Fragment and Structure-Based Drug Discovery for a Class C GPCR: Discovery of the $mGlu_5$ negative allosteric modulator HTL14242 (3-chloro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzonitrile)

John A. Christopher, Sarah J. Aves, Kirstie A. Bennett, Andrew S. Doré, James C. Errey, Ali Jazayeri, Fiona H. Marshall, Krzysztof Okrasa, Maria J. Serrano-Vega, Benjamin G. Tehan, Giselle R. Wiggin, Miles Congreve.*

Heptares Therapeutics Ltd., BioPark, Welwyn Garden City, Hertfordshire, AL7 3AX, U.K.

Table 1. LCMS and ^{1}H NMR QC data for **5-17**, **21-30**.

Compound	MW	LCMS purity[a]	MS data m/z (ESI+)[a]	Retention Time (min)[a]	LCMS method[b]	^{1}H NMR data[a,c]
5	280.3	> 98%	281.2	3.69	Method 1	(300 MHz, DMSO-d_6) δ: 4.31-4.35 (m, 4H), 6.69 (t, *J*=2.1 Hz, 1H), 7.04 (d, *J*=9.3 Hz, 1H), 7.76-7.79 (m, 2H), 7.98 (br s, 1H), 8.25 (br s, 1H), 8.74 (d, *J*=2.6 Hz, 1H), 9.06 (s, 1H)
6	265.2	> 95%	266.0	3.80	Method 1	(400 MHz, DMSO-d_6) δ: ppm 6.73 (dd, *J*=2.7, 1.5 Hz, 1H), 8.04 (d, *J*=0.9 Hz, 1H), 8.09-8.14 (m, 1H), 8.46-8.51 (m, 1H), 8.59 (d, *J*=1.2 Hz, 1H), 8.67 (t, *J*=1.4 Hz, 1H), 8.79 (d, *J*=2.7 Hz, 1H), 9.21 (d, *J*=0.9 Hz, 1H)
7	265.2	> 98%	266.0	3.55	Method 1	(400 MHz, DMSO-d_6) δ: ppm 6.72 (dd, *J*=2.6, 1.6 Hz, 1H), 7.71 (dd, *J*=11.3, 8.8 Hz, 1H), 8.02 (d, *J*=1.5 Hz, 1H), 8.17 (ddd, *J*=8.7, 4.6, 2.3 Hz, 1H), 8.37 (s, 1H), 8.59 (dd, *J*=7.0, 2.3 Hz, 1H), 8.78 (d, *J*=2.8 Hz, 1H), 9.24 (d, *J*=1.0 Hz, 1H)
8	247.3	95%	248.2	3.47	Method 1	(300 MHz, DMSO-d_6) δ: ppm 6.72 (dd, *J*=2.8, 1.7 Hz, 1H), 7.80 (t, *J*=7.8 Hz, 1H), 8.01-8.08 (m, 2H), 8.52 (d, *J*=1.1 Hz, 1H), 8.60 (dt, *J*=8.0, 1.5 Hz, 1H), 8.73-8.78 (m, 2H), 9.19 (d, *J*=1.3 Hz, 1H)
9	240.2	95%	241.2	4.44	Method 1	(400 MHz, DMSO-d_6) δ: ppm 6.73 (dd, *J*=2.8, 1.6 Hz, 1H), 7.47 (tdd, *J*=8.4, 2.8, 0.8 Hz, 1H), 7.65 (dq, *J*=16.0, 6.0 Hz, 1H), 8.03 (dd, *J*=1.6, 0.4 Hz, 1H), 8.10 (ddd, *J*=10.4, 2.4, 1.6 Hz, 1H), 8.15 (ddd, *J*=8.0, 1.6, 0.8 Hz, 1H), 8.43 (d, *J*=1.2 Hz, 1H), 8.78 (dd, *J*=2.8, 0.8 Hz, 1H), 9.18 (d, *J*=1.2 Hz, 1H)

10	270.3	>98%	271.2	4.12	Method 2	(400 MHz, DMSO-d_6) δ: ppm 3.89 (s, 3H), 6.71 (dd, *J*=2.5, 1.8 Hz, 1H), 7.08 (dt, *J*=10.5, 2.3 Hz, 1H), 7.65 (d, *J*=1.8 Hz, 1H), 7.67 (m, 1H), 8.01 (d, *J*=1.0 Hz, 1H), 8.40 (d, *J*=1.0 Hz, 1H), 8.76 (d, *J*=2.8 Hz, 1H), 9.15 (d, *J*=1.0 Hz, 1H)
11	283.3	95 %	284.2	3.22	Method 1	(400 MHz, DMSO-d_6) δ ppm 6.74 (s, 1H), 7.73 (s, 1H), 7.89 (d, *J*=8.9 Hz, 1H), 8.04 (s, 1H), 8.27 (d, *J*=9.5 Hz, 1H), 8.40 (s, 1H), 8.58 (s, 1H), 8.64 (s, 1H), 8.79 (d, *J*=2.1 Hz, 1H), 9.20 (s, 1H)
12	261.3	> 98%	262.2	4.01	Method 2	(400 MHz, DMSO-d_6 + D_2O) δ: ppm 2.47 (s, 3H), 6.71 (dd, *J*=2.7, 1.5 Hz, 1H), 7.86 (s, 1H), 8.00 (d, *J*=1.2 Hz, 1H), 8.39 (s, 1H), 8.42-8.50 (m, 2H) 8.75 (d, *J*=2.4 Hz, 1H), 9.15 (d, *J*=0.9 Hz, 1H)
13	281.7	> 98%	282.0, 284.0	4.26	Method 1	(400 MHz, DMSO-d_6) δ: ppm 6.74 (dd, *J*=2.7, 1.5 Hz, 1H), 8.04-8.06 (m, 1H), 8.30 (dd, *J*=2.0, 1.4 Hz, 1H), 8.60-8.67 (m, 2H), 8.76-8.80 (m, 2H), 9.21 (d, *J*=1.2 Hz, 1H)
14	299.7	> 98%	300.0, 302.0	4.18	Method 1	(400 MHz, DMSO-d_6) δ: ppm 6.71 (s, 1H), 8.00 (s, 1H), 8.36 (s, 1H), 8.48 (s, 1H), 8.49 (s, 1H), 8.74-8.79 (m, 1H), 9.23 (s, 1H)
15	260.3	> 98%	261.2	3.79	Method 1	(400 MHz, DMSO-d_6) δ: ppm 2.48 (s, 3H), 6.70 (dd, *J*=2.5, 1.8 Hz, 1H), 7.78 (br. s, 1H), 7.91 (d, *J*=2.0 Hz, 1H), 7.92 (d, *J*=2.0 Hz, 1H), 8.38 (br. s, 1H), 8.45 (br. s, 1H), 8.57 (d, *J*=2.0 Hz, 1H), 8.75 (d, *J*=5.5 Hz, 1H), 8.91 (d, *J*=2.7 Hz, 1H)

