Supporting information

Abbreviations

Ahx, aminohexanoic acid; BSG, bovine skin gelatin; CL, clearance; DMSO, dimethylsulfoxide; DTT, dithiothreitol; EDTA, (ethylenedinitrilo)tetraacetic acid; GST, glutathione S-transferase; IPTG, isopropylthio-β-galactoside; i.v., intraveneous; Ni-NTA, nickel-nitrilotriacetic acid; PAMPA, parallel artificial membrane permeation assay; PK, pharmacokinetic; PRMT, protein arginine methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; s.c., subcutaneous; TCEP, Tris(2-carboxyethyl)phosphine hydrochloride solution; Tris, Tris(hydroxymethyl)aminomethane; V_{ss}, volume of distribution at steady state**.**

ADME/PK methods

Plasma protein binding. Plasma was used from mixed sex human and pooled male Sprague Dawley rat. Solutions of EPZ020411 (1 µM, 0.5% final DMSO concentration) were prepared in buffer (pH 7.4) and 100% species specific plasma. The experiment was performed using equilibrium dialysis with the two compartments separated by a semi-permeable membrane. The buffer solution was added to one side of the membrane and the plasma solution to the other side. After equilibration, samples were taken from both sides of the membrane. Standards were prepared in plasma and buffer and were incubated at 37°C. Test compound incubations were performed in duplicate. Haloperidol was included in each experiment as control. The protein free and protein containing samples were analyzed by LC-MS/MS using two sets of calibration standards for protein free (7 points) and protein containing solutions (6 points). Samples were quantified using standard curves prepared in the equivalent matrix. The fraction unbound in plasma (f_u) was calculated using the following equation:

$$f_u^{corrected} = \frac{PF}{\{(PC - PF) \times V_{Correction}\} + PF}$$

Where, PC = sample concentration in protein containing side

PF = sample concentration in protein free side

$V_{Correction}$ = correction factor for the volume shift i.e. ratio of the volume of the protein after dialysis to that before dialysis.

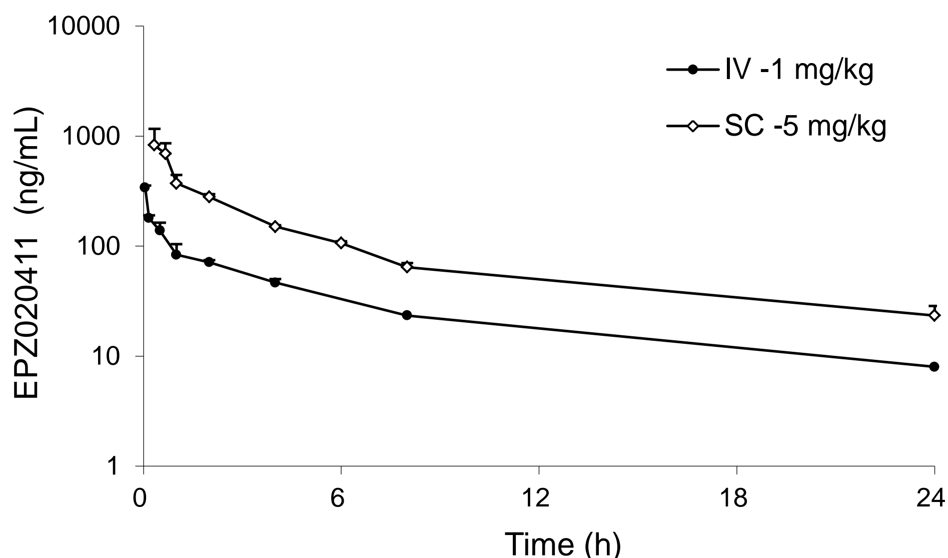
Parallel artificial membrane permeation assay (PAMPA). A solution of hexadecane in hexane was prepared (5% v/v) and added onto the membrane of each well in the filter (donor) plate (Multiscreen filter plate for permeability, Millipore). The donor plates were then allowed to dry to ensure evaporation of hexane. Buffer (pH 7.4) containing DMSO (5%) was added to each well of the acceptor plates.EPZ020411 at 10 µM (5% DMSO) and lucifer yellow (fluorescent integrity marker) were added to the donor plate prior to incubation at room temperature, in a humid environment, for 5 hours. Test compound permeability was assessed in quadruplicate. Desipramine and guanabenz were also run as controls. At the end of the incubation period the donor and acceptor samples were quantified by LC-MS/MS analysis using a 5-point calibration with appropriate dilution of the samples. The apparent permeability coefficient for each compound (P_{app}) was calculated from the following equation:

P_{app}=C*-ln(l-$[drug]_{acceptor}$/$[drug]_{equilibrium}$)

Where, C = V_D*V_A / (V_D +V_A)Area*time

Where, V_D and V_A are the volumes of the donor and acceptor compartments, respectively, area is surface area of the membrane multiplied by the porosity and the equilibrium drug concentration is the concentration of test compound in the total volume of the donor and acceptor compartments.

