



SAPIENZA
UNIVERSITÀ DI ROMA

Lessons in Epigenetics

Antonello Mai

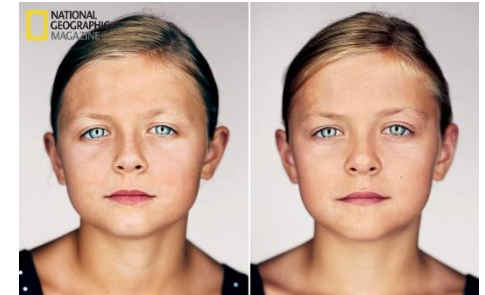
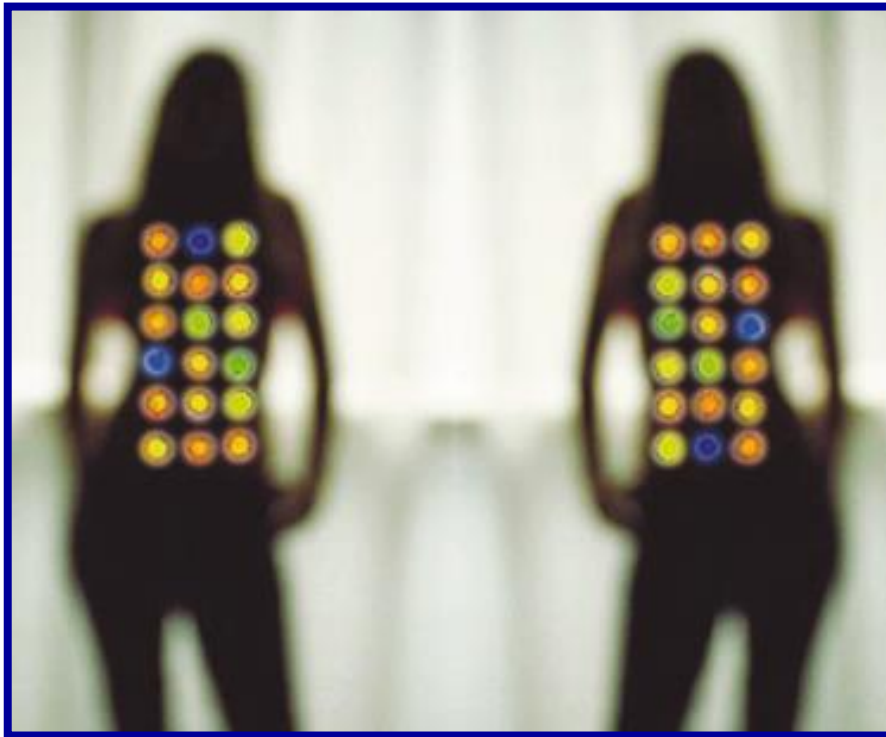
Università degli Studi di Roma *La Sapienza*

Nel mezzo del cammin di nostra vita
mi ritrovai per una selva oscura
ché la diritta via era smarrita.

*Nel mezzo del cammin di nostra vita
mi ritrovai per una selva oscura
ché la diritta via era smarrita.*

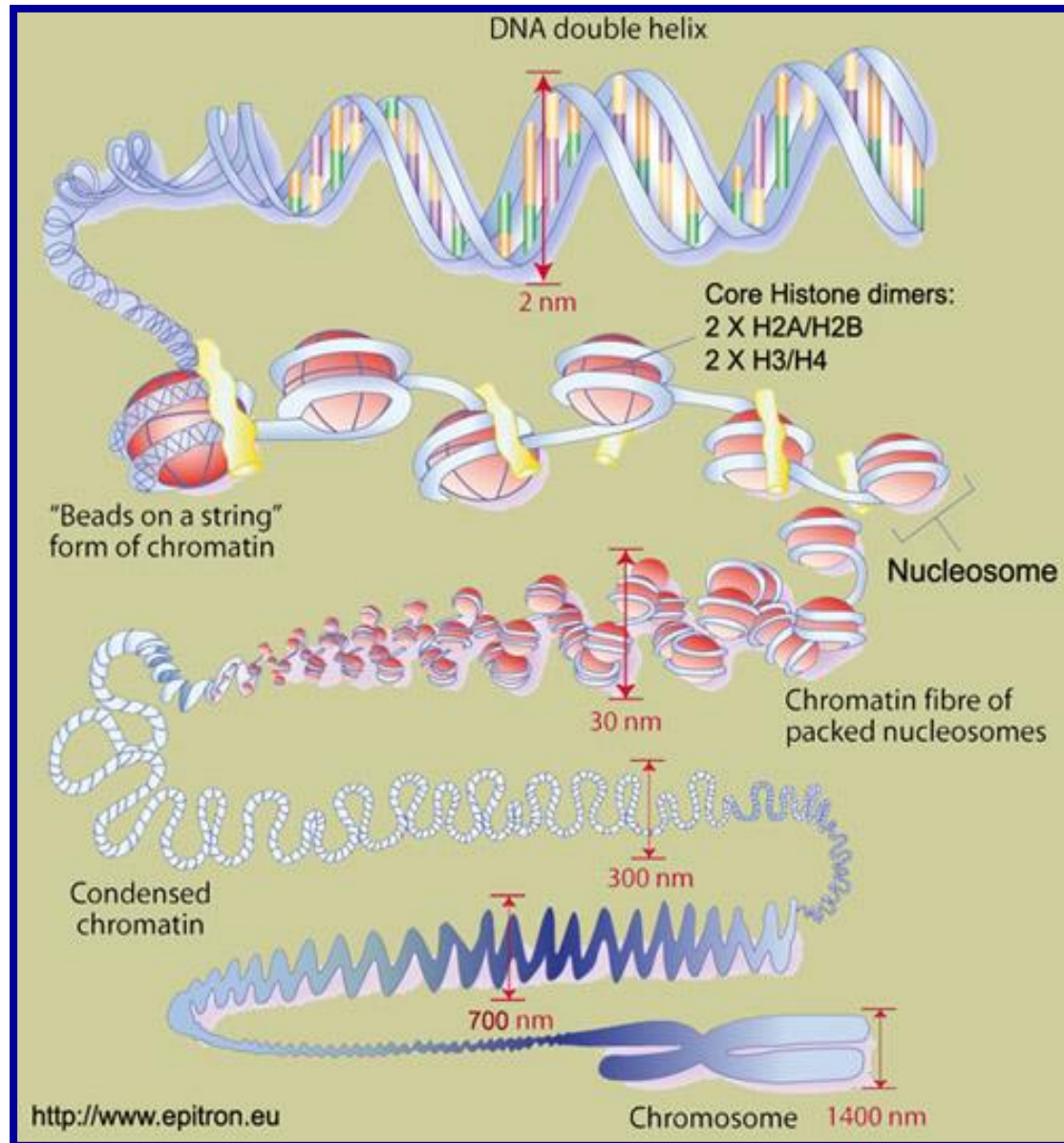
NEL MEZZO DEL CAMMIN DI NOSTRA VITA
MI RITROVAI PER UNA SELVA OSCURA
CHÉ LA DIRITTA VIA ERA SMARRITA.

Genetics or Epigenetics?

