Supporting Information

1*H*-Pyrrolo[3,2-*b*]pyridine GluN2B-selective NMDA antagonists

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Chemistry Experimental Section:

General.

Solvents and reagents were used as supplied by the manufacturer. Concentrated refers to concentrated using a rotary evaporator under reduced pressure.

Unless specified otherwise, normal-phase silica gel column chromatography was performed on silica gel (SiO_2) using prepackaged cartridges and the indicated solvents. Preparative reverse-phase high performance liquid chromatography (HPLC) was performed under one of three conditions:

1) An Agilent HPLC with a Waters XBridge C18 column or Xterra Prep RP_{18} column (5 μm, 30x100 mm or 50x150 mm) and a gradient of 5-99% acetonitrile/water (20 mM NH_4OH) over 12 to 18 min and a flow rate of 30 or 80 ml/min.

2) A Shimadzu LC-8A Series HPLC with an XBridge C18 OBD column (5 μm, 50 x 100mm), mobile phase of 5% ACN in H_2O (both with 0.05% TFA) was held for 1 min, then a gradient of 5-99% ACN over 14 min, then held at 99% ACN for 10 min, with a flow rate of 80 mL/min.

3) A Shimadzu LC-8A Series HPLC with an Inertsil ODS-3 column (3 μm, 30 x 100mm, T = 45 °C), mobile phase of 5% ACN in H_2O (both with 0.05% TFA) was held for 1 min, then a gradient of 5-99% ACN over 6 min, then held at 99% ACN for 3 min, with a flow rate of 80 mL/min.

HRMS was obtained on an Agilent G6230B Time-of-Flight (TOF) mass spectrometer, using a Dual AJS ESI in positive mode with a scan range of 100-1700 amu. The TOF was tuned using

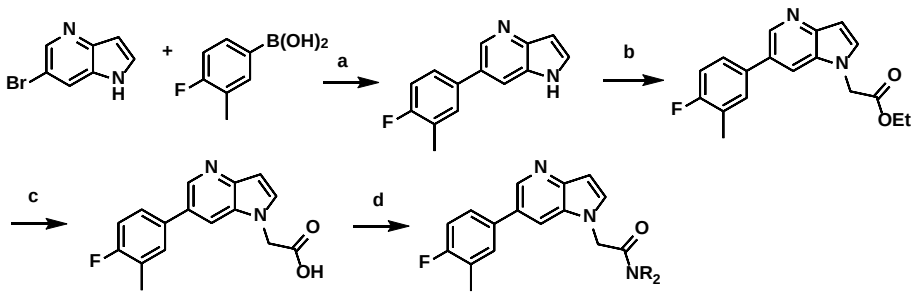
Agilent Technologies ESI-L Low Concentration Tune Mix (G1969-85000). This was diluted 10x with 90% acetonitrile in water and to this mixture, 5 uL of 0.1 mM hexamethoxy-phosphazine (HP-0321) was added. The reference mass solution was made using the Agilent Technologies ES-TOF Reference Mass Solution Kit (G1969-85001), which contains 100 mM ammonium trifluoroacetate ($TFANH_4$), 5mM purine in, and 2.5 mM hexakis (1H, 1H, 3H-tetrafluoropropoxy)phosphazine (HP-0921), all in 90:10 acetonitrile: water. The solution was made by adding 0.1 mL $TFANH_4$, 0.4 mL purine, and 1.0 mL HP-0921 to 1 L of 95:5 acetonitrile:water. This reference solution was continuously infused during the run. Samples were run through an ACE-3 C18 column (3µm, 35 x 2.1 mm), with a mobile phase of 10-98% acetonitrile in 0.01% formic acid over 2 min and then held at 98% acetonitrile for 1 min, at a flow rate of 0.300 mL/min.

^{1}H NMR spectra were recorded on a Bruker Avance I NMR spectrometer operating at 600.13 MHz for ^{1}H with the following spectral parameters: acquisition time = 2.65 s, number of scans = 8, number of data points = 32K and spectral width = 12376 Hz. ^{13}C NMR spectra were taken on the same instrument operating at 150.903 MHz for ^{13}C with the following spectral parameters: acquisition time = 0.87 s, number of scans = 2048, number of data points = 32K and spectral width = 37593 Hz. Measurements were made using 5 mm tubes.

All compounds tested were of a minimum of 95% purity as determined by HPLC. The HPLC method used for purity determinations is as follows: Analytical LCMS was obtained on an Agilent 1100 Series using an ACE C18 column (3µm, 3.0 x 50mm, T = 50 °C) with a mobile phase of 5-99% ACN in 0.05% TFA over 1.5 min and then hold at 99% ACN for 0.5 min, at a flow rate of 2.2 mL/min. MS detector is an Agilent G1956B MSD set in positive mode.

Preparation of intermediates and final products.

Scheme 1. Synthetic route to append amides to 1*H*-pyrrolo[3,2-*b*]pyridines



a) $Pd(dppf)Cl_2$, Cs_2CO_3, dioxane, water, 90 °C; 85%
b) NaH, DMF, 0 °C; then ethyl 2-bromoacetate, rt; 42-87%
c) LiOH, THF, water; 47-79%
d) Secondary Amine, BOP, Et_3N, DCM; 18-39%

General Synthesis of 2-(6-(4-fluoro-3-methylphenyl)-1H-pyrrolo[3,2-b]pyridin-1-yl)acetamides.

Step A: 6-(4-fluoro-3-methylphenyl)-1H-pyrrolo[3,2-b]pyridine. To a solution a 6-bromo-1H-pyrrolo[3,2-b]pyridine (2 g, 10.2 mmol) in dioxane (50 mL) was added (4-fluoro-3-methylphenyl)boronic acid (1.9 g, 12.2 mmol), $Pd(dppf)Cl_2$ (743 mg, 1.02 mmol), Cs_2CO_3 (9.9 g, 30.5 mmol) and water (5 mL). After 16 hours at 90 ºC the reaction mixture was concentrated and purified via silica gel chromatography (0-100% EtOAc in hexanes) to afford the title compound (1.95 g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H), 8.59 (d, *J* = 2.0 Hz, 1H), 7.93 (dd, *J* = 2.1, 0.9 Hz, 1H), 7.71 – 7.62 (m, 2H), 7.59 – 7.51 (m, 1H), 7.24 (dd, *J* = 9.7, 8.5 Hz, 1H), 6.59 – 6.55 (m, 1H), 2.33 (d, *J* = 2.0 Hz, 3H).

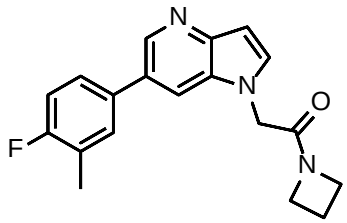
Step B: Ethyl 2-(6-(4-fluoro-3-methylphenyl)-1H-pyrrolo[3,2-b]pyridin-1-yl)acetate. To a solution of intermediate of Step A (1.5 g, 6.6 mmol) in DMF (60 mL) at 0 ºC was added NaH (371 mg, 9.3 mmol, 60% dispersion in oil). The reaction mixture was warmed to room temperature and stirred for 30 minutes and then cooled to 0 ºC followed by the addition of ethyl 2-bromoacetate (0.77 mL, 7 mmol). The reaction mixture was warmed to room temperature and stirred for 12 hours. Water was added and the mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. Purification via silica gel chromatography (0-50% EtOAc in hexanes) gave the title compound (1.8 g, 87%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.65 (d, *J* = 2.0 Hz, 1H), 8.18 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.69 (dd, *J* = 7.7, 2.5 Hz, 1H), 7.67 (d, *J* = 3.3 Hz, 1H), 7.63 – 7.56 (m, 1H), 7.29 – 7.21 (m, 1H), 6.62 (dd, *J* = 3.2, 0.8 Hz, 1H), 5.24 (s, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 2.33 (d, *J* = 1.9 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H).

Step C: 2-[6-(4-Fluoro-3-methyl-phenyl)pyrrolo[3,2-b]pyridin-1-yl]acetic acid. To a solution of intermediate of Step B (700 mg, 2.2 mmol) in THF (40 mL) was added LiOH (107 mg, 4.5 mmol) in water (10 mL) and the reaction mixture was stirred at room temperature for 30 minutes. The reaction mixture was then acidified with 1N HCl and extracted with EtOAc. The pH of the aqueous layer was adjusted to pH 6 and the product precipitated. The solid was collected via filtration and used crude in the next step (300 mg, 47%). MS (ESI): mass calcd. for $C_{16}H_{13}FN_2O_2$, 284.1; m/z found, 285.1 $[M+H]^+$.

General Procedure for Amidation of Carboxylic Acids: To a suspension of intermediate of Step C (1 equiv.) and BOP (1 equiv.) in DCM (3 mL) was added Et_3N (3 equiv.) followed by Secondary Amine (2 equiv.). The crude material was purified by Reverse Phase HPLC to give the final compound (18-39%).

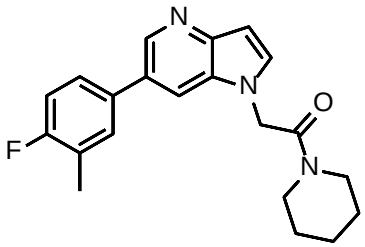
Compounds **5-9** were prepared according to the General Synthesis of 2-(6-(4-fluoro-3-methylphenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-1-yl)acetamides followed by the General Procedure for Amidation of Carboxylic Acids.

Compound **5**:



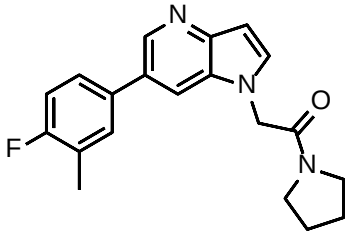
MS (ESI): mass calcd. for $C_{19}H_{18}FN_3O$, 323.1; m/z found, 324.2 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d6) δ 8.62 (d, *J* = 2.0 Hz, 1H), 8.07 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.68 (dd, *J* = 7.6, 2.3 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.27 (dd, *J* = 9.7, 8.5 Hz, 1H), 6.59 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.00 (s, 2H), 4.20 (t, *J* = 7.7 Hz, 2H), 3.91 (t, *J* = 7.8 Hz, 2H), 2.34 (d, *J* = 1.9 Hz, 3H), 2.31 – 2.21 (m, 2H).

Compound **6**:



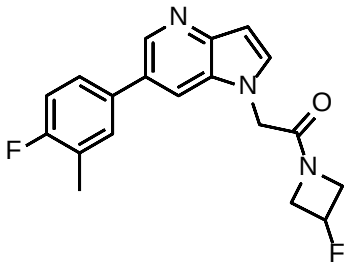
HRMS calcd. for $C_{20}H_{20}FN_3O$ $[M+H]^+$ 352.1820, found 352.1823. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (d, *J* = 1.7 Hz, 1H), 8.76 (s, 1H), 8.03 (d, *J* = 3.3 Hz, 1H), 7.77 (dd, *J* = 7.4, 2.5 Hz, 1H), 7.68 (ddd, *J* = 7.9, 4.8, 2.6 Hz, 1H), 7.38 – 7.31 (m, 1H), 6.82 (dd, *J* = 3.2, 0.9 Hz, 1H), 5.46 (s, 2H), 3.55-3.40 (m, 4H), 2.36-2.34 (m, 3H), 1.65 (s, 4H), 1.47 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.7, 161.7, 160.0, 158.0, 157.8, 139.6, 135.0, 132.6, 132.2, 130.51, 130.47, 128.4, 126.7, 126.6, 125.1, 125.0, 122.1, 115.7, 115.6, 97.2, 47.7, 45.1, 42.5, 39.9, 39.7, 39.6, 39.5, 39.3, 39.2, 39.0, 25.7, 25.0, 23.8, 14.21, 14.19.