Rat pharmacokinetic studies. Male Sprague-Dawley rats (200-230 g; Vital River Laboratory Animal Technology Co., Ltd. n=3 per group) were treated with a single dose of EPZ020411 at 1 mg/kg by intravenous tail vein injection and 5 mg/kg by subcutaneous administration, both formulated in saline, pH 6.5. Animals were fasted overnight and weighed prior to dose administration on the day of dosing. The study was performed in accordance with the AAALAC International guidelines standards. Approximately 300 µL of blood were taken from animals via a jugular vein cannula at pre-specified time intervals (9 time-points) for up to 24 hours post-dose. Blood samples were transferred into K_2-EDTA tubes and stored at -70 ± 10°C prior to protein precipitation and LC-MS/MS analysis. Standard calibration curves were constructed by analyzing a series of control plasma aliquots containing 100 ng/mL omeprazole as internal standard and 2.0 to 1000 ng/mL EPZ020411. Three levels of quality controls were also included in the analysis (3.0 to 800 ng/mL). The concentration of EPZ020411 in each unknown sample was determined by solving the linear calibration curve equation for each corresponding drug/internal standard ratio. Data were analyzed using non-compartmental methods (Phoenix WinNonlin 6.3.0).



Supplementary Figure 1. Mean total blood concentration-time profiles of EPZ020411 after an IV dose of 1 mg/kg and an SC dose of 5 mg/kg in male Sprague-Dawley rats, n=3, mean ± SD.

Cell Culture and Transfection.

A375 (CRL-1619) cells were obtained from ATCC. Cells were cultured in DMEM plus 10% (vol/vol) FBS. PRMT6 (NM_018137, amino acids 2-375) was cloned into BamHI and EcoRI sites of a pcDNA4 HisMAX_A plasmid. Transfection of his-tagged PRMT6 or vector control was carried out using Lipofectamine LTX and Plus reagent (Invitrogen) according to procedures recommended by the manufacturer. Cells were seeded at 200,000 cells/well in 6-well plates. The following day, the cells were concurrently transfected and treated with compound in 0.25% DMSO. Cells were incubated in the presence of increasing concentrations of compound up to 20 µM. Cell pellets were collected after 48 hours of compound treatment.

Cell lysis and Histone Extraction

For isolation of histones from A375 cells, 1-2 x 10^6 cells were harvested at the appropriate time point by centrifugation at 200 x g and lysed by a 5 minute incubation on ice in 250 µl nuclear extraction buffer (10 mM Tris-HCl, 10 mM MgCl2, 25 mM KCl, 1% Triton X-100, 8.6% Sucrose, plus a Roche protease inhibitor tablet 1836145). Nuclei were collected by centrifugation at 600 x g for 5 minutes at 4° C and washed once in TE buffer (pH 7.4). Supernatant was removed and histones extracted for one hour with 0.4 N cold sulfuric acid. Extracts were clarified by centrifugation at 10000 g for 10 minutes at 4°C and transferred to a fresh microcentrifuge tube containing 10X volume of ice cold acetone. Histones were precipitated at -20° C for 2 hours, pelleted by centrifugation at 10000 x g for 10 minutes and resuspended in 75 µl water. Histones were quantified using the BCA protein assay (Pierce 23225).

Western Blot Analysis

Whole cell lysates or histones were separated on 4%–20% Tris-Glycine gels (Invitrogen), transferred to 0.2 µM nitrocellulose membranes and probed with the appropriate primary antibodies blocking buffer. Following primary antibody incubation, membranes were probed with IRDye 800CW Donkey-anti-mouse IgG (Li-COR 926-32212) or Alexa Fluor 680 goat anti-rabbit IgG (Invitrogen A-21076) secondary antibodies and signal was detected using the Li-COR Odyssey Infrared system.

