Electronic Supplementary Information

Development of 1*H*-Pyrazolo[3,4-*b*]pyridines as Metabotropic Glutamate Receptor 5 Positive Allosteric Modulators.

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Experimental Section

Synthetic Chemistry Methods: NMR spectra were run on Bruker Avance spectrometers. Chemical shifts are reported in ppm (δ) with reference to tetramethylsilane (δ=0.0). Purity of all compounds was determined to be ≥95% by analytical HPLC using at least two orthogonal methods. Starting materials were previously described in the literature and/or commercially available unless otherwise noted.

***2-Step, 1-pot method*: 3,4,6-Triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (2)[1]**

3-Phenyl-1*H*-pyrazol-5-amine (0.822 g, 5.17 mmol), 3-oxo-3-phenylpropanenitrile (0.750 g, 5.17 mmol), benzaldehyde (0.527 mL, 5.17 mmol), and Et_3N (1.44 mL, 10.3 mmol) were stirred in DMF (5.2 mL) at 90 °C for 16 h. The volatiles were removed under reduced pressure. Sodium nitrite (1.07 g, 15.5 mmol) and acetic acid (39.6 mL, 692 mmol) were added to the crude material, and the reaction mixture was stirred for 10 min. The volatiles were removed under reduced pressure, and the crude material was subjected to silica gel column chromatography with ethyl acetate in hexanes (10–50%). 3,4,6-Triphenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (1.54 g, 4.14 mmol, 80 % yield) was isolated as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 14.52 (s, 1H), 7.94 (dd, *J*=6.6, 2.9 Hz, 2H), 7.65 - 7.57 (m, 3H), 7.43 - 7.33 (m, 3H), 7.30 - 7.24 (m, 2H), 7.24 - 7.18 (m, 1H), 7.11 - 7.00 (m, 4H). LCMS (method A) t_R, 1.85 min, MS Anal. Calcd. for $[M+H]^+$ $C_{25}H_{17}N_4$: 373.15; found: 373.15.

***SAR to define minimum pharmacophore*:**

1-Methyl-3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (10)

NaH (1.0 mg, 0.02 mmol) was added to a stirred solution of 3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (6.0 mg, 0.02 mmol) in DMF (161 µL) at ambient temperature. The reaction was stirred for 2 min before iodomethane (1.5 µL, 0.02 mmol) was added in a single portion. The reaction was then stirred for 45 min at ambient temperature. The crude reaction mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 1-methyl-3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (4.0 mg, 0.01 mmol, 64 % yield). ^{1}H NMR (500MHz, DMSO-d_6) δ = 8.01 - 7.91 (m, 2H), 7.67 - 7.58 (m, 3H), 7.44 - 7.33 (m, 3H), 7.32 - 7.19 (m, 3H), 7.11 - 6.94 (m, 4H), 4.23 (s, 3H). LCMS (method B) t_R, 2.32 min, MS Anal. Calcd. for $[M+H]^+$ $C_{26}H_{19}N_4$: 387.16; found: 387.00.

3,4,6-Triphenyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile (11)

6-Amino-5-bromo-2,4-diphenylnicotinonitrile

6-Amino-2,4-diphenylnicotinonitrile (543 mg, 2.00 mmol) and NBS (392 mg, 2.20 mmol) were stirred in CH_2Cl_2 (20 mL) at 50 °C for 14 h. The crude reaction mixture was subjected to silica gel column chromatography with ethyl acetate in hexanes (33%). 6-Amino-5-bromo-2,4-diphenylnicotinonitrile (660 mg, 1.89 mmol, 94 % yield) was isolated as a tan solid.

6-Amino-2,4-diphenyl-5-((trimethylsilyl)ethynyl)nicotinonitrile
A mixture of tetrakis (211 mg, 0.18 mmol), copper(I) iodide (70.0 mg, 0.37 mmol), and 6-amino-5-bromo-2,4-diphenylnicotinonitrile (640 mg, 1.83 mmol) in NMP (7.3 mL) was degassed with N_2 (g) for 15 min. Hünig's base (0.96 mL, 5.48 mmol) and trimethylsilylacetylene (0.78 mL, 5.48 mmol) were subsequently added to the reaction mixture before it was heated 70 °C for 23 h. The product was partitioned between ethyl acetate (30 mL) and brine (20 mL), and the layers were separated. The aqueous layer was washed again with ethyl acetate (2 x 20 mL), and the combined organics were dried over sodium sulfate. The solids were filtered away, the volatiles were removed under reduced pressure, and 6-amino-2,4-diphenyl-5-((trimethylsilyl)ethynyl)nicotinonitrile (144 mg, 0.39 mmol, 21 % yield) was isolated using silica gel column chromatography with ethyl acetate in hexanes (10%).

4,6-Diphenyl-1H-pyrrolo[2,3-b]pyridine-5-carbonitrile
6-Amino-2,4-diphenyl-5-((trimethylsilyl)ethynyl)nicotinonitrile (92.0 mg, 0.25 mmol) and potassium ^{t}butoxide (112 mg, 1.00 mmol) were stirred in ^{t}BuOH (1 mL) at 80 °C for 20 h. The reaction mixture was allowed to cool to ambient temperature, and 1N aqueous HCl (4.2 mL) was added. The volatiles were removed under reduced pressure, and the residue was washed with Ethyl acetate and brine. The solids were filtered and washed with ethyl acetate to afford 4,6-diphenyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile (50.0 mg, 0.17 mmol, 68 % yield) as a light tan solid.

3-Bromo-4,6-diphenyl-1H-pyrrolo[2,3-b]pyridine-5-carbonitrile
4,6-Diphenyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile (118 mg, 0.40 mmol) and NBS (85.0 mg, 0.48 mmol) were stirred in DMF (4 mL) at ambient temperature for 16 h. The volatiles were removed under reduced pressure, and the crude material was partitioned between ethyl acetate (50 mL) and sat. aqueous $NaHCO_3$ (30 mL). The organics were subsequently washed with brine (30 mL). The organics were dried over sodium sulfate, the solids were filtered away, and 3-bromo-4,6-diphenyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile (76.0 mg, 0.20 mmol, 51 % yield) was isolated as a tan solid using silica gel column chromatography with dicloromethane.

3,4,6-Triphenyl-1H-pyrrolo[2,3-b]pyridine-5-carbonitrile
3-Bromo-4,6-diphenyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile (37.0 mg, 0.10 mmol), phenylboronic acid (30.0 mg, 0.25 mmol), $PdCl_2$(dppf)-CH_2Cl_2 (12.0 mg, 0.02 mmol), and sodium carbonate (32.0 mg, 0.30 mmol) in a mixture of DME (0.8 mL) and water (0.2 mL) were stirred at 150 °C for 30 min under N_2 (g). Aqueous HCl (0.6 mL, 0.60 mmol) was added, and the volatiles were removed under reduced pressure. The crude material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3,4,6-triphenyl-1*H*-pyrrolo[2,3-*b*]pyridine-5-carbonitrile (3.9 mg, 0.01 mmol, 11 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.08 (br. s., 1H), 8.03 (d, *J*=7.3 Hz, 2H), 7.97 (s, 1H), 7.95 - 7.90 (m, 2H), 7.85 - 7.79 (m, 2H), 7.70 - 7.54 (m, 6H), 7.52 - 7.45 (m, 2H), 7.43 - 7.37 (m, 1H). LCMS (method C) t_R, 4.28 min, MS Anal. Calcd. for $[M+H]^+$ $C_{26}H_{18}N_3$: 372.15; found: 372.1.

