Supplementary Material

Lead Optimization of 5-Aryl Benzimidazolone and Oxindole-Based AMPA Receptor Modulators Selective for TARP γ-8

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Supplementary Table 1. Potency for inhibition of glutamate-evoked responses in calcium flux assays by compound **18**. Data are represented as mean pIC_{50} ± standard deviation of multiple measurements. The numbers in parentheses indicate the number of measurements performed (run in triplicate). Details for all assay conditions are provided in the "AMPA/TARP Calcium Flux Assays" section below.

	Compound **18** (pIC_{50} ± SEM)
GluA1o-γ8	9.74 ± 0.32 (6)
GluA1o+γ8	9.41 (1)
rat(GluA1o-γ8)	9.09 ± 0.34 (2)
mouse(GluA1o-γ8)	9.10 ± 0.32 (2)
monkey(GluA1o+γ8)	9.45 ± 0.34 (2)
GluA1i+γ8	8.96 (1)
GluA2i+γ8	ND
GluA3o+γ8	8.80 (1)
GluA4o+γ8	9.03 (1)
GluA1o+γ8+CNIH2	8.93 (1)
GluA1i	<5 (1)
GluA1o+CNIH2	<5 (1)
GluA1o+γ2	5.03 (1)
GluA1o+γ3	5.24 (1)

GluA1o+γ4	5.45 (1)
GluA1o+γ7	<5 (1)

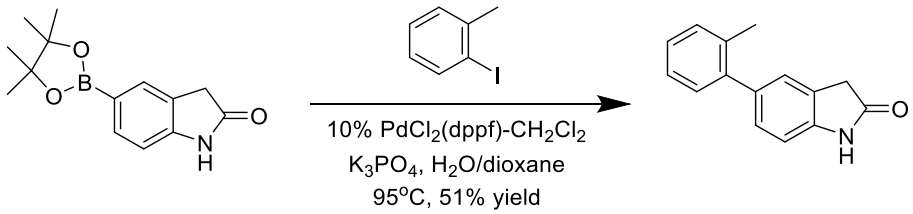
Chemistry Experimental Section:

In obtaining the compounds described in the examples below and the corresponding analytical data, the following experimental procedures and analytical protocols were followed unless otherwise indicated. Anhydrous solvents were obtained from a GlassContour solvent dispensing system. Unless otherwise stated, reaction mixtures were magnetically stirred at room temperature (rt) under a nitrogen atmosphere. Where solutions were "dried," they were generally dried over a drying agent such as Na_2SO_4 or $MgSO_4$. Where mixtures, solutions, and extracts were "concentrated", they were typically concentrated on a rotary evaporator under reduced pressure. Reactions under microwave irradiation conditions were carried out in a Biotage Initiator or CEM Discover instrument. Normal-phase flash column chromatography was performed on silica gel (SiO_2) using prepackaged cartridges, eluting with 2 M NH_3/MeOH in CH_2Cl_2 or EtOAc in hexanes, unless otherwise indicated. Preparative reverse-phase high performance liquid chromatography (HPLC) was performed on an Agilent HPLC with an XBridge™ Prep C18 OBD™ (5 μm, 30 x 100 mm or 50 x 100 mm) column, and a gradient of 10 to 99% acetonitrile/water (20 mM NH_4OH) over 12 to 18 min, and a flow rate of 30 mL/min or 80 mL/min. Occasionally preparative reverse-phase high performance liquid chromatography (HPLC) is referred to as basic HPLC in the text. Preparative reverse-phase high performance liquid chromatography under acidic conditions (acidic HPLC) was performed using a Gilson HPLC with an Inertsil ODS-3 C18, 3μm 30x100mm column at 45 ºC, an acetonitrile/water with

0.05% TFA gradient over 7 min, and a flow rate of 80 mL/min. Mass spectra (MS) were obtained on an Agilent series 1100 MSD using electrospray ionization (ESI) in positive mode unless otherwise indicated. High resolution mass spectrometry data were obtained with a Bruker uTOf detector in positive mode, a Zorbax SB-C18 3.5 µM, 2.1 x 50 mm column at 40 °C, an acetonitrile/water with 0.05% formic acid gradient over 7 min, and a flow rate of 0.3 mL/min. Calculated (calcd.) mass corresponds to the exact mass. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker model DRX spectrometers. The format of the ^{1}H NMR data below is: chemical shift in ppm downfield of the tetramethylsilane reference (multiplicity, coupling constant J in Hz, integration). Chemical names were generated using ChemDraw Ultra 14.0 (CambridgeSoft Corp., Cambridge, MA). Reagents were purchased from commercial suppliers and were used without purification unless otherwise noted.

The synthesis and characterization of compound **3** (JNJ-55511118), compound **12** (JNJ-56022486), and compound [^{3}H] **12** have been reported previously.[1]

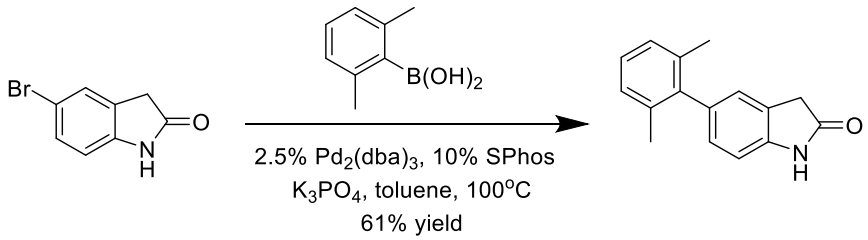
Compound **4**: 5-(o-Tolyl)indolin-2-one.



To a solution of 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indolin-2-one (150 mg, 0.58 mmol), potassium phosphate (246 mg, 1.2 mmol), and 2-iodotoluene (151 mg, 0.69 mmol) in 4:1

dioxane:water (3 mL) was added $PdCl_2(dppf)$-CH_2Cl_2 (42 mg, 0.058 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 95°C for 16 h. After cooling to rt, the reaction mixture was diluted with water and extracted with DCM (x 3). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (0 – 50% EtOAc/hexanes) to afford the title compound (65 mg, 51% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.28 – 7.09 (m, 6H), 6.87 (d, *J* = 7.9 Hz, 1H), 3.52 (s, 2H), 2.23 (s, 3H). HRMS (ESI): *m/z* calcd. for $C_{15}H_{14}NO$ $[M+H]^+$, 224.1076; found, 224.1070.

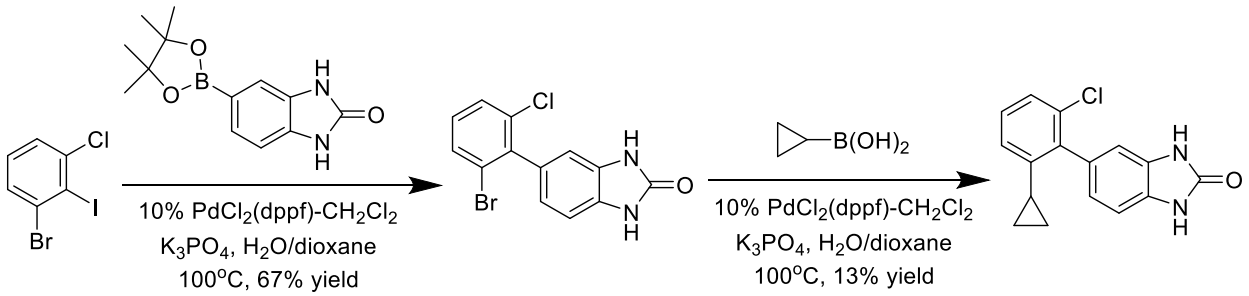
Compound **5**: 5-(2,6-Dimethylphenyl)indolin-2-one.