Compound **7**:



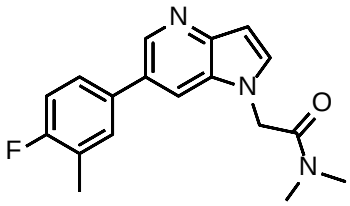
HRMS calcd. for $C_{20}H_{20}FN_3O$ $[M+H]^+$ 338.1663, found 338.1663. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (d, *J* = 1.7 Hz, 1H), 8.79 (s, 1H), 8.01 (d, *J* = 3.2 Hz, 1H), 7.77 (dd, *J* = 7.3, 1.9 Hz, 2H), 7.69 (ddd, *J* = 7.9, 4.9, 2.6 Hz, 1H), 7.38 – 7.31 (m, 1H), 6.82 (dd, *J* = 3.2, 0.7 Hz, 2H), 5.36 (s, 2H), 3.61 (t, *J* = 6.9 Hz, 2H), 3.33 (t, *J* = 6.9 Hz, 2H), 2.35 (d, *J* = 1.7 Hz, 3H), 1.99 (p, *J* = 6.9 Hz, 2H), 1.82 (p, *J* = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.7, 161.6, 160.0, 158.0, 139.3, 135.1, 132.5, 132.2, 130.5, 130.4, 128.4, 126.7, 126.6, 125.1, 125.0, 122.1, 115.7, 115.5, 97.3, 48.3, 45.7, 44.9, 25.6, 23.6, 14.21, 14.19.

Compound **8**:



HRMS calcd. for $C_{19}H_{17}F_2N_3O$ $[M+H]^+$ 342.1412, found 342.1409. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (d, *J* = 2.0 Hz, 1H), 8.08 (dd, *J* = 1.9, 0.7 Hz, 2H), 7.66 (dd, *J* = 7.6, 2.4 Hz, 1H), 7.59 (d, *J* = 3.3 Hz, 1H), 7.57 (ddd, *J* = 7.9, 5.0, 2.6 Hz, 2H), 7.29 – 7.23 (m, 1H), 6.60 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.54 – 5.38 (m, 1H), 5.06 (d, *J* = 5.3 Hz, 2H), 4.59 – 4.47 (m, 1H), 4.36 – 4.19 (m, 2H), 4.03 – 3.91 (m, 1H), 2.33 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.39, 167.38, 161.0, 159.4, 145.4, 141.4, 134.9, 134.8, 133.9, 130.1, 130.0, 129.6, 127.9, 126.13, 126.07, 124.7, 124.6, 115.4, 115.2, 115.0, 101.5, 83.5, 82.2, 57.4, 57.2, 56.0, 55.9, 45.9, 14.21, 14.19.

Compound **9**:

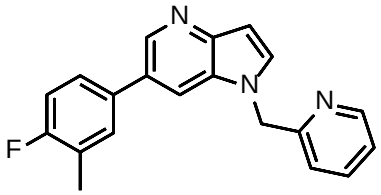


Compound **9** was isolated as a beige powder after silica gel chromatography using a MeOH/CH_2Cl_2 gradient (154 mg, 36%). HRMS calcd. for $C_{18}H_{18}FN_3O$ $[M+H]^+$ 312.1507, found 312.1504. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.61 (d, *J* = 2.0 Hz, 1H), 8.07-8.05 (m, 1H), 7.66 (dd, *J* = 7.5, 2.4 Hz, 1H), 7.58 – 7.54 (m, 2H), 7.28 – 7.22 (m, 1H), 6.58-6.56 (m, 1H), 5.25 (s, 2H), 3.12 (s, 3H), 2.86 (s, 3H), 2.33 (d, *J* = 1.7 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 161.0, 159.4, 145.5, 141.1, 134.94, 134.92, 134.1, 130.01, 129.97, 129.7, 127.6, 126.1, 126.0, 124.7, 124.5, 115.4, 115.2, 115.0, 101.1, 47.2, 35.8, 35.2, 14.21, 14.19.

Compounds **10**-**15** were prepared according to the General Synthesis of 2-(6-(4-fluoro-3-methylphenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-1-yl)acetamides followed by General Nucleophilic Substitution Procedure.

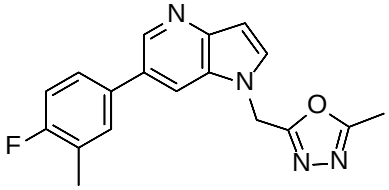
General Nucleophilic Substitution Procedure: To a solution of 6-(4-fluoro-3-methylphenyl)-1*H*-pyrrolo[3,2-*b*]pyridine (1 equiv) in DMF at 0 °C was added NaH (1.5 equiv, 60% dispersion in oil). The reaction mixture was warmed to room temperature and stirred for 30 minutes and then cooled to 0 °C followed by the addition of Alkylation Reagent (1.2 equiv). The reaction mixture was warmed to room temperature and stirred for 12 hours. Water was added and the mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. Purification via silica gel chromatography (EtOAc/hexanes).

Compound **10**:



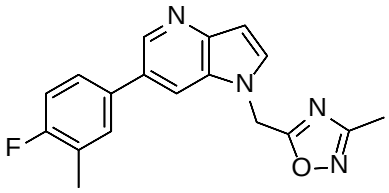
HRMS calcd. for $C_{20}H_{16}FN_3$ $[M+H]^+$ 318.1401, found 318.1400. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.89 – 8.85 (m, 1H), 8.50 – 8.47 (m, 1H), 8.21 (d, *J* = 2.4 Hz, 1H), 7.81 – 7.75 (m, 2H), 7.70 – 7.65 (m, 1H), 7.36 – 7.26 (m, 3H), 6.84 (d, *J* = 3.3 Hz, 1H), 5.79 (s, 2H), 2.33 (d, *J* = 1.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.1, 158.0, 157.8, 155.8, 149.3, 137.3, 132.1, 132.0, 130.5, 130.51, 128.47, 126.63, 126.57, 125.1, 125.0, 123.0, 121.5, 115.7, 115.6, 97.7, 51.0, 14.2.

Compound **11**:



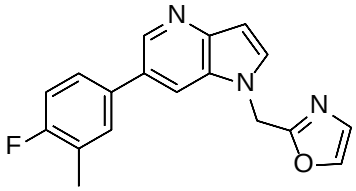
^{1}H NMR (600 MHz, DMSO-d_6) δ 8.67 (d, *J* = 2.0 Hz, 1H), 8.23 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.78 (d, *J* = 3.3 Hz, 1H), 7.68 (dd, *J* = 7.4, 1.9 Hz, 1H), 7.58 (ddd, *J* = 8.0, 5.0, 2.7 Hz, 1H), 7.29 – 7.24 (m, 1H), 6.67 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.86 (s, 2H), 2.44 (s, 3H), 2.33 (d, *J* = 1.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.4, 162.6, 161.1, 159.5, 145.6, 141.9, 134.61, 134.59, 133.1, 130.09, 130.06, 129.0, 128.2, 126.13, 126.08, 124.8, 124.7, 115.5, 115.3, 115.1, 102.5, 14.21, 14.19, 10.3.

Compound **12**:



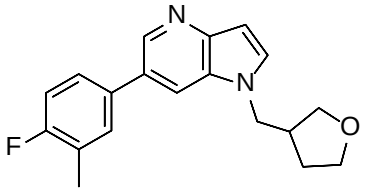
HRMS calcd. for $C_{18}H_{15}FN_4O$, $[M+H]^+$ 323.1303, found 323.1305. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.68 (d, *J* = 2.0 Hz, 1H), 8.29 – 8.25 (m, 1H), 7.79 (d, *J* = 3.5 Hz, 1H), 7.68 (dd, *J* = 7.4, 1.9 Hz, 2H), 7.59 (ddd, *J* = 7.8, 4.9, 2.5 Hz, 1H), 7.26 (dd, *J* = 9.7, 8.5 Hz, 1H), 6.68 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.96 (s, 2H), 2.32 (d, *J* = 1.9 Hz, 3H), 2.27 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 175.4, 167.0, 161.1, 159.5, 145.5, 141.9, 134.6, 134.5, 133.3, 130.1, 130.0, 129.2, 128.2, 126.11, 126.06, 124.8, 124.6, 115.4, 115.3, 115.1, 102.6, 41.4, 14.21, 14.18, 10.9.

Compound **13**:



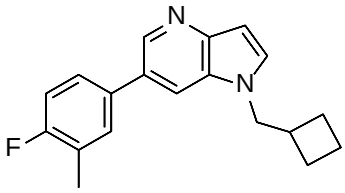
HRMS calcd. for $C_{18}H_{14}FN_3O$, $[M+H]^+$ 308.1194, found 308.1190. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (d, *J* = 2.0 Hz, 1H), 8.21 (dd, *J* = 2.0, 0.9 Hz, 1H), 8.06 (d, *J* = 0.9 Hz, 1H), 7.77 (d, *J* = 3.4 Hz, 1H), 7.67 (dd, *J* = 7.6, 2.5 Hz, 1H), 7.57 (ddd, *J* = 7.9, 4.9, 2.5 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.17 (d, *J* = 0.9 Hz, 1H), 6.65 (dd, *J* = 3.3, 0.8 Hz, 2H), 5.74 (s, 2H), 2.33 (d, *J* = 1.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.1, 159.6, 159.5, 145.6, 141.7, 140.4, 134.68, 134.66, 133.1, 130.1, 130.0, 129.0, 128.1, 127.1, 126.1, 126.0, 124.8, 124.7, 115.5, 115.3, 115.1, 102.2, 42.5, 14.21, 14.19.

Compound **14**:



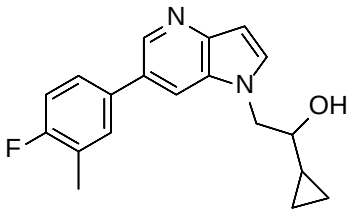
HRMS calcd. for $C_{19}H_{19}FN_2O$, $[M+H]^+$ 311.1554, found 311.1555. ^1H NMR (400 MHz, DMSO-d_6) δ 8.97 – 8.92 (m, 2H), 8.22 (d, *J* = 3.3 Hz, 1H), 7.83 (dd, *J* = 7.4, 2.5 Hz, 1H), 7.78 – 7.72 (m, 1H), 7.39 – 7.31 (m, 1H), 6.83 (d, *J* = 3.2 Hz, 1H), 4.50 – 4.37 (m, 2H), 3.88 – 3.80 (m, 1H), 3.70 – 3.60 (m, 2H), 3.51 – 3.43 (m, 1H), 2.92 – 2.81 (m, 1H), 2.36 (d, *J* = 1.8 Hz, 3H), 1.94 – 1.83 (m, 1H), 1.69 – 1.56 (m, 1H).

Compound **15**:



HRMS calcd. for $C_{19}H_{19}FN_2$, $[M+H]^+$ 295.1605, found 295.1606. ^1H NMR (600 MHz, DMSO-d_6) δ 8.98 – 8.91 (m, 2H), 8.18 (d, *J* = 3.2 Hz, 1H), 7.84 (dd, *J* = 7.5, 2.5 Hz, 1H), 7.78 – 7.72 (m, 1H), 7.39 – 7.32 (m, 1H), 6.80 (dd, *J* = 3.2, 0.8 Hz, 1H), 4.47 (d, *J* = 7.5 Hz, 2H), 2.91 – 2.81 (m, 1H), 2.36 (d, *J* = 1.8 Hz, 3H), 2.00 – 1.76 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.7, 160.1, 158.0, 157.8, 138.2, 134.9, 132.0, 131.8, 130.62, 130.58, 128.4, 126.8, 126.7, 125.1, 125.0, 122.3, 115.7, 115.5, 96.8, 50.9, 35.2, 25.2, 17.5, 14.20, 14.18.

