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Assay Report

Methyltransferases (MT) Inhibitor Assays

Enzymatic Study of Compounds from Client

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Client_MT_Year Month Day

MT Inhibitor Assays

Study Sponsor: Client

Attention:

Address:

Study Director: Henry Zhu, Ph.D.

Testing Facility: BPS Bioscience Inc.
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San Diego, CA 92121
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Study Period:

Report Version: 1

Report Date: Month, Day, Year

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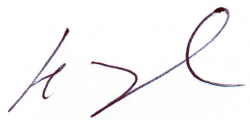
Study Director

_____
Scientist

_____
Date



_____
Henry Zhu, Ph.D.
President

_____
Date

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1. Purpose of the Study

The purpose of the study is to determine the effects of compounds from client on the enzymatic activities of recombinant human G9a, GLP, MLL1, SET7/9, SUV39H1, SUV39H2, PRMT1, PRMT3, PRMT4, PRMT5, PRMT6, PRMT8, NSD1, NSD2, NSD3, EZH1, EZH2, SETD2, SET8, SUV4-20H1, SMYD2, SMYD3, SMYD4, DOT1L, SETDB1, DNMT1, DNMT3A/3L, and DNMT3B/3L using in vitro enzymatic assays.

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2. Materials and Methods

2.1 Materials

Tris Buffered-saline Tween (TBST) Buffer
4×HMT Assay Buffer 1 (BPS catalog number 52160)
4×HMT Assay Buffer 2 (BPS catalog number 52170)
4×HMT Assay Buffer 4 (BPS catalog number 52190)
4×HMT Assay Buffer 5 (BPS catalog number 52191)
4×HMT Assay Buffer 6 (BPS catalog number 52192)
4×HMT Assay Buffer 7Z (BPS catalog number 52193Z)
4×DNMT Assay Buffer 1 (BPS catalog number 52200)
4×DNMT Assay Buffer 2 (BPS catalog number 52201)
SMYD3 Assay Buffer (BPS)
4x Detection buffer 3 (BPS)
AlphaLISA® anti-rabbit IgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen® Glutathione donor beads, 5 mg/ml (PerkinElmer #6765300)
Primary Antibody 1 (BPS 52140A)
Primary Antibody 2 (BPS 52140B2)
Primary Antibody 4 (BPS 52140D)
Primary Antibody 5 (BPS 52140E)
Primary Antibody 6 (BPS 52140F)
Primary Antibody 9 (BPS 52140I)
Primary Antibody 12 (BPS 52140L)
Primary Antibody 15 (BPS 52140O)
Primary Antibody 16 (BPS 52140P)
Primary Antibody 18 (BPS 52140R)
Primary Antibody 23 (BPS 52140W)
Primary Antibody 25 (BPS 52140Y)
Primary Antibody 26 (BPS 52140Z)
HRP-labeled antibody 1 (BPS catalog number 52130H)
HRP-labeled antibody 2 (BPS catalog number 52131H)
Blocking Buffer (BPS catalog number 52100)
HRP chemiluminescent substrate (2 components) (BPS)
White plate pre-coated with methyltransferase substrate
S-adenosylmethionine, SAM (BPS number 52120)
S-adenosylhomocysteine, AdoHcy (BPS)
Sinefungin (BPS)
GSK343 (BPS)
Chaetocin (BPS)
UNC-0646 (BPS)
LLY507 (BPS)
SGC707 (BPS)
A-196 (BPS)
GSK591 (BPS)

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MS023 (BPS)
PFI-2 (BPS)
MEKK2 K260 mutant (BPS).

2.2 Compounds

The test compounds are supplied by Client.

Compound I.D.	Compound Supplied	Stock Concentration	Dissolving Solvent	Test Range (µM)
MS023*	Solution	5 mM	DMSO	0-50 (PRMT1, PRMT4, PRMT6, PRMT8)
A-196*	Solution	5 mM	DMSO	0-50 (SUV4-20H1)
PFI-2*	Solution	5 mM	DMSO	0-50 (SET7/9)
S-adenosyl-homocysteine, AdoHcy*	Solution	50 mM	DMSO	0-500 (MLL, SETD2, SETDB1, SET8, DNMT, SUV39H, PRDM9)
GSK343*	Solution	6 mM	DMSO	0-50 (EZH)
Chaetocin*	Solution	10 mM	DMSO	0-200 (NSD, SMYD4)
UNC-0646*	Solution	3 mM	DMSO	0-10 (G9a, GLP)
LLY507*	Solution	1 mM	DMSO	0-30 (SMYD2)
GSK591*	Solution	5 mM	DMSO	0-50 (PRMT5)
SGC707*	Solution	5 mM	DMSO	0-50 (PRMT3)
MEKK2 K260 mutant**	Solution	0.0056 mM	Buffer	0-2 (SMYD3)

*Reference compounds.
**Reference protein.

2.3 Experimental Conditions

2.3.1 Enzymes and Substrates

N	Assay	Catalog #	Enzyme Lot #	Enzyme Used (ng) / Reaction	Substrate
1	G9a	51001	101020	8	Histone substrate coated on plate /1 µM S-Adenosylmethionine

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2	GLP	51020	120828	25	Histone substrate coated on plate /0.5 µM S-Adenosylmethionine
3	MLL1	51022	120907	150	Histone substrate coated on plate /1 µM S-Adenosylmethionine
4	SET7/9	51010	120314	40	Histone substrate coated on plate /1 µM S-Adenosylmethionine
5	SUV39H1	51070	140114	100	Histone substrate coated on plate /1 µM S-Adenosylmethionine
6	SUV39H2	51080	130122-G4	50	Histone substrate coated on plate /1 µM S-Adenosylmethionine
7	PRMT1	51040	120208	5	Histone substrate coated on plate /0.5 µM S-Adenosylmethionine
8	PRMT3	51043	110912-GC	50	Histone substrate coated on plate /0.5 µM S-Adenosylmethionine
9	PRMT4	51047	110119	200	Histone substrate coated on plate /1 µM S-Adenosylmethionine
10	PRMT5	51045	140131	150	Histone substrate coated on plate /0.5 µM S-Adenosylmethionine
11	PRMT6	51049	120802S-250	100	Histone substrate coated on plate /0.5 µM S-Adenosylmethionine
12	PRMT8	51052	120416	50	Histone substrate coated on plate /0.5 µM S-Adenosylmethionine
13	NSD1	51024	130524-5B (Zn)	2600	Histone substrate coated on plate /30 µM S-Adenosylmethionine
14	NSD2	51026	140221	260	Histone substrate coated on plate /2 µM S-Adenosylmethionine
15	NSD3	51036	130204	1000	Histone substrate coated on plate /5 µM S-Adenosylmethionine
16	SMYD4	51057	160304	2000	Histone substrate coated on plate /4 µM S-Adenosylmethionine
17	PRDM9	N/A	131025	1	Histone substrate coated on plate /0.5 µM S-Adenosylmethionine
18	SMYD3	51110	150304	30	SMYD3 Substrate /10 µM S-Adenosylmethionine
19	Dot1L	51005	120110	500	Histone Octamer /5 µM S-Adenosylmethionine
20	EZH1	51007	130201-EN	500	Histone substrate coated on plate /5 µM S-Adenosylmethionine
21	EZH2	51004	131021	200	Histone substrate coated on plate /5 µM S-Adenosylmethionine
22	SETD2	53019	130625-E3	80	Histone substrate coated on plate /10 µM S-Adenosylmethionine

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23	SET8	51008	111102	2500	Histone substrate coated on plate /70 µM S-Adenosylmethionine
24	SUV4-20H1	51090	130402-10	1500	Histone substrate coated on plate /400 µM S-Adenosylmethionine
25	SMYD2	51014	140918A	500	Histone substrate coated on plate /25 µM S-Adenosylmethionine
26	SETDB1	51055	140721-5	25	Histone substrate coated on plate /1 µM S-Adenosylmethionine
27	DNMT1	51101	130109-1	250	DNMT substrate coated on plate /20 µM S-Adenosylmethionine
28	DNMT3A/3L	51106	130404	300	DNMT substrate coated on plate /20 µM S-Adenosylmethionine
29	DNMT3B/3L	51104	121023	300	DNMT substrate coated on plate /20 µM S-Adenosylmethionine

2.3.2 Assay Conditions

All of the enzymatic reactions were conducted in duplicate at room temperature (DNMTs - 37°C) for 60-960 minutes in a 50 µl mixture containing proper methyltransferase assay buffer, S-adenosylmethionine, enzyme, and the test compound (see 2.2). These 50 µl reactions were carried out in wells of a substrate pre-coated plate.

After enzymatic reactions, the reaction mixtures were discarded and each of the wells was washed three times with TBST buffer, and slowly shaken with Blocking Buffer for 10 minutes.

Wells were emptied, and 100 µl of diluted primary antibody was added. The plate was then slowly shaken for 60 minutes at room temperature.

As before, the plate was emptied and washed three times, and shaken with Blocking Buffer for 10 minutes at room temperature.

After discarding the Blocking Buffer, 100 µl of diluted secondary antibody was added. The plate was then slowly shaken for 30 minutes at room temperature.

As before, the plate was emptied and washed three times, and shaken with Blocking Buffer for 10 minutes at room temperature.

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Blocking Buffer was discarded and a mixture of the HRP chemiluminescent substrates was freshly prepared. 100 µl of this mixture was added to each empty well.

Immediately, the luminescence of the samples was measured in a BioTek Synergy™ 2 microplate reader.

The SMYD3 reactions were conducted in duplicate at 30°C for 180 minutes in a 10 µl mixture containing assay buffer, SMYD3 substrate and enzyme, and the test compound (Section 2.2). These 10 µl reactions were carried out in wells of 384-well Optiplate (PerkinElmer).

After enzymatic reactions, 5 µl of anti-Rabbit Acceptor beads (PerkinElmer, diluted 1:500 with 1x detection buffer) and 5 µl of Primary antibody (BPS, diluted 1:100 with 1x detection buffer) were added to the reaction mix. After brief shaking, plate was incubated for 30 minutes.

Finally, 10 µl of AlphaScreen GSH-conjugated donor beads (Perkin, diluted 1:125 with 1x detection buffer) were added.

In 30 minutes, the samples were measured in AlphaScreen microplate reader (EnSpire Alpha 2390 Multilabel Reader, PerkinElmer).

2.3.3 Data Analysis

Enzyme activity assays were performed in duplicates at each concentration. The luminescence data were analyzed and compared. In the absence of the compound, the intensity (C_e) in each data set was defined as 100% activity. In the absence of enzyme, the intensity (C_0) in each data set was defined as 0% activity. The percent activity in the presence of each compound was calculated according to the following equation: % activity = $(C-C_0)/(C_e-C_0)$, where C= the luminescence in the presence of the compound.

