Supporting Information for Publication

Discovery of a Potent, Selective and Cell-active Dual Inhibitor of Protein Arginine Methyltransferase 4 and Protein Arginine Methyltransferase 6

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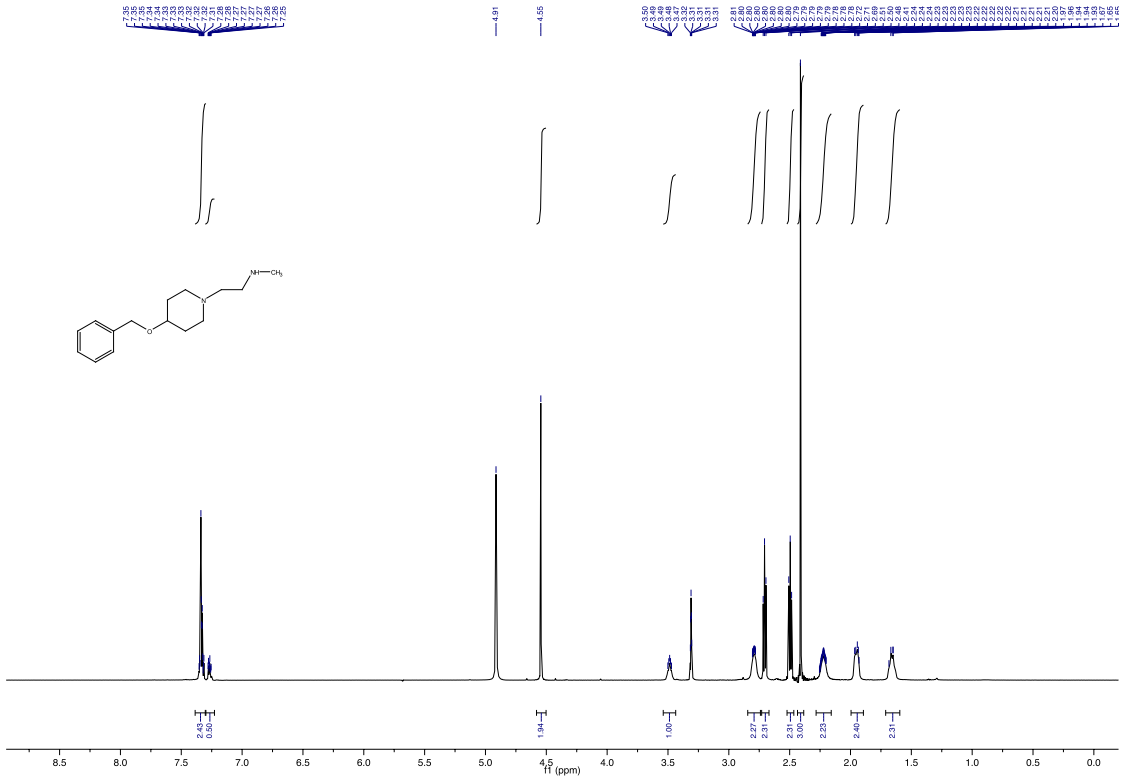
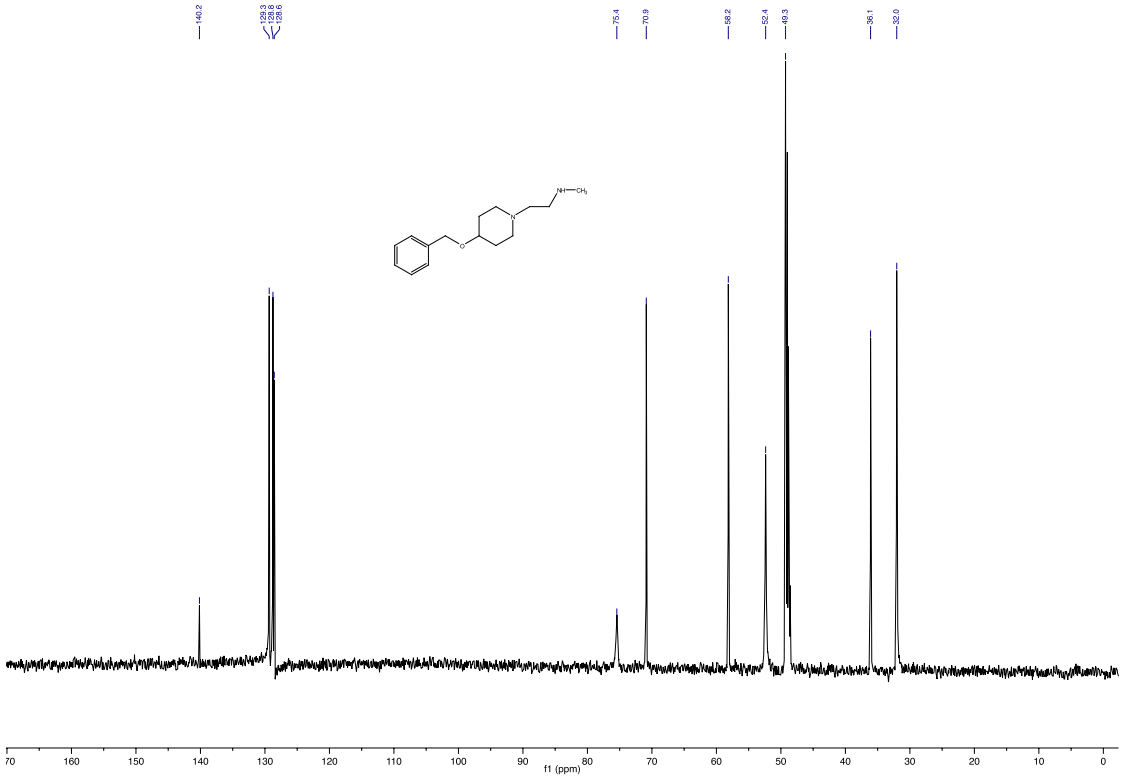
NMR spectra of compound **17**:





Supporting Table S1. Selectivity of 17 against a panel of 9 methyllysine and methylarginine reader proteins. Compound **17** did not stabilize any of these proteins at 200 μM indicating no binding (ΔT_m and $\Delta T_{agg} \leq 1$ °C).

Binder protein	DSF	DSLS
	ΔT_m (°C)	ΔT_{agg} (°C)
UHRF1	0	1
BRPF1	0.7	0.8
WDR5	0	0
HGDF2	-2.5	0.4
TDRD3	NA	-0.4
RBBP1	NA	0.7
FXR1	NA	0.4
EED	NA	-0.2
SND1	NA	0.4

Supporting Table S2. Selectivity of 17 against the CEREP panel. Compound **17** showed no appreciable inhibition (no more than 50% inhibition at 10,000 nM) against 100 GPCRs, ion channels, transporters, and kinases.

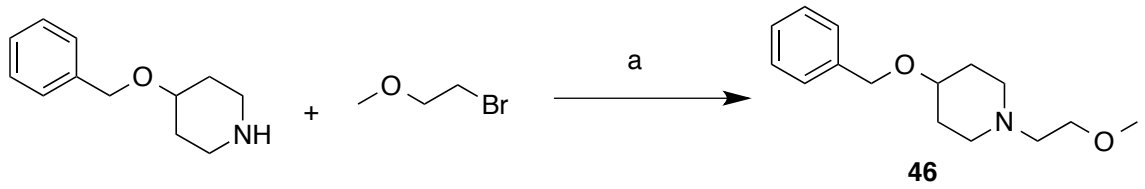
Target	10 μM **17** % Inhibition	Target	10 μM **17** % Inhibition
A1 (h) (antagonist radioligand)	5	Na+ channel (site 2) (antagonist radioligand)	31
A2A (h) (agonist radioligand)	-4	Cl- channel (GABA-gated) (antagonist radioligand)	3
A3 (h) (agonist radioligand)	-11	norepinephrine transporter (h) (antagonist radioligand)	23
alpha 1 (non-selective) (antagonist radioligand)	11	dopamine transporter (h) (antagonist radioligand)	17
alpha 2 (non-selective) (antagonist radioligand)	-3	5-HT transporter (h) (antagonist radioligand)	20
beta 1 (h) (agonist radioligand)	9	Abl kinase (h)	-19
beta 2 (h) (agonist radioligand)	6	Akt1/PKBalpha (h)	5
AT1 (h) (antagonist radioligand)	-4	AurA/Aur2 kinase (h)	-2
BZD (central) (agonist radioligand)	-2	CaMK2alpha (h)	-3
B2 (h) (agonist radioligand)	7	CDC2/CDK1 (h) (cycB)	-2
CB1 (h) (agonist radioligand)	11	CDK2 (h) (cycA)	10
CCK1 (CCKA) (h) (agonist radioligand)	6	CHK1 (h)	10
D1 (h) (antagonist radioligand)	3	CHK2 (h)	3
D2S (h) (antagonist radioligand)	-2	c-Met kinase (h)	-2
ETA (h) (agonist radioligand)	10	EGFR kinase (h)	9
GABA (non-selective) (agonist radioligand)	-8	EphA2 kinase (h)	-2
GAL2 (h) (agonist radioligand)	-8	EphA3 kinase (h)	-12
CXCR2 (IL-8B) (h) (agonist radioligand)	12	EphB4 kinase (h)	8