A solution of (2,6-dimethylphenyl)boronic acid (71 mg, 0.47 mmol), 5-bromoindolin-2-one (50 mg, 0.24 mmol), potassium phosphate (150 mg, 0.71 mmol), dicyclohexyl(2',6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (9.7 mg, 0.024 mmol), and $Pd_2(dba)_3$ (5.4 mg, 0.006 mmol) in toluene (1.0 mL) was degassed with nitrogen for 10 minutes. The reaction mixture was heated at 100 °C for 16 h. After cooling to rt, the reaction was transferred directly to a silica gel column and purified by flash chromatography (0 – 30% EtOAc/hexanes) to provide the title compound (56 mg, 61% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (s, 1H), 7.19-7.06 (m, 3H), 7.06-6.90 (m, 3H), 3.60 (s, 2H), 2.04 (s, 6H). HRMS (ESI): *m/z* calcd. for $C_{16}H_{16}NO$ $[M+H]^+$, 238.1233; m/z found, 238.1226.

The general synthesis of benzimidazolones **6-12** is exemplified by the synthesis of compound **8**. All analogs were made in a similar manner using only minor modifications as noted in the experimentals.

Compound **8**: 5-(2-Chloro-6-cyclopropyl-phenyl)-1,3-dihydrobenzimidazol-2-one.

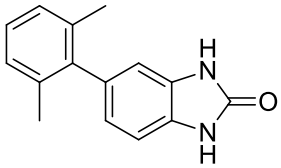


Step A: 5-(2-Bromo-6-chloro-phenyl)-1,3-dihydrobenzimidazol-2-one. To a solution of 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-benzo[d]imidazol-2(3H)-one (1.0 g, 3.8 mmol), potassium phosphate (1.6 g, 7.7 mmol), and 2-bromo-6-chloroiodobenzene (1.5g, 4.6 mmol) in 4:1 dioxane:water (10 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (281 mg, 0.38 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 100 °C for 16 h. After cooling to rt, the reaction mixture was diluted with water and extracted with DCM (x 3). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by trituration with DCM to afford the title compound as a white solid (835 mg, 67% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.71 (s, 2H), 7.72 (dd, J = 8.1, 1.1 Hz, 1H), 7.59 (dd, J = 8.1, 1.1 Hz, 1H), 7.32 (t, J = 8.1 Hz, 1H), 7.01 (dd, J = 7.8, 0.6 Hz, 1H), 6.77 – 6.72 (m, 2H). MS (ESI): mass calcd. for $C_{13}H_8BrClN_2O$, 322.0; m/z found, 322.8 $[M+H]^+$.

Step B: 5-(2-Chloro-6-cyclopropyl-phenyl)-1,3-dihydrobenzimidazol-2-one. To a solution of cyclopropylboronic acid (64 mg, 0.74 mmol), potassium phosphate (210 mg, 0.99 mmol), and 5-(2-bromo-6-chloro-phenyl)-1,3-dihydrobenzimidazol-2-one (160 mg, 0.49 mmol) in 4:1 dioxane:water (2.6 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (36 mg, 0.05 mmol) at once. The

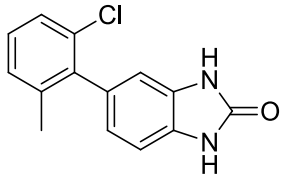
mixture was degassed with nitrogen for 10 minutes and then heated at 100 °C for 19 h. After cooling to rt, the reaction mixture was diluted with water and extracted with DCM (x 3). The crude product was purified by reverse-phase HPLC (Agilent 1100 Series XBridge Prep ^{18}C OBD 5 um, 0.05M NH_4OH in water/MeCN), to afford the title compound as a white solid (18 mg, 13% yield), mp = 260-262°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 2H), 7.38 – 7.21 (m, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 6.88 (dd, *J* = 7.7, 1.3 Hz, 1H), 6.83 – 6.72 (m, 2H), 1.51 (tt, *J* = 8.5, 5.3 Hz, 1H), 0.74 (m, *J* = 6.1, 3.9, 2.5 Hz, 2H), 0.64 (m, *J* = 7.7, 4.7, 2.0 Hz, 2H). HRMS (ESI): *m/z* calcd. for $C_{16}H_{14}ClN_2O$ $[M+H]^+$, 285.0795; found, 285.0789.

Compound **6**: 5-(2,6-Dimethylphenyl)-1,3-dihydrobenzimidazol-2-one.



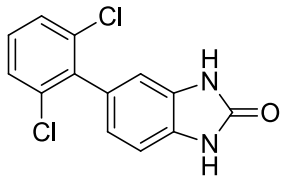
The title compound was prepared in a manner analogous to compound **8**, substituting 2,6-dimethyliodobenzene for 2-bromo-6-chloroiodobenzene and $PdCl_2$(dtbpf) for $PdCl_2$(dppf)-CH_2Cl_2 in Step A. Purification by silica gel chromatography (0 - 100% EtOAc/hexanes) afforded the title compound (22 mg, 24% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.13- 7.02 (m, 4H), 6.86- 6.80 (m, 1H), 6.76 (dd, *J* = 8.0, 1.4 Hz, 1H), 2.00 (s, 6H). HRMS (ESI): *m/z* calcd. for $C_{15}H_{15}N_2O$ $[M+H]^+$, 239.1185; found, 239.1179.

Compound **7**: 5-(2-Chloro-6-methyl-phenyl)-1,3-dihydrobenzimidazol-2-one.



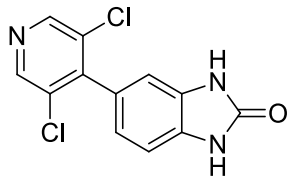
The title compound was prepared in a manner analogous to compound **8**, substituting 1-chloro-2-iodo-3-methylbenzene for 2-bromo-6-chloroiodobenzene in Step A (85 mg, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.69 (s, 1H), 10.65 (s, 1H), 7.41 – 7.31 (m, 1H), 7.31 – 7.21 (m, 2H), 7.06 – 6.95 (m, 1H), 6.77 – 6.63 (m, 2H), 2.04 (s, 3H). HRMS (ESI): *m/z* calcd. for $C_{14}H_{12}ClN_2O$ $[M+H]^+$, 259.0639; found, 259.0633.

Compound **9**: 5-(2,6-Dichlorophenyl)-1,3-dihydrobenzimidazol-2-one.



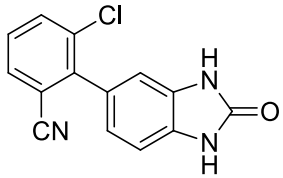
The title compound was prepared in a manner analogous to compound **8**, substituting 1,3-dichloro-2-iodobenzene for 2-bromo-6-chloroiodobenzene in Step A (50 mg, 31% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, *J* = 8.1 Hz, 2H), 7.34 – 7.27 (m, 1H), 7.12 (dd, *J* = 7.7, 0.9 Hz, 1H), 6.91 – 6.85 (m, 2H). HRMS (ESI): *m/z* calcd. for $C_{13}H_9Cl_2N_2O$ $[M+H]^+$, 279.0093; found, 279.0086.

Compound **10**: 5-(3,5-Dichloro-4-pyridyl)-1,3-dihydrobenzimidazol-2-one.