Compound **16**:



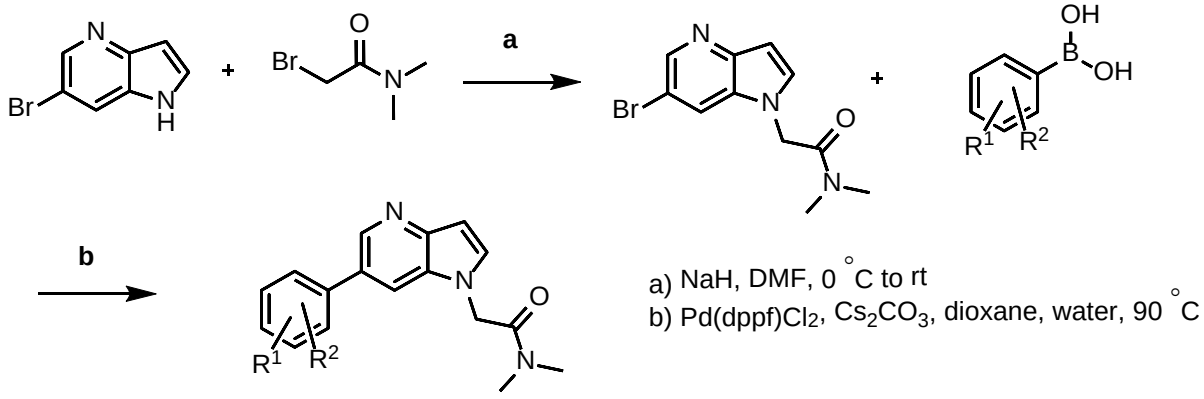
To a solution of Ketone of (prepared by General Nucleophilic Substitution Procedure) (60 mg, 0.19 mmol) in a mixture of THF (2.5 mL) and MeOH (2.5 mL) cooled at 0 °C was added $NaBH_4$ (14 mg, 0.38 mmol). The reaction mixture was stirred at 0 ºC for 30 minutes. The volatiles were evaporated and the residue was taken up in EtOAc and water. The aqueous phase was extracted 2 times with EtOAc. The combined organic layers were washed with water, dried over $MgSO_4$, filtered and evaporated to afford the title compound (39 mg, 64%). MS (ESI): mass calcd. for $C_{18}H_{18}F_2N_2O$, 316.1; m/z found, 317.1 $[M+H]^+$. Analytical HPLC was obtained on a Agilent 1100

Series using Inertsil ODS-3 column (3um, 50 x 3 mm), mobile phase of 5-99% ACN in 0.05% TFA over 1.6 min and then hold at 99% ACN for 0.4 min, at a flow rate of 2.2 mL/min (Temperature = 50 °C). R_t = 0.93 min at 254 nm.

MS (ESI): mass calcd. for $C_{19}H_{19}FN_2O$, 310.1; m/z found, 311.1 $[M+H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ 8.59 (d, *J* = 2.0 Hz, 1H), 8.13 (dd, *J* = 2.1, 0.9 Hz, 1H), 7.71 – 7.65 (m, 2H), 7.62 – 7.55 (m, 1H), 7.29 – 7.22 (m, 1H), 6.55 (dd, *J* = 3.2, 0.9 Hz, 1H), 4.92 (d, *J* = 5.0 Hz, 1H), 4.33 (dd, *J* = 14.2, 4.3 Hz, 1H), 4.24 (dd, *J* = 14.3, 7.1 Hz, 1H), 3.29 – 3.24 (m, 1H), 2.33 (d, *J* = 1.9 Hz, 3H), 0.84 – 0.75 (m, 1H), 0.37 – 0.22 (m, 3H), 0.18 – 0.11 (m, 1H).

General Synthesis of 17-29 and 35.

Scheme 2. Variable Aryl Group Synthesis of 2-(6-(aryl)-1*H*-pyrrolo[3,2-*b*]pyridin-1-yl)acetamides.

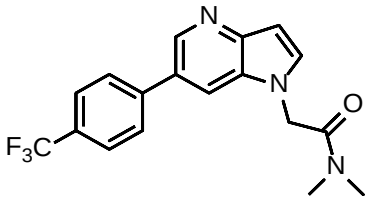


Step A: 2-(6-bromo-1H-pyrrolo[3,2-b]pyridin-1-yl)-N,N-dimethylacetamide. To a solution of 6-bromo-1H-pyrrolo[3,2-b]pyridine (379 mg, 1.92 mmol) in DMF (5 mL) was added sodium hydride (53 mg, 2.3 mmol, 60% dispersion in oil). The reaction mixture was stirred for 30 minutes and 2-bromo-*N,N*-dimethylacetamide (411 mg, 2.48 mmol) was added. The reaction mixture was stirred for 2 hours. Water (20 mL) and ethyl acetate (30 mL) were added. The layers were separated and the water layer was extracted 2x more with ethyl acetate. The combined organic layers were dried ($MgSO_4$). Purification via silica gel chromatography (0-7% 2N NH_3-MeOH / CH_2Cl_2) gave the title compound as a yellow oil (501 mg, 92%). MS (ESI): mass calcd. for $C_{11}H_{12}BrN_3O$, 281.1; m/z found, 282.0 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.72 (s, 1H), 7.29 (d, *J* = 2.7 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 4.87 (s, 2H), 3.10 (s, 3H), 3.01 (s, 3H).

Step B: N,N-dimethyl-2-[6-(Aryl)pyrrolo[3,2-b]pyridin-1-yl]acetamide. A mixture of 2-(6-bromo-1H-pyrrolo[3,2-b]pyridin-1-yl)-N,N-dimethylacetamide (1 equiv), (1.2-1.5 equiv), Cs_2CO_3 (3 equiv),

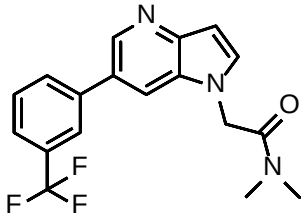
$Pd(dppf)Cl_2$ (0.1 equiv) and water (0-2 mL) in dioxane (3-10 mL) was heated to 90 °C. After 16 hours, the volatiles were removed and a saturated aqueous solution of $NaHCO_3$ was added. The aqueous phase was extracted 3 times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. Purification by Agilent Prep Method X gave the final product.

Compound **17:**



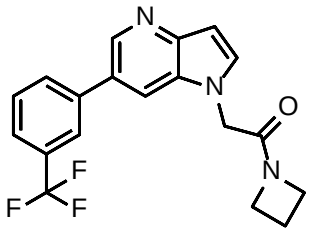
Compound **17** was isolated as a beige solid (212 mg, 68%). MS (ESI): mass calcd. for $C_{18}H_{16}F_3N_3O$; 347.34, found 348.1 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ 8.72 (d, J = 1.9 Hz, 1H), 8.23 – 8.21 (m, 2H), 7.98 (d, J = 8.1 Hz, 2H), 7.85 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 3.2 Hz, 1H), 5.28 (s, 2H), 3.13 (s, 3H), 2.87 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 146.3, 143.0, 141.3, 135.0, 129.8, 127.54, 127.52, 127.3, 126.9, 125.79, 125.77, 125.74, 125.72, 125.3, 123.5, 115.7, 101.3, 47.3, 35.8, 35.3.

Compound **18:**



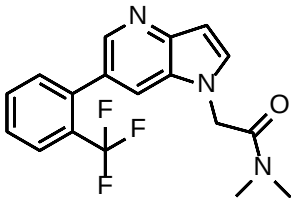
Compound **18** was isolated as a solid (116 mg, 76%). HRMS calcd. for $C_{18}H_{16}F_3N_3O$ $[M+H]^+$ 348.1318, found 348.1319. ^1H NMR (600 MHz, DMSO-d_6) δ 8.71 (d, J = 2.1 Hz, 1H), 8.21 (dd, J = 2.1, 0.9 Hz, 1H), 8.09 – 8.03 (m, 2H), 7.75 – 7.72 (m, 2H), 7.61 (d, J = 3.2 Hz, 1H), 6.61 (dd, J = 3.2, 0.9 Hz, 1H), 5.29 (s, 2H), 3.13 (s, 3H), 2.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 146.1, 141.2, 139.9, 134.8, 131.0, 130.0, 129.9, 129.7, 129.6, 126.9, 123.58, 123.55, 123.2, 123.1, 115.5, 101.2, 47.2, 35.8, 35.2.

Compound **19:**



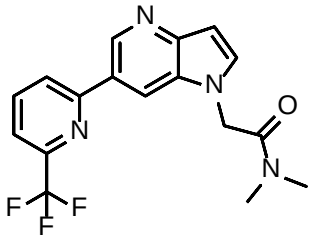
Compound **19** was isolated as a solid (72 mg, 41%). MS (ESI): mass calcd. for $C_{19}H_{16}F_3N_3O$, 359.12; m/z found, 360.1 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 9.09 – 9.03 (s, 1H), 8.94 – 8.86 (s, 1H), 8.24 – 8.12 (m, 3H), 8.10 – 8.01 (d, *J* = 3.2 Hz, 1H), 7.90 – 7.75 (m, 2H), 6.89 – 6.78 (d, *J* = 3.2 Hz, 1H), 5.28 – 5.16 (s, 2H), 4.36 – 4.23 (m, 2H), 3.97 – 3.83 (m, 2H), 2.39 – 2.21 (m, 2H).

Compound **20:**



Compound **20** was isolated as a beige solid (236 mg, 76%). ^1H NMR (600 MHz, DMSO-d_6) δ 8.25 (d, *J* = 1.8 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.84 – 7.81 (m, 1H), 7.76 (t, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.61 (d, *J* = 3.3 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 6.63 – 6.61 (m, 2H), 5.21 (s, 2H), 3.07 (s, 3H), 2.84 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 145.6, 141.9, 138.8, 134.5, 133.1, 132.2, 128.7, 128.1, 127.6, 127.4, 126.04, 126.00, 125.1, 123.3, 117.4, 109.5, 101.2, 47.3, 35.8, 35.2.

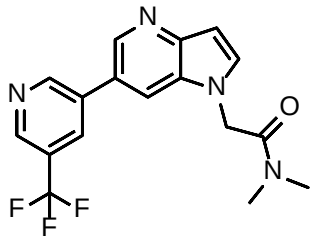
Compound **21:**



Compound **21** was isolated as a beige solid (29 mg, 24%). HRMS calcd. for $C_{17}H_{15}F_3N_4O$, $[M+H]^+$ 349.1271, found 349.1272. ^1H NMR (600 MHz, DMSO-d_6) δ 9.10 (d, *J* = 1.9 Hz, 1H), 8.48-8.46 (m,

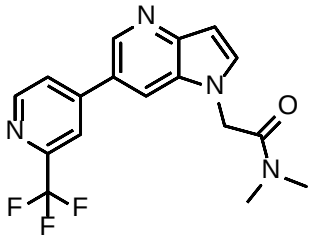
1H), 8.36 (d, *J* = 8.0 Hz, 1H), 8.19 (t, *J* = 7.9 Hz, 1H), 7.84 (d, *J* = 7.4 Hz, 1H), 7.68 (d, *J* = 3.2 Hz, 1H), 6.64 (dd, *J* = 3.2, 0.9 Hz, 1H), 5.32 (s, 2H), 3.15 (s, 3H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 156.4, 147.3, 146.7, 146.5, 146.3, 146.1, 141.5, 139.4, 135.7, 129.5, 125.3, 123.7, 122.5, 120.7, 118.7, 118.6, 115.5, 101.4, 47.3, 35.8, 35.2.

