Supporting Information

Identification of (R)-N-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxamide (CPI-1205), a Potent and Selective Inhibitor of Histone Methyltransferase EZH2, Suitable for Phase I Clinical Trials for B-Cell Lymphomas

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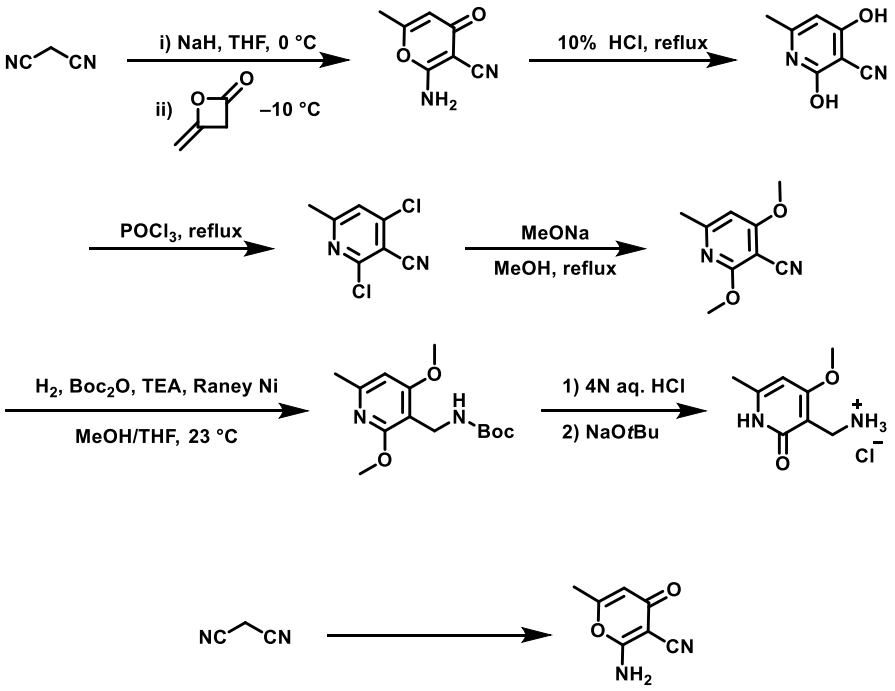
Methods and Materials

All commercial reagents and anhydrous solvents were purchased and used without purification, unless specified. Column chromatography was performed using a Biotage chromatography system on Biotage or Silicycle normal phase silica gel columns. NMR spectra were recorded on a Varian Unity Inova (400 MHz) or an Oxford (Varian, 300 MHz) instrument. LCMS were recorded on an Agilent 1200 series LC connected to an Agilent 6120 MS or Agilent 1100 series LC connected to an Agilent 1956B MS or a Shimadzu LC-MS-2020 system. Preparatory HPLC were performed using a Gilson GX-281 or P230 Gradient System (Elite). Chiral preparatory HPLC were performed using Elite P230 Preparative Gradient System, Thar Prep-80 and Thar SFC X-5 systems.

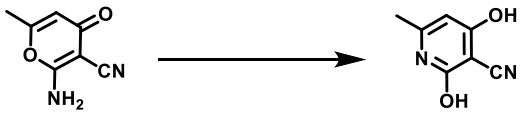
Experimental Procedures

Synthesis of 3-(aminomethyl)-4-methoxy-6-methylpyridin-2(1H)-one (22)



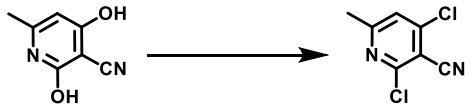
2-Amino-6-methyl-4-oxo-4H-pyran-3-carbonitrile

Malononitrile (110 g, 1.67 mol) was dissolved in dry THF (1000 mL) and cooled in an ice-water bath. NaH (60 % in mineral oil, 67 g, 1.67 mol) was added portion wise, maintaining a reaction temperature below 10 °C, while the reaction flask was continually purged with N_2 gas. After the addition was completed, the mixture was stirred at 0 °C for 0.5 h. 4-Methyleneoxetan-2-one (140 g, 1.67 mol) was then added drop wise at 0 °C. After the addition was completed, the mixture was stirred at –10 °C for 1 h. The reaction mixture was neutralized with 4N HCl and concentrated under vacuum to afford 2-amino-6-methyl-4-oxo-4H-pyran-3-carbonitrile as an orange oil. The crude product was used to next step without further purification.



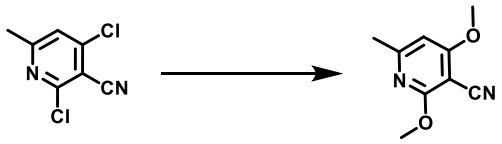
2,4-Dihydroxy-6-methylnicotinonitrile

2-Amino-6-methyl-4-oxo-4H-pyran-3-carbonitrile was dissolved in 4N aq. HCl (2.5 L) and the reaction was heated to reflux for 5 h. The reaction was then cooled to ambient and a precipitate formed. The precipitate was collected via filtration, washed with H_2O (500 mL), ethanol (500 mL) and MTBE (200 mL). The filter cake was dried under high vacuum to afford 2,4-dihydroxy-6-methylnicotinonitrile (165 g, 66%) as a yellow powder.



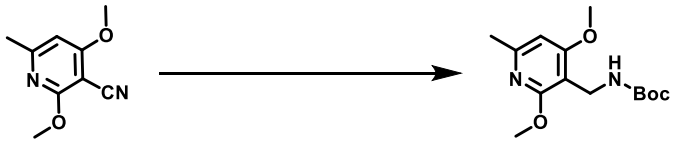
2,4-Dichloro-6-methylnicotinonitrile

2,4-Dihydroxy-6-methylnicotinonitrile (40 g, 266.4 mmol) was dissolved in $POCl_3$ (120 mL) and DMF was added (4 drops). The mixture was heated to reflux for 3 h and was then concentrated under vacuum. The residue was dissolved in EtOAc (2 L) and carefully neutralized by treatment with sat. aq. bicarbonate solution. The mixture was then filtered through Celite, the organic layer separated, dried over sodium sulfate and concentrated in vacuo to provide 2,4-dichloro-6-methylnicotinonitrile (45 g, 90%) as an off-white solid.



