Rational development of 4-aminopyridyl-based inhibitors targeting *Trypanosoma cruzi* CYP51 as anti-Chagas agents

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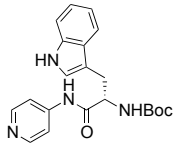
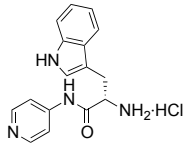
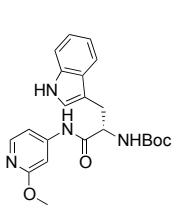


*(S)-tert-butyl (3-(1H-indol-3-yl)-1-oxo-1-(pyridin-4-ylamino)propan-2-yl)carbamate, (**5**)*. To a solution of N-Boc-L-tryptophan (1.0 g, 3.3 mmol), PyBOP (2.0 g, 3.9 mmol), and HOBt (0.29 g) in dry CH_2Cl_2 (20 ml) was slowly added triethylamine (1.5 ml, ca. 4 eq.) at 0 ^oC, and the reaction mixture was stirred and warmed to ambient temperature for 15 min. After cooling to 0 ^oC, 4-aminopyridine (0.39 g, 4.1 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. After completion of the reaction monitored by TLC, ethyl acetate (80 ml) was added to the crude mixture, which was washed with saturated aqueous $NaHCO_3$ (20 ml × 2) and brine (20 ml × 2). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by flash chromatography provided 0.98 g (94%) of 5 as a light yellow solid. R_f = 0.45 (100% EA). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.07 (s, 1H), 8.86 (dd, *J* = 23.3, 11.0 Hz, 1H), 8.36 - 8.23 (m, 2H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 1H), 7.23 (d, *J* = 5.7 Hz, 2H), 7.14 (t, *J* = 7.7 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.98 (s, 1H), 5.57 (q, *J* = 7.5, 6.2 Hz, 1H), 4.67 (s, 1H), 3.40 - 3.15 (m, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.75, 156.25, 150.26, 145.03, 136.47, 127.27, 123.51, 122.34, 119.79, 118.62, 113.90, 111.52, 109.94, 80.82, 56.04, 28.37, 22.61. MS (ESI) 381 *m/z* $[M + H]^+$.

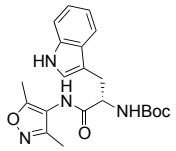


*(S)-2-amino-3-(1H-indol-3-yl)-N-(pyridin-4-yl)propanamide hydrochloric acid, (**6**)*. To a solution of 5 (0.48 g, 1.24 mmol) in dioxane (10 ml) was added 4N HCl in dixoane (5 ml), and the reaction mixture was stirred at room temperature for 12 hours. The solvent was removed with a rotary evaporator, and water was added. The reaction mixture was dried by using lyophilizer to obtain the crude product 6 (0.35 g, 1.09 mmol, 88%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.18 (s, 1H), 11.10 (d, J = 2.5 Hz, 1H), 8.57 (s, 3H), 8.47 (dd, J = 221.3, 7.2 Hz, 3H), 7.71 (d, J = 7.9 Hz, 1H), 7.38 – 7.29 (m, 2H), 7.03 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H), 6.88 (ddd, J = 7.9, 6.9, 1.0 Hz, 1H), 4.64 – 4.42 (m, 1H), 3.44 (qd, J = 14.9, 6.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 170.08, 152.25, 142.45, 136.16, 127.04, 125.32, 121.08, 118.59, 118.45, 114.79, 111.39, 106.06, 53.91, 26.74. MS (ESI) 281 *m/z* $[M + H]^+$.

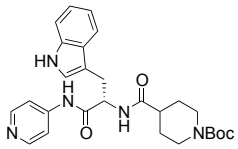


*(S)-tert-butyl (3-(1H-indol-3-yl)-1-((2-methoxypyridin-4-yl)amino)-1-oxopropan-2-yl)carbamate (**7**)*. Compound 7 was obtained by following the procedure for the synthesis of 5 (36%). R_f = 0.36 (50% EA in Hexane). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.36 –

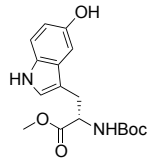
8.15 (m, 1H), 7.94 (d, J = 5.8 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.38 – 7.32 (m, 1H), 7.19 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.10 (ddd, J = 8.0, 7.1, 1.1 Hz, 1H), 7.02 (d, J = 2.4 Hz, 1H), 6.86 (d, J = 1.8 Hz, 1H), 6.74 (dd, J = 5.7, 1.9 Hz, 1H), 5.30 (d, J = 7.6 Hz, 1H), 4.60 (s, 1H), 3.88 (s, 3H), 3.41 – 3.19 (m, 2H), 1.41 (s, 10H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.10, 165.39, 147.51, 146.68, 136.40, 127.27, 123.46, 122.59, 120.07, 118.77, 111.50, 110.27, 108.34, 99.71, 80.97, 53.70, 28.38, 28.11, 22.81. MS (ESI) 411 *m/z* $[M + H]^+$.