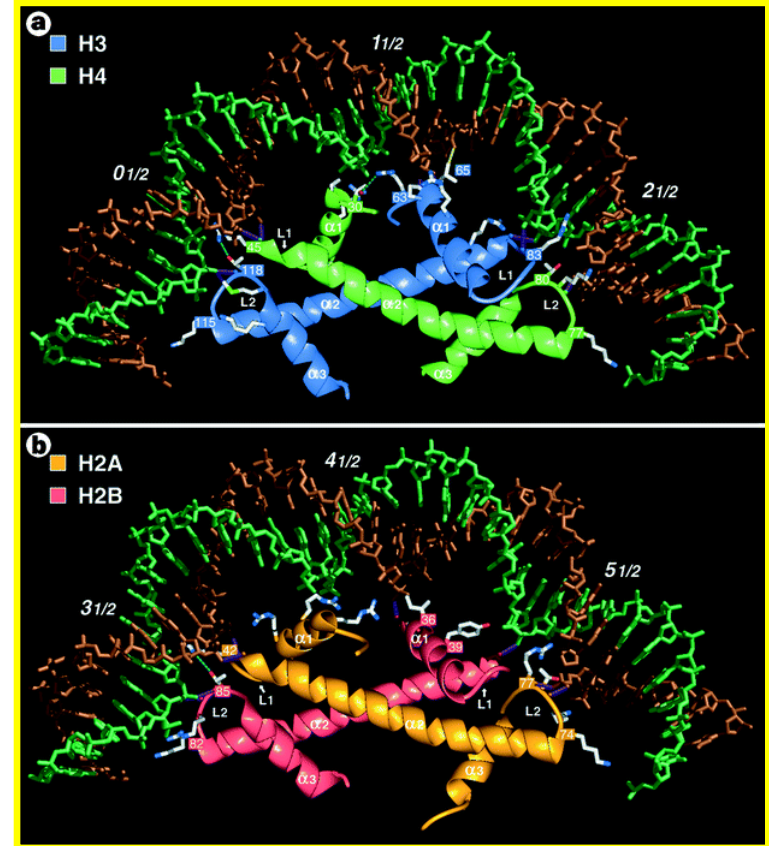
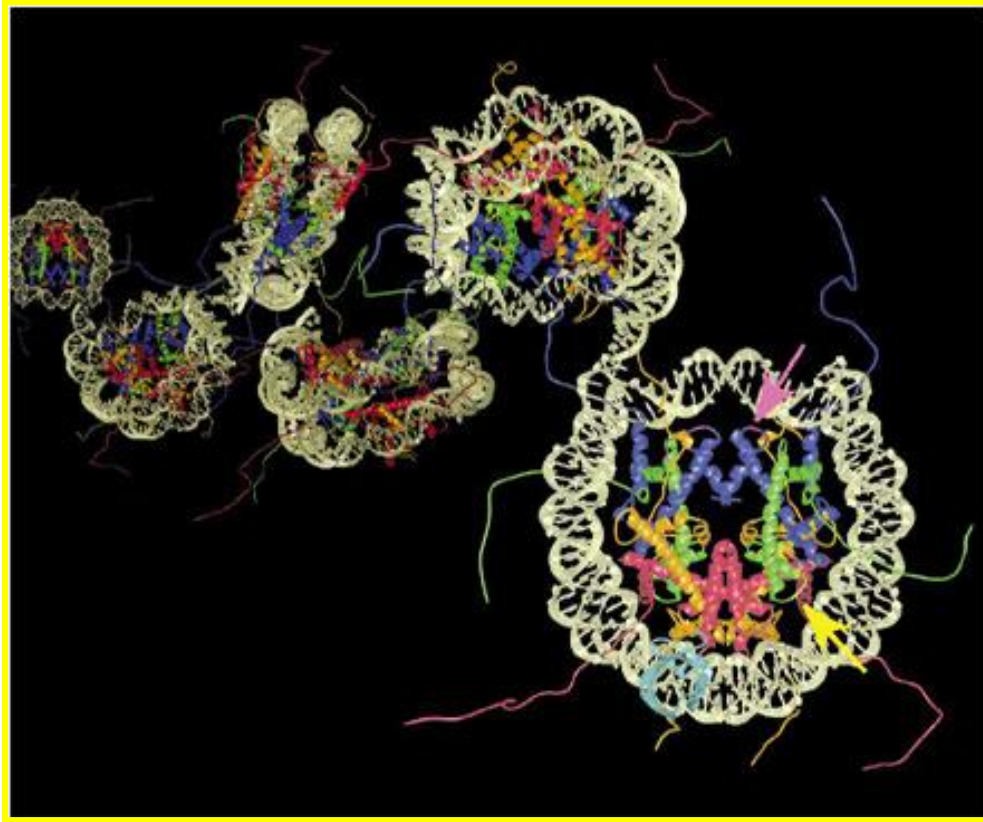


Changes to the genome that do not affect DNA sequence may help to explain differences between genetically identical twins

The chromatin structure

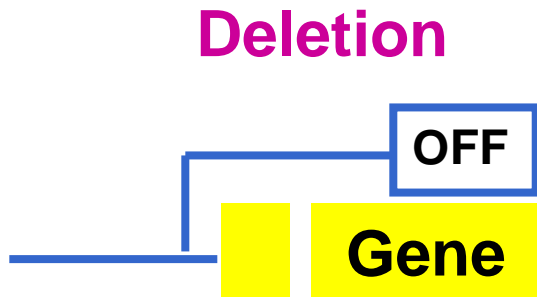
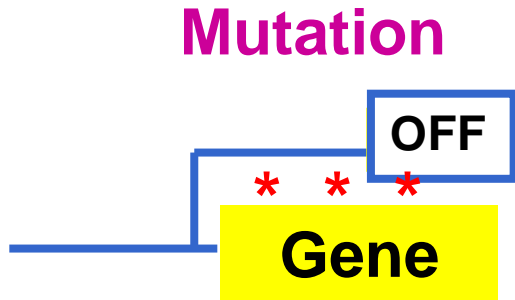


Crystal structure of the nucleosome core



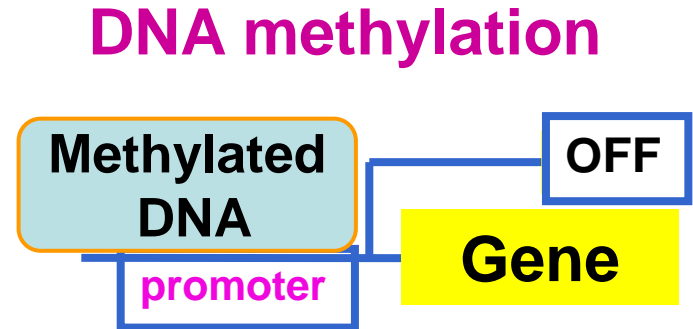
Luger, K. *et al.*, *Nature* **1997**, 389, 251-260.

Genetics



Gene Therapy

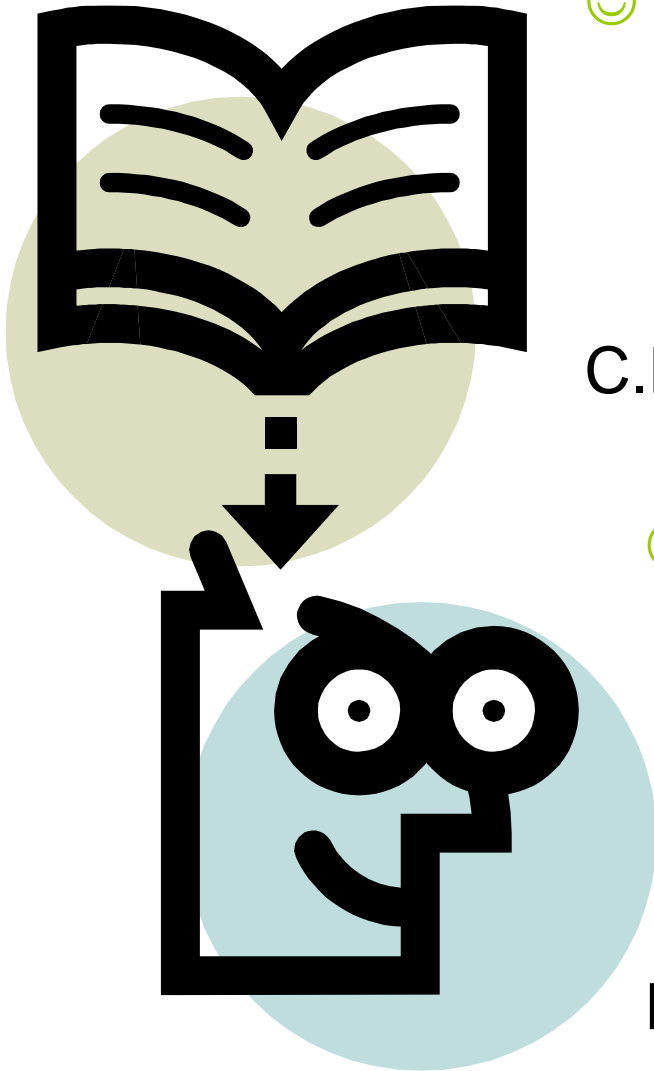
*Epi*Genetics



Epigenetic Therapy

Epigenetics (1997): the study of meiotically/mitotically heritable changes in gene expression that are not caused by alteration of the DNA sequence

Epigenetics: the story



- ☺ Epigenetics (**1942**): the interactions between genes and their products leading to the realisation of the phenotype

C.H. Waddington, *Endeavour* **1942**, 1, 18-20

- ☺ Epigenetics (**1997**): the study of meiotically/mitotically heritable changes in gene expression that are not caused by alteration of the DNA sequence

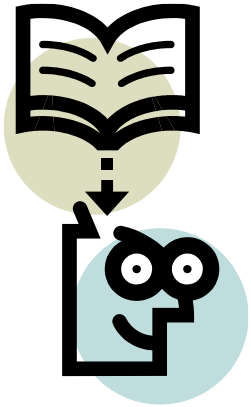
K. Luger et al., *Nature* **1997**, 389, 251-260

☺ 3 major mechanisms in epigenetics:

1) DNA methylation

2) covalent modifications of histones with changes in the chromatin structure

3) siRNA, ncRNA



1964: Reversible acetylation of core

1996: Discovery of the first HAT³⁻⁵ and

1997: X-ray structure of the nucleosome⁷

1999: Discovery of NAD-dependent

2003: A link between histone methylation

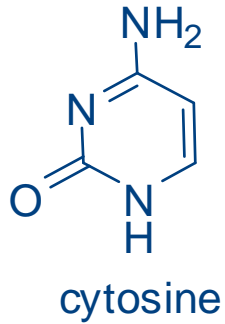
2004: Discovery of the first histone

**2006: Discovery of the histone
demethylating enzyme JM.J¹¹**

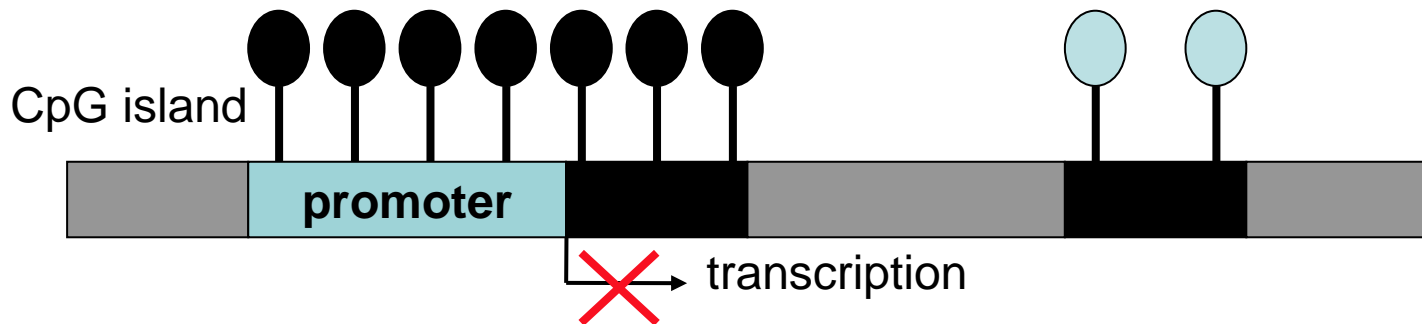
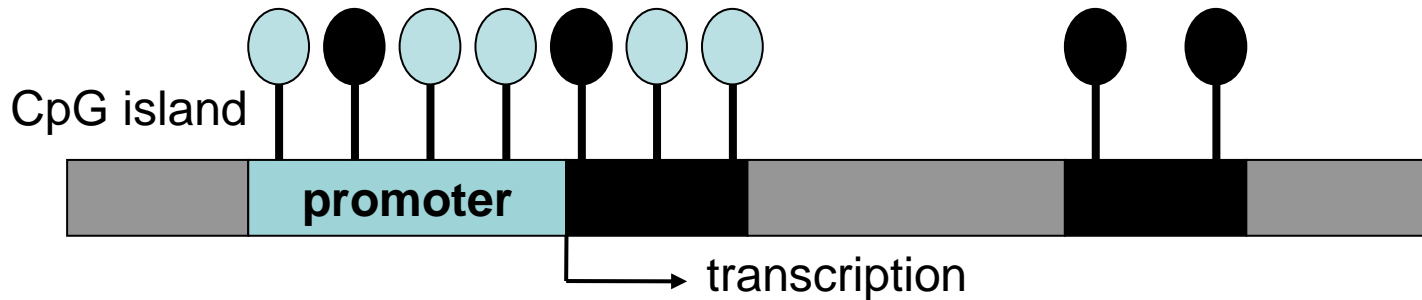
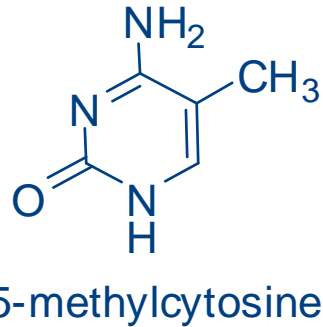
**2012: Discovery of the first *reader* family:
Bromodomain as a valuable target for cancer¹²**

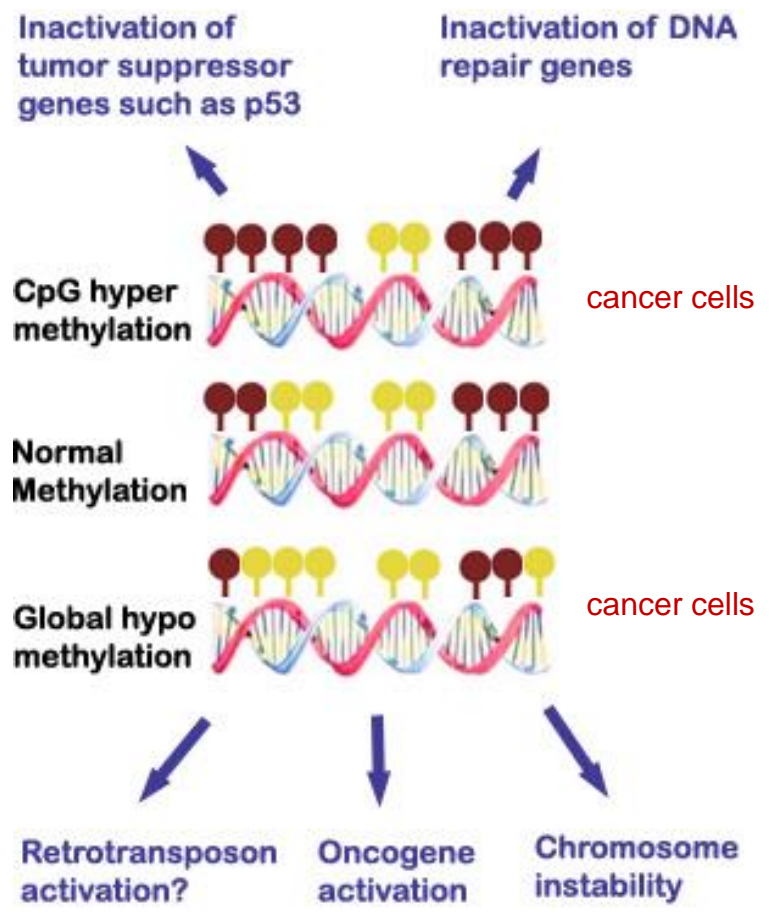
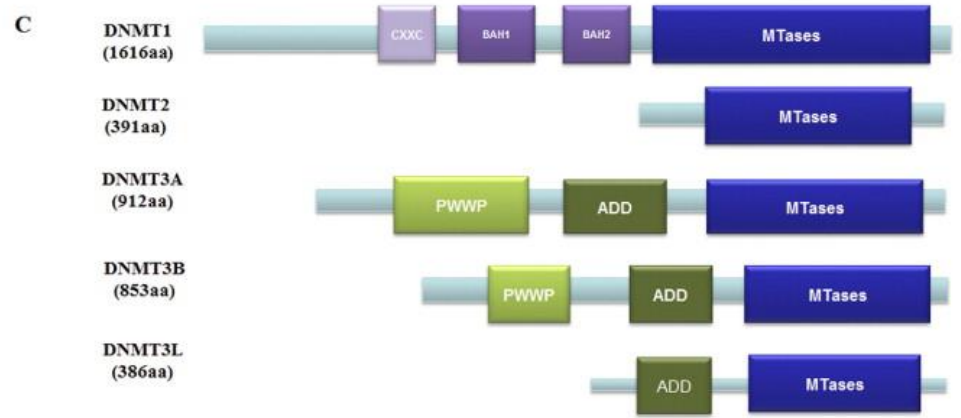
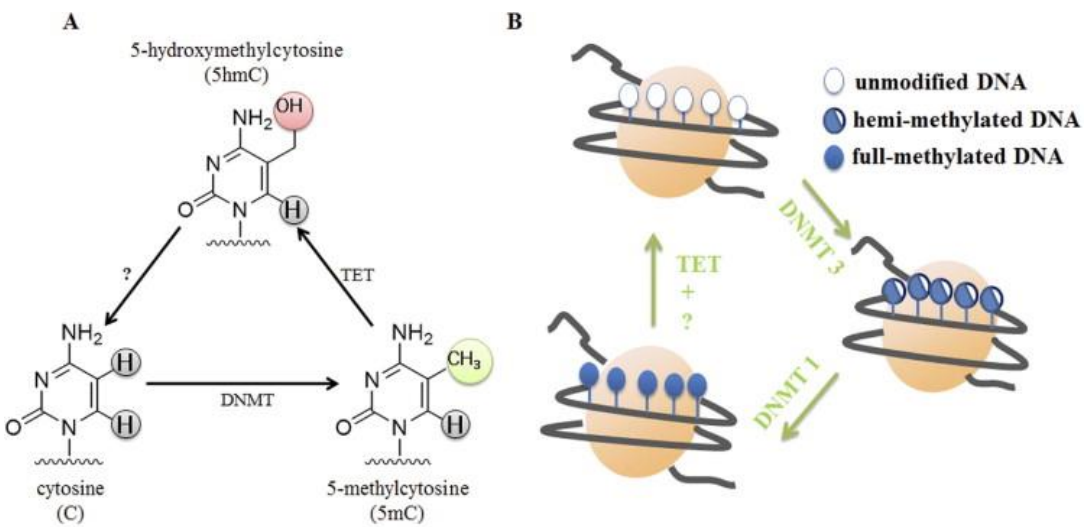
¹²Filippakopoulos, P. *et al.*, *Cell* 2012, 149, 214-231

DNA Methyltransferase inhibitors (DNMTi)

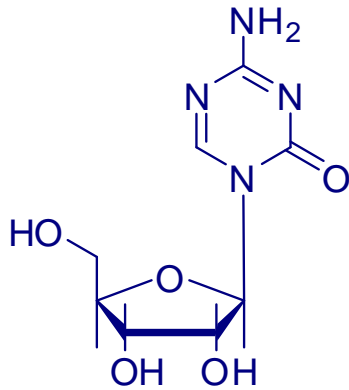


DNMT →



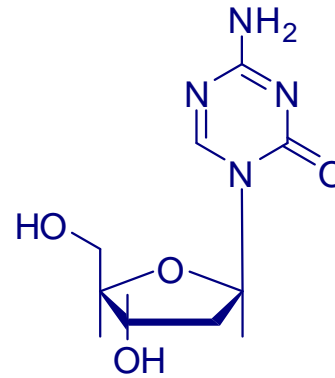


DNMTi: Nucleoside analogues



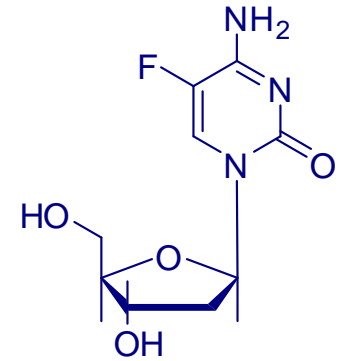
Azacytidine

FDA approved 2004 (myelodysplasia)
Phase I, II, III
(haematological malignancies, HMs)