3,4,6-Triphenyl-1*H*-indazole-5-carbonitrile (12)
2-Bromo-4-cyano-3,5-diphenylaniline
To a solution of 4-cyano-3,5-diphenylaniline (850 mg, 3.14 mmol) in AcOH (15 mL) at ambient temperature was added dropwise a solution of bromine (0.065 mL, 1.26 mmol) in acetic acid (0.5 mL). After 30 min, another portion of bromine (0.065 mL, 1.26 mmol) in acetic acid (0.5 mL) was added dropwise. After 30 min, a final portion of bromine (49 uL) in acetic acid (0.5 mL) was added. After stirring for 20 min, the volatiles were removed under reduced pressure. The resulting residue was suspended in water/ethyl acetate and neutralized with sat. aqueous $NaHCO_3$. The material was only partially soluble in the ethyl acetate/water mixture. The resulting solid was collected by filtration and rinsed with ethyl acetate. The eluent was separated. The organics were washed with brine, dried over MgSO4, filtered, and concentrated. The resulting solid residue was triturated using ethyl acetate, collected by filtration, and combined with the other solids. 2-Bromo-4-cyano-3,5-diphenylaniline (638 mg, 1.64 mmol, 52 % yield) was isolated. ^{1}H NMR (DMSO-d_6) δ = 7.44-7.56 (m, 8H), 7.34-7.39 (m, 2H), 6.88 (s, 1H), 6.54 (br. s., 2H). LCMS (method D) t_R, 1.86 min, MS Anal. Calcd. for $[M+H]^+$ $C_{19}H_{14}BrN_2$: 349.03; found: 349.2.

2-Benzyl-4-cyano-3,5-diphenyl aniline
A screw cap vial was dried, flushed with N_2 (g), and charged with $PdOAc_2$ (3.2 mg, 0.01 mmol) and 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (11.8 mg, 0.03 mmol). After flushing with N_2 (g) for 5 min, the vial was charged sequentially with DMF (1 mL), potassium phosphate (182 mg, 0.86 mmol), 2-bromo-4-cyano-3,5-diphenylaniline (100 mg, 0.29 mmol), and B-benzyl-9-BBN (0.5 M in THF, 1.15 mL, 0.57 mmol). The resulting mixture was placed in a

preheated 80 °C sand bath. After 35 min it was removed from the sand bath. The reaction was diluted with ethyl acetate/water, shaken, treated with celite, and shaken again. The resulting mixture was filtered through celite, and the layers were separated. The organics were washed with 1M NaOH then brine, dried over $MgSO_4$, filtered, and the volatiles were removed under reduced pressure. 2-Benzyl-4-cyano-3,5-diphenyl aniline (75.0 mg, 0.21 mmol, 73 % yield) was isolated using silica gel column chromatography. LCMS (method E) t_R, 3.58 min, MS Anal. Calcd. for $[M+H]^+$ $C_{26}H_{21}N_2$: 361.17; found: 361.3.

3,4,6-Triphenyl-1H-indazole-5-carbonitrile

2-Benzyl-4-cyano-3,5-diphenyl aniline (75.0 mg, 0.21 mmol) was suspended in 8M HCl (1 mL, 8.00 mmol) at ambient temperature. The suspension was cooled to 0 °C and treated with a solution of sodium nitrite (15.8 mg, 0.23 mmol) in water. After the addition was complete, the reaction mixture was stirred for 15 min at that temperature. The reaction was neutralized with an excess of solid sodium acetate, and the resulting mixture was poured into a solution of 2-methyl-2-propanethiol (18.8 mg, 0.21 mmol) in EtOH (2 mL) at 0 °C. The first reaction vial was rinsed with EtOH (2 mL) and added to the second vial. The vial was sealed and shaken rapidly on a vortex mixer to aid in dissolution of the pellet. Within 5 min, the pellet had dissolved. The mixture was poured into ether, washed with water (3X) then brine, dried over $MgSO_4$, filtered, and the volatiles were removed under reduced pressure. The resulting residue was dissolved in DMSO (1 mL) and added to a stirred solution of KO^tBu (187 mg, 1.67 mmol) in DMSO (2 mL). After 30 min, the reaction was poured onto ice, neutralized with 1M HCl, and diluted with ethyl acetate. The layers were separated. The organics were washed with water (2X) then brine, dried over $MgSO_4$, filtered, and the volatiles were removed under reduced pressure. The material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3,4,6-triphenyl-1*H*-indazole-5-carbonitrile (7.3 mg, 0.02 mmol, 9 % yield). LCMS (method E) t_R, 3.47 min, MS Anal. Calcd. for $[M+H]^+$ $C_{26}H_{18}N_3$: 372.15; found: 372.3.

3,4,6-Triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine (13)

(2-Chloro-4,6-diphenylpyridin-3-yl)(phenyl)methanone

3-Benzoyl-4,6-diphenylpyridin-2(1*H*)-one (19.0 mg, 0.05 mmol) was stirred in $POCl_3$ (252 µL, 2.70 mmol) at 100 °C for 16 h. The volatiles were removed under reduced pressure, and the material was used in the next step without purification. The reaction yield was assumed to be 100 %. LCMS (method A) t_R, 1.56 min, MS Anal. Calcd. for $[M]^+$ $C_{24}H_{16}ClNO$: 369.09; found: 369.15.

3,4,6-Triphenyl-1H-pyrazolo[3,4-b]pyridine

(2-Chloro-4,6-diphenylpyridin-3-yl)(phenyl)methanone (20.0 mg, 0.05 mmol) and hydrazine hydrate (20.4 µL, 0.27 mmol) was stirred in ^{n}BuOH (270 µL) at 140 °C for 1 h. The volatiles were removed under reduced pressure. The crude reaction material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine (2.1 mg, 0.01 mmol, 11 % yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ = 13.95 (br. s., 1H), 8.26 (d, *J*=7.5 Hz, 3H), 7.75 (s, 1H), 7.58 - 7.53 (m, 2H), 7.53 - 7.48 (m, 1H), 7.37 - 7.33 (m, 1H), 7.31 (d, *J*=7.2 Hz, 2H), 7.26 - 7.18 (m, 3H), 7.11 (d, *J*=4.2 Hz, 4H). LCMS (method A) t_R, 2.01 min, MS Anal. Calcd. for $[M+H]^+$ $C_{24}H_{18}N_3$: 348.15; found: 348.2.

5-Fluoro-3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine (14)

Selectfluor (61.2 mg, 0.17 mmol) was added to a stirred solution of crude 3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine (30.0 mg, 0.09 mmol) at ambient temperature. The reaction was subsequently stirred at 90 °C for 16 h before the crude reaction mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 5-fluoro-3,4,6-triphenyl-1*H*-pyrazolo[3,4-*b*]pyridine (3.7 mg, 0.01 mmol, 11 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.99 (d, *J*=7.0 Hz, 2H), 7.61 - 7.52 (m, 3H), 7.38 - 7.32 (m, 1H), 7.31 - 7.16 (m, 5H), 7.13 - 7.01 (m, 4H). LCMS (method A) t_R, 2.03 min, MS Anal. Calcd. for $[M+H]^+$ $C_{24}H_{17}FN_3$: 366.14; found: 366.15.