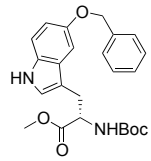
*(S)-tert-butyl (1-((3,5-dimethylisoxazol-4-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamate (**8**)*. Compound 8 was obtained by following the procedure for the synthesis of 5 as a light yellow solid (84%). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.00 – 8.87 (m, 1H), 7.64 (s, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.35 – 7.26 (m, 1H), 7.12 (ddd, J = 8.0, 7.0, 1.1 Hz, 1H), 7.03 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 6.95 (d, J = 2.3 Hz, 1H), 5.50 (d, J = 7.7 Hz, 1H), 4.59 (q, J = 7.2 Hz, 1H), 3.23 (d, J = 6.8 Hz, 2H), 1.96 (s, 3H), 1.84 (s, 3H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.98, 163.62, 157.65, 155.79, 136.38, 127.24, 123.38, 122.11, 119.57, 118.52, 113.07, 111.44, 109.79, 80.36, 55.51, 30.85, 28.23, 10.64, 9.26. MS (ESI) 399 *m/z* $[M + H]^+$.



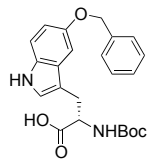
*(S)-tert-butyl 4-((3-(1H-indol-3-yl)-1-oxo-1-(pyridin-4-ylamino)propan-2-yl)carbamoyl)piperidine-1-carboxylate (**9**)*. To a solution of N-Boc-piperidine-4-carboxylic acid (0.082 g, 0.358 mmol) in dry CH_2Cl_2 (5 ml) were added pentafluorophenyl trifluoroacetate (0.06 ml) and $(iPr)_2EtN$ (0.2 ml) at 0 ^oC. The reaction mixture was warm to ambient temperature. After stirring for 30 min., 6 (0.103 g, 0.326 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. After completion of the reaction monitored by TLC, the solvent was removed under reduced pressure. Ethyl acetate (10 ml) was added to the crude mixture, which was washed with saturated aqueous $NaHCO_3$ (2 ml × 2) and brine (2 ml × 2). The organic layer was concentrated in vacuo and directly subjected to purification by flash chromatography to provide the titled product 9 as a light yellow solid (0.12 g, 0.25 mmol, 77%). R_f = 0.24 (100% EA). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.82 (d, J = 2.5 Hz, 1H), 10.53 (s, 1H), 8.49 – 8.36 (m, 2H), 8.22 (d, J = 7.8 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.61 – 7.51 (m, 2H), 7.31 (dd, J = 8.0, 0.9 Hz, 1H), 7.16 (d, J = 2.4 Hz, 1H), 7.05 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 6.96 (ddd, J = 7.9, 7.0, 1.0 Hz, 1H), 4.70 (td, J = 8.4, 5.6 Hz, 1H), 3.88 (t, J = 16.8 Hz, 2H), 3.23 – 2.94 (m, 2H), 2.69 (s, 2H), 2.38 (tt, J = 11.3, 3.7 Hz, 1H), 1.57 (ddd, J = 42.6, 12.7, 3.9 Hz, 2H), 1.38 (s, 11H). ^{13}C NMR (101 MHz, DMSO) δ 174.12, 172.05, 153.81, 150.32, 145.48, 135.99, 127.20, 123.68, 120.90, 118.53, 118.18, 113.37, 111.26, 109.64, 78.53, 54.21, 41.16, 30.67, 28.17, 28.06, 27.67. MS (ESI) 392.4 *m/z* $[M + H]^+$.



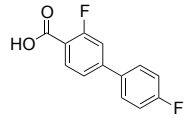
(S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(5-hydroxy-1H-indol-3-yl)propanoate ***(11)***. To a solution of L-5-hydroxyltryptophan (6.66 g, 30.2 mmol) in methanol (100 ml) was slowly added $SOCl_2$ (4.5 ml) at 0 ^oC. After 10 min, the reaction mixture was warmed to ambient temperature and stirred overnight. The solvent was removed under reduced pressure, and CH_2Cl_2 (100 ml) and $(Boc)_2O$ (9.2 g) was added. $(iPr)_2EtN$ (10 ml) was added to the reaction mixture, which was stirred for additional 3 h. Ethyl acetate (300 ml) was added to the crude mixture, which was washed with saturated aqueous $NaHCO_3$ (50 ml × 2) and brine (50 ml × 2). The organic layer was concentrated in vacuo, and the product mixture was purified by flash chromatography to provide the titled product 11 as a brown solid (8.2 g, 24.7 mmol, 82% over 2 steps). ^1H NMR (400 MHz, DMSO-d6) δ 10.52 (d, J = 2.6 Hz, 1H), 8.61 (s, 1H), 7.13 (t, J = 7.9 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.79 (d, J = 2.3 Hz, 1H), 6.59 (dd, J = 8.6, 2.3 Hz, 1H), 4.17 (td, J = 8.5, 5.4 Hz, 1H), 3.60 (s, 3H), 3.33 (s, 1H), 3.07 – 2.84 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 173.03, 155.38, 150.35, 130.64, 127.66, 124.25, 111.77, 111.28, 108.65, 101.86, 78.24, 54.44, 51.72, 28.14, 26.97. MS (ESI) 357 *m/z* $[M + Na]^+$, and 257 *m/z* [M + Na, Boc deprotected]$^+$; 333 *m/z* $[M - H]^-$.



