Supporting Information

Discovery of Substituted 1*H*-Pyrazolo[3,4-*b*]pyridine Derivatives as Potent and Selective FGFR Kinase Inhibitors

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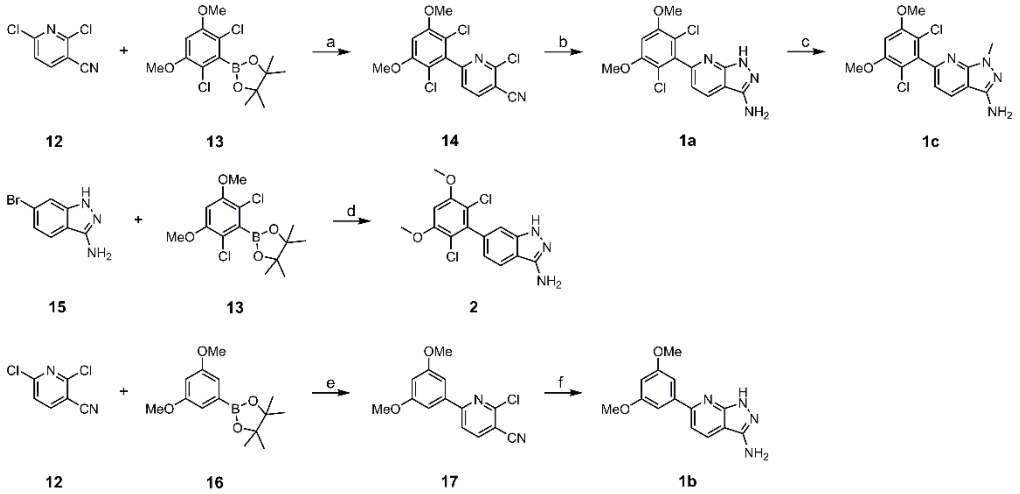
Contents:

1. Chemistry

General Methods. All chemicals and solvents were purchased from commercial sources and used without further purification unless otherwise noted. Reactions were monitored by TLC, using silica gel plates with fluorescence F254 and visualized under UV light. Purification was performed by column chromatography using silica gel (300–400 mesh). All melting points were determined on a Büchi B-510 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Varian Mercury Plus 300 MHz spectrometer or Bruker Avance III 400 MHz spectrometer, and ^{13}C NMR spectra were recorded on Bruker Avance III 126 MHz spectrometer or Bruker Ascend 151 MHz spectrometer. Chemicals shifts (δ) are reported in parts per million, coupling constants (J) values are given in hertz, and peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet. Low-resolution mass spectra (ESI or EI) were recorded on a Finnigan LCQ-DECA spectrometer or a Finnigan/MAT95 spectrometer. High-resolution mass spectra (ESI) were recorded on a Micromass Ultra Q-TOF apparatus. The purity of the final compounds with reported biological data was determined by an Agilent infinity 1260 HPLC system coupled with a diode array detector (DAD) and a ZORBAX Eclipse Plus column (C18, 4.6×150 mm, 5 μm). All tested compounds were purified to ≥95% purity as determined by HPLC.

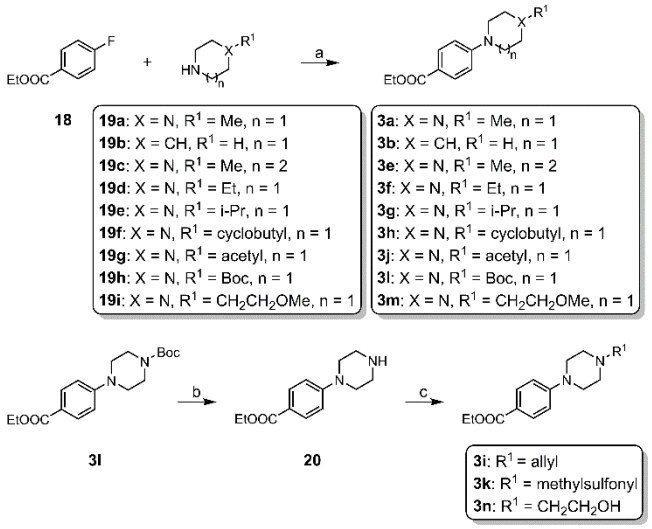
The final compounds were prepared according to the synthetic routes described in Schemes S1–S5.

Scheme S1. Synthesis of Intermediates 1a–c and 2[a]



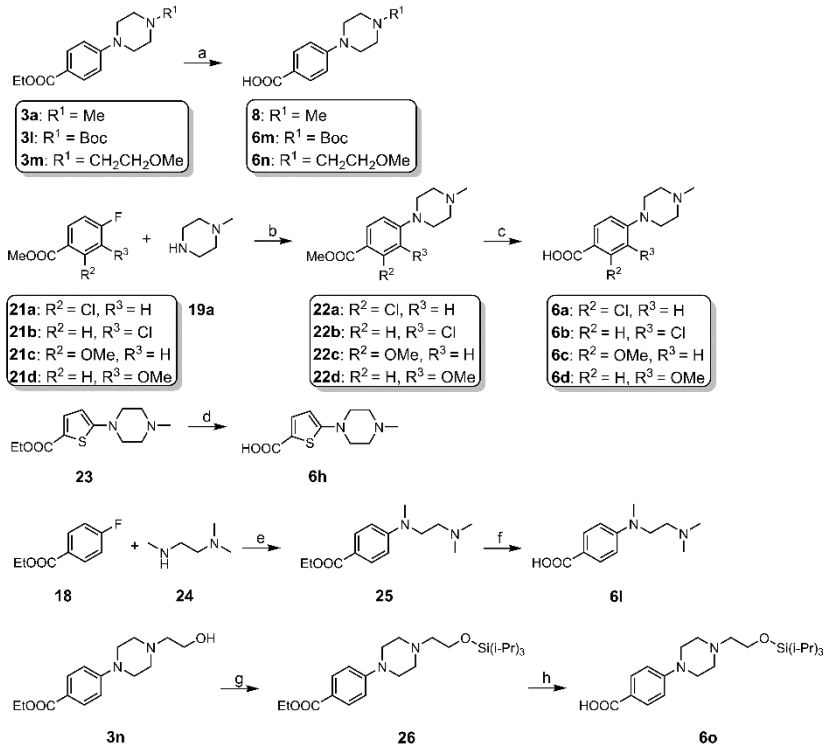
[a]Reagents and conditions: (a) $NaHCO_3$, $Pd(dppf)Cl_2$, $(CH_2OMe)_2$, H_2O, 90 °C, 63%; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, 70 °C, 76%; (c) (i) NaH, DMF, 0 °C; (ii) CH_3I, 0 °C to rt, 80%; (d) Na_2CO_3, $Pd(dppf)Cl_2$, 1,4-dioxane, H_2O, 100 °C, 97%; (e) $NaHCO_3$, $Pd(PPh_3)_4$, Me_2CHOH, H_2O, 90 °C, 79%; (f) $NH_2NH_2{\cdot}H_2O$, EtOH, 70 °C, 83%.

Scheme S2. Synthesis of Intermediates 3a, 3b, and 3e–n[a]



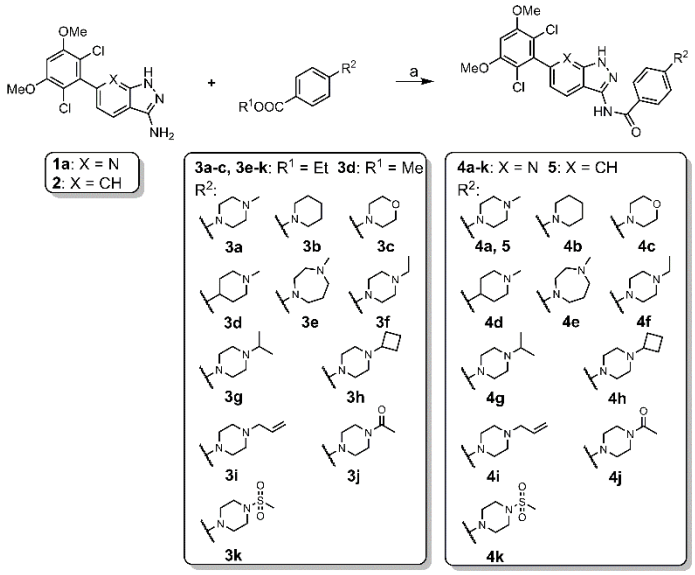
[a]Reagents and conditions: (a) K_2CO_3, DMSO, 120 °C, 58%–88%; (b) CF_3COOH, CH_2Cl_2, rt, 100%; (c) for **3i**, allyl bromide, DIPEA, CH_2Cl_2, rt, 61%; for **3k**, $MeSO_2Cl$, Et_3N, CH_2Cl_2, rt, 84%; for **3n**, $BrCH_2CH_2OH$, K_2CO_3, MeCN, reflux, 51%.

Scheme S3. Synthesis of Intermediates 6a–d, 6h, 6l–o and 8[a]



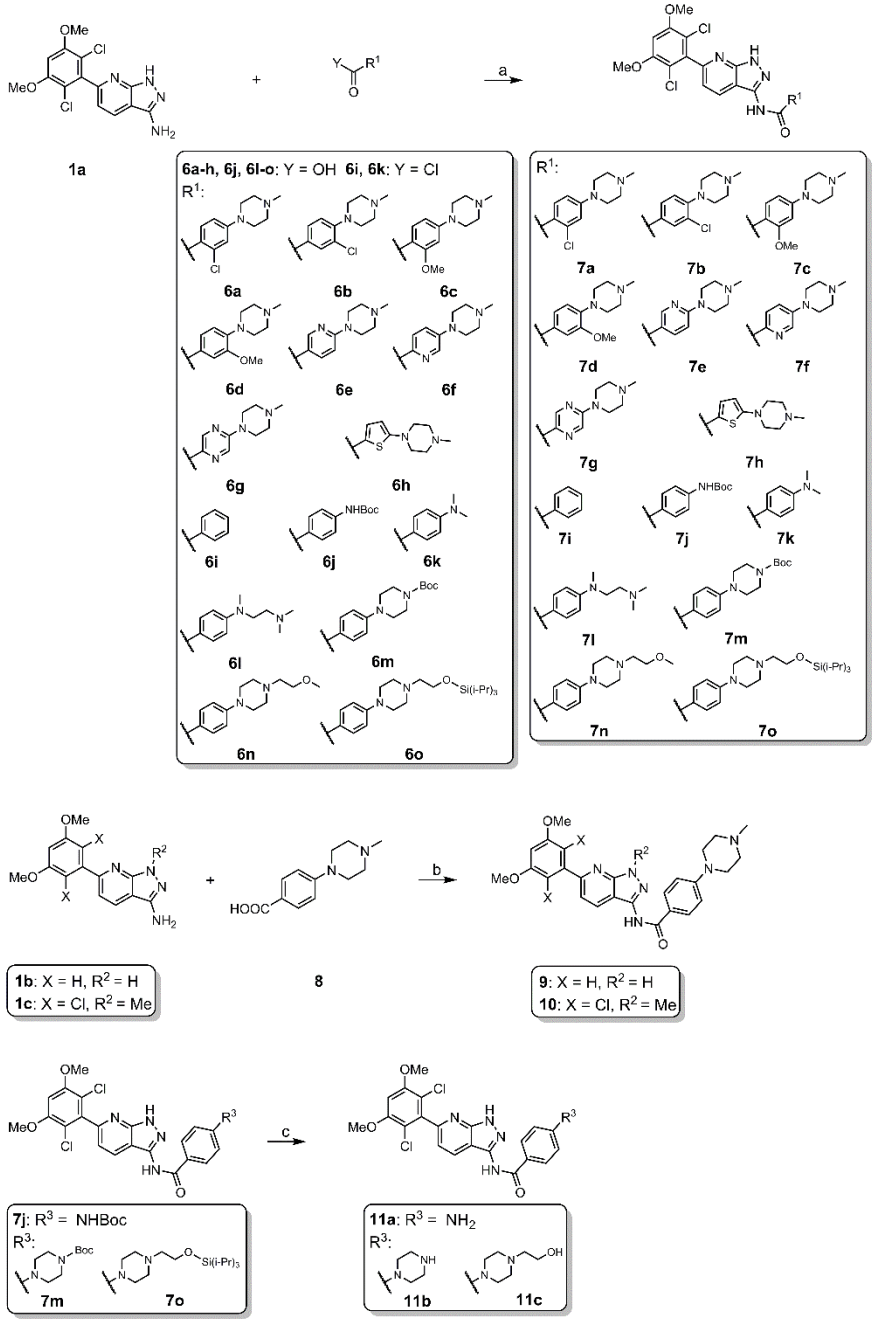
[a]Reagents and conditions: (a) NaOH, H_2O, EtOH, 70 °C, 54%–86%; (b) K_2CO_3, DMSO, 120 °C, 19%–59%; (c) NaOH, H_2O, EtOH, 70 °C, 63%–80%; (d) NaOH, H_2O, THF/EtOH, 70 °C, 68%; (e) K_2CO_3, DMSO, 120 °C, 45%; (f) KOH, H_2O/EtOH, 70 °C, 78%; (g) $(i\text{-Pr})_3SiCl$, 1*H*-imidazole, CH_2Cl_2, 0 °C to rt, 94%; (h) NaOH, H_2O, EtOH, 70 °C, 71%.

Scheme S4. Synthesis of Compounds 4a–k and 5[a]

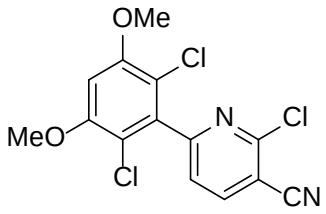


[a]Reagents and conditions: (a) $AlMe_3$, toluene, rt to 60 °C, 4%–28%.

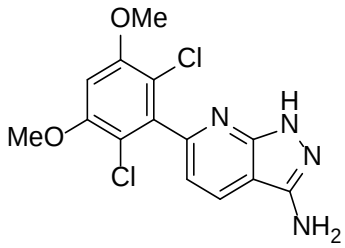
Scheme S5. Synthesis of Compounds 7a–o, 9, 10, and 11a–c[a]



[a]Reagents and conditions: (a) for **7i** and **7k**, DIPEA, THF, 0 °C to rt, 38%–41%; for **7a**–**h**, **7j**, and **7l**–**o**, (i) oxalyl chloride, DMF, CH_2Cl_2, 0 °C to rt; (ii) DIPEA, THF, 0 °C to rt, 18%–60%; (b) (i) oxalyl chloride, DMF, CH_2Cl_2, 0 °C to rt; (ii) DIPEA, THF, 0 °C to rt, 59%–69%; (c) for **11a** and **11b**, CF_3COOH, CH_2Cl_2, rt, 83%–89%; for **11c**, TBAF, THF, 0 °C to rt, 56%.