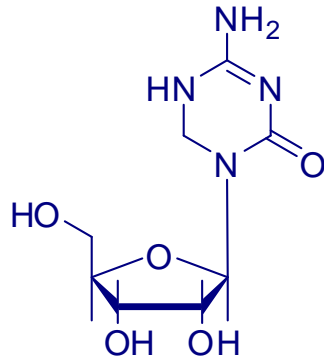
Decitabine

FDA approved 2006 (myelodysplasia)
Phase I, II, III
(HMs, cervical, NSCLC)



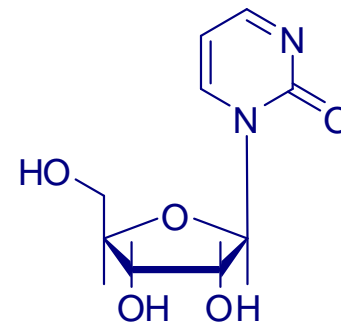
FdCyd

Phase I



DHAC

Phase I, II (ovarian cancer and lymphomas)



Zebularine

Preclinical

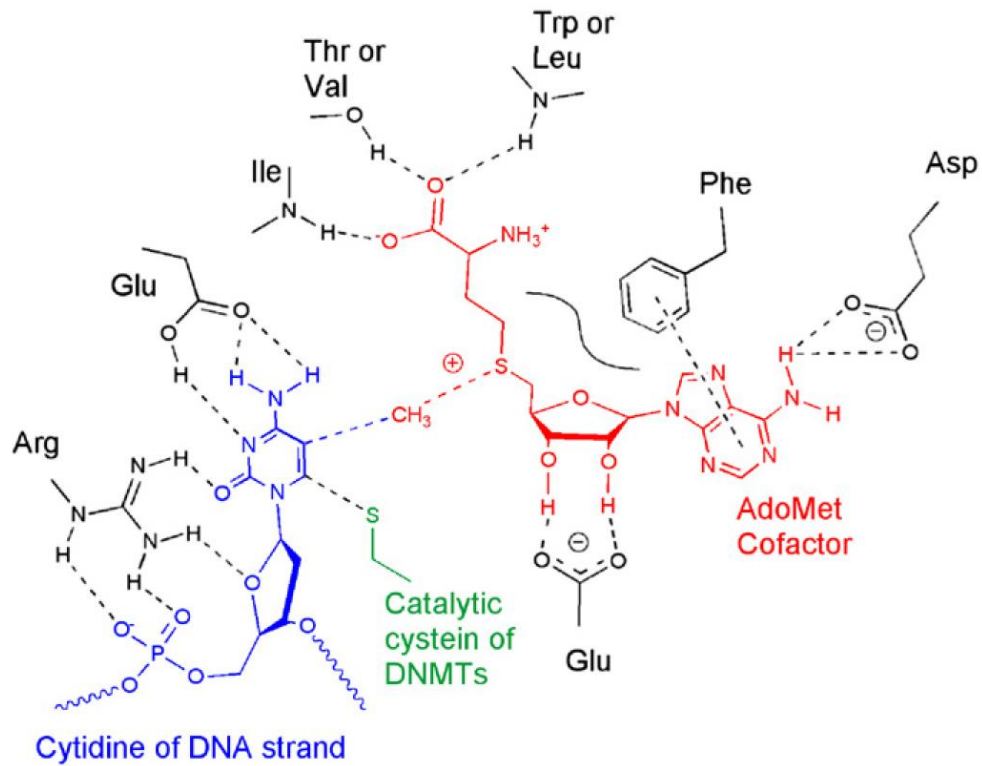
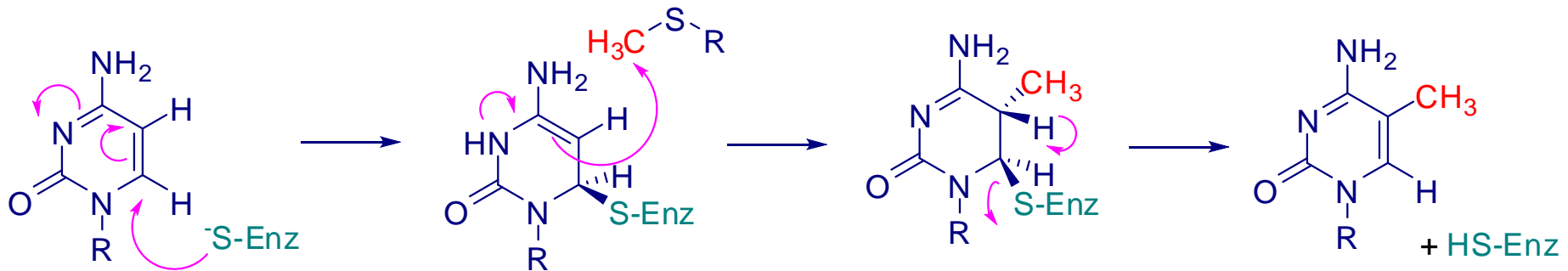


Fig. 4. Schematic representation of the catalytic site of the DNMTs. The targeted cytosine is in blue, the SAM cofactor in red and the catalytic thiolate in green.



Mechanism-based inhibition of DNMTs by cytosine analogues

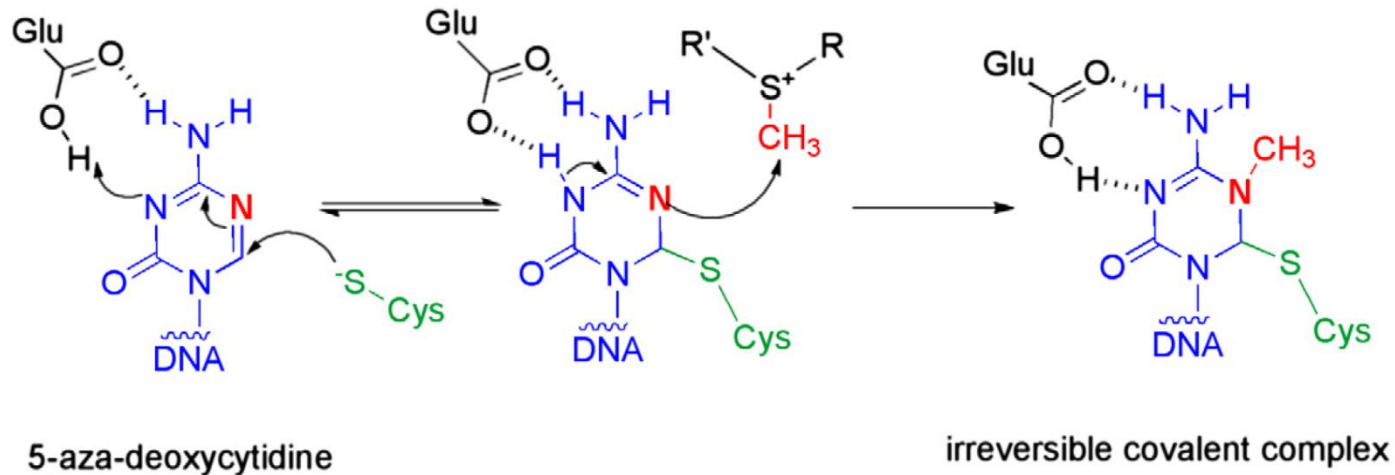
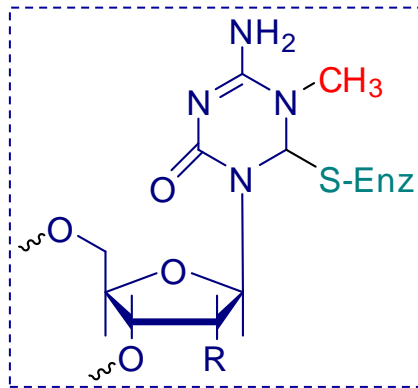
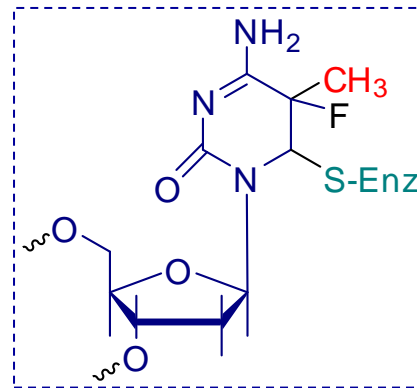


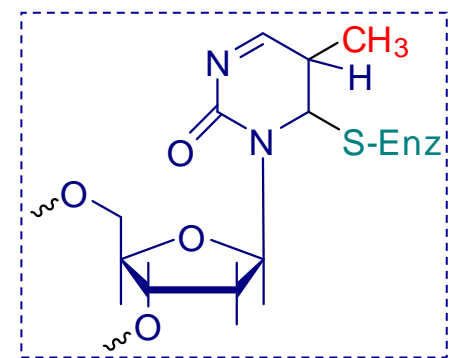
Fig. 8. Mechanism of the 5-azacytidine and 5-aza-2'-deoxycytidine.