Antibodies

The following primary antibodies were used: mouse anti-Histone H3 (CST 3638), mouse anti-Histone H4 (CST 2935), rabbit anti-H3R2me2a (Millipore 05-808), rabbit anti-PRMT6 (AbCam 47244), rabbit anti-R*GGme1 (CST 8015), and mouse anti-GAPDH (Millipore MAB374).

Biochemical assay conditions

Reagents

S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), bicine, Tween-20, dimethylsulfoxide (DMSO), bovine skin gelatin (BSG), and Tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP) were purchased from Sigma-Aldrich at the highest level of purity. ^{3}H-

SAM was purchased from American Radiolabeled Chemicals (St Louis) with a specific activity of 80 Ci/mmol. Streptavidin-coated Flashplates (384-well) were purchased from PerkinElmer. Peptide substrates, based on the amino acid sequence of histone H4, were synthesized by 21st Century Biochemicals (Marlborough, MA) with an N-terminal biotin tag and a C-terminal amide cap and confirmed by LC-MS (PRMT1: Biotin-Ahx-RLARRGGVKRISGLI-NH_2, PRMT6: Biotin-Ahx-RLARRGGVKMe1RISGLI-NH_2, PRMT8: Biotin-Ahx-KPAIRRLARRGGVKR-NH_2, where Ahx is a aminohexanoic acid and KMe1 is a monomethylated lysine).

PRMT expression and purification

Full-length human PRMT1 (NM_001536.5) transcript clone was amplified from an HEK 293 cDNA library, incorporating flanking 5' sequence encoding a FLAG tag (DYKDDDDK). The amplified gene was subcloned into pFastBacI (Life Technologies) modified to encode an N-terminal GST tag and a TEV cleavage sequence. Recombinant baculovirus were generated according to Bac-to-Bac kit instructions (Life Technologies). Protein overexpression was accomplished by infecting exponentially growing High Five insect cell culture at $1.5X10^6$ cells/mL with 1:100 ratio of virus. Infections were carried out at 27°C for 48 hours, harvested by centrifugation, and stored at -80°C for purification. Expressed full-length human GST-tagged PRMT1 protein was purified from cell paste by glutathione sepharose affinity chromatography after equilibration of the resin with 50 mM phosphate buffer, 200 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol, pH 7.8 (Buffer A). GST-tagged PRMT1 was eluted with 50 mM Tris, 2 mM glutathione, pH 7.8, dialyzed in buffer A and concentrated to 1 mg/mL.

Full-length human PRMT6 (NM_018137.2) transcript clone was amplified from an HEK 293 cDNA library, incorporating a flanking 5' sequence encoding a FLAG tag (MDYKDDDDK) and a 3' sequence encoding a hexa His sequence (HHHHHH). The amplified gene was subcloned into pFastBacMam and recombinant baculovirus were generated according to Bac-to-Bac kit instructions. Protein overexpression was accomplished by infecting exponentially growing HEK 293F cell culture at $1.3X10^6$ cells/mL with virus in the presence of 8 mM sodium butyrate. Infections were carried out at 37°C for 48 hours, harvested by centrifugation, and stored at -80°C for purification. Expressed full-length human Flag- and His-tagged PRMT6 protein was purified from cell paste by Ni-NTA agarose affinity chromatography after equilibrating the resin with buffer containing 50 mM Tris, 300 mM NaCl, 10% glycerol, pH 7.8 (Buffer E). The column was washed with 20 mM imidazole in the same buffer and Flag-PRMT6-His was eluted with 150 mM imidazole. Pooled fractions were dialyzed against buffer E and further purified by anti-flag M2 affinity chromatography. Flag-PRMT6-His was eluted with 200 μg/mL FLAG peptide in buffer E. Pooled fractions were dialyzed in 20 mM Tris, 150 mM NaCl, 10% glycerol and 5 mM β-mercaptoethanol, pH 7.8.

