Supporting Information

Discovery of CPI-1612: A Potent, Selective, and Orally Bioavailable EP300/CBP Histone Acetyltransferase (HAT) Inhibitor

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Table of Contents

S1. General Experimental Procedures 1

S2. Materials 1

S3. Instrumentation 1-2

S4. X-ray crystallography 2

S5. Synthesis of Compounds 3-17 2-27

S6. Reference 15. 27-28

S7. In vitro and ADME assays 29-33

S8. Pharmacokinetics 23-37

S9. In vivo experiments 37-48

S10. References 48

S1. General Experimental Procedures

All reactions for the preparation of substrates were performed in standard, dry glassware fitted with rubber septa under an inert atmosphere of nitrogen unless otherwise described. Disposable PTFE syringes were used to transfer liquids and solutions. Concentration of organic and aqueous solutions under reduced pressure was performed using a Buchi rotary evaporator Rotovapor R-215. Silica gel column chromatography was performed using prepacked Biotage silica gel cartridges on a Biotage Isolera One. Thin layer chromatography (TLC) or LCMS (Agilent 1260 Infinity) was used for reaction monitoring, and product detection was performed using pre-coated glass plates covered with 0.20 mm silica gel with fluorescent indicator or by using mass detection by LCMS. TLC plates were visualized by exposure to UV light (λ = 254 nm).

S2. Materials

Reagents were purchased in reagent grade from commercial suppliers and used as received, unless otherwise described. Anhydrous solvents were purchased from Aldrich and used as received, unless otherwise noted.

S3. Instrumentation

^{1}H NMR spectra were recorded on a Brüker 400 MHz or Varian 600 MHz spectrometer, are reported in parts per million downfield from tetramethylsilane, and are referenced to the residual protium resonances of the NMR solvent ($CDCl_3$: 7.26 [$CHCl_3$] or DMSO-d6: 2.50 [DMSO-d5]). Proton-decoupled ^{13}C NMR spectra were recorded on a Brüker 400 MHz or Varian 600 MHz spectrometer, are reported in parts per million downfield from tetramethylsilane, and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$: 77.23 or DMSO-d6: 39.61). Data are represented as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sept = septet, m = multiplet), coupling constants in Hertz (Hz), integration. Liquid chromatography mass spectrometric data were obtained on an Agilent 1260 Infinity II LC / Aglient 6120 Quadropole MS system (Stationary phase: Agilent Zorbax SB-C8 column (4.6 x 75 mm); mobile phase: gradient of 5-95% acetonitrile in water + 0.1% formic acid).

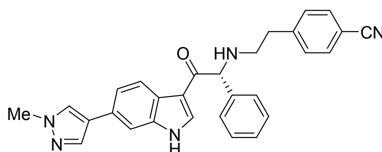
S4. X-ray crystallography

Crystallization method for compounds 1, 12, and 17: To enable crystallization of p300-HAT with AcCoA-competitive compounds, a semi-synthetic construct was prepared following the method of Liu.[2] Crystallization conditions for binary needle-form crystals of P300-HAT-IPL + AcCoA-competitive inhibitor compound **1** used 200 + 150 nL drops, with a protein solution consisting of 0.21 mM P300-HAT-IPL and 0.6 mM compound **1** in a buffer consisting of 20 mM HEPES pH 7.5, 150 mM NaCl, and 1 mM TCEP. The reservoir consisted of 0.2 M Ammonium sulfate, 0.1 M BisTris pH 6, 22% PEG3350. The X-ray structures of **1**, **12**, and **17** have been deposited in the PDB with the codes 6v8b, 6v90, and 6v8n.

Data collection, processing, structure solution, model building, and refinement: Following synchrotron data collection and reduction with XDS, the structures were solved by molecular replacement with Phaser, with 2 chains in the ASU.[5, 6] Iterative cycles of rebuilding were performed in Coot and Refmac5 was used for coordinate refinement. Coordinates and structure factors were deposited in the PDB with codes 6v8b (Compound **1**), 6v90 (Compound **12**), and 6v8n (Compound **17**). Data collection and refinement parameters are presented in Table II (separate file).[7, 8]

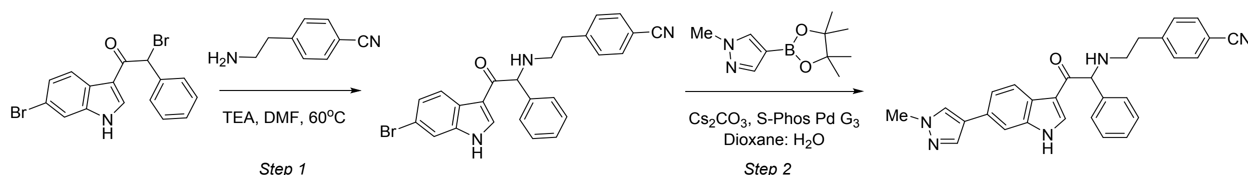
S5. Synthesis of Compounds 3-17.

Compound **3**



(*R*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indol-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile

Compound **3** was prepared according the following scheme and procedures.



<u>Step 1. 4-(2-((2-(6-bromo-1H-indol-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile:</u> A mixture of 2-bromo-1-(6-bromo-1H-indol-3-yl)-2-phenylethan-1-one (0.5 g, 1.27 mmol), 4-(2-aminoethyl)benzonitrile hydrochloride (0.51 g, 2.54 mmol) and Et_3N (0.9 ml, 6.45 mmol) in DMF (10 mL) was heated at 60 °C for 2 hours. After completion of the reaction, the reaction mixture was poured into ice cold water (10 ml) and extracted with ethyl acetate (2 x 20 ml). The combined organic layers were washed with brine (15 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography to afford the title compound (0.52 g, 89%) as an off-white solid.

LCMS: m/z = 458.6 [M+1].

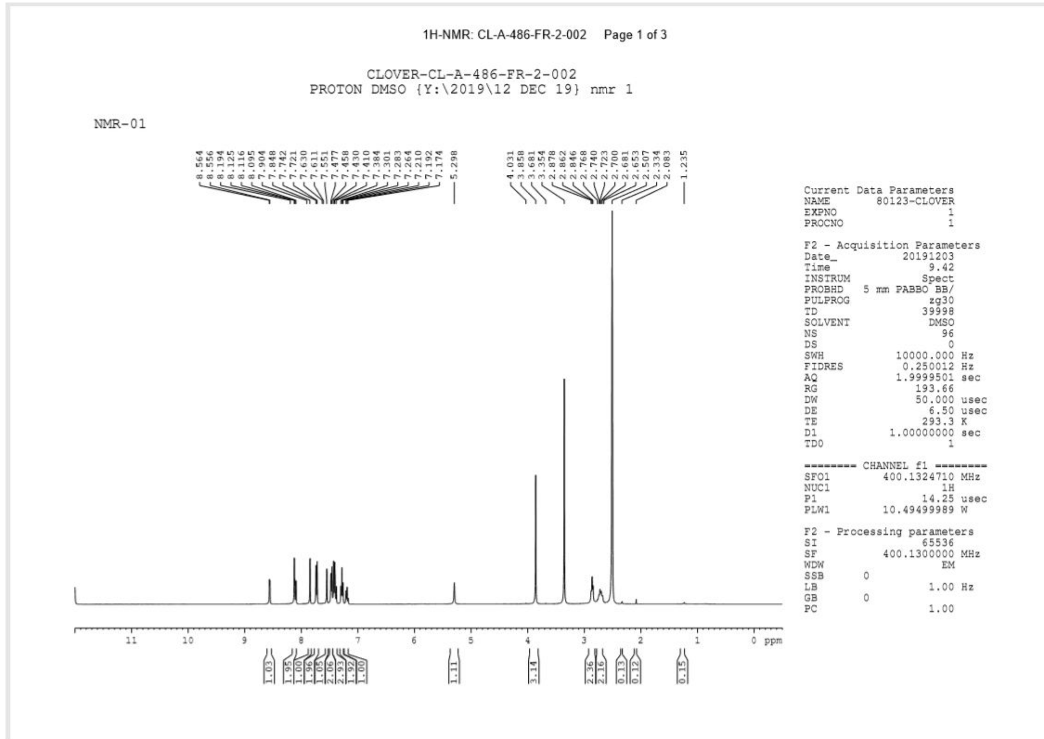
<u>Step 2. (*R*)- and (*S*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indol-3-yl)-2-oxo-1-phenylethyl) amino)ethyl) benzonitrile:</u> A mixture of 4-(2-((2-(6-bromo-1H-indol-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile (0.3 g, 0.65 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.20 g, 0.98 mmol) and cesium carbonate (0.63 g, 1.95 mmol) in dioxane:water (4:1, 5 ml) was degassed by passing a stream of argon through the solution for 20 minutes. S-Phos-Pd G3 catalyst (0.06 g, 0.072 mmol) was added and argon was bubbled through the solution for an additional 10 minutes. The reaction mixture was heated in a sealed tube at 100°C for 2 hours. After completion of reaction, the reaction mixture treated with water (25 ml) and the mixture was extracted with ethyl acetate (2 x 30 ml). The combined organic layers were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography to afford the racemic title compound (0.2 g, 66%) as a solid.

The racemic title compound was resolved by Chiral SFC (CHIRALPAK IB; 35% (MeOH:IPA = 50:50) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

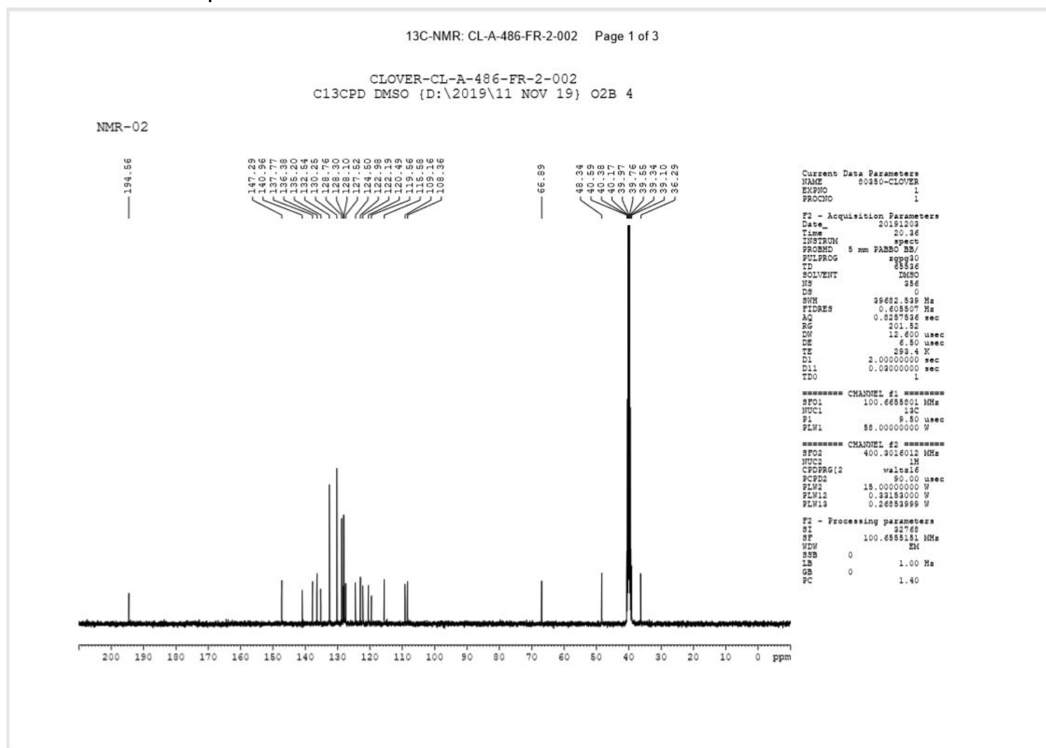
Stereoisomer of **3** (faster-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.68-2.87 (m, 5H), 3.87 (s, 3H), 5.29 (s, 1H), 7.18-7.21 (m, 1H), 7.27-7.31 (m, 2H), 7.39-7.48 (m, 5H), 7.56 (s, 1H), 7.74 (d, J = 8.0 Hz, 2H), 7.85 (s, 1H), 8.10-8.13 (m, 2H), 8.56 (s, 1H), 12.01 (s, 1H). LCMS: m/z = 460.66 [M+1].

Compound **3** (slower-eluting isomer; assigned as the (*R*)-isomer based on analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.72-2.86 (m, 5H), 3.85 (s, 3H), 5.28 (s, 1H), 7.19 (t, J = 7.3 Hz, 1H), 7.28 (t, J = 7.5 Hz, 2H), 7.37-7.47 (m, 5H), 7.54 (s, 1H), 7.73 (d, J = 8.2 Hz, 2H), 7.84 (s, 1H), 8.12 (s, 2H), 8.55 (d, J = 3.1 Hz, 1H), 11.98 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): 194.56, 147.29, 140.96, 137.77, 136.38, 135.20, 132.54, 130.25, 128.76, 128.30, 128.10, 127.52, 124.50, 122.98, 122.19, 120.49, 119.56, 115.58, 109.16, 108.36, 66.89, 48.34, 36.29. LCMS: m/z = 465.3 [M+1]. LCMS: m/z = 460.66 [M+1]. HRMS (ESI): m/z = 460.2125 [M+1]; (exact mass for $C_{29}H_{25}N_5O$ = 459.2059).

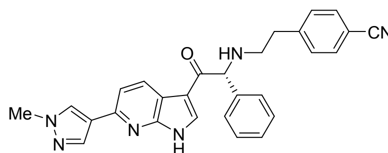
^{1}H NMR for compound **3**:



^{13}C NMR for compound **3**:

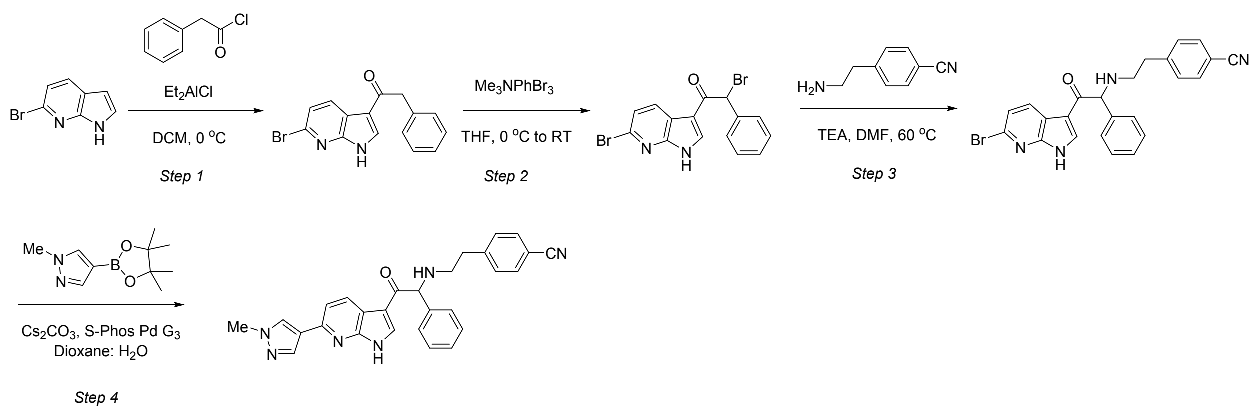


Compound **4**



(*R*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-oxo-1-phenylethyl)amino) ethyl) benzonitrile

Compound **4** was prepared according the following scheme and procedures.



<u>Step 1. 1-(6-bromo-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-phenylethan-1-one</u>: To a stirred solution of 6-bromo-1H-pyrrolo[2,3-b]pyridine (1 g, 5.07 mmol) in dry DCM (90 ml) was added $AlCl_3$ (5.40 g, 40.60 mmol) portion wise at 0°C followed by the drop wise addition of phenyl acetyl chloride (1.09 g, 7.10 mmol) in DCM (10 ml) at 0°C. The resulting reaction mixture was stirred at room temperature for 2 hours. Reaction was monitored by the TLC and saturated sodium bicarbonate solution (50 ml) was added slowly at 0°C. The DCM layer was separated and the aqueous layer was extracted with DCM (2 x 50 ml). The obtained solid was filtered through Büchner funnel and resulting solid was stirred in DCM (50 ml) for 30 minutes, which was then filtered again with Büchner funnel. The combined DCM layer was washed with brine (100 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude solid was triturated with 80:20 ethyl acetate:hexanes. The solid was collected by filtration through Büchner funnel and dried to obtain the title compound as solid (0.85 g, 53%).

^{1}H NMR (400 MHz, DMSO-d6): δ 4.20 (s, 2H), 7.18-7.24 (m, 1H), 7.28-7.35 (m, 4H), 7.43 (d, *J* = 8.0 Hz, 1H), 8.37 (d, *J* = 8.4 Hz, 1H), 8.72 (d, *J* = 2.8 Hz, 1H), 12.78 (s, -NH). LCMS: (Method C-3): R_T 1.74 min; *m/z* 315.3 [M^+] and 317.4 [M^++2].

<u>Step 2. 2-bromo-1-(6-bromo-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-phenylethan-1-one:</u> To a stirred solution of 1-(6-bromo-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-phenylethan-1-one (0.85 g, 2.69 mmol) in THF (40 ml) under an atmosphere of nitrogen was added a solution of trimethylphenylammonium tribromide (1.01 g, 2.69 mmol) in THF (15 ml) drop wise at 0°C. The resulting reaction mixture was warmed to room temperature and stirred for 3 hours. After completion of the reaction, as judged by LCMS, the reaction was quenched with a saturated aqueous solution of sodium bicarbonate (50 ml). The reaction mixture was extracted with ethyl acetate (2 x 50 ml) and the combined organic layers were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was triturated with 80:20 ethyl acetate:hexanes to provide a solid that was collected by filtration to afford the title compound as solid (0.35 g, 33%).

^{1}H NMR (400 MHz, DMSO-d6): δ 6.87 (s, 1H), 7.29-7.35 (m, 1H), 7.37-7.41 (m, 2H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 7.2 Hz, 2H), 8.40 (d, *J* = 8.0 Hz, 1H), 8.82 (d, *J* = 2.8 Hz, 1H), 12.98 (s, -NH). LCMS: *m/z* = 395.1 [M+1].

<u>Step 3. 4-(2-((2-(6-bromo-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)-benzonitrile</u>: A mixture of 2-bromo-1-(6-bromo-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-phenylethan-1-one (0.25 g, 0.63 mmol), 4-(2-aminoethyl)benzonitrile hydrochloride (0.23 g, 1.26 mmol) and Et_3N (0.35 ml, 2.53 mmol) in DMF (5 ml) was heated at 60°C for 4 hours. The reaction mixture was poured into ice-cold water (25 ml) and extracted with ethyl acetate (2 x 20 ml). The combined organic layers were washed with brine (15 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the title compound as solid (0.23 g, 79%).

^{1}H NMR (400 MHz, DMSO-d6): δ 2.68-2.87 (m, 4H), 5.33 (s, 1H), 7.19-7.23 (m, 1H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.40-7.43 (m, 3H), 7.47 (d, *J* = 7.2 Hz, 2H), 7.73 (d, *J* = 8.0 Hz, 2H), 8.37 (d, *J* = 8.0 Hz, 1H), 8.76 (s, 1H), 12.80 (s, -NH). LCMS: *m/z* = 459.2 [M+1].

