Methods

Biochemical activity assays:

PRMT5-MEP50 complex was expressed in Sf9 cells and purified by immobilized metal affinity chromatography following the procedures described previously [1]. Effect of compound 1 and compound 2 on methyltransferase activity of G9a, GLP, SUV39H1, SUV39H2, SETDB1, SETD8, SUV420H1, SUV420H2, SETD7, MLL1 trimeric complex, MLL3 pentameric complex, EZH2 trimeric complex, PRMT1, PRMT3, PRMT4, PRMT5-MEP50 complex, PRMT6, PRMT7, PRMT8, PRMT9, PRDM9, SETD2, SMYD2, and SMYD3 was assessed by monitoring the incorporation of tritium-labeled methyl group to lysine or arginine residues of peptide substrates using Scintillation Proximity Assay (SPA) as previously described [2-4]. Assays were performed in 10 µl reaction volume containing ^{3}H-SAM (cat#NET155V001MC; specific activity range 12−18 Ci/mmol) and at substrate concentrations close to the apparent K_m values for each enzyme. Two concentrations (1 µM and 10 µM) of compound 1 or compound 2 were assessed in all selectivity assays. To stop the enzymatic reactions, 10 µl of 7.5 M guanidine hydrochloride was added, followed by addition of 180 µl of buffer (20 mM Tris-HCl, pH 8.0), mixed and then transferred to a FlashPlate (Cat.# SMP103; Perkin Elmer; www.perkinelmer.com). After mixing, the reaction mixtures in Flash plates were incubated for 2 hours and the CPM counts were measured using a TopCount NXT™ Microplate Scintillation and Luminescence Counter (PerkinElmer Life Sciences). The CPM counts in the absence of compound for each data set were considered as 100% activity. In the absence of the enzyme, the CPM counts in each data set were defined as background (0%).

For DOT1L, NSD1, NSD2, NSD3, ASH1L, DNMT3A/3L, and DNMT3B/3L, a filter-based assay was used. In this assay, 10 µl of reaction mixtures were incubated at 23 ^{o}C for 1 hour, 50 µl of 10% trichloro acetic acid (TCA) was added, mixed and transferred to filter-plates (Millipore; cat.# MSFBN6B10; www.millipore.com). Plates were centrifuged at 2000 rpm (Allegra X-15R - Beckman Coulter, Inc.) for 2 min followed by two additional 10% TCA wash and one ethanol wash followed by centrifugation. Plates were dried and 30 µl MicroO (MicroScint-O; Cat.# 6013611, Perkin Elmer; www.perkinelmer.com) was added to each well, centrifuged and removed. 50 µl of MicroO was added again and CPM was measured using Topcount plate reader.

In the case of PRMT5-MEP50 complex a same SPA assay was used to assess the effect of the compounds following the procedures described previously [1]. In brief, the reaction mixture contained 20 mM Tris-HCl (pH 8.0) containing 0.01% Tween-20 and 5 mM Tris(2-carboxyethyl)phosphine (TCEP), 15 nM PRMT5-MEP50, 0.07 µM biotinylated $H4_{1-24}$ peptide (SGRGKGGKGLGKGGAKRHRKVLRDK-Biotin) and 0.6 µM ^{3}H-SAM.

To determine the mechanism of action (MOA) the competition of the compound 1 with SAM and peptide substrate was assessed separately. To do this the IC_{50} values against COMPOUND 1 at saturating concentrations of $H4_{1-24}$ peptide substrate (2 µM) and varying concentrations of SAM (0.5, 1, 4, 8, 12, 16, 20, and 25×K_m^{app}). To determine the competition with peptide, the SAM concentration was kept at saturation (3.5 µM) and IC_{50} values were determined at varying peptide concentration (0.5, 1, 4, 8, 12, 16, 20, and 25×K_m^{app}).

Surface Plasmon Resonance (SPR):

SPR studies were performed using a Biacore™ T200 (GE Health Sciences Inc.) at 20 °C. Approximately 4400 response units (RU) of biotinylated PRMT5 was captured onto one flow cell of a streptavidin-conjugated SA chip (according to manufacturer's protocol) while another flow cell was left empty for reference subtraction. Compound 1 was dissolved in 100% DMSO (20 mM stock) and diluted to 8 µM before triplicate 3-fold serial dilutions were prepared in 100% DMSO. For SPR analysis the serially titrated compound was diluted 1:20 in HBS-EP (20 mM Hepes (pH 7.4), 150 mM NaCl, 3 mM EDTA, and 0.05% Tween-20) giving a final concentration of 5% DMSO. Kinetic determination experiments were performed using single cycle kinetics with an on time of 90 seconds, off time of 300 seconds, and a flow rate of 100 µl/min. Kinetic curve fittings and K_D calculations were done with a 1:1 binding model and the Biacore T200 Evaluation software (GE Health Sciences Inc.). Using compound 1 to access the binding competence of the immobilized biotin-tagged PRMT5, the results indicate that the protein was 50% functional for binding.

Knockdown

MCF7 cells were grown in DMEM supplemented with 10% FBS, penicillin (100 units/mL) and streptomycin (100 µg/mL). 40% confluent cells were transfected with 50 nM siRNas: control, PRMT6, PRMT5 (Silencer™ Select, ThermoFisher Scientiffic), PRMT1,

PRMT4 (SMARTpool siGENOME, Dharmacon) PRMT3 PRMT7 (siGENOME, Dharmacon) using jetPRIME® transfection reagent (Polyplus), following manufacturer instructions. Protein levels were analysed in westernblot 3 days post transfection, as described below.

PRMT5 cellular assay

MCF7 cells were grown in DMEM supplemented with 10% FBS, penicillin (100 units/ml) and streptomycin (100 µg/ml). 40% confluent cells were treated with different concentrations of compounds or DMSO control for 48 h. The levels of SmBB' and symmetrically dimethylated SmBB' were analyzed in westernblot, as described below.

Western blot

Cells were lysed in 100 µL of total lysis buffer (20 mM Tris-HCl pH 8, 150 mM NaCl, 1 mM EDTA, 10 mM MgCl2, 0.5% TritonX-100, 12.5 U/mL benzonase (Sigma), complete EDTA-free protease inhibitor cocktail (Roche)). After 3 min incubation at RT, SDS was added to final 1% concentration. Total cell lysates were resolved in 4-12% Bis-Tris Protein Gels (Invitrogen) with MOPS buffer (Invitrogen) and transferred in for 1.5h (80 V) onto PVDF membrane (Millipore) in Tris-Glycine transfer buffer containing 20% MeOH and 0.05% SDS. Blots are blocked for 1 h in blocking buffer (5% milk in 0.1% Tween 20 PBS) and incubated with primary antibodies: anti-smBB' (1:1000, Sigma # S06698), anti-Rme2s (1:2000, Cell Signalling #13222), anti-PRMT1(#07-404, Millipore), anti-PRMT3 (SAB1402505, Sigma), anti-PRMT4 (12495, Cell Signalling) anti-PRMT5(ab109451, Abcam), anti-PRMT6 (ab47244, Abcam), anti-PRMT7(ab179822, Abcam), anti-B-actin (ab3280, Abcam) in blocking buffer overnight at 4ºC. After five washes with 0.1% Tween 20 PBS the blots are incubated with goat-anti rabbit (IR800 conjugated, LiCor #926-32211) and donkey anti-mouse (IR 680, LiCor #926-68072) antibodies (1:5000) in Odyssey Blocking Buffer (LiCor) for 1 h at RT and washed five times with 0.1% Tween 20 PBS. The signal is read on an Odyssey scanner (LiCor) at 800 nm and 700 nm.