Compound **22:**



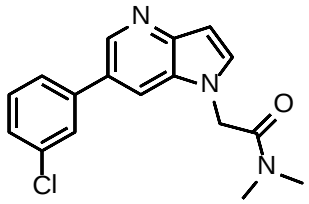
Compound **22** was isolated as a beige solid (48 mg, 39%). HRMS calcd. for $C_{17}H_{15}F_3N_4O$ $[M+H]^+$ 349.1271, found 349.1268. ^{1}H NMR (500 MHz, Methanol-d_4) δ 9.16 (d, *J* = 2.2 Hz, 1H), 8.90 – 8.84 (m, 1H), 8.67 (d, *J* = 1.9 Hz, 1H), 8.49 – 8.46 (m, 1H), 8.25 – 8.22 (m, 1H), 7.60 (d, *J* = 3.3 Hz, 1H), 6.70 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.30 (s, 2H), 3.21 (s, 3H), 2.98 (s, 3H).

Compound **23:**



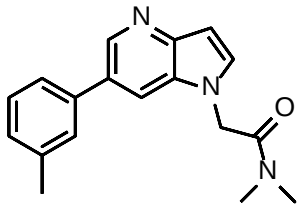
Compound **23** was isolated as a beige solid (28 mg, 23%). HRMS calcd. for $C_{17}H_{15}F_3N_4O$ $[M+H]^+$ 349.1271, found 349.1270. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (d, *J* = 2.0 Hz, 1H), 8.83 (d, *J* = 5.1 Hz, 1H), 8.45 – 8.42 (m, 1H), 8.30 – 8.27 (m, 2H), 8.15 (dd, *J* = 5.2, 1.8 Hz, 1H), 7.69 (d, *J* = 3.2 Hz, 1H), 6.67 – 6.64 (m, 2H), 5.31 (s, 2H), 3.14 (s, 3H), 2.87 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.9, 150.6, 148.2, 147.4, 147.21, 147.17, 141.2, 135.9, 129.6, 124.3, 123.9, 122.6, 120.8, 117.75, 117.73, 115.9, 101.4, 47.3, 35.8, 35.2.

Compound **24:**



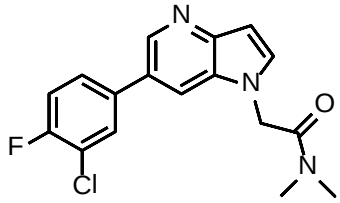
Compound **24** was isolated as a beige solid (58 mg, 64%). HRMS calcd. for $C_{17}H_{16}ClN_3O$ $[M+H]^+$ 314.1055, found 314.1057. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.67 (d, *J* = 2.0 Hz, 1H), 8.17 (dd, *J* = 2.0, 0.8 Hz, 2H), 7.81 (t, *J* = 1.9 Hz, 1H), 7.73 (ddd, *J* = 7.8, 1.7, 1.0 Hz, 2H), 7.59 (d, *J* = 3.3 Hz, 1H), 7.52 (t, *J* = 7.9 Hz, 1H), 7.43 (ddd, *J* = 8.0, 2.1, 1.0 Hz, 1H), 6.59 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.27 (s, 2H), 3.12 (s, 3H), 2.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 146.0, 141.1, 141.0, 134.6, 133.7, 130.7, 129.7, 126.9, 126.8, 126.4, 125.5, 115.3, 101.1, 47.2, 35.8, 35.2.

Compound **25:**



Compound **25** was isolated as a solid (75 mg, 58%). HRMS calcd. for $C_{18}H_{19}N_3O$, $[M+H]^+$ 294.1601, found 294.1599. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (d, *J* = 1.9 Hz, 1H), 8.07 (dd, *J* = 2.1, 0.9 Hz, 1H), 7.58 – 7.48 (m, 3H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.20 – 7.17 (m, 1H), 6.57 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.26 (s, 2H), 3.12 (s, 3H), 2.86 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.1, 145.6, 141.2, 138.8, 138.1, 134.2, 129.9, 128.8, 128.6, 127.7, 127.5, 124.1, 115.1, 101.1, 47.3, 35.9, 35.3, 21.1.

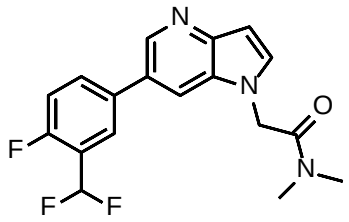
Compound **26:**



Compound **26** was isolated as a solid (4 mg, 7%). MS (ESI): mass calcd. for $C_{17}H_{15}ClFN_3O$, 331.1; m/z found, 332.0 $[M+H]^+$. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.55 (d, *J* = 1.9 Hz, 1H), 8.06 (dd, *J* =

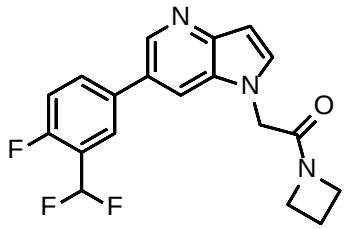
2.0, 0.9 Hz, 1H), 7.83 (dd, *J* = 7.0, 2.3 Hz, 1H), 7.69 – 7.62 (m, 1H), 7.55 (d, *J* = 3.3 Hz, 1H), 7.35 (t, *J* = 8.9 Hz, 1H), 6.67 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.28 (s, 2H), 3.21 (s, 3H), 2.98 (s, 3H).

Compound **27:**



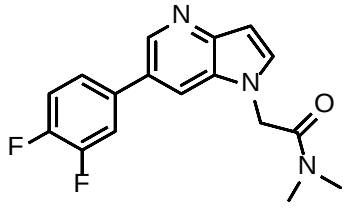
^{1}H NMR (500 MHz, Methanol-d_4) δ 8.59 (d, *J* = 1.9 Hz, 1H), 8.10 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.98 – 7.87 (m, 2H), 7.57 (d, *J* = 3.4 Hz, 1H), 7.42 – 7.33 (m, 1H), 7.07 (t, *J* = 54.7 Hz, 1H), 6.69 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.31 (s, 2H), 3.22 (s, 3H), 3.00 (s, 3H).

Compound **28:**



Compound **28**was isolated as a solid (21 mg, 17%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.59 (d, *J* = 1.9 Hz, 1H), 8.10 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.96 – 7.85 (m, 2H), 7.58 (d, *J* = 3.4 Hz, 1H), 7.40 – 7.33 (m, 1H), 7.19 – 6.93 (m, 1H), 6.68 (dd, *J* = 3.4, 0.9 Hz, 1H), 5.03 (s, 2H), 4.32 – 4.24 (m, 2H), 4.07 (t, *J* = 7.8 Hz, 2H), 2.42 – 2.32 (m, 2H).

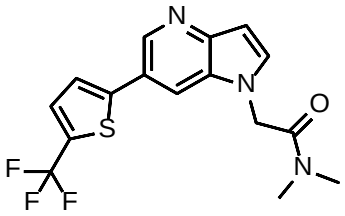
Compound **29**:



Compound **29** was isolated as a beige solid (56 mg, 50%). HRMS calcd. for $C_{17}H_{15}F_2N_3O$ $[M+H]^+$ 316.1256, found 316.1259. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.67 (d, *J* = 2.0 Hz, 1H), 8.17 – 8.13

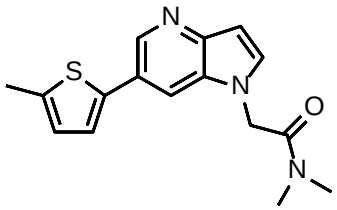
(m, 1H), 7.85 (ddd, *J* = 12.4, 7.8, 2.3 Hz, 1H), 7.64-7.59 (m, 1H), 7.59 (d, *J* = 3.2 Hz, 1H), 7.55 (dt, *J* = 10.6, 8.5 Hz, 1H), 6.59 (d, *J* = 3.2 Hz, 1H), 5.25 (s, 2H), 3.12 (s, 3H), 2.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 150.6, 150.5, 149.6, 149.5, 149.0, 148.9, 148.0, 147.9, 145.9, 141.0, 136.50, 136.47, 136.46, 136.43, 134.6, 129.7, 126.2, 123.46, 123.44, 123.41, 123.39, 117.9, 117.8, 115.7, 115.6, 115.3, 101.1, 47.2, 35.8, 35.2.

Compound **30**:



Compound **30** was prepared according to the Variable Aryl Group Synthesis, where Arylboronic acid in Step B is (5-(trifluoromethyl)thiophen-2-yl)boronic acid (1.5 equiv). The product was isolated as a solid (82 mg, 65%). HRMS calcd. for $C_{16}H_{14}F_3N_3OS$ $[M+H]^+$ 354.0882, found 354.0878. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.69 (d, *J* = 1.9 Hz, 1H), 8.25 (dd, *J* = 1.9, 0.9 Hz, 1H), 7.65 (d, *J* = 3.3 Hz, 1H), 7.59 – 7.56 (m, 1H), 7.54 – 7.51 (m, 1H), 6.71 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.32 (s, 2H), 3.22 (s, 3H), 2.99 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.9, 158.0, 157.8, 146.4, 139.2, 136.5, 131.25, 131.22, 130.1, 127.4, 127.2, 124.1, 123.4, 121.6, 121.0, 116.1, 100.9, 47.5, 35.8, 35.3.

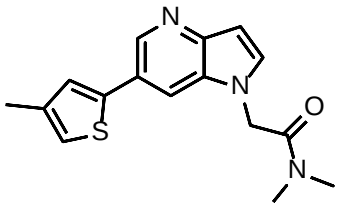
Compound **31:**



Compound **31** was prepared according to the Variable Aryl Group Synthesis, where Arylboronic acid in Step B is (5-methylthiophen-2-yl)boronic acid (1.5 equiv). HRMS calcd. for $C_{16}H_{17}N_3OS$ $[M+H]^+$ 300.1165, found 300.1165. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.82 (d, *J* = 1.8 Hz, 1H), 8.54 (s, 1H), 7.90 (d, *J* = 3.3 Hz, 1H), 7.48 (d, *J* = 3.5 Hz, 1H), 6.93-6.91 (m, 1H), 6.75 (dd, *J* = 3.3, 0.9 Hz, 1H),

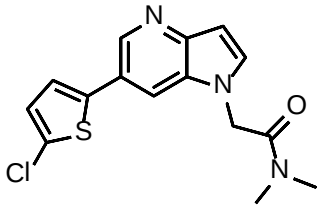
5.39 (s, 2H), 3.13 (s, 3H), 2.88 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.5, 140.3, 138.7, 136.6, 134.6, 132.1, 126.9, 125.2, 123.6, 119.1, 98.1, 47.8, 35.8, 35.2, 15.0.

Compound **32:**



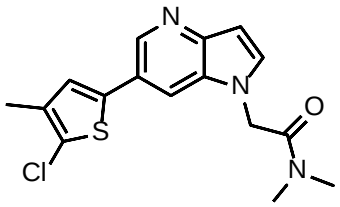
Compound **32** was prepared according to the Variable Aryl Group Synthesis, using (4-methylthiophen-2-yl)boronic acid in Step B (1.5 equiv). HRMS calcd. for $C_{16}H_{17}N_3OS$ $[M+H]^+$ 300.1165, found 300.1164. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60 (d, *J* = 1.9 Hz, 1H), 8.03 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.55 (d, *J* = 3.2 Hz, 1H), 7.37 (d, *J* = 1.4 Hz, 1H), 7.13 – 7.10 (m, 1H), 6.56 (dd, *J* = 3.2, 0.9 Hz, 1H), 5.24 (s, 2H), 3.12 (s, 3H), 2.87 (s, 3H), 2.26 (d, *J* = 1.1 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.1, 145.7, 141.5, 140.0, 138.3, 134.5, 129.7, 125.7, 122.9, 120.3, 113.6, 101.4, 47.3, 35.8, 35.3, 15.5.