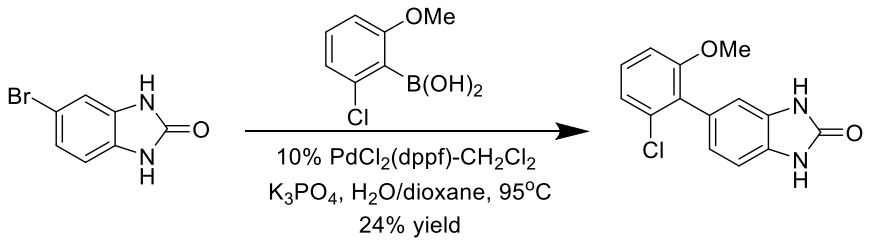
The title compound was prepared in a manner analogous to compound **8**, substituting 3,5-dichloro-4-iodopyridine for 2,6-dimethyliodobenzene in Step A (86 mg, 53% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 10.81 (s, 1H), 8.72 (s, 2H), 7.12 – 7.01 (m, 1H), 6.93 – 6.85 (m, 2H). HRMS (ESI): *m/z* calcd. for $C_{12}H_8Cl_2N_3O$ $[M+H]^+$, 280.0045; found, 281.0039.

Compound **11**: 3-Chloro-2-(2-oxo-1,3-dihydrobenzimidazol-5-yl)benzonitrile.



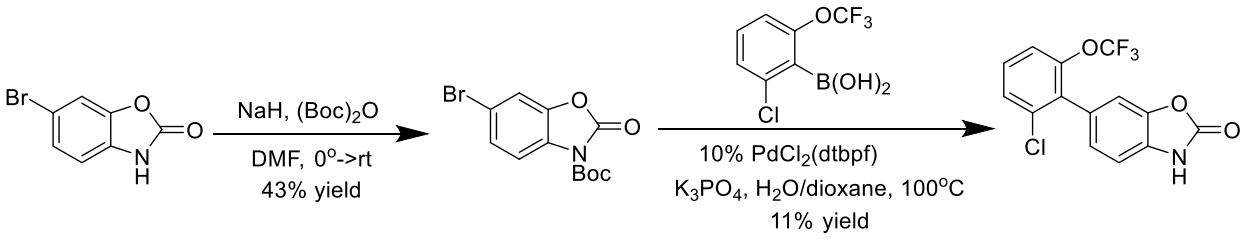
The title compound was prepared in a manner analogous to compound **8**, substituting 2-bromo-3-chlorobenzonitrile for 2,6-dimethyliodobenzene in Step A (48 mg, 23% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 10.80 (s, 1H), 7.92 (ddd, *J* = 8.3, 4.0, 1.2 Hz, 2H), 7.58 (dd, *J* = 8.3, 7.7 Hz, 1H), 7.08 – 7.03 (m, 1H), 6.98 – 6.92 (m, 2H). HRMS (ESI): *m/z* calcd. for $C_{14}H_9ClN_3O$ $[M+H]^+$, 270.0435; found, 270.0429.

Compound **13**: 5-(2-Chloro-6-methoxy-phenyl)-1,3-dihydrobenzimidazol-2-one.



To a solution of (2-chloro-6-methoxyphenyl)boronic acid (158 mg, 0.84 mmol), 5-bromo-1,3-dihydrobenzoimidazol-2-one (150 mg, 0.70 mmol), and potassium phosphate (299 mg, 1.4 mmol), in 4:1 dioxane:water (3.0 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (52 mg, 0.07 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 95°C for 16h. After cooling to rt, water was added and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by reverse-phase HPLC (XBridge C18 column (5µm, 100 x 4.6mm), mobile phase of 10-100% ACN in 20 mM NH_4OH) to afford the title product (47 mg, 24% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 7.34 – 7.24 (m, 1H), 7.10 – 6.97 (m, 3H), 6.91 – 6.83 (m, 2H), 3.70 (s, 3H). HRMS (ESI): *m/z* calcd. for $C_{14}H_{12}ClN_2O_2$ $[M+H]^+$, 275.0588; found, 275.0582.

Compound **14**: 6-[2-Chloro-6-(trifluoromethoxy)phenyl]-3H-1,3-benzo[d]oxazol-2(3H)-one.

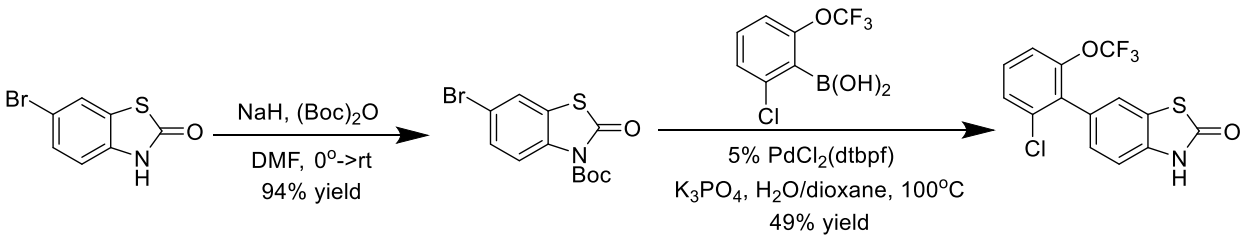


Step A: tert-Butyl 6-bromo-2-oxobenzo[d]oxazole-3(2H)-carboxylate. To a cooled (0°C) solution of 6-bromo-1,3-benzoxazol-2(3H)-one (200 mg, 0.93 mmol) in DMF (4.0 mL) was added sodium hydride (60 wt% in mineral oil, 45 mg, 1.1 mmol) portionwise. The resulting mixture was warmed to rt and stirred for 2h. Di-tert-butyl dicarbonate (306 mg, 1.4 mmol) was added portionwise and stirring was maintained overnight. Water was added and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to afford a waxy solid which was used without further

purification (126 mg, 43% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (d, *J* = 2.0 Hz, 1H), 7.36 (d, *J* = 8.5, 2.0 Hz, 1H), 7.08 (d, *J* = 8.5 Hz, 1H), 1.67 (s, 9H).

Step B: 6-[2-Chloro-6-(trifluoromethoxy)phenyl]-3H-1,3-benzo[d]oxazol-2(3H)-one. To a solution of (2-chloro-6-trifluoromethoxyphenyl)boronic acid (153 mg, 0.64 mmol), tert-butyl 6-bromo-2-oxobenzo[d]oxazole-3(2H)-carboxylate (100 mg, 0.32 mmol), and potassium phosphate (169 mg, 0.80 mmol) in 1:1 dioxane:water (1.4 mL) was added $PdCl_2$(dtbpf) (21 mg, 0.032 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 100°C for 2h. After cooling to rt, water was added and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by silica gel chromatography (0 – 10% EtOAc/hexanes) afforded the title compound (12 mg, 11% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.09 (s, 1H), 7.46 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.37 (t, *J* = 8.1 Hz, 1H), 7.32 – 7.28 (m, 2H), 7.06 – 7.01 (m, 2H). HRMS (ESI): *m/z* calcd. for $C_{14}H_8ClF_3NO_3$ $[M+H]^+$, 330.0146; found, 330.0139.

Compound **15**: 6-[2-Chloro-6-(trifluoromethoxy)phenyl]-3H-1,3-benzothiazol-2-one.