MDM4 Exon5/6 PCR Assay in A375 Tumor Cells:

Culture A375 tumor cells (ATCC), a melanoma cancer cell line, to 70%-90% confluence in T150 flasks with the growth medium (DMEM; HYCLONETM #SH30022 with 10% FBS; GIBCO® 10082-147 or equivalent). Treat the cells with standard trypsin/EDTA

treatment for 3 min and aspirate and wash with PBS to release the adherent cells from the culture flask. Seed the cells into a 96 well plate (Costar 3596) at 5000/well in growth medium (90 µL). Incubate the plate overnight at 37^{o}C and 5% CO2 and treat the cells with test compound at serial dilutions (1:3 10-point serial dilution, final DMSO addition of 0.2%) for 72 hr. On Day 5 after incubation, remove the medium from the culture plates and wash the cells twice with cold PBS (150 µL/well). Prepare Lysis Working Reagent from the TAQMAN® Gene Expression Cells-to-CT Kit (INVITROGENTM cat. # AM1729) by dilution of DNAse 1 into Lysis Solution at 1/100. Add Lysis Working Reagent (50 µL/well) to the cell plates, mix well, and incubate at room temperature for 5 min. Add kit stop solution (5 µL) to each well, mix the wells, and incubate for 2 min at room temperature. Prepare Reverse Transcriptase Master Mix in the following volume ratios; RT buffer: Nuclease-free water: RT Enzyme at 62.5:31.25:6.25. Add RT mix (48 µL/well) to each well of a 96-well NUNC™ plate (THERMO SCIENTIFICTM #260860). Add RT mix (20 µL) and each cell lysate sample (5 µL) into a 384 PCR plate (Clear Optical Reaction Plate, cat. # 4309849, APPLIED BIOSYSTEMS®) quadrant 1 and add a second addition of RT Mix and cell lysate to quadrant 3. For the RT reaction, seal the plates and then place them in a thermal cycler set at 37 °C for 60 min; 95 °C for 5 min; stop at 4 °C. For qPCR, prepare MDM4 exon 5 primers (Life Technologies Hs00967240-m1) and exon 6 primers (Life Technologies Hs00967242-m1) separately in the following volume ratios of RT mix: H2O: exon probe at 10:6:1. Add Exon 5 (17 µL) to odd columns of a 384 PCR plate and add exon 6 (17 µL) to the even columns. Add cDNA (3 µL, from the RT plate) by quadrant stamping to each well of the qPCR plate containing the 17 µL exon primer solution. In this manner, perform qPCR for both exons on cDNAs from a single RT reaction on an individual cell lysate. Seal the plates, spin, and place on a real-time PCR instrument (Life Technologies ViiA7 Real-Time PCR). Run the TAQMAN ® reaction in the following staged cycles: stage 1 (50 °C, 2 min), stage 2 (95 °C, 10 min), stage 3 (95 °C, 15 seconds), stage 4 (60 °C, 60 seconds) with stages 3 and 4 repeated for 40 cycles. Retrieve CT data from the real-time PCR reader and perform the following calculations, where “CPD” is the value from compound-treated samples, and “DMSO” is the minimum control sample, using an Excel template:

(Exon 6 CT CPD – Exon 6 CT DMSO) - (Exon 5 CT CPD – Exon 5 CT DMSO) = ΔCT

$2^{(-\Delta CT)}$ = Fold Change

Using Genedata software, plot the data as Fold Change (y axis) versus Log test compound concentration (x-axis) and calculate the EC_{50} values using a 4-parameter logistic fit.

7-day Proliferation Assay:

On day 1, plate cancer cell line/s (ATCC) at appropriate seeding density in a 96-well assay plate (BD Falcon 35-3219). Incubate the plates in a 37^{o}C incubator (5% CO_2) for 18-24 hr. On day 2, prepare and serially dilute test compounds in medium (1:2 10-point serial dilution) from compound stock solutions (10 mM in 100% DMSO). Add 10 µL of serial diluted test compounds to cell plates (0.2% DMSO final concentration). Incubate cell plates at 37^{o}C/5%CO_2 for 7 days. On day nine, gently remove media in cell plates by flicking and blotting on a stack of paper towels. Add 25 µL of CELL TITER-GLO reagent (reconstituted lyophilized enzyme/substrate mixture) to each well. Incubate plates for 10-20 min at room temperature before reading. Measure luminescence using PERKIN-ELMER ENVISION Multi-label Plate Reader. Analyze this luminescence data by a 4-parameter curve fit using Activity Base, to yield cell proliferation IC_{50} values.

A375 Xenograft Tumor Model:

Grow A375 (melanoma) cells in DMEM/High glucose (HYCLONETM cat. # SH30022) with 10% HI FBS (GIBCO® cat. # 10082-147). Harvest cells and inject subcutaneously onto the rear flank of nude mice (A375: 5 x 106 cells/animal, mixed 1:1 with MATRIGEL®). When tumors are established (approximately 200 mm^3, ~18-21 days after implant), randomize animals, and group them into control and test groups. Formulate the test compound in 1% HEC/0.25% TWEEN®-80/0.05% Antifoam. Administer test compound and vehicles by oral gavage. Determine tumor response by tumor volume measurement (caliper) performed twice a week during the course of treatment and report as the percent inhibition of tumor volume versus the vehicle control group.

Chemistry:

General. Unless otherwise noted, all reagents and solvents were obtained from commercial suppliers and used as obtained. Reactions were monitored using Agilent 1100 or 1200 Series LCMS with ultraviolet light (UV) detection at 215 and 254 nm and a low-resolution electrospray mode (ESI). HRMS data were recorded on an Agilent LCMS TOF (time-of-flight), Model G1969A instrument. The purity was measured using Agilent 1100 or 1200 Series high-performance liquid chromatography (HPLC) with UV detection at 215, 254, and 280 nm (15 min; 1.5 mL/min flow rate), eluting with a binary solvent system A and B using a gradient elution (A, water with 0.1% TFA; B, MeCN with 0.1% TFA). Unless otherwise noted, the purity of all compounds was ≥98%. ^{1}H NMR spectra were recorded ^{1}H NMR spectra were recorded on a Bruker AV-400 (400 MHz) spectrometer at ambient temperature. Chemical shifts were reported in parts per million (δ) and were calibrated using a residual signal from an undeuterated solvent as an internal reference. Data for ^{1}H NMR spectra were reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Multiplicities were reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, or combinations thereof.