Full-length human PRMT8 (NM_019854.4) transcript clone was amplified from an HEK 293 cDNA library and subcloned into pGEX-4T-1 (GE Life Sciences) adding an N-terminal GST tag and a thrombin cleavage sequences. PRMT8 was overexpressed in *E. coli* (BL21(DE3) Gold, Stratagene) by IPTG induction (0.3 mM) at 16°C. The culture was grown for 12 hours, harvested by centrifugation, and stored at -80°C for purification. Expressed full-length human GST-tagged PRMT8 protein was purified from cell paste by glutathione sepharose affinity chromatography after equilibrating the resin with Buffer A. GST-tagged PRMT8 was eluted with 50 mM Tris, 2 mM glutathione, pH 7.8. Pooled fractions were cleaved by thrombin (10U) and dialyzed in buffer A. GST was removed by reloading the cleaved protein sample onto glutathione sepharose column and PRMT8 was collected in the flow-through fractions. PRMT8 was further purified by ceramic hydroxyapatite chromatography. The column was washed with 50 mM phosphate buffer, 100 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol, pH 7.8 and PRMT8 was eluted by 100 mM phosphate in the same buffer. Protein was concentrated and buffer was exchanged to 50 mM Tris, 300 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol, pH 7.8 by ultrafiltration.

PRMT Biochemical Assays

All assays were performed in a buffer containing 20 mM bicine, 1 mM TCEP, 0.005% BSG and 0.002% Tween-20, pH 7.6. Compounds were allowed to incubate with the PRMT enzyme for 30 minutes at room temperature in the 384-well polypropylene plate and reactions were initiated by the addition of a SAM and peptide mix. Final assay conditions were as follows: PRMT1 assays contained 0.75 nM PRMT1, 200 nM ^{3}H-SAM , 1.5 μM fresh, unlabeled SAM, and 20 nM peptide. PRMT6 assays contained 1 nM PRMT6, 200 nM ^{3}H-SAM, 250 nM fresh, unlabeled SAM, and 75 nM peptide. PRMT8 assays contained 1.5 nM, 100 nM ^{3}H-SAM, 500 nM fresh, unlabeled SAM, and 60 nM peptide. SAH was used for the 100% inhibition controls at 1 mM and the final DMSO concentration in all wells was 2%. The assays were stopped by the addition of unlabeled SAM to a final concentration of 400 μM, which dilutes the ^{3}H-SAM to a level where its incorporation into the peptide substrate is no longer detectable. Terminated reactions were transferred to a 384-well Flashplate and the biotinylated peptides were allowed to bind for at least 1 hour before the plate was washed once with 0.1% Tween-20 on a Biotek ELx405 plate washer. The plates were then read in a PerkinElmer TopCount plate reader to measure the quantity of ^{3}H-labeled peptide bound to the Flashplate surface.

Crystallography methods

Purified NΔPRMT6 (4mg/mL in 25mM Tris pH7.8, 200mM NaCl, 1mM TCEP) was incubated with 1mM SAM and 1 mM ligand (final DMSO concentration of 2%) and was used in protein crystallization. 1 μL protein was added to 1 μL well solution containing 0.05 M MgCl, 0.1 M MES pH 6.5, 10% v/v Isopropanol, 5% w/v PEG 4000 and was equilibrated via vapor diffusion with the well solution at 18°C. 100 μL paraffin oil was added to the well reservoir to slow vapor diffusion and improve crystal quality. Crystals were cryoprotected in a solution containing 80% mother liquor and 20% glycerol prior to vitrification in liquid nitrogen. Data reduction and scaling was performed using HKL2000. Structure determination was performed byrigid body refinement using a previously solved structure of NΔPRMT6 (data not shown) utilizing the CCP4 software package. After manual ligand placement, iterative cycles of refinement and

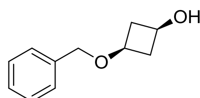
model building were performed using REFMAC5 and COOT, respectively. Data collection and refinement statistics are shown in Supplementary Table 1.

Supplementary Table 1. Crystallography data collection and refinement statistics (numbers in parentheses correspond to last shell statistics)

	Compound 1	**EPZ020411**
Beam line	SSRF BL17U	SSRF BL17U
Space group	P 43	P 43
Unit cella,b,c (**Å**) α,β,γ (°)	99.97 99.97 89.49 90.0 90.0 90.0	99.67 99.67 89.25 90.0 90.0 90.0
Resolution(**Å**)	27.74-2.37 (2.43-2.37)	50.00 – 2.10 (2.18 – 2.10)
R_{merge}	0.126 (0.513)	0.239 (0.846)
Completeness (%)	99.9 (99.8)	99.8 (99.5)
# reflections collected/unique	175172/35875	480862/51056
I/σ	10.5 (2.2)	8.3 (2.5)
R_{factor}/R_{free}	0.181/0.231	0.180/0.221
RMSD bonds (**Å**)	0.010	0.010
RMSD angles (°)	1.444	1.386
PDBID	4Y2H	4Y30