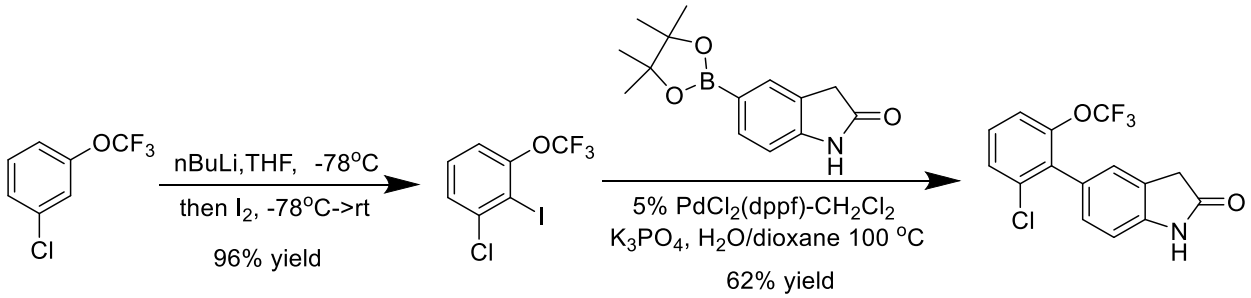


Step A: tert-Butyl 6-bromo-2-oxobenzo[d]thiazole-3(2H)-carboxylate. To a cooled (0°C) solution of 6-bromobenzo[d]thiazol-2(3H)-one (200 mg, 0.87 mmol) in DMF (3.7 mL) was added sodium hydride (60 wt% in mineral oil, 42 mg, 1.0 mmol) portionwise. The resulting mixture was warmed to rt and stirred for 2h. Di-tert-butyl dicarbonate (285 mg, 1.3 mmol) was added portionwise and stirring was maintained overnight. Water was added and the aqueous

layer was extracted with EtOAc. The combined organic extracts were washed with water (x2), dried over Na_2SO_4, and concentrated under reduced pressure to afford a waxy solid which was used without further purification (283 mg, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.9 Hz, 1H), 7.49 (d, *J* = 2.0 Hz, 1H), 7.41 (d, *J* = 8.9, 2.1 Hz, 1H), 1.65 (s, 9H).

Step B: 6-[2-Chloro-6-(trifluoromethoxy)phenyl]-3H-1,3-benzothiazol-2-one. To a solution of (2-chloro-6-trifluoromethoxyphenyl)boronic acid (146 mg, 0.61 mmol), tert-butyl 6-bromo-2-oxobenzo[d]oxazole-3(2H)-carboxylate (100 mg, 0.30 mmol), and potassium phosphate (161 mg, 0.76 mmol) in 1:1 dioxane:water (1.4 mL) was added $PdCl_2$(dtbpf) (9.9 mg, 0.015 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 100°C for 2h. After cooling to rt, water was added and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by silica gel chromatography (0 – 10% EtOAc/hexanes) afforded the title compound (51 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.13 (bs, 1H), 7.49 – 7.44 (m, 1H), 7.39 – 7.27 (m, 3H), 7.23 – 7.17 (m, 2H). MS (ESI): mass calcd. for $C_{14}H_7ClF_3NO_2S$, 345.0; m/z found, 346.0 $[M+H]^+$.

Compound **16**: 5-[2-Chloro-6-(trifluoromethoxy)phenyl]indolin-2-one.

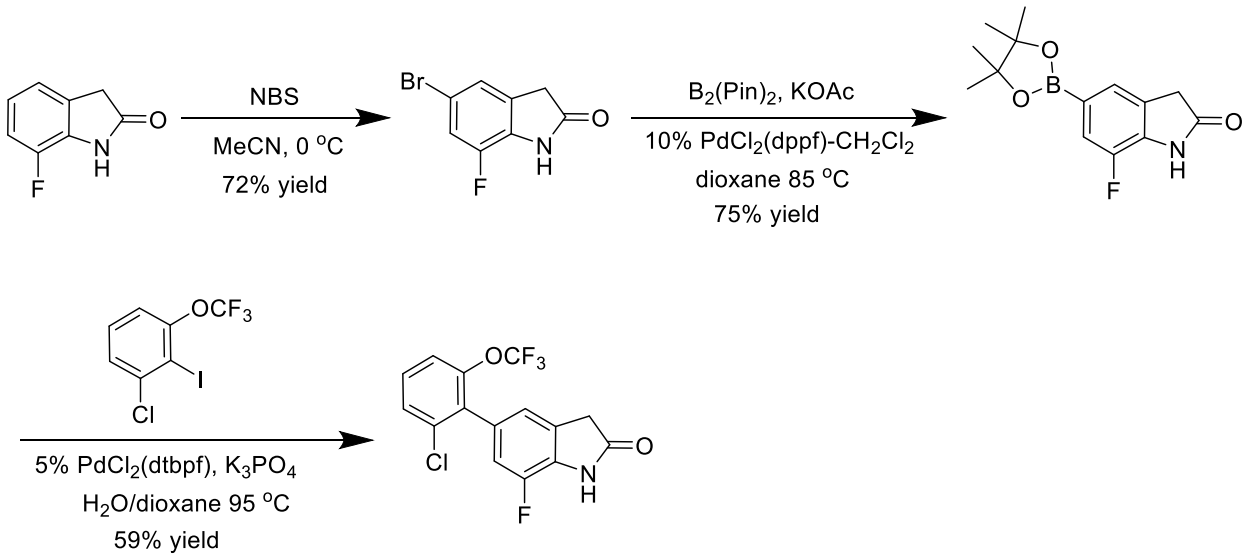


Step A: 1-Chloro-2-iodo-3-(trifluoromethoxy)benzene (compound **25**). To a cooled (-78 °C) solution of 1-chloro-3-(trifluoromethoxy)benzene (100 g, 510 mmol) in THF (500 mL) was added *n*-butyllithium (2.5 M/hexanes, 205 mL, 510 mmol) dropwise over a period of 10 minutes.

Stirring was maintained at -78°C for 1 h, and then a solution of iodine (130 g, 510 mmol) in THF (500 mL) was added dropwise at -78 °C over a period of 30 minutes. After the addition, the temperature was maintained at -78 °C for 1 h and then allowed to warm to rt and stirred for a total of 18 h. The reaction mixture was poured into saturated aqueous Na_2SO_3 and extracted with EtOAc (2 x 1000 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to give the title compound as an oil (159 g, 96% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.32 (t, *J* = 8.1 Hz, 1H), 7.16 (dt, *J* = 8.2, 1.4 Hz, 1H).

Step B: 5-[2-Chloro-6-(trifluoromethoxy)phenyl]indolin-2-one. To a solution of 1-chloro-2-iodo-3-(trifluoromethoxy)benzene (1.5 g, 4.6 mmol), potassium phosphate (1.6 g, 7.7 mmol), and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaboralan-2-yl)indolin-2-one (1.0 g, 3.9 mmol) in 4:1 dioxane:water (30 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (141 mg, 0.19 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 100 °C for 19 h. After cooling to rt, water was added and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by silica gel chromatography (0 – 50% EtOAc/hexanes) afforded the title compound (780 mg, 62% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 7.65 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.57 – 7.44 (m, 2H), 7.16 – 7.02 (m, 2H), 6.92 (d, *J* = 8.0 Hz, 1H), 3.54 (s, 2H). HRMS (ESI): *m/z* calcd. for $C_{15}H_{10}ClF_3NO_2$ $[M+H]^+$, 328.0353; found, 328.0347.

Compound **17**: 5-(2-Chloro-6-(trifluoromethoxy)phenyl)-7-fluoroindolin-2-one.