The values of % activity versus a series of compound concentrations were then plotted using non-linear regression analysis of Sigmoidal dose-response curve generated with the equation $Y=B+(T-B)/1+10^{((LogEC50-X)\times Hill\ Slope)}$, where Y=percent activity, B=minimum percent activity, T=maximum percent activity, X= logarithm of compound and Hill Slope=slope factor or Hill coefficient. The IC_{50} value was determined by the concentration causing a half-maximal percent activity.

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3. Assay Results

3.1. Summary of the Inhibitory Effects of the Compounds on Individual MT Activities

The mean IC_{50} values of the compounds are summarized in Table 3.1.

Table 3.1 Inhibitory Effects of the Compounds on MT Activities (IC_{50} in μM)

	MS023	A-196	PFI-2	AdoHcy	MEKK2 mutant	GSK343	Chaetocin	UNC-0646	LLY507	GSK591	SGC707	SGC0946
G9a								0.039				
GLP								0.016				
MLL1				1.5								
SET7/9			0.081									
SUV39H1				3.4								
SUV39H2				27.8								
PRMT1	0.27											
PRMT3											0.13	
PRMT4	0.18											
PRMT5										0.032		
PRMT6	0.063											
PRMT8	0.025											
NSD1							1.1					
NSD2							0.6					
NSD3							2.4					

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	MS023	A-196	PFI-2	AdoHcy	MEKK2 mutant	GSK343	Chaetocin	UNC-0646	LLY507	GSK591	SGC707	SGC0946
EZH1						0.04						
EZH2						0.004						
SETD2				0.087								
SET8				15								
SUV4-20H1		0.17										
SMYD2									0.31			
SMYD3					0.017							
SMYD4							4.4					
DOT1L												0.06
PRDM9				11.8								
SETDB1				1.7								
DNMT1				0.5								
DNMT3A/3L				1.2								
DNMT3B/3L				1.8								

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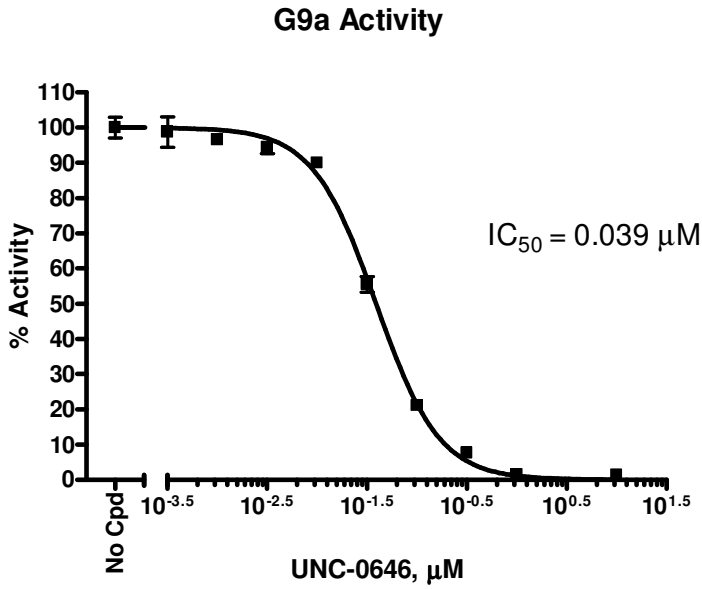
3.2. Results of the Effects of the Compounds on Individual MT Activity

3.2.1. G9a

Table 3.2.1. Data for the Effect of the UNC-0646 on G9a Activity

UNC-0646 [µM]	G9a Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	2130	2255	97	103
0.0003	2074	2257	94	103
0.001	2140	2098	98	96
0.003	2035	2109	93	96
0.010	1983	1976	90	90
0.03	1296	1204	58	53
0.1	488	556	19	23
0.3	209	262	6	9
1.0	90	121	1	2
3.2	44	58	-1	-1
10	99	109	1	2
Background	70	81		



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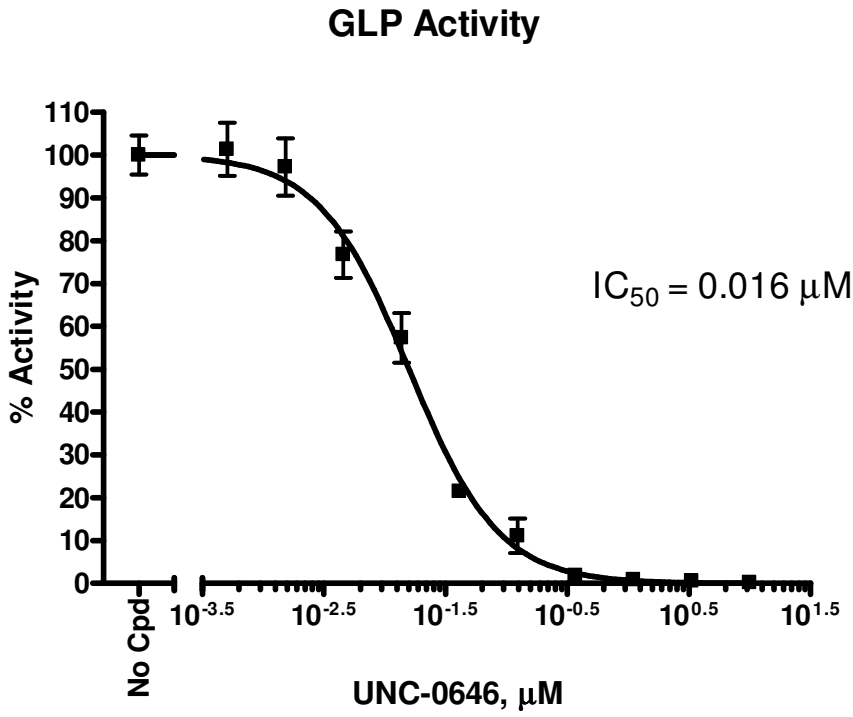
3.2.2. GLP

Table 3.2.2. Data for the Effect of UNC-0646 on GLP Activity

UNC-0646 [μM]	GLP Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	2537	2771	95	105
0.001	2848	2529	108	95
0.002	2410	2754	91	104
0.005	1917	2196	71	82
0.01	1706	1408	63	52
0.04	627	641	21	22
0.1	265	473	7	15
0.4	110	150	1	3
1	119	91	1	0
3	89	106	0	1
10	77	99	0	1
Background	68	98		



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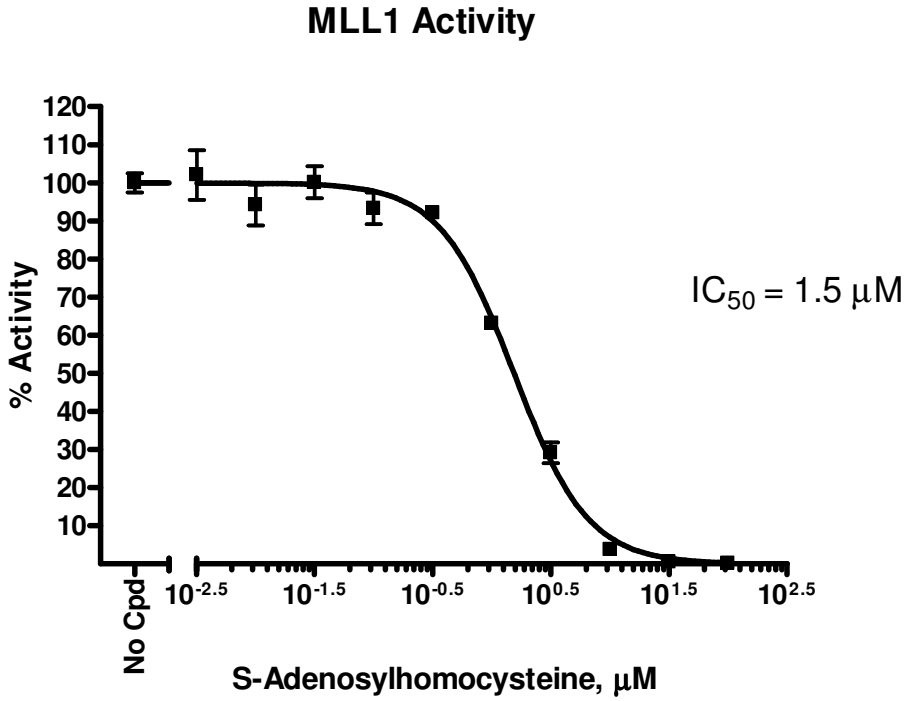
3.2.3. MLL Complex

Table 3.2.3. Data for the Effect of S-adenosylhomocysteine on MLL1 Activity

AdoHcy [μM]	MLL1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	25410	24175	102	98
0.003	23703	26924	96	109
0.01	24680	22021	100	89
0.03	23784	25880	96	104
0.1	22114	24177	89	98
0.3	22851	22821	92	92
1	15242	16093	61	65
3	6583	7932	26	32
10	876	1099	3	4
30	194	143	1	0
100	102	90	0	0
Background	49	30		



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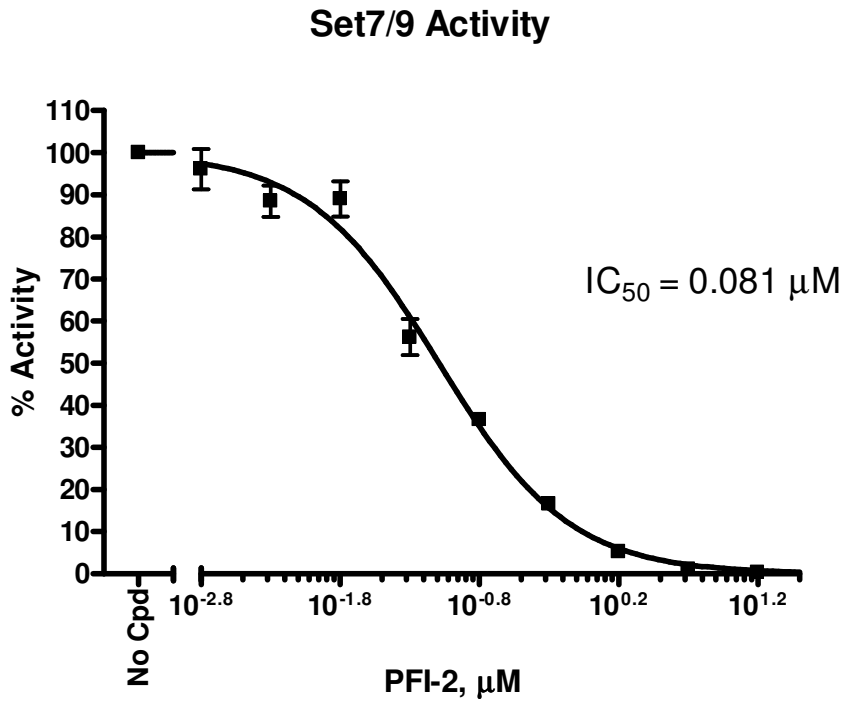
3.2.4. SET7/9