16	260.3	> 98%	261.2	3.80	Method 1	(400 MHz, DMSO-d_6) δ: ppm 2.48 (s, 3H), 6.70 (dd, *J*=2.5, 1.8 Hz, 1H), 7.78 (br. s, 1H), 7.91 (d, *J*=2.0 Hz, 1H), 7.92 (d, *J*=2.0 Hz, 1H), 8.38 (br. s, 1H), 8.45 (br. s, 1H), 8.57 (d, *J*=2.0 Hz, 1H), 8.75 (d, *J*=5.5 Hz, 1H), 8.91 (d, *J*=2.3 Hz, 1H)
17	299.69	> 95%	300.0, 301.8	4.62	Method 1	(400 MHz, DMSO-d_6) δ: ppm 8.20 (d, *J*=4.3 Hz, 1H), 8.28 (br. s, 1H), 8.60 (d, *J*=0.8 Hz, 1H), 8.65 (br. s, 1H), 8.76 (br. s, 1H), 8.89 (d, *J*=4.3 Hz, 1H), 9.21 (d, *J*=1.2 Hz, 1H)
21	292.7	> 98%	293.0, 295.0	4.25	Method 1	(400 MHz, DMSO-d_6) δ: 7.66 (ddd, *J*=7.6, 4.8, 1.2 Hz, 1H), 8.09 (td, *J*=7.8, 1.8 Hz, 1H), 8.30 (dd, *J*=2.0, 1.4 Hz, 1H), 8.52 (dt, *J*=7.9, 0.9 Hz, 1H), 8.61-8.72 (m, 1H), 8.77 (t, *J*=1.5 Hz, 1H), 8.80-8.89 (m, 1H), 9.01 (d, *J*=1.2 Hz, 1H), 9.45 (d, *J*=1.5 Hz, 1H)
22	310.7	> 98%	311.0, 313.0	4.29	Method 1	(400 MHz, $CDCl_3$) δ: ppm 7.43-7.54 (m, 1H), 7.84-7.94 (m, 2H), 8.47 (dd, *J*=6.1, 2.1 Hz, 1H), 8.56 (d, *J*=7.8 Hz, 1H), 8.76 (d, *J*=3.9 Hz, 1H), 8.93 (d, *J*=0.8 Hz, 1H), 9.40 (d, *J*=1.2 Hz, 1H)
23	306.8	> 98%	307.0, 309.0	4.71	Method 1	(400 MHz, DMSO-d_6) δ: ppm 2.44 (s, 3H), 7.87-7.92 (m, 1H), 8.27-8.30 (m, 1H), 8.42 (d, *J*=7.9 Hz, 1H), 8.63-8.69 (m, 2H), 8.75 (t, *J*=1.4 Hz, 1H), 8.96 (d, *J*=1.2 Hz, 1H), 9.41 (d, *J*=1.2 Hz, 1H)
24	301.3	> 98%	302.0	3.91	Method 1	(400 MHz, DMSO-d_6) δ: ppm 8.03 (td, *J*=8.7, 2.7 Hz, 1H), 8.60 (dd, *J*=8.9, 4.6 Hz, 1H), 8.69 (t, *J*=1.4 Hz, 1H), 8.85 (d, *J*=2.7 Hz, 1H), 9.01-9.14 (m, 3H), 9.47 (d, *J*=1.2 Hz, 1H)

25	310.7	> 98%	311.0, 313.0	4.71	Method 1	^{1}H NMR (400 MHz, DMSO) δ: 8.02 (td, *J*=8.7, 2.7 Hz, 1H), 8.29 (dd, *J*=2.0, 1.4 Hz, 1H), 8.57-8.67 (m, 2H), 8.76 (t, *J*=1.5 Hz, 1H), 8.84 (d, *J*=2.7 Hz, 1H), 8.95 (d, *J*=1.5 Hz, 1H), 9.44 (d, *J*=1.2 Hz, 1H); ^{19}F NMR (376 MHz, DMSO) δ: -125.85 (dd, *J*=8.6, 4.6 Hz, 1F); ^{13}C NMR (101 MHz,) δ: 113.1, 114.1, 117.1, 123.6 (d, $^{3}J_{CF}$=5.4 Hz, 1C), 124.6 (d, $^{2}J_{CF}$=19.2 Hz, 1C), 129.7, 131.5, 134.0, 134.9, 138.2 (d, $^{2}J_{CF}$=24.5 Hz, 1C), 139.0, 149.4 (d, 4J_{CF}=3.8 Hz, 1C), 158.9, 160.5 (d, $^{1}J_{CF}$=258.4 Hz, 1C), 160.8, 162.5.
26	317.7	> 98%	318.0, 319.8	4.47	Method 1	(400 MHz, $CDCl_3$) δ: 7.83-7.86 (m, 1H), 8.22 (dd, *J*=8.2, 2.1 Hz, 1H), 8.46-8.53 (m, 2H), 8.76 (d, *J*=8.2 Hz, 1H), 8.89 (d, *J*=1.5 Hz, 1H), 9.04-9.09 (m, 1H), 9.44 (d, *J*=1.2 Hz, 1H)
27	327.2	> 98%	327.0, 329.0	5.14	Method 1	(400 MHz, DMSO-d_6) δ: 8.23 (dd, *J*=8.5, 2.7 Hz, 1H), 8.28-8.32 (m, 1H), 8.52 (d, *J*=8.5 Hz, 1H), 8.65 (t, *J*=1.7 Hz, 1H), 8.76 (t, *J*=1.5 Hz, 1H), 8.88 (d, *J*=2.1 Hz, 1H), 8.97 (d, *J*=1.2 Hz, 1H), 9.45 (d, *J*=1.2 Hz, 1H)
28	328.7	98%	329.0, 331.0	4.68	Method 1	(400 MHz, DMSO-d_6) δ: ppm 8.03 (td, *J*=8.7, 3.1 Hz, 1H), 8.51-8.56 (m, 2H), 8.62 (dd, *J*=8.9, 4.6 Hz, 1H), 8.80 (s, 1H), 8.84 (d, *J*=3.1 Hz, 1H), 9.50 (d, *J*=1.2 Hz, 1H)
29	293.7	> 98%	294.0, 296.0	3.41	Method 1	(400 MHz, $CDCl_3$) δ: ppm 7.75-7.84 (m, 2H), 8.51-8.53 (m, 2H), 8.70-8.74 (m, 1H), 9.16 (d, *J*=1.2 Hz, 1H), 9.37-9.40 (m, 1H), 9.45 (d, *J*=1.5 Hz, 1H)

30	293.7	> 98%	294.0, 296.0	3.65	Method 1	(400 MHz, $CDCl_3$) δ: ppm 7.82-7.85 (m, 1H), 8.47-8.54 (m, 3H), 8.91 (d, *J*=1.2 Hz, 1H), 9.05 (d, *J*=5.2 Hz, 1H), 9.45 (dd, *J*=7.2, 1.4 Hz, 2H)