Azacytidine/Decitabine adduct

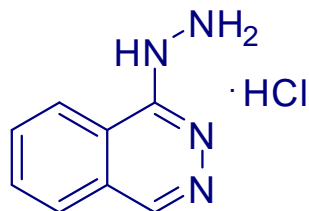


FdCyd adduct

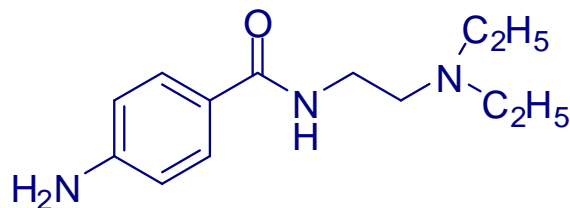


Zebularine adduct
????? reversible!

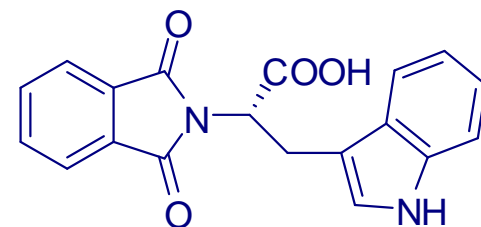
DNMTi: Non-nucleoside analogues



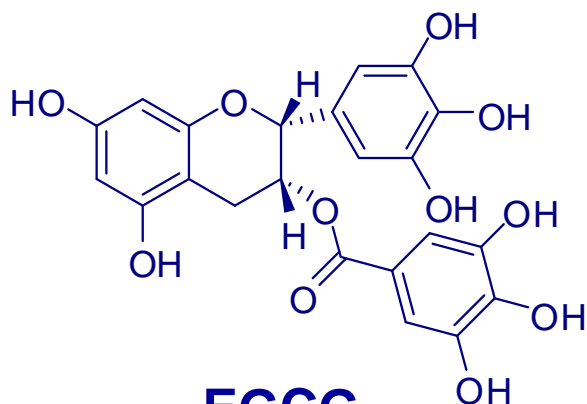
Hydralazine hydrochloride
Phase I (cervical cancer)



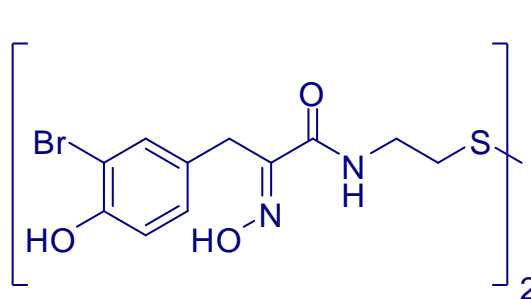
Procainamide
Preclinical



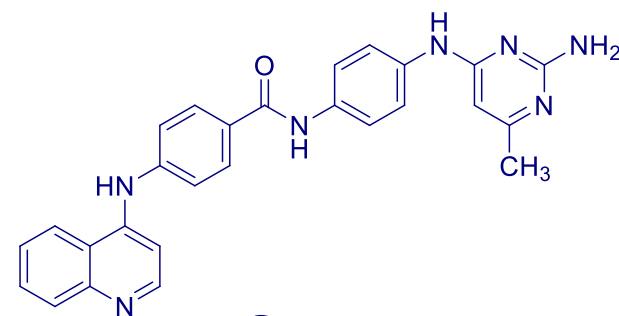
RG108
Preclinical



EGCG
Preclinical



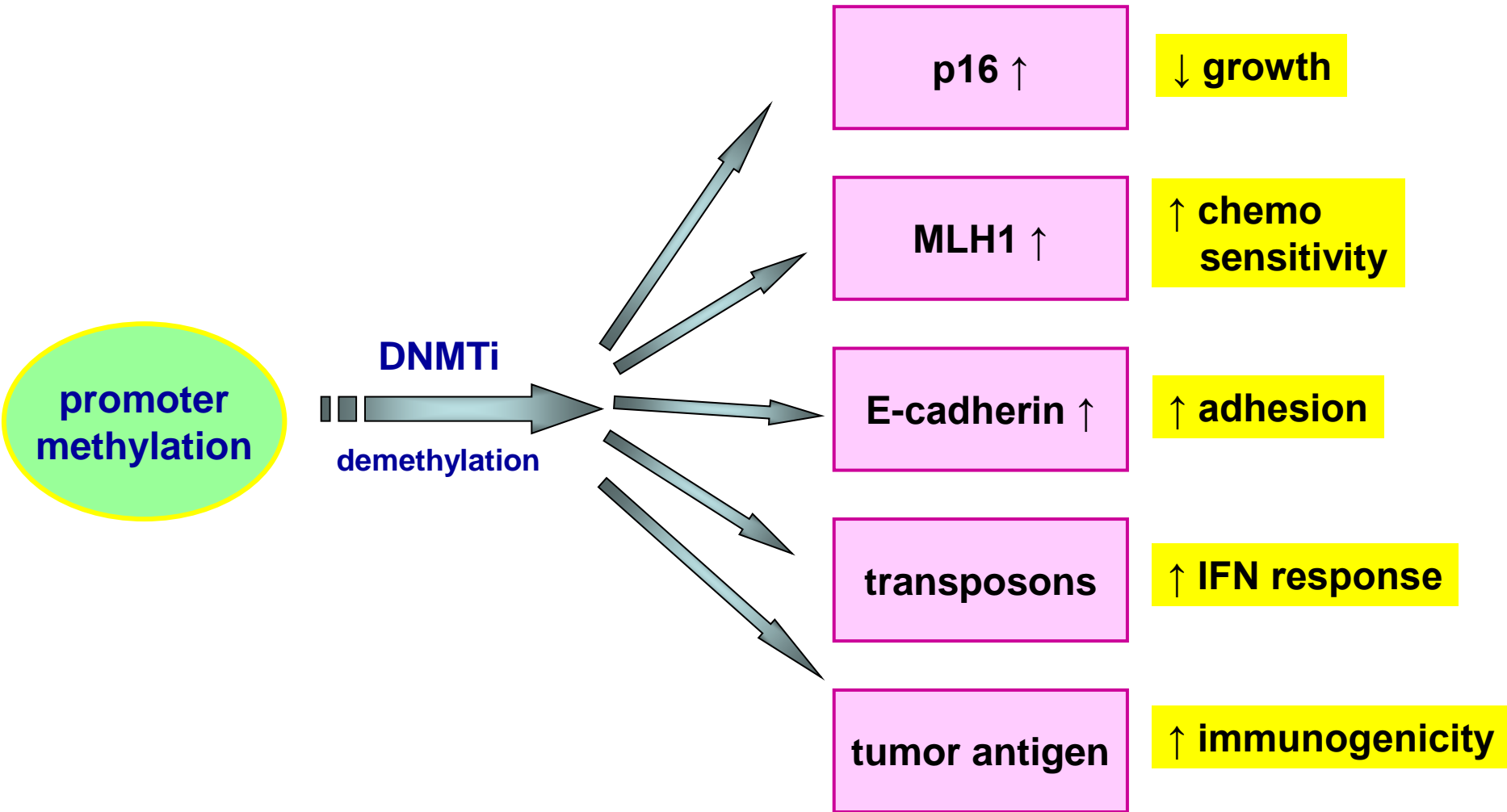
Psammaplin A
Preclinical



SGI-1027

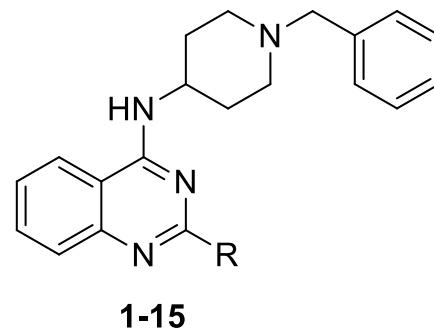
MG98
human DNMT1 antisense oligonucleotide, Phase I (advanced/metastatic solid tumors)