Table 3.2.4. Data for the Effect of PFI-2 on SET7/9 Activity

PFI-2 [μM]	SET7/9 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	55172	56545	99	101
0.002	56353	51001	101	91
0.005	47349	51517	85	92
0.02	52047	47394	93	85
0.05	29076	33845	52	60
0.2	20979	20171	37	36
0.5	9976	8846	18	15
2	3151	3136	5	5
5	880	781	1	1
16	415	371	0	0
50	261	296	0	0
Background	229	221		



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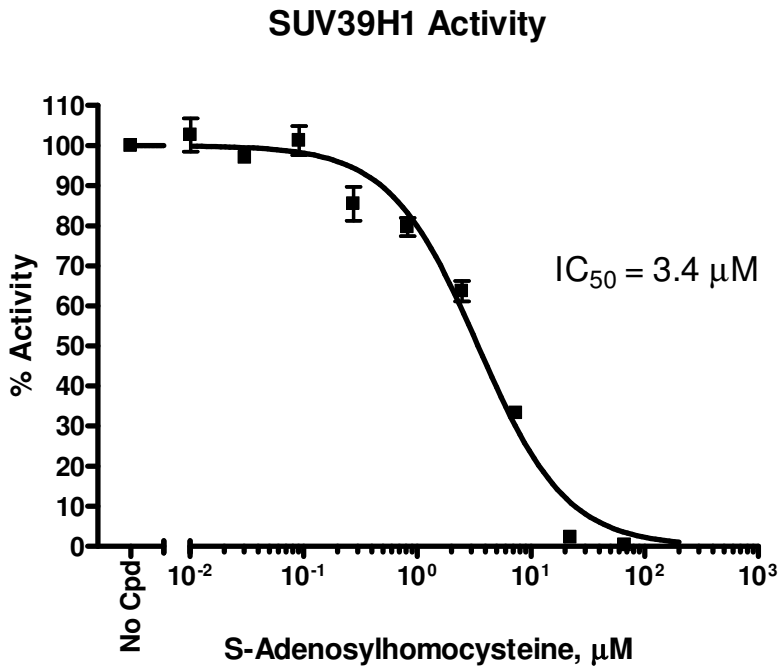
3.2.5. SUV39H1

Table 3.2.5. Data for the Effect of S-adenosylhomocysteine on SUV39H1 Activity

AdoHcy [µM]	SUV39H1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	5612	5453	101	99
0.01	5447	5901	98	107
0.03	5371	5367	97	97
0.1	5399	5800	98	105
0.3	4974	4510	90	81
1	4306	4550	77	82
2	3695	3420	66	61
7	1968	1845	34	32
22	258	183	3	2
67	93	135	0	1
200	82	96	0	0
Background	107	87		



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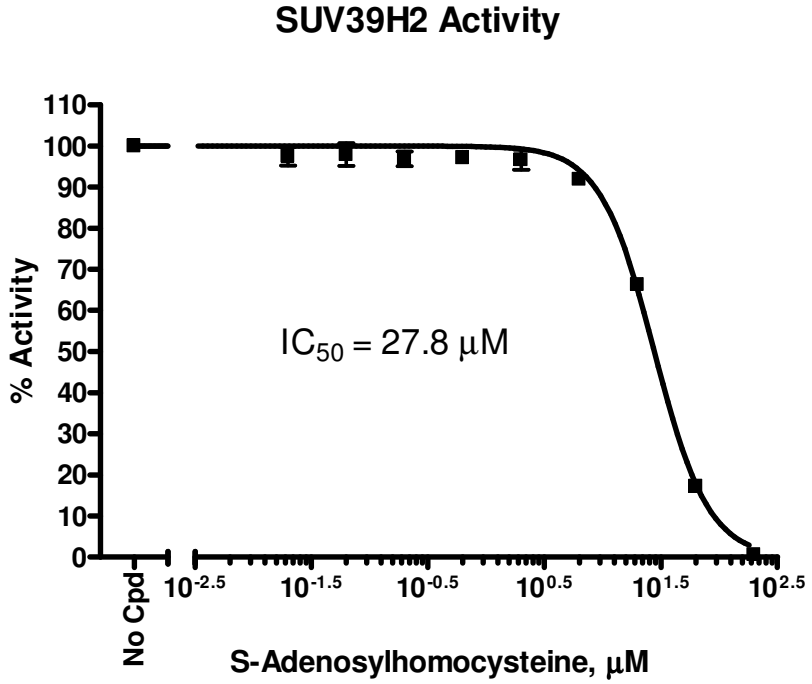
3.2.6. SUV39H2

Table 3.2.6. Data for the Effect of S-adenosylhomocysteine on SUV39H2 Activity

AdoHcy [μM]	SUV39H2 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	5933	6135	98	102
0.02	5752	6019	95	100
0.06	5745	6072	95	101
0.2	5741	5953	95	99
0.6	5839	5885	97	97
2	5690	5980	94	99
6	5551	5545	92	92
20	3976	4080	65	67
60	1143	1086	18	17
200	144	114	1	0
Background	92	97	0	0



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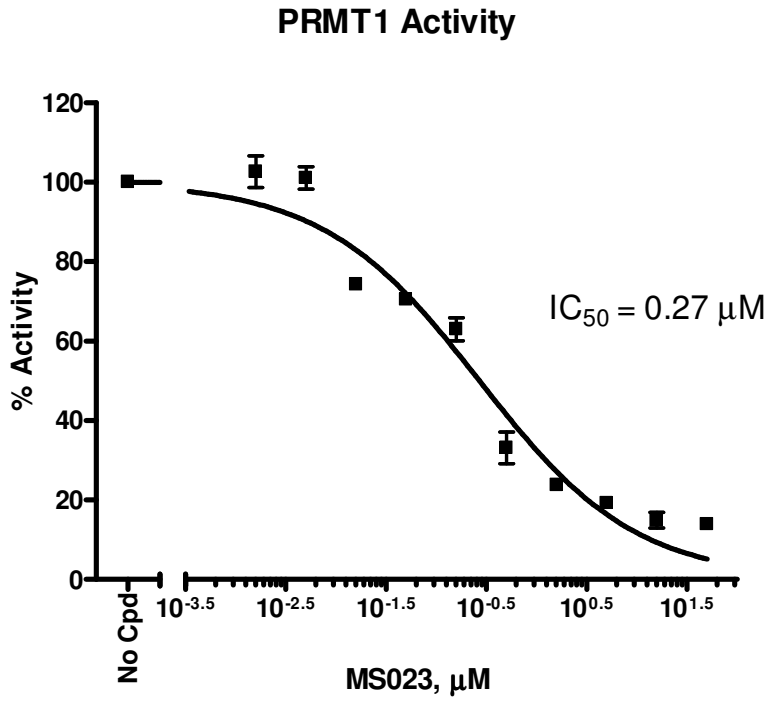
3.2.7. PRMT1

Table 3.2.7. Data for the Effect of MS023 on PRMT1 Activity

MS023 [μM]	PRMT1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	20979	20406	101	99
0.002	22060	20413	107	99
0.005	20335	21495	98	104
0.02	15487	15283	75	74
0.05	14509	14594	70	70
0.2	12464	13655	60	66
0.5	7729	6084	37	29
1.6	5312	4647	25	22
5	4161	3935	20	19
16	3563	2745	17	13
50	3219	2673	15	13
Background	75	72		



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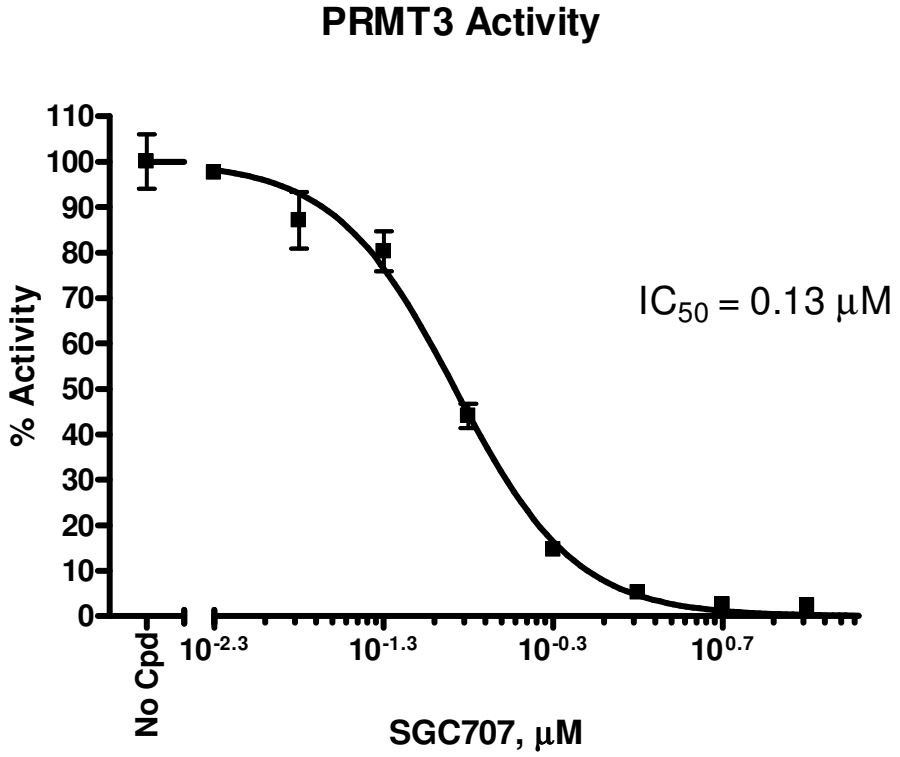
3.2.8. PRMT3

Table 3.2.8. Data for the Effect of SGC707 on PRMT3 Activity

SGC707 [μM]	PRMT3 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	71483	63444	106	94
0.005	66670	65837	99	98
0.02	62984	54603	93	81
0.05	57148	51254	85	76
0.2	28051	31669	41	47
0.5	9321	10836	14	16
2	3613	3805	5	5
5	1444	2470	2	3
16	2008	1587	3	2
50	1188	1399	1	2
Background	287	185		