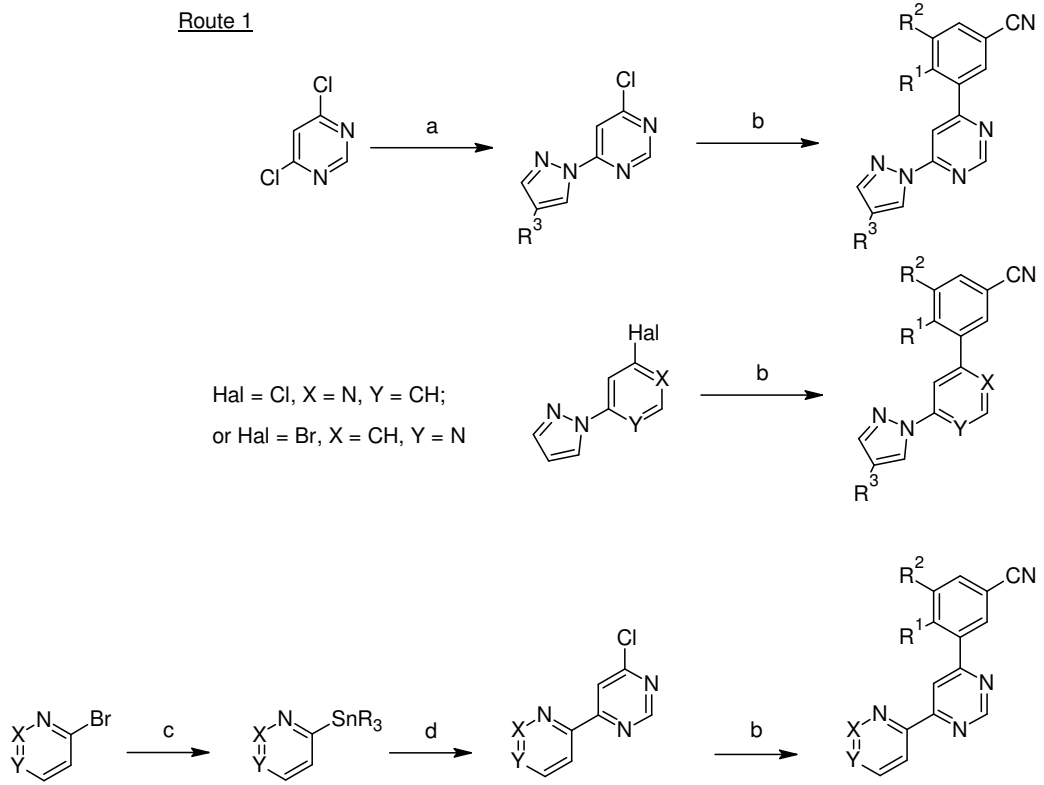
[a] Data generated by Heptares. [b] QC Method 1: LCMS data with electrospray ionisation were generated under the following conditions. Instruments: Agilent 1260 Infinity LC with Diode Array Detector, Agilent 6120B Single Quadrupole MS with API-ES Source; Column: Phenomenex Gemini-NX C-18, 3 micron, 2.0 x 30 mm; Gradient [time (min)/solvent B in A (%)]: 0.00/2, 0.10/2, 8.40/95, 9.40/95, 9.50/2, 10.00/2; Solvents: solvent A = 2.5 L H_2O + 2.5 mL 28% aqueous ammonia solution; solvent B = 2.5 L MeCN + 129 mL H_2O + 2.7 mL 28% aqueous ammonia solution); Injection volume 0.5 µL; UV detection 190 to 400 nM; column temperature 40°C; Flow rate 1.5 mL/min. QC Method 2: LCMS data with electrospray ionisation were generated under the following conditions. Instruments: Waters Alliance 2795, Waters 2996 PDA detector, Micromass ZQ; Column: Phenomenex Gemini-NX C-18, 3 micron, 2.0 x 30 mm; Gradient [time (min)/solvent B in A (%)]: 0.00/2, 0.10/2, 8.40/95, 9.40/95, 9.50/2, 10.00/2; Solvents: solvent A = 2.5 L H_2O + 2.5 mL ammonia solution; solvent B = 2.5 L MeCN + 135 mL H_2O + 2.5 mL 28% aqueous ammonia solution); Injection volume 3 µL; UV detection 230 to 400 nM; column temperature 45°C; Flow rate 1.5 mL/min. [c] All ^1H NMR data are at 300 or 400 MHz as indicated.

Table 2: Synthetic routes for final compounds **5-17**, **21-30**.

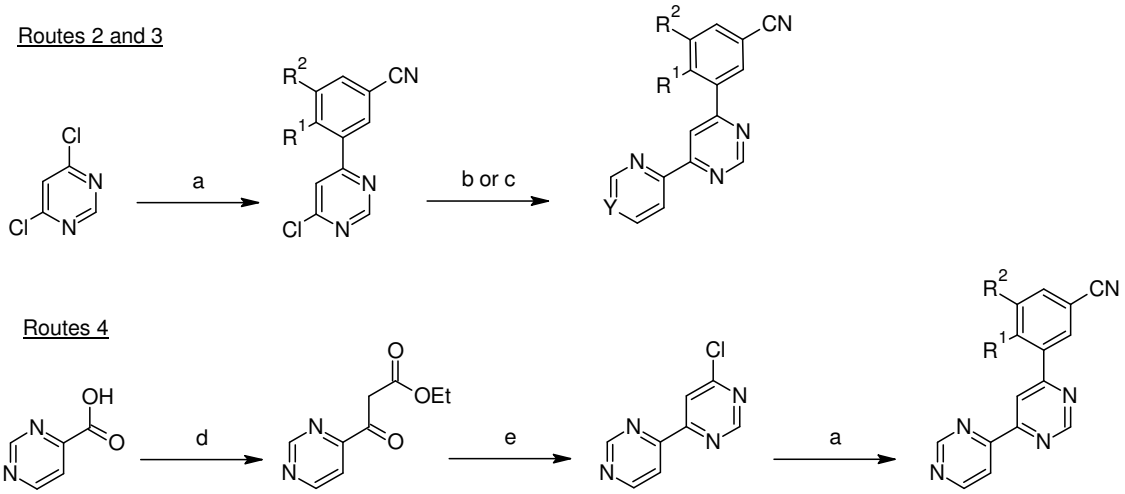
Compound	Route	Compound	Route
5	*a*	**17**	1
6	1	**21**	1
7	1	**22**	1
8	1	**23**	2
9	1	**24**	2
10	1	**25**	2 or 3
11	1	**26**	1
12	1	**27**	2
13	1	**28**	2
14	1	**29**	1
15	1	**30**	4
16	1		

[a] Compound purchased from ChemBridge Corporation, catalogue number 55113728.

Synthetic details for preparation of intermediates and final compounds.