***SAR to determine optimal substitution*:**

6-(4-Chlorophenyl)-3,4-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (15)

A drop of piperidine (1.9 µL, 0.02 mmol) was added to a stirred suspension of benzaldehyde (81.0 µL, 0.80 mmol) and 3-(4-chlorophenyl)-3-oxopropanenitrile (140 mg, 0.78 mmol) in EtOH (779 µL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 4 h. The volatiles were removed under reduced pressure, and to 50 mg of crude product was added 3-phenyl-1*H*-pyrazol-5-amine (29.7 mg, 0.19 mmol) and DMF (187 µL). This new reaction mixture was stirred at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 6-(4-chlorophenyl)-3,4-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (18.5 mg, 0.05 mmol, 24 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.46 (br. s, 1H), 7.98 (d, *J*=8.5 Hz, 2H), 7.69 (d, *J*=8.2 Hz, 2H), 7.43 - 7.38 (m,

1H), 7.35 (d, *J*=7.3 Hz, 2H), 7.30 - 7.24 (m, 2H), 7.24 - 7.18 (m, 1H), 7.10 - 7.05 (m, 2H), 7.05 - 6.98 (m, 2H). LCMS (method B) t_R, 2.42 min, MS Anal. Calcd. for $[M+H]^+$ $C_{25}H_{16}ClN_4$: 407.11; found: 407.0.

6-(3-Chlorophenyl)-3,4-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (16)
A drop of piperidine (1.9 µL, 0.02 mmol) was added to a stirred suspension of benzaldehyde (81.0 µL, 0.80 mmol) and 3-(3-chlorophenyl)-3-oxopropanenitrile (140 mg, 0.78 mmol) in EtOH (779 µL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 4 h. The volatiles were removed under reduced pressure, and to 50 mg of crude product was added 3-phenyl-1*H*-pyrazol-5-amine (29.7 mg, 0.19 mmol) and DMF (187 µL). This new reaction mixture was stirred at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 6-(3-chlorophenyl)-3,4-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (22.1 mg, 0.05 mmol, 27 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.53 (br. s., 1H), 8.00 (s, 1H), 7.92 (d, *J*=7.6 Hz, 1H), 7.71 - 7.62 (m, 2H), 7.43 - 7.38 (m, 1H), 7.36 (d, *J*=7.0 Hz, 2H), 7.31 - 7.25 (m, 2H), 7.25 - 7.19 (m, 1H), 7.10 - 7.05 (m, 2H), 7.05 - 7.00 (m, 2H). LCMS (method B) t_R, 2.30 min, MS Anal. Calcd. for $[M+H]^+$ $C_{25}H_{16}ClN_4$: 407.11; found: 407.10.

6-Cyclopropyl-3,4-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (17)
A drop of piperidine (6.4 µL, 0.06 mmol) was added to a stirred suspension of benzaldehyde (267 µL, 2.63 mmol) and 3-cyclopropyl-3-oxopropanenitrile (280 mg, 2.57 mmol) in EtOH (2.57 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 4 h. The volatiles were removed under reduced pressure, and to 100 mg of crude product was added 3-phenyl-1*H*-pyrazol-5-amine (81.0 mg, 0.51 mmol) and DMF (507 µL). This new reaction mixture was stirred at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 6-cyclopropyl-3,4-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (5.1 mg, 0.01 mmol, 3 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.15 (br. s, 1H), 7.43 - 7.34 (m, 1H), 7.32 - 7.22 (m, 4H), 7.18 (t, *J*=7.5 Hz, 1H), 7.04 (t, *J*=7.6 Hz, 2H), 6.97 (d, *J*=7.3 Hz, 2H), 2.68 - 2.60 (m, 1H), 1.31 - 1.18 (m, 4H). LCMS (method B) t_R, 2.24 min, MS Anal. Calcd. for MH^+ $C_{22}H_{17}N_4$: 337.15; found: 337.15.

4-Cyclopropyl-3,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (18)
A drop of piperidine was added to a stirred suspension of cyclopropanecarbaldehyde (34.1 µL, 0.52 mmol) and 3-oxo-3-phenylpropanenitrile (73.5 mg, 0.51 mmol) in EtOH (0.51 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 16 h. The volatiles were removed under reduced pressure, and to 100 mg of crude product was added 3-phenyl-1*H*-pyrazol-5-amine (81.0 mg, 0.51 mmol) and DMF (507 µL). This new reaction mixture was stirred at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 4-cyclopropyl-3,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (99.3 mg, 0.28 mmol, 56 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.31 (br. s., 1H), 7.87 - 7.79 (m, 2H), 7.79 - 7.73 (m, 2H), 7.62 - 7.55 (m, 3H), 7.55 - 7.47 (m, 3H), 2.49 - 2.43 (m, 1H), 0.68 - 0.61 (m, 2H), 0.60 - 0.54 (m, 2H). LCMS (method B) t_R, 2.20 min, MS Anal. Calcd. for $[M+H]^+$ $C_{22}H_{17}N_4$: 337.15; found: 337.15.

3,6-Diphenyl-4-propyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (19)
3-Phenyl-1*H*-pyrazol-5-amine (329 mg, 2.07 mmol), 3-oxo-3-phenylpropanenitrile (300 mg, 2.07 mmol), butyraldehyde (222 µL, 2.48 mmol), piperidine (5 µL, 0.05 mmol), and Et_3N (576 µL, 4.13 mmol) were stirred in DMF (2.1 mL) at 90 °C for 17 h. The volatiles were removed under reduced pressure. Sodium nitrite (428 mg, 6.20 mmol) and acetic acid (15.9 mL, 227 mmol) were added to the crude material, and the reaction mixture was stirred for 10 min. The volatiles were removed under reduced pressure, and the crude material was subjected to silica gel column chromatography using ethyl acetate in hexanes (0-40%). Purification afforded 3,6-diphenyl-4-propyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (502 mg, 1.48 mmol, 72 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 14.36 (br. s., 1H), 7.88 - 7.82 (m, 2H), 7.67 - 7.61 (m, 2H), 7.61 - 7.53 (m, 6H), 2.99 - 2.90 (m, 2H), 1.49 - 1.31 (m, 2H), 0.63 (t, *J*=7.3 Hz, 3H). LCMS (method B) t_R, 2.25 min, MS Anal. Calcd. for $[M+H]^+$ $C_{22}H_{19}N_4$: 339.16; found: 339.18.

3-(4-Chlorophenyl)-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (20)
A drop of piperidine (8.5 µL, 0.09 mmol) was added to a stirred suspension of benzaldehyde (358 µL, 3.53 mmol) and 3-oxo-3-phenylpropanenitrile (500 mg, 3.44 mmol) in EtOH (3.45 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 16 h. The volatiles were removed under reduced pressure, and to 50 mg of crude product was added 3-(4-chlorophenyl)-1*H*-pyrazol-5-amine (41.5 mg, 0.21 mmol) and DMF (214 µL). This new reaction mixture was stirred at 150 °C for 72 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate.

Purification afforded 3-(4-chlorophenyl)-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (5.1 mg, 0.01 mmol, 6 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.57 (br. s., 1H), 7.97 - 7.90 (m, 2H), 7.63 - 7.58 (m, 3H), 7.48 - 7.41 (m, 1H), 7.39 - 7.34 (m, 2H), 7.34 - 7.28 (m, 2H), 7.13 (d, *J*=8.2 Hz, 2H), 7.03 (d, *J*=8.5 Hz, 2H). LCMS (method B) t_R, 2.30 min, MS Anal. Calcd. for $[M+H]^+$ $C_{25}H_{16}ClN_4$: 407.11; found: 407.07.

3-(3-Chlorophenyl)-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (21)
A drop of piperidine (8.5 µL, 0.09 mmol) was added to a stirred suspension of benzaldehyde (358 µL, 3.53 mmol) and 3-oxo-3-phenylpropanenitrile (500 mg, 3.44 mmol) in EtOH (3.45 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 16 h. The volatiles were removed under reduced pressure, and to 50 mg of crude product was added 3-(3-chlorophenyl)-1*H*-pyrazol-5-amine (41.5 mg, 0.21 mmol) and DMF (214 µL). This new reaction mixture was stirred at 150 °C for 72 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-(3-chlorophenyl)-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (33.7 mg, 0.08 mmol, 37 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.62 (br. s., 1H), 7.97 - 7.90 (m, 2H), 7.64 - 7.57 (m, 3H), 7.48 - 7.42 (m, 1H), 7.41 - 7.37 (m, 2H), 7.36 - 7.30 (m, 2H), 7.29 (d, *J*=7.9 Hz, 1H), 7.15 (t, *J*=7.8 Hz, 1H), 7.12 - 7.06 (m, 1H), 6.94 (s, 1H). LCMS (method B) t_R, 2.28 min, MS Anal. Calcd. for $[M+H]^+$ $C_{25}H_{16}ClN_4$: 407.11; found: 407.07.