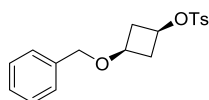
Synthesis of N1,N2-dimethyl-N1-((3-(4-(3-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)cyclobutoxy)phenyl)-1H-pyrazol-4-yl)methyl)ethane-1,2-diamine bis(2,2,2-trifluoroacetate), EPZ020411

Step 1: 3-(benzyloxy)cyclobutanol



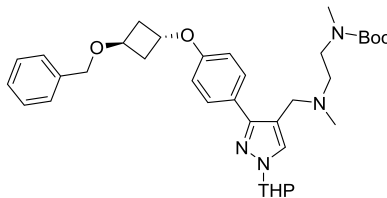
Into a 100-mL round-bottom flask, was placed 3-(benzyloxy)cyclobutan-1-one (7 g, 39.72 mmol, 1.00 equiv), methanol (50 mL). Then the mixture was cooled to 0 degree C and $NaBH_4$ (2.3 g, 62.46 mmol, 1.57 equiv) was added in batches over 10 mins. The resulting solution was stirred overnight at room temperature. The reaction was then quenched by the addition of 50 mL of NH_4Cl (sat. aq.). The resulting mixture was concentrated under vacuum. The resulting solution was extracted with ethyl acetate (50 mL x 5). The organic phase was washed with 3x50 mL of brine (sat.), and then it was collected and dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 6.9 g (97%) of 3-(benzyloxy)cyclobutan-1-ol as light yellow oil.

Step 2: 3-(benzyloxy)cyclobutyl 4-methylbenzenesulfonate



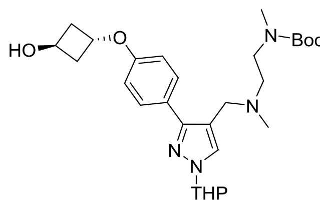
Into a 100-mL round-bottom flask, was placed 3-(benzyloxy)cyclobutan-1-ol (6.5 g, 36.47 mmol, 1.00 equiv), dichloromethane (50 mL), triethylamine (25 mL). Cooled to 0 degree C, this was followed by the addition of a solution of 4-methylbenzene-1-sulfonyl chloride (13.8 g, 72.39 mmol, 1.98 equiv) in dichloromethane (10 mL) by dropwise with stirring over 30 mins. The resulting solution was stirred overnight at room temperature. The resulting solution was diluted with 30 mL of CH_2Cl_2. The resulting mixture was washed with 3x30 mL of brine (sat.). The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0%-10%). The collected fractions were combined and concentrated under vacuum. This resulted in 10.5 g (87%) of 3-(benzyloxy)cyclobutyl 4-methylbenzene-1-sulfonate as a yellow solid.

Step 3: Tert-butyl 2-(((3-(4-(3-(benzyloxy)cyclobutoxy)phenyl)-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-4-yl)methyl)(methyl)amino)ethyl(methyl)carbamate



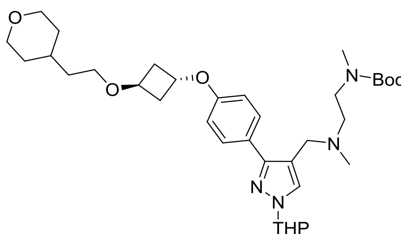
Into a 100-mL round-bottom flask, was placed tert-butyl N-[2-([3-(4-hydroxyphenyl)-1-(oxan-2-yl)-1H-pyrazol-4-yl]methyl(methyl)amino)ethyl]-N-methylcarbamate (9 g, 20.24 mmol, 1.00 equiv), 3-(benzyloxy)cyclobutyl 4-methylbenzene-1-sulfonate (8.1 g, 24.37 mmol, 1.20 equiv), Cs_2CO_3 (20 g, 61.19 mmol, 3.02 equiv) and N,N-dimethylformamide (100 mL). The resulting solution was stirred for 3 h at 100°C in an oil bath. The reaction was then quenched by the addition of 50 mL of water. The resulting solution was extracted with ethyl acetate (50 mL x 3). The resulting mixture was washed with 3x50 mL of brine (sat.). The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0%-20%). The collected fractions were combined and concentrated under vacuum. This resulted in 10.5 g (86%) of tert-butyl N-(2-[[(3-[4-[3-(benzyloxy)cyclobutoxy]phenyl]-1-(oxan-2-yl)-1H-pyrazol-4-yl)methyl](methyl)amino]ethyl)-N-methylcarbamate as yellow oil. LCMS (Method A, ESI): RT= 1.43 min, *m/z* =605.4 $[M+H]^+$.