Reagents and conditions: (a) Pyrazole or 4-fluoropyrazole, K_2CO_3, DMF, rt; (b) Substituted aryl boronic acid or ester, $PdCl_2(dppf)$, Cs_2CO_3 or 1M(aq) Na_2CO_3, 1,4-dioxane / H_2O, 80-90ºC; (c) R_3SnSnR_3 (R = Me or *n*-Bu), $Pd(PPh_3)_4$, DME, 80-110ºC; (b) 4,6-dichloropyrimidine, $Pd(PPh_3)_4$, CuI, PhMe, 110ºC.



Reagents and conditions: (a) Substituted aryl boronic acid or ester, $PdCl_2(dppf)•CH_2Cl_2$, Cs_2CO_3 or 1M(aq) Na_2CO_3, 1,4-dioxane, H_2O, 80-90ºC; (b) Heteroaryl trialkylstannane, $Pd(PPh_3)_4$, CuI, PhMe, 110ºC; (c) (i) 2-Bromo-5-fluoropyridine, *i*-PrMgCl, $ZnCl_2$, THF, rt, (ii) Substituted chloropyrimidine, $Pd(PPh_3)_4$, THF, 50-60ºC; (d) (i) Oxalyl chloride, DMF, 0ºC, (ii) EtOAc, LDA, THF, -78ºC; (e) (i) $H_2NCH=NH•HCl$, NaOMe, MeOH, rt, (ii) $POCl_3$, rt.

Where no preparative routes are included, the relevant intermediate is commercially available and was used without further purification. Room temperature (rt) refers to approximately 20-27 ^oC. ^1H NMR spectra were recorded at 300 MHz or 400 MHz as indicated on Bruker, Varian or JEOL instruments. Chromatography refers to column chromatography performed using 60-120 mesh silica gel and executed under nitrogen pressure (flash chromatography) conditions. TLC for monitoring reactions refers to TLC run using the specified mobile phase and silica gel F254 as a stationary phase from Merck. Microwave-mediated reactions were performed

in Biotage Initiator or CEM Discover microwave reactors. LCMS experiments were carried out using electrospray conditions under the conditions specified in the above table.

Synthesis of 25, HTL14242, by Route 2.

3-(5-Amino-2-chloropyridin-4-yl)-5-chlorobenzonitrile. A mixture of 4,6-dichloropyrimidine (9.0 g, 60.4 mmol), 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (17.6 g, 66.8 mmol) and cesium carbonate (35.0 g, 107.4 mmol) were dissolved in 1,4-dioxane / water (9:1, 50 mL). After degassing with N_2, $Pd(dppf)Cl_2$ (2.20 g, 3.0 mmol) was added and the reaction mixture was stirred at 90°C for 2 h. After cooling to rt the reaction mixture was partitioned between H_2O (250 mL) and EtOAc (150 mL), the phases were separated and the aqueous layer extracted with EtOAc (2 x 150 mL). The combined organic layers were dried (Na_2SO_4), concentrated *in vacuo* and purification by column chromatography (SiO_2, 0 to 10% ethyl acetate in hexane) yielded the title compound (4.0 g, 16.0 mmol) as off-white solid. m/z 250.1 $(M+H)^+$; δ_H (400 MHz; d_6-DMSO) 8.27 (s, 1H), 8.53 (s, 1H), 8.59 (s, 1H), 8.66 (s, 1H), 9.16 (s, 1H).

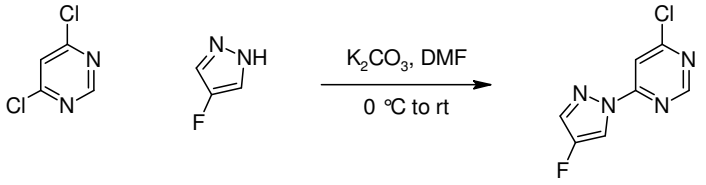
3-Chloro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzonitrile (**25**, HTL14242).

A mixture of 2-bromo-5-fluoropyridine (500 mg, 2.89 mmol) and hexamethylditin (946 mg, 2.89 mmol) in DME (10 mL) was degassed by purging with N_2 for 5 min before the addition of tetrakis(triphenylphosphine)palladium(0) (166 mg, 0.14 mmol). The

reaction mixture was stirred at 110 °C for 16 h before cooling to rt and partitioning between H_2O (50 mL) and EtOAc (25 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2 x 25 mL). The combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo* to yield crude 5-fluoro-2-(trimethylstannyl)pyridine (700 mg) which was used in the subsequent step without characterisation or further purification. 3-Chloro-5-(6-chloropyrimidin-4-yl)benzonitrile (100 mg, 0.39 mmol) and crude 5-fluoro-2-(trimethylstannyl)pyridine (114 mg) were dissolved in toluene (15 mL) and the reaction mixture was degassed by purging with N_2 for 5 min before the addition of tetrakis(triphenylphosphine)palladium(0) (46.2 mg, 0.04 mmol) and copper(I) iodide (7.6 mg, 0.03 mmol). The reaction mixture was stirred at 110 °C for 16 h before cooling to rt and partitioning between H_2O (50 mL) and EtOAc (25 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2 x 25 mL). The combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by gradient flash chromatography, eluting with 0-10% EtOAc in hexane yielded the title compound (37 mg, 0.12 mmol) as a pale yellow solid. *m/z* 311.0, 313.0 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 8.02 (td, *J* = 8.7, 2.7 Hz, 1H) 8.29 (dd, *J* = 2.0, 1.4 Hz, 1H) 8.57-8.67 (m, 2H) 8.76 (t, *J* = 1.5 Hz, 1H) 8.84 (d, *J* = 2.7 Hz, 1H) 8.95 (d, *J* = 1.5 Hz, 1H) 9.44 (d, *J* = 1.2 Hz, 1H); ^{19}F NMR (376 MHz, DMSO) δ: -125.85 (dd, *J* = 8.6, 4.6 Hz, 1F); ^{13}C NMR (101 MHz,) δ: 113.1, 114.1, 117.1, 123.6 (d, $^3J_{CF}$ = 5.4 Hz, 1C), 124.6 (d, $^2J_{CF}$ = 19.2 Hz, 1C), 129.7, 131.5, 134.0, 134.9, 138.2 (d, $^2J_{CF}$ = 24.5 Hz, 1C), 139.0, 149.4 (d, $^4J_{CF}$ = 3.8 Hz, 1C), 158.9, 160.5 (d, $^1J_{CF}$ = 258.4 Hz, 1C), 160.8, 162.5.

Synthesis of intermediates

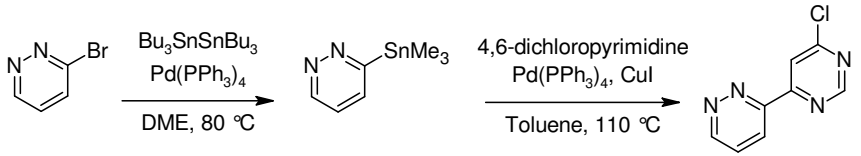
Intermediate 1, 4-chloro-6-(4-fluoro-1H-pyrazol-1-yl)pyrimidine.