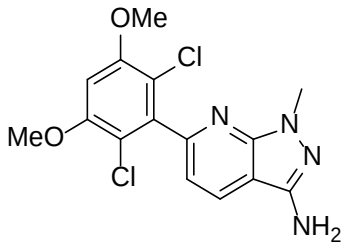


Synthesis of 2-chloro-6-(2,6-dichloro-3,5-dimethoxyphenyl)nicotinonitrile (14). A mixture of 2,6-dichloronicotinonitrile (**12**, 7.923 g, 45.80 mmol), 2-(2,6-dichloro-3,5-dimethoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane[1] (**13**, 18.303 g, 54.96 mmol), Pd(dppf)Cl_2 (3.352 g, 4.58 mmol) and aqueous sodium bicarbonate (1 M, 183 mL) in 1,2-dimethoxyethane (550 mL) was heated at 90 °C under argon for 24 h. After cooling to room temperature, the mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, 80:20 to 60:40) to provide the title compound (9.867 g, 63%) as a white solid, mp 208–209 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 7.9 Hz, 1H), 7.39 (d, J = 7.9 Hz, 1H), 6.66 (s, 1H), 3.96 (s, 6H). MS (EI) *m/z* 342 M^+.

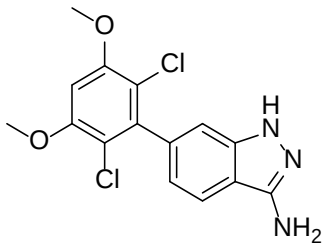


Synthesis of 6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (1a). A mixture of 2-chloro-6-(2,6-dichloro-3,5-dimethoxyphenyl)nicotinonitrile (**14**, 9.864 g, 28.71 mmol) and hydrazine hydrate (85%, 16.4 mL) in ethanol (144 mL) was heated at 70 °C for 11 h. After cooling to room temperature, the mixture was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol + 9% of aqueous ammonia, 98:2 to 97:3) to provide the title compound (7.438 g, 76%) as a yellow solid,

mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H), 8.19 (d, J = 8.1 Hz, 1H), 7.01 (s, 1H), 6.86 (d, J = 8.1 Hz, 1H), 5.64 (s, 2H), 3.97 (s, 6H). MS (ESI) *m/z* 339.2 $[M + H]^+$.

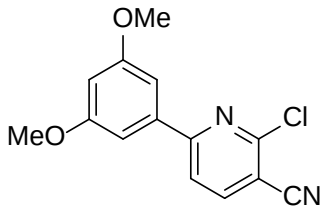


Synthesis of 6-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (1c). To a solution of 6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (**1a**, 339 mg, 1.00 mmol) in anhydrous *N*,*N*-dimethylformamide (1.5 mL) was added sodium hydride (60% in mineral oil, 40 mg, 1.00 mmol) at 0 °C. After the addition, the mixture was stirred for 30 min at 0 °C and iodomethane (64 μL, 1.03 mmol) was added. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of water, and the mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol, 99:1 to 98:2) to provide the title compound (281 mg, 80%) as a yellow solid, mp 219–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, J = 8.0 Hz, 1H), 7.02 (s, 1H), 6.87 (d, J = 8.0 Hz, 1H), 5.76 (s, 2H), 3.97 (s, 6H), 3.72 (s, 3H). MS (ESI) *m/z* 353.2 $[M + H]^+$.

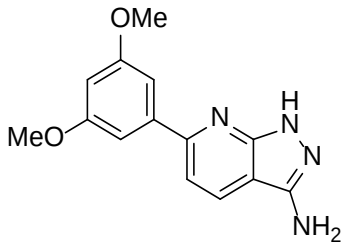


Synthesis of 6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-indazol-3-amine (2). A mixture of 6-bromo-1*H*-indazol-3-amine (**15**, 848 mg, 4.00 mmol), 2-(2,6-dichloro-3,5-

dimethoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**13**, 2.664 g, 8.00 mmol), Pd(dppf)Cl_2 (300 mg, 0.41 mmol) and aqueous sodium carbonate (1 M, 16 mL) in 1,4-dioxane (40 mL) was heated at 100 °C under argon for 18 h. After cooling to room temperature, the mixture was filtered through celite and washed with ethyl acetate. The filtrate was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol + 9% of aqueous ammonia, 99:1 to 98:2) to provide the title compound (1.308 g, 97%) as a yellow solid, mp 235–237 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.45 (s, 1H), 7.73 (d, J = 8.4 Hz, 1H), 6.99 (s, 1H), 6.97 (s, 1H), 6.66 (d, J = 8.2 Hz, 1H), 5.40 (s, 2H), 3.96 (s, 6H). MS (ESI) *m/z* 338.2 $[M + H]^+$.

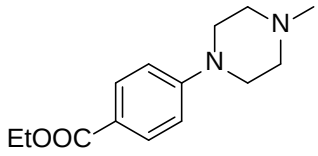


Synthesis of 2-chloro-6-(3,5-dimethoxyphenyl)nicotinonitrile (17). A mixture of 2,6-dichloronicotinonitrile (**12**, 1.384 g, 8.00 mmol), 2-(3,5-dimethoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**16**, 2.325 g, 8.80 mmol), Pd(PPh_3)$_4$ (925 mg, 0.80 mmol) and aqueous sodium bicarbonate (1 M, 22.4 mL) in isopropanol (67.2 mL) was heated at 90 °C under argon for 3 h. After cooling to room temperature, the mixture was concentrated in vacuum and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, 98:2 to 96:4) to provide the title compound (1.740 g, 79%) as a white solid, mp 132–133 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 2.2 Hz, 2H), 6.61 (t, J = 2.2 Hz, 1H), 3.88 (s, 6H). MS (EI) *m/z* 274 M^+.

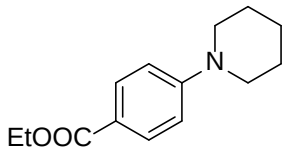


Synthesis of 6-(3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (1b). A mixture of 2-chloro-6-(3,5-dimethoxyphenyl)nicotinonitrile (**17**, 1.738 g, 6.33 mmol) and hydrazine hydrate (85%, 3 mL) in ethanol (30 mL) was heated at 70 °C for 22 h. After cooling to room temperature, the mixture was concentrated in vacuum and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was triturated in dichloromethane (5 mL) to provide the title compound (1.415 g, 83%) as a yellow solid, mp 190–191 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.17 (d, J = 8.3 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 2.2 Hz, 2H), 6.58 (t, J = 2.2 Hz, 1H), 5.58 (s, 2H), 3.83 (s, 6H). MS (ESI) *m/z* 271.2 $[M + H]^+$.

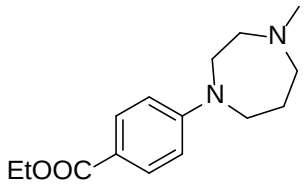
General Procedure A: Synthesis of substituted benzoic acid esters 3a, 3b, 3e–h, 3j, 3l, 3m, 22a–d, and 25. A solution of the appropriate 4-fluorobenzoic acid ester (**18** and **21a**–**d**) (5.00 mmol), amine (**19a**–**i** and **24**) (6.00 mmol) and potassium carbonate (7.50 mmol) in dimethyl sulfoxide (5 mL) was heated at 120 °C for 14–25 h. The resulting solution was diluted with 25 mL of water and partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (for **3a**, **3e**–**h**, **3j**, **3m**, **22a**–**d**, and **25**, dichloromethane/methanol, 99:1 to 97:3; for **3b** and **3l**, petroleum ether/ethyl acetate, 99:1 to 95:5) to give the desired product.



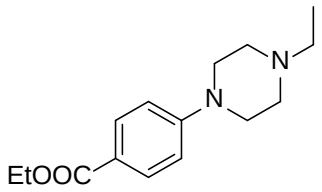
Ethyl 4-(4-methylpiperazin-1-yl)benzoate (3a). The title compound was prepared from **18** and **19a** following general procedure A. Pale yellow solid (88%), mp 74–75 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 4.33 (q, J = 7.1 Hz, 2H), 3.44–3.24 (m, 4H), 2.64–2.51 (m, 4H), 2.35 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 249.3 $[M + H]^+$.



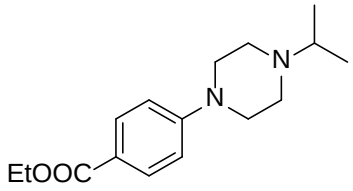
Ethyl 4-(piperidin-1-yl)benzoate (3b). The title compound was prepared from **18** and **19b** following general procedure A. White solid (86%), mp 80–81 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, J = 9.1 Hz, 2H), 6.85 (d, J = 9.1 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.36–3.29 (m, 4H), 1.73–1.60 (m, 6H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 234.3 $[M + H]^+$.



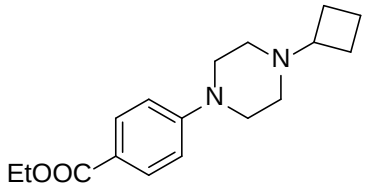
Ethyl 4-(4-methyl-1,4-diazepan-1-yl)benzoate (3e). The title compound was prepared from **18** and **19c** following general procedure A. Yellow oil (58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 9.1 Hz, 2H), 6.65 (d, J = 9.1 Hz, 2H), 4.31 (q, J = 7.1 Hz, 2H), 3.67–3.59 (m, 2H), 3.54 (t, J = 6.3 Hz, 2H), 2.76–2.67 (m, 2H), 2.59–2.51 (m, 2H), 2.38 (s, 3H), 2.08–1.96 (m, 2H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 263.2 $[M + H]^+$.



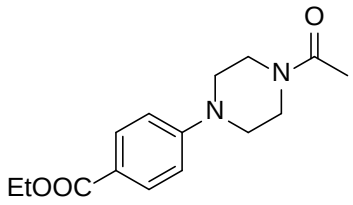
Ethyl 4-(4-ethylpiperazin-1-yl)benzoate (3f). The title compound was prepared from **18** and **19d** following general procedure A. Pale yellow solid (81%), mp 75–76 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.45–3.26 (m, 4H), 2.73–2.55 (m, 4H), 2.47 (q, J = 7.2 Hz, 2H), 1.36 (t, J = 7.1 Hz, 3H), 1.13 (t, J = 7.2 Hz, 3H). MS (ESI) *m/z* 263.2 $[M + H]^+$.



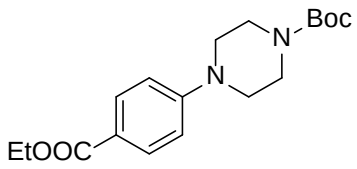
Ethyl 4-(4-isopropylpiperazin-1-yl)benzoate (3g). The title compound was prepared from **18** and **19e** following general procedure A. Pale yellow solid (65%), mp 79–80 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.1 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.47–3.28 (m, 4H), 2.80–2.70 (m, 1H), 2.69–2.61 (m, 4H), 1.36 (t, J = 7.1 Hz, 3H), 1.09 (d, J = 6.5 Hz, 6H). MS (ESI) *m/z* 277.2 $[M + H]^+$.



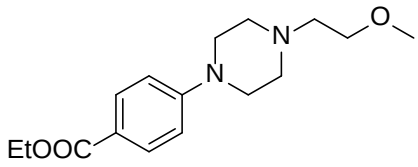
Ethyl 4-(4-cyclobutylpiperazin-1-yl)benzoate (3h). The title compound was prepared from **18** and **19f**[2] following general procedure A. Pale yellow solid (64%), mp 108–109 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.41–3.28 (m, 4H), 2.84–2.71 (m, 1H), 2.56–2.38 (m, 4H), 2.13–2.01 (m, 2H), 1.99–1.83 (m, 2H), 1.79–1.67 (m, 2H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 289.2 $[M + H]^+$.



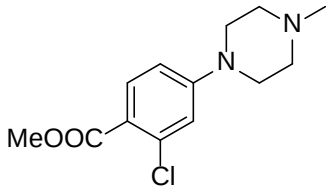
Ethyl 4-(4-acetylpiperazin-1-yl)benzoate (3j). The title compound was prepared from **18** and **19g** following general procedure A. White solid (63%), mp 149–150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 9.1 Hz, 2H), 6.87 (d, J = 9.1 Hz, 2H), 4.33 (q, J = 7.1 Hz, 2H), 3.81–3.73 (m, 2H), 3.67–3.58 (m, 2H), 3.39–3.28 (m, 4H), 2.15 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 299.1 $[M + Na]^+$.



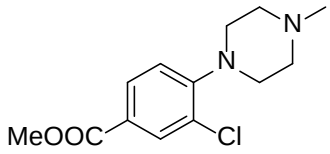
***tert*-Butyl 4-(4-(ethoxycarbonyl)phenyl)piperazine-1-carboxylate (3l).** The title compound was prepared from **18** and **19h** following general procedure A. White solid (58%), mp 120–121 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, J = 8.9 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 4.33 (q, J = 7.1 Hz, 2H), 3.70–3.54 (m, 4H), 3.37–3.21 (m, 4H), 1.48 (s, 9H), 1.37 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 357.1 $[M + Na]^+$.



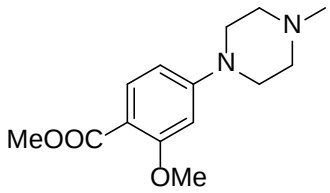
Ethyl 4-(4-(2-methoxyethyl)piperazin-1-yl)benzoate (3m). The title compound was prepared from **18** and **19i** following general procedure A. Pale yellow solid (76%), mp 52–53 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.55 (t, J = 5.5 Hz, 2H), 3.40–3.32 (m, 7H), 2.70–2.60 (m, 6H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 293.2 $[M + H]^+$.