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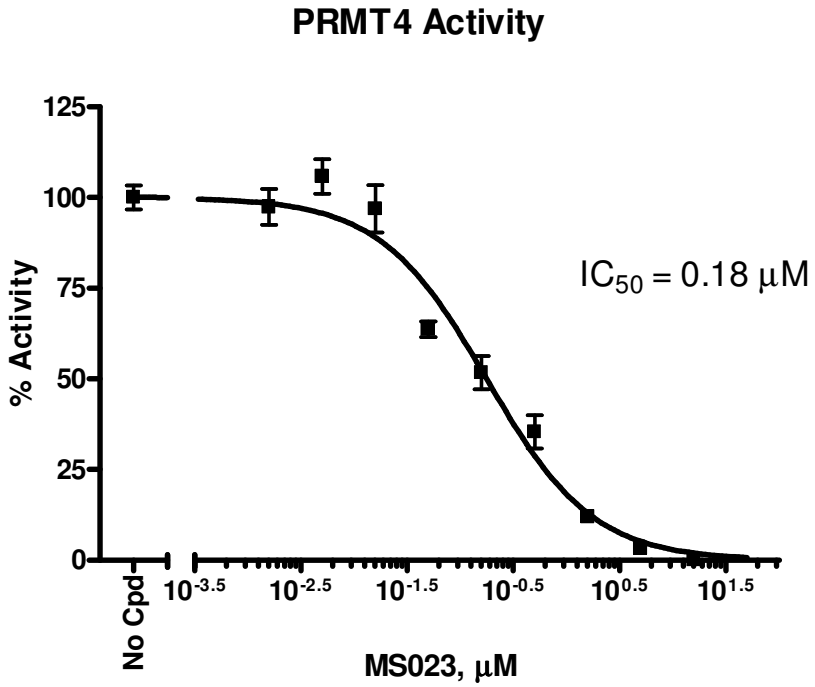
3.2.9. PRMT4

Table 3.2.9. Data for the Effect of MS023 on PRMT4 Activity

MS023 [μM]	PRMT4 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	4600	4311	101	99
0.002	4558	4126	107	99
0.005	4500	4920	98	104
0.02	4606	4034	75	74
0.05	2767	2956	70	70
0.2	2132	2536	60	66
0.5	1416	1818	37	29
1.6	534	644	25	22
5	137	278	20	19
16	33	104	17	13
50	63	50	15	13
Background	55	71		



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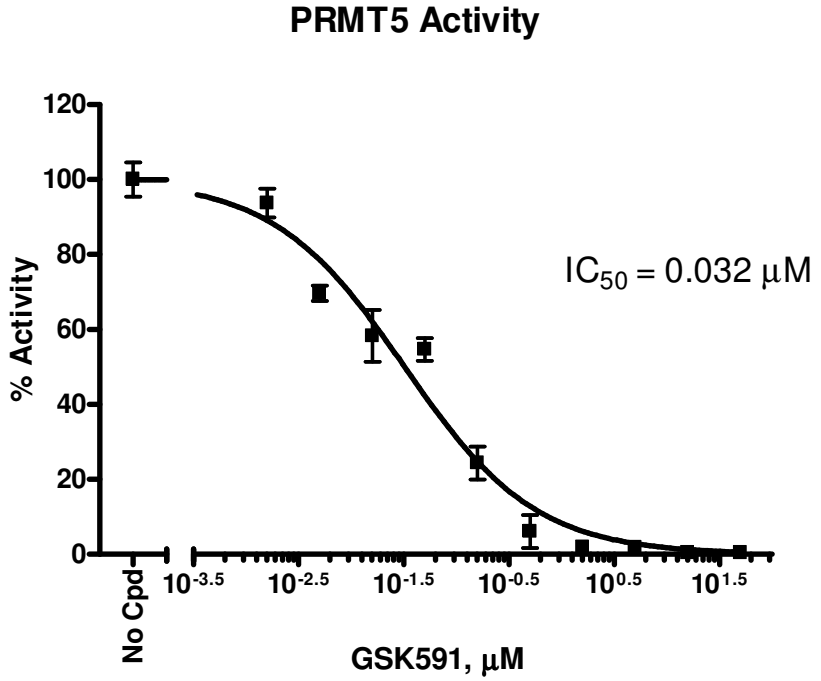
3.2.10. PRMT5

Table 3.2.10. Data for the Effect of GSK591 on PRMT5 Activity

GSK591 [µM]	PRMT5 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	5021	5489	95	105
0.002	4739	5131	90	98
0.005	3599	3810	68	72
0.02	3475	2768	65	51
0.05	3094	2782	58	52
0.2	1610	1162	29	20
0.5	677	227	11	2
1.6	302	165	3	1
5	210	256	1	2
16	147	174	0	1
50	107	213	-1	1
Background	154	124		



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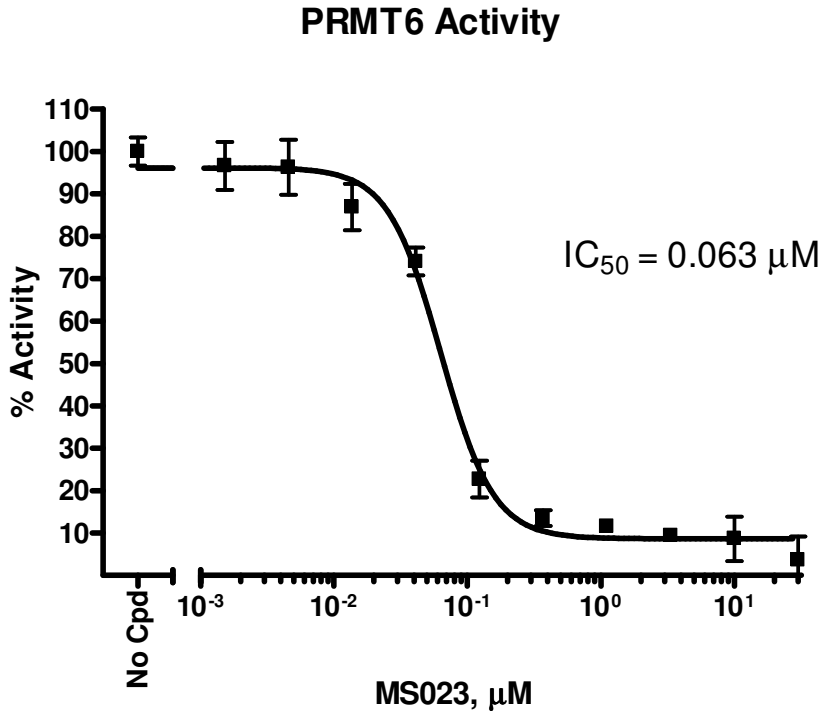
3.2.11. PRMT6

Table 3.2.11. Data for the Effect of MS023 on PRMT6 Activity

MS023 [μM]	PRMT6 Activity (Chemiluminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	35752	33756	103	97
0.002	35444	32004	102	91
0.005	35601	31653	103	90
0.02	32435	29114	92	81
0.05	25890	27875	71	77
0.2	12631	9995	27	18
0.5	7976	9086	12	15
1.6	7584	8252	10	13
5	7429	7135	10	9
16	5462	8633	3	14
50	7203	3823	9	-2
Background	4447	4394		



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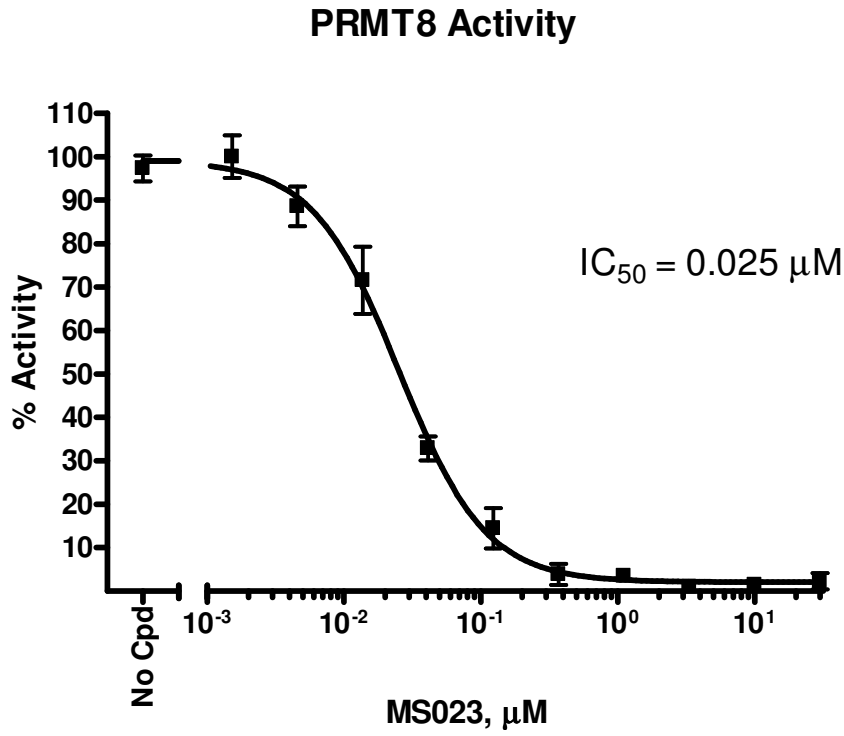
3.2.12. PRMT8

Table 3.2.12. Data for the Effect of MS023 on PRMT8 Activity

MS023 [μM]	PRMT8 Activity (Chemiluminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	46430	43849	100	94
0.002	44180	48443	95	105
0.005	43331	39316	93	84
0.02	37265	30516	79	64
0.05	18190	15776	36	30
0.2	10972	6902	19	10
0.5	5361	3259	6	1
1.6	4126	4149	3	3
5	3693	2435	2	0
16	3704	2820	2	0
50	4416	2810	4	0
Background	2497	2746		



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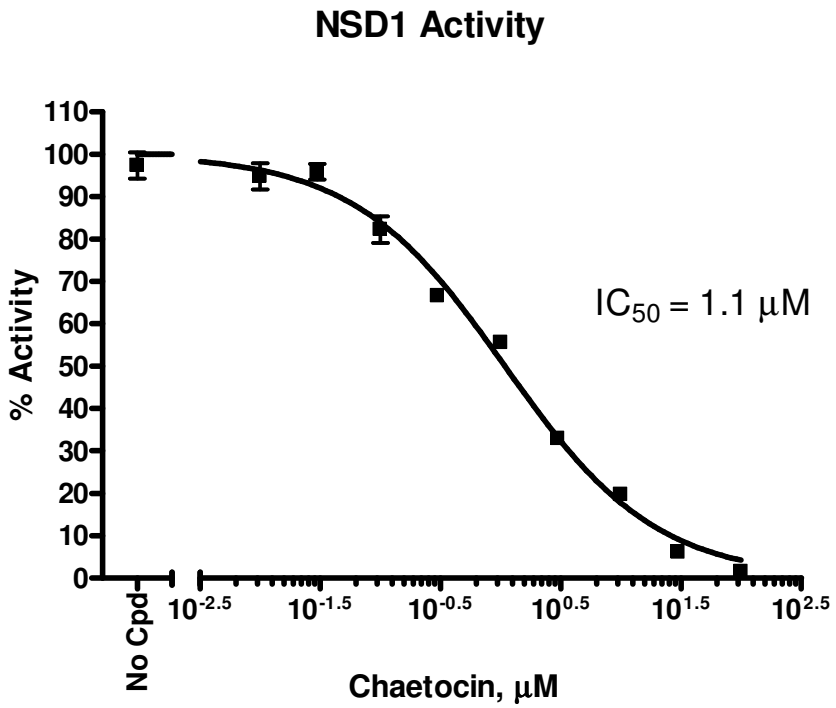
3.2.13. NSD1