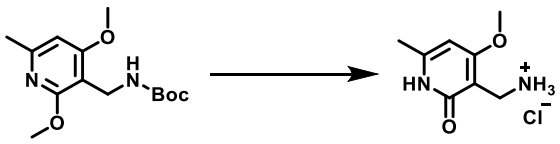
2,4-Dimethoxy-6-methylnicotinonitrile

2,4-Dichloro-6-methylnicotinonitrile (45 g, 240 mmol) was dissolved in MeOH (300 mL). NaOMe (30% in MeOH, 100 mL, 1680 mmol) was added and the mixture was heated at reflux for 4 h. The reaction was then allowed to cool to ambient temperature and the basic solution neutralized by addition of acetic acid. The solvent was removed in vacuo and the residue was treated with H_2O (300 mL) and MTBE (100 mL). The resulting solids were collected by filtration and co-evaporated with THF (300 mL) to give 2,4-dimethoxy-6-methylnicotinonitrile (40 g, 95%) as a dark-yellow solid.



***tert*-Butyl ((2,4-dimethoxy-6-methylpyridin-3-yl)methyl)carbamate**

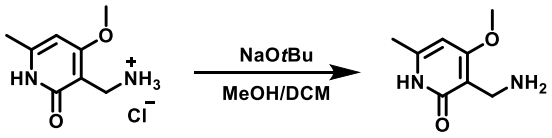
2,4-Dimethoxy-6-methylnicotinonitrile (10.0 g, 56 mmol) was dissolved in THF (260 mL) and MeOH (260 mL). Raney Nickel (wet, 10.0 g), Et_3N (29.0 g, 280 mmol) and Boc_2O (36.8 g, 168 mmol) were added sequentially. The reaction vessel was fitted with a septum, purged with N_2 (g) then purged with H_2(g). A balloon filled with H_2 (g) was then attached to the flask and the reaction was allowed to stir overnight at ambient temperature. The reaction was then filtered through a bed of Celite, washing with DCM/MeOH (1:1) to afford ((2,4-dimethoxy-6-methylpyridin-3-yl)methyl)carbamate (13.9 g, 88 %) as a yellow solid.



3-(aminomethyl)-4-methoxy-6-methylpyridin-2(1H)-one hydrochloride

In a 2 L round bottom flask, *tert*-butyl ((2,4-dimethoxy-6-methylpyridin-3-yl)methyl)carbamate (83 g, 294 mmol) was dissolved in 4 N HCl/H_2O (830 mL). The reaction was heated at reflux for 4.5 h. The reaction was cooled to ambient temperature then concentrated in vacuo to afford a brown oil. The residue was suspended in EtOH (300 mL) for 15 min and a yellow precipitate formed. The precipitate was collected by filtration and the filter cake washed with ethanol (100 mL), MTBE (100 mL) and dried under high vacuum to give fraction 1 of 3-(aminomethyl)-4-methoxy-6-methylpyridin-2-ol (38 g, 63 %) as a yellow powder. The filtrate from fraction 1 was concentrated under vacuum and the residue was precipitated from ethanol (100 mL). The precipitate was filtered, and the filter cake washed with ethanol (100 mL), MTBE (100 mL) and dried under high vacuum to provide fraction 2 of 3-(aminomethyl)-4-methoxy-6-methylpyridin-2-ol (20 g, 33 %) as a yellow powder. The solids were combined to give 3-(aminomethyl)-4-methoxy-6-methylpyridin-2-ol (total quantity: 58 g, 96% yield)

LC-MS m/z 169 $[M+H]^+$.



3-(Aminomethyl)-4-methoxy-6-methylpyridin-2(1H)-one (22)

The following reaction was performed in a single neck 2-L round bottom flask fitted with magnetic stirbar.

To a single neck 2-L round bottom flask containing of 3-(aminomethyl)-4-methoxy-6-methylpyridin-2(1H)-one hydrochloride (94.40 g, 461.30 mmol, 1.0 equiv) was added CH_2Cl_2 (450 mL, 4.8 volume) and MeOH (450 mL, 4.8 volume). To the resulting murky solution was added NaOMe (24.9 g, 461.3 mmol, 1.0 equiv) in several portions. The solution was stirred at room temperature for 1 h before concentrating. The resulting oil was diluted with CH2Cl2, the insoluble material filtered over Celite, and the filter cake was washed with CH_2Cl_2. The filtrate was concentrated to afford 3-(aminomethyl)-4-methoxy-6-methylpyridin-2(1H)-one (60.00 g, 356.70 mmol, 77.3% yield) as off-white soilds that were used in subsequent reactions without further purification.

Compound characterization. The synthesis of the compounds described in the manuscript has been described in the following patent literature: US Patent 9085583 and US Patent Application 20150376190 All compounds used for testing in the biochemical, cellular, and *in vivo* assays were purified to >95% purity, as determined by HPLC and 1H NMR.

(*R*)-1-(1-(1-(Ethylsulfonyl)piperidin-4-yl)ethyl)-*N*-((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1*H*-indole-3-carboxamide (1) (CPI-169):[1]

LC-MS *m/z* 529 $[M+H]^+$;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (s, 1H), 7.82 – 7.66 (m, 2H), 7.62 (d, *J* = 7.8, 1H), 7.17 – 6.99 (m, 2H), 6.15 (s, 1H), 4.32 (d, *J* = 5.1 Hz, 2H), 4.25 – 4.15 (m, 1H), 3.84 (s, 3H), 3.73 – 3.65 (m, 1H), 3.45 – 3.36 (m, 1H), 2.98 (q, *J* = 7.4 Hz, 2H), 2.87 – 2.77 (m, 1H), 2.60 (s, 3H), 2.54 – 2.45 (m, 1H),

2.42 – 2.30 (m, 1H), 2.20 (s, 3H), 2.06 – 1.97 (m, 1H), 1.58 – 1.48 (m, 3H), 1.42 – 1.31 (m, 1H), 1.17 (t, *J* = 7.4 Hz, 3H), 1.13 – 1.00 (m, 1H), 0.83 – 0.73 (m, 1H).