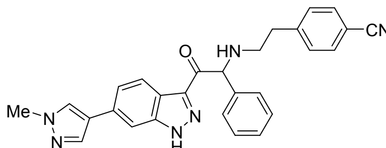
Step 4. (*R*)- and (*S*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl) benzonitrile: A mixture of 4-(2-((2-(6-bromo-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl) benzonitrile (0.20 g, 0.43 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.10 g, 0.52 mmol), and cesium carbonate (0.35 g, 1.09 mmol) in a mixture of dioxane:water (4:1, 10 ml) was degassed for 20 minutes with argon gas. To the reaction mixture, SPhos Pd G3 precatalyst (0.03 g, 0.04 mmol) was added and degassing was continued for another 10 minutes. The reaction mixture was heated in a sealed tube under microwave irradiation at 135°C for 45 minutes. After completion of reaction, the reaction mixture was diluted with water (30 ml) and extracted with ethyl acetate (2 x 25 ml). The combined organic layers were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography to afford the racemic title compound (0.08 g, 40%) as a solid.

The racemic title compound was resolved by Chiral SFC (CHIRALPAK IB; 25% (MeOH:IPA = 50:50) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

Compound **4** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.68-2.86 (m, 4H), 3.89 (s, 3H), 5.31 (s, 1H), 7.18-7.22 (m, 1H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.46-7.52 (m, 3H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.97 (s, 1H), 8.24 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.65 (s, 1H), 12.47 (s, -NH). LCMS: *m/z* = 461.2 [M+1].

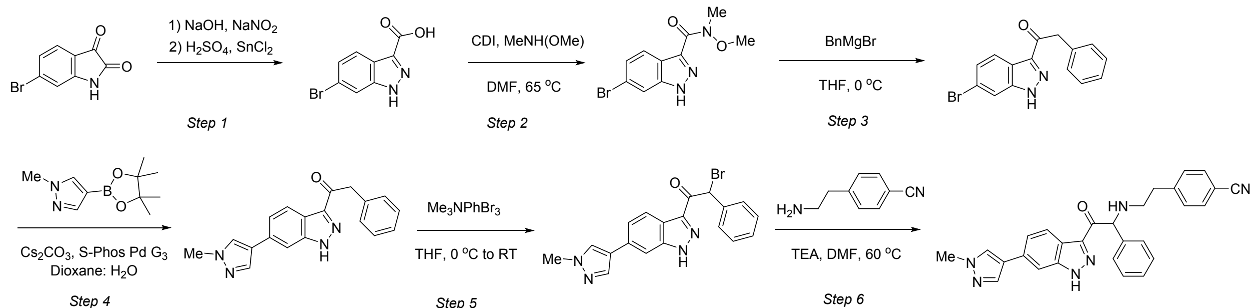
Enantiomer of **4** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.68-2.86 (m, 4H), 3.89 (s, 3H), 5.30 (s, 1H), 7.19-7.22 (m, 1H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.46-7.50 (m, 3H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.97 (s, 1H), 8.23 (s, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 8.63 (s, 1H), 12.46 (s, -NH). LCMS: *m/z* = 461.2 [M+1].

Compound **5**



(*rac*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-3-yl)-2-oxo-1-phenylethyl)amino)-ethyl)benzonitrile

Compound **5** was prepared according the following scheme and procedures.



Step 1. 6-Bromo-1H-indazole-3-carboxylic acid: To a stirred solution of sodium hydroxide (0.48 g, 12.0 mmol) in water (8 ml) was added 6-bromoisatin (2.5 g, 11.0 mmol). The reaction mixture was gently heated until it became dark red. This dark-red solution was cooled to 0°C and mixed with a solution of sodium nitrite (0.76 g, 11 mmol) in water (3 ml) at 0°C. This solution was then added to a rapidly stirred solution of sulphuric acid (1.1 ml, 22.0 mmol) in water (42 ml) via a dropping funnel at 0°C. The rate of addition was maintained such that the temperature of reaction mixture should not rise above 5°C. After completion of addition, the brownish-yellow solution was stirred for 15 minutes. Then a cold solution of stannous chloride dihydrate (6 g, 26.5 mmol) in concentrated hydrochloric acid (10 ml) was added from a dropping funnel to the stirred solution of the diazo-compound. The mixture was stirred for 1 hour after the addition was complete. The crude product, a yellow to brown paste, was collected on a Büchner funnel (1.75 g, 65%) and used for the subsequent step directly without further purification. LCMS: m/z = 239.16 [M-1], 241.20 [M+1].

Step 2. 6-Bromo-N-methoxy-N-methyl-1H-indazole-3-carboxamide: To a stirred solution of 6-bromo-1H-indazole-3-carboxylic acid (1.0 g, 4.16 mmol) in DMF (10 ml) at room temperature was added CDI (0.742 g, 4.58 mmol). The reaction mixture was stirred at 65°C for 1 hour. The reaction was cooled to room temperature and hydroxylamine hydrochloride (0.447 g, 4.58 mmol) was added. The resulting mixture was stirred at 65°C for 12 hours. After completion of the reaction, the reaction was diluted with water (30 ml) and extracted with ethyl acetate (2 x 35 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the title compound (0.35 g, 30%). LCMS: m/z = 284.0 [M+1].

Step 3. 1-(6-Bromo-1H-indazol-3-yl)-2-phenylethan-1-one: To a stirred solution of 6-bromo-N-methoxy-N-methyl-1H-indazole-3-carboxamide (0.284, 1.0 mmol) in THF (3 ml) cooled to 0°C was added benzylmagnesium bromide (2 ml, 2M solution in THF 4.0 mmol). The reaction mixture was stirred at 0°C for 2 hours. After completion of the reaction, the reaction mixture was quenched with saturated aqueous ammonium chloride solution. The aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound (0.29 g, 92 %). LCMS: m/z = 315.0 [M+1].

Step 4. 1-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-3-yl)-2-phenylethan-1-one**:** A suspension of 1-(6-bromo-1H-indazol-3-yl)-2-phenylethan-1-one (0.35 g, 1.11 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.34 g, 1.66 mmol) and cesium carbonate (0.72 g, 2.22 mmol) in a 4:1 mixture of dioxane:water (5 ml) was degassed for 20 min with argon gas. To the reaction mixture, S-phos Pd G3 precatalyst (0.04 g, 0.055 mmol) was added and degassing was continued for another 10 minutes. The reaction mixture was heated in a sealed tube at 100°C for 2 hours. After completion of reaction, the reaction mixture was diluted with water (25 ml) and extracted with ethyl acetate (3 x 25

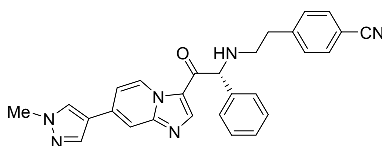
ml). The combined organic layers were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (0.32 g, 92%) as solid. LCMS: *m/z* = 317.0 [M+1].

Step 5. 2-bromo-1-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-3-yl)-2-phenylethan-1-one: To a solution of 1-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-3-yl)-2-phenylethan-1-one (0.38 g, 1.20 mmol) in dry THF (10 ml) under an atmosphere of nitrogen, was added a solution of trimethylphenylammonium tribromide (0.49 g, 1.32 mmol) in THF (3 ml) drop wise at 0°C. The resulting reaction mixture was warmed to room temperature and stirred for 3 hours. The reaction was quenched with a saturated aqueous solution of sodium bicarbonate (25 ml) and the product was extracted with ethyl acetate (3 x 25 ml). The combined organic layers were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was triturated with 80:20 ethyl acetate: hexanes. The solid was collected by filtration to afford the title compound (0.4 g, 84%) as solid. LCMS: *m/z* = 395.0 [M+1].

Step 6. (*rac*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-3-yl)-2-oxo-1-phenylethyl)-amino)ethyl) benzonitrile: A mixture of 2-bromo-1-(6-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-3-yl)-2-phenylethan-1-one (0.05 g, 0.12 mmol), 4-(2-aminoethyl)benzonitrile (0.02 g, 0.15 mmol) and Et_3N (0.035 ml, 0.25 mmol) in DMF (1 ml) was heated at 60°C for 2 hours. After completion of reaction, the reaction mixture was poured into ice-cold water (25 ml) and extracted with ethyl acetate (3 x 25 ml). The combined organic layers were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to afford the title compound (0.012 g, 21%) as a solid.

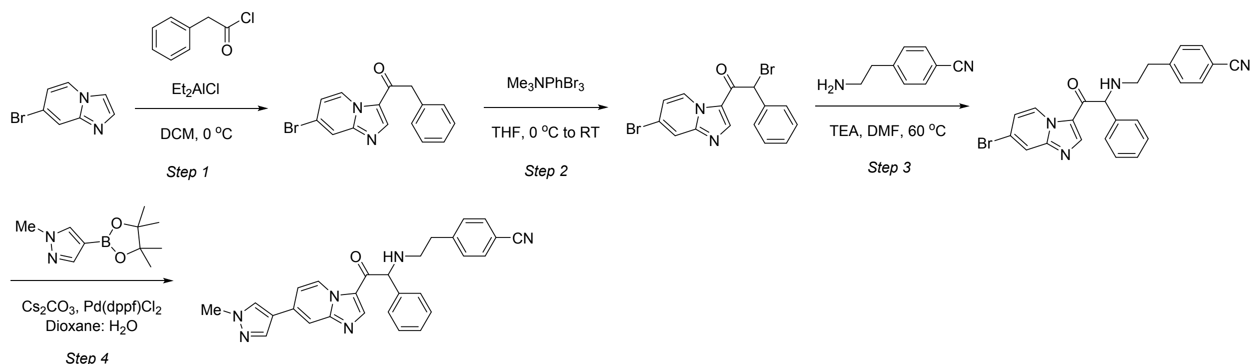
^{1}H NMR (400 MHz, DMSO-d6): δ 2.70-2.80 (m, 2H), 2.84-2.87 (m, 2H), 3.87 (s, 3H), 5.71 (s, 1H), 7.20 (d, J = 7.2 Hz, 1H), 7.28 (t, J = 7.6 Hz, 2H), 7.41-7.44 (m, 4H), 7.55 (d, J = 8.4 Hz, 1H), 7.71-7.74 (m, 3H), 7.97 (d, 1H), 8.08 (d, J = 8.4 Hz, 1H), 8.26 (s, 1H), 13.70 (s, -NH). LCMS: *m/z* = 461.5 [M+1].

Compound **6**



(*R*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)pyrazolo[1,5-a]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile

Compound **6** was prepared according the following scheme and procedures.



Step 1. 1-(7-bromoimidazo[1,2-a]pyridin-3-yl)-2-phenylethan-1-one: To a stirred solution of 7-bromoimidazo[1,2-a]pyridine (0.5 g, 2.53 mmol) in CS_2 (10 ml) under an atmosphere of nitrogen, $AlCl_3$ (0.675 g, 5.07 mmol) was added portion wise at 45°C. The reaction mixture was stirred for 30 minutes at 45°C and phenyl acetyl chloride (0.78 g, 5.07 mmol) was added dropwise. The resulting reaction mixture was stirred at 45°C for 12 hours. The reaction was quenched with a saturated aqueous solution of sodium bicarbonate (20 ml) and the mixture was extracted with ethyl acetate (2 x 30 ml). The combined organic layers were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the title compound (0.14 g, 17%). LCMS: *m/z* = 315.03 [M+1].

Step 2. 2-bromo-1-(7-bromoimidazo[1,2-a]pyridin-3-yl)-2-phenylethan-1-one: To a solution of 1-(7-bromoimidazo[1,2-a]pyridin-3-yl)-2-phenylethan-1-one (0.5 g, 1.58 mmol) in dry THF (20 ml) under an atmosphere of nitrogen, was added a solution of trimethylphenylammonium tribromide (0.65 g, 1.74 mmol) in THF (1.5 ml) drop wise at 0°C. The resulting reaction mixture was warmed to room temperature and stirred for 5 days. The reaction was quenched with saturated sodium bicarbonate solution (25 ml) and the mixture was extracted with ethyl acetate (2 x 30 ml). The combined organic layers were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product obtained was triturated with mixture of 80:20 ethyl acetate:hexanes to provide a solid which was collected by filtration to afford the title compound (0.5 g, LCMS: 20%) which was used for next step without further purification. LCMS: *m/z* = 394.0 [M+1].

Step 3. 4-(2-((2-(7-bromoimidazo[1,2-a]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)-benzonitrile: A mixture of 2-bromo-1-(7-bromoimidazo[1,2-a]pyridin-3-yl)-2-phenylethan-1-one (1 g, 2.53 mmol), 4-(2-aminoethyl)benzonitrile hydrochloride (0.92 g, 5.07 mmol) and Et_3N (1.41 ml, 10.15 mmol) in DMF (10 ml) was heated at 60°C for 3 hours. The reaction mixture was poured into ice-cold water (25 ml) and extracted with ethyl acetate (2 x 40 ml). The combined organic extracts were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography to afford the title compound (0.28 g, LCMS: 41%) as solid. LCMS: *m/z* = 459.5 [M+1].

Step 4. (*R*)- and (S)-4-(2-((2-(7-(1-methyl-1H-pyrazol-4-yl)imidazo[1,2-a]pyridin-3-yl)-2-oxo-1-phenylethyl)amino) ethyl)-benzonitrile: A suspension of 4-(2-((2-(7-bromoimidazo[1,2-a]pyridin-3-yl)-2-

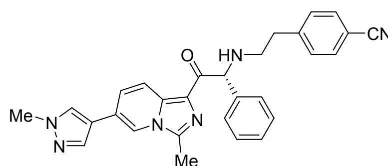
oxo-1-phenylethyl)amino)ethyl)benzonitrile (0.20 g, 0.43 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.11 g, 0.56 mmol), and cesium carbonate (0.42 g, 1.30 mmol) in a mixture of 4:1 dioxane:water (10 ml) were degassed for 20 minutes with argon. To the reaction mixture, Pd(dppf)Cl_2 (0.03 g, 0.043 mmol) was added and degassing was continued for another 10 minutes. The reaction mixture was heated in a sealed tube under microwave irradiation at 90°C for 1 hour. The reaction was cooled to room temperature, diluted with water (30 ml) and extracted with ethyl acetate (2 x 25 ml). The combined organic extracts were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residues was purified by silica gel chromatography to afford the racemic title compound (0.10 g, 50%) as a solid.

The racemic title compound was resolved by Chiral SFC (CHIRALPAK OX-H; 25% MeOH in CO_2 + 0.1% DEA) to furnish the enantiopure compounds. Isomer 1: t_R = 19.1 min, Isomer 2: t_R = 24.2 min.

Compound **6** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.73-2.75 (m, 2H), 2.86-2.89 (m, 2H), 3.89 (s, 3H), 5.36 (s, 1H), 7.23-7.25 (m, 1H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.50-7.55 (m, 3H), 7.73 (d, *J* = 8.0 Hz, 2H), 8.05 (s, 1H), 8.15 (s, 1H), 8.35 (bs, 1H), 8.44 (d, *J* = 8.0 Hz, 1H), 8.90 (s, 1H), 9.44 (d, *J* = 7.2 Hz, 1H). LCMS: *m/z* = 461.4 [M+1].

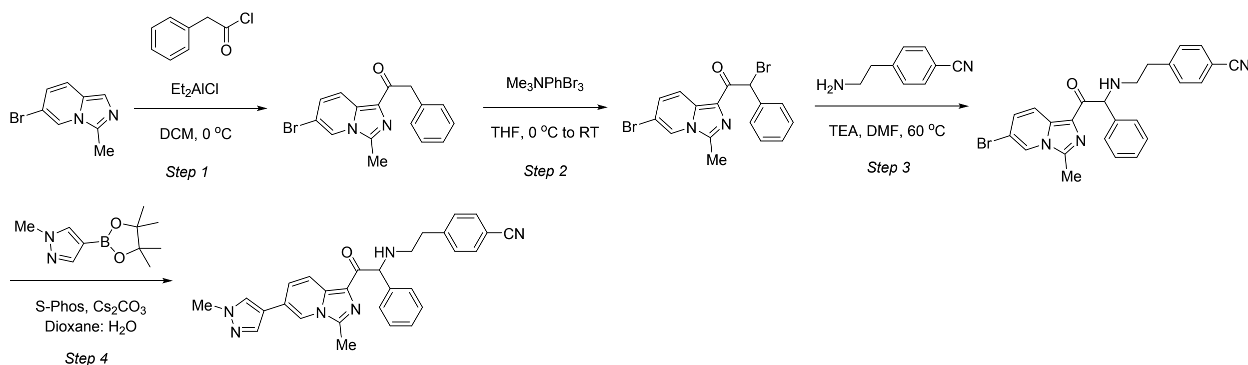
Enantiomer of **6** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.73-2.75 (m, 2H), 2.86-2.88 (m, 2H), 3.90 (s, 3H), 5.37 (s, 1H), 7.23-7.25 (m, 1H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.50-7.55 (m, 3H), 7.74 (d, *J* = 8.0 Hz, 2H), 8.05 (s, 1H), 8.15 (s, 1H), 8.35 (bs, 1H), 8.44 (d, *J* = 8.0 Hz, 1H), 8.89 (s, 1H), 9.44 (d, *J* = 7.2 Hz, 1H). LCMS: *m/z* = 461.5 [M+1].

Compound **7**



(*R*)-4-(2-((2-(3-methyl-6-(1-methyl-1H-pyrazol-4-yl)imidazo[1,5-a]pyridin-1-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile

Compound **7** was prepared according the following scheme and procedures.



Step 1. 1-(6-bromo-3-methylimidazo[1,5-a]pyridin-1-yl)-2-phenylethan-1-one: To a stirred solution of 6-bromo-3-methylimidazo[1,5-a]pyridine (0.8 g, 3.79 mmol) in dry DCM (12 ml), anhydrous aluminum chloride (1.51 g, 11.37 mmol) was added at room temperature. Then a solution of phenyl acetyl chloride (1.75 g, 11.37 mmol) in DCM (4 ml) was added drop wise at room temperature. After completion of reaction, the reaction mixture was quenched with water (50 ml) and extracted with DCM (2 x 50 ml). The combined organic extracts were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the title compound (0.41 g, 32%). LCMS: m/z = 329.07 [M+1].

Step 2. 2-bromo-1-(6-bromo-3-methylimidazo[1,5-a]pyridin-1-yl)-2-phenylethan-1-one: To a solution of 1-(6-bromo-3-methylimidazo [1,5-a]pyridin-1-yl)-2-phenylethan-1-one (0.41 g, 1.24 mmol) in dry THF (16 ml), a solution of trimethylphenylammonium tribromide (0.51 g, 1.37 mmol) in THF (8 ml) was added drop wise at 0°C under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature overnight. Upon completion of the reaction, saturated sodium bicarbonate solution (20 ml) was added slowly and the mixture was extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure and dried to afford the title compound (0.50 g, 98%) as a solid. LCMS: m/z = 408.1 [M+1].

Step 3. 4-(2-((2-(6-bromo-3-methylimidazo[1,5-a]pyridin-1-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile: A mixture of 2-bromo-1-(6-bromo-3-methylimidazo[1,5-a]pyridin-1-yl)-2-phenylethan-1-one (0.52 g, 1.27 mmol), 4-(2-aminoethyl)benzonitrile (0.37 g, 2.55 mmol) and Et_3N (0.25 g, 2.55 mmol) in DMF (5 ml) was heated at 60°C for 3 hours. After completion of reaction, the reaction mixture was poured into ice-cold water (20 ml) and extracted with ethyl acetate (3 x 25 ml). The combined organic extracts were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the title compound (0.25 g, 41%). LCMS: m/z = 473.4 [M+1].