Effects of DNMTi on gene expression

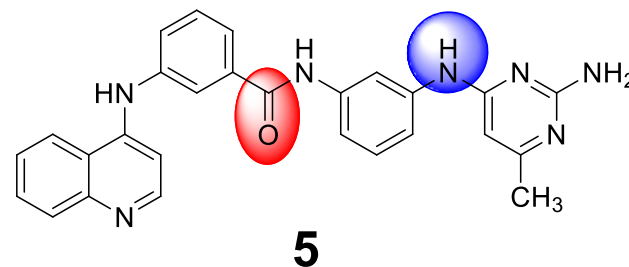


DNMT Inhibition: Our Experience

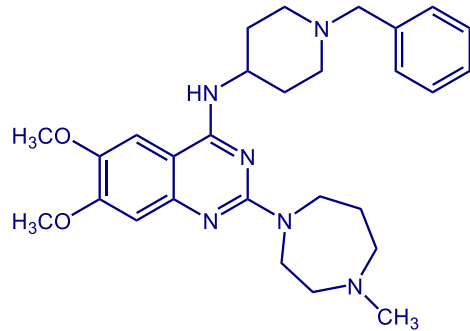
1. – The Quinazoline Story



2. – SGI-1027 analogues with improved activity



From HKMT inhibitors to JMJC inhibitors

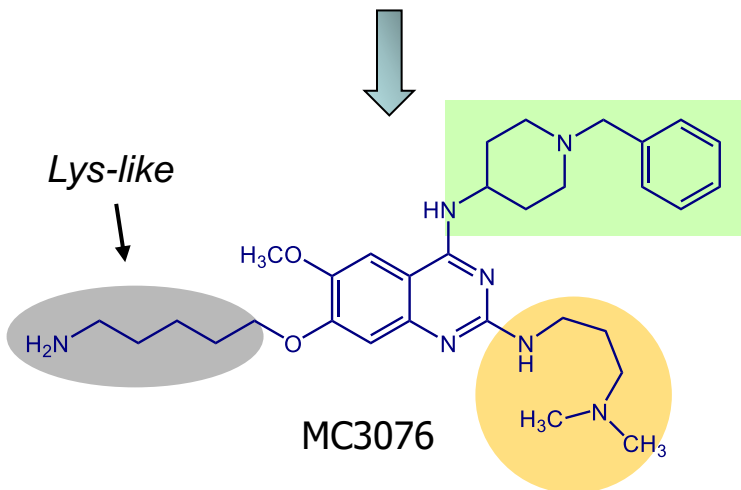


BIX-01294

Kubicek S *et al*, *Mol Cell* 2007, 25, 473

IC₅₀ vs G9a: : 1.7 μM

IC₅₀ vs KIAA1718 (JHDM): 16.5 μM

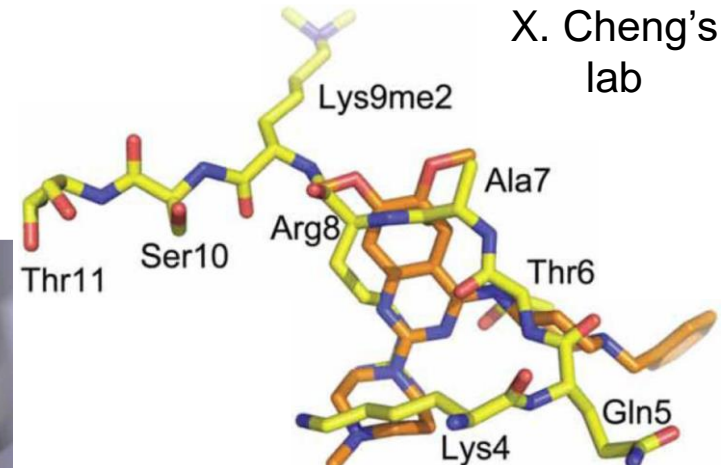
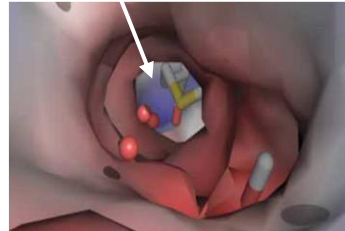


MC3076

IC₅₀ vs G9a: : 0.27 μM

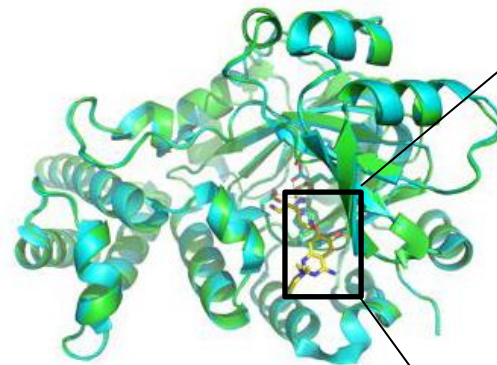
IC₅₀ vs KIAA1718 (JHDM): 3.7 μM

K9 channel empty!



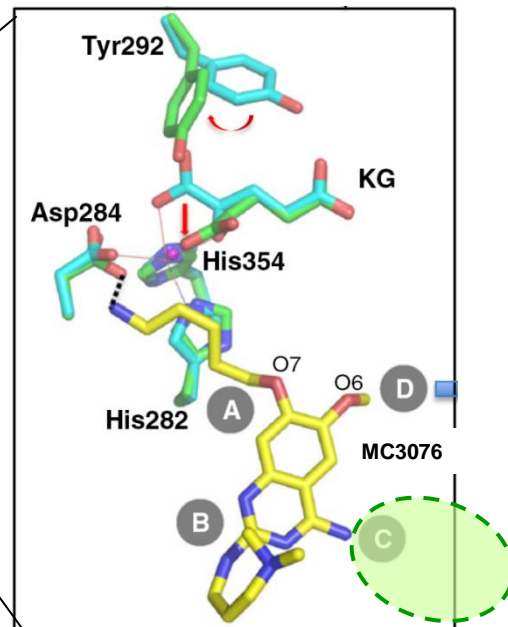
superimposition **GLP-BIX** and **GLP-H3** complexes

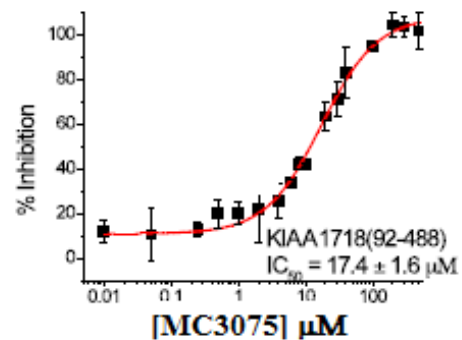
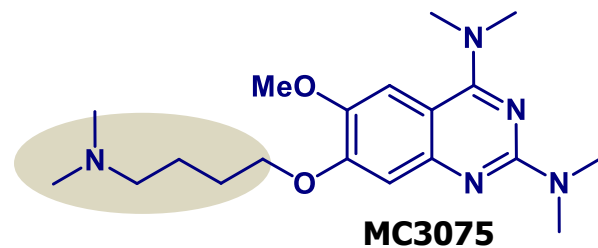
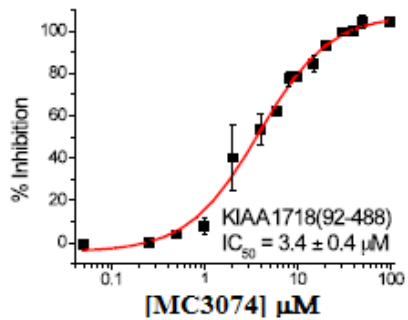
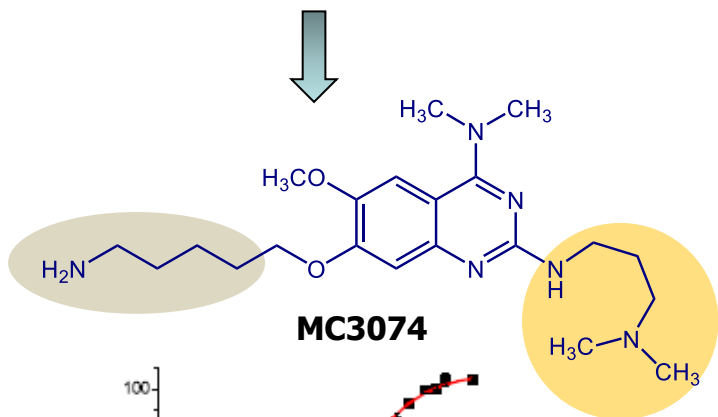
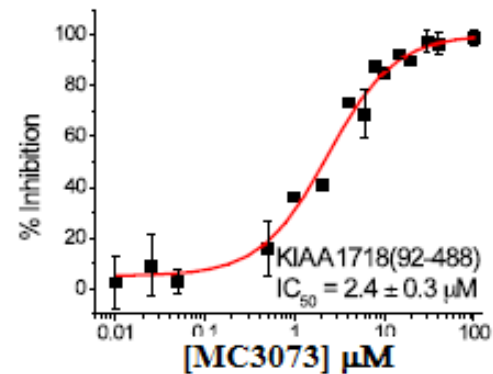
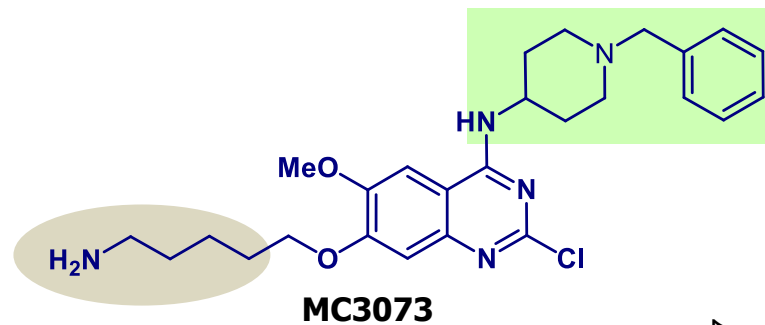
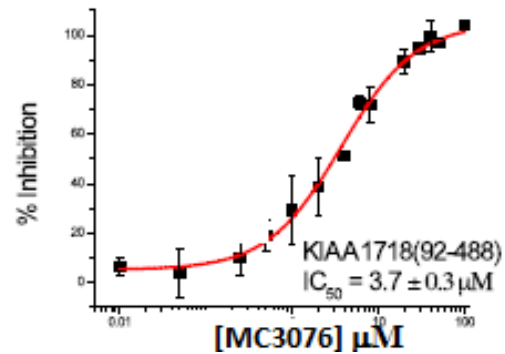
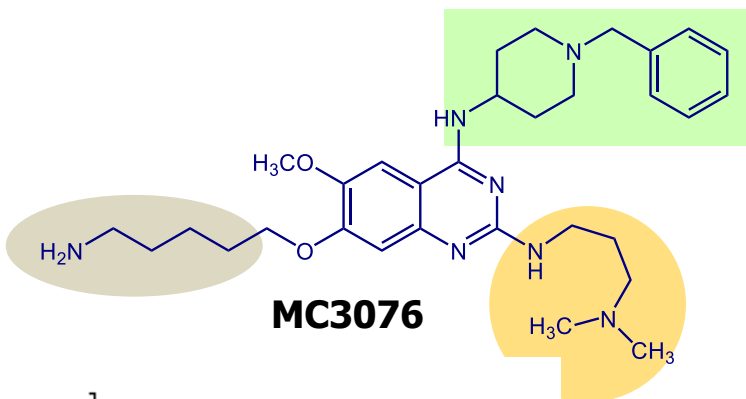
Chang Y *et al.*, *Nature Struct Mol Biol* 2009, 16, 312-317