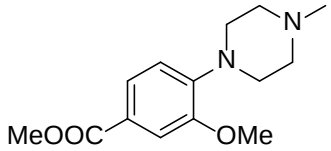
Methyl 2-chloro-4-(4-methylpiperazin-1-yl)benzoate (22a). The title compound was prepared from **21a** and **19a** following general procedure A. Yellow oil (28%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 8.9 Hz, 1H), 6.87 (d, J = 2.6 Hz, 1H), 6.73 (dd, J = 9.0, 2.6 Hz, 1H), 3.87 (s, 3H), 3.38–3.28 (m, 4H), 2.58–2.48 (m, 4H), 2.34 (s, 3H). MS (ESI) *m/z* 269.2 $[M + H]^+$.



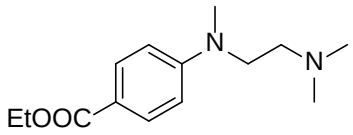
Methyl 3-chloro-4-(4-methylpiperazin-1-yl)benzoate (22b). The title compound was prepared from **21b** and **19a** following general procedure A. Yellow oil (48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J = 2.0 Hz, 1H), 7.88 (dd, J = 8.4, 2.0 Hz, 1H), 7.03 (d, J = 8.5 Hz, 1H), 3.89 (s, 3H), 3.28–3.09 (m, 4H), 2.72–2.53 (m, 4H), 2.37 (s, 3H). MS (ESI) *m/z* 269.3 $[M + H]^+$.



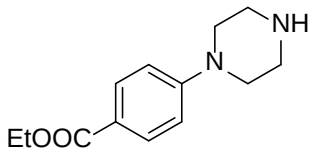
Methyl 2-methoxy-4-(4-methylpiperazin-1-yl)benzoate (22c). The title compound was prepared from **21c** and **19a** following general procedure A. Yellow oil (59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, J = 8.8 Hz, 1H), 6.45 (dd, J = 8.9, 2.0 Hz, 1H), 6.37 (d, J = 1.8 Hz, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.38–3.28 (m, 4H), 2.59–2.50 (m, 4H), 2.35 (s, 3H). MS (ESI) *m/z* 265.3 $[M + H]^+$.



Methyl 3-methoxy-4-(4-methylpiperazin-1-yl)benzoate (22d). The title compound was prepared from **21d** and **19a** following general procedure A. Yellow oil (19%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (dd, J = 8.2, 1.7 Hz, 1H), 7.51 (d, J = 1.6 Hz, 1H), 6.91 (d, J = 8.2 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 3.28–3.08 (m, 4H), 2.69–2.55 (m, 4H), 2.36 (s, 3H). MS (ESI) *m/z* 265.2 $[M + H]^+$.

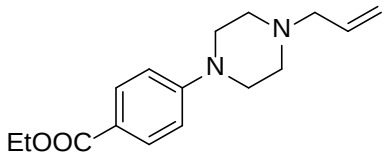


Ethyl 4-((2-(dimethylamino)ethyl)(methyl)amino)benzoate (25). The title compound was prepared from **18** and **24** following general procedure A. Yellow oil (45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, J = 9.1 Hz, 2H), 6.64 (d, J = 9.0 Hz, 2H), 4.31 (q, J = 7.1 Hz, 2H), 3.57–3.46 (m, 2H), 3.03 (s, 3H), 2.52–2.43 (m, 2H), 2.29 (s, 6H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 251.0 $[M + H]^+$.

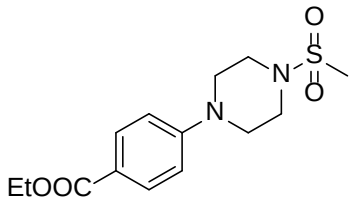


Synthesis of ethyl 4-(piperazin-1-yl)benzoate (20). To a stirred solution of *tert*-butyl 4-(4-(ethoxycarbonyl)phenyl)piperazine-1-carboxylate (**3l**, 970 mg, 2.90 mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (10 mL), and the resulting mixture was stirred for 4 h at room temperature. The mixture was concentrated in vacuum and the residue was basified by the addition of saturated aqueous sodium bicarbonate, followed by extraction with dichloromethane. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to provide the title compound (680 mg, 100%) as a pale yellow solid, mp 94–95 °C. ^{1}H

NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.1 Hz, 2H), 4.33 (q, J = 7.1 Hz, 2H), 3.46–3.21 (m, 4H), 3.19–2.94 (m, 4H), 1.37 (t, J = 7.1 Hz, 3H). MS (ESI) *m/z* 235.2 $[M + H]^+$.

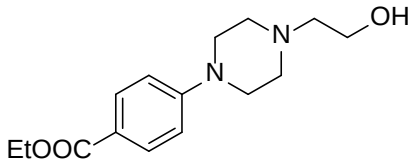


Synthesis of ethyl 4-(4-allylpiperazin-1-yl)benzoate (3i). To a stirred solution of ethyl 4-(piperazin-1-yl)benzoate (**20**, 468 mg, 2.00 mmol) and *N*,*N*-diisopropylethylamine (0.9 mL, 5.17 mmol) in anhydrous dichloromethane (4 mL) was added 3-bromoprop-1-ene (0.2 mL, 2.31 mmol) dropwise under argon, and the resulting mixture was stirred at room temperature overnight. The mixture was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol, 99:1 to 98:2) to provide the title compound (334 mg, 61%) as a pale yellow solid, mp 78–79 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 9.1 Hz, 2H), 6.86 (d, J = 9.1 Hz, 2H), 5.89 (ddt, J = 16.8, 10.2, 6.6 Hz, 1H), 5.27–5.16 (m, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.40–3.30 (m, 4H), 3.06 (d, J = 6.6 Hz, 2H), 2.65–2.55 (m, 4H), 1.36 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 275.2 $[M + H]^+$.

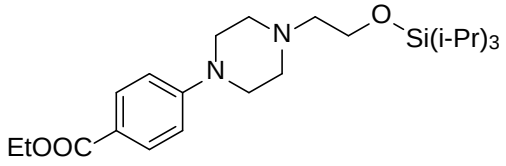


Synthesis of ethyl 4-(4-(methylsulfonyl)piperazin-1-yl)benzoate (3k). To a stirred solution of ethyl 4-(piperazin-1-yl)benzoate (**20**, 234 mg, 1.00 mmol) in anhydrous dichloromethane (3 mL) was added methanesulfonyl chloride (102 μL, 1.32 mmol) and triethylamine (350 μL, 2.51 mmol) dropwise under argon, and the resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of water, and the mixture was extracted with dichloromethane. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated.

The resulting residue was triturated in dichloromethane/methanol (10:1, 3 mL) to provide the title compound (261 mg, 84%) as a white solid, mp 228–229 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81 (d, J = 9.0 Hz, 2H), 7.03 (d, J = 9.0 Hz, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.55–3.40 (m, 4H), 3.29–3.17 (m, 4H), 2.92 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 311.1 $[M - H]^-$.



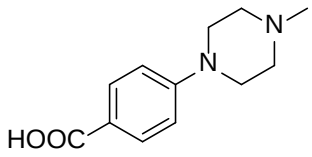
Synthesis of ethyl 4-(4-(2-hydroxyethyl)piperazin-1-yl)benzoate (3n). To a stirred solution of ethyl 4-(piperazin-1-yl)benzoate (**20**, 1.626 g, 6.94 mmol) in acetonitrile (28 mL) was added 2-bromoethan-1-ol (0.5 mL, 7.05 mmol) and potassium carbonate (1.152 g, 8.34 mmol) under argon, and the resulting mixture was refluxed overnight. After cooling to room temperature, the mixture was filtered through celite and washed with dichloromethane. The filtrate was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol, 99:1 to 97:3) to provide the title compound (981 mg, 51%) as a pale yellow solid, mp 92–93 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 8.9 Hz, 2H), 6.87 (d, J = 8.9 Hz, 2H), 4.33 (q, J = 7.2 Hz, 2H), 3.67 (t, J = 5.3 Hz, 2H), 3.37–3.29 (m, 4H), 2.70–2.64 (m, 4H), 2.64–2.56 (m, 2H), 1.37 (t, J = 7.1 Hz, 3H). MS (ESI) m/z 279.2 $[M + H]^+$.



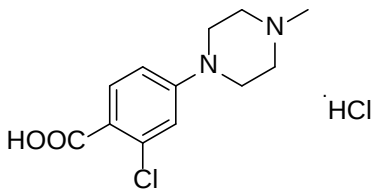
Synthesis of ethyl 4-(4-(2-((triisopropylsilyl)oxy)ethyl)piperazin-1-yl)benzoate (26). To a stirred solution of ethyl 4-(4-(2-hydroxyethyl)piperazin-1-yl)benzoate (**3n**, 557 mg, 2.00 mmol) and 1*H*-imidazole (178 mg, 2.61 mmol) in anhydrous dichloromethane (4 mL) was added triisopropylsilyl chloride (514 μL, 2.40 mmol) dropwise under argon at 0 °C, and the resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of water, and the mixture was

extracted with dichloromethane. The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol, 99:1 to 98:2) to provide the title compound (819 mg, 94%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 8.9 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.88 (t, J = 6.2 Hz, 2H), 3.38–3.27 (m, 4H), 2.74–2.66 (m, 4H), 2.62 (t, J = 6.2 Hz, 2H), 1.36 (t, J = 7.1 Hz, 3H), 1.18–0.92 (m, 21H). MS (ESI) *m/z* 435.3 $[M + H]^+$.

General Procedure B: Synthesis of substituted benzoic acids 8, 6a–d and 6m–o. To a stirred solution of the appropriate benzoic acid ester (1.00 mmol) in ethanol (6.8 mL) was added aqueous sodium hydroxide (1 M, 3.4 mL), and the resulting mixture was heated at 70 °C for 3–5 h. After cooling to room temperature, the mixture was concentrated in vacuum and the residue was dissolved in water. The solution was acidified by the addition of aqueous hydrochloric acid (2 M) at 0 °C. A solid was precipitated which was filtered, washed with cold water, and dried in vacuum to afford the desired product.

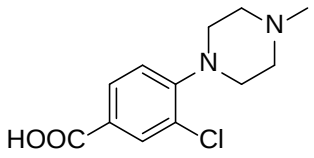


4-(4-Methylpiperazin-1-yl)benzoic acid (8). The title compound was prepared from **3a** following general procedure B. White solid (54%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 3.30–3.18 (m, 4H), 2.46–2.38 (m, 4H), 2.21 (s, 3H). MS (ESI) *m/z* 221.2 $[M + H]^+$.

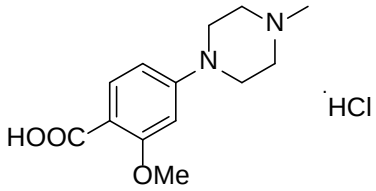


2-Chloro-4-(4-methylpiperazin-1-yl)benzoic acid hydrochloride (6a). The title

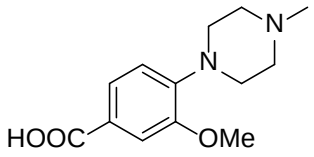
compound was prepared from **22a** following general procedure B. White solid (63%), mp 226 ºC dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.75 (s, 1H), 10.76 (s, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.09 (d, J = 2.4 Hz, 1H), 6.99 (dd, J = 8.8, 2.3 Hz, 1H), 4.26–3.84 (m, 2H), 3.68–3.39 (m, 2H), 3.28–2.90 (m, 4H), 2.79 (s, 3H). MS (ESI) *m/z* 255.2 $[M - Cl]^+$.



3-Chloro-4-(4-methylpiperazin-1-yl)benzoic acid (6b). The title compound was prepared from **22b** following general procedure B. Pale yellow solid (80%), mp 223–224 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92–7.83 (m, 2H), 7.29 (d, J = 8.3 Hz, 1H), 3.34 (s, 8H), 2.80 (s, 3H). MS (ESI) *m/z* 255.2 $[M + H]^+$.

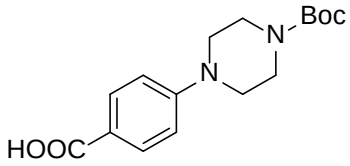


2-Methoxy-4-(4-methylpiperazin-1-yl)benzoic acid hydrochloride (6c). The title compound was prepared from **22c** following general procedure B. White solid (64%), mp 225–226 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.94 (s, 1H), 11.33 (s, 1H), 7.65 (d, J = 9.1 Hz, 1H), 6.59 (d, J = 7.4 Hz, 2H), 4.04 (d, J = 12.6 Hz, 2H), 3.82 (s, 3H), 3.46 (d, J = 11.9 Hz, 2H), 3.32–3.19 (m, 2H), 3.15–2.99 (m, 2H), 2.78 (s, 3H). MS (ESI) *m/z* 251.2 $[M - Cl]^+$.

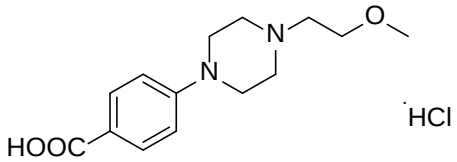


3-Methoxy-4-(4-methylpiperazin-1-yl)benzoic acid (6d). The title compound was prepared from **22d** following general procedure B. Pale yellow solid (68%), mp 210 ºC dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.49 (s, 1H), 7.53 (dd, J = 8.2, 1.6 Hz, 1H),

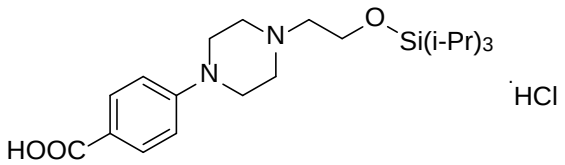
7.45 (d, *J* = 1.6 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 3.84 (s, 3H), 3.62 (d, *J* = 9.6 Hz, 2H), 3.45 (d, *J* = 8.7 Hz, 2H), 3.26–3.05 (m, 4H), 2.77 (d, *J* = 4.6 Hz, 3H). MS (ESI) *m/z* 251.2 $[M + H]^+$.



4-(4-(*tert*-Butoxycarbonyl)piperazin-1-yl)benzoic acid (6m). The title compound was prepared from **3l** following general procedure B. White solid (86%), mp 216 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1H), 7.77 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 9.0 Hz, 2H), 3.51–3.40 (m, 4H), 3.31–3.23 (m, 4H), 1.42 (s, 9H). MS (ESI) *m/z* 305.1 $[M - H]^-$.