Table 3.2.13. Data for the Effect of Chaetocin on NSD1 Activity

Chaetocin [μM]	NSD1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	27938	26238	103	97
0.003	28189	27449	104	101
0.01	27228	25517	101	94
0.03	27175	26176	100	97
0.1	22019	23750	81	88
0.3	18541	18542	68	68
1	15452	15493	57	57
3	9235	9133	34	34
10	5494	5542	20	20
30	1833	1702	7	6
100	550	406	2	1
Background	65	53	0	0



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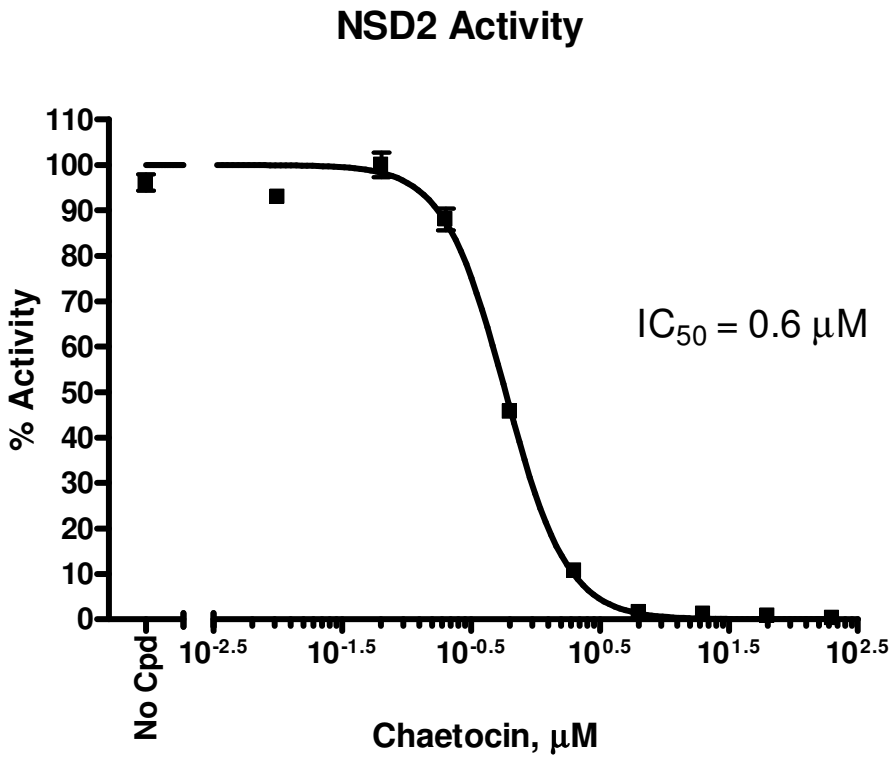
3.2.14. NSD2

Table 3.2.14. Data for the Effect of Chaetocin on NSD2 Activity

Chaetocin [μM]	NSD2 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	15573	14999	98	94
0.020	14832	14728	93	93
0.06	16325	15462	103	97
0.20	14369	13616	90	86
0.6	7339	7271	46	45
2.0	1696	1862	10	11
6	282	373	1	2
20	330	239	1	1
63	335	92	2	0
200	73	190	0	1
Background	94	93	0	0



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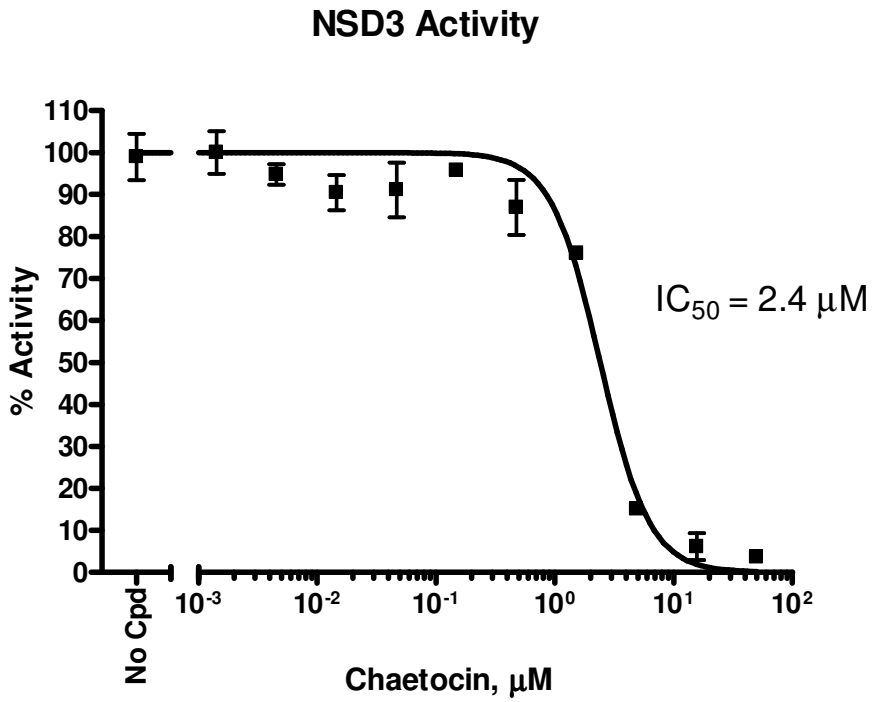
3.2.15. NSD3

Table 3.2.15. Data for the Effect of the compounds on NSD3 Activity

Chaetocin [µM]	NSD3 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	3537	3171	106	94
0.001	3222	3557	96	106
0.005	3297	3136	98	93
0.01	2934	3212	87	96
0.05	2879	3310	86	99
0.1	3290	3204	98	95
0.5	3172	2741	94	81
2	2549	2641	75	78
5	594	554	16	15
16	173	384	3	9
50	198	192	4	4
Background	99	50	1	-1



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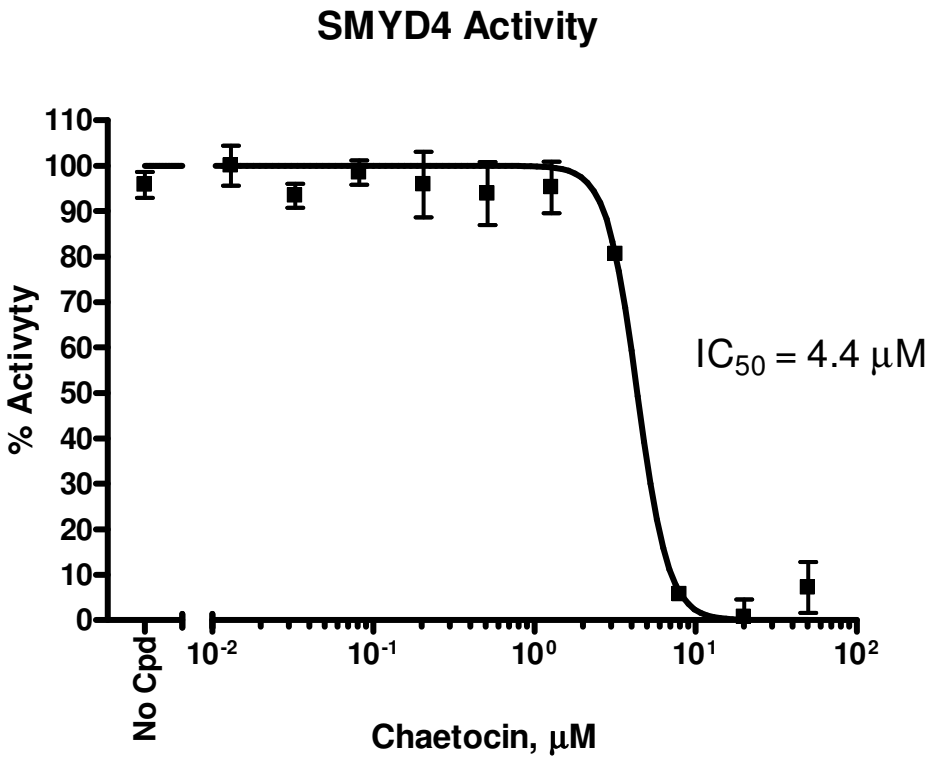
3.2.16. SMYD4

Table 3.2.16. Data for the Effect of Chaetocin on SMYD4 Activity

Chaetocin [μM]	SMYD4 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	1323	1253	103	97
0.01	1395	1286	109	100
0.03	1291	1225	100	95
0.08	1355	1288	106	100
0.2	1379	1199	108	93
0.5	1350	1178	105	91
1.3	1211	1351	94	105
3.2	1092	1104	84	85
8	164	166	6	6
20	152	54	5	-3
50	114	254	2	13
Background	89	100		



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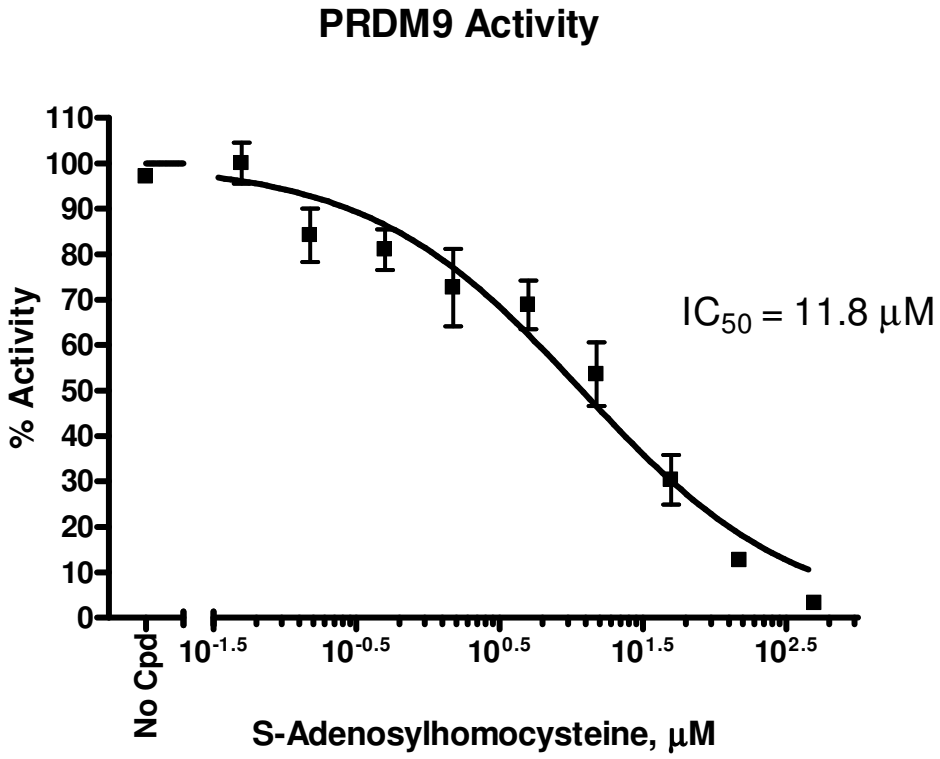
3.2.17. PRDM9