(*R*)-*N*-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(piperidin-4-yl)ethyl)-1*H*-indole-3-carboxamide (2):

LC-MS *m/z* 437 $[M+H]^+$

(*R*)-*N*-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-methylpiperidin-4-yl)ethyl)-1*H*-indole-3-carboxamide (3):

LC-MS *m/z* 451 $[M+H]^+$;

^{1}H NMR (400MHz, DMSO-d_6) δ 11.58 (s, 1 H), 7.76 – 7.65 (m, 2 H), 7.59 (d, *J* = 7.6 Hz, 1 H), 7.11 – 6.99 (m, 2 H), 6.14 (s, 1 H), 4.31 (d, *J* = 5.1 Hz, 2 H), 4.13 (br. s., 1 H), 3.83 (s, 3 H), 2.83 (d, *J* = 10.0 Hz, 1 H), 2.61 – 2.52 (m, 5 H), 2.19 (s, 3 H), 2.09 (s, 4 H), 1.88 (d, *J* = 10.7 Hz, 2 H), 1.53 (d, *J* = 6.7 Hz, 3 H), 1.34 (br. s., 1 H), 1.02 (d, *J* = 8.2 Hz, 1 H), 0.66 (br. s., 1 H).

(*R*)-1-(1-(1-Isobutyrylpiperidin-4-yl)ethyl)-*N*-((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1*H*-indole-3-carboxamide (4):

LC-MS *m/z* 507 $[M+H]^+$;

^{1}H NMR (400MHz, DMSO-d_6) δ 11.59 (s, 1 H), 7.75 (d, *J* = 7.4 Hz, 1 H), 7.72 – 7.67 (m, 1 H), 7.64 (d, *J* = 8.0 Hz, 1 H), 7.14 – 7.01 (m, 2 H), 6.15 (s, 1 H), 4.58 – 4.46 (m, 1 H), 4.32 (d, *J* = 4.9 Hz, 2 H), 4.09 – 3.99 (m, 1 H), 3.84 (s, 3 H), 3.81 – 3.72 (m, 1 H), 3.08 – 2.97 (m, 1 H), 2.92 – 2.81 (m, 1 H), 2.78 – 2.65 (m, 3 H), 2.59 (br. s., 3 H), 2.20 (s, 3 H), 2.03 – 1.90 (m, 1 H), 1.59 – 1.47 (m, 4 H), 1.02 – 0.86 (m, 6 H), 0.78 – 0.69 (m, 1 H).

Isopropyl (*R*)-4-(1-(3-(((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2-methyl-1*H*-indol-1-yl)ethyl)piperidine-1-carboxylate (5):

LC-MS *m/z* 523 $[M+H]^+$;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.59 (br. s., 1 H), 7.74 (d, *J* = 7.8 Hz, 1 H), 7.69 (t, *J* = 4.9 Hz, 1 H), 7.62 (d, *J* = 7.8 Hz, 1 H), 7.13 – 7.01 (m, 2 H), 6.15 (s, 1 H), 4.78 – 4.67 (m, 1 H), 4.32 (d, *J* = 4.9 Hz, 2 H), 4.23 – 4.12 (m, 1 H), 4.12 – 4.02 (m, 1 H), 3.84 (s, 3 H), 3.82 – 3.74 (m, 1 H), 2.79 – 2.66 (m, 1 H), 2.58 (s, 3 H), 2.46 – 2.34 (m, 2 H), 2.20 (s, 3 H), 1.96 – 1.88 (m, 1 H), 1.58 – 1.46 (m, 4 H), 1.15 (d, *J* = 6.0 Hz, 6 H), 0.95 – 0.89 (m, 1 H), 0.74 – 0.65 (m, 1 H).

(*R*)-1-(1-(1-(Dimethylcarbamoyl)piperidin-4-yl)ethyl)-*N*-((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1*H*-indole-3-carboxamide (6):

LC-MS *m/z* 508 $[M+H]^+$

(*R*)-*N*-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(oxetan-3-yl)piperidin-4-yl)ethyl)-1*H*-indole-3-carboxamide (7):

LC-MS *m/z* 493 $[M+H]^+$;

^{1}H NMR (400MHz, DMSO-d_6) δ 11.58 (s, 1 H), 7.76 – 7.65 (m, 2 H), 7.59 (d, *J* = 7.8 Hz, 1 H), 7.10 – 6.99 (m, 2 H), 6.14 (s, 1 H), 4.49 (t, *J* = 6.4 Hz, 1 H), 4.43 (t, *J* = 6.5 Hz, 1 H), 4.37 (t, *J* = 6.1 Hz, 1 H), 4.34 – 4.28 (m, 3 H), 4.21 – 4.10 (m, 1 H), 3.83 (s, 3 H), 3.30 – 3.23 (m, 1 H), 2.75 (br. s., 1 H), 2.71 – 2.64 (m, 1 H), 2.60 (s, 3 H), 2.19 (s, 4 H), 1.90 (br. s., 1 H), 1.75 (br. s., 1 H), 1.53 (d, *J* = 6.9 Hz, 3 H), 1.42 (br. s., 2 H), 1.11 – 0.98 (m, 1 H), 0.72 – 0.63 (m, 1 H).

Ethyl (*R*)-2-(4-(1-(3-(((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)carbamoyl)-2-methyl-1*H*-indol-1-yl)ethyl)piperidin-1-yl)acetate (8):

LC-MS *m/z* 523 $[M+H]^+$;

^{1}H NMR (400MHz, DMSO-d_6) δ 11.59 (br. s., 1 H), 7.81 – 7.65 (m, 2 H), 7.60 (d, *J* = 7.4 Hz, 1 H), 7.16 – 6.98 (m, 2 H), 6.15 (s, 1 H), 4.32 (d, *J* = 4.9 Hz, 2 H), 4.23 – 4.11 (m, 1 H), 4.04 (q, *J* = 7.0 Hz, 2 H), 3.84 (s, 3 H), 2.95 – 2.86 (m, 1 H), 2.60 (s, 5 H), 2.20 (s, 4 H), 1.94 – 1.79 (m, 2 H), 1.54 (d, *J* = 6.9 Hz, 4 H), 1.41 – 1.32 (m, 1 H), 1.15 (t, *J* = 7.1 Hz, 3 H), 1.04 (d, *J* = 6.0 Hz, 2 H), 0.71 - 0.61 (m, 1 H).