(3aS,4S,6R,6aR)-6-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxole-4-carboxylic acid (**4**).
Iodobenzene diacetate (160 g, 496.8 mmol) was added portion wise to an ice-cold mixture of **3** (60.0 g, 184.2 mmol) in acetonitrile (250 mL) and water (180 mL), and was subsequently followed by addition of TEMPO (14 g, 89.6 mmol). The mixture was stirred at room temperature for 1 hr. The solvent was removed under reduced pressure and the crude residue was partitioned between water (100 mL) and EtOAc (300 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic extracts were washed with a 10% aqueous solution of thiosulfate (2x100 mL) and water (2x100 mL). The organic layer was dried over sodium sulfate, and was filtered. The volatiles were removed under reduced pressure to give a pale yellow solid. The crude material was treated with hexanes (400 mL) and stirred at room temperature for 1 hour. The resulting solids were removed by filtration, and were washed with hexanes (100 mL), then dried to give the title compound (66.7 g, 89% purity, 100% crude) as a yellow solid. ES/MS cald for $C_{14}H_{14}ClN_3O_5$ $[M+H]^+$: 339.06, found: 340.00/342.00

((3aS,4S,6R,6aR)-6-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)(morpholino)methanone (**5**).
1,1'-carbonyldiimidazole (37.7 g, 232 mmol) was added in small portions to an ice-cold suspension of **4** (63.7 g, 166 mmol) in CH_2Cl_2 (320 mL). The mixture was allowed to warm to room temperature and stirred for 45 min. Morpholine (21.7g, 249 mmol) was added drop wise, and the mixture was stirred at room temperature for 3 days. The reaction mixture was diluted with CH_2Cl_2 and water (150 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 100mL). The combined organic extracts were washed with water (100 mL) and saturated aqueous sodium chloride (100 mL), and dried over sodium sulfate, filtered. The solvents were removed under reduced pressure to a residue that was purified by silica gel chromatography eluting with a gradient of 20-60% EtOAc/hexanes to give the title compound (34 g, 50% yield) as a brown foam. ES/MS calc for $C_{18}H_{21}ClN_4O_5$ $[M]^+$: 408.12, found: 409.00/411.00.

((3aS,4S,6R,6aR)-6-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)(phenyl)methanone (**6**).
A 3.0 M solution of phenylmagnesium bromide in diethyl ether (1.79 ml, 5.38 mmol) was added dropwise to a solution of **5** (1.00 g, 2.45 mmol) in THF (15 mL) over 2 min at 0 °C. The resulting mixture was stirred at 0 °C for 30 min, and then was quenched by the addition of 1 N aqueous HCl (7.3 mL). After 3 min, the aqueous layer was extracted with CH_2Cl_2 (2x 50mL). The combined organic extracts were washed with brine, and dried over sodium sulfate, and filtered. The volatiles were removed under reduced pressure to a residue that was purified by silica gel chromatography eluting with a gradient of 35-75% of a 10% mixture of MTBE/ CH_2Cl_2 in hexanes over 25 min to give the title compound (893 mg, 91 % yield). ES/MS calc for $C_{20}H_{18}ClN_3O_4$ $[M]^+$: 399.10, found 400.00/402.00

(R)-((3aR,4R,6R,6aR)-6-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)(phenyl)methanol (**7**).
A mixture of **6** (0.866g, 2.17mmol) and (*R*,*R*)-Ts-DENEB™ (70.4 mg, 10.8 mmol) in 5:2 formic acid/triethylamine complex (15 mL) was stirred at room temperature for 4 hours. The mixture was diluted with water (60 mL), and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, and dried over sodium sulfate, and filtered. The volatiles were removed under reduced pressure to a residue that was purified by silica gel chromatography eluting with a gradient of 15-30% acetone in hexanes over 20 min to give the title compound (0.420 g, 48% yield). ES/MS calc for $C_{20}H_{20}ClN_3O_4$ $[M]^+$: 401.11, found: 402.00/404.00

(R)-((3aR,4R,6R,6aR)-6-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)(phenyl)methanol (**8**).
A mixture of **7** (0.399 g, 0.993 mmol) and 7N NH3 in MeOH (15 mL, 105 mmol) were combined in a sealed reaction vessel. The mixture was heated in a Biotage microwave reactor at 100 °C for 8 hr. After cooling to ambient temperature, the solvent was removed under a stream of nitrogen to a residue that was purified by silica gel chromatography eluting with a gradient of 50-100% of a 10% MeOH/MTBE mixture in hexanes over 25 min, to give the title compound (0.295 g, 78% yield). ES/MS calc for $C_{20}H_{22}N_4O_4$ $[M]^+$: 382.16, found: 383.20.

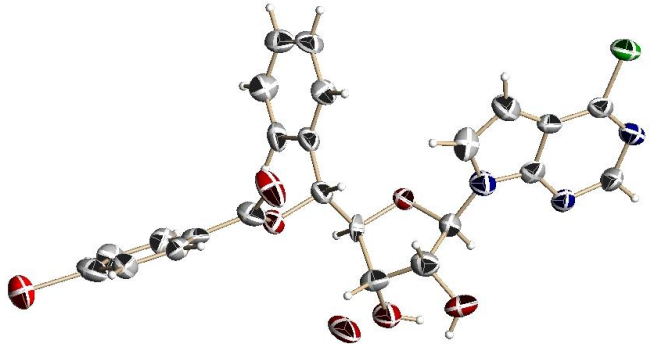
(S)-((3aR,4R,6R,6aR)-6-(4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)(phenyl)methanol (**9**).
Under a N2 atmosphere, Lithium tri-sec-butyl borohydride (1.0 mol/L) in THF (450 µL, 0.45 mmol) was added dropwise to a -78 ˚C solution of **6** (143.0 mg, 0.3577 mmol) in THF (5.1 mL). After stirring for 1 hour, the mixture was quenched by adding saturated NH_4Cl (4 mL). The aqueous was extracted with EtOAc (3x30 mL), and the combined organic layers were washed with saturated NH_4Cl, saturated sodium bicarbonate, then with brine. The organics were dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified by chromatography (5% acetone/hexanes (2 min) then 10% acetone/hexanes (10 min) then 15% acetone/hexanes (15 min) to provide the title compound (86.7mg, 60% yield) as a white powder. ES/MS calc for $C_{20}H_{18}ClN_3O_4$ $[M]^+$: 399.10, found 400.00/402.00.