Selected compounds to demonstrate structural diversity:
3-*Tert*-butyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (22)
A drop of piperidine (8.5 µL, 0.09 mmol) was added to a stirred suspension of benzaldehyde (358 µL, 3.53 mmol) and 3-oxo-3-phenylpropanenitrile (500 mg, 3.44 mmol) in EtOH (3.45 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 16 h. The volatiles were removed under reduced pressure, and to 84 mg of crude product was added 3-(*tert*-butyl)-1*H*-pyrazol-5-amine (50.0 mg, 0.36 mmol) and DMF (359 µL). This new reaction mixture was stirred at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-*tert*-butyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (7.4 mg, 0.02 mmol, 6 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.03 (br. s., 1H), 7.92 - 7.80 (m, 2H), 7.66 - 7.49 (m, 8H), 1.04 (s, 9H). LCMS (method B) t_R, 2.43 min, MS Anal. Calcd. for $[M+H]^+$ $C_{23}H_{21}N_4$: 353.18; found: 353.16.

3-Isopropyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (23)
A drop of piperidine (8.5 µL, 0.09 mmol) was added to a stirred suspension of benzaldehyde (358 µL, 3.53 mmol) and 3-oxo-3-phenylpropanenitrile (500 mg, 3.44 mmol) in EtOH (3.45 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 16 h. The volatiles were removed under reduced pressure, and to 150 mg of crude product was added 5-isopropyl-1H-pyrazol-3-amine (80.0 mg, 0.64 mmol) and DMF (643 µL). This new reaction mixture was stirred at 100 °C for 72 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-isopropyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (53.1 mg, 0.15 mmol, 24 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.01 (s, 1H), 7.89 (s, 2H), 7.67 - 7.60 (m, 5H), 7.60 - 7.55 (m, 3H), 2.72 - 2.60 (m, 1H), 0.99 (d, *J*=7.0 Hz, 6H). LCMS (method B) t_R, 2.12 min, MS Anal. Calcd. for $[M+H]^+$ $C_{22}H_{19}N_4$: 339.16; found: 339.17.

3-Cyclopropyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (24)
A drop of piperidine (8.5 µL, 0.09 mmol) was added to a stirred suspension of benzaldehyde (358 µL, 3.53 mmol) and 3-oxo-3-phenylpropanenitrile (500 mg, 3.44 mmol) in EtOH (3.45 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 16 h. The volatiles were removed under reduced pressure, and to 50 mg of crude product was added 5-cyclopropyl-1H-pyrazol-3-amine (26.4 mg, 0.21 mmol) and DMF (214 µL). This new reaction mixture was stirred at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude mixture was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-cyclopropyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (42.2 mg, 0.12 mmol, 57 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.89 (br. s., 1H), 7.91 - 7.86 (m, 2H), 7.72 - 7.66 (m, 2H), 7.66 - 7.60 (m, 3H), 7.60 - 7.53 (m, 3H), 1.39 - 1.22 (m, 1H), 0.83 (d, *J*=2.7 Hz, 2H), 0.64 (dd, *J*=8.2, 2.4 Hz, 2H). LCMS (method B) t_R, 2.16 min, MS Anal. Calcd. for $[M+H]^+$ $C_{25}H_{17}N_4$: 337.15; found: 337.13.

3-Methyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (25)
3-Methyl-1*H*-pyrazol-5-amine (20.1 mg, 0.21 mmol), 3-oxo-3-phenylpropanenitrile (30.0 mg, 0.21 mmol), benzaldehyde (21.9 mg, 0.21 mmol), and Et_3N (57.6 µL, 0.41 mmol) were stirred in DMF (207 µL) at 90 °C for 16 h. The volatiles were removed under reduced pressure. Sodium nitrite (42.8 mg, 0.62 mmol) and acetic acid (1590 µL, 27.7 mmol) were added to the crude material, and the reaction mixture was stirred for 10 min. The volatiles were removed under reduced

pressure, and the crude material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-methyl-4,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (38.8 mg, 0.12 mmol, 59 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 13.99 (s, 1H), 7.89 (dd, *J*=6.4, 2.7 Hz, 2H), 7.63 (s, 5H), 7.60 - 7.56 (m, 3H), 2.00 (s, 3H). LCMS (method B) t_R, 2.08 min, MS Anal. Calcd. for $[M+H]^+$ $C_{20}H_{15}N_4$: 311.13; found: 311.10.

3-*Tert*-Butyl-6-phenyl-4-(3,3,3-trifluoropropyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (26)
3-(*Tert*-butyl)-1*H*-pyrazol-5-amine (57.5 mg, 0.41 mmol), 3-oxo-3-phenylpropanenitrile (60.0 mg, 0.41 mmol), 4,4,4-trifluorobutanal (44.9 µL, 0.41 mmol), and Et_3N (115 µL, 0.83 mmol) were stirred in DMF (413 µL) at 90 °C for 16 h. The volatiles were removed under reduced pressure. Sodium nitrite (86.0 mg, 1.24 mmol) and acetic acid (3171 µL, 55.4 mmol) were added to the crude material, and the reaction mixture was stirred for 10 min. The volatiles were removed under reduced pressure, and the crude material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-butyl-6-phenyl-4-(3,3,3-trifluoropropyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (11.6 mg, 0.03 mmol, 7 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 13.98 (br. s., 1H), 7.89 - 7.82 (m, 2H), 7.66 - 7.49 (m, 3H), 3.70 - 3.61 (m, 2H), 2.80 (d, *J*=6.7 Hz, 2H), 1.52 (s, 9H). LCMS (method B) t_R, 2.47 min, MS Anal. Calcd. for $[M+H]^+$ $C_{20}H_{20}F_3N_4$: 373.16; found: 373.00.

3-(3-Chlorophenyl)-4-(3,3-difluorocyclobutyl)-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (27)
3-(3-Chlorophenyl)-1*H*-pyrazol-5-amine (40.0 mg, 0.21 mmol), 3-oxo-3-phenylpropanenitrile (30.0 mg, 0.21 mmol), 3,3-difluorocyclobutanecarbaldehyde (24.8 mg, 0.21 mmol), and Et_3N (57.6 µL, 0.41 mmol) were stirred in DMF (207 µL) at 90 °C for 16 h. The volatiles were removed under reduced pressure. Sodium nitrite (42.8 mg, 0.62 mmol) and acetic acid (1574 µL, 0.62 mmol) were added to the crude material, and the reaction mixture was stirred for 10 min. The volatiles were removed under reduced pressure, and the crude material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-(3-chlorophenyl)-4-(3,3-difluorocyclobutyl)-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (16.3 mg, 0.04 mmol, 19 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.54 (br. s., 1H), 7.87 - 7.82 (m, 2H), 7.80 - 7.75 (m, 1H), 7.68 - 7.56 (m, 6H), 4.12 - 4.03 (m, 1H), 3.22 - 3.08 (m, 2H), 2.58 (q, *J*=11.0 Hz, 2H). LCMS (method B) t_R, 2.35 min, MS Anal. Calcd. for $[M+H]^+$ $C_{23}H_{16}ClF_2N_4$: 421.10; found: 421.14.

