

SUPPORTING INFORMATION

Discovery of an *in vivo* Chemical Probe of the Lysine Methyltransferases G9a and GLP

Feng Liu, Dalia Barsyte-Lovejoy, Fengling Li, Yan Xiong, Victoria Korboukh, Xi-Ping Huang,

Abdellah Allali-Hassani, William P. Janzen, Bryan L. Roth, Stephen V. Frye, Cheryl H.

Arrowsmith, Peter J. Brown, Masoud Vedadi, and Jian Jin*

Table of Contents

A.	Table S1: Selectivity of inhibitor 7 versus 50 kinases	S2
B.	Table S2: Selectivity of inhibitor 7 versus 44 GPCRs, ion channels and transporters	S3
C.	Table S3: Binding affinity of inhibitor 7 for selected GPCRs.....	S4
D.	Table S4: Methyltransferase assay components and conditions	S5
E.	¹ H NMR spectrum of inhibitor 7	S6
F.	¹³ C NMR spectrum of inhibitor 7	S7
G.	¹ H NMR spectrum of inhibitor 13	S8
H.	¹³ C NMR spectrum of inhibitor 13	S9

Table S1. Selectivity of inhibitor 7 versus 50 kinases.*

kinase	%inhibition	Kinase	%inhibition	Kinase	%inhibition
ABL	-4	TIE2	-5	JNK2	-19
CSK	7	TRKA	1	MAPKAPK2	-2
EGFR	-5	TYRO3	-2	MST1	-10
EPHA2	-7	AKT1	-24	NEK2	-5
EPHB4	-2	AMPK α 1/ β 1/ γ 1	10	p38 α	4
FGFR1	-10	AurA	4	p70S6K	-22
FLT3	-1	CaMK4	3	PAK2	4
IGF1R	-4	CDK2/CycA2	1	PBK	-8
ITK	-4	CHK1	-2	PDK1	-1
JAK3	-1	CK1 ϵ	-5	PIM1	-1
KDR	-3	DAPK1	-6	PKAC α	-7
LCK	-1	DYRK1B	-1	PKC α	4
MET	-7	Erk2	2	PKD2	-6
PDGFR α	-1	GSK3 β	-9	ROCK1	-21
PYK2	1	HGK	-6	SGK	1
SRC	1	IKK β	-4	TSSK1	-1
SYK	-4	IRAK4	-12		

*% inhibition at 10 μ M

Table S2. Selectivity of inhibitor 7 versus 44 GPCRs, ion channels and transporters.*

Targets	%inhibition	Targets	%inhibition	Targets	%inhibition
5-HT1A	22	α 2B	6	H3	93
5-HT1B	9	α 2C	67	H2	31
5-HT1D	17	β 1	-8	κ opioid	7
5-HT1E	20	β 2	-11	M1	48
5-HT2A	4	β 3	-10	M2	48
5-HT2B	-8	BZP Rat Brain Site	39	M3	26
5-HT2C	22	D1	25	M4	-4
5-HT3	-1	D2	-8	M5	-7
5-HT5A	-4	D3	22	μ opioid	74
5-HT6	1	D4	12	NET	41
5-HT7	26	D5	17	PBR	34
α 1A	44	DAT	18	SERT	1
α 1B	19	δ opioid	5	σ ₁	3
α 1D	59	GABAA	-3	σ ₂	53
α 2A	16	H1	6		

*% inhibition at 1 μ M

Table S3. Binding affinity of inhibitor 7 for selected GPCRs.

Target	α_{1D}	α_{2C}	H ₃	μ opioid	σ_2
K_i (nM)	4,500	> 10,000	45	> 10,000	900

Table S4. Methyltransferase assay components and conditions.

Methyltransferase	Protein (nM)	SAM (μM)	Peptide	Peptide (μM)	Assay method	Incubation time (h)
PRMT3	100	20	H4 (1-24)	7	SPA	1
PRMT5-MEP50	15	2	H4 (1-24)	0.12	SPA	1
SETD8	50	60	H4 (1-24)	40	SPA	1
SETD7	20	2	H3 (1-25)	2	SPA	0.5
G9a	5	8	H3 (1-25)	0.8	SPA	0.25
GLP	5	8	H3 (1-25)	0.6	SPA	0.25
SETDB1	20	15	H3 (1-25)	1.7	SPA	0.25
SUV39H2	10	3	H3 (1-25)	0.5	SPA	0.25
MLL1 complex	20	2	H3 (1-25)	2	SPA	1
PRC2 complex	20	2	H3 (21-44)	1	SPA	1
SUV420H1	500	12	H4 (1-24)K20Me1	3	SPA	1
SUV420H2	1000	9	H4 (1-24)K20Me1	1	SPA	1
SMYD2	30	0.5	P53 (361-380)	3	SPA	1
SMYD3	1000	10	H3-H4 tetramer	1	Filter plate	1
DOT1L	10	1	Nucleosome	1	Filter plate	1
DNMT1	100	2	dsDNA	0.6	SPA	0.5