Compound **33**:



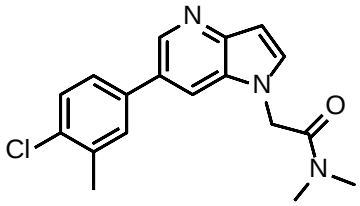
Compound **33** was prepared according to the Variable Aryl Group Synthesis, using (5-chlorothiophen-2-yl)boronic acid in Step B (1.5 equiv). HRMS calcd. for $C_{15}H_{14}ClN_3OS$ $[M+H]^+$ 320.0619, found 320.0616. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60 (d, *J* = 2.0 Hz, 1H), 8.05 (d, *J* = 2.0 Hz, 1H), 7.59 (d, *J* = 3.2 Hz, 1H), 7.41 (d, *J* = 3.9 Hz, 1H), 7.18 (d, *J* = 3.9 Hz, 1H), 6.58 (d, *J* = 3.2 Hz, 1H), 5.24 (s, 2H), 3.12 (s, 3H), 2.87 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.9, 145.9, 141.0, 139.7, 134.9, 129.5, 128.0, 126.6, 123.2, 121.7, 113.8, 101.5, 47.3, 35.8, 35.2.

Compound **34:**



Compound **34** was prepared was prepared according to the Variable Aryl Group Synthesis, using (5-chloro-4-methylthiophen-2-yl)boronic acid Step B (1.5 equiv). HRMS calcd. for $C_{16}H_{16}ClN_3OS$, $[M+H]^+$ 334.0775, found 334.0768. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.56 (d, *J* = 2.0 Hz, 1H), 8.03 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.58 (d, *J* = 3.3 Hz, 1H), 7.37 (s, 1H), 6.57 (dd, *J* = 3.2, 0.9 Hz, 1H), 5.24 (s, 2H), 3.12 (s, 3H), 2.87 (s, 4H), 2.20 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 146.0, 139.6, 138.4, 135.7, 134.9, 129.6, 125.2, 121.8, 121.7, 113.6, 101.5, 47.3, 35.8, 35.3, 13.4.

Compound **35:**



Compound **35** was isolated as a brown solid (219 mg, 73%). HRMS calcd. for $C_{18}H_{18}ClN_3O$ $[M+H]^+$ 327, found 348.1319. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.65 (d, *J* = 2.0 Hz, 1H), 8.11 (d, *J* = 2.0 Hz, 1H), 7.75 (d, *J* = 2.4 Hz, 1H), 7.63 – 7.56 (m, 2H), 7.53-7.51 (m, 1H), 6.59 (d, *J* = 3.2 Hz, 1H), 5.26 (s, 2H), 3.12 (s, 3H), 2.87 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.1, 145.8, 141.1, 137.7, 135.9, 134.4, 132.2, 129.8, 129.5, 129.3, 127.3, 126.0, 115.1, 101.2, 47.3, 35.8, 35.3, 19.7.

3-[^{3}H] 1-(azetidin-1-yl)-2-[6-(4-fluoro-3-methyl-phenyl)pyrrolo[3,2-b]pyridin-1-yl]ethanone (a tritiated version of **5**):

Step A: 3-bromo-6-(4-fluoro-3-methylphenyl)-1H-pyrrolo[3,2-b]pyridine. To a solution of 6-(4-fluoro-3-methylphenyl)-1*H*-pyrrolo[3,2-*b*]pyridine (Step A of General Synthesis of 2-(6-(4-fluoro-3-methylphenyl)-1H-pyrrolo[3,2-b]pyridin-1-yl)acetamides in EXPERIMENTAL PROCEDURES section) (1g, 4.4 mmol) in DMF (45 mL) at room temperature was added NBS (944 mg, 5.3 mmol). After 1 hour, water was added and the reaction mixture was extracted with 60% EtOAc in

hexanes. The combined organic layers were washed with water, dried over $MgSO_4$, filtered and evaporated. Purification via silica gel chromatography (0-100% EtOAc in hexanes) gave the title compound (1.2 g, 89%). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.78 (s, 1H), 8.67 (d, *J* = 2.0 Hz, 1H), 7.98 (d, *J* = 1.9 Hz, 1H), 7.88 (d, *J* = 2.9 Hz, 1H), 7.72 – 7.63 (m, 1H), 7.62 – 7.53 (m, 1H), 7.25 (dd, *J* = 9.7, 8.5 Hz, 1H), 2.33 (d, *J* = 1.9 Hz, 3H).

Step B: 1-(azetidin-1-yl)-2-[3-bromo-6-(4-fluoro-3-methyl-phenyl)pyrrolo[3,2-b]pyridin-1-yl]ethanone. To a solution of intermediate of Step A (200 mg, 0.66 mmol) in DMF (7 mL) at 0 ºC was added NaH (37 mg, 0.92 mmol, 60% dispersion in oil). The reaction mixture was warmed to room temperature and stirred for 30 minutes and then cooled to 0 ºC followed by the addition of a solution of 1-(azetidin-1-yl)-2-bromoethanone (140 mg, 0.78 mmol) in DMF (3 mL). The reaction mixture was warmed to room temperature and stirred for 12 hours. Water was added and the mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. Purification via silica gel chromatography (0-100% EtOAc in hexanes) gave the title compound (178 mg, 68%). MS (ESI): mass calcd. for $C_{19}H_{17}BrFN_3O$, 401.1; m/z found, 402.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.70 (d, *J* = 1.8 Hz, 1H), 8.18 (d, *J* = 1.9 Hz, 1H), 7.80 (s, 1H), 7.71 – 7.67 (m, 1H), 7.64 – 7.56 (m, 1H), 7.28 (dd, *J* = 9.7, 8.5 Hz, 1H), 5.02 (s, 2H), 4.24 (t, *J* = 7.7 Hz, 2H), 3.91 (t, *J* = 7.7 Hz, 2H), 2.34 (d, *J* = 1.9 Hz, 3H), 2.33 – 2.23 (m, 2H).

To a 3mg of 1-(azetidin-1-yl)-2-[3-bromo-6-(4-fluoro-3-methyl-phenyl)pyrrolo[3,2-b]pyridin-1-yl]ethanone.was added 0.3ml methanol, followed by 10wt.% Pd/C 3mg, and the mixture was stirred at room temperature for 2 hrs under tritium gas pressure. Reaction was stop after 2 hrs and filtered. The solvent was removed in vacuo 5X. ^{3}H NMR (300 MHz, Methanol-d4) δ 6.97 (s, 1H). (See Appendix for characterization)(Moravek, Brea CA).

Pharmacological Assays:

Calcium mobilization (FLIPR) assay.

CHO-T-Rex cells expressing Biomyx hNR1a/NR2 clones (BIOMYX Inc., San Diego, CA): Cells are grown to 75-90% confluence before passaging. Cells are lifted using 0.05% Trypsin/EDTA solution. Each confluent T225 flask typically yields enough cells for 2 plates (384-well) at the plating density of 20,000 cells/50µl/well. Twenty-four hours before measurements, the expression of the NMDA receptors in the stable cell line is induced with Tet-On inducible system in the presence of ketamine, a non-selective NMDA receptor blocker. On the day of the experiment, cell culture media is carefully washed and the cells are loaded with Calcium 5 Dye Kit (Molecular Devices) in dye loading buffer containing 137 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM HEPES and 5 mM D-glucose; pH 7.4. After 1h incubation at the room temperature, the dye is washed away with the assay buffer (137 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 0.01 mM EDTA, 10 mM HEPES and 5 mM D-glucose; pH 7.4) In the FLIPR TETRA reader, various concentrations of the test compounds are added to the cells for 5 min while fluorescence is monitored to detect potential agonist activity. Next, co-agonists, glutamate and glycine are added for another 5 minutes. The concentration of glutamate corresponding to ~EC_{80} is used to maximize the assay's signal window and ability to detect NMDA receptor antagonists and negative allosteric modulators. A saturating concentration (10 µM) of glycine is also present in the assay. A non-selective NMDA receptor antagonist, (+)MK-801 is used as a positive control for antagonist activity. The fluorescent signal in the presence of test compounds is quantified and normalized to the signal defined by the appropriate control wells.

Radioligand binding assay.

Rat adult cortex is homogenized in the assay buffer (50 mM Tris; pH 7.4). The resulting cortical membranes containing native NMDA receptors are purified by centrifugation and extensively washed, then re-suspended in the assay buffer. The test compounds, [^{3}H]-**6** and membranes are mixed together and incubated with shaking for 2 hours at room temperature to reach binding equilibrium. Non-specific binding of [^{3}H]-**5** is determined by pre-incubation of brain membranes with 10 µM of compound **1** (CP-101,606). Following the incubation, the bound and unbound tracer is separated by filtration with cell harvester and GF/B filter plates (PerkinElmer) soaked with polyethylenimine. The extent of binding is measured by counting [^{3}H] radioactivity retained on the filters plates with liquid scintillator counter. Binding affinity (equilibrium dissociation

constant K_i) for the test compounds is determined by fitting experimental data with the following model logEC50=log(10^logKi*(1+[Radioligand]/HotKd)) and Y=Bottom + (Top-Bottom)/(1+10^(X-LogEC50)) where [Radioligand] is the concentration of [^{3}H]-**5**, HotKdNM is the equilibrium dissociation constant of [^{3}H]-**6**, Top and Bottom are the curve plateaus in the units of Y axis.

Liver Microsomal Stability.

Microsomal stability studies were conducted on a Biomek® FX Robotic Liquid Handling Workstation (Beckman Coulter, Brea, CA), which consists of a 96-channel pipette head, a 12-postion workstation deck, and a plate incubator. Test compounds (1 µM) were spiked in a reaction mix consisting of 100 mM potassium phosphate buffer (pH 7.4), 3 mM MgCl2, and 0.5 mg/mL liver microsomes from mouse, rat, and human (BD Gentest). The reaction was brought to 37 °C and initiated by adding NADPH to a final concentration of 1 mM. After mixing on the plate-deck, 50 µL aliquots were excised from the reaction plate at 0, 5, 10, 20, 40, and 60 min and quenched with four volumes of acetonitrile spiked with 500 µg/nL of the internal standard phenytoin. Quenched plates were centrifuged at 5700 rpm for 10 min at 4 °C, and supernatant was diluted 1:3 in water before LC/MS/MS analysis.

The compound half-lives were derived from plots of the ln of percent remaining compound over time to determine the intrinsic clearance. The predicted hepatic clearance was derived from the intrinsic clearance value using equations from the well-stirred model (Current Drug Metabolism, 2008, 9, 940-951), where no correction was made plasma protein binding and the blood to plasma concentration ratio was assumed to be one. The extraction ratio (ER) was calculated by dividing the predicted hepatic clearance by species blood flow (Q), where Q is 90, 55, and 21.7 mL/min/kg for mouse, rat and human, respectively.

MDCK/MDR-1 Permeability.

