPINVVALINDTTGTLVASLYTD-------------------------------  222
3B8A_ScHK_P1     190 PLLQNEISKRE-LPIEIVALINDTVGTLIASYYTD-------------------------------  223
2Q2R_TcGlcK      116 IARLSDYPKAL-FPPGHSAILNDLEAGGFGVLAVSDAHVFSEYFGVMWEGTQWRTCEQEPAGSVIG  180
6DA0_NfGlcK      143 EFFYSQLPDTL-FPASKNTFLNDLEASCYGIINVGTNNRLHEFFCPIDALNNYATSQTVR---LSD  204

cons             199    .       :     :::**  .   .    .                               264


3VEY_HsGCK       208 HQCEVGMIVGTGCNACYMEEMQNVELVEG-------DEG---RMCVNTEWGA-------FGDSGEL  256
3O8M_KlHK        223 PQTKMGIIIGTGVNGAYYDVVSGIEKLEGLLPEDIGPDS---PMAINCEYGS-------FDNEHLV  278
3B8A_ScHK_P1     224 PETKMGVIFGTGVNGAFYDVVSDIEKLEGKLADDIPSNS---PMAINCEYGS-------FDNEHLV  279
2Q2R_TcGlcK      181 RGRCLVLAPGTGLGSSLIYY----------------NPPMNQHIVVPLELGS-------QTLPMR-  222
6DA0_NfGlcK      205 TSEYAVLAMGTGLGTGLIVG----------------S-AGGKFNVIPLEAGHVHIATPGVNSEHFK  253

cons             265       :  *** .                                 :  * *                330


3VEY_HsGCK       257 DEFLLEYDRLVDESSANPGQQLYEKLIGGKYMGELVRLVLLRLVDENLLFHGEASEQLRTRGA--F  320
3O8M_KlHK        279 L-PRTKYDVIIDEESPRPGQQAFEKMTSGYYLGEIMRLVLLDLYDSGFIFKDQDISKLKEAYV--M  341
3B8A_ScHK_P1     280 L-PRTKYDVAVDEQSPRPGQQAFEKMTSGYYLGELLRLVLLELNEKGLMLKDQDLSKLKQPYI--M  342
2Q2R_TcGlcK      223 ----KDIDYIQTLHAELKLFPNYENMVSGAGLEFHYRQVVRGSR-----------------PP--C  265
6DA0_NfGlcK      254 E-ERERIEFLSQKIYGGAYPIEYEDICSGRGLEFCYEFEIRNDP-----------------NAVRK  301

cons             331        :              :*.: .*  :    .  :                          396


3VEY_HsGCK       321 ETRFVSQVESDT-GDRKQIYNILS-TLGLRPSTTDCDIVRRACESVSTRAAHMCSAGLAGVINRMR  384
3O8M_KlHK        342 DTSYPSKIEDDPFENLEDTDDLFKTNLNIETTVVERKLIRKLAELVGTRAARLTVCGVSAICDKRG  407
3B8A_ScHK_P1     343 DTSYPARIEDDPFENLEDTDDIFQKDFGVKTTLPERKLIRRLCELIGTRAARLAVCGIAAICQKRG  408
2Q2R_TcGlcK      266 SAGEIAKLASE-----------------G----------DANACKAMKKYHEYLMRVGSEASMALL  304
6DA0_NfGlcK      302 TASQIAESYS-----------------T----------DTYARQAMITHYRYLMKAAQNIA-VLI  338

cons             397  :   :.  .                                  .        .              462
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3VEY_HsGCK       385 ESRSEDVMRI--TVGVDGSVYKLHPS-FKER--F---HASVRRLT-------PSCEITFIE---SE  432
3O8M_KlHK        408 ------YKTA--HIAADGSVFNRYPG-YKEK--A---AQALKDIYNWDVEKMEDHPIQLVA---AE  456
3B8A_ScHK_P1     409 ------YKTG--HIAADGSVYNKYPG-FKEA--A---AKGLRDIYGWTG-DASKDPITIVP---AE  456
2Q2R_TcGlcK      305 --------PL--TIVLVGDNIVNNAFFYRNPQNLKEMHHEALNHEMERF-G-FQSRVSYLRQKKLL  358
6DA0_NfGlcK      339 --------PTCRGVFFAGDNQVFNEDFFKEH--LSILQKELFQTHQKK--H-WLTDLKPYRQMKEY  391

cons             463             :   *.        :::                          :          528


3VEY_HsGCK       433 EGSGRGAAL-----------------------------------VSAVACKKACMLGQ------  455
3O8M_KlHK        457 DGSGVGAAI-----------------------------------IACLTQKRLA---AGKSVGI  482
3B8A_ScHK_P1     457 DGSGAGAAV-----------------------------------IAALSEKRIA---EGKSLGI  482
2Q2R_TcGlcK      359 NLNLMGCYRCGLDLS-------------------------------------------------  373
6DA0_NfGlcK      392 NFNVKGCLQKARELAQLGSTTMVDEKPKAGLKDVLSVAIPTAFFSVLSFFTAKNLY---EK-----  449

cons             529 : .  *.                                                          594


3VEY_HsGCK       456 ---  455
3O8M_KlHK        483 KGE  485
3B8A_ScHK_P1     483 IGA  485
2Q2R_TcGlcK      374 ---  373
6DA0_NfGlcK      450 ---  449

cons             595      597
```