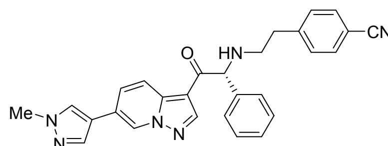
Step 4. (*R*)- and (*S*)-4-(2-((2-(3-methyl-6-(1-methyl-1H-pyrazol-4-yl)imidazo[1,5-a]pyridin-1-yl)-2-oxo-1-phenylethyl) amino)ethyl)benzonitrile: A suspension of 4-(2-((2-(6-bromo-3-methylimidazo[1,5-a]pyridin-1-yl)-2-oxo-1-phenylethyl) amino)ethyl) benzonitrile (0.25 g, 0.53 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.22 g, 1.06 mmol) and cesium carbonate (0.51 g, 1.58 mmol) in a mixture of 4:1 dioxane:water (5 ml) was degassed for 20 minutes with argon. To the reaction mixture, S-Phos Pd G3 precatalyst (0.04 g, 0.05 mmol) was added and degassing was continued for 10 minutes. The reaction mixture was heated in a sealed tube at 90°C for 2 hours. The reaction mixture was diluted with water (10 ml) and extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (10 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the racemic title compound (0.15 g, 59%) as a solid.

The racemic title compound was resolved by Chiral SFC (CHIRALPAK IB; 45% (50:50 IPA:MeOH) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

Enantiomer of **7** (faster-eluting isomer): ^1H NMR (400 MHz, DMSO-d6): δ 2.70 (s, 3H), 2.82-2.85 (m, 4H), 3.88 (s, 3H), 5.63 (d, J = 10 Hz, 1H), 7.17-7.27 (m, 3H), 7.42 (t, J = 6.8 Hz, 4H), 7.62 (d, J = 9.6 Hz, 1H), 7.72 (d, J = 8 Hz, 2H), 8.04 (s, 1H), 8.12 (d, J = 9.2 Hz, 1H), 8.30 (s, 1H), 8.56 (s, 1H). LCMS: m/z = 475.4 [M+1].

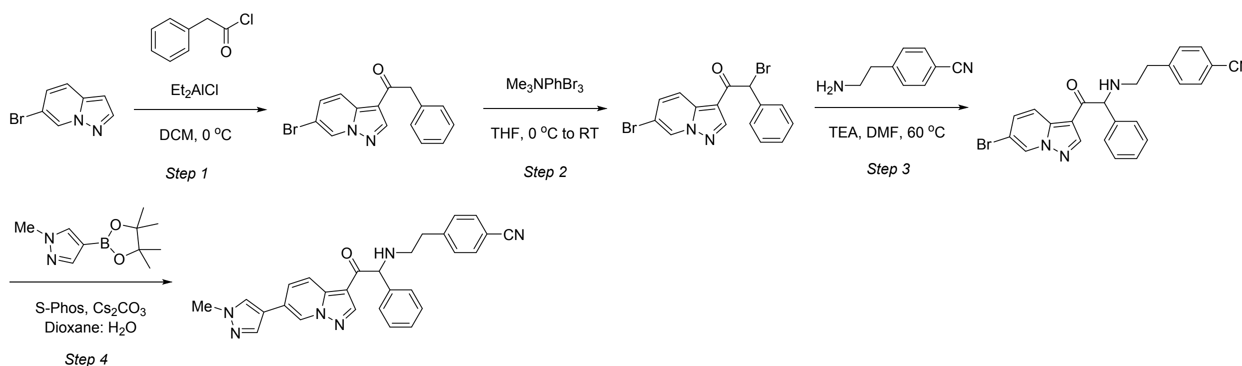
Compound **7** (slower-eluting isomer; assigned as the (*R*)-isomer based on analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.70 (s, 3H), 2.74-2.85 (m, 4H), 3.88 (s, 3H), 5.63 (d, J = 10.4 Hz, 1H), 7.17-7.27 (m, 3H), 7.42 (t, J = 6.8 Hz, 4H), 7.62 (d, J = 9.6 Hz, 1H), 7.72 (d, J = 8 Hz, 2H), 8.04 (s, 1H), 8.12 (d, J = 9.2 Hz, 1H), 8.30 (s, 1H), 8.57 (s, 1H). LCMS: m/z = 475.4 [M+1].

Compound **8**



(*R*)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)pyrazolo[1,5-a]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile

Compound **8** was prepared according the following scheme and procedures.



<u>Step 1. 1-(6-bromopyrazolo[1,5-a]pyridin-3-yl)-2-phenylethan-1-one:</u> To a stirred solution of 6-bromopyrazolo[1,5-a]pyridine (2 g, 10.2 mmol) in dry DCM (24 ml) was added aluminum chloride (4.1 g, 30.5 mmol) portion wise at 0°C . To this mixture phenylacetyl chloride (4 ml, 30.5 mmol) was added drop wise at 0°C and the resulting reaction mixture was stirred at room temperature overnight. Saturated sodium bicarbonate solution (60 ml) was added slowly and the organic layer was separated. The aqueous layer was extracted with DCM (2 x 50 ml). During workup a solid formed, which was filtered through Büchner funnel. The resulting solid was stirred in DCM (50 ml) for 30 minutes, which was filtered again with a Büchner funnel. The combined organic extracts were washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to afford the title compound (1.0 g, 31%). LCMS: *m/z* = 315.3 [M+1].

<u>Step 2. 2-bromo-1-(6-bromopyrazolo[1,5-a]pyridin-3-yl)-2-phenylethan-1-one:</u> To a solution of 1-(6-bromopyrazolo[1,5-a]pyridin-3-yl)-2-phenylethan-1-one (1 g, 3.20 mmol) in dry THF (12 ml) under an atmosphere of nitrogen was added a solution of trimethylphenylammonium tribromide (1.313 g, 3.50 mmol) in THF (6 ml) drop wise at room temperature and the resulting reaction mixture was stirred for 12 hours. Water (35 ml) was added and the reaction mixture was extracted with ethyl acetate (2 x 35 ml). The combined organic extracts were washed with brine (35 ml), dried over anhydrous Na_2SO_4 and

concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography to afford the title compound (1 g, 79%). LCMS: m/z = 395.2 [M+1].

Step 3. 4-(2-((2-(6-bromopyrazolo [1, 5-a]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)-benzonitrile: A mixture of 2-bromo-1-(6-bromopyrazolo[1,5-a]pyridin-3-yl)-2-phenylethan-1-one (0.90 g, 2.28 mmol), 4-(2-aminoethyl)benzonitrile (0.62 g, 3.42 mmol), and triethylamine (1.28 ml, 9.13 mmol) in DMF (10 ml) was heated at 60°C for 2 hours. The reaction mixture was poured into ice-cold water (50 ml) and the mixture was extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to afford the title compound (0.58 g, 55%). LCMS: m/z = 459.5 [M+1].

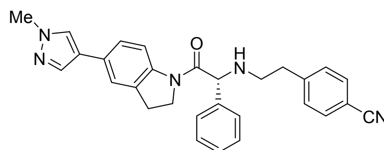
Step 4. (*R*)- and (S)-4-(2-((2-(6-(1-methyl-1H-pyrazol-4-yl)pyrazolo[1,5-a]pyridin-3-yl)-2-oxo-1-phenylethyl)-amino)ethyl)benzonitrile: A suspension of 4-(2-((2-(6-bromopyrazolo[1,5-a]pyridin-3-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile (0.6 g, 1.31 mmol), 1-methylpyrazole-4-boronic acid pinacol ester (0.41 g, 1.97 mmol) and cesium carbonate (1.29 g, 3.92 mmol) in a mixture of 2:1 dioxane-water (18 ml) was purged for 20 minutes with argon. Then, S-Phos Pd G3 precatalyst (0.102 g, 0.131 mmol) was added and purging with argon was continued for another 10 minutes. The reaction mixture was heated at 100°C for 1.5 hours. The reaction mixture was diluted with water (20 ml) and extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the racemic title compound (0.3 g, 50%).

The racemic title compound was resolved by Chiral SFC (CHIRALPAK IB; 30% (50:50 IPA:MeOH) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

Compound **8** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.68 – 2.73 (m, 2H), 2.80 – 2.90 (m, 2H), 3.88 (s, 3H), 5.29 (s, 1H), 7.22-7.25 (m, 1H), 7.31 (t, J = 7.6 Hz, 2H), 7.43 (d, J=8.0 Hz, 2H), 7.48 (d, J = 7.2 Hz, 2H), 7.73 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 9.2 Hz, 1H), 8.05 (s, 1H), 8.21 (d, J = 9.2 Hz, 1H), 8.31 (s, 1H), 8.87 (s, 1H), 9.19 (s, 1H). LCMS: m/z = 461.6 [M+1].

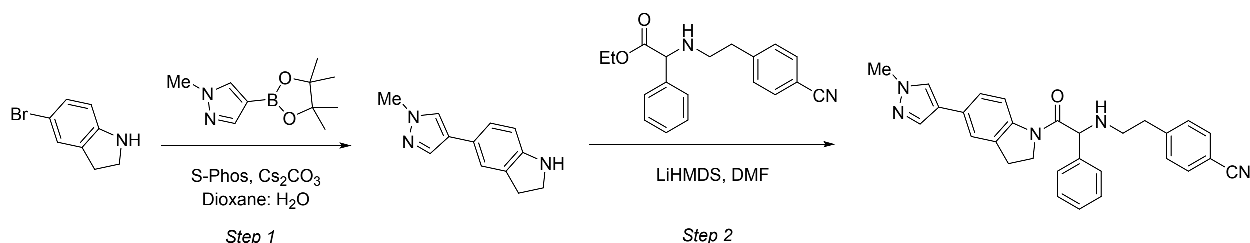
Enantiomer of **8** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.68 – 2.75 (m, 2H), 2.80 – 2.90 (m, 2H), 3.88 (s, 3H), 5.30 (s, 1H), 7.21-7.25 (m, 1H), 7.31 (t, J = 7.6 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 7.2 Hz, 2H), 7.74 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 8.8 Hz, 1H), 8.06 (s, 1H), 8.22 (d, J = 9.2 Hz, 1H), 8.32 (s, 1H), 8.88 (s, 1H), 9.20 (s, 1H). LCMS: m/z = 461.6 [M+ 1].

Compound **9**



(*R*)-4-(2-((2-(5-(1-methyl-1H-pyrazol-4-yl)indolin-1-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile

Compound **9** was prepared according the following scheme and procedures.



Step 1. 5-(1-methyl-1H-pyrazol-4-yl)indoline: A suspension of 5-bromoindoline (0.50 g, 2.52 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.63 g, 3.02 mmol), and cesium carbonate (2.46 g, 7.57 mmol) in a 4:1 mixture of dioxane:water (6 ml) was degassed for 20 minutes with argon. To the reaction mixture, S-Phos Pd G3 precatalyst (0.09 g, 0.13 mmol) was added and degassing was continued for another 10 minutes. The reaction mixture was heated at 100°C for 2 hours. The reaction mixture was diluted with water (25 ml) and extracted with ethyl acetate (2 x 30 ml). The combined organic extracts were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to give the title compound (0.35 g, 70%) as solid.

^{1}H NMR (400 MHz, DMSO-d6): 2.91 (t, *J* = 8Hz, 2H), 3.41 (t, *J* = 8 Hz, 2H), 3.84 (s, 3H), 5.46 (s, 1H, -NH), 6.48 (d, *J* = 8 Hz, 1H), 7.10 (d, *J* = 8 Hz, 1H), 7.23 (s, 1H), 7.66 (s, 1H), 7.89 (s, 1H). LCMS: *m/z* = 200.2 [M+1].

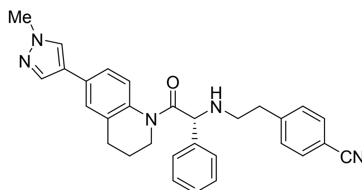
Step 2. (*R*)- and (*S*)-4-(2-((2-(5-(1-methyl-1H-pyrazol-4-yl)indolin-1-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile: To a mixture of 5-(1-methyl-1H-pyrazol-4-yl)indoline (0.2 g, 1.00 mmol) and ethyl 2-((4-cyanophenethyl)amino)-2-phenylacetate (0.2 g, 1.00 mmol) in DMF (2 ml), LiHMDS (2.0 ml, 2.00 mmol; 1*M* in THF) was added under an atmosphere of nitrogen at 0°C. The reaction mixture was then heated to 80°C for 2 hours. After completion of the reaction, the mixture was diluted with ethyl acetate (50 ml) and quenched with water (50 ml) at room temperature. The aqueous layer was extracted with ethyl acetate (2 x 30 ml). The combined organic extracts were washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the racemic title compound (0.22 g, 47%).

The racemic title compound was resolved by Chiral SFC (CHIRALPAK IB; 40% (50:50 IPA:MeOH) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

Enantiomer of **9** (faster-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.68-2.84 (m, 3H), 3.03-3.18 (m, 3H), 3.69-3.76 (m, 1H), 3.84 (s, 3H), 4.35 (d, *J* = 8 Hz, 1H), 4.68 (s, 1H), 7.29-7.44 (m, 9H), 7.74 (d, *J* = 8 Hz, 2H), 7.79 (s, 1H), 8.06-8.11 (m, 2H). LCMS: m/z = 462.4 [M+1].

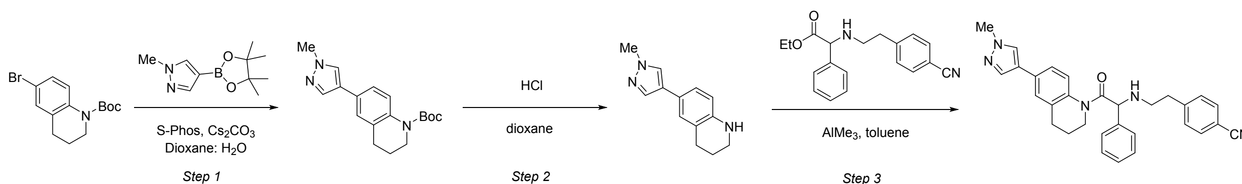
Compound **9** (slower-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.61-2.85 (m, 5H), 3.04-3.14 (m, 2H), 3.69-3.76 (m, 1H), 3.84 (s, 3H), 4.32-4.38 (m, 1H), 4.67-4.70 (m, 1H), 7.27-7.44 (m, 9H), 7.74 (d, *J* = 8 Hz, 2H), 7.80 (s, 1H), 8.06-8.11 (m, 2H). LCMS: m/z = 462.4 [M+1].

Compound **10**



(R)- 4-(2-((2-(6-(1-Methyl-1H-pyrazol-4-yl)-3,4-dihydroquinolin-1(2H)-yl)-2-oxo-1-phenylethyl)amino)-ethyl)benzonitrile

Compound **10** was prepared according the following scheme and procedures.



Step 1. *tert*-Butyl 6-(1-methyl-1H-pyrazol-4-yl)-3,4-dihydroquinoline-1(2H)-carboxylate**:** A suspension of *tert*-butyl 6-bromo-3,4-dihydroquinoline-1(2H)-carboxylate (0.55 g, 1.76 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.439 g, 2.11 mmol), and cesium carbonate (1.43 g, 4.40 mmol) in mixture of 4:1 dioxane:water (10 ml) was purged for 20 minutes with argon gas. S-Phos Pd-G3-precatalyst (0.066 g, 0.08 mmol) was added and purging was continued for another 10 minutes. The reaction mixture was heated at 100°C for 2 hours. The reaction mixture was poured into water (25 ml) and extracted with ethyl acetate (2 x 30 ml). The combined organic extracts were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (0.55 g, 99%) as a solid.

^{1}H NMR (400 MHz, DMSO-d6): 1.08 (s, 9H), 1.81-1.87 (m, 2H), 2.74 (t, J = 6.4 Hz, 2H), 3.63 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 7.29-7.31 (m, 2H), 7.54 (d, J = 9.2 Hz, 1H), 7.80 (s, 1H), 8.07 (s, 1H) ; LCMS: m/z = 314.2 [M+1].

Step 2. 6-(1-Methyl-1H-pyrazol-4-yl)-1,2,3,4-tetrahydroquinoline: To a stirred solution of *tert*-butyl 6-(1-methyl-1H-pyrazol-4-yl)-3,4-dihydroquinoline-1(2H)-carboxylate (0.1 g, 0.31 mmol) in dioxane (1 ml) was added 4M HCl in dioxane (1 ml) dropwise at 0°C. The reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated and neutralized with saturated sodium bicarbonate solution and extracted with ethyl acetate (3 x 10 ml). The combined organic extracts were washed with brine (10 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford the title compound (0.050 g, 73%). LCMS: m/z = 214.2 [M+1].

Step 3. (R)-and (S)- 4-(2-((2-(6-(1-Methyl-1H-pyrazol-4-yl)-3,4-dihydroquinolin-1(2H)-yl)-2-oxo-1-phenylethyl)amino)ethyl)benzonitrile: To a mixture of 6-(1-methyl-1H-pyrazol-4-yl)-1,2,3,4-tetrahydroquinoline (0.05 g, 0.23 mmol) and ethyl 2-((4-cyanophenethyl)amino)-2-phenylacetate (0.060 g, 0.19 mmol) in toluene (0.6 ml) was added trimethylaluminum (0.19 ml, 2M in toluene, 0.39 mmol) under an atmosphere of nitrogen at 0°C. The resulting reaction mixture was heated at 100°C for 2 hours. After completion of the reaction, the mixture was quenched with saturated aqueous sodium bicarbonate (10 ml) and the aqueous layer was extracted with ethyl acetate (2 x 10 ml). The combined organic extracts were washed with brine (10 ml), dried over anhydrous Na_2SO_4, and concentrated under

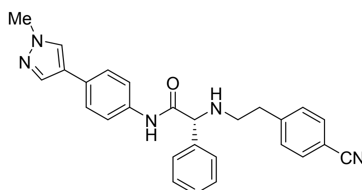
reduced pressure. The resulting residue was purified by silica gel chromatography to afford the racemic title compound (0.03g, 32%).

The racemic compound was resolved by chiral HPLC (CHIRALCEL OJ-H; 15% (50:50 ACN: IPA) in Liquid CO_2 + 0.1% DEA) to furnish the enantiopure compounds as solids.

Compound **10** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.71-1.77 (m, 2H), 2.58-2.79 (m, 6H), 3.36-3.52 (m, 1H), 3.79-3.85 (m, 1H), 3.86 (s, 3H), 4.86 (s, 1H), 6.85-7.19 (m, 2H), 7.25-7.35 (m, 6H), 7.41 (d, , J = 7.6 Hz, 2H), 7.75 (d, , J = 8.0 Hz, 2H), 7.85 (s, 1H), 8.12 (s, 1H). LCMS: m/z = 476.3 [M+1].

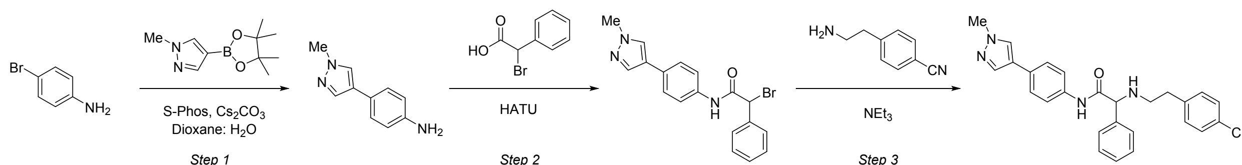
Enantiomer of **10** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.71-1.77 (m, 2H), 2.65-2.79 (m, 6H), 3.36-3.46 (m, 1H), 3.79-3.81 (m, 1H), 3.86 (s, 3H), 4.86 (s, 1H), 6.85-7.15 (m, 2H), 7.25-7.35 (m, 6H), 7.41 (d, , J = 8.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 7.85 (s, 1H), 8.12 (s, 1H). LCMS: m/z = 476.3 [M+1].

Compound **11**



(*R*)-2-((4-Cyanophenethyl)amino)-N-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)-2-phenylacetamide

Compound **11** was prepared according the following scheme and procedures.



