Supplementary Information for Manuscript Entitled Rapid identification of novel allosteric PRC2 inhibitors

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GENERAL EXPERIMENTAL CONDITIONS

All reagents and solvents were sourced from commercial suppliers and used without further purification. All reactions were performed in dried glass reaction tubes or round bottom flasks equipped with a magnetic stir bar under a nitrogen atmosphere; all solvents were anhydrous. Microwave reactors used were biotage initiators.

Normal phase flash chromatography:
Normal phase purifications were performed on an automated Teledyne Isco CombiFlash® Rf, using prepacked Puriflash, High Capacity Silica Columns (50 µm, spherical particles).

NMR spectra:
NMR spectra were obtained on Bruker Avance 500 (500 MHz) system using d_6-DMSO as solvent. Measurements were taken at ambient temperature unless otherwise specified, and the following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; ddd, doublet of doublet of doublet; dq, double of quartets; dt, doublet of triplets; tt, triplet of triplets; p, pentet.

UPLC conditions:
UPLC was carried out using a Waters UPLC fitted with a Waters SQD, SQD2 or QDA mass spectrometer with mass Spec = ESI with positive/negative switching

A:	0.1 % NH_3 in water
B:	acetonitrile
Column:	Waters Acquity CSH™ C18 1.7 µm 2.1 x 50 mm
Gradient:	97% A/3% B to 3% A/97% B over 1.5 min
UV:	220 nm - 320 nm
Temperature:	40 °C

Flow rate: 1 ml/min

High Resolution Mass Spectrometry Accurate Mass condtions:

The High Resolution Mass Spectrometer is run in Electrospray (ESI) +ve or -ve ion mode and automatic MSMS using CID at 35eV is carried out automatically on the two biggest ions generated from MS1.

Mass range:	100 – 1000 amu
Mass Spectrometer:	Orbitrap XL or Waters Xevo Qtof
Gradient:	Acid and Base mobile phase eluent available consisting of A = aqueous 0.1% Formic acid (0.1% ammonium hydroxide) and B= Acetonitrile 0.1% formic acid, (0.1% ammonium hydroxide) and runs a 95%A to 5%A gradient at 0.7mL/min over 4.0 mins. There is a 0.5 min hold and a return to 95%A by 5 minutes.
Total run time:	5 mins
Column:	Waters CSH 50 x 2.1 BEH
Temperature:	45°C.
Injection volume:	1 – 5uL
UV:	220 to 400nm (PDA detector).
Sample preparation:	No greater than 0.5mg/mL solution in DMSO/methanol

Preparative HPLC conditions:

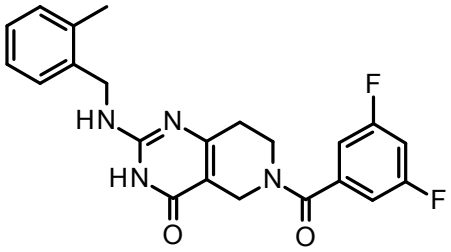
Preparative HPLC was performed on an a fully automated Gilson preparative HPLC-MS system, fitted with a Gilson UV / Vis 151 detector, GX-271 liquid handler and PerkinElmer SQ 300 MS Detector.

Method 1	*acidic conditions*
Column	Waters CSH C18 OBD column, 5µ silica, 30 mm diameter, 100 mm length
Temperature	Ambient
Detection	UV-MS
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	acetonitrile
Gradient initial	0% B, 1 mins- 5% B; 7 mins – 98% B; 9 mins – 98% B; 9.1 mins – 5% B; 10 mins -5% B
Flow rate	50 mL/min
Method 2	*basic conditions*
Column	Waters CSH C18 OBD column, 5µ silica, 30 mm diameter, 100 mm length
Temperature	Ambient
Detection	UV-MS
Mobile Phase A	0.1% NH_3 in water
Mobile Phase B	acetonitrile
Gradient initial	0% B, 1 mins- 5% B; 7 mins – 98% B; 9 mins – 98% B; 9.1 mins – 5% B; 10 mins -5% B
Flow rate	50 mL/min

Purity criteria: Final compounds isolated as singletons with >95% purity based on UPLC and/or 1H NMR.