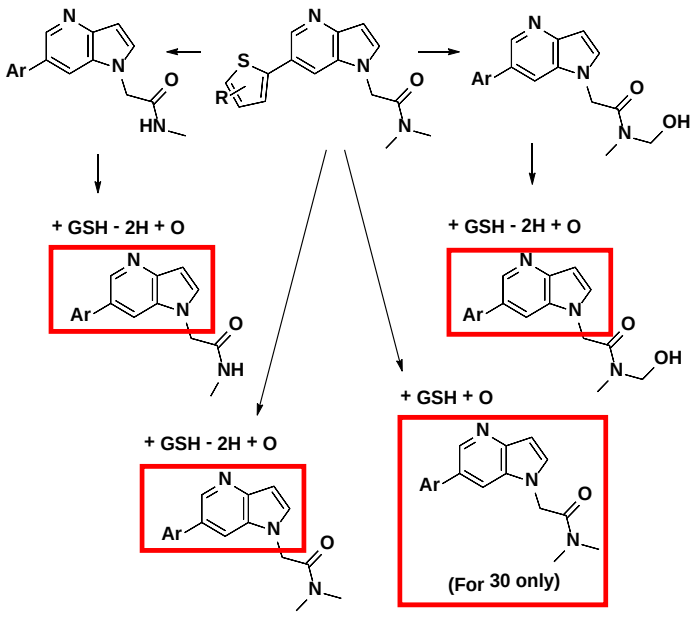
Permeability assays were conducted at Cyprotex according to the company's protocol using the MDCK-MDR1 cell line obtained from the NIH.3 In brief, cells between passage numbers 6 – 30 were seeded onto a Multiscreen plate™ (Millipore) at a cell density of 3.4 x 105 cells/cm2 and cultured for three days before permeability studies were conducted. Test compounds were dissolved as 10 mM DMSO solutions and added to Hanks Balanced Salt Solution (HBSS), pH 7.4 culture media at a final concentration of 5 µM (1 % DMSO v/v). The working solution was applied

to cells on the donor side and incubated at 37 ° C for 60 min to determine the apical (A) to basolateral (B) and B to A permeability, respectively. In addition, A to B permeability was measured in the presence of the PgP inhibitor elacridar (2 μM). All conditions for test compound were conducted in duplicate, and each assay includes the reference markers propranolol (high permeability) and prazosin (PgP substrate). After incubation, samples were processed for LC/MS/MS analyses to determine the apparent permeability coefficient (Papp) of the test compound in the A to B direction in the presence and absence of the PgP inhibitor and in the B to A direction. In addition, the percent recovery was measured for all incubation conditions. The integrity of each monolayer was monitored by examining the permeation of lucifer yellow by fluorimetric analysis.

Glutathione Trapping Assay.

Based on MS/MS spectra, the GSH adducts for **30** and **34** were determined to be formed through a common mechanism, where adducts were localized to either the core or thiophene moiety, through bioactivation of parent molecule or its two major metabolites, the products of *N*-demethylation and methyl hydroxylation (Figure 1). Compound **30** also formed a GSH adduct with a net addition of parent mass + GSH + O, suggestive of an epoxidation mechanism. No adducts were identified in the absence of NADPH.

Scheme 3. Glutathione adducts identified for 30 and 34.



β-Nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) and glutathione (GSH) reduced were purchased from Sigma (St. Louis, MO). Formic acid was purchased from MP Biomedicals (Solon, OH). Human liver microsomes (UltraPool HLM 150, mixed gender, Lot # 38290) and potassium phosphate buffer (pH 7.4) were obtained from Corning (Corning, NY). High-pressure liquid chromatography (HPLC)-grade water and acetonitrile were obtained from EMD Millipore (Billerica, MA). All other reagents were from commercial vendors and were of analytical grade or higher quality. Test compound at 10 µM was incubated with human liver microsomes (1 mg/mL protein concentration) fortified with 5 mM GSH and 3 mM MgCl2 in the presence and absence of 1 mM NADPH. After 60 min at 37º C, the reaction was terminated by adding 1.5 volumes of ice-cold acetonitrile/methanol mixture (3:1, v/v). The samples were centrifuged for 10 min at 2054 *g* to precipitate proteins. Supernatant was evaporated under a stream of nitrogen, reconstituted in a water/acetonitrile mixture (4:1, v/v), and injected onto the LC-MS system. The LC-MS/MS analysis was conducted using a Waters Acquity UPLC system (Milford, MA) with a Waters Acquity PDA eλ detector (205 to 600 nm) interfaced to a LTQ-Orbitrap XL high resolution mass spectrometer (Thermo-Finnigan, San Jose, CA). The Fourier Transform Mass Spectrometer (FTMS) of the LTQ-Orbitrap XL was operated in the positive mode in full scan from 80 – 1000 *m/z* at a resolution of 15,000 and with two MS/MS scan events with a mass list tailored to each compound acquired at a resolution of 7,500 to aide structural identification. Product ions obtained in MS/MS scan events were obtained using either Collision-induced dissociation (CID) or Higher-energy collisional dissociation (HCD). Chromatographic separation was achieved on a Hypersil Gold C18 HPLC column (3 □m, 3 □ 150 mm, Thermo Scientific, Waltham, MA) run at a flow rate of 0.27 mL/min. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). For a typical run, the following gradient elution method was employed: 0-0.3 min, 5% B; 0.3-20.0 min, 5 – 80% B; 20.0-24.5 min, 80% B; 24.5-24.6 min, 80 – 98% B; 24.6-26.5 min, 98% B; 26.5-26.6 min, 5% B and 26.6-28.0 min, 5% B. The LC-MS/MS data was analyzed using Xcalibur MS software (Thermo-Finnigan, San Jose, CA) and Janssen Research & Development internal MS fragmentation prediction software PrISE 2.1.1, developed by Dotmatics Limited.[1]

Plasma Protein Binding.

Table 1. Plasma and brain protein binding for 9, 30 and 34

	Plasma Protein Binding human (% bound)	Plasma Protein Binding rat (% bound)	Brain Protein Binding rat (% bound)
9	96.57	95.53	96.06
30	97.85	98.26	98.27
34	97.96	96.68	99.25

Plasma protein binding was determined by equilibrium dialysis using the RED device (Pierce, catalog # #89810), according to manufacturer's protocol. Compounds were prepared as 100 µM DMSO stocks and spiked into 1 mL of mouse, rat and human plasma (Bioreclamations) to make a final concentration of 1 µM. Plasma (300 µL) was dispensed into wells separated by an 8 KDa-permeable cellulose membrane from wells containing 100 mM potassium phosphate, pH 7.4 (500 µL). Each compound was tested in triplicate. The RED devise was sealed and equilibrium was permitted for 6 h in a 37 °C incubator with gentle agitation at 100 RPM. After incubation, plasma samples were prepared by transferring 10 µL from plasma wells to 90 µL of fresh 100 mM potassium phosphate, pH 7.4, and buffer samples were prepared by transferring 90 µL from buffer wells to 10 µL of naïve plasma. In addition, a reference sample without equilibration was prepared in triplicate by mixing 10 µL of plasma containing 1 µM compound with 90 µL buffer in order to determine compound recovery from the assay. Two-volumes of 1:1 acetonitrile:methanol spiked with the internal standard phenytoin (0.2 µg/mL) were added to reference and samples. Precipitation of plasma protein binding was allowed for 15 min before reference and samples were centrifuge clarified. Supernatant (10 µL) was used for LC/MS/MS analyses.

LC/MS/MS Methodology.

Compounds were quantified on an API4000 MS/MS System (Applied Biosystems, Concord, Ontario, Canada) interfaced with an Agilent 1100 Series HPLC as previously described (Letavic et al, ACS Med. Chem. Lett., 2013, 4, 419–422).

hERG Assay.

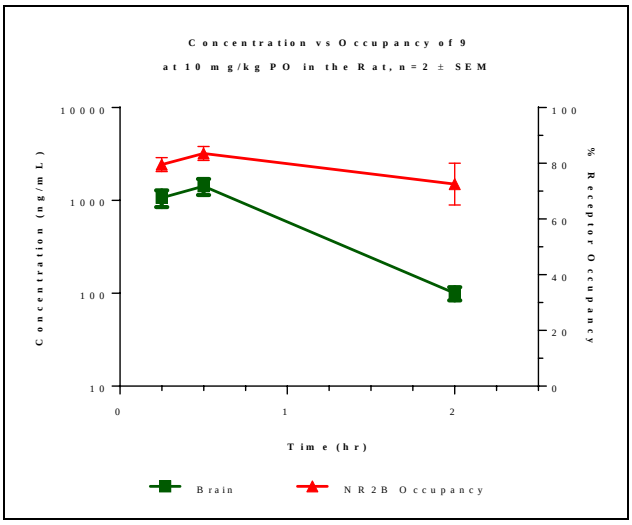
Compounds were tested using a modified version of a [^{3}H]dofetilide binding assay to transfected HEK293 cell membranes (Finlayson et al., *Eur J of Pharmacology* 2001, 430(1), 147-148). Compounds were solubilized at 10mM and serially diluted in DMSO using the PerkinElmer Janus to a working concentration of 100X the final concentration (100% DMSO). Final compound concentrations tested were 10, 3, 1, 0.3, 0.1, 0.03, and 0.01 uM (1% DMSO). Terfenadine was used for non-specific binding determination. hERG HEK293 membranes (Catalog no. C01-1001 from Analytical Biological Services Inc.) in 50mM Tris Base, 10mM KCl, and 1mM MgCl2 6H2O buffer was added to a 96-well plate along with compounds of interest being tested against the hERG channel, followed by addition of 6nM final concentration of [^{3}H]dofetilide (Catalog no. TRQ41166 from Quotient Bioresearch). The plates were incubated at room temperature for 80 minutes. Binding was terminated by filtration onto GF/B filter plates followed by four washes with 50mM Tris Base, 10mM KCl, and 1mM $MgCl_2$-$6H_2O$ buffer. The plates were dried at 50 °C for 1 hour followed by addition of scintillation fluid, then the radioactivity was determined on a TopCount reader.

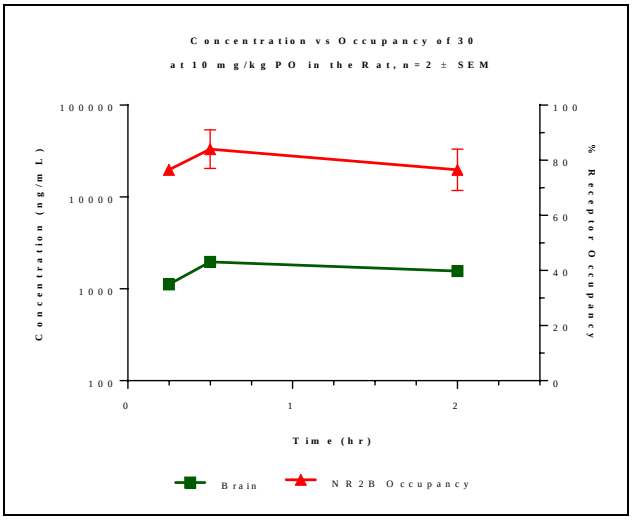
Pharmacokinetic Studies.

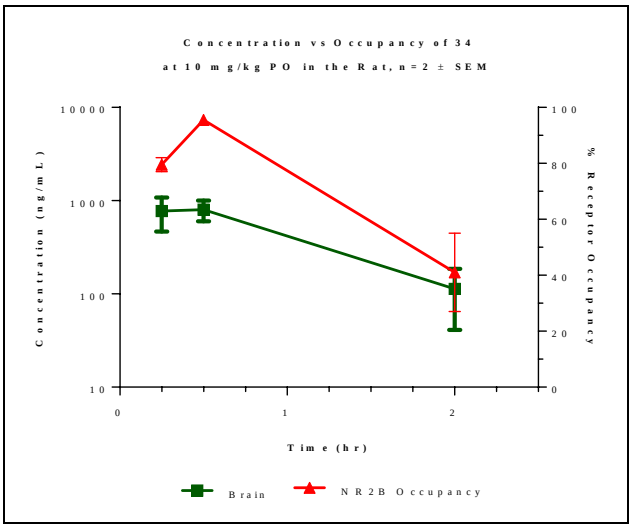
Single dose pharmacokinetic studies in Sprague Dawley rats were conducted following iv (1 mg/kg) and po (5 mg/kg) administration as a solution in either 20% hydroxypropyl-β-cyclodextrin (HP-β-CD) or 1:1 PEG400/H2O. Blood was sampled at predose and at 0.033 (iv), 0.083 (iv), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose. Plasma concentrations were quantitated by LC−MS/MS. Pharmacokinetic parameters were derived from noncompartmental analysis of the plasma concentration vs time data using WinNonlin software (Pharsight, Palo Alto, CA).