Step 1. 4-(1-Methyl-1H-pyrazol-4-yl)aniline: A suspension of 4-bromoaniline (1.0 g, 5.81 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (1.3 g, 6.39 mmol) and cesium carbonate (5.68 g, 17.43 mmol) in a 4:1 mixture of dioxane:water (20 ml) was purged for 20 minutes with argon. S-Phos Pd G3 precatalyst (0.213 g, 0.29 mmol) was added and purging was continued for another 10 minutes. The reaction mixture was heated at 100°C for 2 hours. The reaction mixture was poured into water (15 ml) and extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (10 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (0.965 g, 95 %) as solid. ^{1}H NMR (400 MHz, DMSO-d6): 3.81 (s, 3H), 5.01 (s, 2H, -NH2), 6.54 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 7.63 (s, 1H), 7.86 (s, 1H). LCMS: m/z = 174.2 [M + 1].

Step 2. 2-Bromo-N-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)-2-phenylacetamide: To a stirred solution of 4-(1-methyl-1H-pyrazol-4-yl) aniline (0.95 g, 5.48 mmol) and 2-bromo-2-phenylacetic acid

(1.3 g, 6.03 mmol) in ethyl acetate (10 ml) was added T3P (5.22 g, 8.22 mmol; 50% in ethyl acetate). The reaction mixture was stirred for 30 minutes at room temperature. Then Hunig's base (1.41 g, 10.96 mmol) was added and the reaction mixture was heated at 60°C for 3 hours. The reaction mixture was poured into water (15 ml) and extracted with ethyl acetate (2 x 10 ml). The combined organic extracts were washed with brine (10 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (1.2 g, 59 %) as a solid. ^{1}H NMR (400 MHz, DMSO-d6): 3.85 (s, 3H), 5.79 (s, 1H), 7.38-7.44 (m, 3H), 7.52-7.59 (m, 4H), 7.65 (d, J = 6.8Hz, 2H), 7.82 (s, 1H), 8.09 (s, 1H), 10.54 (s, 1H, -NH). LCMS: m/z = 370.1 [M + 1] and 372.4 [M + 2].

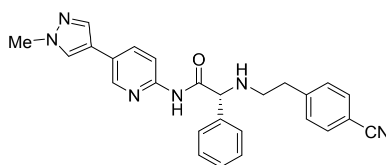
Step 3. (*R*)- and (*S*)-2-((4-Cyanophenethyl)amino)-N-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)-2-phenylacetamide: A mixture of 2-bromo-N-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)-2-phenylacetamide (0.5 g, 1.35 mmol), 4-(2-aminoethyl)benzonitrile hydrochloride (0.296 g, 2.7 mmol) and treithylamine (0.6 ml, 4.05 mmol) in DMF (5 ml) was heated for 2 hours at 60°C. After completion of the reaction, the reaction mixture was poured into ice-cold water (15 ml) and extracted with ethyl acetate (2 x 30 ml). The combined organic extracts were washed with brine (15 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the racemic title compound (0.35 g, 59%) as solid.

The racemic title compound was resolved by chiral HPLC (CHIRALPAK AD-H; 30% (50:50 ACN:IPA in liquid CO_2 + 0.1% DEA) to furnish the enantiopure compounds.

Enantiomer of **11** (faster-eluting enantiomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.76-2.78 (m, 2H), 2.86-2.88 (m, 2H), 3.85 (s, 3H), 4.38 (s, 1H), 7.28-7.37 (m, 3H), 7.45-7.49 (m, 6H), 7.53 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 7.79 (s, 1H), 8.06 (s, 1H), 10.04 (s, 1H). LCMS: m/z = 436.5 [M + 1].

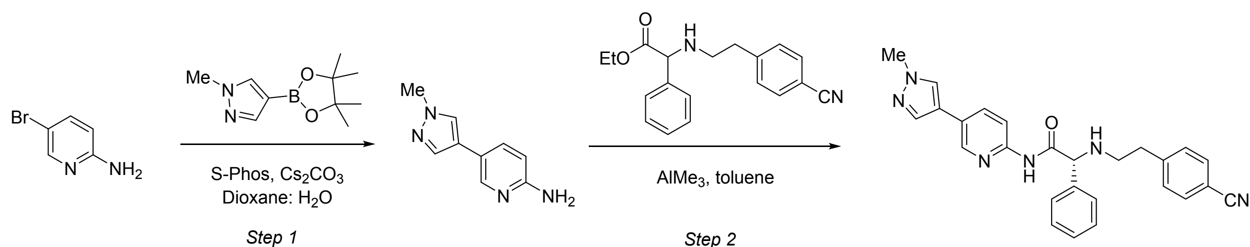
Compound **11** (slower-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.76-2.79 (m, 2H), 2.86-2.89 (m, 2H), 3.85 (s, 3H), 4.38 (s, 1H), 7.28-7.37 (m, 3H), 7.44-7.49 (m, 6H), 7.53 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 7.79 (s, 1H), 8.06 (s, 1H), 10.03 (s, 1H). LCMS: m/z = 436.5 [M + 1].

Compound **12**

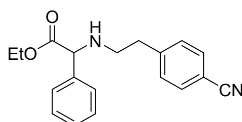


(*R*)-2-((4-cyanophenethyl)amino)-N-(5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-2-phenylacetamide

Compound **12** was prepared according the following scheme and procedures.



Step 1. 5-(1-Methyl-1H-pyrazol-4-yl)pyridin-2-amine: A suspension of 5-bromopyridin-2-amine (18.0 g, 104.04 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (32.47 g, 156.06 mmol) and cesium carbonate (101.75 g, 312.12 mmol) in a 4:1 mixture of dioxane:water (360 ml) were purged for 20 minutes with argon gas. To this mixture, Pd(dppf)Cl_2 (7.61 g, 10.40 mmol) was added and purging was continued for another 10 minutes. The reaction mixture was heated at 80°C for 1.5 hours. The reaction mixture was poured into water (200 ml) and extracted with ethyl acetate (2 x 200 ml). The combined organic extracts were washed with brine (150 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to afford the title compound (15 g, 82 %) as a solid. ^{1}H NMR (400 MHz, DMSO-d6): 3.83 (s, 3H), 5.86 (s, 2H, -NH2), 6.44 (d, J = 8.4 Hz, 1H), 7.20 (dd, J = 8.4 Hz, 2.4 Hz, 1H), 7.70 (s, 1H), 7.95 (s, 1H), 8.14 (d, J = 2.09 Hz, 1H). LCMS: m/z = 175.1 [M+1].



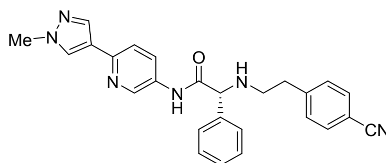
Step 2 (reagent synthesis). Ethyl 2-((4-cyanophenethyl)amino)-2-phenylacetate: A mixture of ethyl 2-bromo-2-phenylacetate (2.0 g, 8.22 mmol), 4-(2-aminoethyl)benzonitrile hydrochloride (2.25 g, 12.33 mmol) and triethylamine (2.50 g, 24.66 mmol) in DMF (20 ml) was heated for 3 hours at 60°C. The reaction mixture was poured into ice-cold water (50 ml) and extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (2.2 g, 86%) as a viscous oil. ^{1}H NMR (400 MHz, DMSO-d6): δ 1.10 (t, J = 7.2 Hz, 3H), 2.62-2.82 (m, 4H), 4.02-4.09 (m, 2H), 4.39 (d, J = 8.4 Hz, 1H), 7.28-7.35 (m, 5H), 7.40 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H). LCMS: m/z = 309.28 [M+1].

Step 2. (*R*)- and (*S*)-2-((4-cyanophenethyl)amino)-N-(5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-2-phenylacetamide: To a stirred solution of 5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-amine (1.0 g, 5.74 mmol) and ethyl 2-((4-cyanophenethyl)amino)-2-phenylacetate (2.12 g, 6.88 mmol) in dry toluene (10 ml) was added trimethylaluminium (5.8 ml, 2M in toluene, 11.48 mmol) at 0°C. The reaction mixture was stirred at 100°C for 2 hours. After completion of the reaction, the reaction mixture was poured into ice-cold water (50 ml) and extracted with ethyl acetate (2 x 100 ml). The combined organic extracts were washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the racemic title compound (0.30 g, 12%).

The racemic title compound was resolved by chiral HPLC (CHIRALCEL OJ-H; 14% MeOH in liquid CO_2 + 0.1% DEA) to furnish the enantiopure compounds.

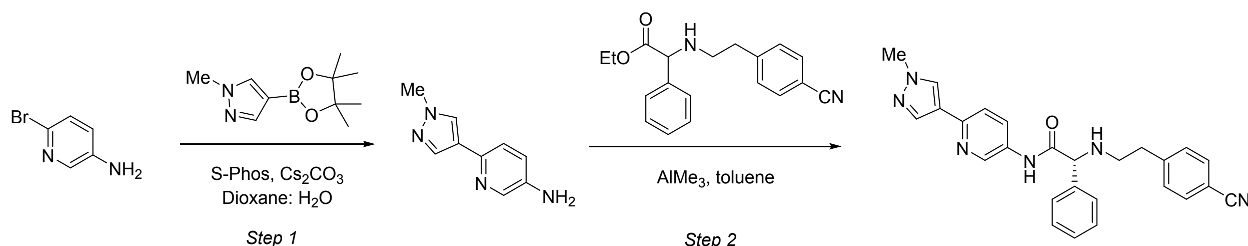
Compound **12** (faster-eluting isomer; assigned as the (*R*)-isomer based on the X-ray co-crystal structures of **12** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.73-2.80 (m, 2H), 2.85-2.88 (m, 3H), 3.86 (s, 3H), 4.53 (d, J = 8.8 Hz, 1H), 7.25-7.29 (m, 1H), 7.32-7.35 (m, 2H), 7.44 (d, J = 8.0 Hz, 4H), 7.73 (d, J = 8.0 Hz, 2H), 7.89 (s, 1H), 7.92-7.95 (m, 1H), 8.02 (d, J = 8.4 Hz, 1H), 8.17 (s, 1H), 8.55 (d, J = 1.6 Hz, 1H), 10.48 (s, 1H). LCMS: m/z = 437.22 [M+1].

Compound **13**



(*R*)-2-((4-cyanophenethyl)amino)-N-(6-(1-methyl-1H-pyrazol-4-yl)pyridin-3-yl)-2-phenylacetamide

Compound **13** was prepared according the following scheme and procedures.



<u>Step 1. 6-(1-methyl-1H-pyrazol-4-yl)pyridin-3-amine:</u> The title compound was prepared in a procedure analogous to that used for the preparation of 5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-amine (step 1 in the synthesis of compound **12**) but starting with 6-bromopyridin-3-amine.

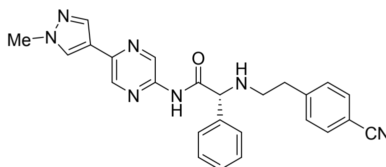
<u>Step 2. (*R*)- and (*S*)-2-((4-cyanophenethyl)amino)-N-(6-(1-methyl-1H-pyrazol-4-yl)pyridin-3-yl)-2-phenylacetamide:</u> To a mixture of 6-(1-methyl-1H-pyrazol-4-yl)pyridin-3-amine (250 mg, 1.44 mmol) and ethyl 2-((4-cyanophen ethyl)amino)-2-phenylacetate (531 mg, 1.72 mmol) in toluene, trimethyl aluminium (2.9 ml, 2.870 mmol; 1 M in toluene) was added under an atmosphere of nitrogen at room temperature. The resulting reaction mixture was heated to 100°C for 2 hours. After completion of the reaction, the mixture was diluted with ethyl acetate (20 ml) and slowly quenched with water (20 ml) at room temperature. The aqueous layer was extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the racemic title compound (150 mg, 30%).

The racemic title compound was resolved by chiral HPLC (CHIRALPAK IB; 55% (50:50 MeOH:IPA) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

Compound **13** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.61-2.82 (m, 2H), 2.87-2.89 (m, 2H), 3.87 (s, 3H), 4.43 (s, 1H), 7.27-7.31 (m, 1H), 7.34-7.39 (m, 2H), 7.44-7.48 (m, 4H), 7.57-7.59 (d, J = 8.8 Hz, 1H), 7.74-7.76 (d, J = 8.0 Hz, 2H), 7.92 (s, 1H), 8.01 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 8.20 (s, 1H), 8.66 (d, J = 2.4 Hz, 1H), 10.34 (s, 1H, -NH). LCMS: m/z = 437.24 [M+1].

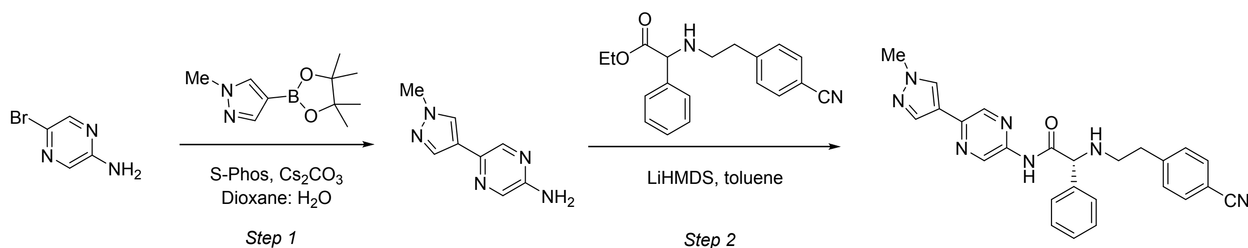
Enantiomer of **13** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.77-2.78 (m, 2H), 2.87-2.89 (m, 2H), 3.87 (s, 3H), 4.42 (s, 1H), 7.27-7.31 (m, 1H), 7.34-7.39 (m, 2H), 7.44-7.48 (m, 4H), 7.59 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.0 Hz, 2H), 7.92 (s, 1H), 8.01 (dd, J = 8.4 Hz, 2.4 Hz, 1H), 8.20 (s, 1H), 8.66 (d, J = 2.0 Hz, 1H), 10.34 (s, 1H, -NH). LCMS: m/z = 437.24 [M+1].

Compound **14**



(*R*)-2-((4-cyanophenethyl)amino)-N-(5-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)-2-phenylacetamide

Compound **14** was prepared according the following scheme and procedures.



Step 1. 5-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-amine: A suspension of 5-bromopyrazin-2-amine (0.20 g, 1.14mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.35 g, 1.72 mmol), and cesium carbonate (1.12 g, 3.44mmol) in a 4:1 mixture of dioxane:water (2 ml) was degassed for 20 minutes with argon. S-Phos Pd G3 precatalyst (0.043 g, 0.057mmol) was added and degassing was continued for 10 minutes. The reaction mixture was heated at 100°C for 1 hour. The reaction mixture was poured into water (15 ml) and extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (0.15 g, 74%) as solid.

LCMS: m/z = 176.1 [M+1].

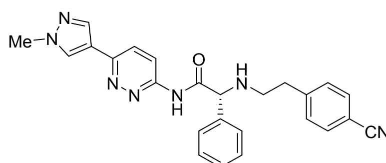
Step 2. (*R*)- and (*S*)-2-((4-cyanophenethyl)amino)-N-(5-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-yl)-2-phenyl acetamide: To a mixture of 5-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-amine (0.12 g, 0.68 mmol) and ethyl 2-((4-cyanophenethyl)amino)-2-phenylacetate (0.21 mg, 0.68 mmol) in DMF (1 ml), was added LiHMDS (1.37 ml, 1.37 mmol; 1M in THF) under an atmosphere of nitrogen at 0°C. The resulting reaction mixture was heated to 50°C for 1 hour. After completion of the reaction, the mixture was diluted with ethyl acetate (20 ml) and slowly quenched with water (30 ml) at room temperature. The aqueous layer was extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (30 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography to afford the racemic title compound (0.09 g, 25%).

The racemic title compound was resolved by chiral HPLC (CHIRALPAK IB; 55% (50:50 MeOH:IPA) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

Compound **14** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.69 (s, 1H), 2.78-2.90 (m, 4H), 3.91 (s, 3H), 4.63 (s, 1H), 7.28-7.38 (m, 3H), 7.45-7.49 (m, 4H), 7.75 (d, J = 8 Hz, 2H), 7.94 (d, J = 9.2 Hz, 1H), 8.06 (s, 1H), 8.26 (d, J = 9.2 Hz, 1H), 8.40 (s, 1H), 11.16 (s, 1H). LCMS: m/z = 438.4 [M+1].

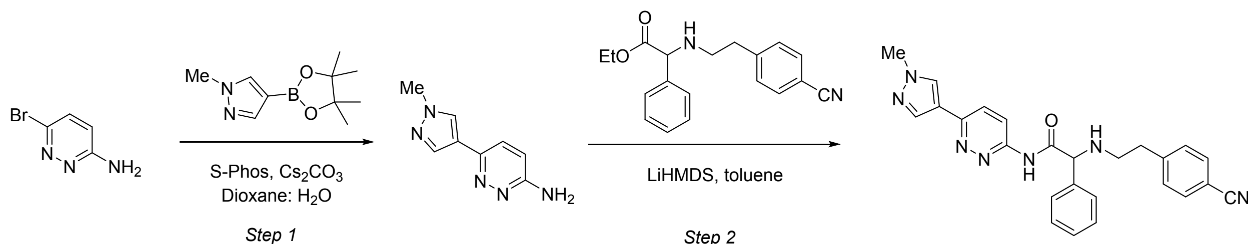
Enantiomer of **14** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.69 (s, 1H), 2.78-2.88 (m, 4H), 3.91 (s, 3H), 4.63 (s, 1H), 7.28-7.49 (m, 7H), 7.74 (d, J = 8 Hz, 2H), 7.94 (d, J = 9.2 Hz, 1H), 8.06 (s, 1H), 8.25 (d, J = 9.2 Hz, 1H), 8.40 (s, 1H), 11.15 (s, 1H). LCMS: m/z = 438.4 [M+1].

Compound **15**



(*R*)-2-((4-cyanophenethyl)amino)-N-(6-(1-methyl-1H-pyrazol-4-yl)pyridazin-3-yl)-2-phenylacetamide

Compound **15** was prepared according the following scheme and procedures.



<u>Step 1. 6-(1-methyl-1H-pyrazol-4-yl)pyridazin-3-amine</u>: A mixture of 6-bromopyridazin-3-amine (0.40 g, 2.29 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.70 g, 3.36 mmol), and cesium carbonate (2.24 g, 1.72 mmol) in a 4:1 mixture of dioxane:water (4 ml) was purged for 20 minutes with argon. S-Phos Pd G3 precatalyst (0.08 g, 0.16 mmol) was added and degassing was continued for another 10 minutes. The reaction mixture was heated at 100°C for 1 hour. The reaction mixture was poured into water (20 ml) and extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were washed with brine (20 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (0.33 g, 82%) as solid.

LCMS: *m/z* = 176.01 [M+1].

<u>Step 2. (*R*)- and (*S*)-2-((4-cyanophenethyl)amino)-N-(6-(1-methyl-1H-pyrazol-4-yl)pyridazin-3-yl)-2-phenyl acetamide</u>: To a mixture of 6-(1-methyl-1H-pyrazol-4-yl)pyridazin-3-amine (0.20 g, 1.14 mmol) and ethyl 2-((4-cyanophenethyl)amino)-2-phenylacetate (0.35 g, 1.14 mmol) in DMF (2 ml) was added LiHMDS (2.2 ml, 2.28 mmol; 1*M* in THF) under an atmosphere of nitrogen at 0°C. The resulting reaction mixture was heated to 50°C for 1 hour. After completion of the reaction, the mixture was diluted with ethyl acetate (20 ml) and slowly quenched with water (30 ml) at room temperature. The aqueous layer

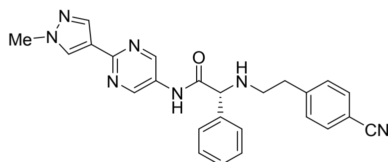
was extracted with ethyl acetate (2 x 30 ml). The combined organic extracts were washed with brine (30 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography to afford the title compound (0.17 g, 35%).