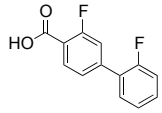
(S)-methyl 3-(5-(benzyloxy)-1H-indol-3-yl)-2-((tert-butoxycarbonyl)amino)propanoate ***(12)***. To a solution of 11 (1.2 g, 3.63 mmol) in acetone (10 ml) were added Cs_2CO_3 (1.5 g) and benzylbromide (0.52 ml). The reaction mixture was stirred overnight at room temperature, and then it was filtered. The filtrate was concentrated under reduced pressure, and it was directly subjected to purification by flash chromatography to yield the product (1.2 g, 2.81 mmol, 77%). R_f = 0.57 (50% EA in Hexane), R_f = 0.09 (20% EA in Hexane). ^1H NMR (400 MHz, DMSO-d6) δ 10.71 (d, J = 2.5 Hz, 1H), 7.49 (d, J = 7.1 Hz, 2H), 7.39 (dd, J = 8.2, 6.5 Hz, 2H), 7.36 – 7.29 (m, 1H), 7.24 (dd, J = 8.5, 1.9 Hz, 2H), 7.13 (dd, J = 6.3, 2.4 Hz, 2H), 6.80 (dd, J = 8.8, 2.4 Hz, 1H), 5.09 (s, 2H), 4.20 (td, J = 8.7, 5.3 Hz, 1H), 3.61 (s, 3H), 3.15 – 2.88 (m, 2H), 1.32 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 172.96, 155.34, 152.13, 137.79, 131.41, 128.34, 127.67, 127.61, 127.48, 124.45, 112.00, 111.56, 109.67, 101.77, 78.17, 69.92, 54.74, 51.73, 28.11, 26.91. MS (ESI) 325 (Boc-deprotected) *m/z* $[M + H]^+$.



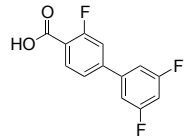
*(S)-3-(5-(benzyloxy)-1H-indol-3-yl)-2-((tert-butoxycarbonyl)amino)propanoic acid (**13**).* To a solution of 12 in ethanol (10 ml) was added 10% NaOH (5 ml) at 0 ^{o}C, and the reaction mixture was stirred for 2 h at 0 ^{o}C. The reaction was monitored by TLC, and after completion of the reaction, 2N HCl was added to the reaction mixture at 0 ^{o}C until white solid precipitated. The white solid was filtered, washed with water, and dried overnight to provide the title product as a white solid (1.1 g, 2.73 mmol, 97%). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.53 (s, 1H), 10.68 (d, J = 2.5 Hz, 1H), 7.48 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.32 (dd, J = 8.4, 6.2 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 7.14 (dd, J = 22.4, 2.4 Hz, 2H), 6.98 (d, J = 8.2 Hz, 1H), 6.79 (dd, J = 8.7, 2.3 Hz, 1H), 5.09 (s, 2H), 4.14 (td, J = 8.8, 4.5 Hz, 1H), 3.16 – 2.86 (m, 2H), 1.32 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d6) δ 173.95, 155.37, 152.09, 137.78, 131.39, 128.33, 127.67, 127.61, 127.57, 124.39, 111.92, 111.48, 110.05, 101.88, 77.95, 69.90, 54.58, 28.14, 26.92. MS (ESI) 409 *m/z* [M - H]$^{-}$.



*3,4'-difluoro-[1,1'-biphenyl]-4-carboxylic acid (**16a**).* The general procedure B was followed using 15 to provide 16a as a white solid (88%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.22 (s, 1H), 7.93 (t, J = 8.0 Hz, 1H), 7.88 – 7.80 (m, 2H), 7.69 – 7.58 (m, 2H), 7.38 – 7.30 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 164.80, 164.77, 163.86, 162.85, 161.41, 160.30, 145.31, 145.22, 134.08, 132.53, 129.28, 129.19, 122.39, 122.36, 117.84, 117.74, 116.07, 115.86, 114.91, 114.68. MS (ESI) 233 *m/z* [M - H]$^{-}$.

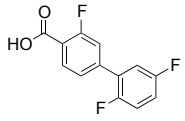


*2',3-difluoro-[1,1'-biphenyl]-4-carboxylic acid (**16b**).* The general procedure B was followed using 15 to provide 16b as a white solid (88%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.31 (s, 1H), 7.96 (t, J = 8.1 Hz, 1H), 7.63 (td, J = 7.9, 1.7 Hz, 1H), 7.50 (tdd, J = 6.6, 5.6, 2.4 Hz, 3H), 7.41 – 7.30 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.74, 164.71, 162.28, 160.26, 159.73, 157.80, 141.30, 141.21, 132.12, 130.94, 130.85, 130.80, 130.78, 126.02, 125.90, 125.16, 125.13, 124.84, 124.80, 124.77, 118.48, 118.38, 117.23, 117.20, 117.00, 116.96, 116.44, 116.21. MS (ESI) 233 *m/z* [M - H]$^{-}$.