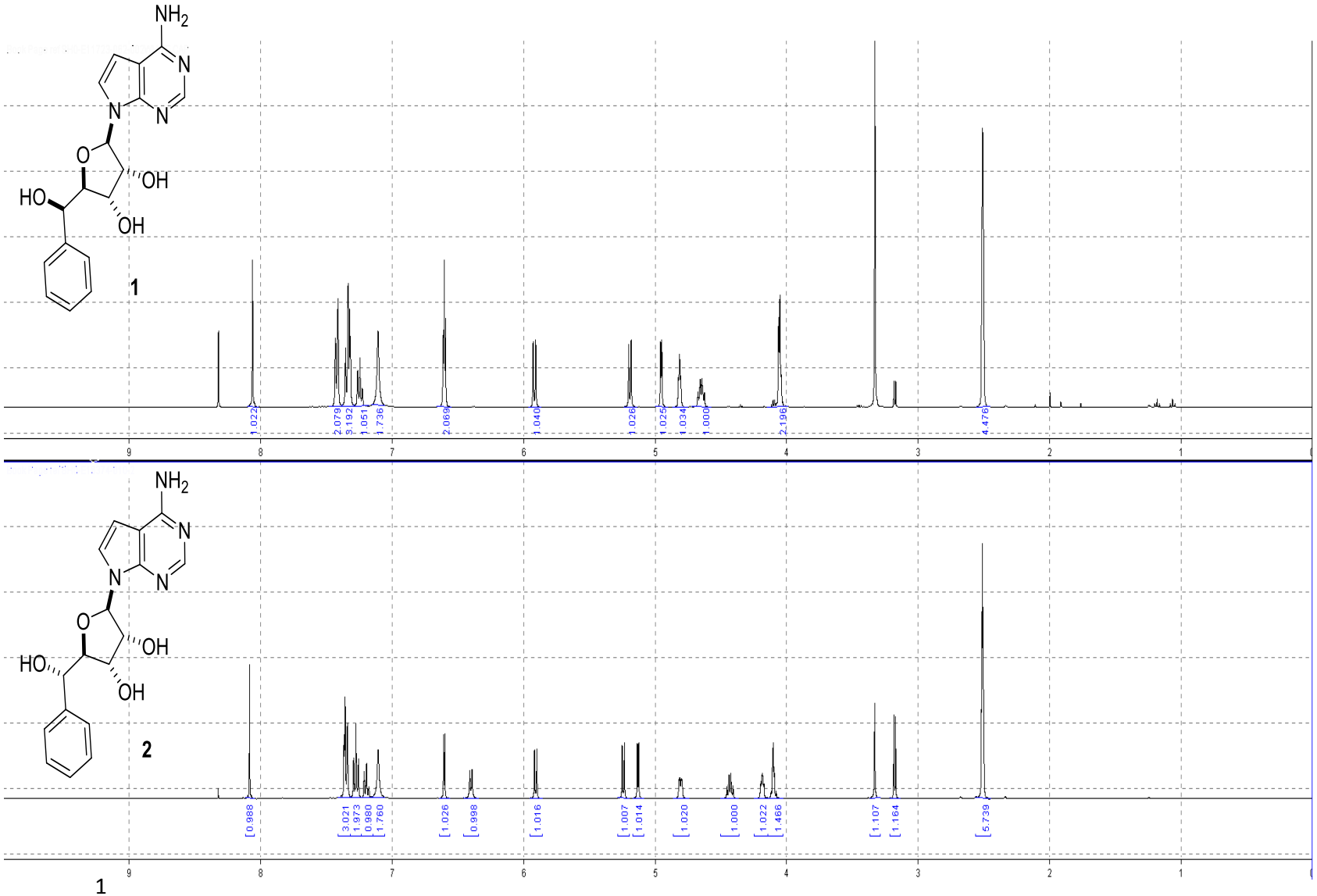
(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((R)-hydroxy(phenyl)methyl)tetrahydrofuran-3,4-diol (**1**).
A solution of **8** (0.293 g,0.766 mmol), 4N HCl in 1,4-dioxane (9.6 mL), and water (3 drops) was stirred at room temperature for 45 min. The mixture was concentrated under reduced pressure to a residue that was purified by silica gel chromatography eluting with a gradient of 50-100% acetone in hexanes over 25 min to give the title compound (0.195 g, 74% yield) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (s, 1H), 7.42 (d, J = 7.3 Hz, 2H), 7.32-7.35 (m, 3H), 7.24 (t, J = 7.2 Hz, 1H), 7.11-7.13 (m, 2H), 6.60 (t, J = 3.4 Hz, 2H), 5.92 (d, J = 7.9 Hz, 1H), 5.19 (d, J = 7.1 Hz, 1H), 4.95 (d, J = 3.9 Hz, 1H), 4.81 (t, J = 3.5 Hz, 1H), 4.04-4.06 (m, 2H), 4.55-4.67 (m, 1H). ES/MS cald for $C_{17}H_{18}N_4O_4$ $[M]^+$: 342.13, found: 343.0; $[\alpha]^D_{25}$ = -138.9° (*c* = 9.9 MeOH).

(2R,3R,4S,5R)-2-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-5-((S)-hydroxy(phenyl)methyl)tetrahydrofuran-3,4-diol (**2**)
A 50 mL Paar glass bottle was charged with **9** (2.50 g, 6.22 mmol), ammonium hydroxide (28 mass%) in H_2O (218 mmol, 30 mL, 35.0 equiv) and 1,4-dioxane (30 mL). The bottle was sealed with a high pressure cap, and the mixture was heated at 90 ˚C for 12 hours. The volatiles were removed under reduced pressure to a residue that was dried under vacuo. Purification by chromatography (220g, silica gel; CH_2Cl_2 (5 min) to 25% B/CH_2Cl_2 (over 20 min); B = 100 mL of 10% 7N NH3 in MeOH (400 mL) and CH_2Cl_2 (500 mL) gave the amine (1.59 g, 66.7% yield) as a light cream foam. ES/MS calc for $C_{20}H_{22}N_4O_4$ $[M]^+$: 382.16, found: 383.20.
A mixture of the amine (1.58 g, 4.13 mmol) in trifluoroacetic acid (207 mmol, 15.8 mL, 50.0 equiv.) and water (1.7 mL) was stirred at rt for 12

hours. The volatiles were removed under reduced pressure to a residue that was redissolved in MeOH (15mL). The pH of the mixture was adjusted to 9-10 with 28% NH_4OH (1.7mL). After stirring for 30 min, the volatiles were removed to a residue that was purified by chromatography (120g, silica gel; CH_2Cl_2 (3 min) to 45% B/CH_2Cl_2 (over 20 min., hold for 20 min); B = 100 mL 7N NH_3 in MeOH/400 mL MeOH/500 mL CH_2Cl_2) to provide **2** (1.01 g, 71.4% yield) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.37-7.34 (m, 3H), 7.28 (t, *J* = 7.5 Hz, 2H), 7.21-7.18 (m, 1H), 7.11 (s, 2H), 6.60 (d, *J* = 3.6 Hz, 1H), 6.40 (d, *J* = 7.1 Hz, 1H), 5.91 (d, *J* = 7.0 Hz, 1H), 5.24 (d, *J* = 6.8 Hz, 1H), 5.13 (d, *J* = 4.3 Hz, 1H), 4.81 (dd, *J* = 2.2, 6.9 Hz, 1H), 4.45-4.41 (m, 1H), 4.18 (td, *J* = 4.6, 1.9 Hz, 1H), 4.12-4.08 (m, 1H), 3.33 (s, 1H), 3.18 (d, *J* = 5.3 Hz, 1H), 2.52-2.50 (m, 6H). ES/MS cald for $C_{17}H_{18}N_4O_4$ $[M]^+$: 342.13, found: 343.0.