The racemic title compound was resolved by chiral HPLC (CHIRALPAK IC; 35% (50:50 ACN:IPA) in hexanes + 0.1% DEA) to furnish the enantiopure compounds.

Compound **15** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.76 (bs, 3H), 2.87 (t, J = 6.8 Hz, 2H), 3.89 (s, 3H), 4.58 (s, 1H), 7.29-7.37 (m, 3H), 7.44-7.47 (m, 4H), 7.75 (d, J = 8.4 Hz, 2H), 8.04 (s, 1H), 8.33 (s, 1H), 8.72 (d, J = 1.2 Hz, 1H), 9.22 (s, 1H), 10.79 (s, 1H, -NH). LCMS: m/z = 438.32 [M+1].

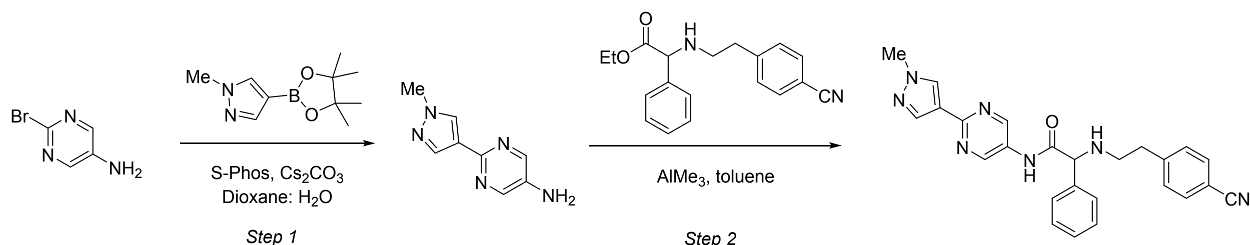
Enantiomer of **15** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.77 (m, 3H), 2.86 (t, 2H), 3.89 (s, 3H), 4.58 (s, 1H), 7.29-7.37 (m, 3H), 7.44-7.47 (m, 4H), 7.74 (d, 2H, J = 8 Hz), 8.04 (s, 1H), 8.33 (s, 1H), 8.72 (s, 1H), 9.22 (s, 1H), 10.79 (s, 1H, -NH). LCMS: m/z = 438.27 [M+1].

Compound **16**



(*R*)-2-((4-cyanophenethyl)amino)-N-(2-(1-methyl-1H-pyrazol-4-yl)pyrimidin-5-yl)-2-phenylacetamide

Compound **16** was prepared according the following scheme and procedures.



Step 1. 2-(1-methyl-1H-pyrazol-4-yl)pyrimidin-5-amine: A suspension of 2-bromopyrimidin-5-amine (0.22 g, 1.29 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.53 g, 2.59 mmol), and cesium carbonate (1.26 g, 3.88 mmol) in a 2:1 mixture of dioxane:water (3 ml) was degassed for 20 minutes with argon. To the reaction mixture, $Pd(dppf)Cl_2$ (0.09 g, 0.13 mmol) was added and degassing was continued for another 10 minutes. The reaction mixture was heated in a sealed tube at 85°C for 2 hours. After completion of reaction, the reaction mixture was poured into water (20 ml) and extracted with ethyl acetate (2 x 25 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product obtained was purified by column chromatography to afford the title compound (0.20 g, 92%) as solid.

LCMS: m/z = 176.1 [M+1].

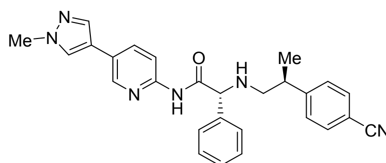
Step 2. (*R*)- and (*S*)-2-((4-cyanophenethyl)amino)-N-(2-(1-methyl-1H-pyrazol-4-yl)pyrimidin-5-yl)-2-phenylacetamide: To a mixture of 2-(1-methyl-1H-pyrazol-4-yl)pyrimidin-5-amine (0.19 g, 1.08 mmol) and ethyl 2-((4-cyanophenethyl)amino)-2-phenylacetate (0.36 g, 1.19 mmol) in toluene (4 ml), trimethylaluminum (2.2 ml, 2.17 mmol, 1M in toluene) was added under an atmosphere of nitrogen at room temperature. The resulting reaction mixture was heated to 100°C for 1 hour. After completion of the reaction, the mixture was diluted with ethyl acetate (20 ml) and slowly quenched with water (20 ml) at room temperature. The above suspension was filtered through a pad of celite and the organic layer was separated and dried over Na_2SO_4. The organic extract was concentrated under reduced pressure to obtain crude compound. The crude compound was purified by silica gel column chromatography to afford the racemic title compound (0.23 g, 49%).

The racemic title compound was resolved by chiral HPLC (CHIRALPAK OJ-H; 20% MeOH in liquid CO_2+ 0.1% DEA) to furnish the enantiopure compounds.

Compound **16** (faster-eluting isomer; assigned as the (*R*)-isomer by analogy to X-ray co-crystal structures of **1**, **12**, and **17** with the EP300 HAT domain): ^{1}H NMR (400 MHz, DMSO-d6): 2.76-2.90 (m, 5H), 3.90 (s, 3H), 4.43 (s, 1H), 7.30-7.48 (m, 7H), 7.75 (d, J = 8 Hz, 2H), 7.96 (s, 1H), 8.30 (s, 1H), 8.95 (s, 2H), 10.45 (s, 1H, -NH). LCMS: m/z = 438.34 [M+1].

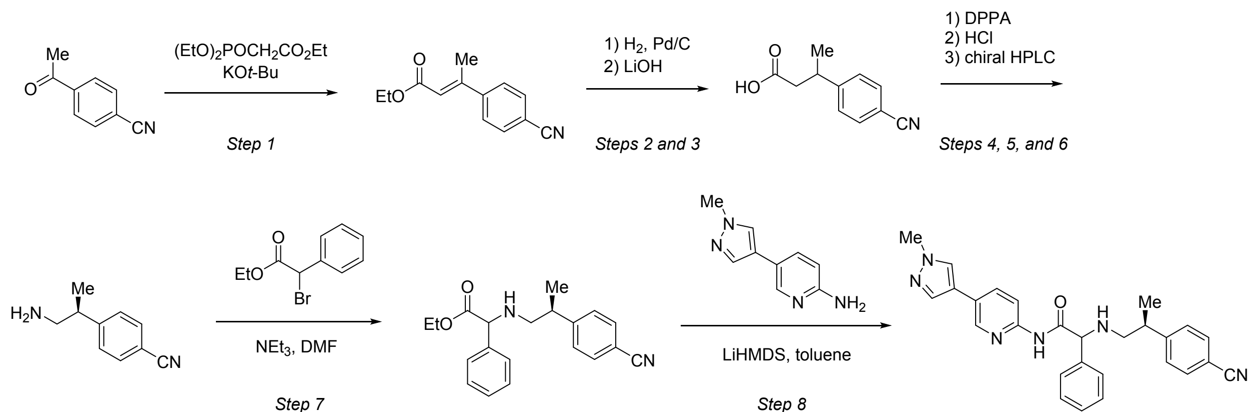
Enantiomer of **16** (slower-eluting isomer): ^{1}H NMR (400 MHz, DMSO-d6): δ 2.68-2.91 (m, 5H), 3.90 (s, 3H), 4.44 (s, 1H), 7.30-7.49 (m, 7H), 7.75 (d, J = 8 Hz, 2H), 7.97 (s, 1H), 8.31 (s, 1H), 8.95 (s, 2H), 10.43 (s, 1H, -NH). LCMS: m/z = 438.34 [M+1].

Compound **17**



(*R*)-2-(((*S*)-2-(4-cyanophenyl)propyl)amino)-N-(5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-2-phenylacetamide

Compound **17** was prepared according the following scheme and procedures.



Step 1. Ethyl (E and Z)-3-(4-cyanophenyl)but-2-enoate: To a stirred solution of potassium *tert*-butoxide (10.09 g, 89.7 mmol) in dry THF (90 ml) was added triethyl phosphonoacetate (20.08 g, 89.7 mmol) at 0°C under an atmosphere of nitrogen. Then the reaction mixture was stirred for 15 minutes at 0°C. The reaction was then warmed to room temperature and stirred for 1 hour. Then 4-acetylbenzonitrile (10.0 g, 69.0 mmol) was added as a solution in THF (50 ml) and the reaction was heated to 70°C for 3 hours. After completion of reaction, the pH of the reaction mixture was adjusted to 3-4 with 1N HCl. The THF was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with brine (50 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford the title compound (8.5 g, 58 %).

^{1}H NMR (400 MHz, DMSO-d6): 1.15 (t, J = 6.8 Hz, 1.5 H), 1.36 (t, J = 6.8 Hz, 3 H), 2.21 (s, 1.5 H), 2.60 (s, 3H), 4.05 (q, J = 7.1 Hz, 1H), 4.27 (q, J = 7.2 Hz, 2H), 6.01 (S, 0.5 H), 6.19 (S, 1H), 7.30-7.71 (m, 6 H).

Step 2. Ethyl 3-(4-cyanophenyl)butanoate: To a stirred solution of ethyl (E, Z) 3-(4-cyanophenyl)but-2-enoate (8.0 g, 37.2 mmol) in a 1:4 mixture of methanol:ethyl acetate (140 ml) was added Pd/C (0.8 g, 10% w/w, 50% moisture). The reaction was stirred at room temperature under an atmosphere of hydrogen gas for 3 hours. The reaction mixture was diluted with ethyl acetate and filtered through a pad of celite. The filtrate was concentrated under reduced pressure to afford the title compound (4.5 g, 56%).

^{1}H NMR (400 MHz, CDCl3): 1.23 (t, J = 7.2 Hz, 3H), 1.33 (d, J = 6.8 Hz, 3H), 2.62 (dd, J = 7.6 Hz,1.2 Hz, 2H), 3.70 (q, J = 7.2 Hz, 1H), 4.07-4.15 (m, 2 H), 7.37 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H).

Step 3. 3-(4-Cyanophenyl)butanoic acid: To a stirred solution of ethyl 3-(4-cyanophenyl)butanoate (4.5 g, 20.71 mmol) in a 4:2:1 mixture of MeOH:THF:H_2O (100 ml) was added LiOH (3.48 g, 82.95 mmol) at 5°C to 10°C. The resulting reaction mixture was stirred at room temperature for 1.5 hours. After completion of reaction, the reaction solvent was evaporated. The residue was dissolved in water (10 ml) and extracted with ethyl acetate (2 x 15 ml). The pH of the aqueous layer adjusted to 3-4 with concentrated HCl. The precipitate that formed was filtered off to afford title compound (3.8 g, 97 %) as a white solid.

^{1}H NMR (400 MHz, DMSO-d6): 1.23 (d, J = 6.8, 3H), 2.58 (d, J = 7.6 Hz, 2H), 3.24 (q, J =7.2, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 12.15 (s, 1H).

Step 4. *tert*-Butyl (2-(4-cyanophenyl)propyl)carbamate: To a stirred solution of 3-(4-cyanophenyl)butanoic acid (5.0 g, 26.45 mmol) in *tert*-butanol (65 ml) was added triethylamine (11.0 ml, 79.36 mmol) at room temperature. Then the reaction mixture was cooled to 5-10°C and DPPA (12.30 g, 44.97 mmol) was added drop wise. After formation of acylazide, the reaction was stirred at 90°C overnight. The reaction mixture was diluted with water (40 ml) and extracted with ethyl acetate (2 x 40 ml). The combined organic extracts were washed with brine (25 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford the title compound (4.5 g, 66 %) as a solid.

^{1}H NMR (400 MHz, DMSO-d6): 1.17 (d, J = 6.8 Hz, 2H), 1.33 (s, 9H), 2.90-3.00 (m, 1H), 3.04-3.15 (m, 2H), 6.91 (t, J = 5.2 Hz,1H, -NH), 7.42 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 7.2 Hz, 2H).

Step 5. 4-(1-Aminopropan-2-yl)benzonitrile hydrochloride: To a stirred solution of *tert*-butyl-(2-(4-cyanophenyl)propyl)carbamate (4.5 g, 17.29 mmol) in methanol (9 ml) was added a solution of 4M HCl in dioxane (10.8 ml, 2.4 vol.) drop wise at 0°C. The resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure to afford the title compound (2.81 g, 83 %) as a solid.

^{1}H NMR (400 MHz, DMSO-d6): 1.28 (d, J = 6.8 Hz, 2H), 3.03 (d, J = 5.6 Hz, 2H), 3.15-3.26 (m, 1H), 7.55 (d, J = 8.0 Hz, 2 H), 7.83 (d, J = 8.0 Hz, 2H), 8.21 (s, 3H). LCMS: m/z = 161.6 [M +1].

4-(1-Aminopropan-2-yl)benzonitrile: 4-(1-aminopropan-2-yl)benzonitrile hydrochloride was treated with an aqueous solution of saturated sodium bicarbonate and extracted with ethyl acetate (3 x 30 ml) to obtained the crude compound as liquid which was further purified by silica gel chromatography (DCM: MeOH = 90:10) to afford the racemic title compound (2.29 g, 83%) a viscous oil. ^{1}H NMR (400 MHz, CDCl3): 1.28 (d, J = 6.8 Hz, 3H), 2.85 (d, J = 5.6 Hz, 3H), 7.34 (d, J = 7.2 Hz, 2 H), 7.63 (d, J = 7.2 Hz, 2H). LCMS: m/z =161.5 [M +1].

Step 6. (*S*)-4-(1-aminopropan-2-yl)benzonitrile: The racemic amine may be resolved in the enantiopure title compound by preparative chiral SFC using a CHIRALPAK AD-H column (250 mm, 50 mm, 5 microns; mobile phase 25% acetonitrile:methanol:dimethylamine (80:20:0.1) in 75% CO_2). The early eluting isomer has been unambiguously assigned as (*S*)-4-(1-aminopropan-2-yl)benzonitrile by obtaining an x-ray co-crystal structure of a truncated form of p300 with compound **17** which was prepared from this material. HPLC retention time of this material matched that from earlier batches of **17** which were prepared using *rac*-4-(1-aminopropan-2-yl)benzonitrile followed by chiral HPLC resolution of all four stereoisomers.

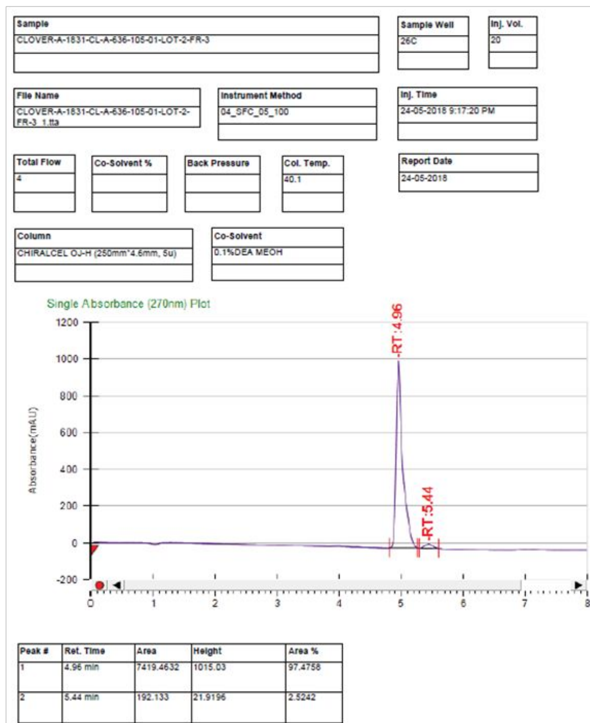
Step 7. (*R, S*)- and (*S, S*)-ethyl 2-((2-(4-cyanophenyl)propyl)amino)-2-phenylacetate: A mixture of ethyl 2-bromo-2-phenylacetate (9.11 g, 37.5 mmol), (*S*)-4-(1-aminopropan-2-yl)benzonitrile (5.0 g, 31.2 mmol) and triethylamine (13.1 ml, 93.7 mmol) in DMF (50 ml) was heated at 60°C for 3 hours. The reaction mixture was poured into ice cold water (150 ml) and extracted with ethyl acetate (2 x 150 ml). The combined organic layers were washed with brine (150 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford a mixture of the title compounds (7.0 g, 70%) as a viscous oil.

^{1}H NMR (400 MHz, DMSO-d6): 1.08 (t, J = 6.8 Hz, 3H), 1.16 (d, J = 6.8 Hz, 3H), 2.35-2.44 (m, 1H), 2.49-2.66 (m, 1H), 2.96 (q, J = 6.8 Hz, 1H), 3.96-4.06 (m, 2H), 4.32 (s, 1H), 7.26-7.42 (m, 7H), 7.74 (t, J = 7.6 Hz, 2H). LCMS: m/z = 323.6 [M+1].

Step 8. (*S*)-2-(((*S*)-2-(4-cyanophenyl)propyl)amino)-N-(5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-2-phenylacetamide and (*R*)-2-(((*S*)-2-(4-cyanophenyl)propyl)amino)-N-(5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-2-phenylacetamide: To a stirred solution of 5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-amine (2.5 g, 14.4 mmol), a 1:1 mixture of (*R, S*)- and (*S, S*)-ethyl 2-((2-(4-cyanophenyl)propyl)amino)-2-phenylacetate (7.0 g, 21.7 mmol) in dry THF (50 ml) was added LiHMDS (37 ml, 1M in THF, 36.2 mmol) at 0°C. The reaction mixture was stirred at room temperature for 1 hour. After completion of the reaction, the reaction mixture was poured into ice-cold water (100 ml) and extracted with ethyl acetate (2 x 75 ml). The combined organic extracts were washed with brine (100 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography to afford a mixture of the title compounds (5.0 g, 51%).

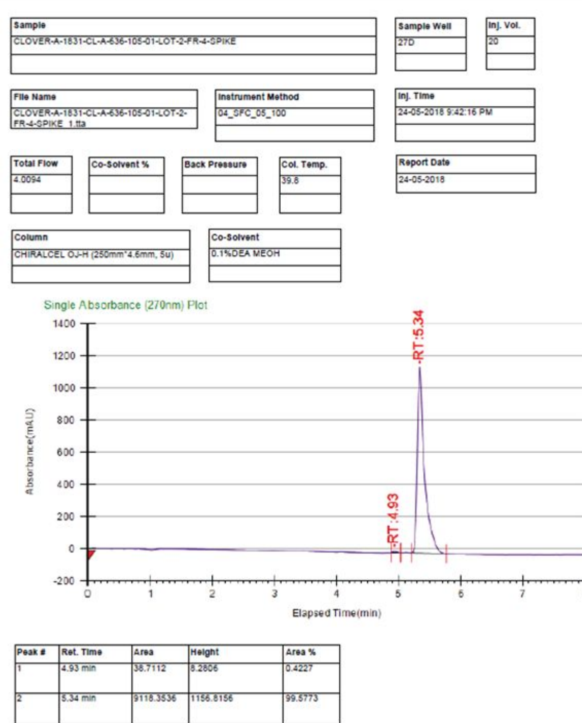
The title compounds were resolved by chiral HPLC (CHIRALCEL OJ-H; 15% MeOH in liquid CO_2 + 0.1% DEA) to obtain compound **17** (slower eluting isomer; t_R = 5.34 min.) and the (*S, S*)-diastereomer (faster eluting isomer; t_R = 4.96 min.).