CCR1 (h) (agonist radioligand)	-1	ERK2 (h) (P42mapk)	46
H1 (h) (antagonist radioligand)	24	FGFR1 kinase (h)	-4
H2 (h) (antagonist radioligand)	1	FGFR2 kinase (h)	4
MC4 (h) (agonist radioligand)	19	FGFR3 kinase (h)	-14
MT1 (ML1A) (h) (agonist radioligand)	6	GSK3beta (h)	2
M1 (h) (antagonist radioligand)	17	HGK (h) (MAP4K4)	-6
M2 (h) (antagonist radioligand)	23	IKKalpha (h)	-2
M3 (h) (antagonist radioligand)	40	IRAK4 (h)	1
NK2 (h) (agonist radioligand)	-7	IRK (h) (InsR)	-10
NK3 (h) (antagonist radioligand)	-15	JAK3 (h)	-8
Y1 (h) (agonist radioligand)	4	JNK1 (h)	-6
Y2 (h) (agonist radioligand)	-15	KDR kinase (h) (VEGFR2)	-7
NTS1 (NT1) (h) (agonist	-7	Lck kinase (h)	4

radioligand)			
delta (DOP) (h) (agonist radioligand)	-10	MAPKAPK2 (h)	10
kappa (KOP) (agonist radioligand)	11	MARK1 (h)	2
mu (MOP) (h) (agonist radioligand)	4	MNK2 (h)	-1
NOP (ORL1) (h) (agonist radioligand)	-5	NEK2 (h)	-1
EP4 (h) (agonist radioligand)	-10	p38alpha kinase (h)	-1
5-HT1A (h) (agonist radioligand)	-2	PAK2 (h)	-2
5-HT1B (antagonist radioligand)	-1	PAK4 (h)	-7
5-HT2A (h) (antagonist radioligand)	13	PDK1 (h)	-5
5-HT2B (h) (agonist radioligand)	10	Pim2 kinase (h)	5
5-HT3 (h) (antagonist radioligand)	5	PKA (h)	7
5-HT5a (h) (agonist radioligand)	-6	PKCbeta 2 (h)	-1
5-HT6 (h) (agonist radioligand)	11	PLK1 (h)	29
5-HT7 (h) (agonist radioligand)	16	RAF-1 kinase (h)	1
sst (non-selective) (agonist radioligand)	-1	ROCK1 (h)	1
VPAC1 (VIP1) (h) (agonist radioligand)	-5	SGK1 (h)	-7
V1a (h) (agonist radioligand)	12	SIK (h)	6
Ca2+ channel (L, verapamil site) (phenylalkylamine) (antagonist radioligand)	19	Src kinase (h)	0
KV channel (antagonist radioligand)	-24	TAOK2 (TAO1) (h)	28
SKCa channel (antagonist radioligand)	-8	TRKA (h)	3

Supporting Table S3. Selectivity of 17 against the PDSP panel. Compound **17** showed no more than 50% inhibition at 10,000 nM against 41 targets and >50% inhibition at 10,000 nM against 3 targets in the panel.