KIAA1718-MC3076 complex

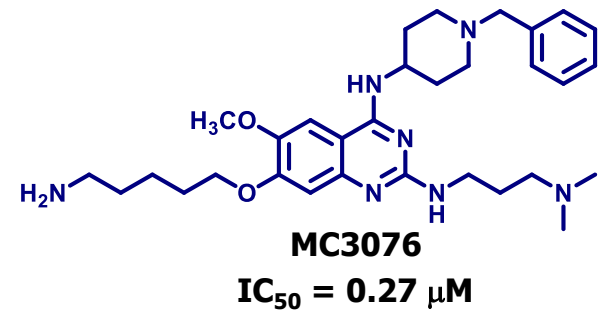
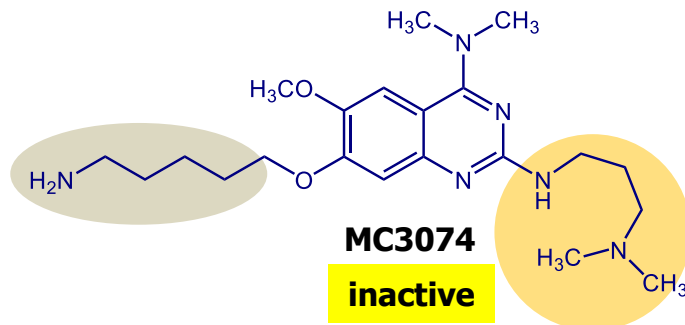
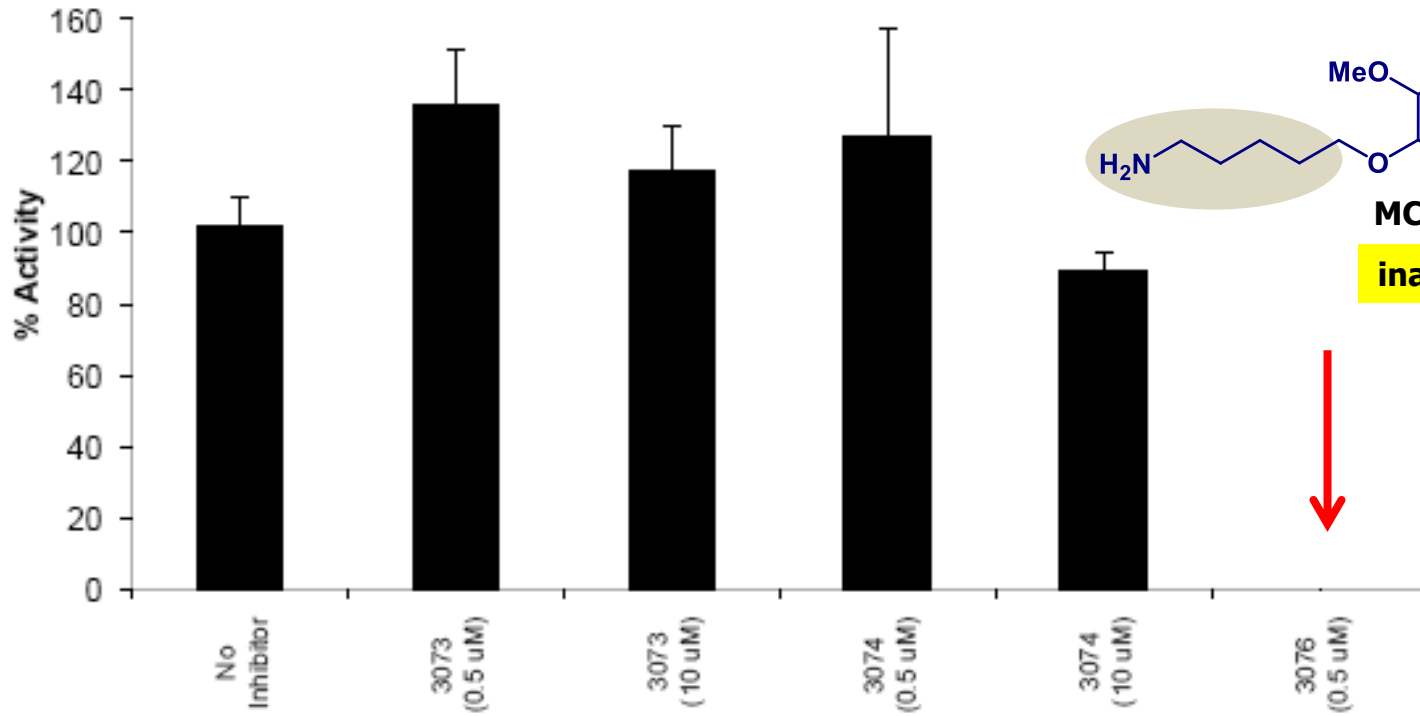
Upadhyay AK *et al*,
J Mol Biol 2012, 416, 319-327



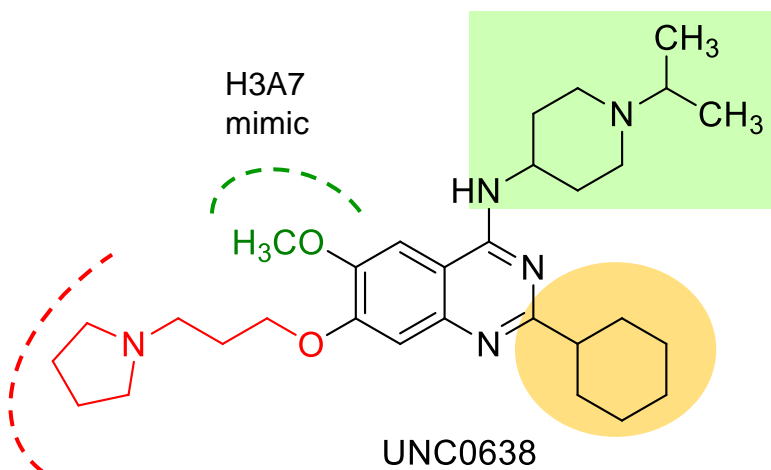


KIAA/JMJ-selective inhibitors

% GLP residual activity



Quinazoline-based DNMT inhibitors



Vedadi M *et al*, *Nat Chem Biol* 2011, 7, 566

H3K9me1/2 mimic

IC₅₀

G9a = 0.012 μM

GLP = 0.019 μM

SUV39H1, SUV39H2,

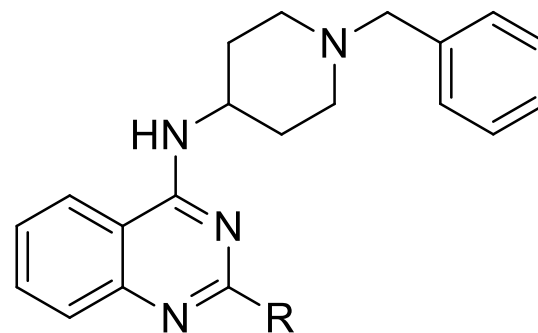
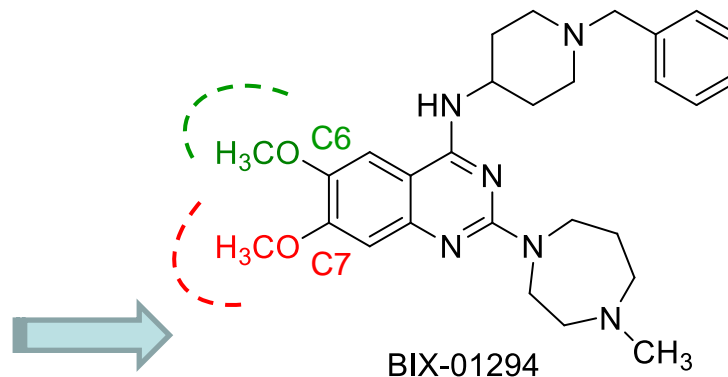
SETD7, SETD8,

MLL, EZH2,

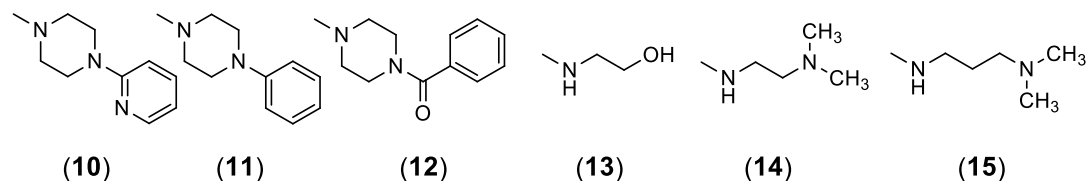
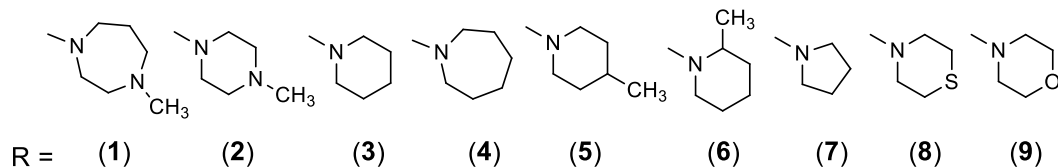
PRMT1, PRMT3 = >10 μM