(*R*)-*N*-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(3,3,3-trifluoropropyl)piperidin-4-yl)ethyl)-1*H*-indole-3-carboxamide (9):

LC-MS *m/z* 533 $[M+H]^+$;

^{1}H NMR (400MHz, DMSO-d_6) δ 11.60 (br. s., 1 H), 7.78 – 7.66 (m, 2 H), 7.60 (d, *J* = 8.2 Hz, 1 H), 7.13 – 7.00 (m, 2 H), 6.15 (s, 1 H), 4.32 (d, *J* = 4.9 Hz, 2 H), 4.22 – 4.09 (m, 1 H), 3.84 (s, 3 H), 3.03 – 2.91 (m, 1 H), 2.73 – 2.64 (m, 1 H), 2.60 (s, 3 H), 2.48 – 2.31 (m, 5 H), 2.20 (s, 3 H), 2.01 – 1.85 (m, 2 H), 1.58 – 1.46 (m, 4 H), 1.36 – 1.29 (m, 1 H), 1.08 – 0.98 (m, 1 H), 0.73 – 0.62 (m, 1 H).

(*R*)-1-(1-(1-(2,2-Difluoropropyl)piperidin-4-yl)ethyl)-*N*-((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1*H*-indole-3-carboxamide (10):

LC-MS *m/z* 515 $[M+H]^+$;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (s, 1 H), 7.79 – 7.65 (m, 2 H), 7.60 (d, J = 8.0 Hz, 1 H), 7.16 – 6.99 (m, 2 H), 6.15 (s, 1 H), 4.38 – 4.25 (m, 2 H), 4.21 – 4.10 (m, 1 H), 3.84 (s, 3 H), 3.03 – 2.90 (m, 1 H), 2.75 – 2.54 (m, 6 H), 2.25 – 2.11 (m, 5 H), 1.88 (br. s., 2 H), 1.66 – 1.45 (m, 6 H), 1.43 – 1.26 (m, 1 H), 1.12 – 0.97 (m, 1 H), 0.70 – 0.61 (m, 1 H).

(*R*)-*N*-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(2,2,3,3-tetrafluoropropyl)piperidin-4-yl)ethyl)-1*H*-indole-3-carboxamide (11):

LC-MS *m/z* 551 $[M+H]^+$

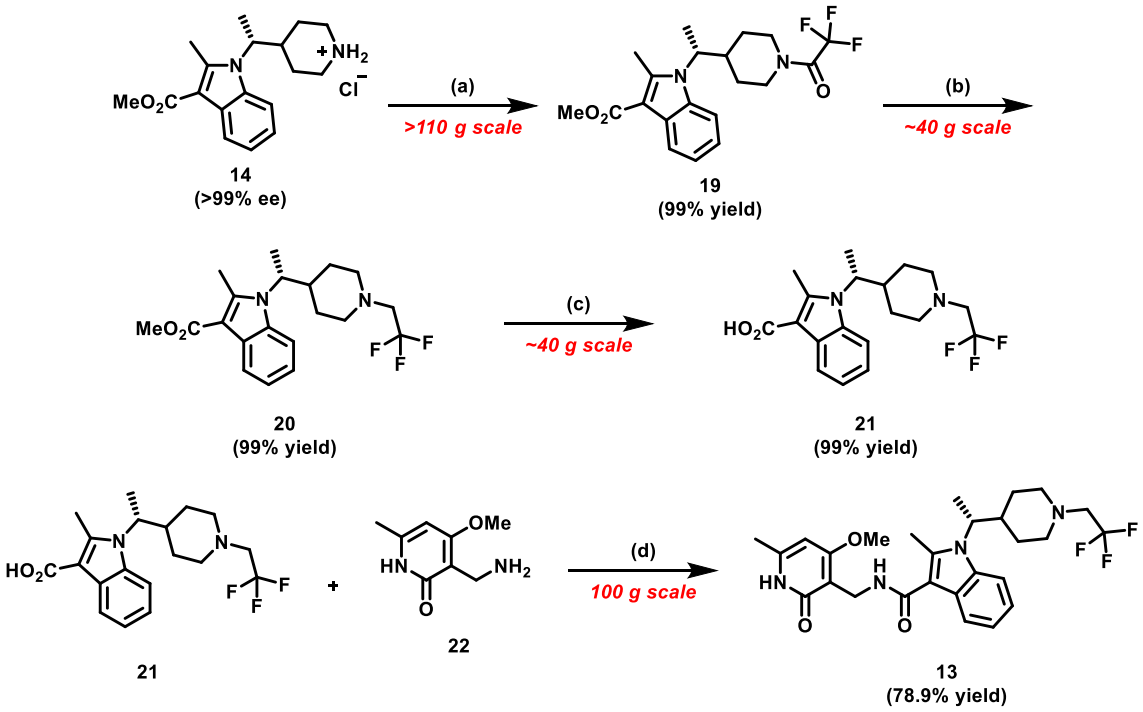
^{1}H NMR (400 MHz, Acetone-d_6) δ 12.06 (br. s, 1 H), 7.89 (d, *J* = 7.9 Hz, 1 H), 7.70 (t, *J* = 4.9 Hz, 1 H), 7.62 (d, *J* = 8.1 Hz, 1 H), 6.99 – 7.12 (m, 2 H), 6.15 – 6.48 (m, 2 H), 4.50 – 4.61 (m, 2 H), 4.18 – 4.30 (m, 1 H), 3.93 (s, 3 H), 3.04 (d, *J* = 11.5 Hz, 1 H), 2.85 – 2.96 (m, 2 H), 2.73 – 2.83 (m, 2 H), 2.71 (s, 3H), 2.28 – 2.43 (m, 2 H), 2.23 (br. s., 3 H), 2.00 (d, *J* = 12.8 Hz, 1H), 1.62 (d, *J* = 7.0 Hz, 3 H), 1.46 (dq, *J* = 12.2, 3.9 Hz, 1 H), 1.11 – 1.23 (m, 1 H), 0.79 (d, *J* = 12.8 Hz, 1 H).