3-(3-Chlorophenyl)-4-(3,3-difluorocyclobutyl)-6-(3-fluorophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (28)
3-(3-Chlorophenyl)-1*H*-pyrazol-5-amine (35.6 mg, 0.18 mmol), 3-(3-fluorophenyl)-3-oxopropanenitrile (30.0 mg, 0.18 mmol), 3,3-difluorocyclobutanecarbaldehyde (22.1 mg, 0.18 mmol), and Et_3N (51.3 µL, 0.37 mmol) were stirred in DMF (184 µL) at 90 °C for 16 h. The volatiles were removed under reduced pressure. Sodium nitrite (38.1 mg, 0.55 mmol) and acetic acid (1390 µL, 24.3 mmol) were added to the crude material, and the reaction mixture was stirred for 10 min. The volatiles were removed under reduced pressure, and the crude material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-(3-chlorophenyl)-4-(3,3-difluorocyclobutyl)-6-(3-fluorophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (1.3 mg, 0.003 mmol, 2 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.77 (s, 1H), 7.71 - 7.57 (m, 6H), 7.48 - 7.40 (m, 1H), 4.09 (t, *J*=8.8 Hz, 1H), 3.22 - 3.09 (m, 2H), 2.66 - 2.54 (m, 2H). LCMS (method F) t_R, 2.12 min, MS Anal. Calcd. for $[M+H]^+$ $C_{23}H_{15}ClF_3N_4$: 439.09; found: 439.3.

3-(3-Chlorophenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (29)
3-(3-Chlorophenyl)-1*H*-pyrazol-5-amine (400 mg, 2.07 mmol), 3-oxo-3-phenylpropanenitrile (300 mg, 2.07 mmol), cyclopropanecarbaldehyde (154 µL, 2.07 mmol), and Et_3N (576 µL, 4.13 mmol) were stirred in DMF (2.1 mL) at 90 °C for 16 h. The volatiles were removed under reduced pressure. Sodium nitrite (428 mg, 6.20 mmol) and acetic acid (15.6 mL, 273 mmol) were added to the crude material, and the reaction mixture was stirred for 10 min. The volatiles were removed under reduced pressure, and the crude material was subjected to silica gel column chromatography with ethyl acetate in hexanes (0–30%). Purification afforded 3-(3-chlorophenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (500 mg, 1.35 mmol, 65 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.41 (br. s., 1H), 7.86 - 7.80 (m, 3H), 7.76 (dt, *J*=7.1, 1.6 Hz, 1H), 7.61 - 7.51 (m, 5H), 2.48 - 2.45 (m, 1H), 0.73 - 0.66 (m, 2H), 0.62 - 0.57 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d_6) δ = 159.98, 154.67, 152.15, 144.68, 137.75, 135.74, 132.69, 129.87, 129.71, 129.14, 128.99, 128.34, 128.24, 128.21, 117.61, 111.88, 103.05, 14.01, 8.75. IR (film): 3228 (s), 3052 (w), 2228 (m), 1581 (s), 1555 (s), 1503 (w), 1447 (m), 1284 (m) cm^{-1}. HRMS (ESI): *m/z* $[M+H]^+$ Calcd. for $C_{22}H_{16}N_4Cl$: 371.1058; found: 371.1053.

3-(3-Chloro-4-fluorophenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (30)
4-Cyclopropyl-6-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile
Piperidine (138 mL, 1.39 mmol) was added to a stirred suspension of cyclopropylcarbaldehyde (4.26 mL, 57.1 mmol) and 3-oxo-3-phenylpropanenitrile (8.08 g, 55.7 mmol) in EtOH (55.7 mL) under N_2 (g) at ambient temperature. The reaction mixture was then heated to 50 °C for 16 h. The volatiles were removed under reduced pressure, and to 4.87 g of crude product was added 1H-pyrazol-5-amine (2.05 g, 24.7 mmol) and DMF (10.0 mL). This new reaction mixture was stirred at

150 °C for 16 h. The crude reaction mixture was diluted with ethyl acetate (150 mL) and brine (50 mL), and the layers were separated. The organic layer was washed with brine (30 mL) and dried over sodium sulfate. The solids were filtered away, and the volatiles were removed under reduced pressure to afford 4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile. Sodium nitrite (5.11 g, 74.0 mmol) and acetic acid (40.0 mL) were added to the crude material, and the reaction mixture was stirred for 16 h. The volatiles were removed under reduced pressure, and the crude material was subjected to silica gel column chromatography with ethyl acetate in hexanes (0–30%). Purification afforded 4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile. LCMS (method D) t_R, 2.61 min, MS Anal. Calcd. for $[M+H]^+$ $C_{16}H_{13}N_4$: 261.11; found: 261.2.

3-Bromo-4-cyclopropyl-6-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile
To a brown suspension of 4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (3.64 g, 13.98 mmol) in DCM (130 mL) was added NBS (2.74 g, 15.38 mmol) at ambient temperature. The resulting mixture was stirred at 45 °C for 2 h. The reaction was allowed to cool to ambient temperature and was diluted with THF (300 mL) and Et_2O (300 mL). The resulting solution was washed with sat. aqueous sodium thiosulfate (100 mL) and brine (100 mL), dried over magnesium sulfate, the solids were filtered away, and the volatiles were removed under reduced pressure. The crude material was subjected to silica gel column chromatography with ethyl acetate in hexanes (0–50%). Purification afforded 3-bromo-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (2.60 g, 7.67 mmol, 55 % yield) as a yellow powder. LCMS (method D) t_R, 3.01 min, MS Anal. Calcd. for $[M+H]^+$ $C_{16}H_{12}BrN_4$: 339.02; found: 339.1.

3-(3-Chloro-4-fluorophenyl)-4-cyclopropyl-6-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile
3-Bromo-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (30.0 mg, 0.09 mmol), (3-chloro-4-fluorophenyl)boronic acid (46.3 mg, 0.27 mmol), $PdCl_2$(dppf)-CH_2Cl_2 adduct (10.8 mg, 0.01 mmol), and sodium carbonate (46.9 mg, 0.44 mmol) were stirred in DME (2 mL) and water (0.5 mL) at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-(3-chloro-4-fluorophenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (17.2 mg, 0.04 mmol, 50 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 14.38 (br. s., 1H), 8.00 (dd, *J*=7.0, 1.8 Hz, 1H), 7.88 - 7.78 (m, 3H), 7.62 - 7.51 (m, 4H), 2.49 - 2.45 (m, 1H), 0.77 - 0.68 (m, 2H), 0.65 - 0.56 (m, 2H). LCMS (method F) t_R, 2.92 min, MS Anal. Calcd. for $[M]^+$ $C_{22}H_{15}ClFN_4$: 388.09; found: 388.1.

3-(4-Chloro-3-(trifluoromethyl)phenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (31)
3-Bromo-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (70.0 mg, 0.21 mmol), (4-chloro-3-(trifluoromethyl)phenyl)boronic acid (139 mg, 0.62 mmol), $PdCl_2$(dppf)-CH_2Cl_2 adduct (25.3 mg, 0.03 mmol), and sodium carbonate (109 mg, 1.03 mmol) were stirred in DME (6604 µL) and water (1651 µL) at 150 °C for 16 h. The volatiles were removed under reduced pressure, and the crude material was partitioned between ethyl acetate (25 mL) and brine (25 mL). The layers were separated, and the organics dried over sodium sulfate. The solids were filtered away, the volatiles removed under reduced pressure, and the resulting material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-(4-chloro-3-(trifluoromethyl)phenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (13.6 mg, 0.03 mmol, 15 % yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ = 14.46 (br. s., 1H), 8.24 (s, 1H), 8.14 (d, *J*=8.1 Hz, 1H), 7.88 (d, *J*=8.3 Hz, 1H), 7.84 (dd, *J*=6.1, 2.7 Hz, 2H), 7.61 - 7.55 (m, 3H), 2.50 - 2.45 (m, 1H), 0.74 - 0.68 (m, 2H), 0.65 - 0.59 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.5, 155.0, 153.0, 144.1, 138.3, 135.4, 133.9, 132.0, 131.2, 130.3, 129.7, 128.9, 128.9, 128.8, 127.0 (q, *J*=30.5 Hz), 118.1, 112.4, 103.9, 14.6, 9.4. LCMS (method A) t_R, 2.01 min, MS Anal. Calcd. for $[M+H]^+$ $C_{23}H_{15}ClF_3N_4$: 439.09; found: 439.15.