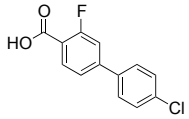


*3,3',5'-trifluoro-[1,1'-biphenyl]-4-carboxylic acid (**16c**).* The general procedure B was followed using 15 to provide 16c as a white solid (83%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.32 (s, 1H), 7.94 (t, J = 8.0 Hz, 1H), 7.77 (dd, J = 12.2, 1.8 Hz, 1H), 7.71 (dd, J = 8.2, 1.8 Hz, 1H), 7.65 – 7.53 (m, 2H), 7.32 (tt, J = 9.3, 2.3 Hz, 1H). ^{13}C NMR (101 MHz,

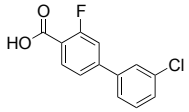
DMSO) δ 164.67, 164.64, 164.15, 164.01, 162.73, 161.70, 161.57, 160.18, 143.55, 141.10, 132.49, 122.76, 122.72, 119.05, 118.95, 115.49, 115.25, 110.51, 110.44, 110.32, 110.25, 104.37, 104.11, 103.85. MS (ESI) 251 *m/z* [M - H]$^-$.



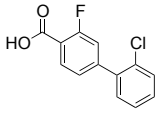
*2',3,5'-trifluoro-[1,1'-biphenyl]-4-carboxylic acid (**16d**)*. The general procedure B was followed using 15 to provide 16d as a white solid (82%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.35 (s, 1H), 7.96 (t, J = 7.9 Hz, 1H), 7.54 (dddd, J = 11.4, 8.1, 3.2, 1.7 Hz, 3H), 7.42 (ddd, J = 10.1, 9.1, 4.6 Hz, 1H), 7.38 – 7.30 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.67, 164.63, 162.23, 159.67, 159.54, 157.15, 157.12, 156.45, 154.01, 140.05, 139.96, 132.13, 127.32, 124.91, 124.87, 124.84, 118.99, 118.89, 118.15, 118.06, 117.90, 117.81, 117.40, 117.36, 117.25, 117.19, 117.16, 117.13, 117.10, 117.01, 116.94, 116.91. MS (ESI) 251 *m/z* [M - H]$^-$.



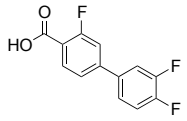
*4'-chloro-3-fluoro-[1,1'-biphenyl]-4-carboxylic acid (**16e**)*. The general procedure B was followed using 15 to provide 16e as a white solid (> 90%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.20 (s, 1H), 7.93 (q, J = 8.3 Hz, 1H), 7.85 – 7.75 (m, 2H), 7.71 – 7.60 (m, 2H), 7.59 – 7.52 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.75, 164.72, 162.85, 160.30, 145.01, 144.93, 136.41, 133.80, 132.58, 129.06, 128.84, 122.44, 122.40, 118.18, 118.08, 114.99, 114.76. MS (ESI) 249 *m/z* [M - H]$^-$.



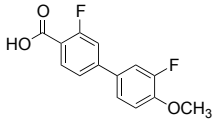
*3'-chloro-3-fluoro-[1,1'-biphenyl]-4-carboxylic acid (**16f**)*. The general procedure B was followed using 15 to provide 16f as a white solid (61%), which was further purified by HPLC. ^{1}H NMR (400 MHz, DMSO-d6) δ 13.31 (s, 1H), 7.94 (t, J = 8.0 Hz, 1H), 7.85 (d, J = 2.0 Hz, 1H), 7.79 – 7.61 (m, 3H), 7.56 – 7.47 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.77, 164.74, 162.82, 160.27, 144.74, 144.65, 139.74, 133.96, 132.55, 130.90, 128.67, 126.84, 125.77, 122.69, 122.66, 118.50, 118.40, 115.31, 115.08. MS (ESI) 249 *m/z* [M - H]$^-$.



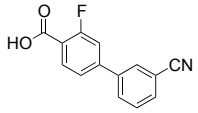
*2'-chloro-3-fluoro-[1,1'-biphenyl]-4-carboxylic acid (**16g**)*. The general procedure B was followed using 15 to provide 16g as a white solid (94%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.24 (s, 1H), 7.94 (t, J = 7.9 Hz, 1H), 7.65 – 7.57 (m, 1H), 7.50 – 7.44 (m, 3H), 7.44 – 7.34 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.77, 164.74, 161.91, 159.36, 144.86, 144.77, 137.61, 131.75, 131.36, 131.06, 130.16, 130.00, 127.68, 125.49, 125.45, 118.58, 118.47, 117.91, 117.67. MS (ESI) 249 *m/z* [M - H]$^-$.



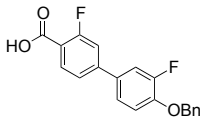
*3,3',4'-trifluoro-[1,1'-biphenyl]-4-carboxylic acid (**16h**)*. The general procedure B was followed using 15 to provide 16h as a white solid (78%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.29 (s, 1H), 7.98 – 7.88 (m, 2H), 7.74 – 7.62 (m, 3H), 7.56 (dt, J = 10.6, 8.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.72, 164.69, 162.79, 160.24, 151.07, 150.95, 148.71, 148.63, 148.51, 144.01, 143.92, 135.14, 132.52, 124.10, 124.03, 124.00, 122.56, 122.52, 118.39, 118.29, 118.21, 118.03, 116.43, 116.25, 115.21, 114.97. MS (ESI) 251 *m/z* [M - H]$^{-}$.