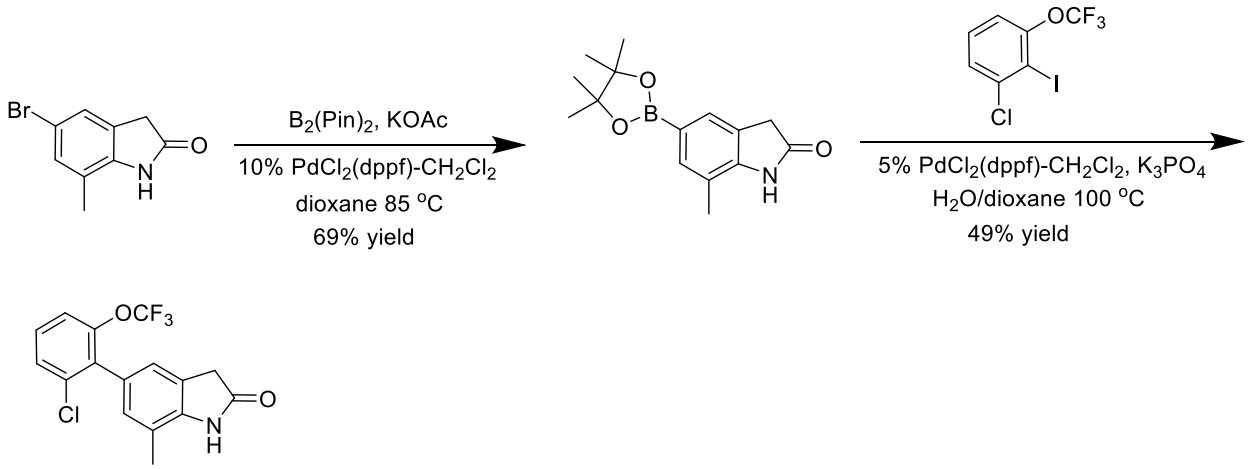
Step A: 5-Bromo-7-fluoroindolin-2-one (compound **29**). To a cooled (0°C) solution of 7-fluoroindolin-2-one (1.0 g, 6.6 mmol) in acetonitrile (50 mL) was added N-bromosuccinimide (1.2 g, 6.6 mmol). The reaction mixture was warmed to rt and stirred overnight. The resulting suspension was filtered through a pad of celite, and the filter cake was washed with EtOAc (10 mL). The filtrate was concentrated in vacuo to provide the title compound, which needed no further purification (1.1 g, 72% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ 11.01 (br, s, 1H), 7.42 (d, J = 10 Hz, 1H), 7.28 (s, 1H), 3.59 (s, 2H).

Step B: 7-Fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indolin-2-one (compound **32**). To a solution of 5-bromo-7-fluoroindolin-2-one (585 mg, 2.54 mmol) in dioxane (8.0 mL) was added potassium acetate (500 mg, 5.0 mmol), bis(pinacolato)diboron (775 mg, 3.05 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (186 mg, 0.25 mmol). The solution was degassed with nitrogen and then heated at 85 °C for 2h. After cooling to rt, the reaction mixture was diluted with brine and extracted with EtOAc (x 2). The combined organic extracts were dried over Na_2SO_4, concentrated, and the crude product was triturated with DCM to provide the title compound as a white solid (535 mg, 75% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.02 (s, 1H), 7.37 – 7.26 (m, 1H), 7.30 – 7.20 (m, 1H), 3.57 – 3.56 (m, 2H), 1.28 (s, 12H). MS (ESI): mass calcd. for $C_{14}H_{17}BFNO_3$, 277.1; m/z found, 278.1 $[M+H]^+$.

Step C: 5-(2-Chloro-6-(trifluoromethoxy)phenyl)-7-fluoroindolin-2-one. To a solution of 1-chloro-2-iodo-3-(trifluoromethoxy)benzene (58 mg, 0.18 mmol), potassium phosphate (77 mg,

0.36 mmol), and 7-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indolin-2-one (50 mg, 0.18 mmol) in 4:1 dioxane:water (2.0 mL) was added $PdCl_2$(dtbpf) (5.9 mg, 0.009 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 95 °C for 40 minutes. After cooling to rt, water was added and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by silica gel chromatography (0 – 50% EtOAc/hexanes) afforded the title compound (62 mg, 59% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.65 (dd, J = 8.1, 1.2 Hz, 1H), 7.56 (t, J = 8.2 Hz, 1H), 7.53 – 7.46 (m, 1H), 7.08 (dd, J = 10.8, 1.4 Hz, 1H), 6.99 (d, J = 1.5 Hz, 1H), 3.64 (t, J = 1.0 Hz, 2H). HRMS (ESI): *m/z* calcd. for $C_{15}H_9ClF_4NO_2$ $[M+H]^+$, 346.0259; found, 346.0252.

Compound **18**: 5-[2-Chloro-6-(trifluoromethoxy) phenyl]-7-methyl-indolin-2-one.

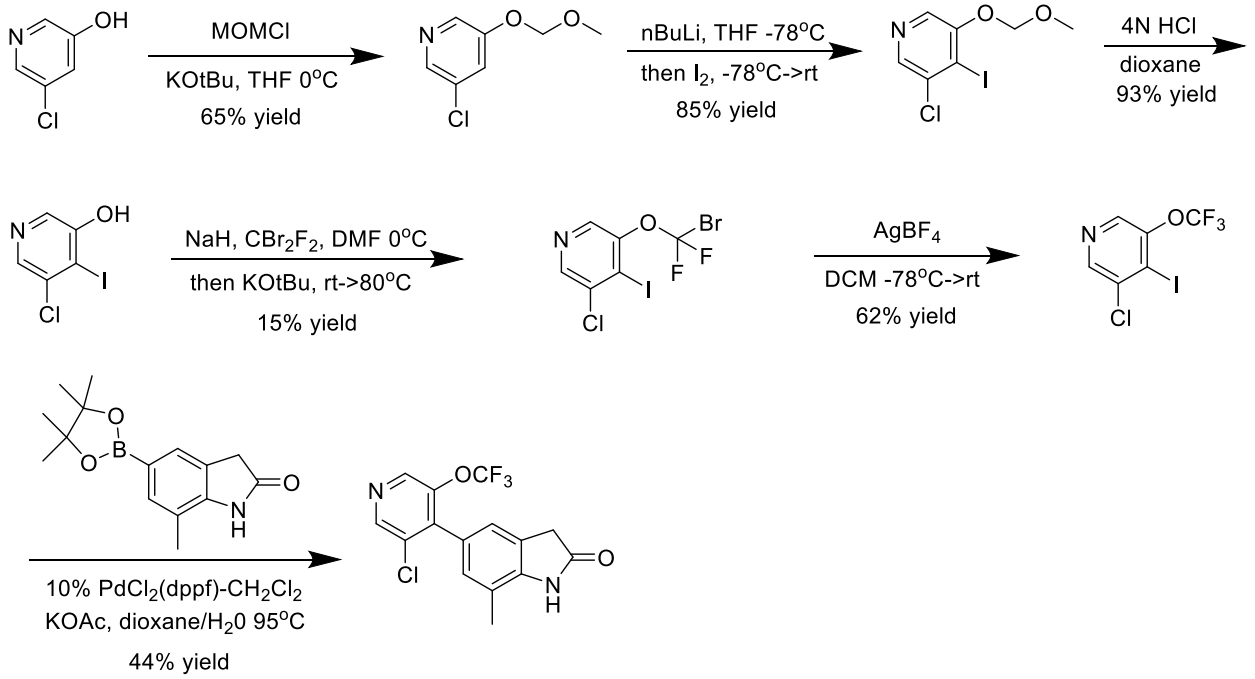


Step A: 7-Methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indolin-2-one (compound **33**). To a solution of 5-bromo-7-methylindolin-2-one (2.0 g, 8.9 mmol) in dioxane (29 mL) was added potassium acetate (1.7 g, 18 mmol), bis(pinacolato)diboron (2.7 g, 11 mmol) and $PdCl_2$(dppf)-CH_2Cl_2 (650 mg, 0.88 mmol). The solution was degassed with nitrogen and then heated at 85 °C for 2h. After cooling to rt, the reaction mixture was diluted with brine and extracted with EtOAc (x 2). The combined organic extracts were dried over $MgSO_4$, filtered, and