3-(4-Chloro-3-methoxyphenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (32)
3-Bromo-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (30.0 mg, 0.09 mmol), (4-chloro-3-methoxyphenyl)boronic acid (49.5 mg, 0.27 mmol), $PdCl_2$(dppf)-CH_2Cl_2 adduct (10.8 mg, 0.01 mmol), and sodium carbonate (47 mg, 0.44 mmol) were stirred in DME (2.0 mL) and water (0.5 mL) at 150 °C for 1.5 h. The volatiles were removed under reduced pressure, and the crude material was partitioned between ethyl acetate (25 mL) and brine (25 mL). The layers were separated, and the organics dried over sodium sulfate. The solids were filtered away, the volatiles removed under positive N_2 (g) pressure, and the resulting material was subjected to reversed phase HPLC with an acetonitrile/water mixture with 10 mM aqueous ammonium acetate. Purification afforded 3-(4-chloro-3-methoxyphenyl)-4-cyclopropyl-6-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (17.7 mg, 0.04 mmol, 47 % yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.37 (br. s., 1H), 7.86 - 7.79 (m, 2H), 7.61 - 7.54 (m, 4H), 7.52 (s, 1H), 7.35 (d, *J*=7.9 Hz, 1H), 3.93 (s, 3H), 0.72 (d, *J*=8.2 Hz, 2H), 0.61 (d, *J*=5.2 Hz, 2H). LCMS (method E) t_R, 2.34 min, MS Anal. Calcd. for $[M+H]^+$ $C_{23}H_{18}ClN_4O$: 401.12; found: 401.4.

LC/MS HPLC methods:

method A: Column: Phenomenex Luna 30 x 2.0 mm 3um; Mobile Phase A: 10:90 acetonitrile:water with 0.1% TFA; Mobile Phase B: 90:10 acetonitrile:water with 0.1% TFA; Temperature: 40 °C; Gradient: 0-100% B over 2 min; Flow: 1 mL/min.

method B: Column: Phenomenex Luna 30 x 2.0 mm 3um; Mobile Phase A: 10:90 methanol:water with 0.1% TFA; Mobile Phase B: 90:10 methanol:water with 0.1% TFA; Temperature: 40 °C; Gradient: 0-100% B over 2 min; Flow: 1 mL/min.

method C: Column: Phenomenex Luna 50 x 2.0 mm 3um; Mobile Phase A: 10:90 methanol:water with 0.1% TFA; Mobile Phase B: 90:10 methanol:water with 0.1% TFA; Temperature: 40 °C; Gradient: 0-100% B over 4 min; Flow: 0.8 mL/min.

method D: Column: Phenomenex Luna C18 30 x 2.0 mm 3um; Mobile Phase A: 10:90 acetonitrile:water with 0.1% TFA; Mobile Phase B: 90:10 acetonitrile:water with 0.1% TFA; Temperature: 40 °C; Gradient: 0-100% B over 2 min; Flow: 1 mL/min.

method E: Column: Phenomenex Luna C18 50 x 2.0 mm 3um; Mobile Phase A: 10:90 acetonitrile:water with 0.1% TFA; Mobile Phase B: 90:10 acetonitrile:water with 0.1% TFA; Temperature: 40 °C; Gradient: 0-100% B over 4 min; Flow: 0.8 mL/min.

method F: Column: Waters BEH C18 50 x 2.0 mm 1.7um; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 40 °C; Gradient: 0-100% B over 4 min; Flow: 1 mL/min.

Metabolic Stability – 10 min Incubation with Human and Mouse Liver Microsomes

Methods and Materials

Incubation with Liver Microsomes

Test compound is received as a 3.5 mM stock solution in 100% DMSO. Compound is diluted to create a 50 µM acetonitrile (ACN) solution containing 1.4% DMSO, which is then used as a 100x stock for incubation with microsomes. Each compound is tested in duplicate separately in each of two species in the Metabolic Stability-Human and Mouse assay suite. Compound, NADPH and liver microsome solutions are combined for incubation in three steps:

1) 152 µL of liver microsome suspension, protein concentration of 1.1 mg/mL in 100 mM NaP_i, pH 7.4, 6.6 mM $MgCl_2$ buffer, is pre-warmed at 37 °C.
2) 1.7 µL of 50 µM compound (98.6% ACN, 1.4% DMSO) is added to the same tube and pre-incubated at 37 °C for 5 min.
3) The reaction is initiated by the addition of 17 µL of pre-warmed 10 mM NADPH solution in 100 mM NaP_i, pH 7.4.

Reaction components are mixed well, and 75 µL are immediately transferred into 150 µL quench/stop solution (zero-time point, T_0). Reactions are incubated at 37 °C for 10 min and then an additional 75 µL aliquot is transferred into 150 µL quench solution. Acetonitrile containing 100 µM DMN (a UV standard for injection quality control), is used as the quench solution to terminate metabolic reactions.

Quenched mixtures are centrifuged at 1500 rpm (~500 X g) in an Allegra X-12 centrifuge, SX4750 rotor (Beckman Coulter Inc., Fullerton,CA) for 15 min to pellet denatured microsomes. A volume of 90 µL of supernatant extract, containing the mixture of parent compound and its metabolites, is then transferred to a separate 96-well plate for UV-LC/MS-MS analysis to determine the per cent of parent compound that is remaining in the mixture.

Metabolic Stability Assay - Reaction Components	
Reaction Components	Final Concentration in the Metabolic Stability Assay
Compound (Substrate)	0.5 µM
NaPi Buffer, pH 7.4	100 mM
DMSO	0.014%
Acetonitrile	0.986%
Microsomes (human, mouse) (BD/Gentest)	1 mg/mL protein
NADPH	1.0 mM
$MgCl_2$	6.66 mM
37 °C Incubation time	0 min and 10 min
Quench/Stop Solution (ACN+100 µM DMN)	150 µL
Sample of Reaction	75 µL
Sedimentation of Denatured Microsomes	15 min
UV-LC/MS analysis of supernatant	0.17 µM

Sample Analysis – Reagents and Materials

A. *Materials* Assay Optimization
- Axygen round well plates 1.1mL, Axygen Scientific, P-DW-11-C.
- Axygen sealing mat, Axygen Scientific, AM-2ML-RD.

B. *Solvents*
- Acetonitrile, 'Baker Analyzed' HPLC Solvent. J.T. Baker, 9017-03.
- Water, 'Baker Analyzed' HPLC Solvent. J.T. Baker, 4218-03.
- Formic Acid, 98% GR ACS. EMD, FX0440-5.
- Ammonium Acetate, CAS # 631-61-8

C. *Columns*
- Analytical column:

- Acentis ® Express C18, 2.0 cm x 2.1 mm, 2.7 µm
- Phenomenex Gemini C6-Phenyl, 2.0cm x 2.1 mm, 3 µm (for use with basic mobile phase)

D. *Mobile Phases & Solutions*
- (A) - 98:2 Water:Acetonitrile, 2mM ammonium acetate, 0.2% formic acid.
- (B) - 2:98 Water:Acetonitrile, , 0.2% formic acid.
- (C) - 0.1% ammonium hydroxide in water
- (D) - 0.1% ammonium hydroxide in acetonitrile

Sample Analysis – Instruments

A. Mass Spectrometers
- Thermo Quantum Ultra triple-quadrupole mass spectrometer with heated electrospray (H-ESI) ionization source.