*3,3'-difluoro-4'-methoxy-[1,1'-biphenyl]-4-carboxylic acid (**16i**)*. The general procedure B was followed using 15 to provide 16i as a white solid (> 90%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.18 (s, 1H), 7.90 (t, J = 8.0 Hz, 1H), 7.78 – 7.57 (m, 4H), 7.27 (t, J = 8.8 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 164.80, 164.77, 162.95, 160.40, 152.96, 150.53, 147.85, 147.74, 144.88, 144.79, 132.48, 130.31, 130.24, 123.41, 123.38, 121.93, 121.89, 117.50, 117.40, 114.57, 114.46, 114.38, 114.22, 56.13. MS (ESI) 263 *m/z* [M - H]$^{-}$.

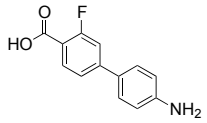


*3'-cyano-3-fluoro-[1,1'-biphenyl]-4-carboxylic acid (**16j**)*. The general procedure B was followed using 15 to provide 16j as a white solid (85%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.25 (s, 1H), 8.29 (t, J = 1.8 Hz, 1H), 8.12 (dt, J = 8.1, 1.4 Hz, 1H), 7.96 (t, J = 8.0 Hz, 1H), 7.90 (dt, J = 7.7, 1.3 Hz, 1H), 7.78 (dd, J = 12.2, 1.7 Hz, 1H), 7.74 – 7.66 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.73, 164.70, 162.83, 160.27, 144.09, 144.01, 138.70, 132.61, 132.34, 131.81, 130.78, 130.28, 122.79, 122.75, 118.79, 118.69, 118.57, 115.48, 115.24, 112.31. MS (ESI) 240 *m/z* [M - H]$^{-}$.

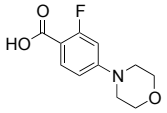


*4'-(benzyloxy)-3,3'-difluoro-[1,1'-biphenyl]-4-carboxylic acid (**16k**)*. The general procedure B was followed using 15 to provide 16k as a white solid (72%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.16 (s, 1H), 7.90 (t, J = 8.0 Hz, 1H), 7.74 (dd, J = 12.8, 2.3 Hz, 1H), 7.70 – 7.56 (m, 3H), 7.51 – 7.45 (m, 2H), 7.45 – 7.39 (m, 2H), 7.39 – 7.32 (m, 2H), 5.26 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.79, 164.75, 162.93, 160.37, 153.24, 150.81, 146.82, 146.71, 144.80, 136.36, 132.46, 130.65, 128.52, 128.11, 127.81, 123.32,

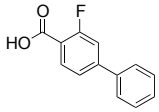
123.29, 121.95, 121.92, 117.55, 117.45, 115.70, 114.76, 114.57, 114.49, 114.26, 70.25. MS (ESI) 339 *m/z* $[M - H]^-$.



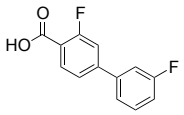
*4'-amino-3-fluoro-[1,1'-biphenyl]-4-carboxylic acid (**16l**)*. The general procedure B was followed using 15 to provide 16l as a yellow solid (56%). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.84 (t, J = 8.2 Hz, 1H), 7.58 – 7.40 (m, 4H), 6.71 – 6.59 (m, 2H), 5.30 (d, J = 227.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.97, 164.94, 163.23, 160.68, 149.93, 147.20, 147.11, 132.42, 127.74, 124.18, 120.52, 120.49, 115.49, 115.39, 114.04, 112.67, 112.44. MS (ESI) 230 *m/z* $[M - H]^-$.



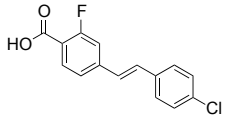
*2-fluoro-4-morpholinobenzoic acid (**16m**)*. The procedure for the synthesis of 20 was followed using 22 to provide 16m as a white solid (95%). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.50 (s, 1H), 7.71 (t, J = 8.9 Hz, 1H), 6.82 – 6.69 (m, 2H), 3.70 (dd, J = 5.9, 3.9 Hz, 4H), 3.31 – 3.24 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 164.86, 164.83, 164.43, 161.90, 155.47, 155.36, 133.08, 133.05, 109.04, 107.26, 107.16, 100.94, 100.67, 65.71, 46.63. MS (ESI) 224 *m/z* $[M - H]^-$.



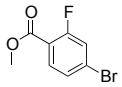
*3-fluoro-[1,1'-biphenyl]-4-carboxylic acid (**16n**)*. The general procedure B was followed using 15 to provide 16n as a white solid (39%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.28 (s, 1H), 7.94 (t, J = 8.0 Hz, 1H), 7.77 – 7.60 (m, 5H), 7.54 (td, J = 8.3, 6.3 Hz, 1H), 7.33 – 7.23 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.75, 164.72, 163.88, 162.81, 161.45, 160.26, 144.88, 144.78, 140.00, 139.94, 132.51, 131.11, 131.03, 123.15, 123.12, 122.64, 122.60, 118.46, 118.35, 115.70, 115.49, 115.25, 115.01, 114.00, 113.77. MS (ESI) 215 *m/z* $[M - H]^-$.



