Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Synthesis, Activity and Metabolic Stability of Non-Ribose Containing Inhibitors of Histone Methyltransferase DOT1L

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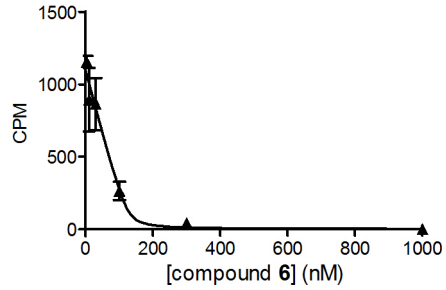

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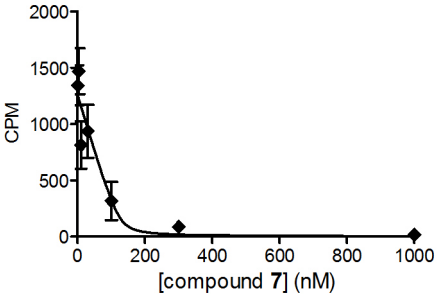

Figure S1. Fitting curves for compounds **6** (A) and **7** (B) using Morrison tight binding model in Prism 5.0, from which the K_i values were obtained.

Experimental Section

All reagents were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). All compounds were characterized by ^{1}H spectrum on a Varian (Palo Alto, CA) 400-MR spectrometer. The purities were determined by a Shimadzu Prominence HPLC using a Zorbax C18 column (4.6 x 250 mm; methanol:water = 70:30; flow rate = 1 mL/min; monitored at 254 and 280 nm). The purities of compounds **6**, **7** and *epi*-**6** were found to be >95%. Identities of compounds **6**, **7** and *epi*-**6** were confirmed with high resolution mass spectra (HRMS) using a ThermoFisher LTQ-Orbitrap mass spectrometer. Control compounds **4** and SAH were from the same batches of our previous studies.[1,2]

Scheme 1:

Compound 8. To a solution of *D*-ribose (7.5 g, 50 mmol) in cyclohexanone (50 mL) was added concentrated H_2SO_4 (0.5 mL). The reaction mixture was stirred for 6 h at room temperature, after which the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with Saturated $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a light brown oil, which was dissolved in THF (100 mL). Vinylmagnisum bromide (250 mL, 1 M in THF) was added dropwise at -78 $^{\circ}$C. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction was quenched by adding saturated NH_4Cl (40 mL) and the aqueous layer was extracted with EtOAc (3 x 40 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified with column chromatography (silica gel, EtOAc/Hexanes 2:1) to give **8** as a colorless oil (8.65 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.16-6.03 (m, 1 H), 5.40 (d, J = 17.6 Hz, 1 H), 5.30 (d, J = 10.8 Hz, 1 H), 4.39-4.35 (m, 1 H), 4.14-4.12 (m, 1 H), 4.07-4.04 (m, 1 H), 3.97-3.91 (m, 2 H), 3.77-3.72 (m, 1 H), 1.66-1.39 (m, 10 H).

Compound 9. To a solution of **8** (2.32 g, 9 mmol) in MeOH/H_2O (80 mL, 5:1) was added $NaIO_4$ (3.86 g, 18 mmol) slowly at 0 ^{0}C. The reaction was warmed to room temperature and stirred for 1 h. Upon filtering off the solid, MeOH was removed under reduced pressure. EtOAc (80 mL) was added, washed

with brine (20 mL), dried over Na_2SO_4, and concentrated to give an aldehyde, which was used for next step without further purification. To a suspension of Ph_3PCH_3Br (8.0 g, 22.5 mmol) in THF (45 mL) was added *t*-BuOK (2.52 g, 22.5 mmol) slowly at 0 °C. After stirred for 1h at room temperature, the reaction mixture was cooled to 0 °C and a solution of the crude aldehyde in THF (9 mL) was added. The reaction mixture was stirred overnight and quenched by adding water (25 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, concentrated, and purified with column chromatography (silica gel, EtOAc/Hexanes 1:6) to give **9** as a colorless oil (1.77 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.14-5.98 (m, 2 H), 5.46-5.22 (m, 4 H), 4.64 (m, 1 H), 4.18 (m, 1 H), 4.01 (m, 1 H), 1.79 (d, J = 4.4 Hz, 1 H), 1.67-1.37 (m, 10 H).