(*R*)-1-(1-(1-(2,2-Difluoroethyl)piperidin-4-yl)ethyl)-N-((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1*H*-indole-3-carboxamide (12):

LC-MS *m/z* 501 $[M+H]^+$;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (br. s., 1 H), 7.77 – 7.66 (m, 2 H), 7.60 (d, *J* = 7.6 Hz, 1 H), 7.14 – 7.00 (m, 2 H), 6.15 (s, 1 H), 6.06 (t, *J* = 55.7 Hz, 1 H), 4.32 (d, *J* = 4.9 Hz, 2 H), 4.15 (br. s., 1 H), 3.84 (s, 3 H), 3.03 – 2.93 (m, 2 H), 2.73 – 2.62 (m, 3 H), 2.60 (s, 3 H), 2.26 – 2.10 (m, 4 H), 1.93 – 1.79 (m, 1 H), 1.59 – 1.46 (m, 4 H), 1.41 – 1.29 (m, 1 H), 1.11 – 0.97 (m, 1 H), 0.67 (br. s., 1 H).

Experimental procedures for the 100 g scale synthesis of CPI-1205 (13)

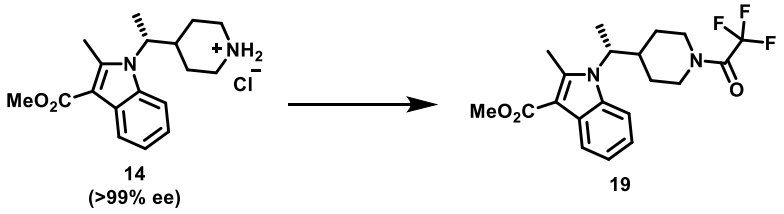


Reagents and Conditions: (a) 2,2,2-Trifluoroacetic anhydride (1.4 equiv), *i*-PrNEt$_2$ (2.5 equiv), CH_2Cl_2, 0 °C; (c) BH_3•THF (2.2 equiv), THF, 70 °C; (d) 6N aq. NaOH (6.0 equiv), EtOH, 85 °C; (c) CDI (1.3 equiv), THF, 60 °C

Methyl (R)-2-methyl-1-(1-(piperidin-4-yl)ethyl)-1H-indole-3-carboxylate hydrochloride (14):

The synthesis of methyl (R)-2-methyl-1-(1-(piperidin-4-yl)ethyl)-1H-indole-3-carboxylate hydrochloride (**14**) was previously disclosed. Please see the following reference 2 below.[2]

LC-MS *m/z* 301 $[M+H]^+$.

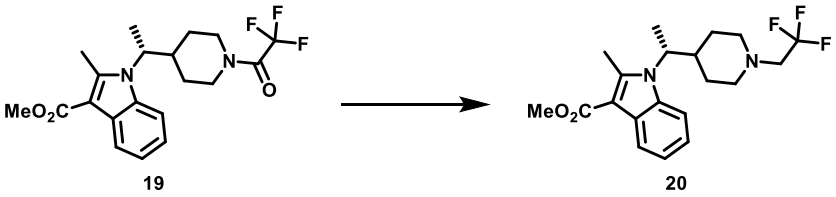


Methyl (R)-2-methyl-1-(1-(1-(2,2,2-trifluoroacetyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylate (19):

The following reaction was performed in a single necked 2-L round bottom flask equipped with a stirbar and fitted with liquid addition funnel.

To a 2-L round bottom flask, equipped with a magnetic stirbar and fitted with a liquid addition funnel, was added a solution of (R)-methyl 2-methyl-1-(1-(piperidin-4-yl)ethyl)-1H-indole-3-carboxylate hydrochloride (100.0 g, 296.9 mmol, 1.0 equiv) in CH_2Cl_2 (800 mL, 8 volume). The reaction mixture was cooled (0 °C), followed by the addition of *N*-ethyl-*N*-isopropylpropan-2-amine (129.3 mL, 742.2 mmol, 2.5 equiv) in one-portion. 2,2,2-trifluoroacetic anhydride (57.8 mL, 415.6 mmol, 1.4 equiv) was charged into the addition funnel and subsequently introduced to the cooled (0 °C) reaction mixture over a period of 30 min. After complete addition of 2,2,2-trifluoroacetic anhydride, the resultant reaction mixture was stirred for an additional 30 min at 0 °C or until LCMS analysis indicated complete consumption of starting material. The mixture was partitioned with water and warmed to ambient temperatures. The bi-phasic mixture was transferred to a separatory funnel, the organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford crude methyl (R)-2-methyl-1-(1-(1-(2,2,2-trifluoroacetyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylate (125.8 g, 317.35 mmol) as a foam. The resultant product was used in the subsequent reaction without further purification.

LRMS *m/z* 419 $[M+H]^+$



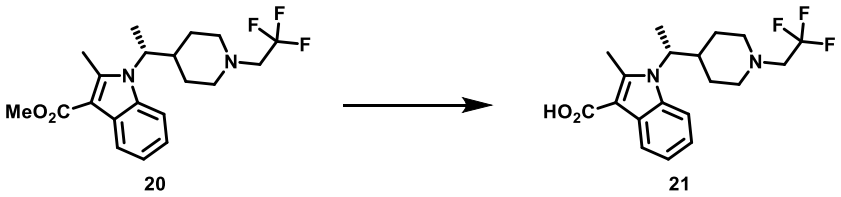
Methyl (R)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylate (20):

The corresponding reduction of the trifluoroacetamide with BH_3·THF was processed in three separate batches (~40 g of material per run). The following reaction was performed in a three-neck 1-L round bottom flask equipped with a magnetic stirbar, fitted with a liquid addition funnel and a reflux condenser.

To a 1-L round bottom flask was added a solution of (R)-methyl 2-methyl-1-(1-(1-(2,2,2-trifluoroacetyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylate (40.18 g, 101.36 mmol, 1.0 equiv) in THF (200 mL, 5 volume), and the resultant solution was cooled to 0 °C. The addition funnel was charged with BH_3·THF complex (1 M in THF, 223.0 mL, 223.0 mmol, 2.2 equiv) and the BH_3·THF complex was

introduced to the cooled reaction mixture. After complete addition of BH_3·THF, the solution was stirred at 0 °C for 15 min and subsequently warmed to room temperature. After 15 min at ambient temperatures, the reaction mixture was heated to 50 °C until complete consumption of the amide was observed as determined by LCMS analysis (typically ~4 h).