Table 3.2.17. Data for the Effect of S-adenosylhomocysteine on PRDM9 Activity

AdoHcy [µM]	PRDM9 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	22045	21630	101	99
0.05	21484	23489	98	108
0.15	17653	20258	81	93
0.5	19256	17265	88	79
1.5	14499	18281	66	84
5	14355	16736	65	76
15	10591	13721	48	62
50	5759	8196	26	37
150	3271	2801	14	12
500	1027	841	4	3
Background	193	255	0	0



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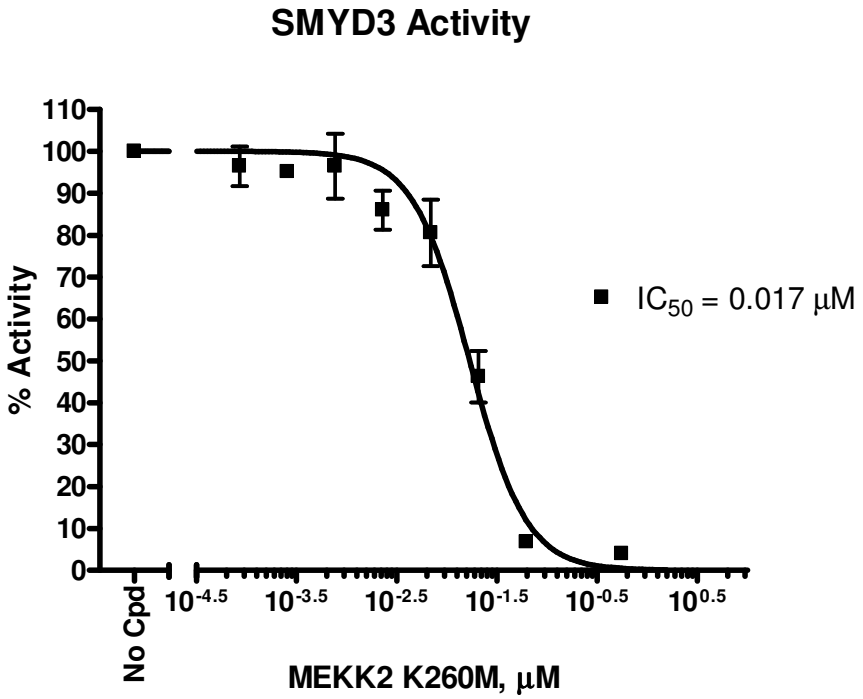
3.2.18. SMYD3

Table 3.2.18. Data for the Effect of the inibitor on SMYD3 Activity

MEKK2 mutant [μM]	SMYD3 Activity (A-counts)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	21095	20717	101	99
0.0001	21119	19379	101	92
0.0003	20101	19928	96	95
0.0008	21693	18818	104	89
0.002	17449	19175	81	91
0.007	15846	18773	73	88
0.02	9831	12110	40	52
0.06	3406	3952	5	8
0.2	1733	1732	-4	-4
0.6	3139	3200	4	4
2	2682	1037	1	-8
Background	2343	2514		



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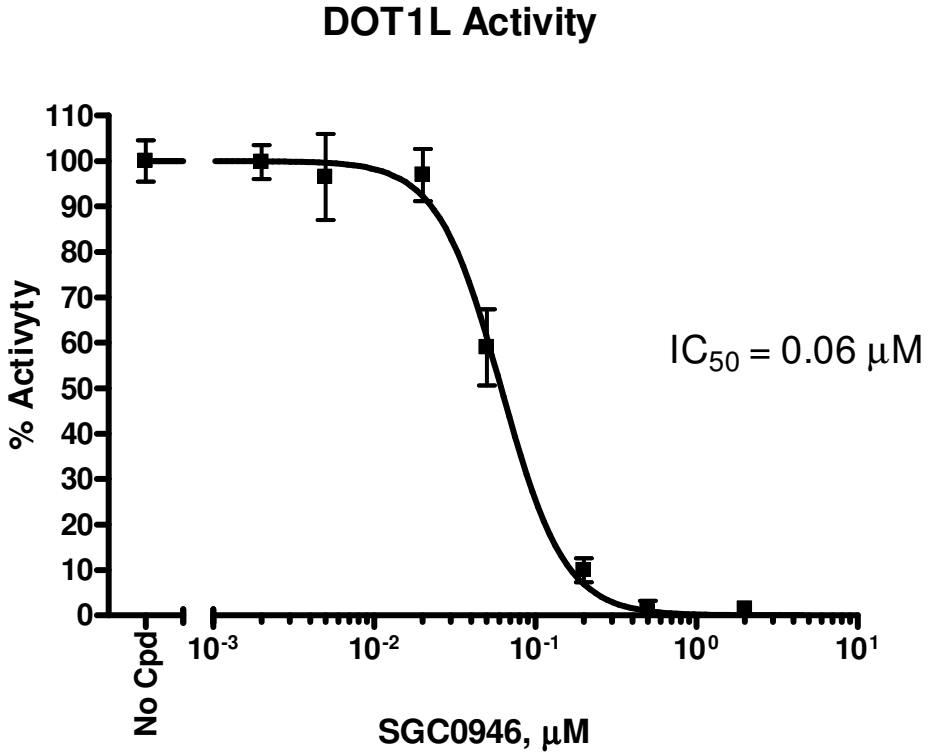
3.2.19. Dot1L

Table 3.2.19. Data for the Effect of SGC0946 on Dot1L Activity

SGC0946 [μM]	Dot1L Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	1537	1677	95	105
0.002	1661	1546	104	96
0.005	1698	1408	106	87
0.02	1472	1647	91	103
0.05	1106	850	67	51
0.2	266	185	13	7
0.5	63	122	-1	3
2	100	91	2	1
5	87	52	1	-1
Background	82	65		



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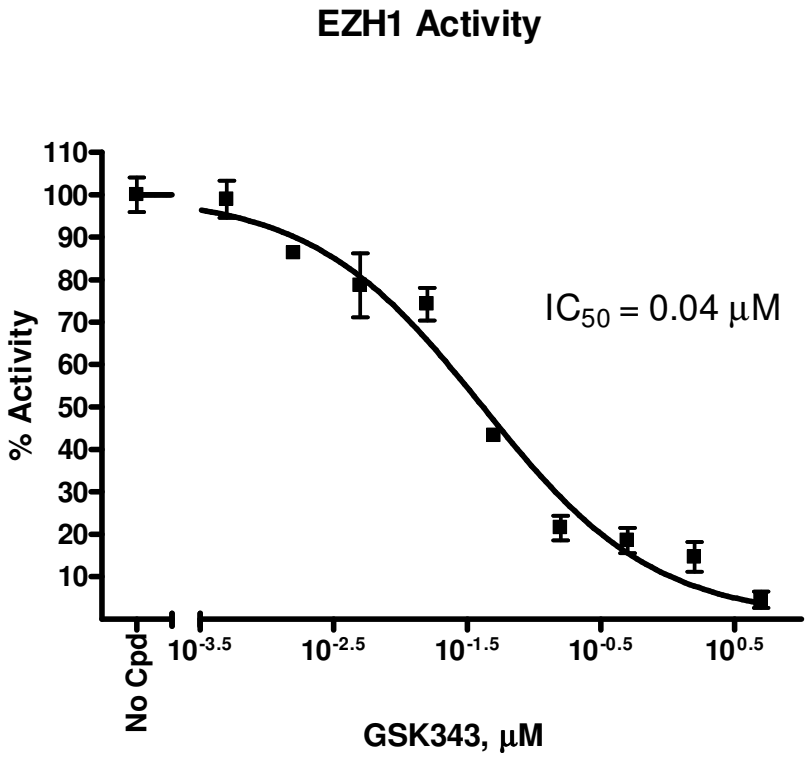
3.2.20. EZH1

Table 3.2.20. Data for the Effect of GSK343 on EZH1 Activity

GSK343 [μM]	EZH1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	1148	1237	96	104
0.001	1228	1133	103	95
0.002	1049	1037	87	86
0.005	878	1042	71	86
0.02	870	954	70	78
0.05	566	584	42	44
0.2	370	307	24	19
1	273	339	15	22
2	226	302	11	18
5	175	134	6	3
Background	94	115		



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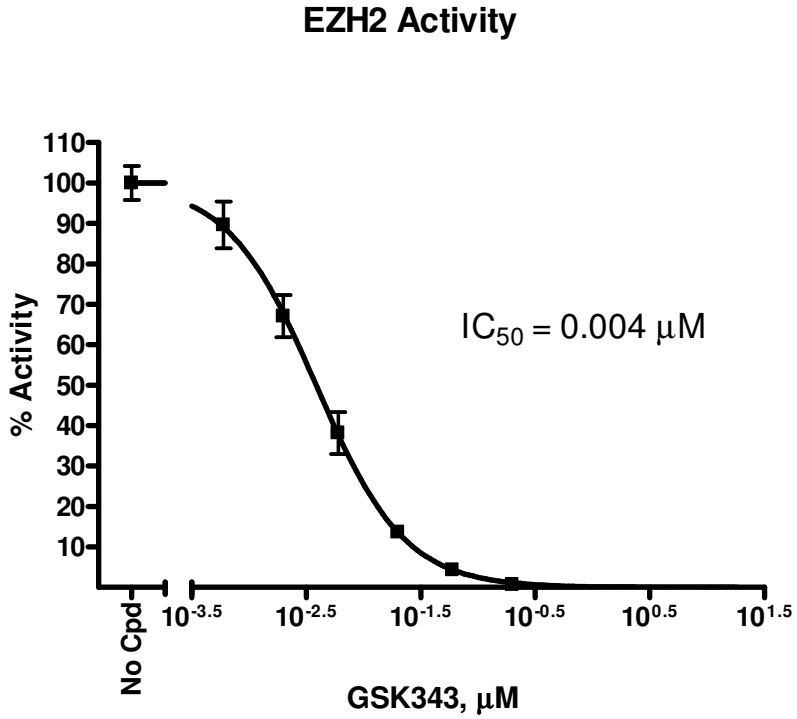
3.2.21. EZH2