concentrated in vacuo. 100 mL of 1:1 EtOAc/hexanes were added to the crude product and the resulting precipitate was filtered to provide the title compound (2.4 g, 69% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 7.32 (s, 1H), 7.31 (s, 1H), 3.47 (s, 2H), 2.19 (s, 3H), 1.26 (s, 12H). MS (ESI): mass calcd. for $C_{15}H_{20}BNO_3$, 273.1; m/z found, 274.1 $[M+H]^+$.

Step B: 5-[2-Chloro-6-(trifluoromethoxy) phenyl]-7-methyl-indolin-2-one. To a solution of 1-chloro-2-iodo-3-(trifluoromethoxy)benzene (909 mg, 2.82 mmol), potassium phosphate (1.09 g, 5.13 mmol), and 7-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indolin-2-one (700 mg, 2.56 mmol) in 4:1 dioxane:water (10 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (94 mg, 0.13 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 100°C for 2h. After cooling to rt, water was added and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by silica gel chromatography (0 – 50% EtOAc/hexanes) afforded the title compound as a white solid (876 mg, 49% yield), mp = 172-177°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.57 (s, 1H), 7.63 – 7.61 (m, 1H), 7.52 (t, J = 8.2 Hz, 1H), 7.48 – 7.45 (m, 1H), 6.93 (d, J = 1.7 Hz, 1H), 6.91 – 6.86 (m, 1H), 3.54 (s, 2H), 2.23 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.8, 146.9, 142.3, 134.4, 134.2, 130.0, 129.8, 128.5, 125.9, 125.2, 122.9, 119.7, 118.3, 119.8 (q, J = 257.4 Hz), 36.0, 16.4. HRMS (ESI): *m/z* calcd. for $C_{16}H_{12}ClF_3NO_2$ $[M+H]^+$, 342.0509; found, 342.0503.

Compound **19**: 5-[3-Chloro-5-(trifluoromethoxy)-4-pyridyl]-7-methyl-indolin-2-one.



Step A: 3-Chloro-5-(methoxymethoxy)pyridine. To a cooled (0 °C) solution of 5-chloropyridin-3-ol (5g, 38.6 mmol) in dry THF (50 mL) was added potassium *tert*-butoxide (21.6g, 193 mmol), and the reaction mixture was then warmed to rt and stirred for 30 minutes. The reaction was again cooled to (0 °C) and chloromethyl methyl ether (7.4 mL, 96.5 mmol) was added dropwise. After the addition, the mixture was stirred for another 3h at rt. The reaction mixture was diluted with water and extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound as an oil, which was used without further purification (4.3g, 65% yield). MS (ESI): mass calcd. for $C_7H_8ClNO_2$, 173.0; m/z found, 174.0 $[M+H]^+$.

Step B: 3-Chloro-4-iodo-5-(methoxymethoxy)pyridine (compound **25**). To a cooled (-78 °C) solution of 3-chloro-5-(methoxymethoxy)pyridine (3.4g, 19.7 mmol) in THF (20 mL) was added *n*-butyllithium (2.5 M/hexanes, 13 mL, 142 mmol) dropwise over a period of 20 minutes. Stirring was maintained at -78 °C for 1 h, and then a solution of iodine (6.6 g, 26 mmol) in THF (20 mL) was added dropwise at -78 °C over a period of 30 minutes. After the addition, the resultant mixture was then allowed to warm to rt and stirred for another 30 minutes. The reaction mixture was poured into saturated aqueous Na_2SO_3 and extracted with EtOAc (2 x 200 mL). The

combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to give the title compound (2.8g, 47% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 8.18 (s, 1H), 5.40 (s, 2H), 3.44 (s, 3H). MS (ESI): mass calcd. for $C_7H_7ClINO_2$, 298.9; m/z found, 300.0 $[M+H]^+$.

Step C: 5-Chloro-4-iodopyridin-3-ol (compound **24**). 3-Chloro-4-iodo-5-(methoxymethoxy)pyridine (2.8g, 9.2 mmol) was added to 4N HCl/dioxane (15 mL) and the mixture was stirred at rt for 24 hours. The resulting yellow precipitate was filtered and washed with DCM to provide the title compound (2.2 g, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.10 (s, 1H), 7.29 (s, 1H). MS (ESI): mass calcd. for C_5H_3ClINO, 254.9; m/z found, 255.9 $[M+H]^+$.

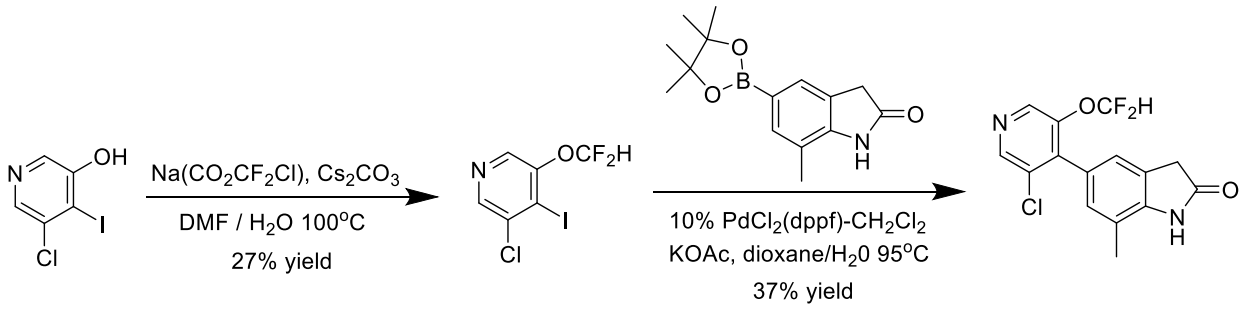
Step D: 3-(Bromodifluoromethoxy)-5-chloro-4-iodopyridine. To a cooled (0 °C) solution of 5-chloro-4-iodopyridin-3-ol (1.6g, 6.3 mmol) in DMF (20 mL) was added sodium hydride (1.3g, 31.3 mmol) portion wise. The mixture was then warmed to rt, stirred vigorously for 1 h. The reaction mixture was cooled to 0 °C, then a solution of dibromodifluoromethane (4.6 mL, 50.1 mmol) in DMF (5.0 mL) was added, followed by portion wise addition of potassium *tert*-butoxide (2.1g, 18.8 mmol). The mixture was heated to 80 °C for 24 h in a sealed vial. After cooling to rt, the crude reaction mixture was diluted with water, and extracted with EtOAc (x 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. Purification (FCC, SiO_2; 0-50% EtOAc/hexanes) provided the title compound (350 mg, 15% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 8.48 (t, J = 1.6 Hz, 1H). MS (ESI): mass calcd. for $C_6H_2BrClF_2INO$, 382.8; m/z found, 383.8 $[M+H]^+$.