(*S*, *S*)-diastereomer



Peak #	Ret. Time	Area	Height	Area %
1	4.96 min	7419.4632	1015.03	97.4758
2	5.44 min	192.133	21.9196	2.5242

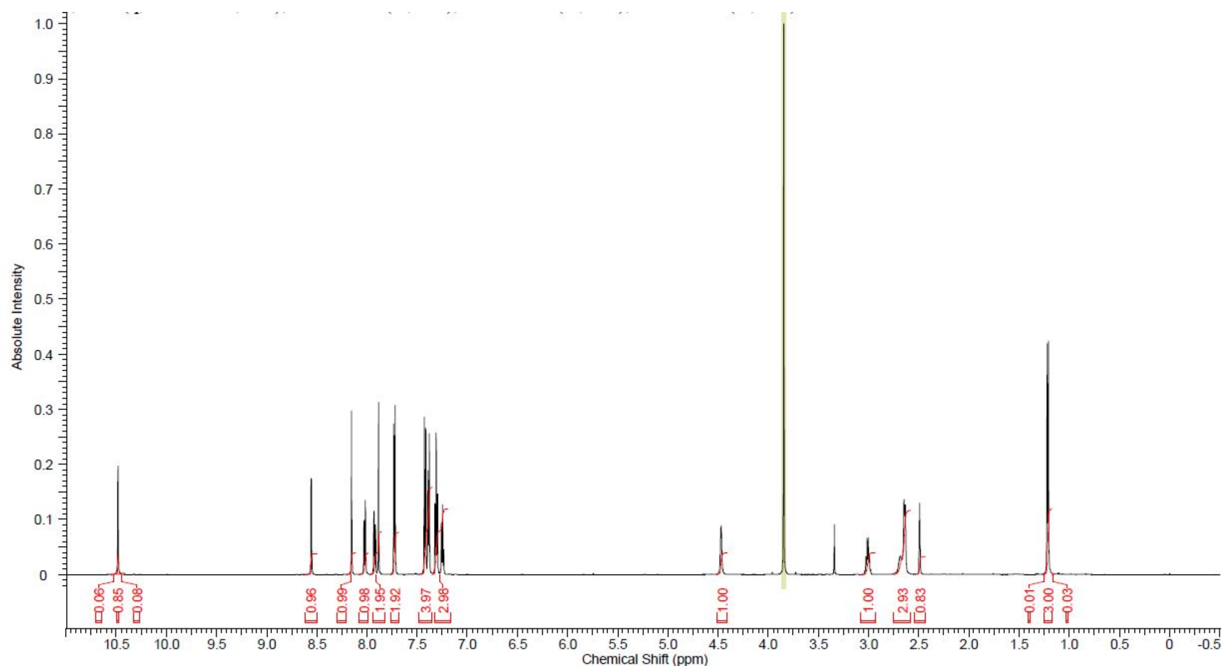
Compound **17** – CPI-1612



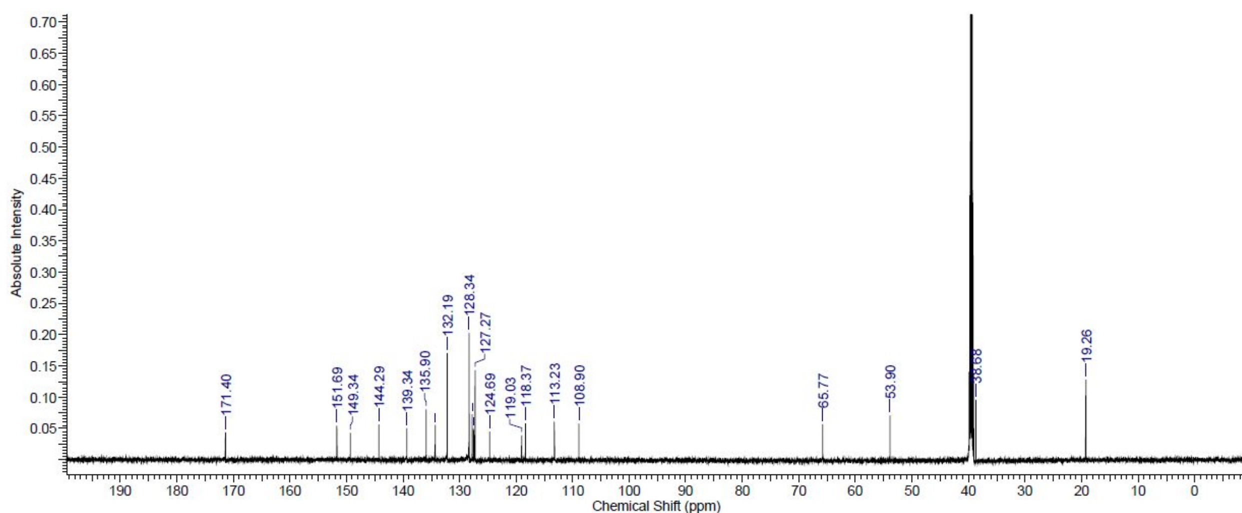
Peak #	Ret. Time	Area	Height	Area %
1	4.93 min	38.7112	5.2806	0.4227
2	5.34 min	9118.3536	1156.8156	99.5773

Compound **17** (slower-eluting isomer; assigned as the (*R*)-isomer based on the X-ray co-crystal structure **17** with the EP300 HAT domain): ^{1}H NMR (600 MHz, DMSO-d6): 1.23 (d, J = 6.8 Hz, 3H), 2.64-2.69 (m, 3H), 3.02 (q, J = 6.8 Hz, 1H), 3.86 (s, 3H), 4.47 (d, J = 7.6 Hz, 1H), 7.24-7.45 (m, 7H), 7.75 (d, J = 8.4 Hz, 2H); 7.90 (s, 1H), 7.92-8.03 (m, 2H), 8.18 (s, 1H), 8.56 (d, J = 1.6 Hz, 1H), 10.52 (s, -NH, 1H). ^{13}C NMR (150 MHz, DMSO-d6): 19.26, 38.68, (one peak obscured by DMSO), 53.90, 65.77, 108.90, 113.23, 118.37, 119.03, 124.69, 127.27, 127.51, 127.75, 128.31, 128.34, 132.19, 134.32, 135.90, 139.34, 144.29, 149.34, 151.69, 171.40. LCMS: m/z = 451.5 [M+1].

^{1}H NMR for compound **17**:



^{13}C NMR for compound **17**:



S6. Reference 15. The compounds and their associated data below are provided here to support the statement made in the manuscript concerning the rationale of adding a methyl group to compound **12** to afford compound **17**. In all cases we have examined, addition of the methyl group at the carbon atom adjacent to the aromatic group that is part of the phenethylamine side-chain provides approximately a 2- to 3-fold improvement in the EP300 HAT domain SPA assay. However, this modification does not always reduce the *in vitro* clearance (e.g. in the case of **21** to **22**), as it does for the case of **12** to **17** and

18 to **19**. Compounds in the table below were prepared by procedures that are analogous to those used for the preparation of compounds **3**, **11**, and **17**.

Compound	Structure	EP300 HAT SPA IC_{50} (μM)	MLM, HLM Cl (μL/min/mg)
18		0.25	52, 89
19		0.13	30, 61
20[a]		0.13	102, 109
21		0.24	15, 16
22		0.12	37, 27
23[a]		0.20	76, 127
24		0.33	
25		0.46	
26		1.8	

[a]Compound is a single enantiomer with unknown relative stereochemistry at the starred position.

S7. In vitro assays.

Data Analysis: For assays described below IC_{50}s were calculated by non-linear least squares four parameter fits of the form:

$$\% \, Inhibition = Max - \frac{Max - Min}{1 + \left(\frac{[Cmpd]}{IC50}\right)^n}$$

where % inhibition is the observed inhibition at varying [Cmpd], Max is the uninhibited (just DMSO) control, Min is the no enzyme control and n is the Hill coefficient. Analyses were carried out using either ActivityBase (IDBS), Genedata Screener (Genedata) or GraphPad Prism8 (GraphPad). For biochemical assays data are a minimum of N=2 and associated errors are reported as $\pm$ Standard Deviation (SD).

SPA Assays: The activity of the compounds described herein as p300/CBP HAT inhibitors may be readily determined using a scintillation proximity assay (SPA) methodology (Udenfriend, S.; Gerber, L.; Nelson, N. Scintillation Proximity Assay: A Sensitive and Continuous Isotopic Method for Monitoring Ligand/Receptor and Antigen/Antibody Interactions. *Anal. Biochem.* **1987**, *161*, 494-500). In particular, the compounds of the following examples had activity in reference assays by exhibiting the ability to inhibit the acetylation of histone peptide by a truncated form of the p300 enzyme (p300 HAT).

The p300 HAT domain (residues 1287-1666) was expressed and purified with an N-terminal His tag from Escherichia coli cells. The expressed protein was purified by Ni2+ affinity, followed by anion exchange chromatography. Appropriate fractions were pooled and buffer exchanged into 20mM Hepes pH 7.5, 150mM NaCl, and 1mM TCEP.

Compounds of interest solubilized in DMSO were stamped in a Greiner black 384-well plate in a 10-point duplicate dose response using an Echo 550 (Labcyte). p300-HAT domain purified in-house (aa 1287-1666) was diluted to 6nM in reaction buffer (50mM Tris pH 8.0, 100mM NaCl, 1mM DTT, 0.069mM Brij-35, 0.1mM EDTA, 0.1mg/mL BSA), combined with 4.14µM AcCoA (Sigma-Aldrich) and 0.46µM 3H-AcCoA (PerkinElmer), and 12.5µL added to each well and incubated for 30min at RT. Reactions were initiated with 12.5µL 2µM biotinylated H3(1-21) peptide (New England Peptide) and run for 1hr at RT, then quenched with 20µL stop solution (200mM Tris pH 8.0, 200mM EDTA, 2M NaCl, 160µM anacardic acid). 35µL of the reaction volume was transferred to a 384-well streptavidin FlashPlate (PerkinElmer) using a Bravo liquid handler (Velocity 11) and incubated for 1.5hr at RT. Plates were aspirated, washed with 95µL wash buffer (15mM Tris pH 8.5, 0.069µM Brij-35), aspirated, sealed, and scintillation counts read on a Topcount (PerkinElmer). Data were analyzed in Genedata to determine inhibitor IC_{50} values. Data were analyzed in either Genedata Screener or Prism8.

The full length p300 SPA and full length CBP assays were run following the same protocol as p300 HAT SPA assay, but used 6nM purified full length p300 (purchased from Active Motif) in place of the purified p300-HAT domain.

H3K18Ac MSD Assay: In a typical experiment the p300 HAT inhibitory activity inside cells of the compounds described herein was determined in accordance with the following experimental method. 20k HCT-116 cells per well are plated in 75µL RPMI+10% FBS media the night before treatment. Compounds plated in DMSO at 4x final concentration are resuspended in 30µL RPMI+10% FBS, then

25µL is combined with corresponding wells containing cells. Treated cells are incubated for 2hr at 37°C, then lysed in 500µL final volume and frozen at -80°C. MSD plates (Meso Scale Discovery) are coated overnight at 4°C with 60µL 1:500 α-total histone antibody (Millipore MAB3422) in PBS. Plates are then blocked with 5% BSA in TBST shaking at RT for 1hr, washed, and 30µL lysate added to each well for 2hr shaking at RT. Plates are washed and 25µL 1:216 α-H3K18ac antibody (CST 9675) in PBS added, then incubated for 1hr shaking at RT. Plates are washed again, then 25µL 1:1000 Sulfo-Tag goat α-rabbit antibody (Meso Scale Discovery R32Ab-1) in PBS is added for 1hr shaking at RT. Plates are washed once more, then 150µL 1x Read Buffer (MSD #R92TD-3) is added to all wells and read on MSD SECTOR Imager 2400 using the conventional read setup.

JEKO-1 cell culture and viability assay: The Jeko-1 cell line was obtained from ATCC and grown in suspension in RPMI + GlutaMAX (1X) 1640 medium containing phenol red, and further supplemented with 1% Penicillin-Streptomycin (P/S) and 20% Fetal Bovine Serum (FBS). For cell viability assays, 15,000 cells were plated per well of a 96-well plate containing different dilutions of compound for 72 hours. Relative cell numbers were assessed by Cell Titer-Glo (CTG) luminescent cell viability assay (Promega) using an Envision instrument (Perkin Elmer). Prism 6.0 (Graphpad Software, 2013) was used for curve fitting and GI50 calculations.

Kinetic solubility: *1. Preparation of stock solutions* - The stock solutions of test compounds and control compound progesterone were prepared in DMSO at the concentrations of 10 mM. *2. Procedure for solubility determination* - 15 µL of stock solution (10 mM) of each sample was placed in order into their proper 96-well rack. 485 µL of PBS pH 7.4 was added into each vial of the cap-less Solubility Sample plate. The assay was performed in singlet. Add one stir stick to each vial and seal using a molded PTFE/Silicone plug. Then the solubility sample plate was transferred to the Eppendorf Thermomixer Comfort plate shaker and shaked at 25°C at 1100 rpm for 2 hours. After completion of the 2 hours, plugs were removed and the stir sticks were removed using a big magnet, the samples from the Solubility Sample plate were transferred into the filter plate. Using the Vacuum Manifold, all the samples were filtered. Aliquot of 5 µL was taken from the filtrate followed by addition of 495 µL of a mixture of H2O and acetonitrile containing internal standard (1:1). A certain proportion of ultrapure water was used to dilute the diluent according to the peak shape. The dilution factor was changed according to the solubility values and the LC-MS signal response. *3. Preparation of 3 µM standards (STD)* - From the 10 mM DMSO STD plate, 6 µL was transferred into the remaining empty plate, and then 194 µL of DMSO was added to that plate to have a STD concentration of 300 µM. From the 300 µM DMSO STD plate, 5 µL was transferred into the remaining empty plate, and then 495 µL of a mixture of H2O and acetonitrile containing internal standard (1:1) was added to that plate to have a final STD concentration of 3 µM. A certain proportion of ultrapure water was used to dilute the diluent according to the peak shape. The concentrations of the standard samples were changed according to the LC-MS signal response. *4. Procedure for sample analysis* - The plate was placed into the well plate autosampler. The samples were evaluated by LC-MS/MS analysis. *5. Data analysis* - All calculations were carried out using Microsoft Excel. The filtrate was analyzed and quantified against a standard of known concentration using LC coupled with mass spectral peak identification and quantitation. Solubility values of the test compound and control compound were calculated as follows:

$$[\text{Sample}] = (\text{area ratio}_{\text{sample}} \times \text{Inj. Vol. STD} \times \text{DF}_{\text{sample}} \times [\text{STD}])/(\text{area ratio}_{\text{STD}} \times \text{Inj. Vol.}_{\text{sample}})$$

Any value of the compounds that was not within the specified limits was rejected and the experiment was repeated.

Mouse Liver Microsome Stability: 1. The master solution was prepared according to Table 5.

Table 5. Preparation of Master Solution			
Reagent	Stock Concentration	Volume	Final Concentration
Phosphate buffer	100 mM	210 µL	100 mM
Microsomes	20 mg/mL	12.5 µL	1 mg/mL

2. Add 222.5 µL of master solution and 25 µL of the 10 mM NADPH to the incubation plates. Pre-warm for 10 min. 3. The reaction was started with the addition of 2.5 µL of 100 µM control compound or test compound solutions. Verapamil was used as positive control in this study. The final concentration of control compound and test compounds were 1 µM. 4. Aliquots of 25 µL were taken from the reaction solution at 0.5, 5, 10, 15, 20 and 30 minutes. The reaction was stopped by the addition of 5 volumes of cold acetonitrile with IS (100 nM alprazolam, 200 nM caffeine and 100 nM tolbutamide). Samples were centrifuged at 3, 220 g for 30 minutes. Aliquot of 100 µL of the supernatant was mixed with 100 µL of ultra-pure H_2O and then used for LC-MS/MS analysis. *5. Data Analysis* - All calculations were carried out using Microsoft Excel. Peak areas were determined from extracted ion chromatograms. The slope value, k, was determined by linear regression of the natural logarithm of the remaining percentage of the parent drug vs. incubation time curve. The in vitro half-life (in vitro t1/2) was determined from the slope value: *in vitro* $t_{1/2} = -(0.693/k)$. Conversion of the in vitro t1/2 (min) into the in vitro intrinsic clearance (in vitro CLint, in µL/min/mg protein) was done using the following equation (mean of duplicate determinations): in vitro $Cl_{int} = (0.693/t_{1/2})*$(volume of incubation (µL)/amount of proteins (mg)). *6. Data Processing Rules* **-** The rules for data processing are shown in Table 6.

Table 6. Rules for data processing	
≥80% at 30 min	If T-test with $p<0.05$ is obtained, report the calculated CLint value; When the calculated Clint value < 6.8, then report < 6.8 instead of calculated value.
	If T-test with $p<0.05$ is not obtained, then report < 6.8 for CLint value when all the other data points fall in the range of 80%~120% (one data point within the range of 70%~130% is accepted, otherwise the experiment should be repeated).
<80% at 30 min	Always remove from the calculation all points with < 10% left of 0.5 min sample, but leave at least 2 points
	If T-test with $p<0.05$ is obtained, report the calculated CLint value.
	If T-test with $p<0.05$ is not obtained, the experiment must be repeated.