Ex vivo radioligand binding autoradiography.

Figure 1. GluN2B occupancy and brain concentrations of **9**, **30** and **34** over a 2 hour timecourse.


Concentration vs Occupancy of 9
at 10 mg/kg PO in the Rat, n=2 ± SEM
Concentration (ng/mL)
% Receptor Occupancy
Time (hr)
Brain
NR2B Occupancy


Concentration vs Occupancy of 30
at 10 mg/kg PO in the Rat, n=2 ± SEM
Concentration (ng/mL)
% Receptor Occupancy
Time (hr)
Brain
NR2B Occupancy


Concentration vs Occupancy of 34
at 10 mg/kg PO in the Rat, n=2 ± SEM
Concentration (ng/mL)
% Receptor Occupancy
Time (hr)
Brain
NR2B Occupancy

Animal work was done in accordance with the Guide Care for and Use of Laboratory Animals adopted by the US National Institutes of Health. Animals were allowed to acclimate for 7 days after receipt. They were group housed in accordance with institutional standards, received food and water ad libitum and were maintained on a 12 hour light/dark cycle. Male Sprague Daley Rats approximately 300-400 grams in body weight were used. The animals were euthanized using CO_2 and decapitated at the indicated timepoints after drug administration. For time course studies 2 animals per time point over 3 time points were used. For dose response studies, three animals per dose over 7-10 doses were tested. Brains were rapidly frozen and twenty micron thick tissue sections were prepared for autoradiography[2]. Sections were briefly incubated with the radiotracer 3-[^{3}H] 1-(azetidin-1-yl)-2-[6-(4-fluoro-3-methyl-phenyl)pyrrolo[3,2-*b*]pyridin-1-yl]ethanone. Ex vivo GluN2B labelling was expressed as the percentage of GluN2B labelling in corresponding brain areas of vehicle-treated animals.

References:

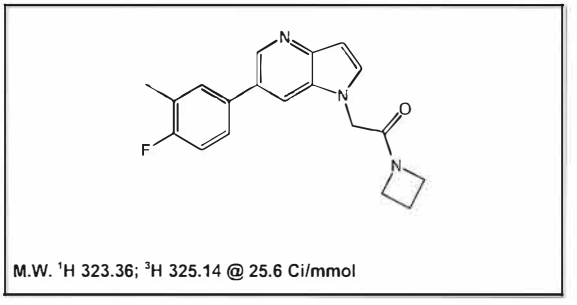
(1) Hill, A.W.; Mortishire-Smith, R.J. Automated assignment of high-resolution collisionally activated dissociation mass spectra using a systematic bond disconnection approach. *Rapid Commun. Mass Spectrom.* **2005**, *19(21)*, 3111-3118.

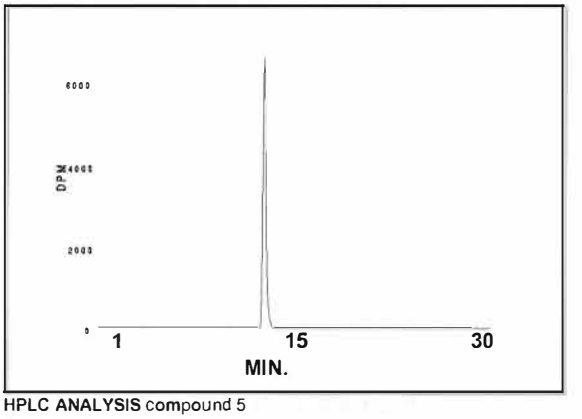
(2) Lord B, Wintmolders C, Langlois X, Nguyen L, Lovenberg T, Bonaventure P. Comparison of the ex vivo receptor occupancy profile of ketamine to several NMDA receptor antagonists in mouse hippocampus. *Eur. J. Pharmacol*. **2013**, *715*, 21–25.

Appendix

Characterization Data

Compound 5, [^{3}H]-





HPLC ANALYSIS compound 5
Date and Time: 10/15/2018 3:42:52 P Unit 04 Radio

Peak #	Area %	Time	Area
1	0.18	12.17330	22.93116
2	99.64	12.57330	12585.18716
3	0.02	13.31000	2.22367
4	0.17	13.61000	20.90681
Totals	100.00		12631.24880

Specific Activity: 25.6 Ci/mmol

Concentration: 1.0 mCi/ml; 12.70 µg/ml

Radiochemical Purity: 99.6%

Column: Phenomenex Prodigy ODS(2) 4.6 x 250mm, 5µm

Flow Rate: 1 ml/min.

Mobile Phase: A: Water with 0.1% TFA
B: Acetonitrile
0-20min. 0-100% B
Hold to 30min.

A) HPLC

Concentrations and volumes:

Standard solution concentration was 1.0 mg/ml.

Compound 5, [^{3}H]- concentration was 1.0 mCi/ml.

Volume of standard alone injection was 1.0 µl.

Volume of **Compound 5, [^{3}H]- a**lone injection was 0.5 µl.

B) Mass spectrometry (LC/MS)– positive mode

MS of Compound 5, [^{3}H]-

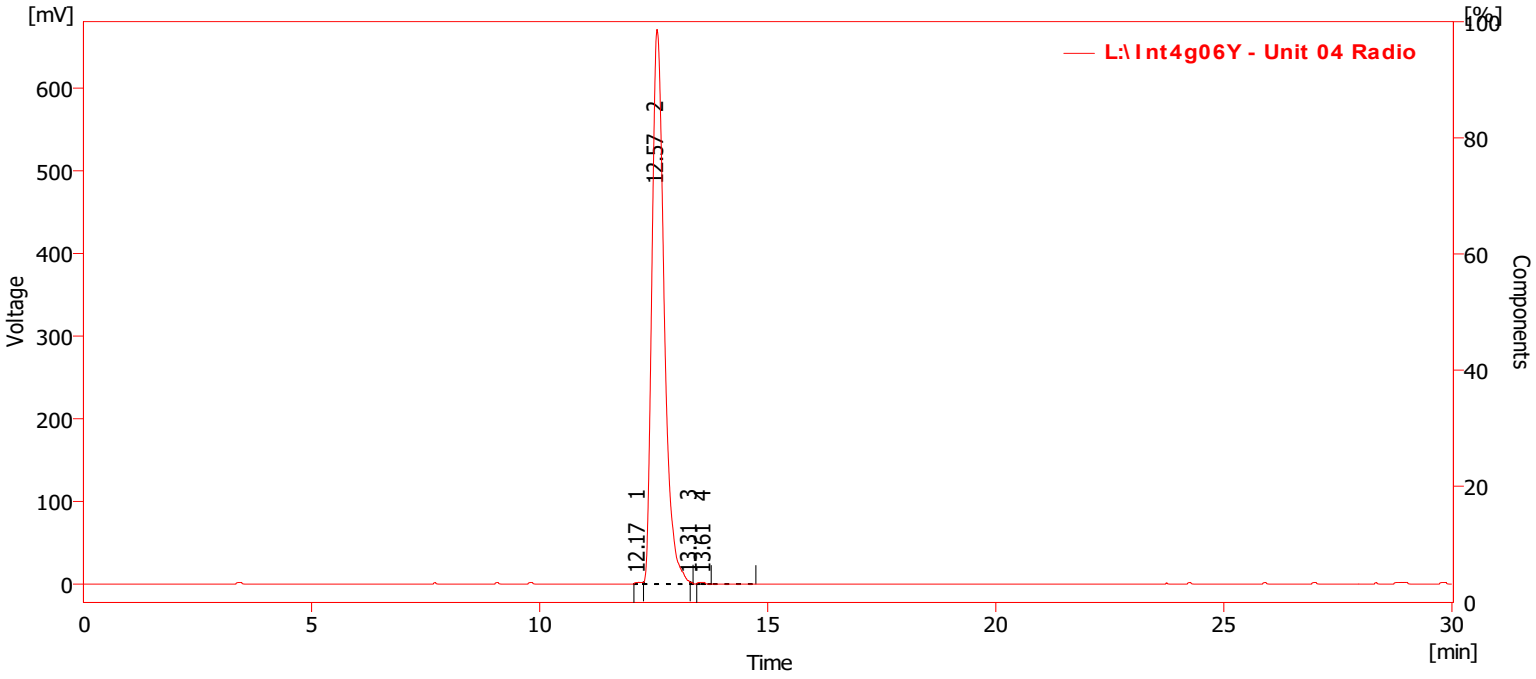
Compound 5, [3H]-
Lot 591-075-0256-A-20181012-JPL

Chromatogram Info:

File Name	: L:\Int4g06Y	File Created	: 10/16/2018 8:39:18 AM
Origin	: Acquired, Acquisition started 10/15/2018 3:12:51 PM	Acquired Date	: 10/15/2018 3:42:52 PM
Project	: Work1		

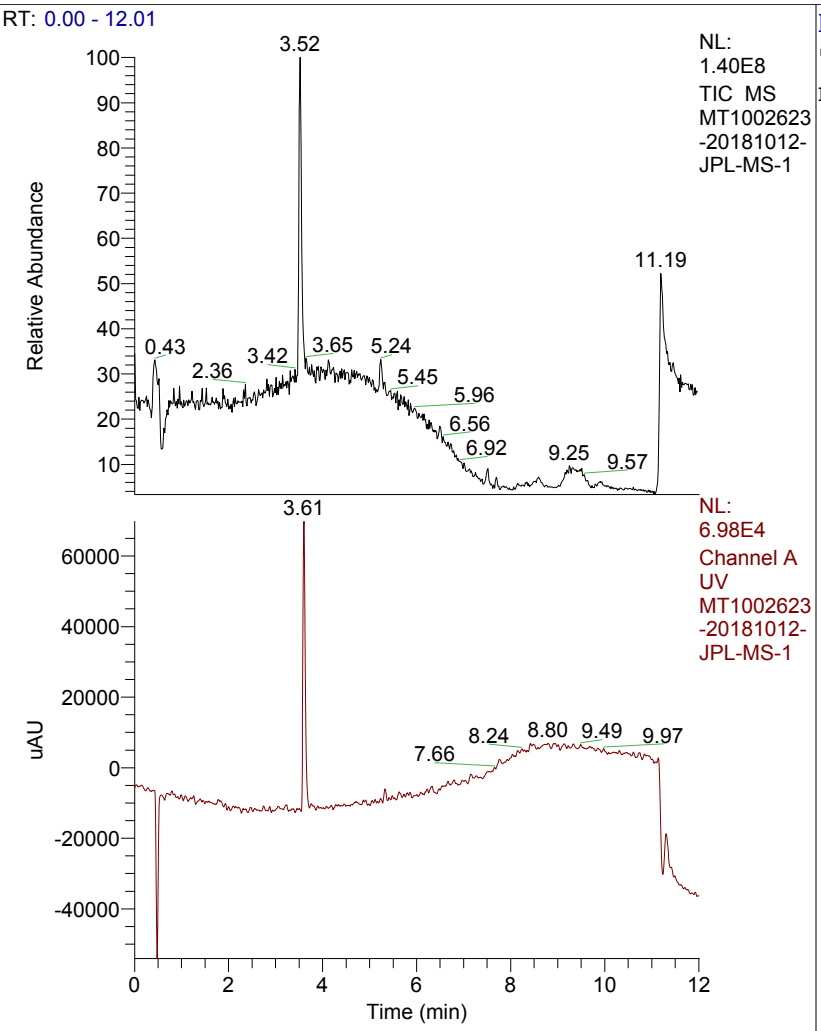
Method : MT1002623
Description : Radiochemical trace of 3H material alone
Created : 8/22/2018 12:37 PM
Detection : Radiochemical