Compound 10. A solution of **9** (1.1 g, 4.91 mmol) in CH_2Cl_2 (100 mL) was degassed, followed by addition of 2nd generation Grubbs' catalyst (172 mg, 5 mmol%). The reaction mixture was stirred overnight at room temperature and concentrated under reduced pressure to give a brown dark residue, which was dissolved in CH_2Cl_2 (20 mL). Dess-Martin periodinane (DMP, 3.11 g, 7.34 mmol) and $NaHCO_3$ (619 mg, 7.34 mmol) were added into the solution. After 2 h, the reaction was quenched by adding saturated $Na_2S_2O_3$ (30 mL) and ether (100 mL) and stirred for 30 min. The separate organic layer was dried over Na_2SO_4, concentrated and the residue purified with column chromatography (silica gel, EtOAc/Hexanes 1:8) to give **10** as a colorless oil (821 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (dd, J = 2.4, 3.6 Hz, 1 H), 6.20 (d, J = 5.2 Hz, 1 H), 5.27-5.24 (m, 1 H), 4.45 (d, J = 5.2 Hz, 1 H), 1.67-1.37 (m, 10 H).

Compound 11. To a suspension of CuBr-Me_2S complex (57 mg) in THF (15 mL) was added vinylmagnesium bromide (4.06 mL, 4.1 mmol, 1 M solution in THF) slowly at -78 °C. After 15 min, a solution of **10** (630 mg, 3.25 mmol), chlorotrimethylsilane (845 μL), and hexamethylphosphoramide (1.45 mL) in THF (5 mL) was added slowly. The reaction was further stirred at -78 °C for 5 h, allowed to warm to 0 °C and quenched with saturated NH_4Cl (10 mL). The aqueous layer was extracted with

EtOAc (3 x 10 mL) and the combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, concentrated under reduced pressure, and purified with column chromatography (silica gel, EtOAc/Hexanes 1:10) to give the 1,4-addition product as a white oil [641mg, 89%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.81 (m, 1 H), 5.14-5.06 (m, 2 H), 4.60 (d, J = 4.8 Hz, 1 H), 4.17 (d, J = 5.2 Hz, 1 H), 3.10 (t, J = 4.8 Hz, 1 H), 2.82 (dd, J = 19.2, 8.8 Hz, 1 H), 2.31-2.22 (m, 1 H), 1.65-1.37 (m, 10 H)].

To a solution of the product thus obtained (666 mg, 3.0 mmol) in MeOH (40 mL) was added $CeCl_3 \cdot 7H_2O$ (780 mg, 2.1 mmol) and $NaBH_4$ (224.2 mg, 6 mmol) at -15 °C. The reaction mixture was warmed to room temperature and stirred for 1h. Upon removal of the solvent, water was added to the residue and pH adjusted to 5 with acetic acid. The product was extracted with EtOAc (3 x 15 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, concentrated, and purified with column chromatography (silica gel, EtOAc/Hexanes 1:4) to give **11** as a colorless oil (671mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (m, 1 H), 5.19-5.02 (m, 2 H), 4.48-4.46 (m, 1 H), 4.07-4.02 (m, 1 H), 2.76-2.70 (m, 1 H), 2.48 (d, J = 7.2 Hz, 1 H), 1.90-1.86 (m, 2 H), 1.69-1.35 (m, 10 H).

Compound 12. To a solution of **11** (300 mg, 1.34 mmol), 6,6-di-Boc-protected adenine (898 mg, 2.7 mmol) and Ph_3P (807 mg, 3.1 mmol) in THF (20 mL) was added diisopropyl azodicarboxylate (DIAD, 625 μL) dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 3 days. Upon removal of the solvent, the residue was subjected to a flash column chromatography (silica gel, EtOAc/Hexanes 1:3) to give **12** as a white foam (530 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (s, 1 H), 8.09 (s, 1 H), 5.94 (m, 1 H), 5.21-5.14 (m, 2 H), 5.12-5.06 (m, 1 H), 4.83-4.79 (m, 1 H), 4.64-4.62 (m, 1 H), 2.83-2.79 (m, 1 H), 2.57-2.47 (m, 1 H), 1.91-1.87 (m, 2 H), 1.67-1.37 (m, 10 H), 1.44 (s, 18 H).