Table 3.2.21. Data for the Effect of GSK343 on EZH2 Activity

GSK343 [μM]	EZH2 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	26546	28871	96	104
0.0006	23240	26446	84	95
0.002	17170	20062	62	72
0.006	12073	9196	43	33
0.02	3705	4016	13	14
0.06	1313	1242	4	4
0.2	300	234	1	1
0.6	95	54	0	0
2	95	56	0	0
6	78	74	0	0
20	78	58	0	0
Background	80	88	0	0



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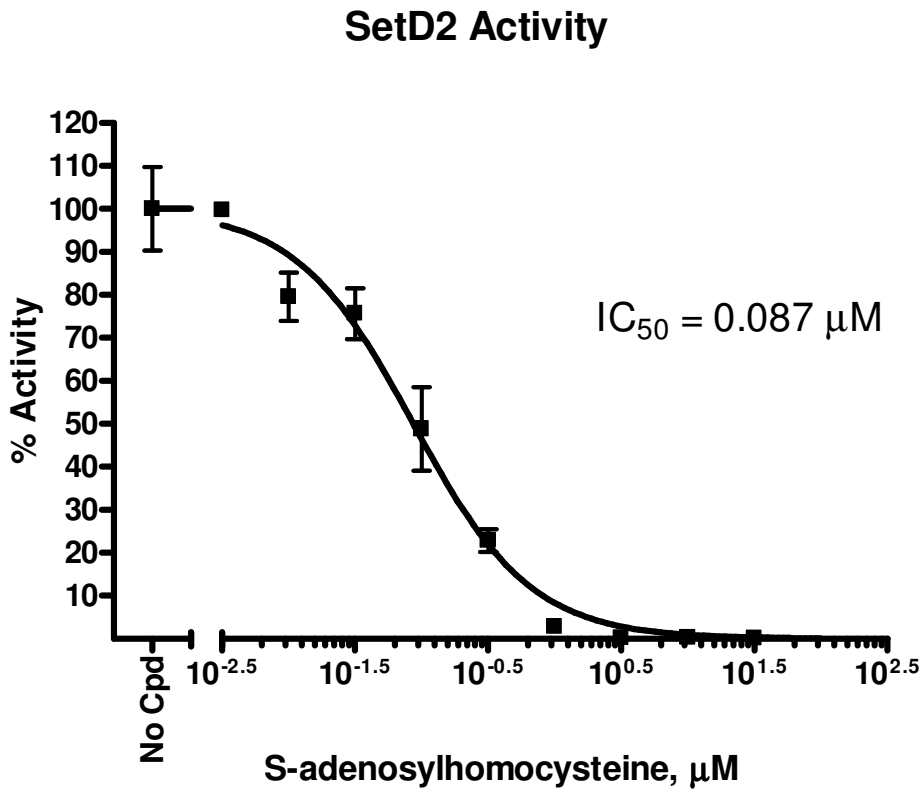
3.2.22. SETD2

Table 3.2.22. Data for the Effect of S-adenosylhomocysteine on SetD2 Activity

AdoHcy [µM]	SetD2 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	7895	9584	90	110
0.003	8752	8678	100	99
0.010	6484	7454	74	85
0.032	7141	6117	82	70
0.100	5148	3465	59	39
0.32	2289	1831	25	20
1.0	311	338	3	3
3.2	74	119	0	0
10.0	82	128	0	1
31.6	69	112	0	0
100	85	79	0	0
Background	73	92		



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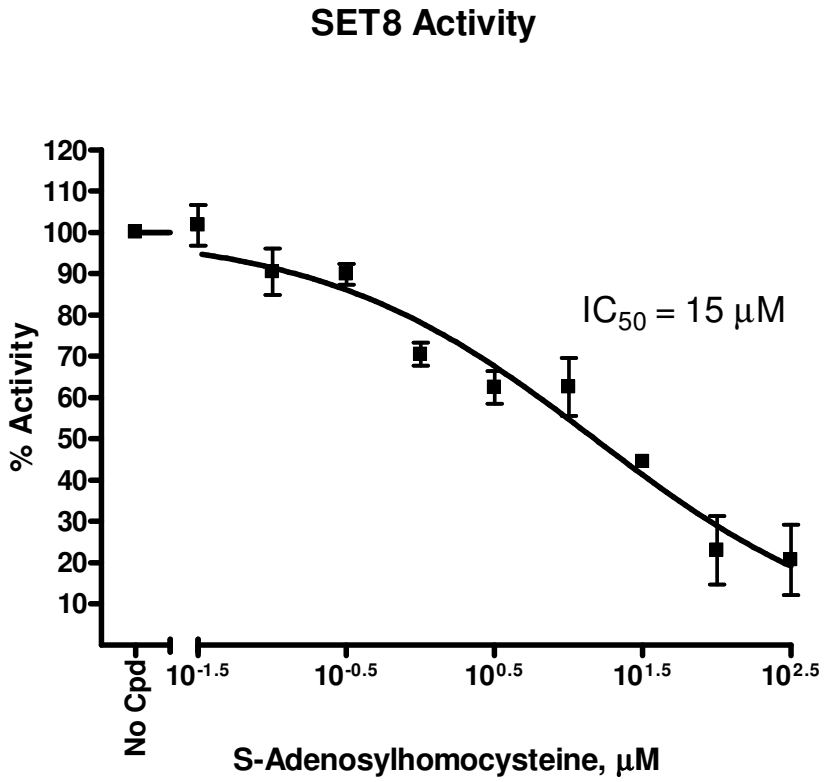
3.2.23. SET8

Table 3.2.23. Data for the Effect of S-adenosylhomocysteine on SET8 Activity

AdoHcy [μM]	SET8 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	544	538	101	99
0.03	527	570	97	107
0.1	524	475	96	85
0.3	486	508	87	92
1.0	425	401	73	68
3.2	395	361	66	58
10	409	348	70	55
32	305	295	46	43
100	243	171	31	15
317	234	160	29	12
Background	114	101		



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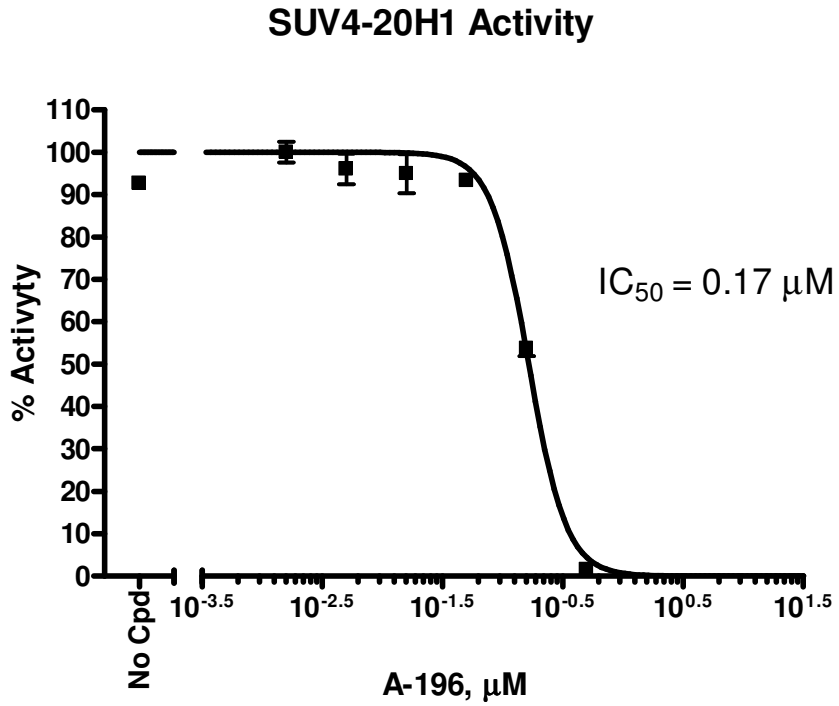
3.2.24. SUV4-20H1

Table 3.2.24. Data for the Effect of A-196 on SUV4-20H1 Activity

A-196 [μM]	SUV4-20H1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	42786	43786	92	94
0.002	45566	47847	98	102
0.005	43175	46556	92	100
0.02	42182	46484	90	100
0.05	44245	42965	95	92
0.2	25911	24282	55	52
0.5	784	837	1	2
1.6	72	44	0	0
5	67	63	0	0
16	54	29	0	0
50	57	22	0	0
Background	113	137		



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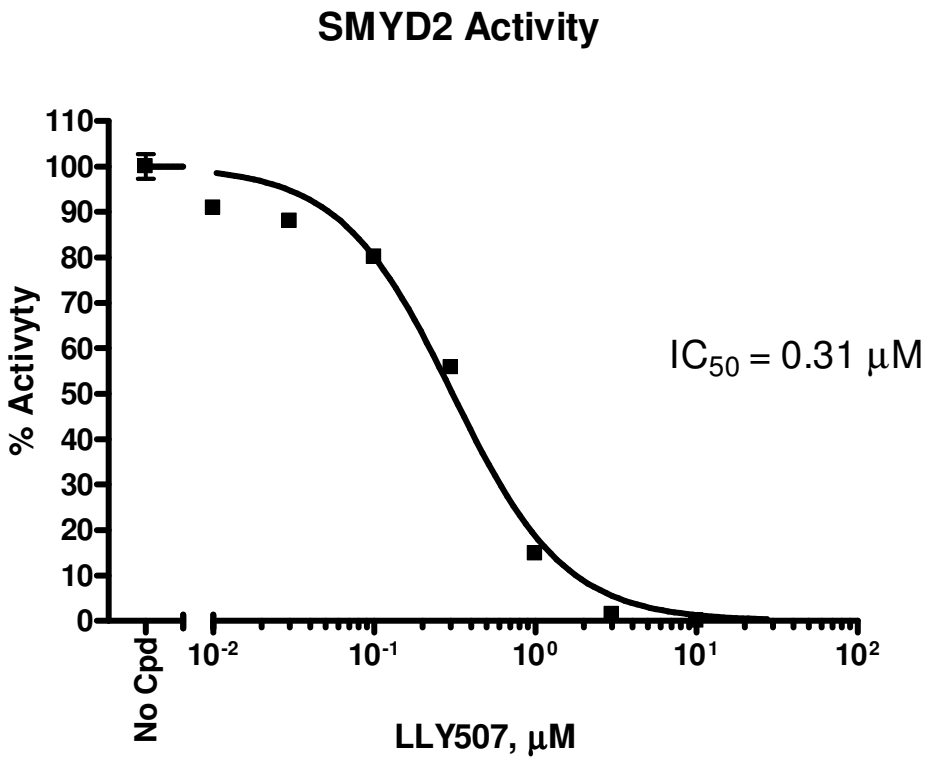
3.2.25. SMYD2