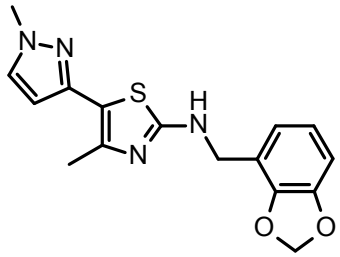
SYNTHESIS AND CHARACTERISATION OF COMPOUNDS

6-(3,5-difluorobenzoyl)-2-(o-tolylmethylamino)-3,5,7,8-tetrahydropyrido[4,3-d]pyrimidin-4-one



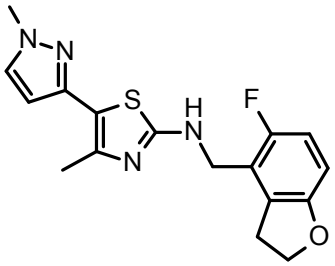
1H NMR (500 MHz, DMSO) 2.30 (s, 3H), 2.48-2.55 (m, 2H), 3.4 – 3.53 (m, 1H), 3.73 – 3.92 (m, 1H), 3.96 – 4.16 (m, 1H), 4.21 – 4.37 (m, 1H), 4.43 (d, *J* = 5.5 Hz, 2H), 6.58 – 6.8 (m, 1H), 7.13 – 7.2 (m, 3H), 7.2 – 7.27 (m, 3H), 7.38 (s, 1H), 10.77 (s, 1H); **m/z:** ES+ [M+H]+ 411; **HRMS (ESI):** calculated for $C_{22}H_{20}N_4O_2F_2$ [M+H]+: 411.1633, found 411.1636, error 0.7 ppm.

N-(1,3-benzodioxol-4-ylmethyl)-4-methyl-5-(1-methylpyrazol-3-yl)thiazol-2-amine



1H NMR (500 MHz, DMSO) 2.03 (s, 3H), 3.71 (s, 3H), 4.42 (d, *J* = 5.7 Hz, 2H), 6.04 (s, 2H), 6.29 (d, *J* = 1.9 Hz, 1H), 6.82 – 6.89 (m, 3H), 7.45 (d, *J* = 1.9 Hz, 1H), 8.16 (t, *J* = 5.7 Hz, 1H); **m/z:** ES+ [M+H]+ 329; **HRMS (ESI):** calculated for $C_{16}H_{16}N_4O_2S$ [M+H]+: 329.1067, found 329.1064, error 0.8 ppm.

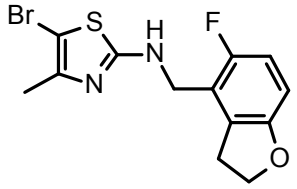
N-[(5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl]-4-methyl-5-(1-methylpyrazol-3-yl)thiazol-2-amine



Into a microwave vial with stirring bar was added Xphos G2 Pd (19.26 mg, 0.01 mmol), potassium carbonate (72.5 mg, 0.52 mmol), 1-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (43.6 mg, 0.21 mmol), 5-bromo-N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methylthiazol-2-amine (60 mg, 0.17 mmol), 1,4-dioxane (1192 µl) and water (397 µl). The mixture was degassed with nitrogen for 2 mins and sealed. The reaction mixture was stirred at 100 °C for 1 hour in the microwave reactor and cooled to room temperature. The reaction mixture was diluted with DCM (50 mL) and water (50

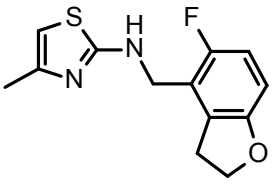
mL), separated and the aqueous layer extracted with DCM (2 x 30 ml). The combined organics were washed with water (50 ml) and brine (25 ml), dried over $MgSO_4$, filtered and evaporated to dryness to afford crude product as a brown gum. The crude product was purified by preparative HPLC (Waters CSH C18 OBD column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methyl-5-(1-methyl-1H-pyrazol-3-yl)thiazol-2-amine (33.0 mg, 54.8 %) as a white solid. **1H NMR** (500 MHz, DMSO) 2.28 (s, 3H), 3.28 – 3.32 (m, 2H), 3.80 (s, 3H), 4.40 (d, *J* = 5.3 Hz, 2H), 4.55 (t, *J* = 8.7 Hz, 2H), 6.29 (d, *J* = 2.3 Hz, 1H), 6.67 (dd, *J* = 8.6, 3.9 Hz, 1H), 6.85 – 6.96 (m, 1H), 7.68 (d, *J* = 2.2 Hz, 1H), 7.86 (t, *J* = 5.4 Hz, 1H); **13C NMR** (126 MHz, DMSO) 17.14, 29.06, 38.83, 40.11, 71.97, 103.27, 108.54 (*J* = 8.5), 111.98, 114.22 (*J* = 24.7), 122.94 (*J* = 18.4), 129.37 (*J* = 5.1), 132.41, 143.94, 144.57, 155.65 (*J* = 236.1), 156.09, 165.28; **m/z:** ES+ [M+H]+ 345; **HRMS (ESI):** calculated for $C_{17}H_{17}N_4OFS$ [M+H]+: 345.1185, found 345.1179, error 1.7 ppm.