Compound 13. A solution of compound **12** (1.08 g, 2mmol) in CH_2Cl_2 (10 mL) was cooled to -78 °C, into which O_3 was bubbled until the solution became light blue. Upon removal of the excess O_3 by passing N_2, Me_2S (5 mL) was added into the solution. The reaction mixture was warmed slowly to room

temperature over 1 h and concentrated to give a syrup, which was dissolved in MeOH (8 mL). $NaBH_4$ (148 mg, 4 mmol) was added into the solution at 0 °C and stirred for 1 h before adding water. The product was extracted with EtOAc (3 x 10 mL) and the combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colorless oil. It was dissolved in CH_2Cl_2 (4.5 mL) at 0 °C, and trifluoroacetic acid (TFA, 0.5 mL) was added dropwise. After stirring for 2 h at room temperature, the solvent was removed under reduced pressure and CH_2Cl_2 (10 mL) was added. This step was repeated for 3 times and the resulting residue was purified with column chromatography (silica gel, 5% methanol in ethyl acetate) to give **13** as a white solid (635mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1 H), 7.88 (s, 1 H), 5.01 (t, J = 6.4 Hz, 1 H), 4.81-4.79 (m, 1 H), 4.69-4.68 (m, 1 H), 4.44 (br, 1 H), 3.82-3.79 (m, 2 H), 2.42 (m, 1 H), 1.80-1.78 (m, 2 H), 1.68-1.37 (m, 10 H).

Compound 14. To a solution of **13** (553 mg, 1.6 mmol), phthalimide (470 mg, 3.2 mmol) and Ph_3P (837 mg, 3.2 mmol) in THF (15 mL) was added diisopropyl azodicarboxylate (646 mg, 3.2 mmol) slowly at 0 °C. After stirring overnight at room temperature, the solvent was evaporated and the resulting residue was purified with column chromatography (silica gel, EtOAc) to give the product (698 mg, 92%) as a white foam, which was refluxed with hydrazine monohydrate (480 mg, 9.6 mmol) in EtOH (15 mL) for 2 h. Upon cooling and filtering off an insoluble material, the filtrate was concentrated under reduced pressure and purified with column chromatography (silica gel, EtOAc:methanol (9:1) containing 1% triethylamine) to give a primary amine as a syrup (500 mg, 99%). To a solution of the product (690 mg, 2.0 mmol) in MeOH (20 mL) were added HOAc (1.16 mL), acetone (2.0 mL) and $NaBCNH_3$ (730 mg, 10.0 mmol) at 0 °C. After stirring overnight, the solvent was removed under reduced pressure. Water (10 mL) was added and the product was extracted with EtOAc (6 x 20 mL). The combined organic phases were dried over Na_2SO_4, concentrated under reduced pressure, and purified with column chromatography (silica gel, EtOAc:methanol (9:1) containing 1% triethylamine) to give **14** as a white oil (733 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1 H), 7.88 (s, 1 H), 4.94-4.93 (m, 1 H), 4.58-4.57 (m, 1H), 4.46-4.45 (m, 1 H), 4.06 (m, 1 H),

2.84-2.82 (m, 2 H), 2.41-2.39 (m, 2 H), 2.12-2.10 (m, 1 H), 1.66-1.35 (m, 10 H), 1.09 (d, *J* = 6.0 Hz, 6 H)

Compound 15. Compound **14** (1.55 g, 4.0 mmol) was refluxed with methyl acrylate (2 mL) in MeOH (12 mL) for 3 days to produce a 1,4-adduct in almost quantitative yield. The crude product was reduced with LiAlH4 (152 mg, 4.0 mmol) in THF (25 mL) at - 15 °C for 1h and room temperature for 3 h. The reaction mixture was quenched by adding of EtOAc at 0 °C and further stirred for 2 h. The precipitation was filtered off and thoroughly washed with EtOAc. The combined organic phases were evaporated under reduced pressure and purified with column chromatography (silica gel, EtOAc:methanol 9:1) to give compound **15** as a white oil (1.6 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1 H), 7.82 (s, 1 H), 4.96-4.93 (m, 1 H), 4.57-4.56 (m, 1H), 4.45-4.43 (m, 1 H), 4.07 (m, 1 H), 3.76-3.74 (m, 2 H), 3.19-3.16 (m, 1 H), 2.72-2.46 (m, 4 H), 2.05-1.99 (m, 1 H), 1.82-1.37 (m, 14 H), 1.04 (d, *J* = 6.4 Hz, 3 H), 1.03 (d, *J* = 6.4 Hz, 3 H).