Step 4: Tert-butyl 2-(((3-(4-(3-hydroxycyclobutoxy)phenyl)-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-4-yl)methyl)(methyl)amino)ethyl(methyl)carbamate



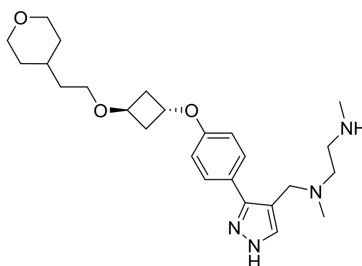
Into a 1-L round-bottom flask, was placed tert-butyl N-(2-[[(3-[4-[3-(benzyloxy)cyclobutoxy]phenyl]-1-(oxan-2-yl)-1H-pyrazol-4-yl)methyl](methyl)amino]ethyl)-N-methylcarbamate (3 g, 4.96 mmol, 1.00 equiv), THF (500 mL), 10% Palladium carbon (3 g) and hydrochloric acid (12N, 0.7 mL). Then hydrogen (gas) was introduced into mixture and maintained at 2 atm. The resulting solution was stirred for 4 h at room temperature. The solids were filtered out. The pH value of the solution was adjusted to 8 with K_2CO_3 (sat. aq.). The resulting solution concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0%-75%). The collected fractions were combined and concentrated under vacuum. This resulted in 2.13 g (83%) of tert-butyl N-[2-[([3-[4-(3-hydroxycyclobutoxy)phenyl]-1-(oxan-2-yl)-1H-pyrazol-4-yl]methyl)(methyl)amino]ethyl]-N-methylcarbamate as light yellow oil. LCMS (Method B, ESI): RT = 0.99 min, *m/z* =515.4 $[M+H]^+$

Step 5: Tert-butyl methyl(2-(methyl((1-(tetrahydro-2H-pyran-2-yl)-3-(4-(3-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)cyclobutoxy)phenyl)-1H-pyrazol-4-yl)methyl)amino)ethyl)carbamate



Into a 50-mL 3-necked round-bottom flask, was placed tert-butyl N-[2-[([3-[4-(3-hydroxycyclobutoxy)phenyl]-1-(oxan-2-yl)-1H-pyrazol-4-yl]methyl)(methyl)amino]ethyl]-N-methylcarbamate (500 mg, 0.97 mmol, 1.00 equiv),N,N-dimethylformamide (10 mL). The temperature was cooled to 0°C. To this was added sodium hydride (120 mg, 5.00 mmol, 5.15 equiv, 60% in mineral oil) in batches. The mixture was stirred for 1 h at R.T. Then to the mixture was added 4-(2-bromoethyl)oxane (470 mg, 2.43 mmol, 2.51 equiv). The resulting solution was stirred overnight at room temperature. The reaction was then quenched by the addition of 30 mL of water. The resulting solution was extracted with 3x30 mL of ethyl acetate. The resulting mixture was washed with 3x30 mL of brine. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0%-60%). The collected fractions were combined and concentrated under vacuum. This resulted in 450 mg (74%) of tert-butyl N-methyl-N-[2-[methyl([[1-(oxan-2-yl)-3-(4-[3-[2-(oxan-4-yl)ethoxy]cyclobutoxy]phenyl)-1H-pyrazol-4-yl]methyl])amino]ethyl]carbamate as yellow oil. LCMS (Method A, ESI): RT = 1.37min, *m/z* =627.4 [M+H].

Step 6: N1,N2-dimethyl-N1-((3-(4-(3-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)cyclobutoxy)phenyl)-1H-pyrazol-4-yl)methyl)ethane-1,2-diamine bis(2,2,2-trifluoroacetate), EPZ020411