Intermediate 1

A mixture of 4,6-dichloropyrimidine (908 mg, 4.57 mmol), 4-fluoropyrazole (551 mg, 6.40 mmol) and K_2CO_3 (884 mg, 6.40 mmol) in DMF (20 mL) was allowed to warm from 0ºC to rt with stirring over 17 h. After addition to H_2O (30 mL) and stirring for 5 min a white solid was isolated by filtration. Purification by gradient flash chromatography, eluting with 0-10% EtOAc in isohexane yielded the title compound (622 mg, 3.13 mmol) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, *J*=3.9 Hz, 1H), 7.94 (d, *J*=0.8 Hz, 1H), 8.40 (d, *J*=3.9 Hz, 1H), 8.79 (s, 1H).

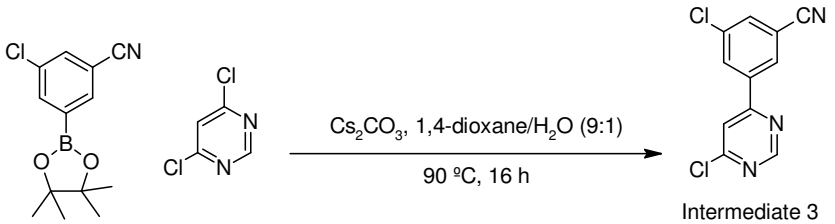
Intermediate 2, 3-(6-chloropyrimidin-4-yl)pyridazine.



Intermediate 2

The title compound (250 mg, 1.30 mmol) was prepared in two steps from 3-bromopyridazine (500 mg, 3.14 mmol), hexabutylditin (1.23 mL, 2.43 mmol) and 4,6-dichloropyrimidine (300 mg, 2.01 mmol) using the methods of **25**, HTL14242, and was used immediately in the next step without characterization.

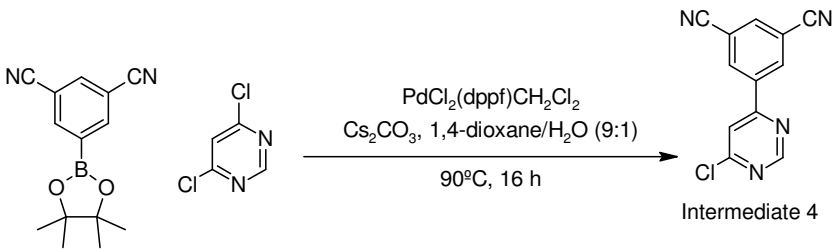
Intermediate 3, 3-chloro-5-(6-chloropyrimidin-4-yl)benzonitrile.



Intermediate 3

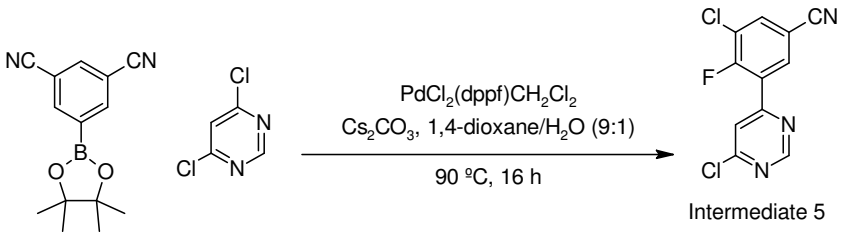
The title compound (4.0 g, 16.0 mmol) was prepared from 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (17.6 g, 66.8 mmol) and 4,6-dichloropyrimidine (9.0 g, 60.4 mmol) using the methods of **25**, HTL14242. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.27 (s, 1H), 8.53 (s, 1H), 8.59 (s, 1H), 8.66 (s, 1H), 9.16 (s, 1H).

Intermediate 4, 5-(6-chloropyrimidin-4-yl)benzene-1,3-dicarbonitrile.



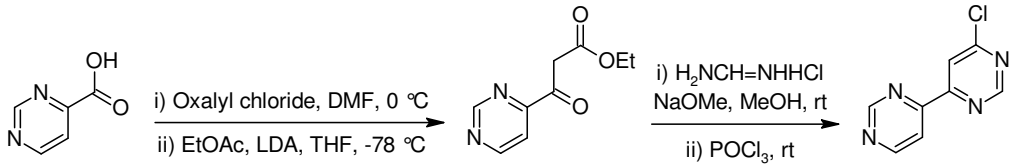
The title compound (1.70 g, 7.06 mmol) was prepared from 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isophthalonitrile (5.60 g, 22.0 mmol) and 4,6-dichloropyrimidine (3.28 g, 22.0 mmol) using the methods of **25**, HTL14242. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.60 (d, *J*=1.2 Hz, 1H), 8.70-8.74 (m, 1H), 9.00 (d, *J*=1.5 Hz, 2H), 9.22 (d, *J*=0.9 Hz, 1H).

Intermediate 5, 3-chloro-5-(6-chloropyrimidin-4-yl)-4-fluorobenzonitrile.



The title compound (110 mg, 0.41 mmol) was prepared from 3-chloro-4-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (245 mg, 0.87 mmol) and 4,6-dichloropyrimidine (200 mg, 1.34 mmol) using the methods of **25**, HTL14242. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.21 (s, 1H), 8.43-8.48 (m, 1H), 8.51-8.58 (m, 1H), 9.26 (d, *J*=1.2 Hz, 1H).

Intermediate 6, 6-chloro-4,4'-bipyrimidine.



Intermediate 6

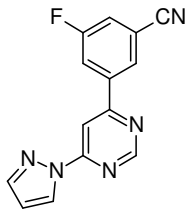
4-Pyrimidinecarboxylic acid (3.0 g, 24.2 mmol) in DMF (0.01 mL) and CH_2Cl_2 (60 mL) was cooled to 0 ºC before the dropwise addition over 10 min of oxalyl chloride (2.7 mL, 31.5 mmol). After stirring at rt for 2 h the reaction mixture was concentrated *in vacuo* and the resulting crude acid chloride redissolved in THF (10 mL). Separately, EtOAc (8.3 mL, 84.6 mmol) was dissolved in THF (30 mL) and cooled to -78 ºC before the dropwise addition of LDA (36.0 mL of a 2 M solution in THF, 72.0 mmol). After stirring at -78 ºC for 1 h the THF solution of the crude acid chloride was added and the mixture stirred at -78 ºC for 3 h. Aqueous HCl (1N, 25 mL) was added, followed by H_2O (100 mL) and EtOAc (100 mL), and the phases were separated. The aqueous phase was extracted with EtOAc (3 x 100 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by gradient flash chromatography, eluting with 0-8% EtOAc in hexane yielded ethyl 3-oxo-3-(pyrimidin-4-yl)propanoate (0.40 g, 2.06 mmol) as a white solid. TLC: R_f 0.6, Hexane / EtOAc 4:1.