1-(3-((((1*R*,2*R*,3*S*,4*R*)-4-adenosyl-2,3-dihydroxycyclopentyl)methyl)(isopropyl)amino)propyl)-3-(4-(*tert*-butyl)phenyl)urea (6). Using steps xii and xiii (Scheme 1) described above, compound **15** (700 mg, 1.6 mmol) was subjected to a Mitsunobu reaction with phthalimide, followed by treatment with hydrazine, to give compound **16** as a white foam (640 mg, 91%). To a solution of **16** (222 mg, 0.5 mmol) in CH_2Cl_2 (4 mL), triethylamine (77 µL, 0.55 mmol) and 4-*tert*-butylphenylisocyante (200 µL, 0.5 mmol) were added at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1h. Upon removal of the solvent, the residue was purified with column chromatography (silica gel, EtOAc:methanol 20:1) to give the cyclohexanone protected **6** (294 mg, 95%) as a white solid, which was treated with HCl (1.0 mL, 4 M in dioxane) in MeOH (3.0 mL) for 12 h. After removal of the solvent, the solid was washed with ethyl acetate (3 x 3 mL) to give compound **6** as a white powder (265 mg, 92%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.38-8.19 (m, 2 H), 7.29-7.18 (m, 4 H), 5.40 (br, 2 H), 4.78-4.72 (m, 1 H), 4.36-4.34 (m, 1 H), 4.01-3.98 (m, 1 H), 3.91-3.89 (m, 1 H), 3.81-2.99 (m, 7 H),

2.38-2.31 (m, 2 H), 198-1.88 (m, 3 H), 1.40-1.16 (16 H). HRMS (ESI) $[M+H]^+$ Calcd for $C_{28}H_{43}N_8O_3^+$: 539.3453, Found: 539.3450.

Scheme 2:

Compound 19. A solution of compound **18** (1.53 g, 3.6 mmol), prepared according to a published procedure from *D*-ribose,[3] in CH_2Cl_2 (13 mL) was degassed followed by addition of 2nd generation Grubbs' catalyst (118 mg). The reaction mixture was refluxed overnight. Additional 2nd generation Grubbs' catalyst (59 mg) was added to increase the yield and the reaction was continued for 6 h. Upon removal of the solvent, the residue was purified with column chromatography (silica gel, EtOAc/hexanes 1:6) to give **19** (1.45 g, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75-36 (m, 10 H), 5.98-5.97 (m, 1 H), 5.77-5.75 (m, 1 H), 5.36-5.34 (m, 1 H), 4.55 (d, J = 4.8 Hz, 1 H), 4.01 (d, J = 9.6 Hz, 1 H), 3.70 (d, J = 9.6 Hz, 1 H), 3.23 (br, 1 H), 1.36 (s, 3 H), 1.28 (s, 3 H), 1.08 (s, 9 H).

Compound 20. To a solution of compound **19** (4.42 g, 10.4 mmol) in DMF (40 mL) was added 4 Å molecular sieve (4.4 g) and pyridinium dichromate (PDC, 8.4 g, 20.8 mmol). The reaction mixture was stirred for 12 h at room temperature. The precipitation was filtered off and washed with EtOAc. The filtrate was washed with water (20 mL) and brine (20 mL), dried over Na2SO4, concentrated under reduced pressure, and purified with column chromatography (silica gel, EtOAc/hexanes 1:6) to give **20** as a colorless oil (3.83 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75-36 (m, 10 H), 6.33 (s, 1 H), 4.96 (d, J = 5.2 Hz, 1 H), 4.70 (d, J = 18.4 Hz, 1 H), 3.50 (d, J = 18.4 Hz, 1 H), 3.49 (d, J = 5.2 Hz, 1 H), 1.35 (s, 3 H), 1.34 (s, 3 H), 1.07 (s, 9 H).

Compound 21. To a solution of **20** (1.77 g, 4.2 mmol) in MeOH (20 mL) was added $CeCl_3 \cdot 7H_2O$ (1.32 g, 3.5 mmol) and $NaBH_4$ (311 mg, 8.4 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1h. Upon removal of the solvent, water (10 mL) was added and pH adjusted to 5 with acetic acid. The product was extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, concentrated under reduced pressure, and purified with column chromatography (silica gel, EtOAc/Hexanes 1:4) to give **21** as a colorless oil (1.75 g, 98%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68-7.37 (m, 10 H), 5.82 (s, 1 H), 4.74 (t, *J* = 5.6 Hz, 1 H), 4.55-4.53 (m, 1 H), 4.38 (d, *J* = 15.2 Hz, 1 H), 4.28 (d, *J* = 15.2 Hz, 1 H), 1.35 (s. 3 H), 1.33 (s, 3 H), 1.06 (s, 9 H).