After complete consumption of the starting material (as judged by LCMS analysis), the addition funnel was charged with MeOH (250 mL, ~6 volume) and the reaction mixture was cooled to 0 °C. To the cooled reaction mixture was slowly added MeOH. Vigorous evolution of gas (H_2 (g)) was observed and the rate of addition of MeOH was periodically adjusted to prevent excessive increase of the internal temperature above 10 °C. After complete addition of MeOH, the solution was warmed to ambient temperatures and finally heated to reflux (60 °C–65 °C) for 1 h or until LCMS analysis revealed complete breakdown of the amine-boronate species. After cooling to room temperature, the reaction solution was concentrated *in vacuo* and low boiling alkyl borate impurities were azeotropically distilled from MeOH (3x). Methyl (R)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylate (101.36 mmol, *assumed quantitative yield*) was isolated as light brown foam and used in the subsequent transformation without further purification.

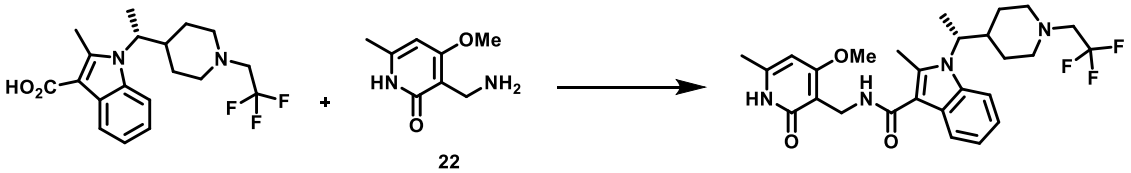


(R)-2-Methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylic acid (21):

The following reaction was performed in a single necked 1-L round bottom flask equipped with a stirbar and fitted with a reflux condenser.

To a 1-L round bottom flask containing a solution of (R)-methyl 2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylate (39.3 g, 102.8 mmol, 1.0 equiv) in EtOH (400 mL, 10 volume) was added aqueous 6 N sodium hydroxide (85.67 mL, 514 mmol, 5.0 equiv). The solution was heated to reflux for 8 h during which the formation of black precipitates were observed. The reaction mixture was cooled to room temperature and the precipitates were filtered over a pad of Celite (**Caution:** the resultant black precipitates are a potentially flammable palladium species!). The filtrate was subsequently concentrated (until 1–2 volume of solvent remained) and the resultant thick slurry was diluted with water and cooled to 0 °C. To the cooled (0 °C) bi-phasic solution was *carefully* added 6 N HCl (in portions) until pH of 4–5 for the aqueous layer was achieved. Solid precipitates formed upon acidification and were subsequently dissolved in EtOAc. The biphasic mixture was transferred to a separatory funnel, the organic layer was separated, and the aqueous layer (pH 4–5) was extracted with EtOAc (3x). The combined organic layers were washed with water (2x), brine, dried over Na_2SO_4, and concentrated to afford crude (R)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylic acid (101.4 mmol, assumed quantitative yield) was isolated as light brown foam and used in the subsequent transformation without further purification.

(R)-N-((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxamide (13):



The following reaction was performed in a one-pot operation in a neck 3-L round bottom flask fitted with a thermocouple, reflux condenser, and overhead stirrer.

To a round bottom flask containing a solution of (R)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxylic acid (95.0 g, 257.9 mmol, 1.0 equiv) in THF (1 L, 10 volume) was added CDI (54.4 g, 335.2 mmol, 1.3 equiv). The resulting solution was heated to 60 °C. Upon heating the reaction mixture, the evolution of gas (CO_2 (g)) was observed. The mixture was heated (60 °C) until complete conversion of the acid to the acyl-imidazole (typically 1 h) was detected by LCMS. To the reaction mixture was added 3-(aminomethyl)-4-methoxy-6-methylpyridin-2(1H)-one (65.1 g, 386.8 mmol, 1.5 equiv) in one portion and the resulting heterogeneous mixture was allowed to heat to reflux. After 24 h, the reaction mixture was cooled to room temperature and partitioned between water and CH_2Cl_2. The cloudy bi-phasic layers were filtered through Celite to remove insoluble emulsive material before further processing. The resulting clear bi-phasic layers were transferred to a separatory funnel, the organic phase was separated and aqueous phase (pH ~14) was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with water (2x), brine, dried over Na_2SO_4, and concentrated *in vacuo* to yield a light brown foam. The foam (dissolved in CH_2Cl_2, divided into 4 x 30 g portions for chromatographic purposes) was purified by silica gel chromatography (Biotage 340 g, isocratic elution with 100% EtOAc (2 column volumes), then gradient elution 2% MeOH : 98% EtOAc to 25% MeOH : 75% EtOAc, and finally isocratic 25% MeOH : 75% EtOAc). The appropriate fractions were collected and concentrated to afford (R)-N-((4-methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxamide (105.0 g, 202.5 mmol, 78.9 % yield).

LRMS *m/z* 519 $[M+H]^+$;

^{1}H NMR (500MHz, DMSO-d_6) δ 11.59 (s, 1 H), 7.74 (d, *J* = 7.6 Hz, 1 H), 7.71 – 7.66 (m, 1 H), 7.61 (d, *J* = 7.8 Hz, 1 H), 7.13 – 7.01 (m, 2 H), 6.15 (s, 1 H), 4.32 (d, *J* = 4.9 Hz, 2 H), 4.22 – 4.12 (m, 1 H), 3.84 (s, 3 H), 3.15 – 2.95 (m, 3 H), 2.75 – 2.66 (m, 1 H), 2.60 (s, 3 H), 2.39 – 2.31 (m, 1 H), 2.20 (s, 3 H), 2.05 – 1.98 (m, 1 H), 1.92 – 1.84 (m, 1 H), 1.56 – 1.46 (m, 4 H), 1.42 – 1.32 (m, 1 H), 1.11 – 1.01 (m, 1 H), 0.69 – 0.62 (m, 1 H).