Step E: 3-Chloro-4-iodo-5-(trifluoromethoxy)pyridine (compound **27**). To a cooled (-78 °C) solution of 3-(bromodifluoromethoxy)-5-chloro-4-iodopyridine (306 mg, 0.8 mmol) in DCM (20 mL) was added silver tetrafluoroborate (341 mg, 1.7 mmol) portionwise. The reaction mixture was then warmed to rt and stirred for 20h. To the crude reaction mixture was added saturated sodium bicarbonate solution (5 mL) and the mixture was filtered. The filtrate was extracted with DCM (x 2); the combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. Purification (FCC, SiO_2; 0-50% EtOAc/hexanes) provided the title compound as white solid (160 mg, 62% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 8.53 (q, J = 1.4 Hz, 1H).

Step F: 5-[3-Chloro-5-(trifluoromethoxy)-4-pyridyl]-7-methyl-indolin-2-one. To a solution of 3-chloro-4-iodo-5-(trifluoromethoxy)pyridine (53 mg, 0.16 mmol), potassium phosphate (70 mg,

0.33 mmol), and 7-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indolin-2-one (45 mg, 0.16 mmol) in 4:1 dioxane:water (2.0 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (12 mg, 0.016 mmol) at once. The mixture was degassed with nitrogen for 10 minutes and then heated at 95°C for 2h. After cooling to rt, water was added and the aqueous layer was extracted with DCM. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was triturated with DCM to afford the title compound as a white solid (56 mg, 44% yield), mp = 195-198°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.82 (s, 1H), 8.74 (q, J = 1.4 Hz, 1H), 7.05 (d, J = 1.7 Hz, 1H), 7.04 – 6.98 (m, 1H), 3.57 (s, 2H), 2.25 (s, 3H). HRMS (ESI): *m/z* calcd. for $C_{15}H_{11}ClF_3N_2O_2$ $[M+H]^+$, 343.0462; m/z found, 343.0456.

Compound **20**: 5-(3-Chloro-5-(difluoromethoxy)pyridin-4-yl)-7-methylindolin-2-one.



Step A: 3-Chloro-4-iodo-5-(difluoromethoxy)pyridine (compound **26**). To a solution of 5-chloro-4-iodopyridin-3-ol (1g, 3.9 mmol) in DMF (16 mL) was added a solution of sodium chlorodifluoroacetate (1.2g, 7.8 mmol) in water (4 mL), followed by the addition of Cs_2CO_3 (3.8g, 12.0 mmol). The reaction mixture was then heated to 100 °C for 24 h. After cooling to rt, the crude mixture was diluted with water and extracted with EtOAc, washed with brine and the combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. Purification by silica gel chromatography (0-50% EtOAc/hexanes) provided the title compound (326 mg, 27% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.31 (t, J = 1.0 Hz, 1H), 7.40 (t, J = 72.5 Hz, 1H).

Step B: 5-[3-Chloro-5-(difluoromethoxy)-4-pyridyl]-7-methyl-indolin-2-one. To a solution of 3-chloro-4-iodo-5-(difluoromethoxy)pyridine (800 mg, 2.62 mmol), potassium phosphate (900 mg, 4.24 mmol), and 7-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indolin-2-one (575 mg, 2.11 mmol) in 4:1 dioxane:water (25 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (160 mg, 0.22 mmol) at once. The mixture was degassed with argon for 10 minutes and then heated at 95°C for 4h. After cooling to rt, water was added and the aqueous layer was extracted with EtOAc. The organic layer was washed successively with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (0-33% EtOAc/hexanes), followed by trituration with diethyl ether to afford the title compound as a white solid (250 mg, 37% yield), mp = 196-201°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.66 (s, 1H), 8.54 (t, J = 1.0 Hz, 1H), 7.21 (t, J = 73.0 Hz, 1H), 7.02 (d, J = 1.6 Hz, 1H), 7.00 – 6.95 (m, 1H), 3.55 (s, 2H), 2.24 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.7, 146.0, 145.8 (t, J = 2.7 Hz), 143.0, 140.0, 139.0, 131.1, 129.8, 125.4, 123.7, 122.6, 118.4, 116.1 (t, J = 260.7 Hz), 36.0, 16.5. HRMS (ESI): *m/z* calcd. for $C_{15}H_{12}ClF_2N_2O_2$ $[M+H]^+$, 325.0555; found, 325.0550.

AMPAR/TARP Calcium Flux Assays:

Mammals express four AMPA-receptor subunits, GluA1-GluA4. Each GluA subunit can be expressed in multiple splice variants; the two most prominent splice variants are called *flip* and *flop*, denoted "i" and "o", respectively. A clonal cell line stably expressing the human GluA1o-γ8 fusion construct under geneticin selection in HEK-293 was established for the primary calcium flux assay as described previously.[1] All other combinations of GluA subunits and TARPs were performed using co-transfections of the respective plasmids into HEK-293F cells. AMPA receptors formed by co-transfections are designated with the 'plus' symbol (e.g. GluA1i co-transfected with TARP γ-8 is referred to as GluA1i+γ8). For assays with transiently-transfected cells, cells were generated by bulk transfection. Prior to transfection, 293-F cells were cultured in Freestyle-293 Expression Medium (Gibco) at 0.5-2 million cells/mL in shaker flasks at 37°C and 8% CO_2 at 120 rpm. Cell suspension volume did not exceed ¼ of the flask volume. At the time of transfection, cells were diluted to 1 million/ml with FreeStyle-293 medium. Cell viability was above 90% for transfections to be considered successful. Transfection was performed by combining same amount of pAdvantage vector and target DNA. Total DNA was 50μg per 40mL transfection. The DNA ratio of AMPA receptor to TARPs was 4:1. The transfection reagent was Freestyle MAX (Invitrogen). Cells were seeded into 384-well poly-lysine-coated plates at 15k cells/ well at 16-24 hours after transfection, and used for assays 24-48 after transfection. The calcium flux assay was performed as follows. HEK-293 cells stably expressing the GluA and TARP constructs were grown in monolayers in 384-well polylysine-coated microtiter plates. Cell plates were washed with assay buffer (135 mM NaCl, 4 mM KCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES, pH 7.4, 300 mOs) using a Biotek EL405 plate washer. The cells were then loaded with a calcium-sensitive dye according to the

manufacturers' instructions (Calcium-5 or Calcium-6; Molecular Devices, Sunnyvale, CA) combined with the test compounds at a range of concentrations. Calcium flux following the addition of 15μM glutamate was monitored using a FLIPR Tetra (Molecular Devices, Sunnyvale, CA). The fluorescent response in each well was normalized to the response of negative and positive control wells. The negative control wells had no added compounds, and the positive control wells had been incubated with 50 μM CP-465022.[2] The responses (R) to glutamate as functions of the test compound concentrations (x) were fitted to a four-parameter logistic function $R = A_2 + (A_1 - A_2)/(1 + (x/x_0)^p)$. The fitted parameter corresponding to the midpoint (x_0) was taken to be the potency of inhibition of the compound (IC50; 50% inhibitory concentration). Potency is expressed as $\mathrm{pIC50} = -\log_{10}(\mathrm{IC50[M]})$.

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 10^5 cells/cm^2 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 (P_{app}) 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.

Plasma Protein Binding:

Plasma protein binding was determined by equilibrium dialysis using the RED Device (Thermo Scientific, Rockford, IL) consisting of a Teflon base block and RED Device inserts comprising two (sample and buffer) side-by-side chambers separated by a dialysis membrane. 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.