Compound 22. To a solution of compound **21** (545 mg, 1.28 mmol), 6-chloropurine (297 mg, 1.92 mmol) and Ph_3P (671 mg, 2.56 mmol) in THF (16 mL) was added DIAD (528 µL, 2.56 mmol) dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. Upon removal of the solvent, the residue was subjected to column chromatography (silica gel, EtOAc/Hexanes 1:3) to give **22** as a colorless oil (681 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88 (s, 1H), 7.90 (s, 1 H), 7.67-7.37 (m, 10 H), 5.83 (s, 1 H), 5.64 (s, 1 H), 5.28 (d, *J* = 5.2 Hz, 1 H), 4.72 (d, *J* = 5.2 Hz, 1 H), 4.48 (dd, *J* =12.4 , 6.8 Hz, 2 H), 1.41 (s, 3 H), 1.37 (s, 3 H), 1.08 (s, 9 H).

Compound 23. Compound **22** (616 mg, 1.1 mmol) was dissolved in 7 M NH_3 in MeOH (3 mL) and heated to 100 °C in a sealed pressure flask overnight. After cooling, the solvent was removed and the residue was dissolved in THF (6 mL). tetrabutylammonium fluoride (1.6 mmol, 1.6 mL as a 1 M THF solution) was added and stirred for 2 h at room temperature. Upon removal of solvent, the residue was subjected to column chromatography (silica gel, 5% methanol in ethyl acetate) to give compound **23** as a white foam (313 mg, 93%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.18 (s, 1 H), 7.97 (s, 1 H), 7.01 (br, 2 H), 5.74 (s, 1 H), 5.42 (s, 1 H), 5.38-5.37 (m, 1 H), 5.03 (br, 1 H), 4.68-4.67 (m, 1 H), 4.18 (s, 2 H), 1.39 (s, 3 H), 1.28 (s, 3 H).

1-(3-((((3*R*,4*S*,5*R*)-3-adenosyl-4,5-dihydroxycyclopent-1-en-1-yl)methyl)(isopropyl)amino)propyl)-3-(4-(tert-butyl)phenyl)urea (7). Using steps xii and xviii (Scheme 1) described in the making of **6**, compound **23** (487.0 mg, 1.2 mmol) was converted to give compound **7** as a white powder (440 mg, 60%). ^{1}H NMR (400 MHz, D_2O): δ (rotamer: 1:1) 8.17, 8.16 (s, 1 H), 8.10, 8.06 (s, 1 H), 7.04-6.79 (m, 4 H), 6.33, 6.34 (s, 1 H), 5.44-5.39 (m, 1 H), 4.28-4.19 (m, 1 H), 4.06-3.97 (m, 1 H), 3.83-3.58 (m, 2 H), 3.52-3.48 (m, 1 H), 3.43-3.9 (m, 1 H), 3.26-3.18 (m, 3 H), 1.98-1.86 (m, 2 H), 1.34-1.24 (m, 6 H), 1.05 (s, 9 H). HRMS (ESI) $[M+H]^+$ Calcd for $C_{28}H_{41}N_8O_3^+$: 537.3296, Found: 537.3292.

1-(3-((((1*S*,2*R*,3*S*,4*R*)-4-adenosyl-2,3-dihydroxycyclopentyl)methyl)(isopropyl)amino)propyl)-3-(4-(*tert*-butyl)phenyl)urea (*epi*-6). To a solution of compound **7** (35 mg, 0.057 mmol) in MeOH (4 mL) was added 10% Pd/C (5 mg). The reaction mixture was stirred at room temperature with a hydrogen balloon for 2 h. The reaction mixture was filtered through a 0.2 μm syringe filer and washed with methanol. The filtrate was concentrated under reduced pressure to afford *epi*-**6** as a white solid (32 mg, 91%). ^{1}H NMR (400 MHz, D_2O): δ (rotamer: 1:1) 8.35, 8.34 (s, 1 H), 8.30, 8.29 (s, 1 H), 7.39 (d, J = 10.4 Hz, 1 H), 7.18 (d, J = 10.4 Hz, 1 H), 4.78-4.76 (m, 1 H), 3.63-3.57 (m, 2 H), 3.37-2.98 (m, 7 H), 1.99-1.91 (m, 1 H), 1.82-1.78 (m, 2 H), 1.38-1.18 (m,17 H). HRMS (ESI) $[M+H]^+$ Calcd for $C_{28}H_{43}N_8O_3^+$: 539.3453, Found: 539.3449.