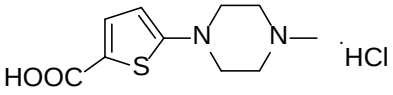


4-(4-(2-Methoxyethyl)piperazin-1-yl)benzoic acid hydrochloride (6n). The title compound was prepared from **3m** following general procedure B. White solid (73%), mp 175 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.44 (s, 1H), 10.77 (s, 1H), 7.81 (d, *J* = 8.9 Hz, 2H), 7.04 (d, *J* = 9.0 Hz, 2H), 4.00 (d, *J* = 12.7 Hz, 2H), 3.81–3.70 (m, 2H), 3.56 (d, *J* = 11.6 Hz, 2H), 3.36 (s, 3H), 3.29–3.03 (m, 4H). MS (ESI) *m/z* 265.2 $[M - Cl]^+$.

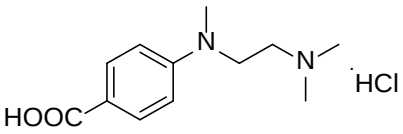


4-(4-(2-((Triisopropylsilyl)oxy)ethyl)piperazin-1-yl)benzoic acid hydrochloride (6o). The title compound was prepared from **26** following general procedure B. White solid (71%), mp 229–230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.42 (s, 1H), 11.19 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 4.12 (t, *J* = 4.9 Hz, 2H), 4.03 (d,

J = 11.7 Hz, 2H), 3.60 (d, *J* = 10.9 Hz, 2H), 3.43–3.15 (m, 6H), 1.17–0.97 (m, 21H). MS (ESI) *m/z* 407.2 $[M - Cl]^+$.



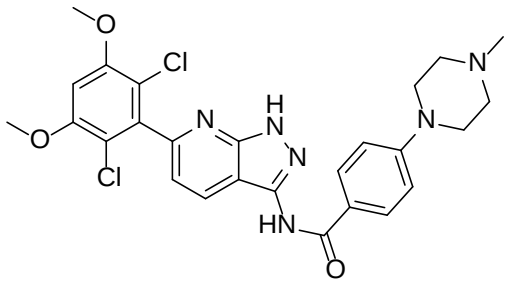
Synthesis of 5-(4-methylpiperazin-1-yl)thiophene-2-carboxylic acid hydrochloride (6h). To a stirred solution of ethyl 5-(4-methylpiperazin-1-yl)thiophene-2-carboxylate[3] (**23**, 305 mg, 1.20 mmol) in tetrahydrofuran/ethanol (2:1, 12 mL) was added aqueous sodium hydroxide (2 M, 6 mL), and the resulting mixture was heated at 70 °C for 6 h. After cooling to room temperature, the mixture was concentrated in vacuum and the residue was dissolved in water. The solution was acidified by the addition of concentrated hydrochloric acid (37%) at 0 °C. A solid was precipitated which was filtered, washed with cold water, and dried in vacuum to afford the title compound (215 mg, 68%) as a yellow-green solid, mp 213–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.46 (s, 1H), 11.12 (s, 1H), 7.47 (d, *J* = 4.2 Hz, 1H), 6.31 (d, *J* = 4.2 Hz, 1H), 3.91–3.18 (m, 8H), 2.78 (s, 3H). MS (ESI) m/z 227.2 $[M - Cl]^+$.



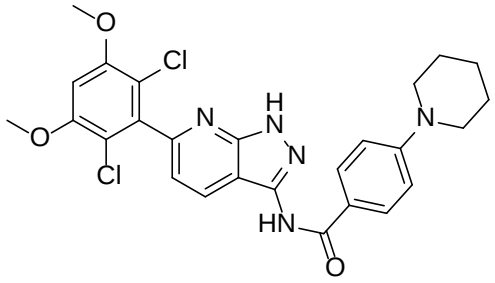
Synthesis of 4-((2-(dimethylamino)ethyl)(methyl)amino)benzoic acid hydrochloride (6l). To a stirred solution of ethyl 4-((2-(dimethylamino)ethyl)(methyl)amino)benzoate (**25**, 300 mg, 1.20 mmol) in ethanol/water (2:1, 9 mL) was added potassium hydroxide (270 mg, 4.81 mmol), and the resulting mixture was heated at 70 °C for 3 h. After cooling to room temperature, the mixture was concentrated in vacuum and the residue was dissolved in water. The solution was acidified by the addition of concentrated hydrochloric acid (37%) at 0 °C. A solid was precipitated which was filtered, washed with cold water, and dried in vacuum to afford the title compound (243 mg, 78%) as a pale yellow solid, mp 219–

221 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (s, 1H), 10.78 (s, 1H), 7.76 (d, J = 8.9 Hz, 2H), 6.83 (d, J = 9.0 Hz, 2H), 3.89–3.74 (m, 2H), 3.26–3.12 (m, 2H), 3.00 (s, 3H), 2.79 (s, 6H). MS (ESI) m/z 223.1 $[M - Cl]^+$.

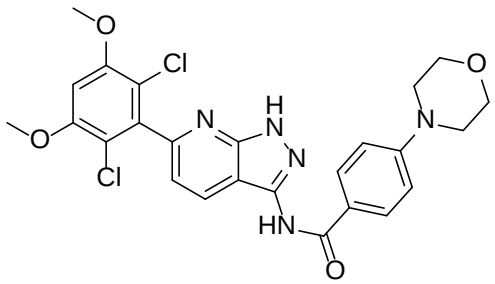
General Procedure C: Synthesis of target compounds 4a–k and 5. A 1.6 M solution of trimethylaluminum in toluene (0.4 mL), was added dropwise to a stirred suspension of the appropriate amine (**1a** and **2**) (0.20 mmol) and benzoic acid ester (**3a**–**k**) (0.20 mmol) in anhydrous toluene (1 mL). The solution was then heated at 60 °C for 22-72 h. After cooling to room temperature, the reaction was quenched by the addition of methanol, and the mixture was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol + 9% of aqueous ammonia, 99:1 to 95:5) to give the desired product.



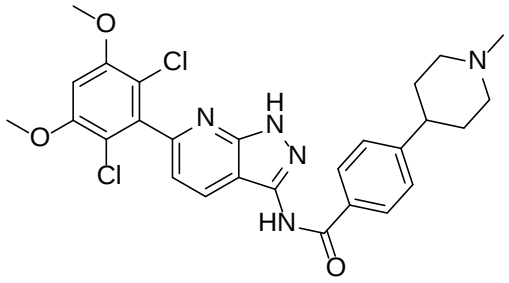
***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-methylpiperazin-1-yl)benzamide (4a).** The title compound was prepared from **1a** and **3a** following general procedure C. White solid (17%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.38 (s, 1H), 10.82 (s, 1H), 8.38 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.9 Hz, 2H), 7.13–6.96 (m, 4H), 3.98 (s, 6H), 3.32–3.25 (m, 4H), 2.48–2.39 (m, 4H), 2.23 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.9, 154.6, 154.4 (2C), 152.0, 151.5, 140.1, 139.4, 133.2, 129.6 (2C), 123.3, 117.1, 114.2 (2C), 112.4 (2C), 107.9, 98.4, 56.8 (2C), 51.8 (2C), 44.4 (2C), 42.0. HRMS (ESI) *m/z* calcd for $C_{26}H_{27}Cl_2N_6O_3$ $[M+H]^+$ 541.1516, found 541.1516. HPLC purity: 97.00%.



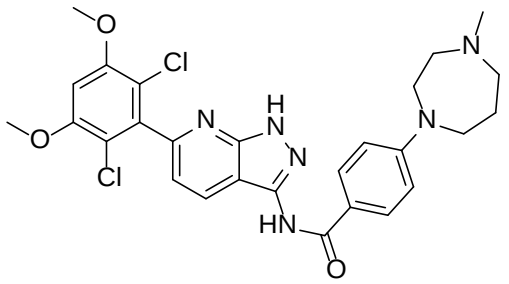
***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(piperidin-1-yl)benzamide (4b).** The title compound was prepared from **1a** and **3b** following general procedure C. Yellow solid (13%), mp 175 °C dec. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.30 (s, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.81 (s, 1H), 7.87 (d, J = 8.6 Hz, 2H), 7.15 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 8.7 Hz, 2H), 6.66 (s, 1H), 3.97 (s, 6H), 3.44–3.24 (m, 4H), 1.93–1.65 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 153.4, 151.5, 140.3, 139.4, 133.3, 129.6 (2C), 121.2, 117.0, 113.4 (2C), 112.4 (2C), 107.9, 98.4, 56.8 (2C), 48.1 (2C), 24.9 (2C), 24.0. HRMS (ESI) *m/z* calcd for $C_{26}H_{26}Cl_2N_5O_3$ $[M+H]^+$ 526.1407, found 526.1413. HPLC purity: 95.47%.



***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-morpholinobenzamide (4c).** The title compound was prepared from **1a** and **3c**[4] following general procedure C. Yellow solid (18%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.38 (s, 1H), 10.83 (s, 1H), 8.39 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.16–6.95 (m, 4H), 3.98 (s, 6H), 3.85–3.68 (m, 4H), 3.31–3.18 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 153.4, 151.5, 140.2, 139.4, 133.2, 129.5 (2C), 122.6, 117.0, 113.3 (2C), 112.4 (2C), 107.9, 98.4, 65.9 (2C), 56.8 (2C), 47.2 (2C). HRMS (ESI) *m/z* calcd for $C_{25}H_{24}Cl_2N_5O_4$ $[M+H]^+$ 528.1200, found 528.1192. HPLC purity: 95.05%.

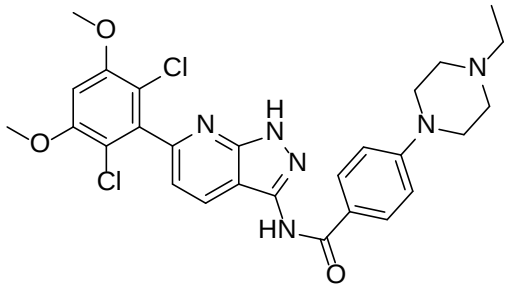


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(1-methylpiperidin-4-yl)benzamide (4d).** The title compound was prepared from **1a** and **3d**[5] following general procedure C. Yellow solid (17%), mp 220 °C dec. ^{1}H NMR (300 MHz, CD_3OD) δ 8.53 (d, J = 8.3 Hz, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.3 Hz, 2H), 7.14 (d, J = 8.3 Hz, 1H), 6.95 (s, 1H), 3.99 (s, 6H), 3.56 (d, J = 12.2 Hz, 2H), 3.07 (dd, J = 13.1, 11.4 Hz, 2H), 3.01–2.93 (m, 1H), 2.87 (s, 3H), 2.21–1.92 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.2, 154.6, 154.4 (2C), 151.5, 148.7, 139.8, 139.3, 133.1, 131.7, 128.4 (2C), 126.8 (2C), 117.2, 112.4 (2C), 107.8, 98.4, 56.8 (2C), 53.6 (2C), 42.8, 38.7, 29.8 (2C). HRMS (ESI) *m/z* calcd for $C_{27}H_{28}Cl_2N_5O_3$ $[M+H]^+$ 540.1564, found 540.1576. HPLC purity: 96.60%.

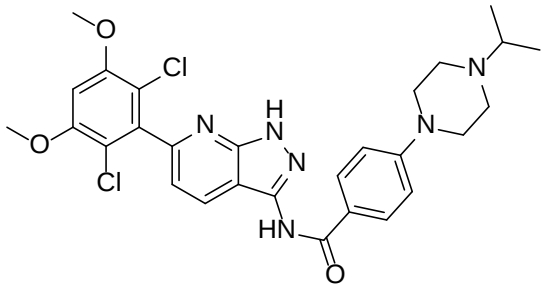


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-methyl-1,4-diazepan-1-yl)benzamide (4e).** The title compound was prepared from **1a** and **3e** following general procedure C. White solid (14%), mp 223 °C dec. ^{1}H NMR (300 MHz, CD_3OD) δ 8.50 (d, J = 8.3 Hz, 1H), 7.99 (d, J = 8.9 Hz, 2H), 7.12 (d, J = 8.3 Hz, 1H), 6.94 (s, 1H), 6.91 (d, J = 9.0 Hz, 2H), 3.99 (s, 6H), 3.88–3.80 (m, 2H), 3.65 (t, J = 6.2 Hz, 2H), 3.28–3.23 (m, 2H), 3.20–3.11 (m, 2H), 2.77 (s, 3H), 2.29–2.17 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 151.5, 151.2, 140.3, 139.4, 133.3, 129.8 (2C), 119.9, 117.0, 112.4 (2C), 110.7 (2C), 107.9, 98.4, 56.8

(2C), 56.3, 55.5 (2C), 47.2, 44.2, 24.8. HRMS (ESI) *m/z* calcd for $C_{27}H_{29}Cl_2N_6O_3$ $[M+H]^+$ 555.1673, found 555.1678. HPLC purity: 97.26%.

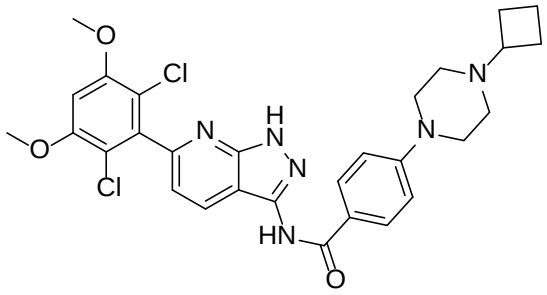


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-ethylpiperazin-1-yl)benzamide (4f).** The title compound was prepared from **1a** and **3f** following general procedure C. White solid (15%), mp 244 °C dec. ^{1}H NMR (300 MHz, CD_3OD) δ 8.51 (d, J = 8.3 Hz, 1H), 7.99 (d, J = 8.9 Hz, 2H), 7.12 (d, J = 8.3 Hz, 1H), 7.08 (d, J = 9.0 Hz, 2H), 6.94 (s, 1H), 3.99 (s, 6H), 3.52–3.39 (m, 4H), 2.84–2.68 (m, 4H), 2.59 (q, J = 7.3 Hz, 2H), 1.19 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 153.2, 151.5, 140.2, 139.4, 133.3, 129.5 (2C), 122.1, 117.0, 113.5 (2C), 112.4 (2C), 107.9, 98.4, 56.8 (2C), 51.9 (2C), 51.5, 46.7 (2C), 11.8. HRMS (ESI) *m/z* calcd for $C_{27}H_{29}Cl_2N_6O_3$ $[M+H]^+$ 555.1673, found 555.1673. HPLC purity: 97.67%.