Column :
Mobile Phase :
Flow Rate :
Note :
Temperature :
Pressure :



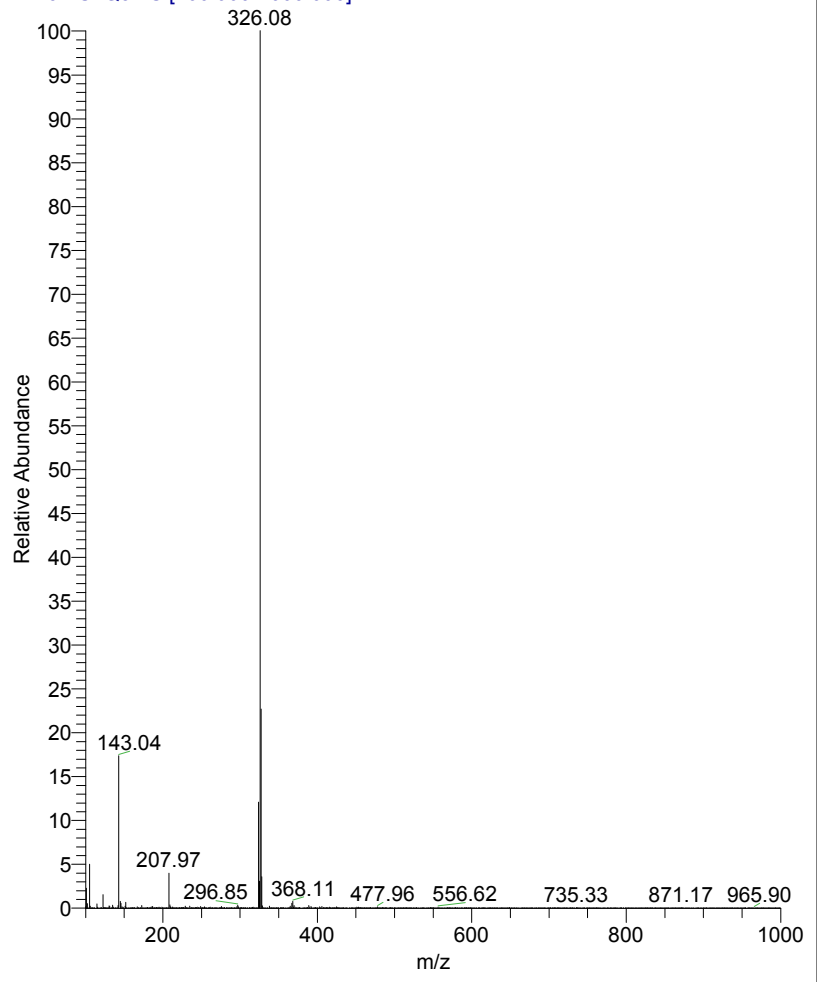
Result Table (Uncal - L:\Int4g06Y - Unit 04 Radio)

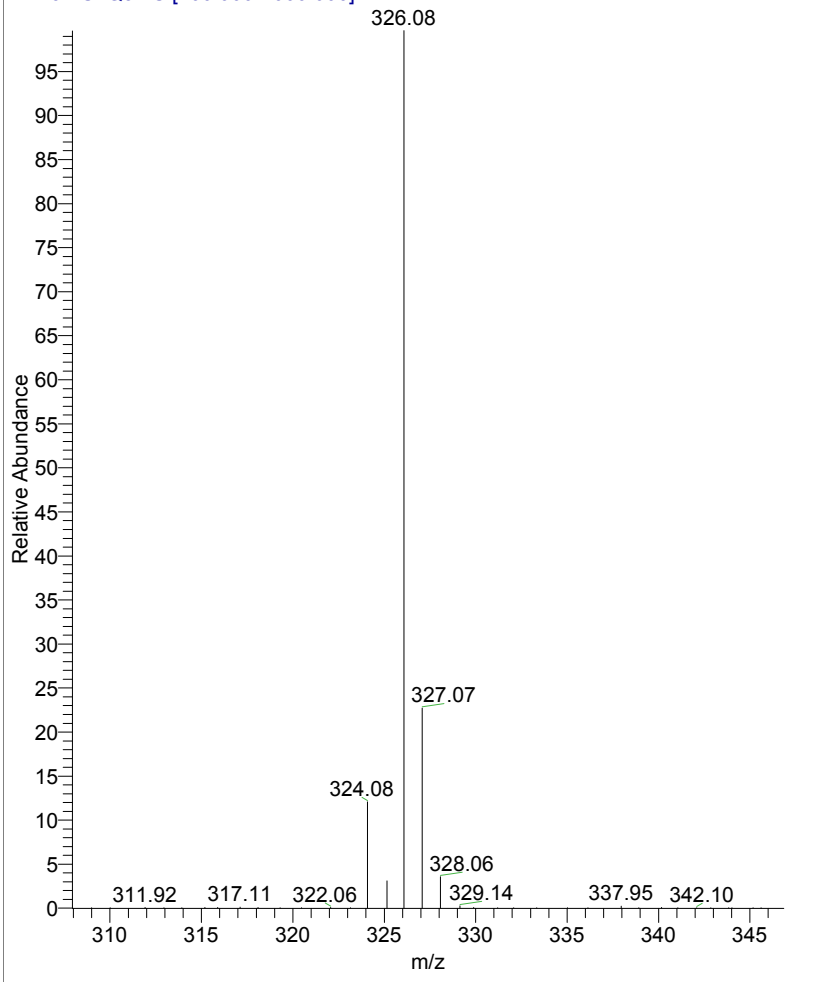
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12.173	22.931	2.478	0.18	0.4	0.20	
2	12.573	12585.187	671.114	99.64	99.3	2.46	
3	13.310	2.224	0.420	0.02	0.1	0.05	
4	13.610	20.907	1.984	0.17	0.3	0.18	
	Total	12631.249	675.997	100.00	100.0		



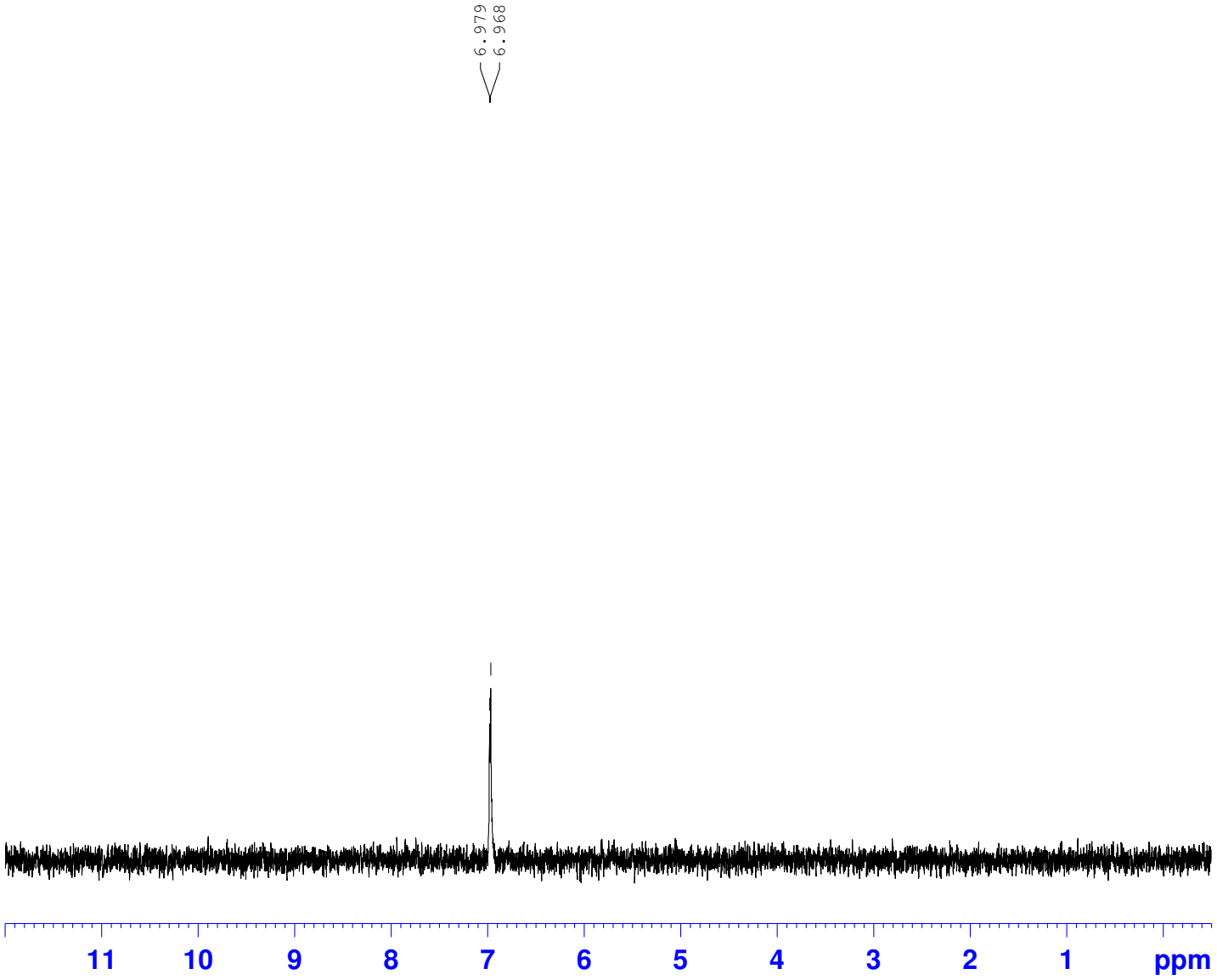
MT1002623-20181012-JPL-MS-1#200 RT: 3.52
T: + c ESI Q3MS [100.00-2000.00]
m/z= 318.28-336.11

m/z	Intensity	Relative
319.30	3.7	0.00
320.47	2125.8	0.00
322.06	33595.9	0.05
323.15	14553.8	0.02
324.08	8738182.0	12.05
325.15	2219279.0	3.06
326.08	72528760.0	100.00
327.07	16454100.0	22.69
328.06	2579690.8	3.56
329.14	175291.3	0.24
329.89	36337.6	0.05
331.20	26198.6	0.04
332.08	5794.5	0.01
333.33	583.9	0.00
335.01	17559.4	0.02





MT1002623 3H NMR in MeOD NS=120000
Batch# 20181012-JPL



NAME	MT1002623	
EXPNO	3	
PROCNO	1	
Date_	20181022	
Time	4.03	
INSTRUM	spect	
PROBHD	5 mm DUX 3H-1H	
PULPROG	zg	
TD	16384	
SOLVENT	MeOD	
NS	120000	
DS	2	
SWH	6172.839	Hz
FIDRES	0.376760	Hz
AQ	1.3271540	sec
RG	46341	
DW	81.000	usec
DE	6.00	usec
TE	300.0	K
D1	2.00000000	sec
TD0	1	

======== CHANNEL f1 ========		
NUC1	3H	
P1	10.00	usec
PL1	2.00	dB
SFO1	320.1321857	MHz
SI	32768	
SF	320.1305850	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	