5-bromo-N-[(5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl]-4-methyl-thiazol-2-amine



To a stirred solution of N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methylthiazol-2-amine (475 mg, 1.80 mmol) in DCM (15 ml) was added 1-bromopyrrolidine-2,5-dione (352 mg, 1.98 mmol). The resultant reaction mixture was stirred at room temperature for 30 mins. The reaction mixture was diluted with DCM (100 mL) and water (100 mL) and the layers were separated. The aqueous layer was extracted with futher DCM (2 x 100 ml) and the combined organic layers were further washed with water (100 mL) and brine (50 ml), dried over $MgSO_4$, filtered and evaporated to afford crude product as a dark brown solid. The crude product was purified by flash silica chromatography (24 g column), elution gradient 0 to 30 % EtOAc in heptane. Pure fractions were evaporated to dryness to afford 5-bromo-N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methylthiazol-2-amine (350 mg, 56.7 %) as a pale yellow solid. **1H NMR** (500 MHz, DMSO) 2.08 (s, 3H), 3.28 (t, *J* = 8.7 Hz, 2H), 4.37 (d, *J* = 5.4 Hz, 2H), 4.55 (t, *J* = 8.8 Hz, 2H), 6.68 (dd, *J* = 8.6, 3.9 Hz, 1H), 6.91 (dd, *J* = 10.1, 8.7 Hz, 1H), 8.03 (t, *J* = 5.4 Hz, 1H); **m/z:** ES+ [M+H]+ 343.

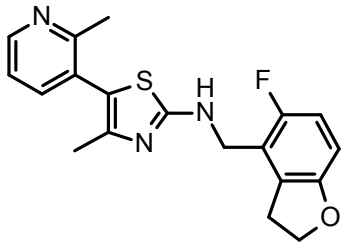
N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methylthiazol-2-amine



Toluene (36 ml) was added to Brettphos Pd G3 (477 mg, 0.53 mmol), 2-bromo-4-methylthiazole (750 mg, 4.21 mmol), sodium tert-butoxide (1053 mg, 10.95 mmol), and (5-fluoro-2,3-dihydrobenzofuran-4-yl)methanamine (1056 mg, 6.32 mmol) under nitrogen, split across 3 microwave vials. Each vial was degassed for 2 minutes and stirred at 100 °C for

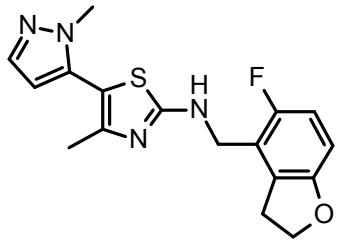
1 hour in the microwave reactor and cooled to room temperature. The combined reaction mixture was diluted with water (150 mL), and extracted with EtOAc (3 x 150 ml). The combined organic layers were washed with water (150 ml) and brine (75 ml), dried over $MgSO_4$, filtered and evaporated to afford crude product as a dark orange gum. The crude product was purified by flash silica chromatography (80 g column), elution gradient 0 to 50 % EtOAc in heptane. Pure fractions were evaporated to dryness to afford N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methylthiazol-2-amine (399 mg, 35.8 %) as a brown solid. **1H NMR** (500 MHz, DMSO) 2.10 (d, *J* = 1.1 Hz, 3H), 3.29 (d, *J* = 8.7 Hz, 2H), 4.37 (d, *J* = 5.3 Hz, 2H), 4.54 (t, *J* = 8.8 Hz, 2H), 6.15 (d, *J* = 1.1 Hz, 1H), 6.66 (dd, *J* = 8.6, 3.9 Hz, 1H), 6.90 (dd, *J* = 10.2, 8.7 Hz, 1H), 7.75 (t, *J* = 5.4 Hz, 1H); **m/z:** ES+ [M+H]+ 265.