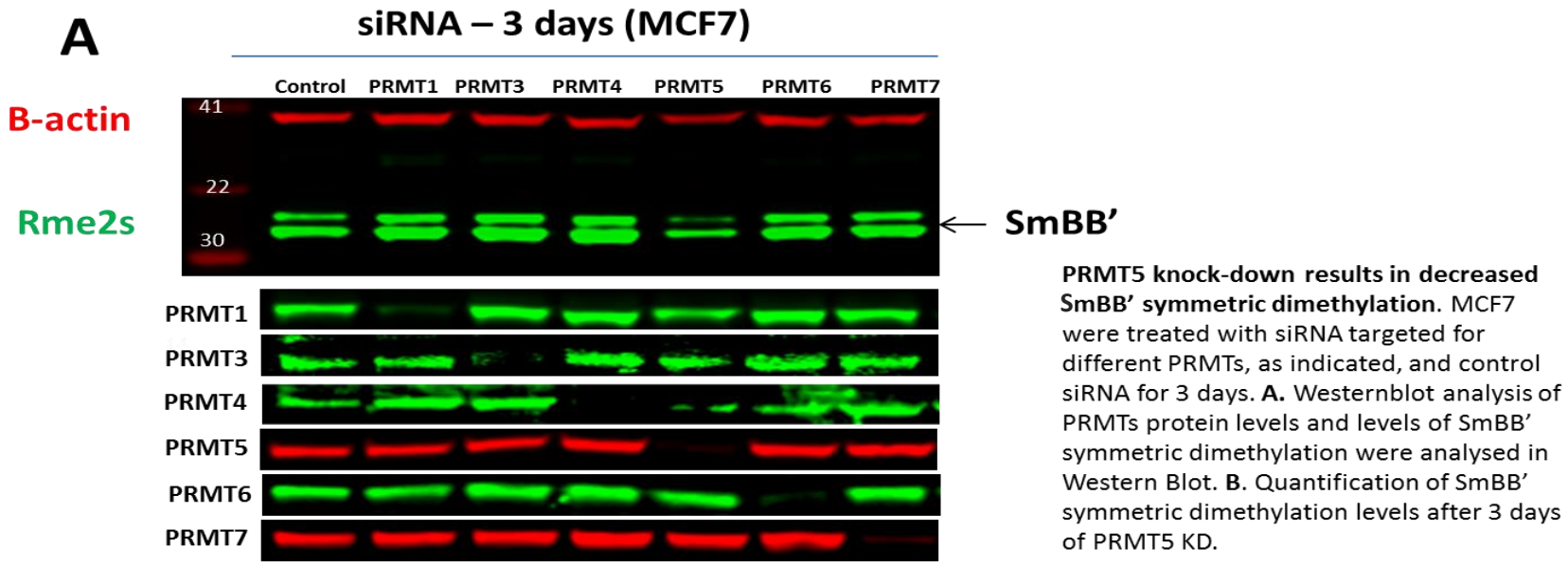
ORTEP for para-bromobenzoate derivative of Compound **1** that established the relative configuration of the 5′-OH by single crystal X-ray crystallography



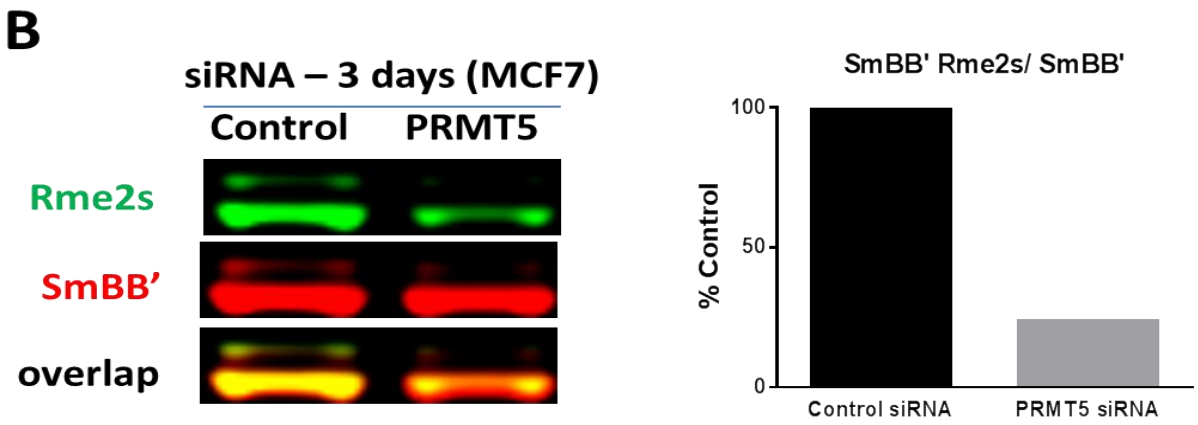


^{1}H NMR Spectra of Compounds **1** and **2**

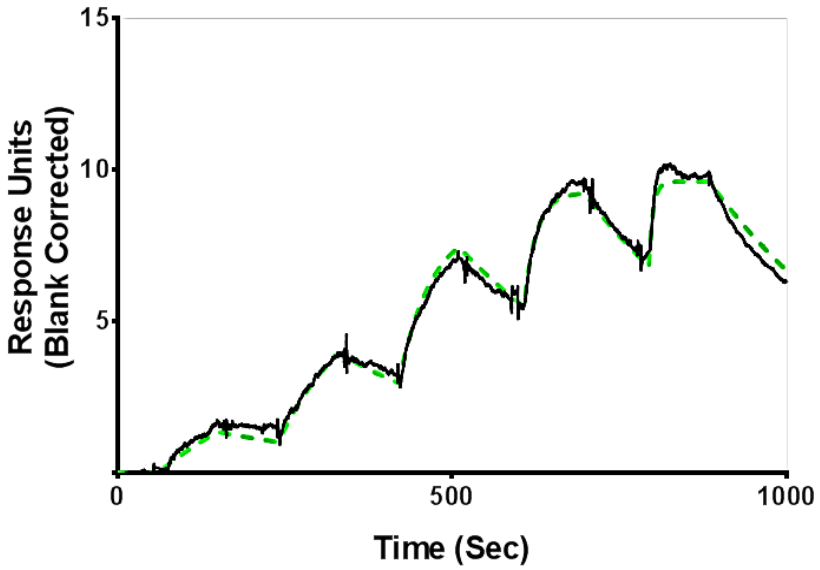
Knockdown Data:

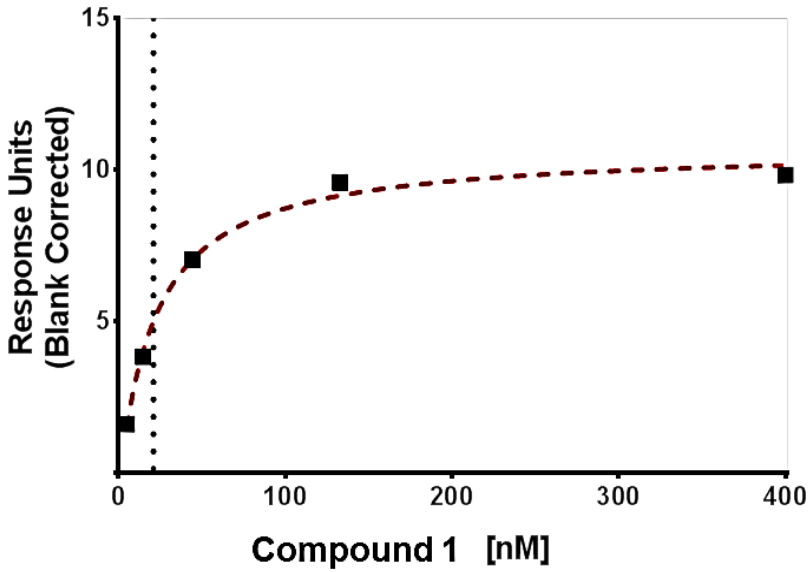


PRMT5 knock-down results in decreased SmBB' symmetric dimethylation. MCF7 were treated with siRNA targeted for different PRMTs, as indicated, and control siRNA for 3 days. **A.** Westernblot analysis of PRMTs protein levels and levels of SmBB' symmetric dimethylation were analysed in Western Blot. **B.** Quantification of SmBB' symmetric dimethylation levels after 3 days of PRMT5 KD.

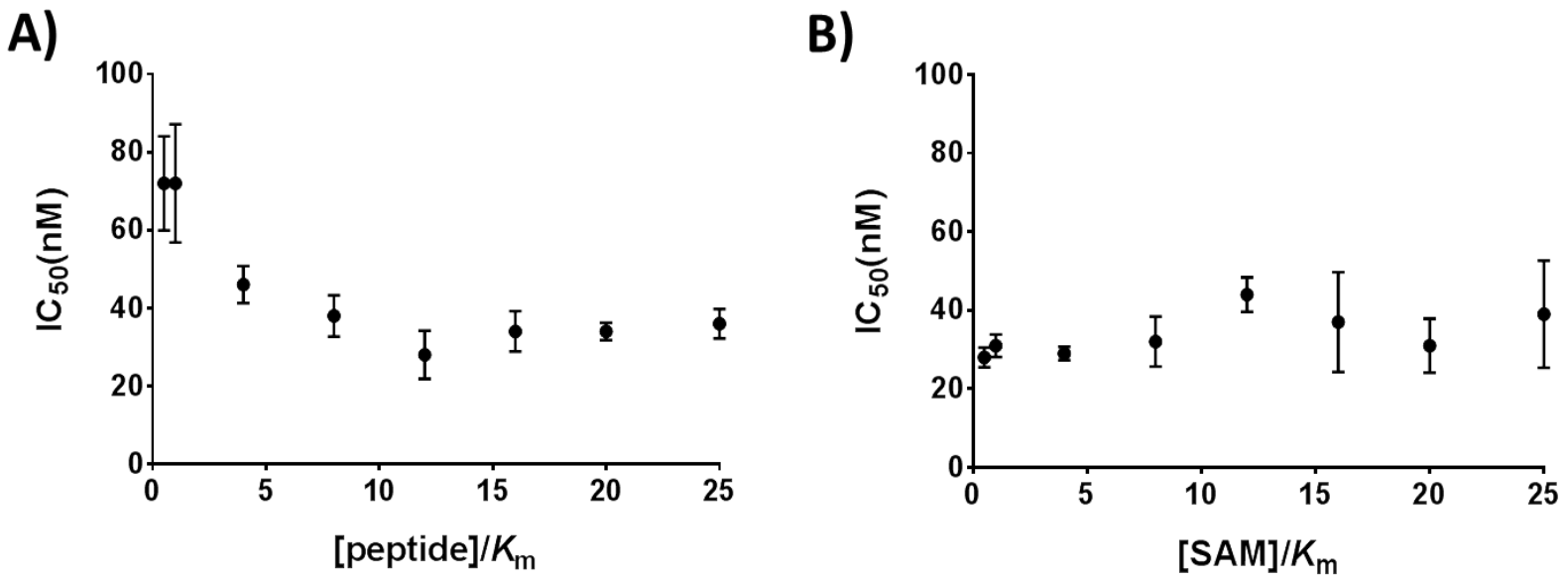


SPR and MOA Data:

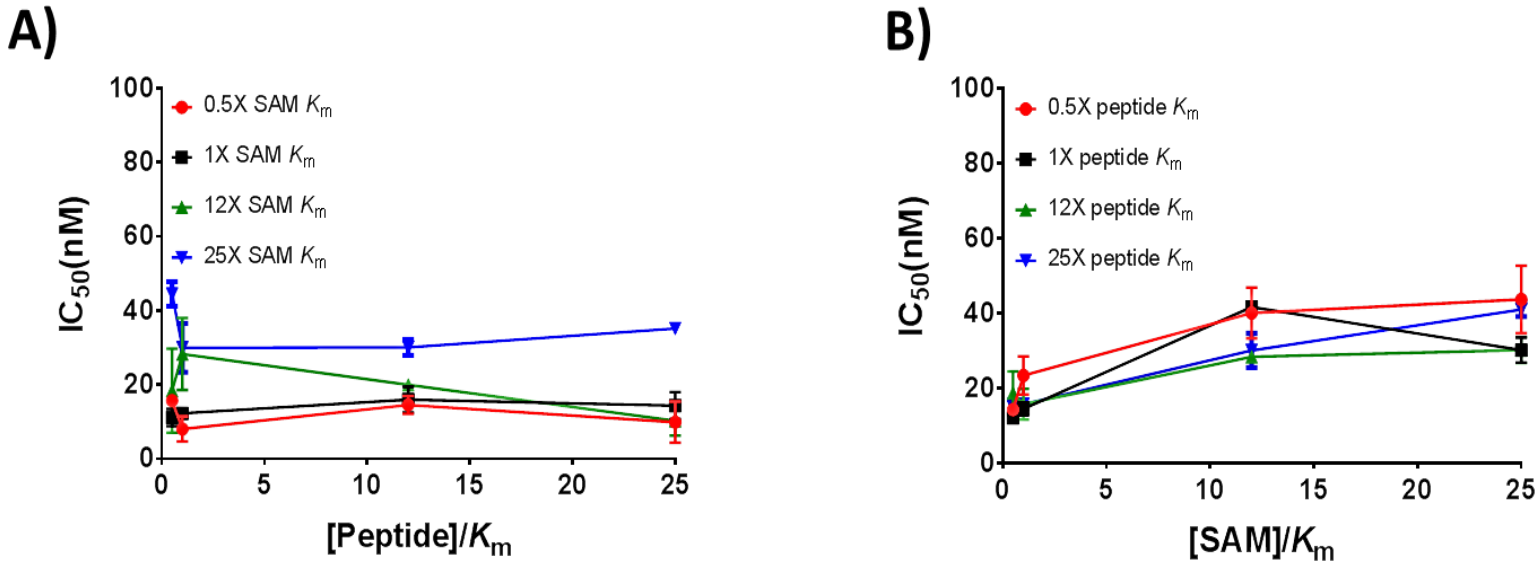




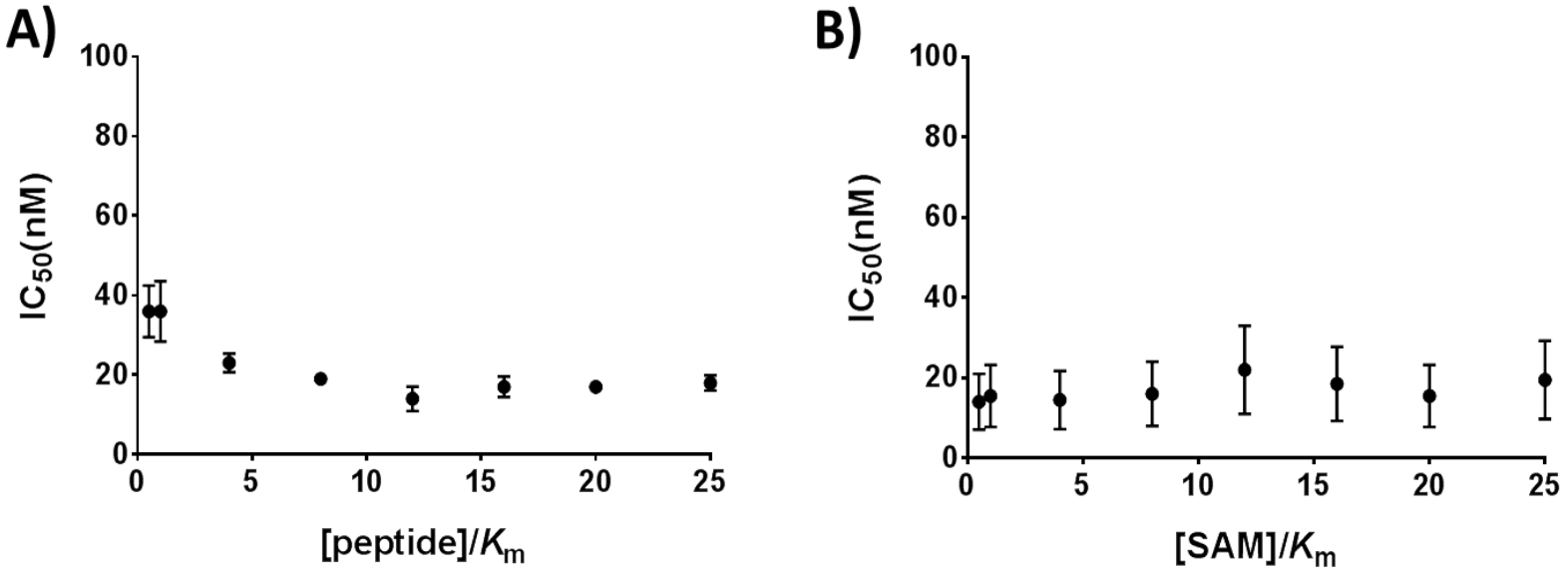
Assessing the binding of compound 1 to PRMT5-MEP50 by Surface Plasmon Resonance (SPR). Binding of compound 1 to PRMT5-MEP50 complex was confirmed by SPR with a K_D value of 6 ± 2 nM (*n*=3), k_{on} of 3.9 ± 0.4 x 10^5 $M^{-1}s^{-1}$ and k_{off} of 2.2 ± 0.8 x 10-3 s^{-1}. Experiments were performed in triplicate in HBS-EP (20 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% Tween-20), 5% DMSO on SA Chip using 90 s on time, 300 s off time and flow rate of 100 µl/min at 20 °C.



Mechanism of action (MOA) of compound 1. MOA of compound 1 was assessed by determining the IC_{50} values in the presence of various concentrations of peptide (A) and SAM (B). No dramatic change in IC_{50} values upon increasing the concentration of each substrate to as high as 25x of the respective K_m values does not support competitive inhibition with respect to either substrate. Experiments were performed in triplicate. SAM concentration was kept at saturation (3.5 µM) and IC_{50} values were determined at various peptide concentrations (0.5, 1, 4, 8, 12, 16, 20, and 25×K_m). To assess possible competition with SAM the peptide concentration was kept at saturation (2 µM) and IC_{50} values were determined at various SAM concentrations (0.5, 1, 4, 8, 12, 16, 20, and 25×K_m). The data supports apparent non-competitive patterns with respect to both substrates.



Mechanism of action (MOA) of compound 1 at various concentrations of second substrate. The competition of compound 1 with peptide (A) and SAM (B) was investigated at various concentrations of SAM and peptide, respectively. Experiments were performed in triplicate. To assess the SAM-dependent peptide competition, SAM concentration was changed to 0.5, 1, 12, and 25 ×K_m (0.3, 0.6, 7.2, and 15 µM) and IC_{50} values were determined at various peptide concentrations (0.5, 1, 12, and 25 ×K_m). To test the possibility of the peptide-dependent SAM-competition, the peptide concentration was varied to 0.5, 1, 12, and 25 ×K_m (0.04, 0.07, 0.84, and 1.1 µM) and IC_{50} values were determined at various SAM concentrations (0.5, 1, 12, and 25 ×K_m). No significant change was observed.



Mechanism of action (MOA) of compound 1. MOA of compound 1 was assessed by determining the IC_{50} values in the presence of various concentrations of peptide (A) and SAM (B). No dramatic change in IC_{50} values upon increasing the concentration of each substrate to as high as 25x of the respective K_m values does not support competitive inhibition with respect to either substrate. Experiments were performed in triplicate. SAM concentration was kept at saturation (3.5 µM) and IC_{50} values were determined at various peptide concentrations (0.5, 1, 4, 8, 12, 16, 20, and 25 ×K_m). To assess possible competition with SAM the peptide concentration was kept at saturation (2 µM) and IC_{50} values were determined at various SAM concentrations (0.5, 1, 4, 8, 12, 16, 20, and 25 ×K_m). The data supports apparent non-competitive patterns with respect to both substrates.