Enzyme inhibition. Expression, purification and inhibition of recombinant human DOT1L (catalytic domain 1 – 472) were performed according to our previous published methods.[1,2] In brief, compounds with concentrations ranging from 1 nM to 100 μM were incubated with DOT1L (100 nM), 1.5 μM oligo-nucleosome in 20 μL of 20 mM Tris buffer (containing 1 mM EDTA, 0.5 mM DTT and 50 μg/mL BSA, pH = 8.0) for 10 min. 0.76 μM (= K_m) of ^{3}H-SAM (10 Ci/mM; Perkin-Elmer) was added to initiate the reaction. After 30 min at 30 ºC, the reaction was stopped by adding SAH (100 μM). 15 μL of reaction mixture was transferred to P81 filter paper (Whatman) that binds histone H3 protein, washed 3x with 50 mM $NaHCO_3$, dried, and placed into a scintillation vial containing scintillation cocktail (2 mL), which was measured with a Beckman LS-6500 scintillation counter. K_i values were calculated using the Morrison tight binding model fitting or standard sigmoidal dose response curve fitting (for less potent inhibitors) in Prism 5.0. Enzyme inhibition assays for PRMT1, CARM1 and SUV39H1 were performed using our previous methods.[1,2]

Western blot. MV4-11 cells in the exponential growth phase were incubated in the presence of increasing concentrations of a compounds (0.025 - 15.625 μM). Cells (2×10^6) were harvested at day 4 and histones were extracted with the EpiQuik^{TM} total histone extraction kit (Epigentek) according to the

manufacturer's protocol. Equal amounts of histones (2 μg) were separated with SDS-PAGE and transferred to a piece of PVDF membrane. The blots were incubated with primary antibodies against dimethylated H3K79 and Histone H3 (Cell Signaling), followed by secondary antibody (anti-rabbit IgG) coupled with horseradish peroxidase (HRP), and detected with Supersignal West Dura substrate (Thermo Scientific).

Metabolic stability testing. For microsome stability assay, pooled human liver microsomes were purchased from Invitrogen. To a 100 mM phosphate buffer solution (450 μL) containing microsomes (the final concentration of 0.5 mg/mL) and $MgCl_2$ (5 mM) was add 5 μL of 200 μM a test compound or verapamil (as the control compound) at 37°C. 50 μL of NADPH (1 mM final concentration) solution in the same buffer was added to initiate the reaction. Aliquots of 50 μL were taken from the reaction solution at 0, 15, 30, 45 and 60 min. The reaction was stopped by the addition of 3 volumes of methanol. Samples were centrifuged at 16,000 g for 10 minutes to precipitate protein. Aliquot of 100 μL of the supernatant was used for LC/MS/MS analysis to determine the remaining amount of the test compound, using Shimadzu HPLC [Phenomenex 5μ C18 (2.0×50 mm) column; Mobile phase: 0.1% formic acid in acetontrile (A) and 0.1% formic acid in water (B)] followed by AB Sciex API4000 mass spectrometer [parameters: ion source, Turbo spray; ionization model, ESI; scan type, MRM; collision gas, 6 L/min; curtain gas, 30 L/min; nebulize gas, 50 L/min; auxiliary gas, 50 L/min; temperature, 500 °C; ionspray voltage, +5500 v (positive MRM)]. All experiments were performed in duplicate.

For human plasma stability assay, to human plasma (500 μL) was added a test compound to a final concentration of 5 μM and incubated at 37°C at approximately 60 rpm on an orbital shaker. Aliquots of 50 μL were taken from the reaction solution at 0, 15, 30, 45 and 60 minutes. The reaction was stopped by the addition of 6 volumes of cold acetonitrile. Samples were centrifuged at 20,000 g for 15 minutes to precipitate protein. An aliquot of 150 μL of the supernatant was used to determine the remaining amount of the test compound, using the same LC/MS/MS method described above. All experiments were performed in duplicate.

References for Supporting Information

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(3) Michel, B. Y.; Strazewski, P. Synthesis of (-)-neplanocin A with the highest overall yield via an efficient Mitsunobu coupling. *Tetrahedron*, **2007**, *63*, 9836-9841.