Table 3.2.25. Data for the Effect of LLY507 on SMYD2 Activity

LLY507 [μM]	SMYD2 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	44733	47219	97	103
0.001	42089	45818	92	100
0.003	42963	41993	93	91
0.01	41317	42270	90	92
0.03	41054	39842	89	87
0.1	36132	37592	79	82
0.3	25956	25409	56	55
1	6741	7043	15	15
3	755	697	1	1
10	90	83	0	0
30	51	49	0	0
Background	85	87	0	0



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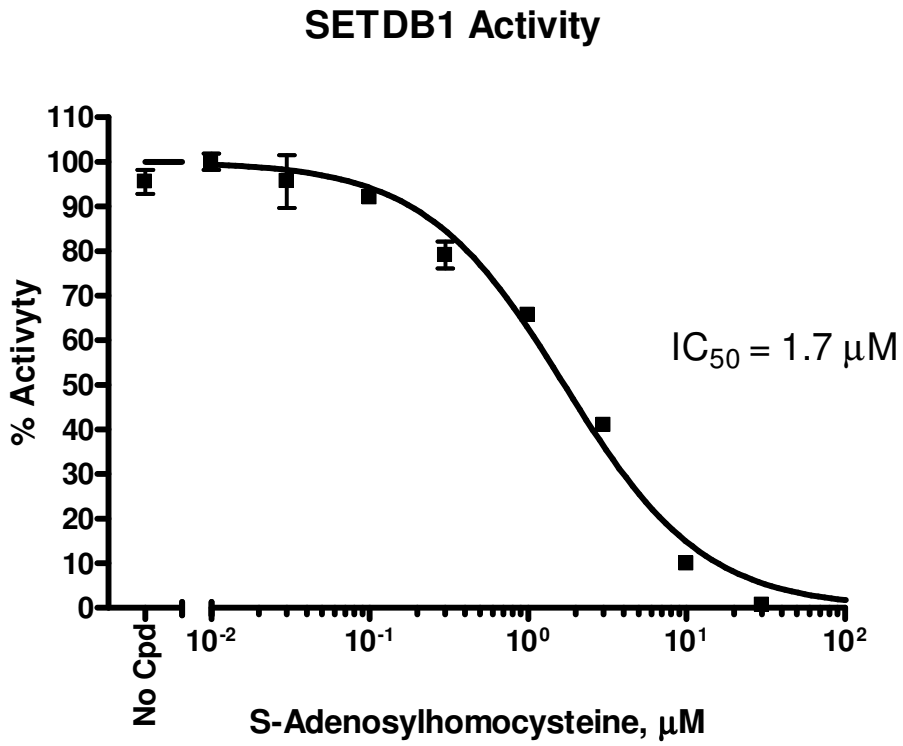
3.2.26. SETDB1

Table 3.2.26. Data for the Effect of S-adenosylhomocysteine on SETDB1 Activity

AdoHcy [µM]	SETDB1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No compound	3032	2869	103	97
0.01	3032	3142	103	107
0.03	2774	3132	94	106
0.1	2868	2822	97	96
0.3	2544	2362	86	80
1	2011	2082	68	70
3	1252	1348	41	45
10	326	395	9	12
30	75	83	1	1
100	55	53	0	0
316	35	52	-1	0
Background	31	87		



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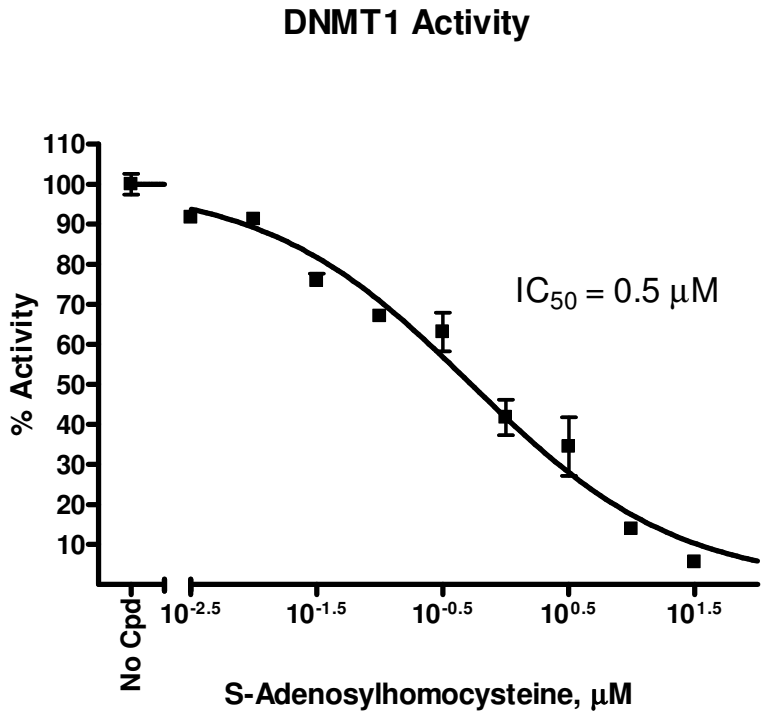
3.2.27. DNMT1

Table 3.2.27. Data for the Effect of S-adenosylhomocysteine on DNMT1 Activity

AdoHcy [µM]	DNMT1 Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	1427	1361	103	97
0.003	1290	1288	92	92
0.01	1286	1280	91	91
0.03	1110	1066	78	74
0.1	979	972	67	67
0.3	864	987	58	68
1	598	711	37	46
3	655	469	42	27
10	315	286	15	13
32	191	201	5	6
100	139	101	1	0
Background	114	135		



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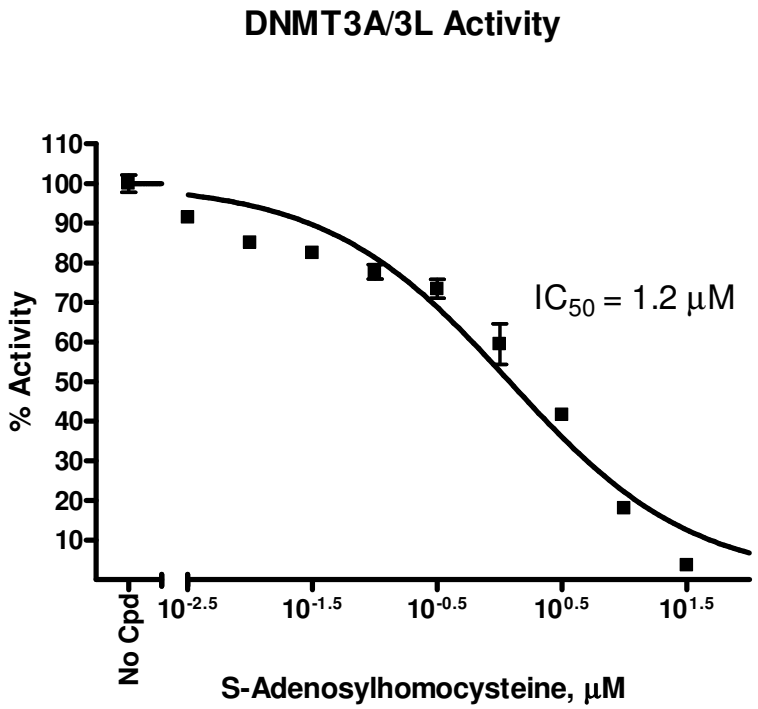
3.2.28. DNMT3A/3L

Table 3.2.28. Data for the Effect of S-adenosylhomocysteine on DNMT3A/3L Activity

AdoHcy [µM]	DNMT3A/3L Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	1567	1634	98	102
0.003	1456	1486	90	92
0.01	1393	1355	86	84
0.03	1336	1336	82	82
0.1	1237	1291	76	79
0.3	1163	1235	71	76
1	1066	911	65	54
3	700	738	40	43
10	380	346	19	17
32	140	151	3	4
100	100	71	1	0
Background	99	83		



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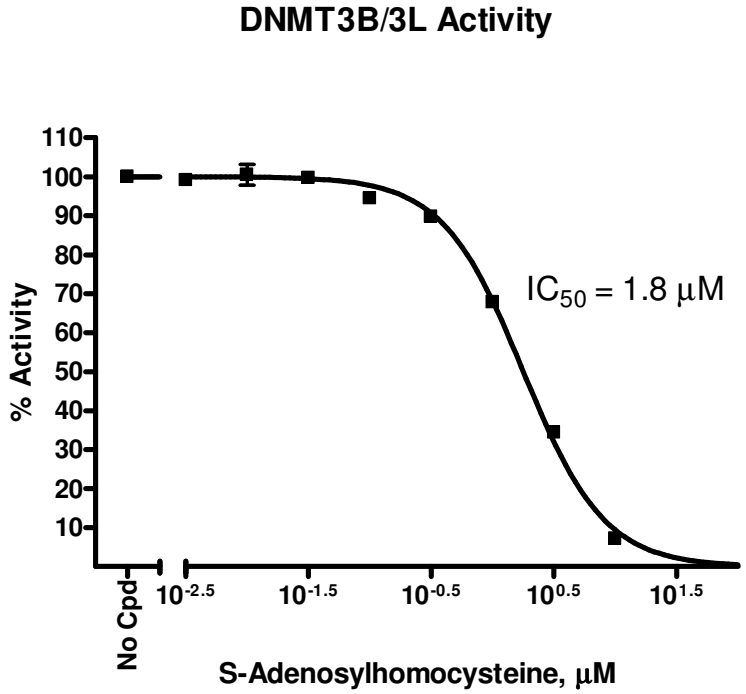
3.2.29. DNMT3B/3L

Table 3.2.29. Data for the Effect of S-adenosylhomocysteine on DNMT3B/3L Activity

AdoHcy [μM]	DNMT3B/3L Activity (Luminescence)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	1493	1494	100	100
0.003	1479	1483	99	99
0.01	1538	1463	103	98
0.03	1511	1467	101	98
0.1	1427	1402	95	94
0.3	1350	1347	90	90
1	1016	1059	66	69
3	550	578	33	35
10	181	177	7	7
32	50	47	0	0
100	41	35	0	0
Background	74	82		



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4. Quality Assurance Statement

I certify that the results presented in this report were generated using the materials and methods mentioned and that these results reflect the Raw Data.

_____
Henry Zhu, Ph.D.
President

_____
Date