Into a 50-mL round-bottom flask, was placed tert-butyl N-methyl-N-[2-[methyl([[1-(oxan-2-yl)-3-(4-[3-[2-(oxan-4-yl)ethoxy]cyclobutoxy]phenyl)-1H-pyrazol-4-yl]methyl])amino]ethyl]carbamate (450 mg, 0.72 mmol, 1.00 equiv), trifluoroacetic acid (3 mL), dichloromethane (3 mL). The resulting solution was stirred for 2 h at room temperature. The resulting mixture was concentrated under vacuum. The crude product was purified by Prep-HPLC with the following conditions (1#-Pre-HPLC-005(Waters)): Column, Atlantis Prep OBD T3 Column,19*150mm,5um,; mobile phase, water with 0.05% TFA and CH_3CN (up to 3.0% in 10 min, up to 100.0% in 1 min, hold 100.0% in 1 min); Detector, UV 254 nm. This resulted in 198.8 mg (41%) of methyl[2-(methylamino)ethyl][[3-(4-[3-[2-(oxan-4-yl)ethoxy]cyclobutoxy]phenyl)-1H-pyrazol-4-yl]methyl]amine bis(trifluoroacetic acid) as light yellow oil. ^{1}H-NMR (300 MHz, D_2O): δ 7.89(s, 1H), 7.43(d, *J*=4.5Hz, 2H), 6.98(d, *J*=4.4Hz, 2H), 4.94-4.87(m, 1H), 4.42(s, 2H), 4.31-4.23(m, 1H), 3.90-3.85(m, 2H), 3.47-3.34(m, 4H), 3.20(s, 4H), 2.61(s, 3H), 2.56(s, 3H), 2.48-2.37(m, 4H), 1.65-1.57(m, 3H), 1.50-1.44(m, 2H), 1.29-1.14(m, 2H) *ppm*. LCMS (Method A, ESI): RT = 1.23min, *m/z* =443.3$[M+H]^+$.

LCMS Method A

<u>**Instrument Information**</u>: SHIMADZU LCMS-2010EV
<u>**Analysis Conditions**</u>:

LC Parameters:
Column: Shim-pack XR-ODS 2.2um 3.0*50mm
Mobile Phase A: Water/0.05% TFA
Mobile Phase B: Acetonitrile/0.05% TFA
Gradient: 5% to 100% B in 2.0 minutes, 100% B for 1.1 minutes, 100% to 5% B in 0.2 minutes, then stop.
Flow Rate: 1.0mL/min
Column Temperature: 40°C
Detector: 254nm and ELSD
Sample Preparation: 1 mg/mL in methanol
Injection Volume: 1 µL
Report: Area Normalized Purity

MS Parameters:

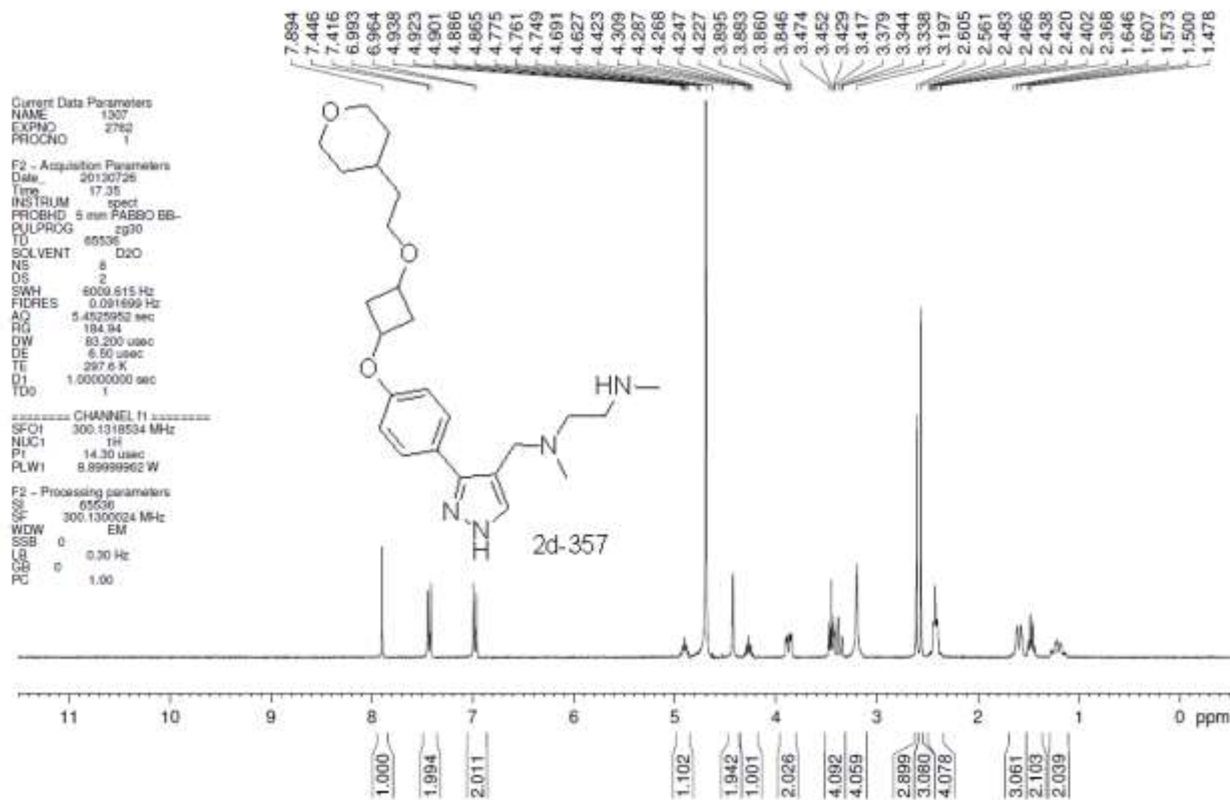
Interface: ESI (Positive)
Interface Voltage: 4.5kv
Heat Block: 250°C
Nebulizing Gas: 1.50 L/min
Scan Range: 90-900 (m/z)
Detector voltage: 1.5kv