Ethyl 3-oxo-3-(pyrimidin-4-yl)propanoate (1.2 g, 6.18 mmol), sodium methoxide (1.33 g, 24.6 mmol) and formamidine hydrochloride (1.0 g, 12.4 mmol) were dissolved in MeOH (20 mL) and stirred at rt for 24 h before concentration *in vacuo*. H_2O (50 mL) and EtOAc (50 mL) were added and the phases were separated. The aqueous phase was extracted with EtOAc (3 x 50 mL) and the combined organic phases were dried (Na_2SO_4), and concentrated *in vacuo* to yield crude [4,4'-bipyrimidin]-6-ol (200 mg) which was used in the next step without further purification. TLC: R_f 0.1, EtOAc.

Crude [4,4'-Bipyrimidin]-6-ol (120 mg, 0.69 mmol) was dissolved in phosphorus(V) oxychloride (4.0 mL, 42.9 mmol) and stirred at rt for 14 h. The mixture was neutralized to approximately pH 7 with saturated aqueous $NaHCO_3$ solution (30 mL) at 0 °C and stirred for 15 min before the addition of EtOAc (300 mL) and H_2O (100 mL). The phases were separated, the aqueous phase was extracted with EtOAc (300 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. Purification by gradient flash chromatography, eluting with 0-9% EtOAc in hexane yielded the title compound (42 mg, 0.22 mmol) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.43 (dd, *J*=5.2, 1.2 Hz, 1H), 8.49 (d, *J*=1.2 Hz, 1H), 9.13 (d, *J*=4.9 Hz, 1H), 9.29 (d, *J*=0.9 Hz, 1H), 9.45 (d, *J*=1.2 Hz, 1H).

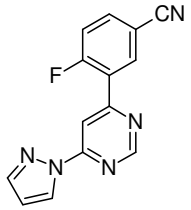
Synthesis of final compounds by Route 1.

6, 3-fluoro-5-[6-(1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzonitrile.



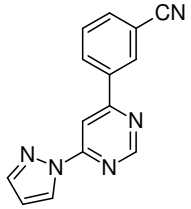
The title compound (1.4 g, 5.3 mmol) was prepared from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (1.0 g, 5.53 mmol) and (3-cyano-5-fluorophenyl)boronic acid (1.1 g, 1.24 mmol) at 80 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

7, 4-fluoro-3-[6-(1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzonitrile.



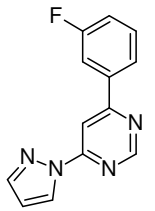
The title compound (115 mg, 0.43 mmol) was prepared from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (180 mg, 1.00 mmol) and (5-cyano-2-fluorophenyl)boronic acid (197 mg, 1.19 mmol) at 80 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

8, 3-[6-(1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzonitrile.



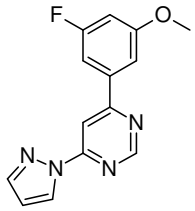
The title compound (45 mg, 0.18 mmol) was prepared from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (120 mg, 0.66 mmol) and 3-cyanophenylboronic acid (117 mg, 0.80 mmol) at 100 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

9, 4-(3-fluorophenyl)-6-(1*H*-pyrazol-1-yl)pyrimidine



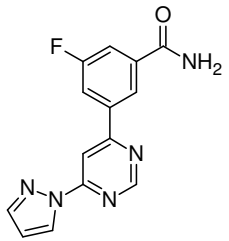
The title compound (40 mg, 0.17 mmol) was prepared from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (90 mg, 0.50 mmol) and 3-fluorophenylboronic acid (83 mg, 0.60 mmol) at 100 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

10, 4-(3-fluoro-5-methoxyphenyl)-6-(1*H*-pyrazol-1-yl)pyrimidine



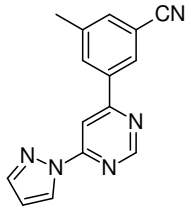
The title compound (91 mg, 0.34 mmol) was prepared from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (90 mg, 0.50 mmol) and 3-fluoro-5-methoxyphenylboronic acid (102 mg, 0.60 mmol) at 80 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

11, 3-fluoro-5-[6-(1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzamide



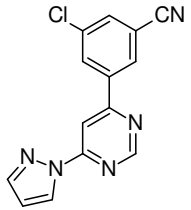
The title compound (155 mg, 0.55 mmol) was prepared from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (150 mg, 0.83 mmol) and 3-carbamoyl-5-fluorophenylboronic acid (182 mg, 1.00 mmol) at 100 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

12, 3-methyl-5-[6-(1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzonitrile.



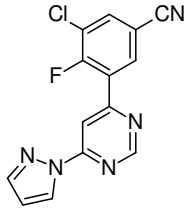
The title compound (27 mg, 0.10 mmol) was prepared from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (225 mg, 1.25 mmol) and (3-cyano-5-methylphenyl)boronic acid (200 mg, 1.24 mmol) at 90 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

13, 3-chloro-5-[6-(1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzonitrile



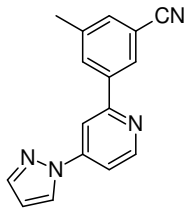
The title compound (26 mg, 0.09 mmol) was synthesized from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (130 mg, 0.72 mmol) and 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (190 mg, 0.72 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

14, 3-chloro-4-fluoro-5-[6-(1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzonitrile



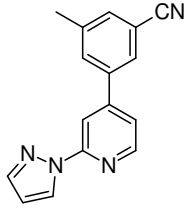
The title compound (61 mg, 0.20 mmol) was synthesized from 4-chloro-6-(1*H*-pyrazol-1-yl)pyrimidine (169 mg, 0.60 mmol) and 3-chloro-4-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (90 mg, 0.50 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

15, 3-methyl-5-[4-(1*H*-pyrazol-1-yl)pyridin-2-yl]benzonitrile



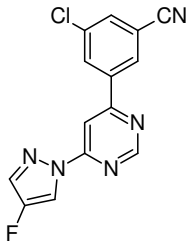
The title compound (99 mg, 0.38 mmol) was synthesized from 2-chloro-4-(1*H*-pyrazol-1-yl)pyridine (90 mg, 0.50 mmol) and 3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (112 mg, 0.50 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

16, 3-methyl-5-[2-(1*H*-pyrazol-1-yl)pyridin-4-yl]benzonitrile



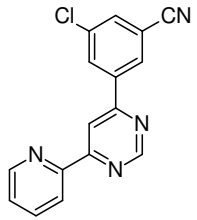
The title compound (100 mg, 0.38 mmol) was synthesized from 4-bromo-2-(1*H*-pyrazol-1-yl)pyridine (112 mg, 0.50 mmol) and 3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (112 mg, 0.50 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

17, 3-chloro-5-[6-(4-fluoro-1*H*-pyrazol-1-yl)pyrimidin-4-yl]benzonitrile



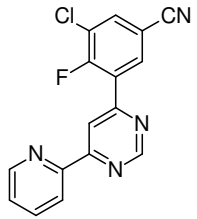
The title compound (37 mg, 0.12 mmol) was synthesized from 4-chloro-6-(4-fluoro-1*H*-pyrazol-1-yl)pyrimidine (Intermediate 1, 144 mg, 0.73 mmol) and 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (248 mg, 0.94 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

21, 3-chloro-5-[6-(pyridin-2-yl)pyrimidin-4-yl]benzonitrile



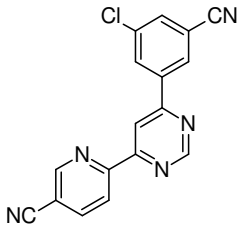
The title compound (45 mg, 0.15 mmol) was synthesized from 4-chloro-6-(pyridin-2-yl)pyrimidine (200 mg, 1.04 mmol) and 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (275 mg, 1.04 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

22, 3-chloro-4-fluoro-5-[6-(pyridin-2-yl)pyrimidin-4-yl]benzonitrile.