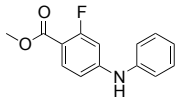
*3,3'-difluoro-[1,1'-biphenyl]-4-carboxylic acid (**16o**)*. The general procedure B was followed using 15 to provide 16o as a white solid (83%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.32 (s, 1H), 7.94 (t, J = 8.0 Hz, 1H), 7.79 – 7.59 (m, 4H), 7.54 (td, J = 8.0, 6.1 Hz, 1H), 7.37 – 7.19 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.78, 164.75, 163.89, 162.83, 161.47, 160.28, 144.87, 144.78, 140.03, 139.95, 132.54, 131.12, 131.04, 123.16, 123.14, 122.65, 122.61, 118.49, 118.39, 115.72, 115.51, 115.26, 115.02, 114.01, 113.79. MS (ESI) 233 *m/z* $[M + H]^+$.



*(E)-4-(4-chlorostyryl)-2-fluorobenzoic acid (**17**)*. A reaction mixture of 15 (0.22 g, 1.0 mmol), 4-chlorostyrene (0.17 ml, 1.42 mmol), $Pd(OAc)_2$ (11.8 mg, 5 mol%), $P(o\text{-tolyl})_3$ (35.2 mg, 10 mol %), and Et_3N (0.5 ml) in dry DMF (5 mL) was stirred under microwave heating (100 °C) for 2 h. After cooling, the palladium catalyst was removed by filtration through Celite pad. To the filtrate was added 2N HCl (aq) to acidify the product mixture. The product mixture was diluted with ethyl acetate/THF (30/30 mL) and washed with brine (20 mL × 2). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting product was purified by flash chromatography to yield 17 (0.20 g, 0.733 mmol, 73%) as a light yellow solid. R_f = 0.51 (100% EA). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.19 (s, 1H), 7.87 (t, J = 8.0 Hz, 1H), 7.71 – 7.61 (m, 2H), 7.61 – 7.40 (m, 5H), 7.33 (d, J = 16.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.77, 164.74, 162.87, 160.32, 143.65, 143.56, 135.31, 132.83, 132.38, 131.00, 128.84, 128.60, 127.11, 122.56, 122.53, 117.80, 117.70, 114.22, 113.99. MS (ESI) 275 *m/z* $[M - H]^-$.

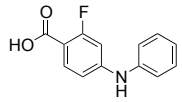


*Methyl 4-bromo-2-fluorobenzoate (**18**)*. To a solution of 15 (13.1 g, 60.0 mmol) in methanol (60 ml) was slowly added $SOCl_2$ (12 ml) at 0 °C. After stirring overnight (ca. 12 h) at ambient temperature, the solvent was removed under reduced pressure, and the reaction mixture was basified with saturated $NaHCO_3$ at 0 °C. Ether (200 ml) was added to the crude mixture, which was washed with saturated $NaHCO_3$ (100 ml × 2) and brine (50 ml × 2). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The product mixture was purified by flash chromatography to provide the titled product 18 as a white solid (13.4 g, 57.7 mmol, 96%). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.86 – 7.78 (m, 1H), 7.39 – 7.30 (m, 2H), 3.92 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.35, 164.31, 163.06, 160.43, 133.30, 128.10, 128.00, 127.71, 127.67, 120.96, 120.71, 117.86, 117.76, 52.64. MS (ESI) 233/235 *m/z* $[M + H]^+$.

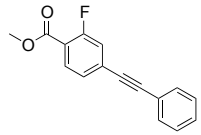


*Methyl 2-fluoro-4-(phenylamino)benzoate (**19**)*. A reaction mixture of 18 (0.103 g, 0.442 mmol), aniline (0.05 ml), $Pd(OAc)_2$ (8 mg, 0.035 mmol), BINAP (32.8 mg, 0.052 mmol), and Cs_2CO_3 (0.156 g) in dry toluene (5 mL) was stirred under microwave heating (100 °C) for 2 h. After cooling, the palladium catalyst was removed by filtration through Celite pad. The product mixture was diluted with ethyl acetate/THF (30/30 mL) and washed with water (10 ml × 2) and brine (10 mL × 2). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting product was purified by flash chromatography to yield 19 (0.20 g, 0.733 mmol, 73%) as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.81 (t, J = 8.6 Hz, 1H), 7.40 – 7.31 (m, 2H), 7.22 – 7.14 (m, 2H), 7.11 (td, J = 7.4, 1.2 Hz, 1H), 6.74 – 6.63 (m, 2H), 6.33 – 6.18 (m, 1H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.24, 164.97, 162.68, 150.36,

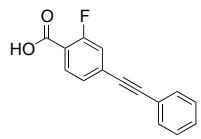
150.25, 140.08, 133.76, 133.74, 129.72, 124.11, 121.48, 110.64, 110.62, 108.77, 108.67, 102.00, 101.73, 51.94. MS (ESI) 246 *m/z* $[M + H]^+$.