PK and ADME Data:

Compound 1: Summary of ADME and PK Properties

MW	342
HTSA pH7.4 solubility (mg/ml)	0.677
MDCK % A-B Transport	Moderate
CYP2D6, 2C9, 3A4 % Inhibition	3, 5, 3
Human, Dog, Rat, Mouse % Metab	28, 13, 26, 4.5
AUC (ng*hr/ml)	7943
Cmax (ng/ml)	3646
T1/2 (Hours)	3.41
Unbound Cmax (nM)	5754
CL (ml/min/kg)	12.6
Vd,ss (ml/kg)	1819
Fraction Unbound (mouse)	0.54
Bioavail (%F)	50
clogP, clogD	0.8, 0.3

10 mg/kg PO (mouse)

•Plasma clearance was slow at approximately 0.25x hepatic blood flow (Qh,mouse=90ml/min/kg), and is consistent with the metabolic stability.

•Volume of distribution was less than 5 L/kg and is consistent with the physchem properties of the compound.

•Oral bioavailability was 50% suggesting that the compound is absorbed and does not undergo significant first pass metabolism.

•Exposures were similar following IP and PO dosing suggesting that the compound is absorbed and is not metabolized by the gut.

•Exposures increased proportionally with dose following IP and PO administration.

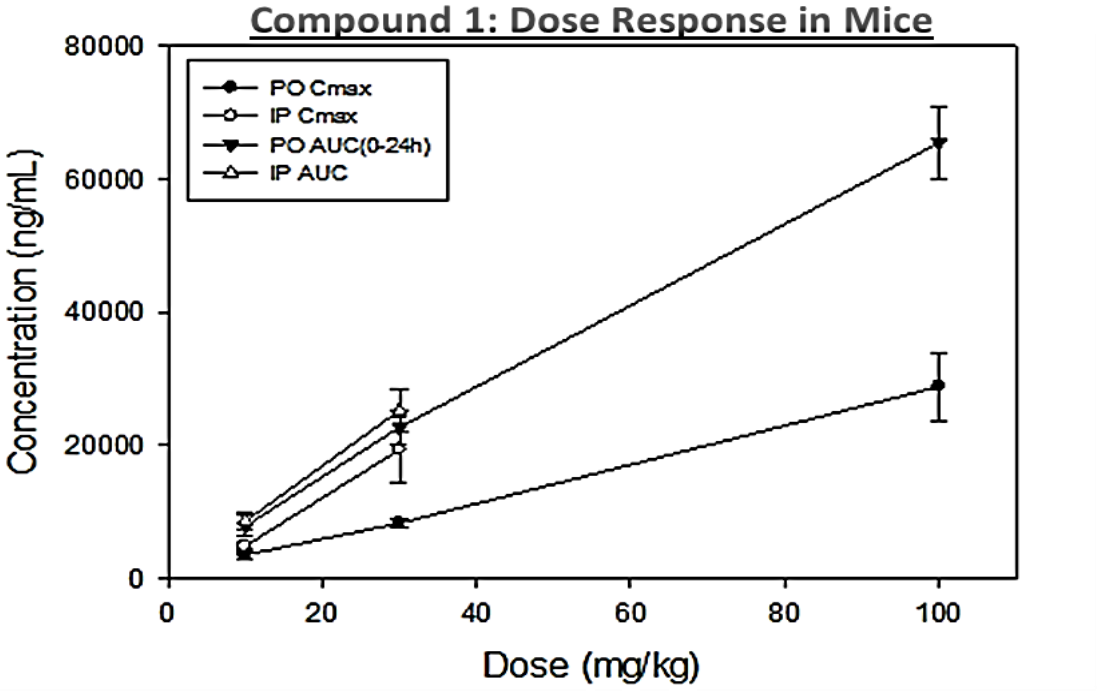


Table S1 Data collection and refinement statistics

	Native
Data collection	
Space group	I222
Cell dimensions	
a, *b*, *c* (Å)	102.79, 139.08, 178.96
α, β, γ (°)	90, 90, 90
Resolution (Å)	2.80 (2.80-2.95)
R_{merge}	0.11 (0.69)
$I / \sigma I$	13.3 (3.1)
Completeness (%)	99.9 (99.9)
Redundancy	7.4 (7.5)
Refinement	
Resolution (Å)	2.8
No. reflections	31922
R_{work} / R_{free}	0.168/0.230
No. atoms	
PRMT5	7267
Compound 1	25
Water	282
B-factors	
PRMT5	73.8
Compound 1	45.5
Water	56.1
R.m.s deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.18

References:

[1] D. Smil, M.S. Eram, F. Li, S. Kennedy, M.M. Szewczyk, P.J. Brown, D. Barsyte-Lovejoy, C.H. Arrowsmith, M. Vedadi, M. Schapira, Discovery of a Dual PRMT5–PRMT7 Inhibitor, ACS Med. Chem. Lett., 6 (2015) 408-412.

[2] D. Barsyte-Lovejoy, F. Li, M.J. Oudhoff, J.H. Tatlock, A. Dong, H. Zeng, H. Wu, S.A. Freeman, M. Schapira, G.A. Senisterra, E. Kuznetsova, R. Marcellus, A. Allali-Hassani, S. Kennedy, J.-P. Lambert, A.L. Couzens, A. Aman, A.-C. Gingras, R. Al-Awar, P.V. Fish, B.S. Gerstenberger, L. Roberts, C.L. Benn, R.L. Grimley,

M.J.S. Braam, F.M.V. Rossi, M. Sudol, P.J. Brown, M.E. Bunnage, D.R. Owen, C. Zaph, M. Vedadi, C.H. Arrowsmith, (R)-PFI-2 is a potent and selective inhibitor of SETD7 methyltransferase activity in cells, Proceedings of the National Academy of Sciences, 111 (2014) 12853-12858.

[3] M.S. Eram, Y. Shen, M.M. Szewczyk, H. Wu, G. Senisterra, F. Li, K.V. Butler, H.Ü. Kaniskan, B.A. Speed, C. dela Seña, A. Dong, H. Zeng, M. Schapira, P.J. Brown, C.H. Arrowsmith, D. Barsyte-Lovejoy, J. Liu, M. Vedadi, J. Jin, A Potent, Selective, and Cell-Active Inhibitor of Human Type I Protein Arginine Methyltransferases, ACS Chem. Biol., 11 (2016) 772-781.

[4] M.S. Eram, S.P. Bustos, E. Lima-Fernandes, A. Siarheyeva, G. Senisterra, T. Hajian, I. Chau, S. Duan, H. Wu, L. Dombrovski, M. Schapira, C. Arrowsmith, M. Vedadi, Trimethylation of Histone H3 Lysine 36 by Human Methyltransferase PRDM9 Protein, J. Biol. Chem., 289 (2014) 12177-12188.