Mouse Plasma Protein Binding Assay: *1. Preparation of Compound Working Solutions* - To prepare 10 mM control compound warfarin and test compounds, weigh the appropriate amount of powder and add the corresponding volume DMSO, respectively. 1 mM working solution: Add 10 µL of 10 mM stock solution into 90 µL of DMSO. *2. Preparation of Buffer Solution pH 7.4* - A basic solution was prepared by dissolving 14.2 g/L Na_2HPO_4 and 8.77 g/L NaCl in deionized water and the solution could be stored at 4°C for up to 7 days. An acidic solution was prepared by dissolving 12.0 g/L NaH_2PO_4 and 8.77 g/L NaCl in deionized water and the solution could be stored at 4°C for up to 7 days. The basic solution

was titrated with the acidic solution to pH 7.4 and store at 4°C for up to 7 days. pH was checked on the day of experiment and was adjusted if outside specification of 7.4 ± 0.1. *3. Preparation of plasma* - Set the temperature of water bath to 37°C. Thaw the frozen Plasma (stored at -80°C) immediately in a 37°C water bath. *4. Preparation of operation plate* - Soak the dialysis membranes in ultrapure water for 60 minutes to separate strips, then in 20% ethanol for 20 minutes, finally in dialysis buffer for 20 minutes. Load the prepared membranes into the dialysis device and install the device again following manufacturers guidelines. Turn on air bath and allow to pre-heat to 37°C. *5. Preparation of control sample at 0 hour* - Add 597 µL of blank plasma solution into each vial of a new plastic plate or separate plastic tube by addition of 3 µL of the working solution of test compound, vortex at 1000 rpm for 2 minutes. The final percent volume of organic solvent is 0.5% and the final concentration for test compound is 5 µM. Immediately transfer 50 µL of the spiked plasma solution suspension to a 96-well plate to act as T=0 control sample. The samples are treated the same as the samples after incubation. Place all remaining spiked plasma solution in the incubator for the duration of the study. *6. Stability determination of test compound in plasma solution* - At the same time, the remaining spiked plasma solution sample in the plastic plate or separate plastic tube is incubated for 6 hours at 37°C with 5% CO_2 in the CO_2 incubator. At T=6 hours, transfer 50 µL of the original spiked plasma solution suspension to the 96-well plate for analysis. *7. Procedure for equilibrium dialysis* - Assemble the dialysis set up following the manufacturer's instructions. Load cells with 120 µL of plasma sample and dialyzed against equal volume of dialysis buffer (PBS). The assay is performed in duplicate. Cover the unit with gas permeable lid and incubate for 6 hours at 37°C at 100 rpm with 5% CO_2 on an orbital shaker in the CO_2 incubator. At the end of incubation, remove lid and pipette 50 µL of post-dialysis samples from both buffer and plasma solution chambers into separated 96-well plate for analysis, respectively.
8. Procedure for sample preparation - Add 50 µL of plasma solution to the buffer samples, and an equal volume of PBS to the collected plasma solution samples. Shake the plate at 1000 rpm for 2 minutes and add 400 µL of acetonitrile containing an appropriate internal standard (IS) to precipitate protein and release compound. Vortex at 1000 rpm for 10 minutes. Centrifuge for 30 minutes at 3,220 g. Transfer 250 µL of the supernatant to new 96-well plates and centrifuge again (3,220 g, 30 minutes). Then transfer 100 µL of the supernatant to new 96-well plates for analysis. Add 100 µL of distilled water to each sample and mix for analysis by LC-MS/MS. *9. Data Analysis* - All calculations are carried out using Microsoft Excel. Determine the concentrations of test compound and control compound in the buffer and plasma solution chambers. Calculate the percentages of test compound(s) and control compound bound as follows:

% Unbound = (Area ratio buffer chamber / Area ratio plasma solution chamber) × 100%

% Bound = 100 % - % Unbound

% Recovery = (Area ratio buffer chamber + Area ratio plasma solution chamber) / (Area ratio Total sample) × 100 %

% Remaining = Area ratio 6hr / Area ratio 0hr × 100%

PAMPA Permeability Assay: *1. Preparation of PBS (100 mM phosphate, pH = 7.4 ± 0.05)* - 2.6 g KH_2PO_4 and 18.5 g $K_2HPO_4 \cdot 3H_2O$ were dissolved in 1000 mL of ultra pure water, mixed thoroughly. The pH was adjusted to 7.40 ± 0.05, using either 1 M sodium hydroxide or 1M hydrochloric acid. *2. Methods*: *2.1 Preparation of Donor Solution* - a. 0.2 mM working solution was prepared by diluting 10 mM stock solution with DMSO. b.10 µM donor solution (5% DMSO) was prepared by diluting 20 µL of working

solution with 380 µL PBS. *2.2.* 150 µL of 10 µM donor solutions to each well of the donor plate, whose PVDFmembrane was precoated with 5 µL of 1% lecithin/dodecane mixture. Duplicates were prepared. *2.3.* 300 µL of PBS was added to each well of the PTFE acceptor plate. *2.4.* The donor plate and acceptor plate were combined together and incubated for 4h at room temperature with shaking at 300 rpm. *2.5.* Preparation of T0 sample: 20 µL donor solution was transferred to new well followed by the addition of 250 µL PBS (DF: 13.5), 130 µL ACN (containing internal standard) as T0 sample. *2.6.* Preparation of acceptor sample: The plate was removed from incubator. 270 µL solution was transferred from each acceptor well and mixed with 130 µL ACN (containing internal standard) as acceptor sample. *2.7.* Preparation of donor sample: 20 µL solution was transferred from each donor well and mixed with 250 µL PBS (DF: 13.5), 130 µL ACN (containing internal standard) as donor sample. *2.8.* Acceptor samples and donor samples were all analysed by LC/MS/MS. *2.9.* The equation used to determine permeability rates (Pe) was displayed as follow.

$P_e = C \times (-\ln(1-([drug]_{acceptor}/[drug]_{equilibrium})) \times 10^{-7}$, where $C = ((V_D \times V_A)/((V_D + V_A) \times Area \times time))$

$V_D = 0.15$ mL; $V_A = 0.30$ mL; Area = 0.28 cm^2; time = 14400 s.

$[drug]_{acceptor} = (Aa/Ai \times DF)_{acceptor}$; $[drug]_{donor} = (Aa/Ai*DF)_{donor}$; Aa/Ai: Peak area ratio of analyte and internal standard; DF: Dilution factor.

S8. Pharmacokinetics

Cassette Mouse Pharmacokinetics: Pharmacokinetic studies were conducted on a single day in male CD-1 mice using three animals per route of administration (IV and PO). Studies were conducted in a cassette fashion with up to 5 compounds per cassette. All PK parameters were calculated based on actual dosage administered to each animal. For IV dosing, a dose volume of 5 mL/kg in DMSO:PEG400:water (1:3:6) was administered for a target dose of 0.5 mg/kg. For oral dosing, a dose volume of 10 mL/kg in DMSO:PEG400:water (1:3:6) was administered for a target dose of 2.5 mg/kg.

Blood samples (0.03 mL) were collected from the dorsal metatarsal vein of the mice at 0.083, 0.25, 0.5, 1, 2, 4, and 8h (IV) and 0.25, 0.5, 1, 2, 4, 8h (PO) post dose. The samples were treated with heparin and centrifuged at 4000 x g for 5 minutes in 4 °C centrifuge to obtain plasma, and plasma aliquots of each sample were either analyzed immediately or were stored at -75 °C until analyzed. The desired serial concentrations of working solutions were achieved by diluting stock solution of analyte with 50% acetonitrile in water solution. 3 µL of working solutions (5, 10, 20, 50, 100, 500, 1000, 5000, 10000 ng/mL) were added to 30 µL of the blank CD1 Mouse plasma to achieve calibration standards of 0.5~1000 ng/mL (0.5, 1, 2, 5, 10, 50, 100, 500, 1000 ng/mL) in a total volume of 33 µL. Four quality control samples at 1 ng/mL, 2 ng/mL, 50 ng/mL and 800 ng/mL for plasma were prepared independently of those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards. 33 µL standards, 33 µL QC samples and 33 µL unknown samples（30 µL plasma with 3 µL blank solution） were added to 200 µL of acetonitrile containing IS mixture for precipitating protein respectively. Then the samples were vortexed for 30 s. After centrifugation at 4 degree Celsius, 4700 rpm for 15 min. The supernatant was diluted at a ratio of 1:2 with H2O (V/V, 1:2), then 3 µL of the diluted supernatant was injected into the LC/MS/MS system for quantitative analysis. WinNonlin (PhoenixTM, version 8.0) or other similar software was used for pharmacokinetic calculations. Oral bioavailability for each oral study animal was obtained by calculating the ratio of oral

dose normalized area under the curve (0-∞) to the mean IV dose-normalized area under the curve (0-∞).

Single Mouse Pharmacokinetics: Pharmacokinetic studies were conducted on a single day in male CD-1 mice using three animals per route of administration (IV and PO). All PK parameters were calculated based on actual dosage administered to each animal. For IV dosing, a 0.2 mg/mL dosing solution of compound **17** in DMSO:PEG400:water (1:3:6) was administered for a target dose of 1.0 mg/kg. For oral dosing, a 0.5 mg/mL dosing solution of compound **17** in DMSO:PEG400:water (1:3:6) was administered for a target dose of 5.0 mg/kg

Analysis was performed in a similar fashion as described previously.

Brain PK (mouse) of compound 17: Compound **17** was administered orally to eighteen male CD-1 mice (5.0 mg/kg; 0.5 mg/mL solution of 17 in DMSO:PEG400:water (1:3:6)). Three mice were euthanized at 6 time points (0.25, 0.5, 1, 2, 4, and 8 hours post dose). Plasma, brain, and CSF levels of compound 17 were then determined as follows. *Blood Samples Processing and Storage:* 1) Approximately 0.3 mL blood was collected at each time point. The 0.3 mL of whole blood of each sample was transferred into plastic microcentrifuge tubes with Heparin as anticoagulant, and then centrifuged at 4 degrees 3000 x g for 5 minutes to obtain plasma; 2) The samples were stored in a freezer at -75±15°C prior to analysis. *CSF Samples Processing and Storage:* 1) The animals were fully exsanguinated with a rising concentration of CO_2 gas prior to CSF and brain collection. 2) For CSF collection, an incision was made by scissors on the neck of the mice, and the foramen magnum was exposed to the sight of scientist. Using a syringe with a venoclysis needle to sample the CSF, the tip of the needle was inserted into the membrane of foramen magnum about 1-2 mm, and then the stopcock was pulled a small length of the syringe to make a negative pressure to transfer the CSF fluid to the tube. While watching the fluid in the tube and pulling the stopcock slowly to obtain the desired amount of CSF, the needle was stopped and split and the syringe to ensure there is no negative pressure in the tube. Then cut off the needle and collect the CSF fluid. If blood was observed in the tube, the draw was stopped immediately and the needle was separated from the syringe, and the blood and CSF were separated to ensure that there was no blood in the CSF fluid. Then the CSF fluid was collected. *Brain Samples Processing and Storage:* 1) The mice were fully exsanguinated prior to brain collection. Procedure: open chest cavity, perform a gentle iv saline flush (saline flush volume ~ 10 mL) from left ventricle with the animal placed head down at a 45 degree angle to facilitate blood removal. 2) Brain samples were collected at the appropriate time points and put into preweighed tubes, and the samples were stored at -75±15°C until analysis. 3) The brain samples were weighed and homogenated with PBS by brain weight (g) to PBS volume (mL) ratio 1:3 before analysis (W/V, 1:3). The actual concentration is the detected value corrected by a multiple of 4x.

Concentrations of compound **17** in the plasma, brain and CSF samples were analyzed using a LC-MS/MS method. WinNonlin (PhoenixTM, version 8.0) or other similar software was used for pharmacokinetic calculations.

Plasma concentration-time data after PO dose of Compound **17**						
Data highlighted in blue were used for $T_{1/2}$ calculation						
PO Dose	5 mg/kg					
Time	Concentration (ng/mL)			Mean	SD	CV
(h)	Case A	Case B	Case C	(ng/mL)	(ng/mL)	(%)

0.25	634	641	990	755	204	27.0
0.5	565	346	480	464	110	23.8
1	427	390	438	418	25	6.01
2	293	243	95.5	211	103	48.8
4	42.1	19.1	20.4	27.2	12.9	47.5
8	7.37	7.62	4.52	6.50	1.72	26.5

PO Dose	5 mg/kg		
Plasma PK parameters after PO dose of **17**		Unit	
$T_{1/2}$		h	1.26
T_{max}		h	0.250
C_{max}		(ng/mL)	755
AUC_{last}		(h*ng/mL)	1087
AUC_{Inf}		(h*ng/mL)	1099
$AUC_{_\%Extrap}$_obs		%	1.08
MRT_{Inf}_obs		h	1.48
AUC_{last}/D		(h*mg/mL)	217

Brain concentration-time data after PO dose of Compound **17**						
Data highlighted in blue were used for $T_{1/2}$ calculation						
PO Dose	5 mg/kg					
Time	Concentration (ng/mL)			Mean	SD	CV
(h)	Case A	Case B	Case C	(ng/mL)	(ng/mL)	(%)
0.25	233	194	244	223	26	11.8
0.5	272	136	181	197	69	35.2
1	171	155	182	169	14	8.19
2	92.4	45.6	44.4	60.8	27.4	45.0
4	13.5	9.08	9.16	10.6	2.5	23.8
8	2.74	2.19	2.47	2.46	0.27	11.1

Brain PK parameters after PO dose of Compound **17**			
PO Dose	5 mg/kg		
PK parameters		Unit	
$T_{1/2}$		h	1.36
T_{max}		h	0.250
C_{max}		(ng/mL)	223
AUC_{last}		(h*ng/mL)	384
AUC_{Inf}		(h*ng/mL)	389
$AUC_{_\%Extrap}$_obs		%	1.24
MRT_{Inf}_obs		h	1.48
AUC_{last}/D		(h*mg/mL)	76.9

AUClast ratio (brain/plasma)		0.353
AUCInf ratio (brain/plasma)		0.354

CSF concentration-time data after PO dose of **17**						
Data highlighted in blue were used for $T_{1/2}$ calculation						
PO Dose	5 mg/kg					
Time	Concentration (ng/mL)			Mean	SD	CV
(h)	Case A	Case B	Case C	(ng/mL)	(ng/mL)	(%)
0.25	5.92	3.16	4.34	4.47	1.38	31.0
0.5	9.46	2.02	7.27	6.25	3.82	61.2
1	3.25	2.07	4.45	3.26	1.19	36.5
2	1.12	BLOQ	1.34	1.23	NA	NA
4	BLOQ	BLOQ	BLOQ	BLOQ	NA	NA
8	BLOQ	BLOQ	BLOQ	BLOQ	NA	NA

Rat Pharmacokinetics: Pharmacokinetic studies were conducted on a single day in male SD rats using three animals per route of administration (IV and PO). All PK parameters were calculated based on actual dosage administered to each animal. For IV dosing, a 0.2 mg/mL dosing solution of compound **17** in DMSO:PEG400:water (1:3:6) was administered for a target dose of 1.0 mg/kg. For oral dosing, a 0.5 mg/mL dosing solution of compound **17** in 0.5% methylcellulose + 0.5% tween80 in water was administered for a target dose of 5.0 mg/kg.

Analysis was performed in a similar fashion as described previously.

Dog Pharmacokinetics: Pharmacokinetic studies were conducted on a single day in male beagle dogs using three animals per route of administration (IV and PO). All PK parameters were calculated based on actual dosage administered to each animal. For IV dosing, a 0.25 mg/mL dosing solution of compound **17** in DMSO:PEG400:water (1:3:6) was administered for a target dose of 0.5 mg/kg. For oral dosing, a 0.2 mg/mL dosing solution of compound **17** in 0.5% methylcellulose + 0.5% tween80 in water was administered for a target dose of 1.0 mg/kg.

Blood samples (0.3 mL) were collected from a peripheral vein (not the dosing vein) of the dogs at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24h (IV) and 0.25, 0.5, 1, 2, 4, 8, and 24h (PO) post dose. The samples were treated with EDTA-K2 and centrifuged at 2000 x g for 10 minutes in 4 °C centrifuge to obtain plasma, and plasma aliquots of each sample were either analyzed immediately or were stored at -75 °C until analyzed. The desired serial concentrations of working solutions were achieved by diluting stock solution of analyte with 50% acetonitrile in water solution. 5 µL of working solutions (5,10, 20, 50, 100, 500, 1000, 5000, 10000 ng/mL) were added to 50 µL of the blank Beagle Dog plasma to achieve calibration

standards of 0.5~1000 ng/mL (0.5,1, 2, 5, 10, 50, 100, 500, 1000 ng/mL) in a total volume of 55 µL. Five quality control samples at 1 ng/mL, 2 ng/mL, 5 ng/mL,50 ng/mL and 800 ng/mL for plasma were prepared independently of those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards. 55 µL standards, 55 µL QC samples and 55 µL unknown samples （50 µL plasma with 5 µL blank solution were added to 200 µL of acetonitrile containing IS mixture for precipitating protein respectively. Then the samples were vortexed for 30 s. After centrifugation at 4 degree Celsius, 4700 rpm for 15 min.The supernatant was diluted with ultrapure water at a ratio of 1:2 (V/V, 1:2),then 2 µL of diluted supernatant was injected into the LC/MS/MS system for quantitative analysis. WinNonlin (PhoenixTM, version 8.0) or other similar software was used for pharmacokinetic calculations. Oral bioavailability for each oral study animal was obtained by calculating the ratio of oral dose normalized area under the curve (0-∞) to the mean IV dose-normalized area under the curve (0-∞).

S9. In vivo experiments

Pharmacokinetic – Pharmacodynamic (PK/PD) Assay:

1. Study Objective

The objective of the project was to evaluate the PK/PD of CPI-0701612 on female C57BL/6 mice and Balb/c nude mice. Not all data from this study is reported in the manuscript.

2. Experimental Design

Table 1. Groups and sampling information.

Group	n	Treatment	Dose (mg/kg)	Schedule	Plasma for PK	Blood for FACS	Blood for PBMC	Spleen
3	5	Vehicle	--	PO, single dose	--	2h, n=5	2h, n=5	2h, n=5
	5				--	6h, n=5	6h, n=5	6h, n=5
	5				--	--	10h, n=5	10h, n=5
4	3	Compound **17**	7.5	PO, single dose	6h, n=3	6h, n=3	6h, n=3	6h, n=3
	3				10h, n=3	--	10h, n=3	10h, n=3
5	3	Compound **17**	2.5	PO, single dose	2h, n=3	2h, n=3	2h, n=3	2h, n=3
	3				6h, n=3	6h, n=3	6h, n=3	6h, n=3
	3				10h, n=3	--	10h, n=3	10h, n=3
6	3	Compound **17**	1	PO, single dose	2h, n=3	2h, n=3	2h, n=3	2h, n=3
	3				6h, n=3	6h, n=3	6h, n=3	6h, n=3
	3				10h, n=3	--	10h, n=3	10h, n=3

3. Materials

3.1. Animals and Housing Condition

3.1.1. Animals

Species: Mus Musculus
Strain: C57BL/6 mice
Age: 6-8 weeks
Sex: female
Body weight: 18-20 g
Number of animals: Balb/c nude 24 mice. C57BL/6 39 mice.
Animal supplier: Shanghai Lingchang biological science and technology Co., Ltd.

3.1.2. Housing condition

The mice were kept in individual ventilation cages at constant temperature and humidity with 3-5 animals in each cage. Temperature: 20~26 ^{o}C, Humidity: 40-70%.

Cages: Made of polycarbonate. The size is 300 mm x 180 mm x 150 mm. The bedding material is corn cob, which was changed twice per week.
Diet: Animals had free access to irradiation sterilized dry granule food during the entire study period.
Water: Animals had free access to sterile drinking water.
Cage identification: The identification labels for each cage contained the following information: number of animals, sex, strain, date received, treatment, study number, group number and the starting date of the treatment.
Animal identification: Animals were marked by ear coding.

4. Test Article

Product identification: Compound **17**
Provider: Constellation Pharmaceuticals
Lot number: 2
Formula: $C_{27}H_{26}N_6O$, MW: 450.546, Purity: 99%
Physical description: White powder
Package and storage condition: 100 mg/vial, stored at 4℃

5. Experimental Methods

5.1. Group Assignment

Mice were assigned into groups using randomized block design based upon their body weight data. This ensured that all the groups were comparable at the baseline.

5.2. Testing Articles Formulation Preparation

The compounds were prepared according to the protocols provided by the sponsor. The formulation information was shown below.

Table 2 Formulation preparation

Compounds	Preparation	Concentration (mg/ml)	Storage
Vehicle	DMSO/PEG400/H_2O, v/v/v,1/3/6	-	4ºC
Compound **17** 7.5 mg/kg	Dissolve 4.11 mg CPI-0701612 in 0.54 ml DMSO, add 1.62 ml PEG400 and mix well, add 3.24 ml H2O, mix well to make a clear solution.	0.75	4ºC
Compound **17** 2.5 mg/kg	Dilute 1 ml 7.5 mg/kg CPI-0701612 solution with 2 ml vehicle, mix well.	0.25	4ºC
Compound **17** 1 mg/kg	Dilute 0.4 ml 7.5 mg/kg CPI-0701612 solution with 2.6 ml vehicle, mix well.	0.10	4ºC

(The formulations were prepared before dosing and used freshly as soon as possible.)

5.3. Observations

The protocol and any amendment(s) or procedures involving the care and use of animals in this study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec prior to conduct. During the study, the care and use of animals was conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). After treatment starts, the animals were checked daily for morbidity and mortality. At the time of routine monitoring, the animals were checked for any effects of treatments on normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights were measured every day), eye/hair matting and any other abnormal effect. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

5.4. Sample Collection and Study Endpoint

The mice were euthanized at the time points shown in Table 1. 500ul whole blood was collected and placed in EDTA-2K tubes. 50 ul blood was centrifuged at 2,000 x g (4,600 rpm), 4 ºC for 15 min and plasma was stored at -80ºC before PK analysis. Remaining 400 ul blood was used for PBMC preparation and FACS assay. Spleen was collected from each mouse, snap frozen and stored at -80ºC before shipping back to the sponsor.