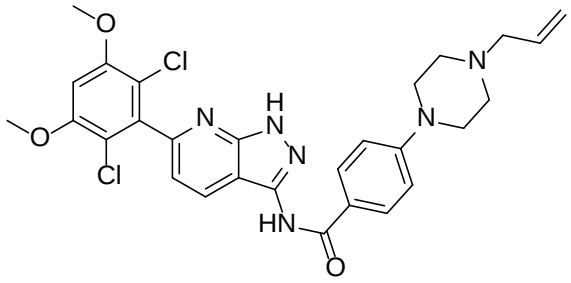


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-isopropylpiperazin-1-yl)benzamide (4g).** The title compound was prepared from **1a** and **3g** following general procedure C. White solid (10%), mp 235 °C dec. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.82 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 8.4 Hz, 2H),

7.14 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.64 (s, 1H), 3.96 (s, 6H), 3.59–3.43 (m, 4H), 3.08–2.97 (m, 1H), 2.96–2.80 (m, 4H), 1.22 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 151.5 (2C), 140.2, 139.4, 133.2, 129.5 (2C), 121.0, 117.0, 113.7 (2C), 112.4 (2C), 107.9, 98.4, 56.8 (3C), 47.5 (4C), 17.5 (2C). HRMS (ESI) *m/z* calcd for $C_{28}H_{31}Cl_2N_6O_3$ $[M+H]^+$ 569.1829, found 569.1830. HPLC purity: 97.62%.

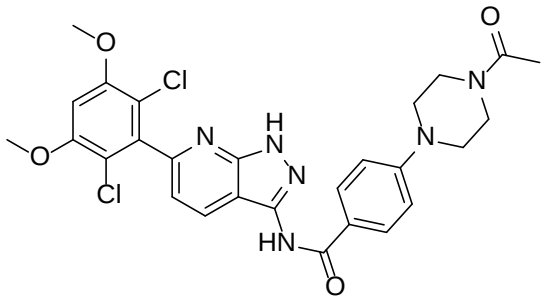


4-(4-Cyclobutylpiperazin-1-yl)-*N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)benzamide (4h). The title compound was prepared from **1a** and **3h** following general procedure C. White solid (6%), mp 198 °C dec. ^{1}H NMR (300 MHz, CD_3OD) δ 8.51 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 8.9 Hz, 2H), 7.18–7.08 (m, 3H), 6.95 (s, 1H), 3.99 (s, 6H), 3.60–3.48 (m, 4H), 3.10–3.05 (m, 1H), 3.04–2.89 (m, 4H), 2.32–2.22 (m, 2H), 2.19–2.09 (m, 2H), 1.92–1.81 (m, 2H). HRMS (ESI) *m/z* calcd for $C_{29}H_{31}Cl_2N_6O_3$ $[M+H]^+$ 581.1829, found 581.1829. HPLC purity: 96.04%.

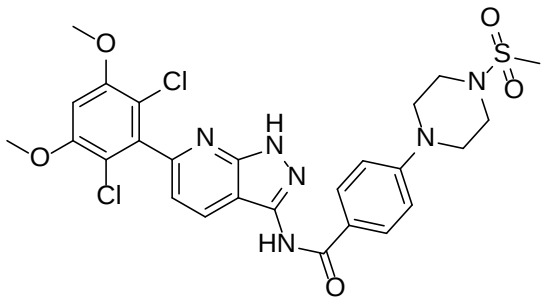


4-(4-Allylpiperazin-1-yl)-*N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)benzamide (4i). The title compound was prepared from **1a** and **3i** following general procedure C. White solid (7%), mp 182 °C dec. ^{1}H NMR (300 MHz, CD_3OD) δ 8.51 (d, *J* = 8.3 Hz, 1H), 7.98 (d, *J* = 8.9 Hz, 2H), 7.12 (d, *J* =

8.3 Hz, 1H), 7.07 (d, *J* = 9.0 Hz, 2H), 6.95 (s, 1H), 5.94 (ddt, *J* = 16.8, 10.1, 6.6 Hz, 1H), 5.35–5.22 (m, 2H), 3.99 (s, 6H), 3.49–3.38 (m, 4H), 3.12 (d, *J* = 6.7 Hz, 2H), 2.76–2.60 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 151.5 (2C), 140.2, 139.4, 133.2 (2C), 129.5 (2C), 120.5, 117.0, 114.0, 113.6 (2C), 112.4 (2C), 107.9, 98.4, 58.2, 56.8 (2C), 52.3 (2C), 46.7 (2C). HRMS (ESI) *m/z* calcd for $C_{28}H_{29}Cl_2N_6O_3$ $[M+H]^+$ 567.1673, found 567.1678. HPLC purity: 95.08%.

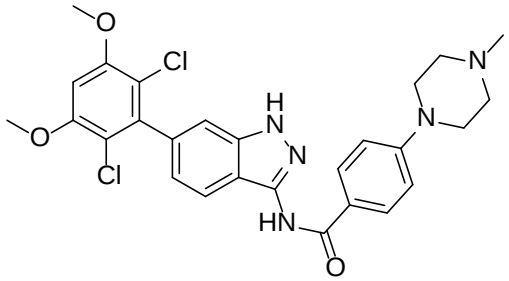


4-(4-Acetylpiperazin-1-yl)-*N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)benzamide (4j). The title compound was prepared from **1a** and **3j** following general procedure C. Yellow solid (28%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.37 (s, 1H), 10.82 (s, 1H), 8.38 (d, *J* = 8.3 Hz, 1H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.13–6.99 (m, 4H), 3.98 (s, 6H), 3.70–3.53 (m, 4H), 3.43–3.36 (m, 2H), 2.05 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.4, 165.0, 154.5, 154.4 (2C), 153.0, 151.5, 140.2, 139.4, 133.3, 129.5 (2C), 122.4, 117.0, 113.7 (2C), 112.4 (2C), 107.9, 98.4, 56.8 (2C), 47.1, 46.8, 45.1, 40.4, 21.2. HRMS (ESI) *m/z* calcd for $C_{27}H_{27}Cl_2N_6O_4$ $[M+H]^+$ 569.1465, found 569.1462. HPLC purity: 95.28%.



***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-**

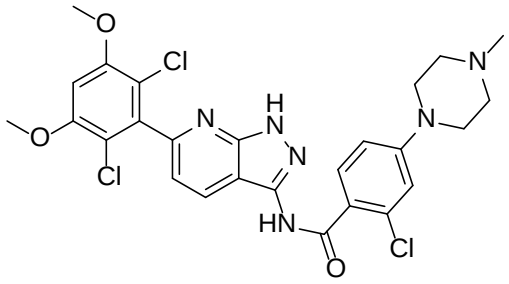
(methylsulfonyl)piperazin-1-yl)benzamide (4k). The title compound was prepared from **1a** and **3k** following general procedure C. White solid (19%), mp >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.39 (s, 1H), 10.86 (s, 1H), 8.38 (d, J = 8.3 Hz, 1H), 8.03 (d, J = 8.7 Hz, 2H), 7.12–7.06 (m, 3H), 7.05 (s, 1H), 3.98 (s, 6H), 3.51–3.40 (m, 4H), 3.29–3.21 (m, 4H), 2.93 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.9, 154.5, 154.4 (2C), 152.7, 151.5, 140.2, 139.4, 133.2, 129.6 (2C), 122.8, 117.0, 114.1 (2C), 112.4 (2C), 107.9, 98.4, 56.8 (2C), 46.8 (2C), 45.0 (2C), 33.9. HRMS (ESI) *m/z* calcd for $C_{26}H_{27}Cl_2N_6O_5S$ $[M+H]^+$ 605.1135, found 605.1140. HPLC purity: 95.41%.



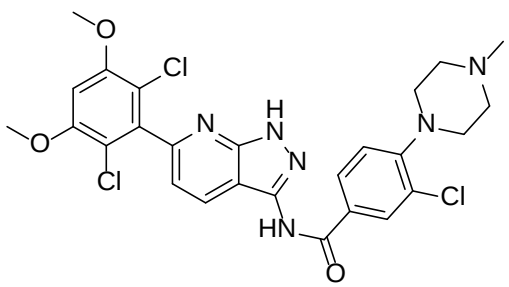
***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-indazol-3-yl)-4-(4-methylpiperazin-1-yl)benzamide (5).** The title compound was prepared from **2** and **3a** following general procedure C. White solid (4%), mp 218 °C dec. ^{1}H NMR (300 MHz, CD_3OD) δ 7.98 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.4 Hz, 1H), 7.28 (s, 1H), 7.06 (d, J = 8.9 Hz, 2H), 6.92 (dd, J = 8.4, 0.9 Hz, 1H), 6.88 (s, 1H), 3.97 (s, 6H), 3.46–3.35 (m, 4H), 2.73–2.53 (m, 4H), 2.37 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.2, 154.3 (2C), 153.0, 140.9, 140.7, 140.4, 135.0, 129.4 (2C), 122.7, 122.2, 121.0, 116.6, 113.6 (2C), 113.0 (2C), 110.7, 97.8, 56.7 (2C), 54.0 (2C), 46.5 (2C), 45.3. HRMS (ESI) *m/z* calcd for $C_{27}H_{28}Cl_2N_5O_3$ $[M+H]^+$ 540.1564, found 540.1561. HPLC purity: 97.22%.

General Procedure D: Synthesis of target compounds 7a–o, 9, and 10. Oxalyl chloride (66 μL, 0.78 mmol) and anhydrous *N*,*N*-dimethylformamide (2 μL, 0.026 mmol) were added to a stirred suspension of the appropriate benzoic acid (**6a**–**h**, **6j**, **6l**–**o,** and **8**) (0.26 mmol) in anhydrous dichloromethane (4 mL) under argon at 0 °C. The reaction mixture was stirred for 4 h at room temperature. The mixture was concentrated in vacuum and a solid was obtained. The acyl chloride was dissolved in anhydrous

tetrahydrofuran (3 mL) and then the solution formed was added to a solution of the appropriate amine (**1a**–**c**) (0.20 mmol) and *N*,*N*-diisopropylethylamine (136 μL, 0.78 mmol) in anhydrous tetrahydrofuran (1 mL) under argon at 0 °C. The reaction mixture was stirred at room temperature overnight. The mixture was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (for **7a**–**h**, **7k**–**o**, **9**, and **10**, dichloromethane/methanol + 9% of aqueous ammonia, 99:1 to 94:6; for **7i** and **7j**, dichloromethane/methanol, 99:1 to 97:3) to give the desired product.

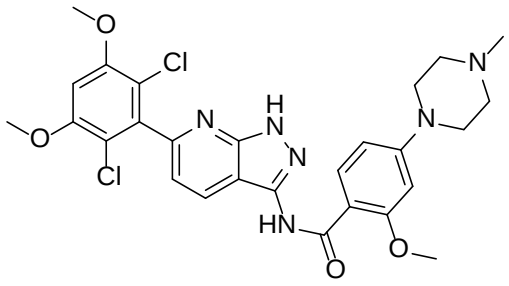


2-Chloro-*N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-methylpiperazin-1-yl)benzamide (7a). The title compound was prepared from **1a** and **6a** following general procedure D. White solid (30%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.40 (s, 1H), 10.95 (s, 1H), 8.44 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 8.9 Hz, 1H), 7.10 (d, J = 8.2 Hz, 1H), 7.05 (s, 1H), 7.03 (s, 1H), 6.98 (d, J = 8.9 Hz, 1H), 3.98 (s, 6H), 3.31–3.20 (m, 4H), 2.48–2.39 (m, 4H), 2.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.2, 154.6, 154.4 (2C), 152.6, 151.4, 139.5, 139.3, 133.0, 131.9, 130.4, 124.3, 117.1, 114.9, 112.5, 112.4 (2C), 107.4, 98.4, 56.8 (2C), 54.2 (2C), 47.0 (2C), 45.7. HRMS (ESI) *m/z* calcd for $C_{26}H_{26}Cl_3N_6O_3$ $[M+H]^+$ 575.1126, found 575.1123. HPLC purity: 95.80%.

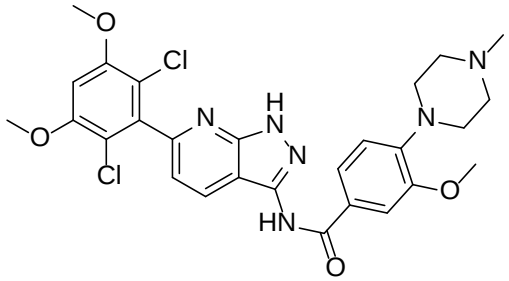


3-Chloro-*N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-

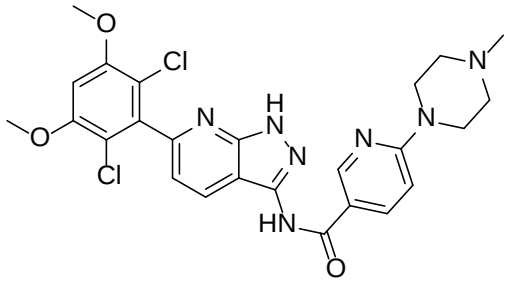
3-yl)-4-(4-methylpiperazin-1-yl)benzamide (7b). The title compound was prepared from **1a** and **6b** following general procedure D. White solid (59%), mp 191 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.46 (s, 1H), 11.12 (s, 1H), 8.39 (d, J = 8.3 Hz, 1H), 8.17 (d, J = 2.1 Hz, 1H), 8.06 (dd, J = 8.5, 2.0 Hz, 1H), 7.29 (d, J = 8.5 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.05 (s, 1H), 3.98 (s, 6H), 3.23–3.08 (m, 4H), 2.81–2.57 (m, 4H), 2.36 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.8, 154.6, 154.4 (2C), 151.8, 151.5, 139.7, 139.3, 133.1, 130.3, 128.2, 128.1, 126.7, 120.4, 117.2, 112.4 (2C), 107.8, 98.4, 56.8 (2C), 54.3 (2C), 49.8 (2C), 45.1. HRMS (ESI) *m/z* calcd for $C_{26}H_{26}Cl_3N_6O_3$ $[M+H]^+$ 575.1126, found 575.1125. HPLC purity: 97.33%.