Materials and Methods for Structural Biology Experiments

The human PRC2 core was expressed and purified as previously described.[3] For crystallization, PRC2 complex was prepared with trimethylated K116 Jarid2 peptide (residues 111-121) and compound **10**. Complex was prepared at a protein concentration of 4.8 mg/ml with 1:5 molar ratio Jarid2 peptide, and 1:1.5 molar ratio compound. Crystals were grown at 18°C using the vapour diffusion technique in MRC sitting drop plates (Hampton Research). Drops were prepared by mixing equal volumes of PRC2 protein

with reservoir solution containing 400mM Ammonium Citrate pH 7.5 and 25 % PEG 3350. Crystals were transferred into reservoir solution containing 20% ethylene glycol prior to flash cooling in liquid nitrogen. Diffraction data was collected at Diamond Light Source on beamline IO4 at 0.9795 Å. Data were indexed and integrated using the XDS[4] program and scaled using SCALA. Phases were generated by Phaser[5] molecular replacement (search model PDB code 5HYN). Electron density was improved by non-crystallographic symmetry averaging using the CCP4 programs Parrot and DM.[6,7] Standard refinement was performed with refmac5[8] and CNS[9] was used to generate composite omit maps. Manual model building was carried out in Coot.[10] Statistics for data collection and refinement are presented in Supporting Information Table 1. The PRC2 inhibitor complex crystallized with the same unit cell as that observed for the equivalent complex with oncogenic peptide and SAH, and with four essentially equivalent copies in the asymmetric unit. However, the 2,2-difluoropropyl containing analogue (**10**) buries 650 Å^2 of surface area compared with only 450 Å^2 for SAH. Figures were created with Pymol (Delano Scientific; http://pymol.sourceforge.net/).

Table 1. Data collection and refinement statistics

	5LS6
Data collection	
Wavelength (Å)	0.9795
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	131.31, 170.27, 275.54
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	68.84-3.47 (3.56-3.47)
R_{sym} or R_{merge}	0.30 (1.81)
I / σI	6.9 (1.3)
$CC_{1/2}$ (%)	99.0 (63.0)
Completeness (%)	99.9 (99.9)
Redundancy	6.8 (7.0)
Refinement	
Resolution (Å)	68.84-3.47
No. reflections	76561
R_{work} / R_{free}	0.228/0.311
No. atoms	
Protein	33534
JARIDme3	340
Compound (9)	148
Ligand (Zn)	32
B-factors	
Protein	103.02
JARIDme3	112.21
Compound (9)	80.18
Ligand (Zn)	69.57
R.m.s. deviations	
Bond lengths (Å)	0.011

Bond angles (°)	1.636

EZH2 Biochemical Assays

PRC2 and biotinylated oligonucleosomes were prepared in-house. H3K27me3 activator peptide (RKQLATKAARK(Me3)SAPATGGVKKP-NH2) was prepared by custom synthesis (New England Peptide, Gardner, MA) and SAH was purchased from Sigma-Aldrich. [3H]-SAM and streptavidin SPA plates (FlashPlate®) were purchased from Perkin-Elmer Corp (Waltham, MA). All other reagents were of reagent grade or better and purchased from commercial sources.

Radioactive assays were read on a TopCount (Perkin-Elmer Corp.) using settings according to manufacturers' recommendations. Data were graphed and analyzed with GraphPad Prism 5.0 (GraphPad Software, Inc, La Jolla, CA) using curve fitting analyses appropriate to the experimental type.

PRC2 (wt or Y641N mutant), biotinylated nucleosome, H3K27me3 activator peptide and compound (in DMSO) were incubated in 50 mM Tris, pH 8.5, 5 mM MgCl2, 1 mM DTT, 70 uM Brij-35, and 0.1 mg/mL BSA for 30 minutes. Reaction was initiated by addition of [3H]-SAM to final conditions of 5 nM PRC2, 200 nM nucleosome (concentration expressed as H3), activator peptide (3.6 μM) and 200 nM [3H]-SAM in a total volume of 25 μl in 384 well Greiner plates. For compound analysis assays were either single point or ten point dose-responses with final total DMSO of 0.8 or 1.6% (v/v). Typically assays were run for 60 minutes with <35% substrate turnover. After reaction assays were quenched by addition of 20 μl of 2 mM SAH and 200 mM EDTA in 50 mM Tris, pH 8.5. Reactions were transferred to streptavidin-coated FlashPlates, incubated for 2 h, aspirated, washed, and read on a TopCount.

Selectivity data against a panel of HMT's and DNMT's

		Compound IC50* (M)		
Methyltransferase:	**Substrate:**	**CPI-1205 (13)**	**SAH**	**Chaetocin**
DNMT1	Poly dl-dC		2.39E-07	ND
DNMT3a	Lambda DNA		4.82E-07	ND
DNMT3b	Lambda DNA		5.16E-08	ND
DNMT3b/DNMT3L	Lambda DNA		1.18E-07	ND
DOT1L	Nucleosomes		1.93E-06	ND
G9a	Histone H3 (1-21)		8.70E-06	ND
GLP	Histone H3 (1-21)		1.30E-05	ND
MLL1	Nucleosomes		3.47E-06	ND
MLL2	Core Histone		2.27E-05	ND
MLL3	Core Histone		3.95E-05	ND
MLL4	Core Histone		2.01E-06	ND
NSD1	Nucleosomes		3.61E-05	2.18E-07
NSD2	Nucleosomes		9.88E-06	1.25E-07
NSD2 (E1099K)	Nucleosomes		4.47E-06	7.05E-08
PRDM9	Histone H3			4.74E-06
PRMT1	Histone H4		7.73E-07	ND
PRMT3	Histone H4		5.57E-06	ND
PRMT4	Histone H3		2.99E-07	ND
PRMT5	Histone 2A		6.07E-05	ND
PRMT5/MEP50	Histone 2A		3.23E-06	ND
PRMT6	Histone H3		2.05E-06	ND
PRMT8	Histone H4		7.37E-07	ND
SET1B	Core Histone		1.80E-05	ND
SET7/9	Core Histone		5.89E-05	ND
SET8	Nucleosomes		2.37E-04	ND
SETD2	Histone H4		1.22E-05	ND
SMYD2	Histone H4		9.89E-06	ND
SUV39H1	Histone H3		1.58E-04	ND
SUV39H2	Histone H3		3.02E-05	ND
SUV420H1TV2	Nucleosomes		1.63E-05	ND

* Empty cells indicate no inhibition or compound activity that could not be fit to an IC50 curve. Top concentration = 10 μM.