Brain Tissue Binding:

Brain tissue binding was assessed by equilibrium dialysis using the RED device similar to the procedure described for plasma protein binding. Rat brain tissue homogenate, prepared in PBS buffer, pH 7.4 (1:10, w/v), was spiked with compound DMSO stock solution to yield a final concentration of 5 µM. The dialysis was carried out in a shaking incubator at 37 °C for 5 h in triplicate. After incubation, 25 µL of homogenate or 50 µL of buffer was extracted with 50 µL of DMSO and 300 µL of acetonitrile and analyzed by LC/MS/MS using the calibration curves across an appropriate concentration range and quality control samples. The apparent unbound fraction ($f_{u,app}$) was derived from the formula:

$$fu, app = \frac{[\mathrm{A}]buffer}{[\mathrm{A}]homogenate}$$

where $[A]_{homogenate}$ and $[A]_{buffer}$ are the concentrations measured in the homogenate and buffer, respectively. The unbound fraction in undiluted brain ($f_{u,\ brain}$) was calculated from the formula

$$fu, brain = \frac{fu, app}{\mathrm{D} + fu, app - \mathrm{D} * fu, app}$$

where D is the dilution factor of 10.

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 $MgCl_2$, and 0.5 mg/mL liver microsomes from mouse, rat, dog, monkey 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, where protein binding in plasma and microsomal proteins is assumed to be similar and the blood to plasma concentration ratio is assumed to be one. The hepatic extraction ratio (ER) was calculated by dividing the predicted hepatic clearance by species blood flow (Q), where Q is 150, 70, 31, 44, and 21.7 mL/min/kg for mouse, rat dog, monkey, and human, respectively.[4]

Glutathione (GSH) Trapping Assay:

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 $MgCl_2$ 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 μm, 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.[5]

Solubility in Aqueous Fluids:

Solubility in simulated gastric (SGF, 0.2% NaCl in 0.1 N HCl, pH 1.2) and intestinal fluids (FasSIF, 0.029 M phosphate buffer, 5 mM sodium taurocholate, and 1.5 mM lecithin, pH 6.8) was investigated. Compound was dissolved in DMSO solutions at a concentration of 10 mM and was used for the solubility experiment. DMSO solutions (20 μL) are dispensed in 96-well plates, and the solvent is removed by evaporation using a Caliper TurboVap 96 set at 30 °C. Buffers (400 μL) of interest are added to the residual solids, and the resulting mixtures are stirred at room temperature for 3 days using magnetic stir bars. The samples are then filtered using an AcroPrep 1 mL 96 filter plate, and the supernatant is analyzed for compound concentration, against external standards.

Metabolite Identification in Hepatocytes:

Pooled from 20 donors cryopreserved human hepatocytes (lot number JGH), hepatocytes from male gender cynomolgus monkey (lot number HTK), beagle dog (lot number KLI), Sprague-Dawley rat (lot number OOY), and CD-1 mouse (lot number ENA) were purchased from Celsis-IVT (Baltimore, MD). All hepatocytes were stored in liquid nitrogen until use. Hepatocyte thawing procedures were performed in accordance with vendor protocols, and cell viability was

> 70 % for all species based upon Trypan blue measurement. Test compound (10 µM) was incubated with hepatocytes (1 million cells/mL) in KHB media for 4 h at 37 °C in a tissue culture incubator. The reaction was terminated by the addition of 1.5 volumes of ice-cold acetonitrile/methanol mixture (3:1, v/v). The samples were centrifuged for 10 min at 2191 g to precipitate proteins, and supernatant was diluted 1:1 in water prior to injecting a 100 µL aliquot onto the LC/MS system. The LC/MS/MS analysis was conducted using Surveyor HPLC system interfaced to a UV detector (214, 254 and 280 nm) and 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 set for four scan events. The first scan event, a full scan from 200 – 1000 m/z acquired at a resolution of 15,000, was followed by three data dependent MS/MS scan events acquired at a resolution of 7,5000 to aide structural identification of metabolites. Chromatographic separation was achieved on a Zorbax SB-C18 column (4.6 x 150 mm, 3.5 µm particle size) using a gradient elution method (0-5 min, 5% B; 5-45 min, 5-85% B; 45-49.9 min, 85% B; 49.9-50 min, 85-98 %; 50-55 min, 98% B; 55.0-55.1 min, 98-5% B; and 55.1-60 min, 5% B) at a flow rate of 0.3 mL/min. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The HPLC-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.[5] The assignment and localization of metabolites is determined from the observed molecular weights from full scan mass spectra and through interpretation of fragment ions produced for metabolite relative to unchanged drug from MS/MS spectra.

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/H_2O. 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).

Blood Brain Barrier Measurements:

Animals were dosed by oral administration of a suspension in 0.5% hydroxypropyl methylcellulose (HPMC). The animals were euthanized using carbon dioxide and decapitated at specified time points after drug administration. Brains were rapidly frozen on powdered dry ice and stored at -80°C before sectioning. Plasma samples were also collected for bioanalysis. Twenty-micron-thick tissue sections at the level of the hippocampus were prepared for determination of receptor occupancy by autoradiography.

Ex Vivo Receptor Occupancy:

Receptor occupancy was assessed by *ex vivo* autoradiography using the AMPAR/TARP γ-8 negative modulator [^{3}H] JNJ-56022486, [^{3}H-**12**]. Coronal and sagittal brain tissue slices of 20-micron thickness were prepared for autoradiography as previously described.[6] Tissue sections were not washed prior to incubation, to avoid dissociation of the drug-receptor complex. Three

adjacent brain slices from the same animal were collected per slide. Two brain slices were used to measure total binding, and the third was evaluated for non-specific binding. Total binding was measured by incubating sections with 5nM [^{3}H] JNJ-56022486 in 50mM Tris HCl containing 0.1% BSA (pH 7.4) at room temperature. Non-specific binding was measured in the adjacent brain section by the addition of 10 μM 5-[2-(4-fluorophenyl)-8-(4-hydroxy-1-piperidyl)imidazo[1,2-a]pyrazin-3-yl]indolin-2-one, a structurally distinct AMPAR/TARP γ-8 negative modulator, to the incubation medium. Incubation was restricted to 10 minutes to minimize dissociation of the drug from the receptor. To stop the incubation, sections were rinsed in 50 mM Tris HCl containing 0.1% BSA on ice 4 times for 10 min per rinse, followed by 2 rapid dips in ice cold deionized water, then dried under a stream of cold air.

Digitized images were acquired with a β-Imager DFine or TRacer (Biospacelab, Paris, France), and the levels of bound radioactivity were determined by using the Beta vision program to count the number of β-particles emerging from the hippocampus. The binding signal was expressed in femtomoles per milligram of tissue equivalent (fmol/mteq). Specific binding (SB) for each animal was determined by subtracting non-specific binding from the average total binding calculated from two adjacent brain slices. Specific binding values from vehicle treated animals were used to establish the range for receptor occupancy. All values from animals treated with drug were then expressed in % receptor occupancy vs. vehicle (i.e. 100 – SB value/average vehicle SB*100). Receptor occupancy values <0% were normalized to 0%, and receptor occupancy values >100% were normalized to 100%. For dose-response experiments, the percentage of receptor occupancy at T_{max} was plotted against dosage, and the sigmoidal log dose-effect curve of best fit was calculated by nonlinear regression analysis, using the GraphPad Prism program (San Diego, CA).

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