***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-2-methoxy-4-(4-methylpiperazin-1-yl)benzamide (7c).** The title compound was prepared from **1a** and **6c** following general procedure D. Pale yellow solid (25%), mp 220 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.36 (s, 1H), 10.28 (s, 1H), 8.58 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 7.05 (s, 1H), 6.69 (dd, J = 9.1, 1.7 Hz, 1H), 6.64 (s, 1H), 4.05 (s, 3H), 3.98 (s, 6H), 3.48–3.34 (m, 4H), 2.27 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.8, 159.0, 154.9, 154.7, 154.4 (2C), 151.6, 139.8, 139.3, 133.8, 132.5, 117.0, 112.4 (2C), 109.9, 107.1, 106.8, 98.4, 97.4, 56.8 (2C), 56.2, 54.2 (2C), 46.7 (2C), 45.5. HRMS (ESI) *m/z* calcd for $C_{27}H_{29}Cl_2N_6O_4$ $[M+H]^+$ 571.1622, found 571.1605. HPLC purity: 95.22%.

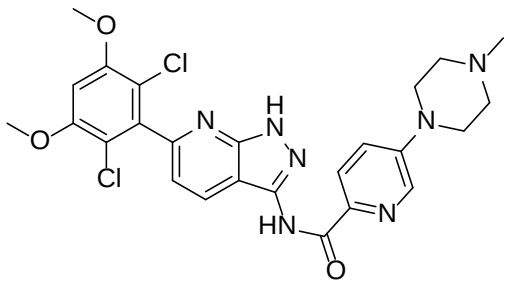


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-3-methoxy-4-(4-methylpiperazin-1-yl)benzamide (7d).** The title compound was prepared from **1a** and **6d** following general procedure D. Pale yellow solid (39%), mp 194 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.44 (s, 1H), 11.04 (s, 1H), 8.40 (d, J = 8.2 Hz, 1H), 7.79–7.65 (m, 2H), 7.15–6.97 (m, 3H), 3.99 (s, 6H), 3.90 (s, 3H), 3.28–3.06 (m, 4H), 3.00–2.68 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.9, 154.6, 154.4 (2C), 151.5, 151.0, 143.8, 140.1, 139.3, 133.3, 126.8, 121.5, 117.4, 117.1, 112.4 (2C), 111.3, 107.8, 98.4, 56.8 (2C), 55.7, 53.8 (2C), 48.3 (2C), 44.3. HRMS (ESI) *m/z* calcd for $C_{27}H_{29}Cl_2N_6O_4$ $[M+H]^+$ 571.1622, found 571.1616. HPLC purity: 95.34%.

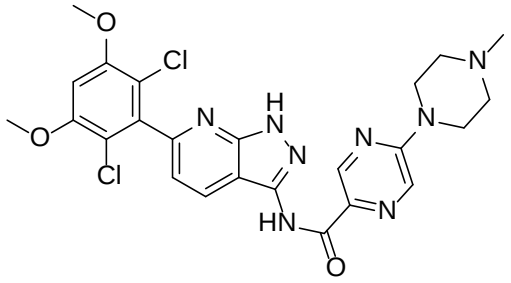


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-6-(4-methylpiperazin-1-yl)nicotinamide (7e).** The title compound was prepared from **1a** and **6e**[6] following general procedure D. Pale yellow solid (33%), mp 224 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.42 (s, 1H), 10.98 (s, 1H), 8.88 (d, J = 2.4 Hz, 1H), 8.40 (d, J = 8.3 Hz, 1H), 8.25 (dd, J = 9.0, 2.4 Hz, 1H), 7.08 (d, J = 8.3 Hz, 1H), 7.05 (s, 1H), 7.02 (d, J = 9.1 Hz, 1H), 3.98 (s, 6H), 3.93–3.52 (m, 4H), 3.10–2.76 (m, 4H), 2.58 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 159.5, 154.6, 154.4 (2C), 151.5, 148.9, 139.9, 139.3, 137.5, 133.2, 118.2, 117.1, 112.4 (2C), 107.8, 106.1, 98.4, 56.8 (2C), 52.5 (2C), 43.1, 42.3 (2C). HRMS (ESI) *m/z* calcd for $C_{25}H_{26}Cl_2N_7O_3$ $[M+H]^+$

542.1469, found 542.1469. HPLC purity: 95.03%.

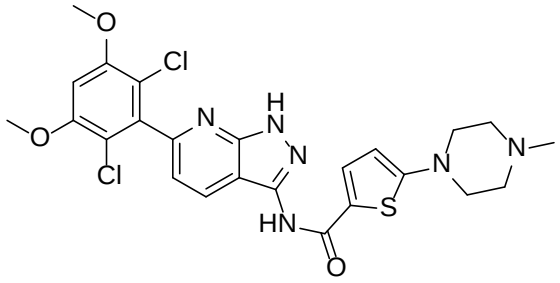


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-5-(4-methylpiperazin-1-yl)picolinamide (7f).** The title compound was prepared from **1a** and **6f**[7] following general procedure D. White solid (21%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.48 (s, 1H), 10.64 (s, 1H), 8.56 (d, J = 8.3 Hz, 1H), 8.46 (d, J = 2.3 Hz, 1H), 8.04 (d, J = 8.8 Hz, 1H), 7.55 (dd, J = 8.7, 2.3 Hz, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.05 (s, 1H), 3.98 (s, 6H), 3.71–3.41 (m, 4H), 3.11–2.75 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.6, 154.7, 154.4 (2C), 151.6, 148.1, 139.3, 139.2, 138.3, 135.5, 133.2, 123.1, 121.2, 117.2, 112.4 (2C), 107.2, 98.4, 56.8 (2C), 52.9 (2C), 45.2 (2C), 44.0. HRMS (ESI) *m/z* calcd for $C_{25}H_{26}Cl_2N_7O_3$ $[M+H]^+$ 542.1469, found 542.1472. HPLC purity: 97.19%.

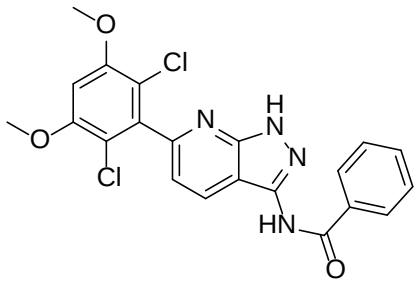


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-5-(4-methylpiperazin-1-yl)pyrazine-2-carboxamide (7g).** The title compound was prepared from **1a** and **6g**[6] following general procedure D. White solid (23%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.47 (s, 1H), 10.56 (s, 1H), 8.79 (d, J = 1.1 Hz, 1H), 8.51 (d, J = 8.3 Hz, 1H), 8.42 (d, J = 0.7 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.05 (s, 1H), 3.98 (s, 6H), 3.87–3.68 (m, 4H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.3, 155.2, 154.7, 154.4 (2C), 151.6, 142.4, 139.3, 139.0, 133.0, 131.7,

129.2, 117.2, 112.4 (2C), 107.6, 98.4, 56.8 (2C), 53.7 (2C), 45.1, 43.4 (2C). HRMS (ESI) *m/z* calcd for $C_{24}H_{25}Cl_2N_8O_3$ $[M+H]^+$ 543.1421, found 543.1424. HPLC purity: 96.31%.

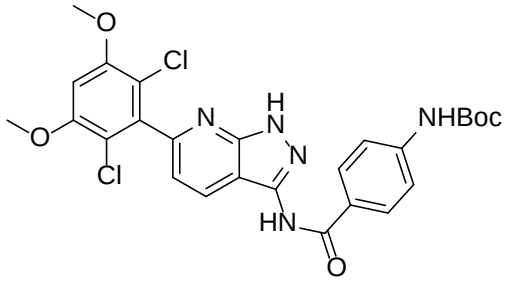


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-5-(4-methylpiperazin-1-yl)thiophene-2-carboxamide (7h).** The title compound was prepared from **1a** and **6h** following general procedure D. Yellow solid (21%), mp 236 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.39 (s, 1H), 10.92 (s, 1H), 8.39 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 4.0 Hz, 1H), 7.06 (d, J = 9.4 Hz, 2H), 6.36 (d, J = 3.8 Hz, 1H), 3.98 (s, 6H), 3.71–3.41 (m, 4H), 3.27–3.00 (m, 4H), 2.72 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.3, 160.2, 154.6, 154.4 (2C), 151.5, 139.8, 139.3, 133.3, 131.4, 123.3, 117.0, 112.4 (2C), 107.6, 106.3, 98.4, 56.8 (2C), 51.4 (2C), 47.2 (2C), 42.3. HRMS (ESI) *m/z* calcd for $C_{24}H_{25}Cl_2N_6O_3S$ $[M+H]^+$ 547.1080, found 547.1083. HPLC purity: 95.88%.

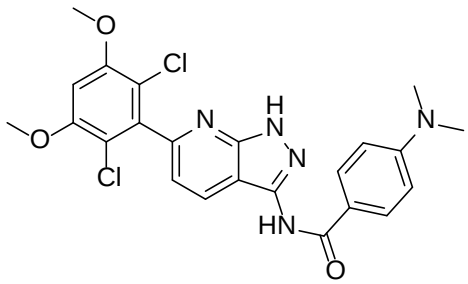


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)benzamide (7i).** The title compound was prepared from **1a** and **6i** following general procedure D. White solid (41%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.47 (s, 1H), 11.14 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 7.2 Hz, 2H), 7.68–7.49 (m, 3H), 7.10 (d, J = 8.2 Hz, 1H), 7.05 (s, 1H), 3.99 (s, 6H). ^{13}C NMR (126 MHz, DMSO-

d_6) δ 165.5, 154.6, 154.4 (2C), 151.5, 139.8, 139.3, 133.4, 133.1, 132.0, 128.5 (2C), 128.0 (2C), 117.2, 112.4 (2C), 107.8, 98.4, 56.8 (2C). HRMS (ESI) *m/z* calcd for $C_{21}H_{17}Cl_2N_4O_3$ $[M+H]^+$ 443.0672, found 443.0670. HPLC purity: 99.76%.

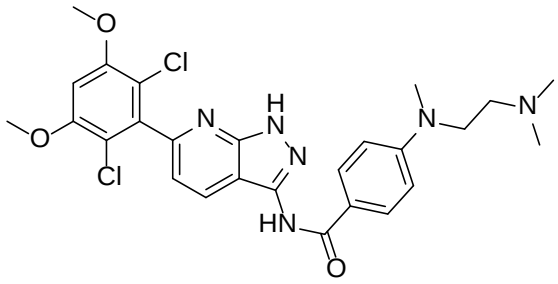


***tert*-Butyl (4-((6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)phenyl)carbamate (7j).** The title compound was prepared from **1a** and **6j**[8] following general procedure D. Pale yellow solid (45%), mp 190–192 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.86 (s, 1H), 9.12 (d, J = 4.3 Hz, 1H), 8.84 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 8.4 Hz, 1H), 6.81 (s, 1H), 6.61 (s, 1H), 3.95 (s, 6H), 1.54 (s, 9H). MS (ESI) *m/z* 558.0 $[M + H]^+$.

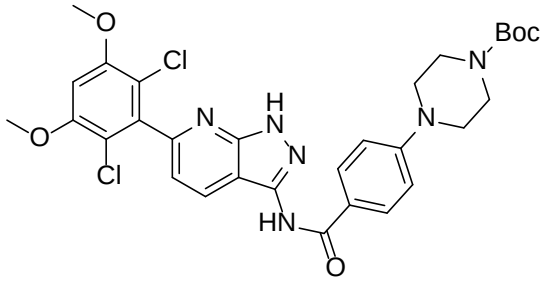


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(dimethylamino)benzamide (7k).** The title compound was prepared from **1a** and **6k** following general procedure D. Pale yellow solid (38%), mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.35 (s, 1H), 10.73 (s, 1H), 8.39 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 9.4 Hz, 2H), 6.77 (d, J = 8.9 Hz, 2H), 3.98 (s, 6H), 3.02 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.2, 154.5, 154.4 (2C), 152.6, 151.5, 140.4, 139.4, 133.3, 129.5 (2C), 119.6, 116.9, 112.4 (2C), 110.8 (2C), 108.0, 98.4, 56.8 (4C). HRMS (ESI) *m/z* calcd for $C_{23}H_{22}Cl_2N_5O_3$ $[M+H]^+$ 486.1094, found 486.1093. HPLC

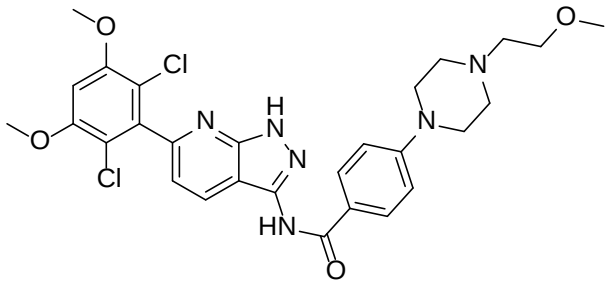
purity: 97.18%.



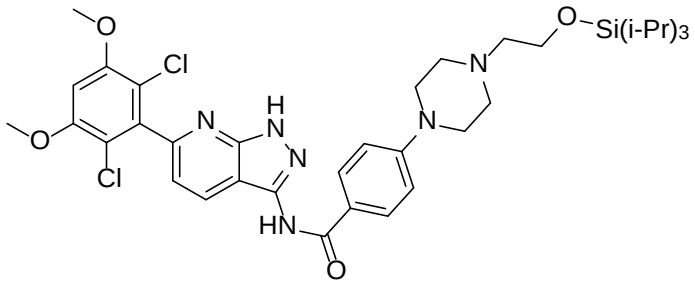
***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-((2-(dimethylamino)ethyl)(methyl)amino)benzamide (7l).** The title compound was prepared from **1a** and **6l** following general procedure D. White solid (18%), mp 209–210 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.34 (s, 1H), 10.71 (s, 1H), 8.38 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 8.6 Hz, 2H), 7.06 (d, J = 9.1 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 3.98 (s, 6H), 3.56 (t, J = 6.4 Hz, 2H), 3.01 (s, 3H), 2.29 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.1, 154.5, 154.4 (2C), 151.5, 151.4, 140.4, 139.4, 133.3, 129.7 (2C), 119.4, 116.9, 112.4 (2C), 110.6 (2C), 108.0, 98.4, 56.8 (2C), 55.2, 49.1, 45.2 (2C), 38.2. HRMS (ESI) *m/z* calcd for $C_{26}H_{29}Cl_2N_6O_3$ $[M+H]^+$ 543.1673, found 543.1677. HPLC purity: 97.61%.