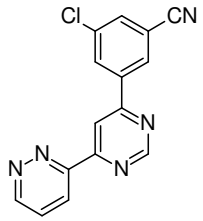
The title compound (35 mg, 0.11 mmol) was synthesized from 4-chloro-6-(pyridin-2-yl)pyrimidine (96 mg, 0.50 mmol) and 3-chloro-4-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (169 mg, 0.60 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

26, 6-[6-(3-chloro-5-cyanophenyl)pyrimidin-4-yl]pyridine-3-carbonitrile.



The title compound (35 mg, 0.11 mmol) was synthesized from 6-(6-chloropyrimidin-4-yl)pyridine-3-carbonitrile (170 mg, 0.78 mmol) and 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (207 mg, 0.79 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

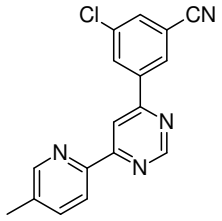
29, 3-chloro-5-[6-(pyridazin-3-yl)pyrimidin-4-yl]benzonitrile.



The title compound (20 mg, 0.07 mmol) was synthesized from 3-(6-chloropyrimidin-4-yl)pyridazine (Intermediate 2, 250 mg, 1.30 mmol) and 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (315 mg, 1.20 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

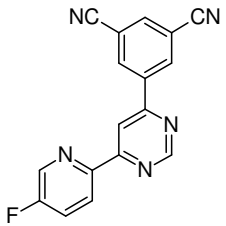
Synthesis of final compounds by Route 2.

23, 3-chloro-5-[6-(5-methylpyridin-2-yl)pyrimidin-4-yl]benzonitrile



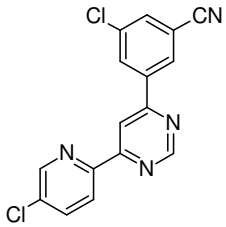
The title compound (45 mg, 0.15 mmol) was synthesized from 3-chloro-5-(6-chloropyrimidin-4-yl)benzonitrile (Intermediate 3, 90 mg, 0.36 mmol) and 5-methyl-2-(tributylstannyl)pyridine (151 mg, 0.40 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

24, 5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzene-1,3-dicarbonitrile



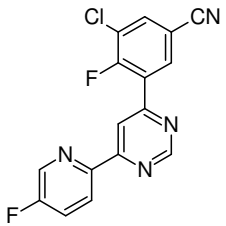
The title compound (950 mg, 3.15 mmol) was synthesized in two steps from 5-(6-chloropyrimidin-4-yl)benzene-1,3-dicarbonitrile (Intermediate 4, 1.50 g, 6.23 mmol) and 2-bromo-5-fluoropyridine (3.0 g, 17.0 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

27, 3-chloro-5-[6-(5-chloropyridin-2-yl)pyrimidin-4-yl]benzonitrile



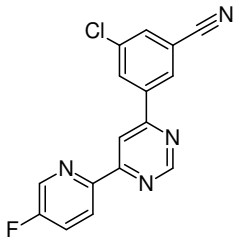
The title compound (50 mg, 0.15 mmol) was synthesized from 3-chloro-5-(6-chloropyrimidin-4-yl)benzonitrile (Intermediate 3, 130 mg, 0.52 mmol) and 5-chloro-2-(tributylstannyl)pyridine (250 mg, 0.62 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

28, 3-chloro-4-fluoro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzonitrile



The title compound (45 mg, 0.14 mmol) was synthesized from 3-chloro-5-(6-chloropyrimidin-4-yl)-4-fluorobenzonitrile (Intermediate 5, 100 mg, 0.37 mmol) and 5-fluoro-2-(trimethylstannyl)pyridine (97 mg, 0.37 mmol) using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

Synthesis of 25, HTL14242, 3-chloro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzonitrile by Route 3.

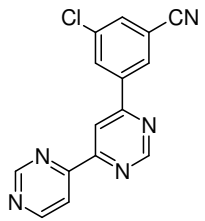


To a dried flask under nitrogen was added *i*-PrMgCl (2M in THF, 11.2 mL, 22.4 mmol) and 2-bromo-5-fluoropyridine (3.94 g, 22.4 mmol) in THF (40 mL). After stirring at rt for 3.5 h $ZnCl_2$ (0.5 M in THF, 51.2 mL, 25.6 mmol) was added dropwise maintaining the temperature below 25°C and the mixture was stirred for a further 1 h. Separately, under nitrogen to a solution of 3-chloro-5-(6-

chloropyrimidin-4-yl)benzonitrile (Intermediate 3, 4.00 g, 16.0 mmol) in THF (80 mL) was added tetrakis(triphenylphosphine)palladium (924 mg, 0.80 mmol) and the previously prepared zincate solution was then added dropwise and the mixture was heated to 50-60°C for 18 h. After cooling to rt the mixture was evaporated to approximately 10% of its original volume, diluted with EtOAc (400 mL) and washed with water (500 mL). The aqueous phase was then extracted with EtOAc (3 x 400 mL) and the combined organic phases were washed with brine (500 mL), dried (anhydrous Na_2SO_4), filtered and concentrated *in vacuo*. Purification by flash chromatography, eluting with 5% EtOAc in hexane yielded the title compound (2.97 g, 9.56 mmol) as a white solid. The purified compound was combined with product batches from other reactions (17.6 g total material) and re-crystallised from hot EtOAc to yield the title compound (12.5 g, 40.2 mmol) as a white solid. See main manuscript for ^{1}H, ^{19}F and ^{13}C NMR data.

Synthesis of final compounds by Route 4.

30, 3-(4,4'-bipyrimidin-6-yl)-5-chlorobenzonitrile.



The title compound (27 mg, 0.09 mmol) was prepared from 6-chloro-4,4'-bipyrimidine (Intermediate 6, 40 mg, 0.21 mmol) and crude 3-chloro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (50 mg) at 100 ºC using the methods of **25**, HTL14242. See Table 1 for LCMS and ^{1}H NMR data.

Table 3. Crystallographic statistics.