Target	10 μM **17** % Inhibition (K_i)	Target	10 μM **17** % Inhibition (K_i)
5-HT1A	2	D2	33
5-HT1B	1	D3	2
5-HT1D	5	D4	17
5-ht1e	11	D5	3
5-HT2A	5	DAT	45
5-HT2B	8	DOR	-1
5-HT2C	-4	GABAA	-10
5-HT3	8	H2	7
5-ht5a	17	H3	88 (87 nM)
5-HT6	7	H4	-14
5-HT7	7	KOR	-10
Alpha1A	6	M1	3
Alpha1B	7	M2	3
Alpha1D	14	M3	16
Alpha2A	18	M4	13
Alpha2B	-4	M5	-6
Alpha2C	10	MOR	7
Beta1	-15	NET	42
Beta2	-11	PBR	38
Beta3	-14	SERT	16
BZP Rat Brain Site	-5	Sigma 1	97 (64 nM)
D1	-10	Sigma 2	85 (574 nM)

Supporting Scheme S1. Synthesis of 46.



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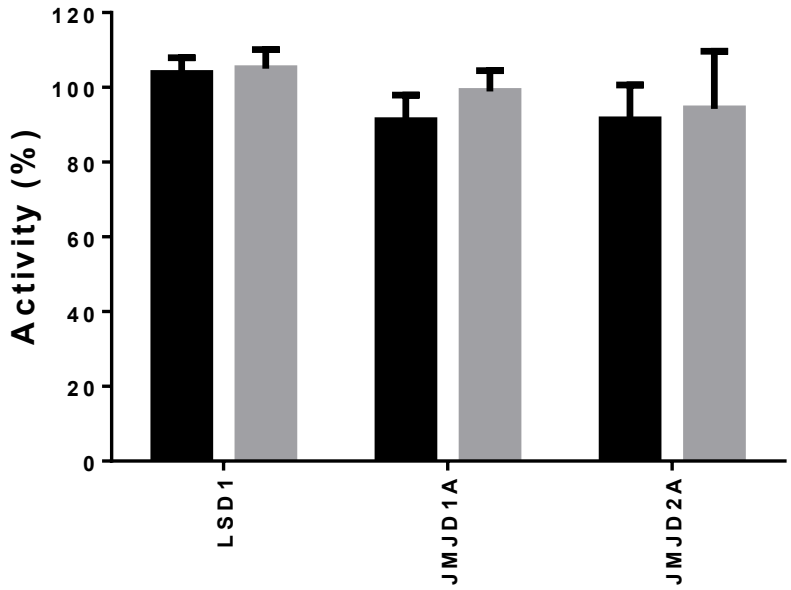
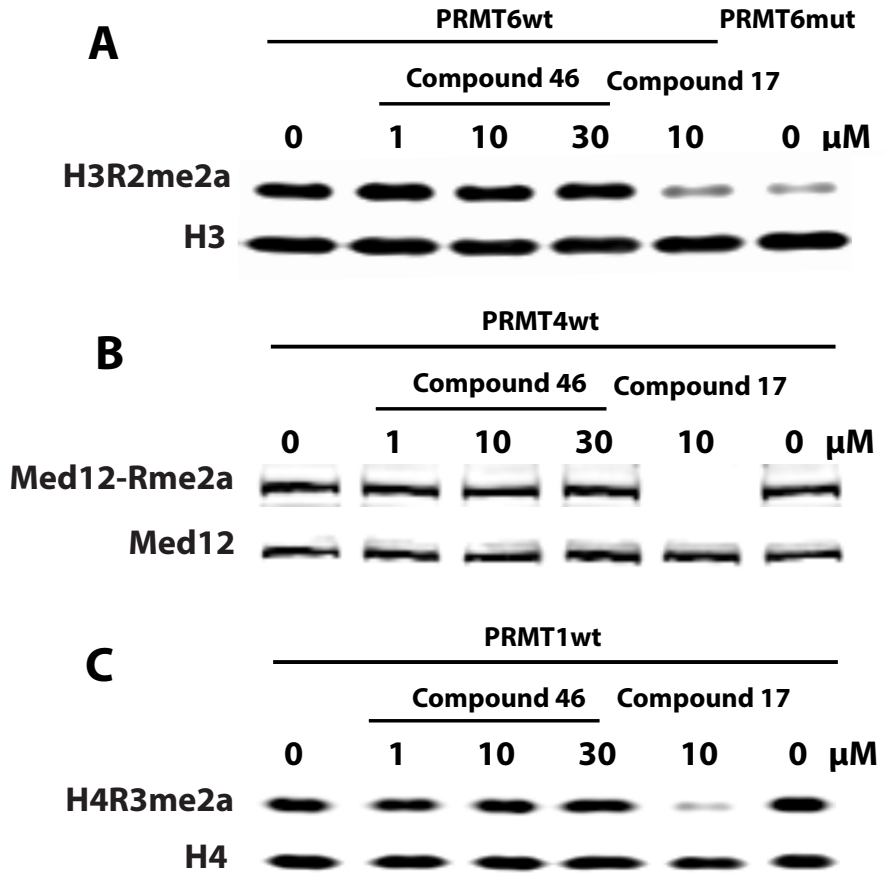
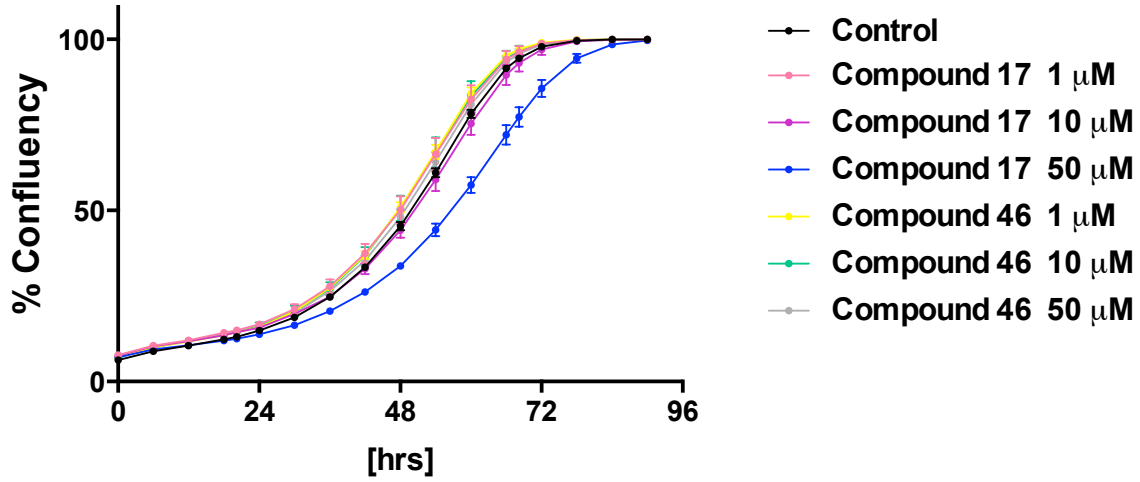
Reagents and conditions: (a) K_2CO_3, KI, CH_3CN, reflux, 20%.