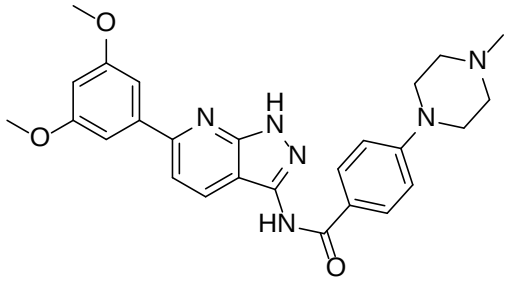
***tert*-Butyl 4-(4-((6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)phenyl)piperazine-1-carboxylate (7m).** The title compound was prepared from **1a** and **6m** following general procedure D. Yellow solid (35%), mp 188–190 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.13 (s, 1H), 8.85 (d, J = 8.4 Hz, 1H), 8.74 (s, 1H), 7.91 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.9 Hz, 2H), 6.66 (s, 1H), 3.97 (s, 6H), 3.70–3.53 (m, 4H), 3.45–3.25 (m, 4H), 1.50 (s, 9H). MS (ESI) *m/z* 627.1 $[M + H]^+$.



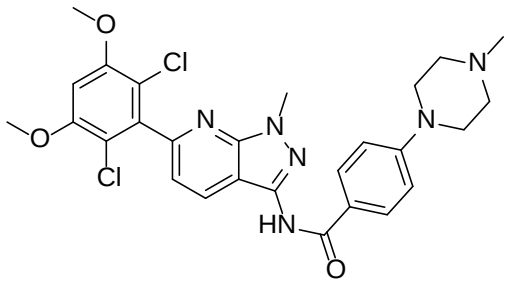
***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-(2-methoxyethyl)piperazin-1-yl)benzamide (7n).** The title compound was prepared from **1a** and **6n** following general procedure D. White solid (60%), mp >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.38 (s, 1H), 10.81 (s, 1H), 8.38 (d, J = 8.3 Hz, 1H), 8.00 (d, J = 8.8 Hz, 2H), 7.12–6.96 (m, 4H), 3.98 (s, 6H), 3.48 (t, J = 5.6 Hz, 2H), 3.32–3.27 (m, 4H), 3.25 (s, 3H), 2.66–2.52 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 153.3, 151.5, 140.2, 139.4, 133.3, 129.5 (2C), 122.0, 117.0, 113.4 (2C), 112.4 (2C), 107.9, 98.4, 70.0, 58.0, 57.0, 56.8 (2C), 52.9 (2C), 46.9 (2C). HRMS (ESI) *m/z* calcd for $C_{28}H_{31}Cl_2N_6O_4$ $[M+H]^+$ 585.1778, found 585.1780. HPLC purity: 95.45%.



***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-(2-((triisopropylsilyl)oxy)ethyl)piperazin-1-yl)benzamide (7o).** The title compound was prepared from **1a** and **6o** following general procedure D. Yellow solid (43%), mp 152–154 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.50 (s, 1H), 8.88 (s, 1H), 8.85 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.15 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 9.0 Hz, 2H), 6.65 (s, 1H), 3.96 (s, 6H), 3.90 (t, J = 6.2 Hz, 2H), 3.40–3.29 (m, 4H), 2.78–2.68 (m, 4H), 2.64 (t, J = 6.2 Hz, 2H), 1.15–0.99 (m, 21H). MS (ESI) *m/z* 727.2 $[M + H]^+$.

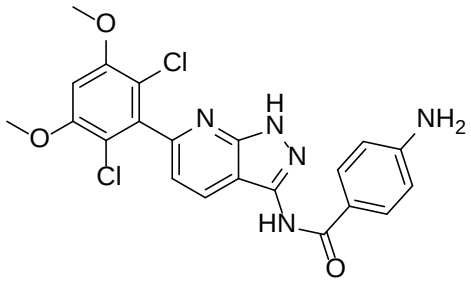


***N*-(6-(3,5-Dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-methylpiperazin-1-yl)benzamide (9).** The title compound was prepared from **1b** and **8** following general procedure D. White solid (69%), mp >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.32 (s, 1H), 10.77 (s, 1H), 8.37 (d, J = 8.6 Hz, 1H), 7.99 (d, J = 9.0 Hz, 2H), 7.76 (d, J = 8.6 Hz, 1H), 7.31 (d, J = 2.2 Hz, 2H), 7.02 (d, J = 9.1 Hz, 2H), 6.62 (t, J = 2.2 Hz, 1H), 3.85 (s, 6H), 3.32–3.25 (m, 4H), 2.48–2.41 (m, 4H), 2.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.9, 160.8 (2C), 155.3, 153.2, 152.1, 140.8, 140.1, 133.5, 129.5 (2C), 122.1, 113.6, 113.4 (2C), 107.8, 105.1 (2C), 101.5, 55.4 (2C), 54.4 (2C), 46.8 (2C), 45.8. HRMS (ESI) *m/z* calcd for $C_{26}H_{29}N_6O_3$ $[M+H]^+$ 473.2296, found 473.2299. HPLC purity: 98.57%.

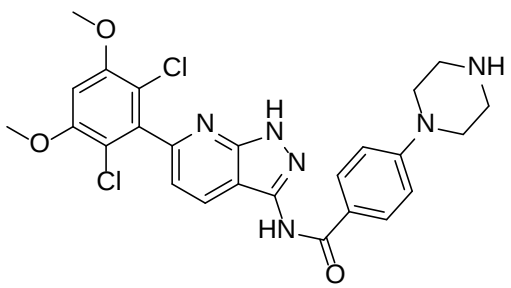


***N*-(6-(2,6-Dichloro-3,5-dimethoxyphenyl)-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-methylpiperazin-1-yl)benzamide (10).** The title compound was prepared from **1c** and **8** following general procedure D. Pale yellow solid (59%), mp 236 ºC dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.43 (d, J = 8.2 Hz, 1H), 8.04 (d, J = 8.2 Hz, 2H), 7.22–6.98 (m, 4H), 4.07 (s, 3H), 3.99 (s, 8H), 3.21–2.86 (m, 4H), 2.67 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.8, 154.7, 154.5 (2C), 149.8, 139.2, 138.9, 133.8, 129.6 (2C), 123.1, 117.0, 114.1 (2C), 112.5 (2C), 109.5, 108.2, 98.5, 56.9 (2C), 51.8 (2C), 44.5 (2C), 42.0, 33.4. HRMS (ESI) *m/z* calcd for $C_{27}H_{29}Cl_2N_6O_3$ $[M+H]^+$

555.1673, found 555.1673. HPLC purity: 97.17%.

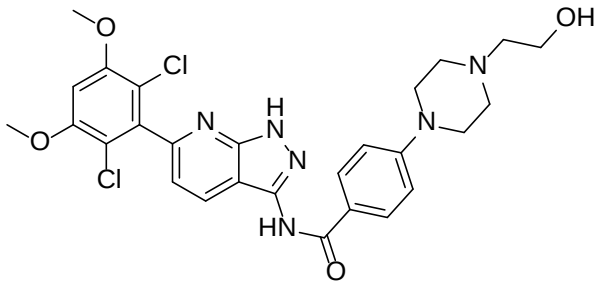


Synthesis of 4-amino-*N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)benzamide (11a). To a stirred solution of *tert*-butyl (4-((6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)phenyl)carbamate (**7j**, 475 mg, 0.85 mmol) in dichloromethane (8 mL) was added trifluoroacetic acid (1.2 mL), and the resulting mixture was stirred at room temperature overnight. The mixture was concentrated in vacuum. The residue was dissolved in methanol, and the solution was basified by the addition of aqueous ammonia. The mixture was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol + 9% of aqueous ammonia, 96:4 to 94:6) to provide the title compound (232 mg, 60%) as a white solid, mp >250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.32 (s, 1H), 10.60 (s, 1H), 8.37 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.0 Hz, 2H), 6.61 (d, J = 8.5 Hz, 2H), 5.82 (s, 2H), 3.98 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.3, 154.4 (3C), 152.5, 151.5, 140.5, 139.4, 133.3, 129.8 (2C), 119.6, 116.9, 112.6 (2C), 112.4 (2C), 108.0, 98.3, 56.8 (2C). HRMS (ESI) *m/z* calcd for $C_{21}H_{18}Cl_2N_5O_3$ $[M+H]^+$ 458.0781, found 458.0781. HPLC purity: 96.26%.



Synthesis of *N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-

***b*]pyridin-3-yl)-4-(piperazin-1-yl)benzamide (11b).** To a stirred solution of *tert*-butyl 4-(4-((6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)carbamoyl)phenyl)piperazine-1-carboxylate (**7m**, 32 mg, 0.051 mmol) in dichloromethane (0.5 mL) was added trifluoroacetic acid (80 μL), and the resulting mixture was stirred at room temperature overnight. The mixture was concentrated in vacuum. The residue was dissolved in methanol, and the solution was basified by the addition of aqueous ammonia. The mixture was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol + 9% of aqueous ammonia, 97:3 to 95:5) to provide the title compound (24 mg, 89%) as a yellow solid, mp 218 ºC dec. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.39 (s, 1H), 10.84 (s, 1H), 8.38 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 8.9 Hz, 2H), 7.12–6.97 (m, 4H), 3.98 (s, 6H), 3.52–3.20 (m, 4H), 3.07–2.90 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 153.1, 151.5, 140.2, 139.4, 133.2, 129.5 (2C), 122.6, 117.0, 113.7 (2C), 112.4 (2C), 107.9, 98.4, 56.8 (2C), 46.2 (2C), 43.9 (2C). HRMS (ESI) *m/z* calcd for $C_{25}H_{25}Cl_2N_6O_3$ $[M+H]^+$ 527.1360, found 527.1368. HPLC purity: 95.09%.



Synthesis of *N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-(2-hydroxyethyl)piperazin-1-yl)benzamide (11c). A 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (0.1 mL), was added dropwise to a stirred solution of *N*-(6-(2,6-dichloro-3,5-dimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-4-(4-(2-((triisopropylsilyl)oxy)ethyl)piperazin-1-yl)benzamide (**7o**, 39 mg, 0.054 mmol) in anhydrous tetrahydrofuran (0.5 mL) under argon at 0 °C. The reaction mixture was stirred at room temperature overnight. The mixture was concentrated in vacuum. The resulting residue was purified by silica gel chromatography (dichloromethane/methanol, 96:4 to 94:6) to provide the title

compound (25 mg, 81%) as a white solid, mp 246 °C dec. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.37 (s, 1H), 10.81 (s, 1H), 8.38 (d, J = 8.3 Hz, 1H), 8.00 (d, J = 8.9 Hz, 2H), 7.10–6.98 (m, 4H), 4.47 (s, 1H), 3.98 (s, 6H), 3.59–3.51 (m, 2H), 3.31 (s, 4H), 2.56 (s, 4H), 2.48–2.37 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.0, 154.5, 154.4 (2C), 153.3, 151.5, 140.2, 139.4, 133.3, 129.5 (2C), 122.0, 117.0, 113.4 (2C), 112.4 (2C), 107.9, 98.4, 60.2, 58.5, 56.8 (2C), 52.9 (2C), 46.9 (2C). HRMS (ESI) *m/z* calcd for $C_{27}H_{29}Cl_2N_6O_4$ $[M+H]^+$ 571.1622, found 571.1616. HPLC purity: 97.26%.

2. Biological Evaluation

2.1 ELISA kinase assay

The effects of the compounds on the activities of various tyrosine kinases were determined using enzyme-linked immunosorbent assays (ELISAs) with purified recombinant proteins. Briefly, 20 μg/mL poly (Glu,Tyr)4:1 (Sigma, St Louis, MO, USA) was pre-coated in 96-well plates as a substrate. A 50-μL aliquot of 10 μmol/L ATP solution diluted in kinase reaction buffer (50 mmol/L HEPES [pH 7.4], 50 mmol/L $MgCl_2$, 0.5 mmol/L $MnCl_2$, 0.2 mmol/L Na_3VO_4, and 1 mmol/L DTT) was added to each well; 1 μL of various concentrations of indicated compounds diluted in 1% DMSO (v/v) (Sigma, St Louis, MO, USA) were then added to each reaction well. DMSO (1%, v/v) was used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 49 μL of kinase reaction buffer. After incubation for 60 min at 37°C, the plate was washed three times with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Anti-phosphotyrosine (PY99) antibody (100 μL; 1:500, diluted in 5 mg/mL BSA T-PBS) was then added. After a 30-min incubation at 37°C, the plate was washed three times, and 100 μL horseradish peroxidase-conjugated goat anti-mouse IgG (1:2000, diluted in 5 mg/mL BSA T-PBS) was added. The plate was then incubated at 37°C for 30 min and washed 3 times. A 100-μL aliquot of a solution containing 0.03% H_2O_2 and 2 mg/mL o-phenylenediamine in 0.1 mol/L citrate buffer (pH 5.5) was added. The reaction was terminated by the addition of 50 μL of 2 mol/L H_2SO_4 as the color changed, and the

plate was analyzed using a multi-well spectrophotometer (SpectraMAX 190, from Molecular Devices, Palo Alto, CA, USA) at 490 nm. The inhibition rate (%) was calculated using the following equation: [1-(A490/A490 control)]×100%. The IC_{50} values were calculated from the inhibition curves in two separate experiments.

2.2 Kinase profiling

The activity of **7n**, at a concentration of 1 μmol/L using an ATP concentration of 10 μM, was screened against total 71 human protein kinases, which include 16 human protein kinases using ELISA kinase assay depicted as before and a panel of 55 human protein kinases by Eurofins using the Eurofins Kinase Profiler Selectivity Testing Service (Table S1).