*2-Fluoro-4-(phenylamino)benzoic acid (**20**).* To a solution of 19 (0.091 g, 0.372 mmol) in methanol (10 ml) was added 10% NaOH (10 ml), and the reaction mixture was stirred for 1 h at 50 °C. After completion of the reaction, the reaction mixture was cooled to ambient temperature, and 2N HCl was added to the reaction mixture until white solid precipitated. The white solid was diluted with ethyl acetate (60 ml) and washed with brine (10 ml × 2). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting product was purified by flash chromatography to yield 20 (0.0782 g, 0.338 mmol, 91%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.52 (s, 1H), 8.94 (s, 1H), 7.72 (t, J = 8.8 Hz, 1H), 7.44 – 7.28 (m, 2H), 7.27 – 7.12 (m, 2H), 7.11 – 6.97 (m, 1H), 6.83 (dd, J = 8.8, 2.2 Hz, 1H), 6.72 (dd, J = 13.9, 2.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.80, 164.77, 164.43, 161.89, 150.25, 150.14, 140.64, 133.61, 133.58, 129.44, 122.63, 120.05, 110.12, 107.85, 107.75, 100.99, 100.73. MS (ESI) 230 *m/z* $[M - H]^-$.

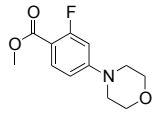


*Methyl 2-fluoro-4-(phenylethynyl)benzoate (**21a**).* A reaction mixture of 18 (0.239 g, 1.03 mmol), ethynylbenzene (0.16 ml), $Pd(OAc)_2$ (20 mg, 0.089 mmol), BINAP (67.2 mg, 0.107 mmol), Et_3N (0.2 ml), and CuI (ca. 10 mg) in dry toluene (5 mL) was stirred under microwave heating (110 °C) for 2 h. After cooling, the palladium catalyst was removed by filtration through Celite pad. The product mixture was diluted with ethyl acetate/THF (40/40 mL) and washed with water (10 ml × 2) and brine (10 mL × 2). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting product was purified by flash chromatography to yield 21a (0.202 g, 0.800 mmol, 77%) as a light yellow solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.92 (t, J = 7.8 Hz, 1H), 7.62 – 7.47 (m, 2H), 7.41 – 7.33 (m, 4H), 7.29 (dd, J = 11.2, 1.5 Hz, 1H), 3.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.58, 164.55, 162.98, 160.39, 132.28, 132.26, 131.95, 129.96, 129.85, 129.23, 128.63, 127.30, 127.27, 122.36, 120.04, 119.80, 118.34, 118.24, 93.42, 87.49, 87.46, 52.56. MS (ESI) 255 *m/z* $[M + H]^+$.

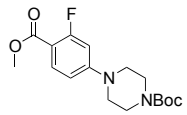


*2-Fluoro-4-(phenylethynyl)benzoic acid (**21b**).* The procedure for the synthesis of 20 was followed using 21a to provide 21b as a white solid (90%). ^{1}H NMR (400 MHz, DMSO-d6) δ 13.43 (s, 1H), 7.90 (t, J = 7.9 Hz, 1H), 7.60 (dt, J = 6.5, 2.3 Hz, 2H), 7.53 (dd, J = 11.3, 1.5 Hz, 1H), 7.47 (td, J = 5.0, 1.8 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 164.47,

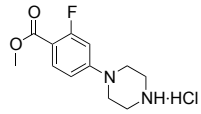
164.44, 162.12, 159.56, 132.34, 131.64, 129.54, 128.88, 128.28, 128.17, 127.48, 127.45, 121.39, 119.64, 119.49, 119.39, 92.73, 87.40, 87.37. MS (ESI) 239 *m/z* $[M - H]^-$.



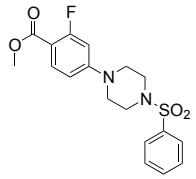
*Methyl 2-fluoro-4-morpholinobenzoate (**22**).* A reaction mixture of 18 (0.23 g, 0.972 mmol), morpholine (0.12 ml, 1.38 mmol), $Pd(OAc)_2$ (10.9 mg, 5 mol%), BINAP (71.6 mg, 10 mol %), and Cs_2CO_3 (0.34 g, 1.04 mol) in dry toluene (5 mL) was stirred under microwave heating (60 °C) for 10 h. After cooling, the palladium catalyst was removed by filtration through Celite pad. The product mixture was diluted with ethyl acetate (30 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The resulting product was purified by flash chromatography to yield 21a as a white solid (0.19 g, 0.784 mmol, 81%). R_f = 0.60 (50% EA). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.77 (t, J = 8.8 Hz, 1H), 6.56 (dd, J = 8.9, 2.5 Hz, 1H), 6.45 (dd, J = 14.5, 2.5 Hz, 1H), 3.82 (s, 2H), 3.80 – 3.74 (m, 4H), 3.25 – 3.17 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.94, 164.75, 164.71, 162.38, 155.65, 155.54, 133.27, 133.24, 109.01, 108.99, 107.76, 107.66, 101.29, 101.03, 66.32, 51.71, 47.17. MS (ESI) 240 *m/z* $[M + H]^+$.