DATA COLLECTION	**$mGlu_5$-14**	**$mGlu_5$-25**
Number of crystals	9	9
Space Group	C121	C121
Cell Dimensions a, b, c, (Å)	142.5, 43.8, 81.4	143.6, 43.7, 81.9
Cell Angles α, β, γ (°)	90, 99, 90	90, 99, 90
Resolution (Å)	3.10	2.60
R_{merge} *	0.194 (0.522)	0.152 (0.723)
I / σ I *	7.0 (2.4)	7.0 (2.0)
Completeness (%) *	91.4 (93.4)	95.5 (96.5)
Redundancy *	3.0 (3.0)	3.3 (3.3)
REFINEMENT		
Resolution (Å)	30.0-3.10	30.0-2.60
No. Reflections	8,350	14,884
R_{work} / R_{free} (%)	23.4 / 28.6	24.2 / 28.5
No. atoms		
Protein	3,204	3,196
Ligand	21	22
B-factors		
Protein ($Å^2$)	48.3	37.0

Ligand (Å^2)	43.6	24.7
R.m.s. deviations		
Bond lengths (Å)	0.002	0.002
Bond Angles (°)	0.649	0.616
Ramachandran Plot: Preferred (%)	96.8	98.0
Allowed (%)	3.2	2.0
Outlier (%)	0.0	0.0

*Statistics in parentheses refer to outer resolution shell.

Selectivity data for 25, HTL14242

Table 4. Cross-screening data for **25**, HTL14242: GPCR, kinase, ion-channel and transporter targets (data generated at Eurofins, www.eurofinspanlabs.com/Panlabs). Data are expressed as percent inhibition of specific binding or activity at 10 μM **25**.

Monoamine Oxidase MAO-A	30%	Glutamate, AMPA	0%
Nitric Oxide Synthase, Neuronal (nNOS)	-8%	Glutamate, Kainate	12%
Protein Serine/Threonine Kinase, AKT1 (PRKBA)	3%	Glutamate, NMDA, Glycine	2%
Protein Serine/Threonine Kinase, AURKA (Aurora-A)	20%	Melanocortin MC_4	33%
Protein Serine/Threonine Kinase, CDK2/CCNA2 (cdk2/cyclin A)	-8%	Muscarinic M1	8%
Protein Serine/Threonine Kinase, CHK1	-20%	Neuropeptide Y Y_1	-6%
Protein Serine/Threonine Kinase, PRKCA (PKCα)	1%	Nicotinic Acetylcholine α	-2%
Adenosine A_{2A}	47%	Opiate μ (OP3, MOP)	25%
Adrenergic β_2	-14%	Purinergic P2Y	-3%
Bradykinin B_1	-2%	Serotonin (5-hydroxytryptamine) 5-HT_{1A}	9%
Calcium Channel N-type	6%	Serotonin (5-hydroxytryptamine) 5-HT_{1B}	2%
Cannabinoid CB_1	17%	Serotonin (5-hydroxytryptamine) 5-HT_{2C}	-6%
Dopamine D_1	7%	Tachykinin NK_2	10
$GABA_{B1A}$	-15%	Transporter, Norepinephrine (NET)	31
$GABA_{B1B}$	-10%	Transporter, Serotonin (5-hydroxytryptamine) (SERT)	-2

Table 5. Cross-screening data for **25**, HTL14242: mGlu receptor profiling. (data generated at Euroscreen, www.euroscreen.com). Data are from recombinant human mGlu receptors using Aequorin, cAMP HTRF™ or GTPγS functional assays and are expressed as percent activation (agonist and PAM modes) or percent inhibition (antagonist/NAM mode) at 10 or 15 μM **25**.

Receptor	Mode	Screening concentration	% Activation or Inhibition
$mGluR_1$	Agonist	15 μM	< 1%
$mGluR_1$	PAM	10 μM	< 1%
$mGluR_1$	Antagonist/NAM	10 μM	< 1%
$mGluR_2$	Agonist	15 μM	21%
$mGluR_2$	PAM	10 μM	1 %
$mGluR_2$	Antagonist/NAM	10 μM	24%
$mGluR_3$	Agonist	15 μM	< 1%
$mGluR_3$	PAM	10 μM	< 1%
$mGluR_3$	Antagonist/NAM	10 μM	47%
$mGluR_4$	Agonist	15 μM	< 1%
$mGluR_4$	PAM	10 μM	< 1%
$mGluR_4$	Antagonist/NAM	10 μM	12%
$mGluR_5$	Agonist	15 μM	< 1%
$mGluR_5$	PAM	10 μM	< 1%
$mGluR_5$	Antagonist/NAM	10 μM	97%
$mGlu_6$	Agonist	15 μM	< 1%
$mGlu_6$	PAM	10 μM	18%
$mGlu_6$	Antagonist/NAM	10 μM	3%
$mGlu_7$	Agonist	15 μM	7%
$mGlu_7$	PAM	10 μM	16%
$mGlu_7$	Antagonist/NAM	10 μM	< 1%

$mGlu_8$	Agonist	15 μM	3%
$mGlu_8$	PAM	10 μM	14%
$mGlu_8$	Antagonist/NAM	10 μM	< 1%

***Ex vivo* autoradiography**

Sprague Dawley rats (male; 250-300 g) were dosed orally with either vehicle (10% DMA + 10% Solutol HS15 + 80% (10% aqueous (2-hydroxypropyl)-β-cyclodextrin) or **25**, HTL14242 (1, 3 and 10 mg/kg). One hour post-dose, animals were sacrificed and whole brains removed, rinsed and blot dried. A coronal block was cut containing the hippocampus and divided along the mid-line and rapidly frozen in isopentane for sectioning and autoradiography. Coronal half-brain sections were cut 20 μm thick, approximately 4 mm posterior to the bregma, to incorporate the hippocampal CA3 region. Three adjacent sections were mounted onto slides and incubated with 2 nM [^{3}H]-M-MPEP (total binding) or 2 nM [^{3}H]-M-MPEP and 10 μM fenobam (non-specific binding, Tocris Bioscience, catalogue number 2386) for 10 min at rt. Binding was terminated by aspiration and washing with ice-cold assay buffer (4 x 5 min) and sections allowed to air dry. Levels of bound radioactivity in the sections were determined using a beta imager over a 16 h period. Occupancy was determined as mean specific binding with the vehicle treated control taken as 100%.

Mouse marble burying assay

Prior to each test, 24 small glass marbles (diameter ~10 mm) were evenly spaced and arranged in a grid-like fashion across the surface of the bedding. Thirty minutes before the test, 75 Male CD-1 mice (n=15/group) were orally administrated (po) with vehicle (10% of Solutol HS15 + 90% of 10%w/v aqueous HPβCD) or **25**, HTL14242 (1, 3, 10 or 30 mg/kg). After 30 min pre-treatment time, mice were placed individually in a cage containing the marbles. Thirty minutes later the animals were removed from the cages, and the number of marbles buried by at least 2/3 into sawdust bedding were counted and recorded.