ND Indicates compound not tested against enzyme

MOA Cell Assay

Ten different doses of each test compound (in a series of 3-fold dilutions) were plated in duplicate 384-well tissue culture treated plates (Catalog # 781080; Greiner Bio One; Monroe, North Carolina). HeLa cells grown in culture were trypsinized and counted using a Countess® cell counter (Catalog # C10281; Life Technologies, Grand Island, NY). Cell were diluted to 67,000 cells per mL in10% DMEM (Catalog # 10569-010 Life Technologies, Grand Island, NY) and 15µL (1,000 cells) were plated into each well using the Biotek MicroFloTM Select Dispenser (BioTek Instruments, Inc. Vermont, USA),) of the 384-well plate. Plates were incubated at 37 °C /5% CO_2 for 72 hrs. One of the duplicate plates was processed for AlphaLISA and the other for viability.

5µL per well Cell-Histone Lysis buffer (1X) (Catalog # AL009F1 Perkin Elmer; Waltham, MA) was added to the plate processed for AlphaLISA and incubated at RT for 30 minutes on a plate shaker with low speed (Model# 4625-Q Thermo Scientific; Waltham, MA). 10µL per well Histone Extraction buffer (catalog # AL009F2; Perkin Elmer; Waltham, MA) was added and the plate further incubated at RT for 20 min on plate shaker with low speed. 10µL per well of a 5X mix of anti-K27me3 acceptor beads and biotinylated anti-Histone H3 (C-ter) antibody (diluted to 3nM final) (Catalog #AL118 Perkin Elmer; Waltham, MA) was added. The acceptor beads and anti-Histone H3 were diluted in 1X Histone Detection buffer (Catalog # AL009F3 Perkin Elmer; Waltham, MA). The plate was sealed with an aluminum plate sealer and incubated at 23^0C for 60 min. Finally, 10µL per well of 5X solution of Streptavidin donor beads (Catalog #6760002 Perkin Elmer; Waltham, MA) (20µg/mL final in 1X Histone Detection Buffer) was added, the plate sealed with Aluminum plate sealer and incubated at 23 °C for 30 min. The plates were read using an EnVision- Alpha Reader (model # 2104 Perkin Elmer; Waltham, MA).

Cell viability was assayed by adding 15 µL of Cell Titer Glo ((Catalog #G7571 Promega Madison, WI) to each well with cells with media. The plates were incubated at RT for 15 – 20 minutes on a plate shaker at low speed. The plates were then read using an EnVision- Alpha Reader (model # 2104 Perkin Elmer; Waltham, MA).

Data from both assays was analyzed using Assay Assistant (Constellation Pharmaceuticals In-house product) and Activity Base (IDBS Ltd, Surrey, UK) template. Data files were imported to Assay Assistant and assay conditions were specified. A unique Analysis ID was created and the data files exported to Activity Base. An analysis template was created on Activity Base to measure dose-dependent inhibition of H3K27me3 mark and cell viability respectively. Readout of DMSO wells were used to normalize the data. Resulting curves were fitted using Activity base software Model 205 (IDBS Ltd, Surrey, UK). The data was checked for quality, validated and integrated in excel format using SARview (IDBS Ltd, Surrey, UK).

KARPAS-422 Efficacy Experiment with CPI-1205 (13)

CB-17 SCID mice were inoculated subcutaneously at the right flank with the exponentially growing KARPAS-422 tumor cells (5×10^6) in 0.2 mL of PBS with Matrigel (1:1) at WuXi (Shanghai). The treatments were started when the average tumor size reached approximately 300 mm^3 (~23 days after inoculation). Each group consisted of 10 randomly assigned tumor-bearing mice. The mice were dosed with vehicle (10% DMSO + 60% PEG400 + 30% ddH_2O) or 160 mg/kg of **CPI-1205 (13)** (PO, BID) until the tumor volume reached 2000 mm^3 as per IACUC guidelines. Tumor size was measured three

times a week using a caliper, and the tumor volume (V) was expressed in mm3 using the formula: $V = 0.5a \times b^2$ where "a" and "b" are the long and short diameters of the tumor, respectively. The mice were weighed every day. TGI % was calculated according to the following equation: TGI (%) = $\{1-(T_1-T_0) / (C_1-C_0)\} \times 100$, where C_1- mean tumor volume of control mice at time t; T_1 -mean tumor volume of treated mice at time t; C_0-mean tumor volume of control mice at time 0; T_0-means tumor volume of treated mice at time 0. At study termination the tumor samples were collected and analyzed by MSD ELISA for H3K27me3 levels. The levels of **CPI-1205 (13)** were measured in plasma and tumor collected from the animals by validated pharmacokinetic methods.

PK/PD Experiments with CPI-1205

CB-17 SCID mice were inoculated subcutaneously at the right flank with the exponentially growing KARPAS-422 tumor cells (10 x 106) in 0.2 mL of PBS with Matrigel (1:1) at WuXi (Shanghai). The treatments were started when the average tumor size reached approximately 200-300 mm^3. The mice were dosed with vehicle (10% DMSO + 60% PEG400 + 30% ddH_2O) or with 160 mg/kg of **CPI-1205 (13)** (PO, BID) for 17 days. At the end of the study plasma and tumor were collected at 1, 6 or 12 h post last dose. The levels of **CPI-1205 (13)** in the plasma and tumor were measured with validated pharmacokinetic using LC-MS. Part of the tumors were homogenized and histones were extracted from the tumor lysates. The levels of H3K27me3 and total Histone H3 in the tumors were assessed using MSD-ELISA on a SECTOR Imager 2400 with appropriate sulfo-tagged antibodies.

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