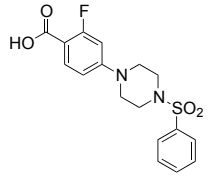
*tert-Butyl 4-(3-fluoro-4-(methoxycarbonyl)phenyl)piperazine-1-carboxylate (**23**).* The procedure for the synthesis of 22 was followed using N-boc-piperazine to provide 23 as a white solid (91%). R_f = 0.27 (20% EA in Hexane). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.82 (t, J = 8.8 Hz, 1H), 6.60 (dd, J = 8.9, 2.5 Hz, 1H), 6.49 (dd, J = 14.5, 2.5 Hz, 1H), 3.86 (s, 3H), 3.60 – 3.52 (m, 4H), 3.33 – 3.26 (m, 4H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.97, 164.76, 164.72, 162.41, 155.29, 155.18, 154.52, 133.36, 133.32, 109.40, 109.37, 107.61, 107.51, 101.63, 101.36, 80.14, 51.70, 46.98, 28.35. MS (ESI) 339 *m/z* $[M + H]^+$.



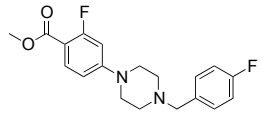
*Methyl 2-fluoro-4-(piperazin-1-yl)benzoate hydrochloride (**24**).* The procedure for the synthesis of 3c was followed using 23 to provide 24 as a crude material (>90%). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.71 (s, 2H), 7.73 (t, J = 8.8 Hz, 1H), 6.93 – 6.77 (m, 2H), 3.77 (s, 3H), 3.62 (dd, J = 6.6, 4.0 Hz, 4H), 3.14 (t, J = 4.8 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 164.27, 163.83, 163.79, 161.73, 154.56, 154.44, 133.00, 132.97, 109.84, 106.72, 106.61, 101.75, 101.48, 51.71, 43.55, 42.00. MS (ESI) 239 *m/z* $[M + H]^+$.



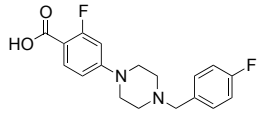
*Methyl 2-fluoro-4-(4-(phenylsulfonyl)piperazin-1-yl)benzoate (**25a**)*. The procedure for the synthesis of 3c was followed using 24 and benzenesulfonyl chloride to provide 25a as a white solid (81%). R_f = 0.42 (20% EA in Hexane). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.81 – 7.71 (m, 3H), 7.71 – 7.62 (m, 3H), 6.80 – 6.70 (m, 2H), 3.75 (s, 3H), 3.45 (dd, J = 6.3, 3.9 Hz, 4H), 2.97 (t, J = 5.1 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 164.18, 163.75, 163.71, 161.65, 154.68, 154.56, 134.57, 133.46, 132.88, 132.84, 129.51, 127.58, 109.71, 106.29, 106.19, 101.52, 101.25, 51.59, 45.90, 45.36. MS (ESI) 379 *m/z* $[M + H]^+$.



*2-Fluoro-4-(4-(phenylsulfonyl)piperazin-1-yl)benzoic acid (**25b**)*. The procedure for the synthesis of 20 was followed using 25a to provide 25b as a white solid (98%). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.54 (s, 1H), 7.83 – 7.70 (m, 3H), 7.70 – 7.60 (m, 3H), 6.79 – 6.66 (m, 2H), 3.43 (t, J = 5.1 Hz, 4H), 2.97 (t, J = 5.1 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 164.77, 164.73, 164.34, 161.80, 154.50, 154.39, 134.58, 133.47, 133.16, 133.13, 129.52, 127.59, 109.69, 107.56, 107.46, 101.67, 101.40, 46.03, 45.38. MS (ESI) 363 *m/z* $[M - H]^-$.



*Methyl 2-fluoro-4-(4-(4-fluorobenzyl)piperazin-1-yl)benzoate (**26a**)*. The procedure for the synthesis of 12 was followed using 24 and 4-fluorobenzyl bromide to provide 26a as a white solid (88%). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.70 (t, J = 8.9 Hz, 1H), 7.35 (dd, J = 8.3, 5.6 Hz, 2H), 7.21 – 7.10 (m, 2H), 6.83 – 6.67 (m, 2H), 3.76 (s, 3H), 3.49 (s, 2H), 3.33 (t, J = 4.9 Hz, 4H), 2.45 (t, J = 4.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO) δ 164.34, 163.86, 163.82, 162.51, 161.81, 160.10, 155.36, 155.25, 134.04, 134.01, 132.82, 132.79, 130.75, 130.67, 115.03, 114.82, 109.22, 105.50, 105.39, 100.80, 100.53, 60.90, 51.95, 51.50, 46.35. MS (ESI) 347 *m/z* $[M + H]^+$.



*2-Fluoro-4-(4-(4-fluorobenzyl)piperazin-1-yl)benzoic acid (**26b**)*. The procedure for the synthesis of 20 was followed using 26a to provide 26b as a white solid (>90%). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.65 (s, 1H), 7.89 – 7.53 (m, 3H), 7.45 – 7.14 (m, 2H), 7.04 – 6.56 (m, 2H), 4.36 (d, J = 4.6 Hz, 2H), 4.05 (d, J = 13.9 Hz, 2H), 3.56 – 3.20 (m, 4H), 3.18 – 2.95 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.74, 164.71, 164.39, 163.95,

161.85, 161.50, 153.86, 153.75, 133.90, 133.81, 133.28, 125.84, 115.81, 115.60, 109.82, 108.09, 107.99, 101.95, 101.68, 57.49, 49.54, 43.63. MS (ESI) 333 *m/z* $[M + H]^+$.