LCMS Method B

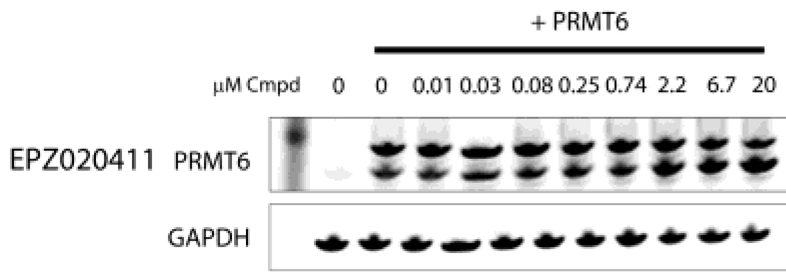
Instrument Information: SHIMADZU UPLCMS-2020EV
Analysis Conditions:

LC Parameters:
Column: Shim-pack XR-ODS 50mm*2.0mm 1.6um
Mobile Phase A: Water /0.1% FA
Mobile Phase B: Acetonitrile /0.05% FA
Gradient: 5% to 100% B in 2.0 minutes, 100% B for 1.1 minutes, 100% to 5% B in 0.1 minutes, then stop.
Flow Rate: 0.7 mL/min
Column Temperature: 40°C
Detector: PDA and ELSD
Sample Preparation: 1 mg/mL in Acetonitrile
Injection Volume: 1 µL
Report: Area Normalized Purity

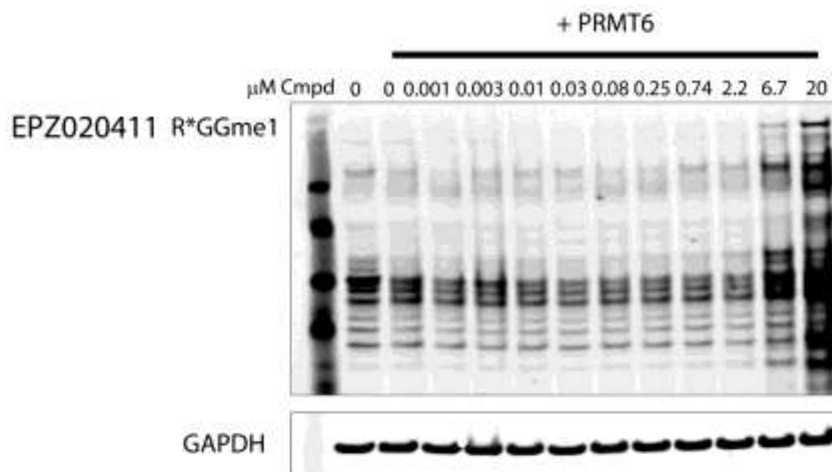
MS Parameters:
Interface: ESI (Positive)
Interface Voltage: Tuning File
Heat Block: 250°C
Nebulizing Gas: 1.50 L/min
Scan Range: 90-900 (m/z)
Detector voltage: 0.85kv



Supplementary Figure 2. 1H NMR spectrum of EPZ020411.



Supplementary Figure 3. Treatment with EPZ020411 for 48 hours does not impact levels of PRMT6 protein in his-PRMT6-transfected A375 cells.



Supplementary Figure 4. Treatment with EPZ020411 for 48 hours has a minimal effect on methylation at the R*GG motif in his-PRMT6-transfected A375 cells (IC50 = 7.1 µM).