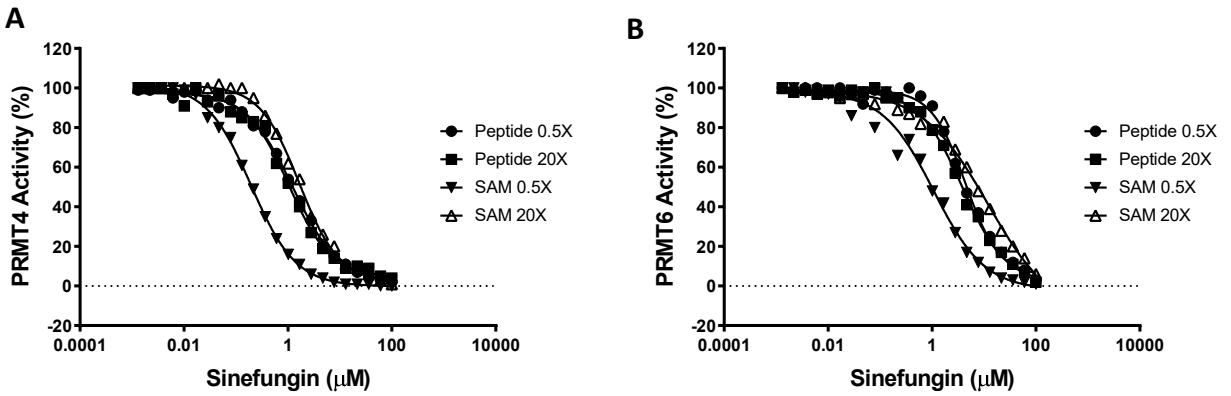
Supporting Figure S1. Selectivity of 17 against KDMs. The inhibitory effect of **17** was tested against KDM1A (LSD1), KDM3A (JMJD1A), and KDM4A (JMJD2A) at two compound concentrations of 1 μM (■) and 10 μM (■).



Supporting Figure S2. Effect of 46 on inhibiting PRMT6 and PRMT4 in cells. Compound **46** was tested in triplicates and representative gels are shown. (A) Compound **46** does not inhibit PRMT6 methyltransferase activity in HEK293 cells. HEK293 cells were transfected with FLAG-tagged PRMT6 (wt) or its catalytically inactive mutant V86K/D88A (mut) and treated with **46** at indicated concentrations for 20 h. H3R2me2a and total H3 levels were determined by Western blot. (B) Compound **46** does not inhibit endogenous PRMT4 methyltransferase activity in HEK293 cells. HEK293 cells were treated with **46** at indicated concentrations for 72 h and Med12-Rme2a and total Med12 levels were determined by Western blot. (C) Compound **46** does not inhibit endogenous PRMT1 methyltransferase activity in HEK293 cells while compound **17** reduces cellular levels of H4R3me2a. HEK293 cells were treated with **46** or **17** at indicated concentrations for 72 h and H4R3me2a and total H4 levels were determined by Western blot.



Supporting Figure S3. Effect of 17 and inactive control 46 on cell growth. HEK293 cells were grown in presence of indicated concentrations of probes for 4 days. Cell confluency was measured using IncuCyte™ ZOOM live cell imaging device. The values are MEAN ± SEM (n = 4).



Supporting Figure S4. Mechanism of inhibition of PRMT4 and PRMT6 activity by sinefungin. Along with attempts to determine the mechanism of action (MOA) of MS049, we used sinefungin as a control. Here we show that SAM competitive mechanism of action of sinefungin in inhibiting PRMT4 (**A**) and PRMT6 (**B**) can easily be confirmed by determining IC_{50} values at SAM concentrations ranging from below K_m (0.5x K_m) to saturation (20x K_m) which resulted in an increase in IC_{50} values from 0.2 μM to 1.7 μM with PRMT4 and 1 μM to 12 μM for PRMT6. For both proteins no significant change in IC_{50} values were observed when the concentration of peptide substrate was increased from 0.5x the K_m of peptide substrate to 20x K_m of peptide (IC_{50} values of 1.2 μM and 1.1 μM for PRMT4 and 4.3 and 3.8 μM for PRMT6) indicating that sinefungin doesn't compete with peptide substrate as expected.