B. HPLC Pump(s)
- Shimadzu 10AD VP
- Shimadzu 20AD

C. Autosampler
- Leap CTC HTS Pal with twin arm

D. Stream Selector
- Cohesive VIM

Sample Analysis - Structural Integrity Pre-Analysis

The Metabolic Stability structural integrity pre-analysis is used to assess the purity of compounds being assayed. Compounds are received in 96-well plates as 57 µL of a 3.5 mM DMSO solution. The 3.5 mM compound DMSO stock solutions are diluted 18-fold with a solution containing equal volumes of acetonitrile, isopropanol, and MilliQ-H_2O. The resulting solutions (200 µM) are analyzed for structural integrity by LC-UV/MS on a Thermo LCQ Deca XP Plus ion trap mass spectrometer, using a Waters XBridge C18, 5 µm, 2 x 50 mm column with a Waters Sentry 2.1 mm guard column, and the LC conditions described in the table below, with a 5 µL injection and a flow rate of 1 mL/min. The acquired data reflect purity by UV absorbance at 220 nm. Only results for those compounds with purity greater than 50% are reported.

Metabolic Stability - Structural Integrity Gradient		
Gradient Time (min)	A %	B%
0.00	100	0
4.00	0	100
5.00	0	100
5.10	100	0
6.00	100	0

Sample Analysis – Incubated Samples

MS/MS condition optimization is conducted on a Thermo TSQ Quantum triple-qudrupole mass spectrometer equipped with a heated-electrospray (H-ESI) source by automated infusion to obtain the SRM transitions and their corresponding collision energy values. Compound solutions at a concentration of 20 µM in 1:1 methanol:water are infused at a flow rate of 60 µL/min, then combined with the mobile phase at a flow rate of 500 µL/min before being introduced into the source. All compounds are optimized first using mobile phase A and B (50% A and 50% B), and if necessary, using mobile phase C and D (also with a 50:50 composition). The optimized parameters, including polarity, tube lens voltage, SRM transition and collision energy, are stored in a Microsoft Access database.

The mass spectrometric conditions obtained from automated infusion are used to analyze incubation samples from the Metabolic Stability assay. Acquisition of multiple injection chromatogram was employed by grouping all the sample injections of a compound into a single data file. Assay samples received contain internal standard: 100 nM alprenolol and 300 nM tolbutamide. Table 1 below lists the MRM transitions and concentrations for the internal standards.

Table 1. Internal Standard

	Polarity	Precursor Ion	Product Ion	Collision Energy	Tube Lens	Conc. (nM)
Alprenolol	Positive	250	116	16	93	100
Tolbutamide	Negative	269	170	19	103	300

Table 2 below lists the gradient conditions for assay sample analysis. The gradient may be modified and the column may be changed to obtain an optimum chromatogram for some compounds if needed. The analysis is conducted on a dual arm Aria LX-2 system. The injection volume is 15 µL and the flow rate is 0.9 mL/min. The MS acquisition window starts at 0.18 min for a total MS acquisition time of 0.25 min for each injection. All samples are injected with the gradient using mobile phase A and B first. If necessary (for instance, for chromatographic reasons), samples are re-injected using mobile phase C and D onto Phenomenex Gemini columns. All LC-MS/MS analysis parameters are captured electronically in the raw data files.

Table 2. Sample Analysis Gradient

Gradient Time (min)	Sec	Flow Rate (mL/min)	A % (or C%)	B% (or D%)	Grad
0.0	6	0.9	95	5	Step
0.1	6	0.9	0	100	Ramp
0.2	9	1.1	0	100	Step
0.35	1	0.9	95	5	Step
0.37	18	0.9	95	5	Step

Data Analysis
Peak integration and data review is performed using GMSU QuickCalc software. Using StarScreen, the % remaining calculation is performed by comparing the LC-MS/MS peak area ratio of analyte to internal standard from the T_{10min} samples to those from the T_{0min} samples for each compound.

Metabolic ID Studies

Sample Type Met ID: **29** (30 uM) was incubated in liver microsomes (human and rat) supplemented with NADPH (1 mM), GSH (5 mM) or NADPH+GSH (5 mM) for 0 and 45 min. Reactions were terminated by adding 1 volume of acetonitrile, and proteins were removed by centrifugation. The supernatants were saved for HPLC/UV/MS analysis. Semi-quantitative analysis: at a lower substrate concentration (0.5 uM), parent drug disappearance and metabolite formation over 10 min were assessed based on mass spectrometric responses (obtained from the incubations with the substrate concentration as 0.5 uM) and UV/MS peak area (obtained with reference to the incubations of 30 uM substrate concentration).

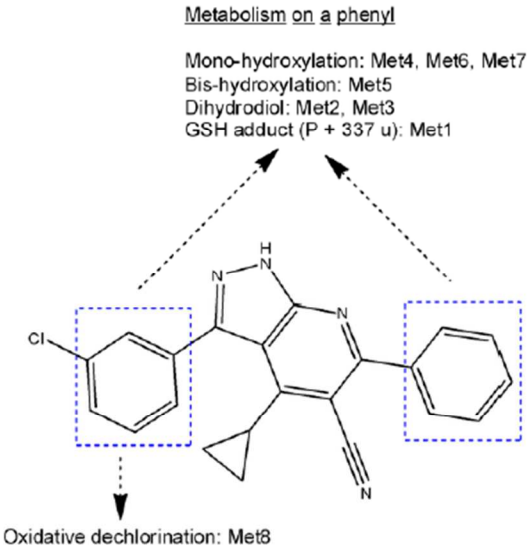
The 45-min incubations at 30 µM were used for metabolite identification, while the incubations at 0.5 µM carried out for up to
10 min were intended to provide measurements of parent drug disappearance and rank ordering of metabolite formation.

HPLC Conditions Waters Aquity binary solvent manager
Solvents Solvent A = 5% MeCN in H_2O with 0.1% formic acid; Solvent B = MeCN
Flow rate 500 µL/min
Column Acquity UPLC BEH (1.7 µm) 100 × 2.00 mm column maintained at 40 C
Elution gradient Time (min) 0 1 6.5 10 11 11.5 13

Solvent B% 5 5 35 100 100 5 5
PDA detection Waters Aquity PDA detector at 255 nm
MS Conditions Waters Xevo QTOF mass spectrometer with an electrospray source operated at a positive ionization mode
Parameters Capillary voltage = 3 kV, cone voltage = 30 V, extraction cone = 1.7 V, source temp = 125 C; desolvation temp = 250 C; high-energy MS trap collision; voltage = 25 - 40 V
Scan Full scan and MSe scan
Lock spray Leucine enkephalin (1 ng/mL) at an infusion rate of 30 µL/min; fragment ions at m/z 556.277 and 278.224 monitored

Results: 29 (at 0.5 µM) exhibited high metabolic clearance in both HLM (9% remaining) and RLM (23% remaining) during a 10-min incubation. Metabolism mainly occurred on the right-hand phenyl and/or the left-hand side chlorophenyl; however, the MS/MS spectra could not be used to further narrow down the soft spot(s). Metabolites included mono-hydroxylation products (Met4, Met6, and Met7), a bis-hydroxylation product (Met5), and two dihydrodiols (Met2 and Met3). Met8 was proposed as a product of oxidative dechlorination. Both Met7 and Met8 were detected only in incubations at the higher substrate concentration. Deuterium-hydrogen exchange was used to rule out *N*-oxidation.