5.5. Protocol for PBMC Preparation and FACS Assay

5.5.1. Panel design for FACS

Fluorochrome	Panel 1	Cell subpopulation
FITC	CD3	T
BV421	Live/Dead	-
PE	H3K27ac	-

5.5.2. Antibody information

Fluorochrome	Mol. Target	Cat No.	Clone#	Lot No.	Dilution
FITC	CD3	BD-553061	145-2C11	6337889	1:100

PE	H3K27ac	Cell Signaling-15562S	NA	2	1:20
BV421	Live/Dead	Biolegend-423114	NA	B220982	1:1000

5.5.3. Red Blood Cell (RBC) Lysis of Whole blood

1) Add 8 mL of 1X RBC Lysis Buffer (BD Lysing buffer) per 0.4 mL of mouse blood.
2) Gently vortex or pipette each tube immediately after adding the lysing solution
3) Incubate for 15 minutes at RT, protect from light (no more than 15 minutes). Then add 20 mL DPBS into the tube for breaking up the lysing reaction.
4) Centrifuge at 350 xg for 5 min at RT. Decant supernatant.
5) Add 30 ml DPBS and centrifuge again at 350 xg for 5 min at RT. Decant supernatant.
6) Resuspend the pellet in the appropriate volume of staining buffer and perform a cell count and viability analysis.

5.5.4. PBMC lysis for RNA extraction

1) Take out about 2/3 of the total PBMCs and spin down at 350 xg for 5 min at RT.
2) Decant supernatant and add 350µL/sample RLT buffer (1:100 β-ME).
3) Homogenize by gently pipetting up and down and immediately freeze down sample on dry ice.
4) Transfer the samples to -80°C freezer before sending back to the sponsor.

5.5.5. Live/Dead staining

1) Take out 2e+5 cells from the rest PBMCs for FACS.
2) Wash the cells twice with DPBS, spin (400 xg for 5 min, RT) and flick.
3) Dilute Live/Dead viability dye (Biolegend-423114) 1:1000 in DPBS.
4) Add 100ul diluted Live/Dead viability dye in each sample.
5) Incubate the plate for 20min at RT, protect from light.

5.5.6. Surface staining

1) Wash cells twice with 200 µL staining buffer, spin (400 xg for 5 min, RT) and flick.
2) Add 5 µL of Fc block to 45 µL /well cells and resuspend cells using a pipette, incubate 5 min at RT in the dark.
3) C57 mice samples make surface stain solution by adding CD3 Ab (1:50 dilution) staining buffer. Antibody surface staining solution is 50 µL/well. The total volume is 100 µL/well (50 µL cells with Fc Block +50 µL Ab staining solution). Nude mice samples add 50uL staining buffer without CD3 antibody. The total volume is 100 µL/well (50 µL cells with Fc Block +50 µL staining buffer).
4) Incubate the plate for 30min in a refrigerator, protect from light.
5) Wash the cells with staining buffer (first wash top up with 150 µL, then spin and flick and wash again with 200 µL).
6) Proceed to intracellular staining.

5.5.7. Intracellular staining protocol

Nuclear: Use Ebioscience FoxP3 and Transcroption factor Staining Buffer Set (00-5523-00)

1) Fix and perm the cells in 1× Ebioscience FoxP3/transcription factor staining buffer set per manufacturer's instructions.
   Note: Dilute Fix/perm buffer 1:4 in assay diluent (1 part Fix/Perm buffer, 3 parts diluent)
2) Add 100 µL 1× FoxP3 Fix/Perm buffer to each well and immediately resuspend the cells by

pipetting up and down, incubate 45 min at in a refrigerator, protect from light.

3) Top up wells with 100µL 1× perm buffer (1:10 dilution in water), spin down (450 xg for 5 min) and wash once more with 200 µL 1× perm buffer. Resuspended with staining buffer and stay in a refrigerator overnight.
H3K27ac staining:
4) Block the cells by adding rabbit gamma globulin (Jackson Immunoresearch) and incubate at RT for 5 min.
Note: >stock = 11 mg/ml - dilute 1:1000 and add 100uL/well (~0.05 ug/well). Mix the cells with pipette tip to dislodge pellet
5) Wash the cells with 200 µL perm buffer.
6) Make intracellular staining solution containing anti-H3K27acetyl as below.
> Dilute anti-H3K27acetyl in 1:20 in 1× perm buffer for 2e+5 cells**.**
7) Add 100ul intracellular staining solution/well, pipette up and down and incubate for 45 min in a refrigerator, protect from light.
8) Spin down and wash twice with 200 uL perm buffer at 450 xg for 5 min.
9) Resuspended with staining buffer and run FACS.

5.5.8. Sample Acquisition

Acquire 1e+4 events (gated on - cell of interest/singlet/CD3+) (C57BL/6 mice).

6. PK Analysis Summary

Table 3. Summary for PK analysis result

Group	Sample time	Mean Concentration in plasma (ng/mL) ± SEM
C57BL/6 mice Compound **17** 7.5 mg/kg, PO, single dose	6h	25±6
	10h	6±1
C57BL/6 mice Compound **17** 2.5 mg/kg PO, single dose	2h	31±2
	6h	4±1
	10h	ND
C57BL/6 mice Compound **17** 1 mg/kg PO, single dose	2h	10±3
	6h	ND
	10h	ND

JEKO-1 Xenograft Study: This study was run at WuXi AppTec (Shanghai) Co., Ltd., 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, China.

1. Study Objective: The objectives of the project were to evaluate the in vivo therapeutic efficacy of CPI-compound 17 and compound 3 in treatment of female Balb/c nude mice bearing Jeko-1 xenograft tumors.

2. Experimental Design:

Group	n	Treatment	Dose (mg/kg)	Dosing Route	Dosing Schedule
1	8	Vehicle	--	P.O.	BID*27 days
5	8	Compound **17**	0.50	P.O.	BID*27 days
7	8	Compound **3**	10	P.O.	D1-12: BID D16-20: BID, 2 days on 3days off D21-27: BID, 3 days on 2days off

Treatment was suspended for 3 days when group average body weight loss >8% or individual mouse lost >15% body weight.

3. Materials

3.1. Animals and Housing Condition

3.1.1. Animals

Species: Mus Musculus
Strain: Balb/c nude mice
Age: 6-8 weeks
Sex: Female
Body weight: 18-22 g
Number of animals: 95 mice (plus 39 spare mice)
Animal supplier: Shanghai Lingchang Laboratory Animal Co., LTD.
3.1.2. Housing condition

The mice were kept in individual ventilation cages at constant temperature and humidity with 4 animals in each cage. Temperature: 20~26 °C. Humidity: 40-70%. *Cages:* Made of polycarbonate. The size is 300 mm x 180 mm x 150 mm. The bedding material is corn cob, which is changed twice per week. *Diet:* Animals had free access to irradiation sterilized dry granule food during the entire study period. *Water:* Animals had free access to sterile drinking water. *Cage identification:* The identification labels for each cage contained the following information: number of animals, sex, strain, data received, treatment, study number, group number and the starting date of the treatment. *Animal identification:* Animals were marked by ear coding.

3.2. Test Articles Information

Product identification: Compound **17**
Provider: Constellation Pharmaceuticals
Lot number: 3
Formula: $C_{27}H_{26}N_6O$, FW: 450.546 Purity: 99%
Physical description: white powder
Package and storage condition: 100 mg/vial, stored at 4℃
Product identification: Compound **3**
Provider: Constellation Pharmaceuticals
Lot number: 1
Formula: $C_{29}H_{25}N_5O$ FW: 459.553 Purity: 97%
Physical description: white powder
Package and storage condition: 442 mg/vial, stored at 4℃

4. Experimental Methods and Procedures

4.1. Cell Culture: The Jeko-1 tumor cells were maintained in vitro in RPMI1640 medium supplemented with 20% heat inactivated fetal bovine serum at 37ºC in an atmosphere of 5% CO2 in air. The tumor cells were routinely subcultured twice weekly. The cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

4.2. Tumor Inoculation and Group Assignment: Each mouse was inoculated subcutaneously at the right flank with Jeko-1 tumor cells (10 x 106) in 0.2 ml of PBS mixed with Matrigel (50:50) for tumor development. The treatment was started on day 18 after tumor inoculation. 56 mice were selected and assigned into 7 groups with the tumor volume reaching 139 mm3. The testing articles were administrated to the mice according to Table 1.

4.3. Testing Article Formulation Preparation

Table 2. Formulation preparation

Compounds	Preparation	Con.(mg/ml)	Storage
Vehicle	DMSO/PEG400/H_2O, v/v/v, 1/3/6	-	4ºC
Compound **17**	Dilute 0.352 ml Compound **17** stock with 1.760 ml vehicle; shake up to make a clear solution.	0.05	fresh
Compound **3**	Dissolve 12.7 mg Compound **3** in 1.232 ml DMSO, ultrasonic to make the compound dissolve completely, add 3.696 ml PEG400 and vortex to mix well, add 7.391 ml H_2O, stir and ultrasonic to make a clear solution.	1	4ºC

(Compound **17** formulations were prepared freshly just before dosing; Compound **3** formulations were prepared every day.)

4.4. Observations: All the procedures related to animal handling, care and the treatment in the study were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). At the time of routine monitoring, the animals were daily checked for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption, body weight gain/loss,eye/hair matting and any other abnormal effect as stated in the protocol. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset. Animals that were observed to be in a continuing deteriorating condition or their tumor size exceeding 3000 mm^3 were euthanized prior to death or before reaching a comatose state.

4.5. Tumor Measurements and the Endpoints: For the study, the major endpoint was to see if the tumor growth could be delayed or mice could be cured. Tumor size was measured three times weekly in two dimensions using a caliper, and the volume was expressed in mm3 using the formula: V = 0.5 a x b2 where a and b are the long and short diameters of the tumor, respectively. The tumor sizes are then used for the calculations of both T/C and TGI values. The T/C value (in percent) is an indication of antitumor effectiveness; T and C are the mean volume of the treated and control groups on day 26. TGI was calculated for each group using the formula: TGI (%) = [1-(Ti-T0)/ (Vi-V0)] ×100; Ti is the average tumor volume of a treatment group on day26, T0 is the average tumor volume of the treatment group on the day of treatment start, Vi is the average tumor volume of the vehicle control group on day 26

with Ti, and V0 is the average tumor volume of the vehicle group on the day of treatment start. In this study, mice from all groups were taken down for samples collection on PG-D27 after the last administration.

4.6. Statistical Analysis: Summary statistics, including mean and the standard error of the mean (SEM), are provided for the tumor volume of each group at each time point. Statistical analysis of difference in tumor volume and tumor weight among the groups was conducted on the data obtained on day 26 and day 27, respectively. A one-way ANOVA was performed to compare tumor volume and tumor weight among groups. All data were analyzed using Prism. $p < 0.05$ was considered to be statistically significant.

4.7. Sample Collection:

1) Plasma collection for PK: Approximately 50µl blood was collected and placed in EDTA-2K tubes (1.5 ml tube containing 2µl of 0.5M EDTA-2K). Anticoagulant blood was centrifuged at 2,000 x g (4,600 rpm), 4 ºC for 15 min. Plasma was stored at -80 ºC before analysis.
2) Blood collection for hematology and PBMC: 100µl whole blood was collected in anticoagulant tubes and diluted with 400µl PBS, then applied for the hematology analysis on ADVIA 2120i. Remaining 300-400µl whole blood was used for FACS analysis and PBMC preparation. PBMC pellets were suspended in RLT buffer for sending back to the sponsor.
3) Tumor collection: Tumor samples were collected in less than 1 minute after blood collection. Four tumors from each group were split into 3 pieces, one for PK, one for tumor lysate preparation and protein concentration quantification, one for backup. All remaining tumors were snap-frozen in liquid nitrogen and then transferred to -80oC before analysis or shipped to the sponsor.
4) Spleen collection: Spleen samples were collected in less than 1 minute after blood collection. Samples were snap-frozen in liquid nitrogen and then transferred to -80oC before shipping back to the sponsor.

4.8. Plasma & Tumor Pharmacokinetics Assay: The collected plasma and tumor samples were sent for pharmacokinetics analysis at WuXi DMPK department. 1) The plasma and tumor samples were analyzed by LC/MS/MS with a detection range of 1 to 3000 ng/ml. 2) The snap frozen tumor was homogenized with 9 fold 30% ACN, the homogenate samples were analyzed by LC/MS/MS with a detection range of 1 to 3000 ng/ml.

4.9. PBMC Preparation and FACS Assay on Blood Samples:

4.9.1. Panel design for FACS assay

Fluorochrome	Panel 1	Cell subpopulation
BV421	Live/Dead	-
PE	H3K27ac	-

4.9.2. Antibody information

Fluorochrome	Mol. Target	Cat No.	Lot No.	Dilution
BV421	Live/Dead	Biolegend-423114	B220982	1:1000
PE	H3K27ac	Cell Signaling-15562S	2	1:20

4.9.3. Red Blood Cell (RBC) Lysis of Whole blood:

1) Add 8 mL of 1X RBC Lysis Buffer (BD Lysing buffer) per 0.4 mL of mouse blood.

2) Gently vortex or pipette each tube immediately after adding the lysing solution
3) Incubate for 15 minutes at RT, protect from light (no more than 15 minutes). Then add 20 mL DPBS into the tube for breaking up the lysing reaction.
4) Centrifuge at 350 xg for 5 min at RT. Decant supernatant.
5) Add 30 ml DPBS and centrifuge again at 350 xg for 5 min at RT. Decant supernatant.
6) Resuspend the pellet in the appropriate volume of staining buffer and perform a cell count and viability analysis.

4.9.4. PBMC lysis for RNA extraction:

1) Take out about 2/3 of the total PBMCs and spin down at 350 xg for 5 min at RT.
2) Decant supernatant and add 350μL/sample RLT buffer (1:100 β-ME).
3) Homogenize by gently pipetting up and down and immediately freeze down sample on dry ice.
4) Transfer the samples to -80°C freezer before sending back to the sponsor.

4.9.5. Live/Dead staining:

1) Take out 2e+5 cells from the rest PBMCs for FACS.
2) Wash the cells twice with DPBS, spin (400 xg for 5 min, RT) and flick.
3) Dilute Live/Dead viability dye (Biolegend-423114) 1:1000 in DPBS.
4) Add 100ul diluted Live/Dead viability dye in each sample.
5) Incubate the plate for 20min at RT, protect from light.

4.9.6. Surface staining:

1) Wash cells twice with 200 μL staining buffer, spin (400 xg for 5 min, RT) and flick.
2) Add 5 μL of Fc block to 45 μL /well cells and resuspend cells using a pipette, incubate 5 min at RT in the dark.
3) Add 50uL staining buffer. The total volume is 100 μL/well (50 μL cells with Fc Block +50 μL staining buffer).
4) Incubate the plate for 30min in a refrigerator, protect from light.
5) Wash the cells with staining buffer (first wash top up with 150 μL, then spin and flick and wash again with 200 μL).
6) Proceed to intracellular staining.

4.9.7. Intracellular staining protocol:

Nuclear: Use Ebioscience FoxP3 and Transcroption factor Staining Buffer Set (00-5523-00)
1) Fix and perm the cells in 1× Ebioscience FoxP3/transcription factor staining buffer set per manufacturer's instructions.
Note: Dilute Fix/perm buffer 1:4 in assay diluent (1 part Fix/Perm buffer, 3 parts diluent)
2) Add 100 μL 1× FoxP3 Fix/Perm buffer to each well and immediately resuspend the cells by pipetting up and down, incubate 45 min at in a refrigerator, protect from light.
3) Top up wells with 100μL 1× perm buffer (1:10 dilution in water), spin down (450 xg for 5 min) and wash once more with 200 μL 1× perm buffer.Resuspended with staining buffer and stay in a refrigerator overnight.
H3K27ac staining:

4) Block the cells by adding rabbit gamma globulin (Jackson Immunoresearch) and incubate at RT for 5 min.

Note: >stock = 11 mg/ml - dilute 1:1000 and add 100uL/well (~0.05 ug/well). Mix the cells with pipette tip to dislodge pellet

5) Wash the cells with 200 µL perm buffer.

6) Make intracellular staining solution containing anti-H3K27acetyl as below.

> Dilute anti-H3K27acetyl in 1:20 in 1× perm buffer for 2e+5 cells.

7) Add 100ul intracellular staining solution/well, pipette up and down and incubate for 45 min in a refrigerator, protect from light.

8) Spin down and wash twice with 200 uL perm buffer at 450 xg for 5 min.

9) Resuspended with staining buffer and run FACS.

4.9.8. Sample Acquisition:

Acquire 1e+4 events (gated on - cell of interest/singlet Live cells) for each sample. The raw data were sent to the sponsor for analysis.

5. Results

5.1. Mortality, Morbidity, and Body Weight Gain or Loss:

Animal body weight was monitored regularly as an indirect measurement of toxicity. Supplement food was supplied to all groups, to help keep their body weight since the treatment beginning.

For Compound **3** at 10mg/kg treatment group, dosing schedule was also suspended for 3 days since PG-D13 when average body weight loss >8%. The treatment for these groups was resumed after that, but the dosing schedules were changed to give the mice some holidays. The detailed dosing schedule is shown in Table 1.

5.2. Tumor Volume Measurement

Mean tumor volume over time of female Balb/c nude mice bearing Jeko-1 xenograft tumors is shown in Table 4, data are represented as Mean ± SEM.

Table 4. Tumor volume measurements (mm^3).

Group	**1**	5	**6**	**7**
Days	Vehicle	Compound **17** 0.5mg/kg	Compound **17** 0.25mg/kg	Compound **3** 10 mg/kg
0	141±10	139±10	139±9	140±10
3	181±13	187±12	174±12	186±14
5	250±26	247±15	237±14	258±18
7	304±35	281±26	286±27	280±17
10	402±46	298±28	340±37	300±20
12	485±55	319±40	368±42	328±22
14	588±73	342±45	365±48	341±18
17	749±91	386±61	358±71	381±33
19	902±116	414±77	378±95	424±50
21	1055±129	424±88	409±123	432±79
24	1303±167	472±113	492±189	472±127

26	1408±305	551±160	573±256	576±173

5.3. Tumor Growth Inhibition Analysis

The inhibition rates of Compound **17** and Compound **3** on Jeko-1 xenograft tumor growth are calculated with tumor volume data on PG-D26. The calculation results are shown in Table 5.

Table 5. Tumor growth inhibition analysis (TGI and T/C).

Group	Treatment	Tumor Size (mm^3)	TGI (%)	T/C (%)	*p* value
1	Vehicle	1408±305	--	--	--
5	Compound **17** - 0.5mg/kg	551±160	67.48	39.14	*$p<0.05$
7	Compound **3** - 10 mg/kg	576±173	65.62	40.89	*$p<0.05$

5.6. Pharmacokinetics Analysis Results:

The average concentrations of test compounds in plasma and tumor samples are shown in Table 6.

Table 6. Mean concentration in plasma (ng/ml) and tumor (ng/g)

Group	Time point	Concentration in plasma (ng/ml)±SEM	Concentration in tumor (ng/g)±SEM
Compound **17** 0.5 mg/kg	2h PG-D1	16±1	
	2h PG-D27	27±4	29±5
Compound **3** 10 mg/kg	2h PG-D1	59±12	
	2h PG-D27	134±18	787±65

S10. References

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