Table S1. Kinase Selectivity Profile of Compound 7n

Kinase	Inhibit rate (%)	Kinase	Inhibit rate (%)
PDGFR-α(h)	32% @ 1 μM	EphB3(h)	18% @ 1 μM
PDGFR-β(h)	22% @ 1 μM	EphB4(h)	9% @ 1 μM
Ret(h)	24% @ 1 μM	ErbB2(h)	1% @ 1 μM
c-Src(h)	40% @ 1 μM	FAK(h)	46% @ 1 μM
ErbB4/HER4(h)	3% @ 1 μM	Fer(h)	43% @ 1 μM
c-Met/HGFR(h)	36% @ 1 μM	Fes(h)	48% @ 1 μM
ALK(h)	0% @ 1 μM	Flt3(h)	36% @ 1 μM
ABL(h)	0% @ 1 μM	IRR(h)	-3% @ 1 μM
EGFR(h)	21% @ 1 μM	IRAK1(h)	12% @ 1 μM
EPH-A2(h)	4% @ 1 μM	IRAK4(h)	14% @ 1 μM
IGF1R(h)	38% @ 1 μM	JAK1(h)	21% @ 1 μM
ALK1(h)	-4% @ 1 μM	JAK2(h)	12% @ 1 μM
ALK2(h)	1% @ 1 μM	JAK3(h)	8% @ 1 μM
ALK4(h)	-1% @ 1 μM	LIMK1(h)	9% @ 1 μM
ALK6(h)	2% @ 1 μM	LRRK2(h)	2% @ 1 μM

A-Raf(h)	3% @ 1 μM	LTK(h)	27% @ 1 μM
Axl(h)	17% @ 1 μM	Mer(h)	20% @ 1 μM
BRK(h)	21% @ 1 μM	MLK1(h)	27% @ 1 μM
B-Raf(h)	6% @ 1 μM	MLK2(h)	20% @ 1 μM
CSK(h)	24% @ 1 μM	MuSK(h)	25% @ 1 μM
c-RAF(h)	-1% @ 1 μM	Pyk2(h)	52% @ 1 μM
DDR2(h)	35% @ 1 μM	RIPK2(h)	12% @ 1 μM
EGFR(L858R)(h)	11% @ 1 μM	Ron(h)	-8% @ 1 μM
EGFR(L861Q)(h)	14% @ 1 μM	Ros(h)	1% @ 1 μM
EGFR(T790M)(h)	30% @ 1 μM	Rse(h)	35% @ 1 μM
EphA1(h)	-12% @ 1 μM	Src(1-530)(h)	5% @ 1 μM
EphA3(h)	-12% @ 1 μM	Syk(h)	27% @ 1 μM
EphA4(h)	0% @ 1 μM	TAK1(h)	8% @ 1 μM
EphA5(h)	-1% @ 1 μM	Tec(h) activated	-2% @ 1 μM
EphA7(h)	12% @ 1 μM	TGFBR1(h)	-1% @ 1 μM
EphA8(h)	1% @ 1 μM	Txk(h)	48% @ 1 μM
EphB1(h)	10% @ 1 μM	ZAK(h)	6% @ 1 μM
EphB2(h)	8% @ 1 μM	ZAP-70(h)	6% @ 1 μM

2.3 Cell culture

Human lung cancer cell line H1581, human acute myelogenous leukemia cancer cell line KG1 and human gastric cancer cell lines SNU16 and KATOIII were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Human bladder cancer cell line RT112 was purchased from DSMZ-German collection of microorganisms and cell cultures. All the cell lines were routinely maintained in media according to the suppliers' recommendations.

2.4 Cell proliferation assay

Cells were seeded in 96-well cell culture plates. On the day when seeding, the cells were exposed to various concentrations of compounds and further cultured for 72h at 37°C. Cell proliferation was then determined using Cell Counts Kit-8 (CCK8). The IC_{50} values were calculated by concentration-response curve fitting using the four-parameter method.

2.5 Pharmacokinetic studies

Test compounds were subjected to pharmacokinetic studies on male ICR mice with three animals in each group. Compounds **4e**, **7n**, and **11c** were administered either as a solution in 2.5% DMSO/40% PEG400/57.5% Saline by intravenous injection (2 mg/kg) or as a suspension in PEG300/D5W (2: 1, v/v) by oral gavage (12.5 mg/kg). Positive drug AZD4547 was administered either as a solution in 2.5% DMSO/40% PEG400/57.5% Saline by intravenous injection (2 mg/kg) or as a suspension in 0.1% Tween-80 in water by oral gavage (12.5 mg/kg). Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 h time points following intravenous dosing and at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h following oral dosing. The concentration of test compounds were determined by high pressure liquid chromatography/tandem mass spectrometry (LC-MS/MS). Relevant pharmacokinetic parameters were derived by noncompartmental analysis (WinNonlin version 5.2), and they are summarized in Table S2.

Table S2. Pharmacokinetic Properties of Compounds 4e, 7n and 11c in ICR Mice[a]

Compd	Dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	CL (mL/h/kg)	V_d (mL/kg)	MRT_{0-t} (h)	$MRT_{0-\infty}$ (h)	*F* (%)
4e	2 (*iv*)	3.47	0.083	869.77	1831.33	1843.43	1084.93	5427.18	3.63	3.80	–
	12.5 (*po*)	2.90	2	499.65	4601.82	4618.74	–	–	3.63	3.80	40.21
7n	2 (*iv*)	3.13	0.083	825.69	1999.16	2004.95	997.53	4510.04	2.81	2.89	–
	12.5 (*po*)	2.76	1	1648.10	8990.41	9009.61	–	–	2.81	2.89	71.95

11c	2 (*iv*)	3.74	0.083	796.28	1068.08	1075.58	1859.47	10025.36	3.27	3.45	–
	12.5 (*po*)	3.38	1	801.97	3491.51	3509.55	–	–	3.27	3.45	52.30
AZD4	2 (*iv*)	2.29	0.083	1882.86	1953.05	2076.72	963.06	3175.24	1.76	2.33	–
547	12.5 (*po*)	2.55	4	1350.03	9112.93	9127.04	–	–	1.76	2.33	74.66

[a]n = 3 animals/group. Data are the mean values.

2.6 *In vitro* plasma protein binding study

The *in vitro* plasma protein binding of compounds **4e**, **7n**, **11c** and AZD4547 were determined using equilibrium dialysis assays in mouse plasma. Propranolol was used as a positive control. The stock solutions of test compounds were prepared to concentrations of 500 μM. The stock solution of positive control was prepared to the concentration of 100 μM. An aliquot (5 μL) of stock solutions of test compounds and positive control were added to 495 μL blank plasma respectively. The final concentrations of test compounds were 5 μM and the final concentration of positive control was 1 μM. The prepared samples (300 μL) were transferred into the plasma chamber, and dialysis buffer (PBS, 500 μL) was added into the buffer chamber. The unit was sealed and incubated at 37 °C on an orbital shaker at approximately 100 rpm for 4 h. Experiments for all the samples were conducted in triplicate.

After incubation, an aliquot (50 μL) of each post-dialysis sample was collected from the buffer chamber and the plasma chamber. PBS (50 μL) was added into the plasma samples, while plasma (50 μL) was added into the buffer sample. A three-fold volume of methanol containing internal standard was added to both of the mixed samples to precipitate the plasma protein. After that, the samples were centrifuged at 14,000 rpm for 5 min to precipitate protein. The concentrations in the supernatants (100 μL) of the samples were determined by LC-MS/MS. The protein binding rates were calculated according to the following formula:

%Free = (Concentration from buffer chamber/Concentration from plasma chamber) × 100%

%Bind = 100% – %Free

The plasma protein binding rates of compounds **4e**, **7n**, **11c** and AZD4547 at a concentration level of 5 μM were 99.6%, 99.6%, 99.5% and 99.3% in mouse plasma, respectively. The plasma protein binding rate of propranolol at a concentration level of 1 μM was 80.4% in mouse plasma.

2.7 Western blot analysis

KG1 and KATOIII cells were treated with the indicated dose of compound **7n** for 2 h at 37 °C and then lysed in 1×sodium dodecyl sulfate (SDS) sample buffer. The cell lysates were subsequently resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were probed with the appropriate primary antibodies [phospho-FGFR1, FGFR1, phospho-FGFR2, FGFR2, phosphor-ERK, ERK, PLCγ, phosphor-PLCγ, GAPDH (all from Cell Signaling Technology, Beverly, MA, USA)] and then with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG. The immunoreactive proteins were detected using an enhanced chemilluminescense detection reagent (Thermo Fisher Scientific, Rockford, IL, USA).

2.8 *In vivo* antitumor activity assay

Female nude mice (4–5 weeks) were housed at five or six mice per cage in a specific pathogen free room with a 12 h light/dark schedule at 25±1 °C; the animals were fed an autoclaved chow diet and water ad libitum. All the animal experiments were performed according to the institutional ethical guidelines of animal care.

H1581 cells at a density of 5×10^6 were first implanted subcutaneously into the right flank of each mouse and then allowed to grow to 700–800 mm^3, which was defined as a well-developed tumor. The well-developed tumors were cut into 1.5 mm^3 fragments and transplanted subcutaneously into the right flank of nude mice using a tracer. When the tumor volume reached 115 mm^3, the mice were randomly assigned into control and treatment groups. The control groups were given vehicle alone, and the treatment groups received compound **7n** at the indicated doses via oral administration once daily for 21 days. AZD4547 was a positive drug. Compound **7n** was formulated as a

suspension in PEG300/D5W (2: 1, v/v) and AZD4547 was formulated as a suspension in 0.1% Tween-80 in water. The sizes of the tumors were measured twice per week using micro calipers, and meanwhile body weights were measured using electronic balance. The tumor volume (TV) was calculated as follows: $TV = (\text{length} \times \text{width}^2)/2$. The tumor volume shown was obtained on the indicated days as the median tumor volume ± SEM for indicated groups of mice. The relative tumor volume values, RTV, were measured on the final day of the study for the drug-treated mice compared with the vehicle-treated mice and were calculated as $RTV = V_t/V_0$, while V_0 is the tumor volume at day 0, V_t is the tumor volume measured each time point. The percentage of tumor volume inhibition values (TGI) was also measured. Significant differences between the treated versus the control groups ($P \leq 0.05$) were determined using Student's t test. Figure S1 shows the mean body weight changes in the course of treatment.

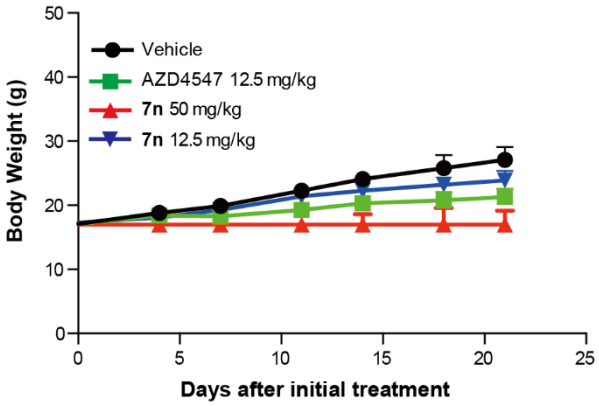


Figure S1. Body weight measurements during the treatment. Results are expressed as the mean ± SEM (n = 6 for the inhibitor-treated group, n = 12 for the vehicle control group).

3. Molecular Modeling

Structure of the kinase domain of FGFR1 ((PDB ID: 4WUN[9]) was prepared by using the Protein Preparation module in Maestro 9.0[10]. Both bond orders and hydrogen atoms were assigned and water molecules were removed. H-bond assignment and Impref

minimization were performed subsequently. The size of the grid box was set to 20 Å×20 Å×20 Å, centered on native ligand (AZD4547) located in the kinase domain of FGFR1. A re-docking evaluation illustrated Glide 5.5[11] could successfully reproduce the active conformation (RMSD = 0.293 Å.). Glide extra-precision (XP) mode[12] was adopted when docking compound **4a** into the kinase domain of FGFR1. Figure 3 were generated with PyMOL (Version 1.3r1)[13].

References

(1) Kamei, T.; Ishibashi, A.; Shimada, T. Metal-free halogenation of arylboronate with *N*-halosuccinimide. *Tetrahedron Lett.* **2014**, *55*, 4245–4247.

(2) Davenport, A. J.; Hallett, D. J. Piperazine and aminopyrolidine compounds as histamine H3 receptor antagonists. WO2010/133544 A1, 2010.

(3) Foote, K. M.; Theoclitou, M. E.; Thomas, A. P.; Buttar, D. Novel compounds. WO2008/075068 A2, 2008.

(4) Qiao, J. X.; Wang, T., C.; Wang, G. Z. Lactam-containing cyclic diamines and derivatives as factor Xa inhibitors. WO2004/082687 A1, 2004.

(5) Heiser, U.; Sommer, R.; Ramsbeck, D.; Meyer, A.; Hoffmann, T.; Boehme, L.; Demuth, H. U. Heterocylcic derivatives as inhibitors of glutaminyl cyclase. WO2011/029920 A1, 2011.

(6) Rheinfelden, G. G.; Zbinden, K. G.; Olsberg, R. N.; Basel, H. S. TAAR1 ligands. US2009/0036452 A1, 2009.

(7) Liu, G. Azolyl urea compounds and methods of use thereof. WO2011/150198 A1, 2011.

(8) Mu, F.; Coffing, S. L.; Riese, D. J.; Geahlen, R. L.; Verdier-Pinard, P.; Hamel, E.; Johnson, J.; Cushman, M. Design, synthesis, and biological evaluation of a series of lavendustin A analogues that inhibit EGFR and Syk tyrosine kinases, as well as tubulin polymerization. *J. Med. Chem.* **2001**, *44*, 441–452.

(9) Yosaatmadja, Y.; Patterson, A. V.; Smaill, J. B.; Squire, C. J. The 1.65 Å resolution structure of the complex of AZD4547 with the kinase domain of FGFR1 displays

exquisite molecular recognition. *Acta Crystallogr. D Biol. Crystallogr.* **2015**, *71*, 525–533.

(10) *Maestro*, version 9.0; Schrödinger, LLC: New York, NY, 2009.

(11) *Glide*, version 5.5; Schrödinger, LLC: New York, NY, 2009.

(12) Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein−ligand complexes. *J. Med. Chem.* **2006**, *49*, 6177–6196.

(13) *The PyMOL molecular graphics system*, version 1.3r1; Schrödinger, LLC: New York, NY, 2010.