In NADPH- and GSH-supplemented incubations, one glutathione adduct, Met1, was detected in HLM incubations, but not in RLM incubations . It was formed via bis-hydroxylation and GSH conjugation. The bioactivation site is unknown. In HLM incubations, the dihydrodiol product (Met3) formed most rapidly. In RLM incubations, the hydroxylation metabolite (Met4) was more prominent. (v5) Conclusion: **29** was unstable in both HLM and RLM incubations in MetXpress studies. The main pathways were oxidation on the right-hand phenyl and/or left-hand chlorophenyl. Detection of dihydrodiols and a GSH adduct indicated that the phenyl(s) underwent bioactivation, likely via arene oxidation.



Biological Methods

I. mGluR5 FLIPR assay - Positive Modulator Mode. Test compounds are incubated with cells in the presence of dye for 60 minutes prior to being read on the FLIPR platform (Molecular Devices). A Ca++ signal is induced in the assay plates via the delivery of an ~EC_{10} concentration of the endogenous agonist L-glutamate; images are acquired at 1 Hz for 100 sec post-delivery of agonist stimulus. Positive modulator activity (i.e. the ability of test compounds to increase the Ca++ response to a sub-maximal concentration of agonist) is normalized to a saturating concentration of a known mGluR5 PAM run in each assay plate. An EC_{50} concentration of test compounds is derived from 4-parameter logistic curve fits of transformed fluoresence data via proprietary software suite.

II. mGluR5 FLIPR assay - Agonist Mode. Cell plates prepared as above are challenged with direct addition of test compounds (rather than an EC_{10} of agonist delivered in the presence of compounds, as in modulator mode) to assess

intrinsic ability S13 to induce Ca++ release. Agonist activity is normalized to a saturating concentration of a known mGluR5 agonist run in each assay plate. An EC_{50} concentration of test compounds is derived from 4-parameter logistic curve fits of transformed fluoresence data via proprietary software suite.

III. Novel Object Recognition Assay (NOR)

Subjects: Studies were conducted in group housed (n = 3-4), male C57Bl/6 mice (25-30 g; Taconic Laboratories, NY) held in colony rooms maintained at constant temperature (21 ± 2 °C), and humidity (50 ± 10%) and illuminated for 12 hours per day (lights on at 0600 hours). Animals had *ad libitum* access to food and water throughout the studies. Behavioral studies were conducted between 0700 and 1400 hours. Animals were maintained in accordance with the guidelines of the Animal Care and Use Committee of the Bristol-Myers Squibb Company, the "Guide for Care and Use of Laboratory Animals" and the guidelines published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Research protocols were approved by the Bristol-Myers Squibb Company Animal Care and Use Committee.

Methods: NOR methods originally established by Ennaceur and Delacour in rat.[2] The novel object recognition task measures long term memory for objects. The procedure followed a 3 day protocol as follows: day 1 (habituation): individual mice were habituated to the testing chamber (a white PVC box 48 cm long x 38 cm wide x 20 cm high with brown linoleum flooring) for 15 min then returned to their home cage; day 2 (training): individual mice were returned to the testing chamber containing 2 identical objects and allowed to explore for 15 min; day 3 (testing): individual mice were returned to the testing chamber 24 h later containing 1 familiar object encountered in the previous training session and one novel object and allowed to explore for 10 min. Animal behavior was video recorded during both training and testing and the amount of time spent exploring the objects determined using Cleversys software. Object exploration was only scored when the animal's nose was within 1 cm of the object. The chamber and objects were thoroughly cleaned with 25% ethanol between subjects. Subjects (n=12-14/group) were dosed i.p. with either vehicle (10% Cremaphor, 10% DMSO, 80% saline; 10 ml/kg) or BMT-145027 at 10 and 30 mg/kg 60 min prior to the training session and tested for recognition memory 24 h later. Results showed that vehicle treated animals showed no preference for the novel object as anticipated. Subjects treated with BMT-145027 at 30 mg/kg showed a significant preference for the novel object.

Total drug exposure data for satellite animals

Compound	Route of Admin	Dose (mg/kg)	Sample Time (min)	Plasma Conc. (nM)		Brain Conc. (nM)		Brain /Plasma
				Mean	SE	Mean	SE	
BMT-145027	IP	10	60	564	78	630	93	1.2
		30		2776	251	1442	116	0.5

IP = intraperitoneal; SE = standard error

Plasma protein binding in mouse = 99.5%

IV. mGluR5 Binding Protocol: 3H-MPEP and 3H-MPEPy Binding

Frozen Preparation of membranes: Radioligand binding assays were performed on membrane homogenates prepared from an HEK293 cell line stably-expressing human metabotropic glutamate receptor subtype 5 (hu-mGluR5/HEK). Adherent cells were removed from flasks using versene and washed. The cell pellet was homogenized using a polytron in 4 °C homogenization buffer (50 mM Tris (pH 7.2), 10 mM $MgCl_2$, 2 mM EGTA and a protease inhibitor cocktail), and then centrifuged for 30 minutes at 32,000xg at 4 °C. The resulting pellet was again homogenized and spun down. The final pellet was again resuspended in homogenization buffer, the protein measured and adjusted, and aliquots of membrane suspension frozen quickly in a dry ice/EtOH bath and stored at -70°C.

[^{3}H]-MethoxyPEPy Binding: [^{3}H]-MethoxyPEPy filtration binding was performed based upon the method of Patel.[3] Frozen aliquots of membrane homogenate were thawed, homogenized and resuspended at 50 µg/well protein in assay buffer (50 mM Tris-HCl (pH 7.5 @ 25 °C), 5 mM $MgCl_2$, 0.005 % Triton-X 100 and 0.1% (v/v) Sigma Protease Inhibitor Cocktail). Non-specific binding was defined with 10 µM unlabeled MPEP. Competition binding experiments were performed using a single concentration (5 nM) of [^{3}H]-MethoxyPyEP in the presence of increasing concentrations of test compound. The reaction was terminated by the addition of 5 ml of ice-cold wash buffer (50 mM Tris-HCl (pH 7.5 @ 4 °C) and 0.1% BSA) and rapid filtration through a Brandel Cell Harvester using Whatman GF/B filters presoaked in 0.5 % PEI. The filter is punched into a 96 well flex-plate and scintillation cocktail is added to each well. The plate is allowed to soak for 8 h and

then read on the micro-beta counter. IC_{50} values were determined using non-linear regression four-parameter logistic equation, y = A + ((B-A)/(1+((C/x)^D))) where A=0% inh, B=100%.

Dissociation experiments to show the effects of test compounds on the K_{off} rates of [^{3}H]-MethoxyPEPy were conducted as follows. hu-mGluR5/HEK membranes were incubated to equiblibrium with 5 nM [^{3}H]-MethoxyPEPy (~ 30 min) at which time 10 uM of cold MPEP and 1 uM of test compound were added at staggered additions (between 0 and 45 min). After 45 min the reaction was terminated. Off rates for control and test compound treated membranes were calculated from curves generated through Graphpad Prism v5.0 using the One Phase Exponential Decay equation. Statistical analysis was performed using Dunnett's Multiple Comparison Test.

	K_{off} (% of control)			
Compound	Rep # 1	Rep #2	Rep #3	Avg
Control	100	100	100	100
BMS-955829	98	102	99	100
28	118	116	114	116
21	n.d.	117	111	114

References

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(3) Patel, S.; Krause, S. M.; Hamill, T.; Chaudhary, A.; Burns, D. H; Gibson, R. A. In vitro characterization of [3H]MethoxyPyEP, and mGluR5 selective ligand. *Life Sci.* **2003**, *73*, 371–379.