N-[(5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl]-4-methyl-5-(2-methyl-3-pyridyl)thiazol-2-amine



Into a microwave vial with stirring bar was added Xphos G2 Pd (8.02 mg, 5.10 µmol), potassium carbonate (30.2 mg, 0.22 mmol), 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (19.15 mg, 0.09 mmol), 5-bromo-N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methylthiazol-2-amine (25 mg, 0.07 mmol), 1,4-dioxane (497 µl) and water (166 µl). The mixture was degassed with nitrogen for 2 mins and sealed. The reaction mixture was stirred at 100 °C for 1 hour in the microwave reactor and cooled to room temperature. The reaction micture was diluted with DCM (50 mL) and water (50 mL), separated and the aqueous layer extracted with DCM (2 x 30 ml). The combined organics were washed with water (50 ml) and brine (25 ml), dried over $MgSO_4$, filtered and evaporated to dryness to afford crude product as a black gum. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness and repurified by flash silica chromatography (4 g column), elution gradient 30 to 100 % EtOAc in heptane. Pure fractions were evaporated to dryness to afford N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methyl-5-(2-methylpyridin-3-yl)thiazol-2-amine (22.00 mg, 85 %) as a white gum. **1H NMR** (500 MHz, DMSO) 1.94 (s, 3H), 2.40 (s, 3H), 3.35 (t, *J* = 8.8 Hz, 2H), 4.41 (d, *J* = 5.3 Hz, 2H), 4.57 (t, *J* = 8.7 Hz, 2H), 6.68 (dd, *J* = 8.6, 3.9 Hz, 1H), 6.84 – 6.98 (m, 1H), 7.25 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.61 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.93 (t, *J* = 5.3 Hz, 1H), 8.43 (dd, *J* = 4.8, 1.7 Hz, 1H); **13C NMR** (126 MHz, DMSO) 16.05, 23.53, 29.03, 40.09, 71.98, 108.60 (*J* = 8.6), 114.18, 114.24 (*J* = 24.7), 121.60, 122.82 (*J* = 18.4), 127.48, 129.52 (*J* = 5.2), 139.47, 145.14, 148.74, 155.69 (*J* = 236.2), 156.06, 157.67, 166.73; **m/z:** ES+ [M+H]+ 356; **HRMS (ESI):** calculated for $C_{19}H_{18}N_3OFS$ [M+H]+: 356.1233, found 356.1227, error 1.7 ppm.

N-[(5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl]-4-methyl-5-(2-methylpyrazol-3-yl)thiazol-2-amine



Into a microwave vial with stirring bar was added Xphos G2 Pd (19.26 mg, 0.01 mmol), potassium carbonate (72.5 mg, 0.52 mmol), 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (43.6 mg, 0.21 mmol), 5-bromo-N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methylthiazol-2-amine (60 mg, 0.17 mmol), 1,4-dioxane (1192 µl) and water (397 µl). The mixture was degassed with nitrogen for 2 mins and sealed. The reaction mixture was stirred at 100 °C for 1 hour in the microwave reactor and cooled to room temperature. The reaction micture was diluted with DCM (50 mL) and water (50 mL), separated and the aqueous layer extracted with DCM (2 x 30 ml). The combined organics were washed with water (50 ml) and brine (25 ml), dried over $MgSO_4$, filtered and evaporated to dryness to afford crude product (98 mg) as a brown gum. The crude product was purified by preparative HPLC (Waters CSH C18 OBD column, 5µ silica, 30 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford N-((5-fluoro-2,3-dihydrobenzofuran-4-yl)methyl)-4-methyl-5-(1-methyl-1H-pyrazol-5-yl)thiazol-2-amine (36.0 mg, 59.8 %) as a cream solid. **1H NMR** (500 MHz, DMSO) 2.04 (s, 3H), 3.33 – 3.37 (m, 2H), 3.70 (s, 3H), 4.42 (d, *J* = 5.3 Hz, 2H), 4.56 (t, *J* = 8.7 Hz, 2H), 6.28 (d, *J* = 1.8 Hz, 1H), 6.68 (dd, *J* = 8.6, 3.9 Hz, 1H), 6.86 – 6.98 (m, 1H), 7.45 (d, *J* = 1.8 Hz, 1H), 8.10 (t, *J* = 5.4 Hz, 1H); **13C NMR** (126 MHz, DMSO) 16.44, 29.02, 37.32, 40.09, 71.98, 104.63, 108.29, 108.65 (*J* = 8.7), 114.25 (*J* = 24.5), 122.66 (*J* = 18.3), 129.48 (*J* = 5.3), 134.16, 138.48, 148.06, 155.67 (*J* = 236.5), 156.07, 167.55; **m/z:** ES+ [M+H]+ 345; **HRMS (ESI):** calculated for $C_{17}H_{17}N_4OFS$ [M+H]+: 345.1185, found 345.1179, error 1.7 ppm.