JMJD2E = 4.5 μM

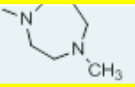
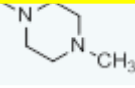
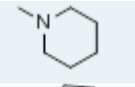
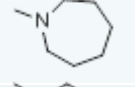
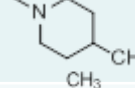
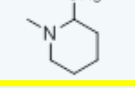
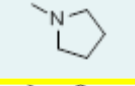
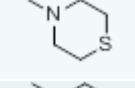
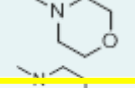
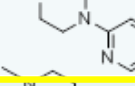
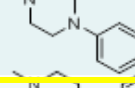
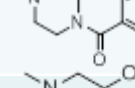
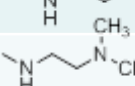
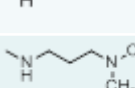

DNMT1 = 107 μM

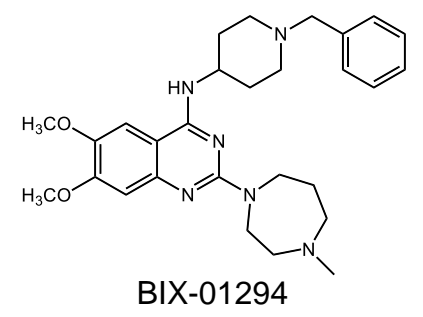
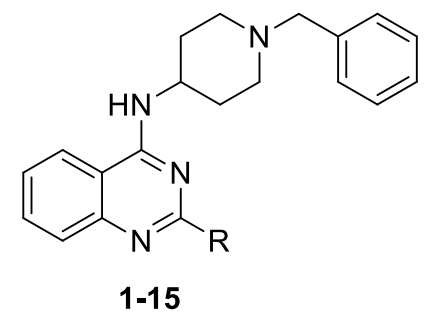


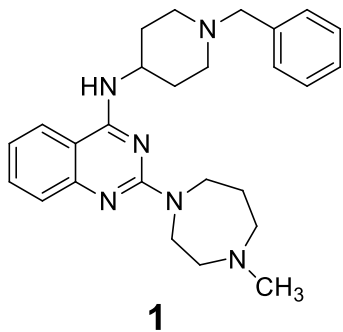
1-15



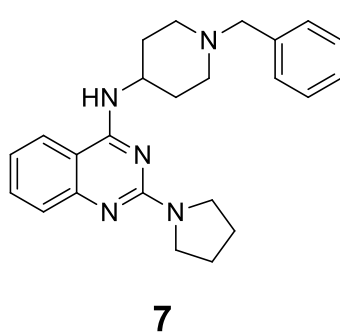
Rotili D *et al*, *PloS One* 2014, 9, e96941

compd	R	% inhibition			
		hDNMT1 100 μ M	catalytic hDNMT3A		
			100 μ M	33 μ M	10 μ M
1		0			
2		0			
3		0			
4		0			
5		0			
6		0			
7		47 \pm 2			
8		0			
9		0			
10		0			
11		0			
12		0			
13		0			
14		0			
15		0			
BIX		35 \pm 8			

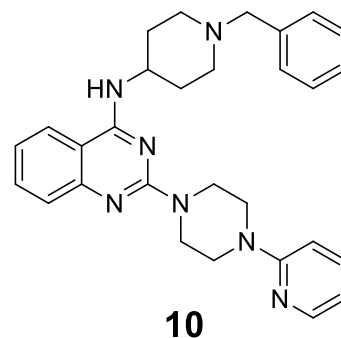




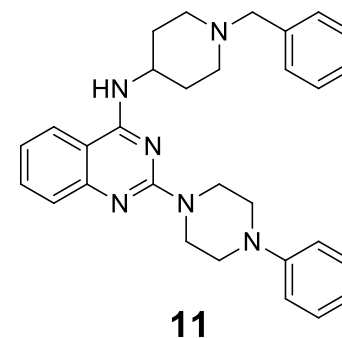
$IC_{50} = 10 \pm 2.5 \mu M$



$IC_{50} = 35 \pm 12 \mu M$

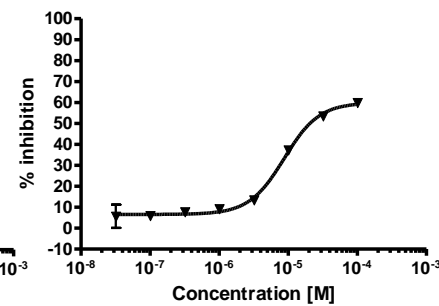
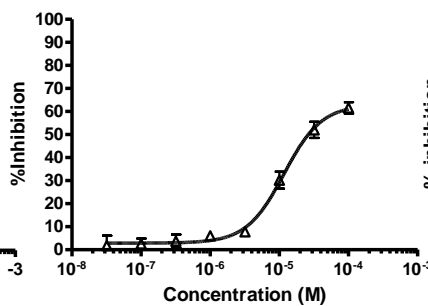
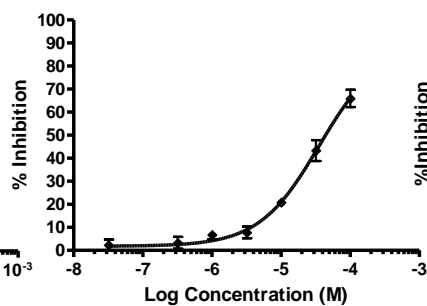
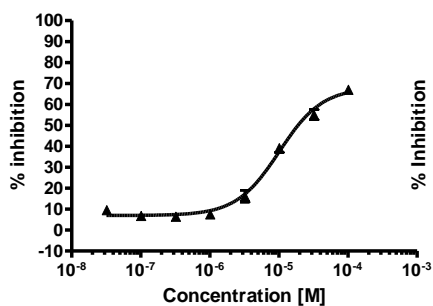


$IC_{50} = 12 \pm 4 \mu M$



$IC_{50} = 9 \pm 2 \mu M$

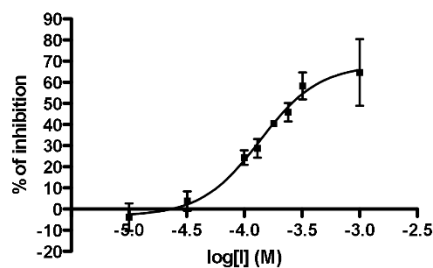
DNMT3A^a



$IC_{50} = 144 \pm 35 \mu M$

DNMT1^a

0% inhibition
at 100 μM



0% inhibition
at 100 μM

0% inhibition
at 100 μM

DNMT3A-
selective
inhibitors

G9a^b

$IC_{50} = >400 \mu M$

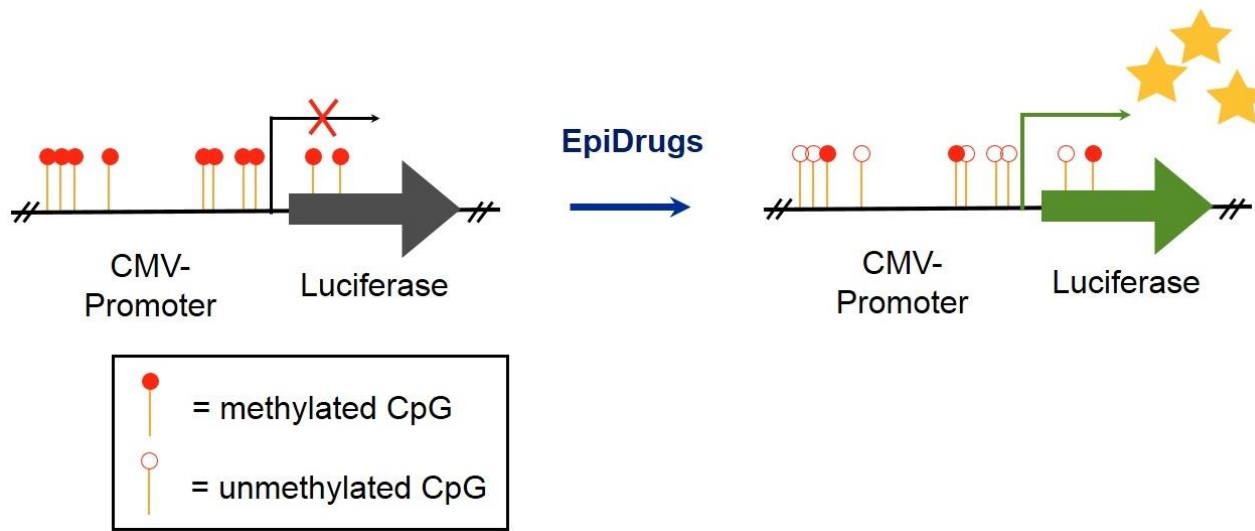
$IC_{50} = 643.0 \mu M$

$IC_{50} = >400 \mu M$

$IC_{50} = >400 \mu M$

^aValues are means of at least three experiments. ^bCompounds were tested in a 10-dose IC_{50} mode with 2-fold serial dilution starting at 400 μM . For 4, 13 and 14 it was no possible to determine IC_{50} values.

A

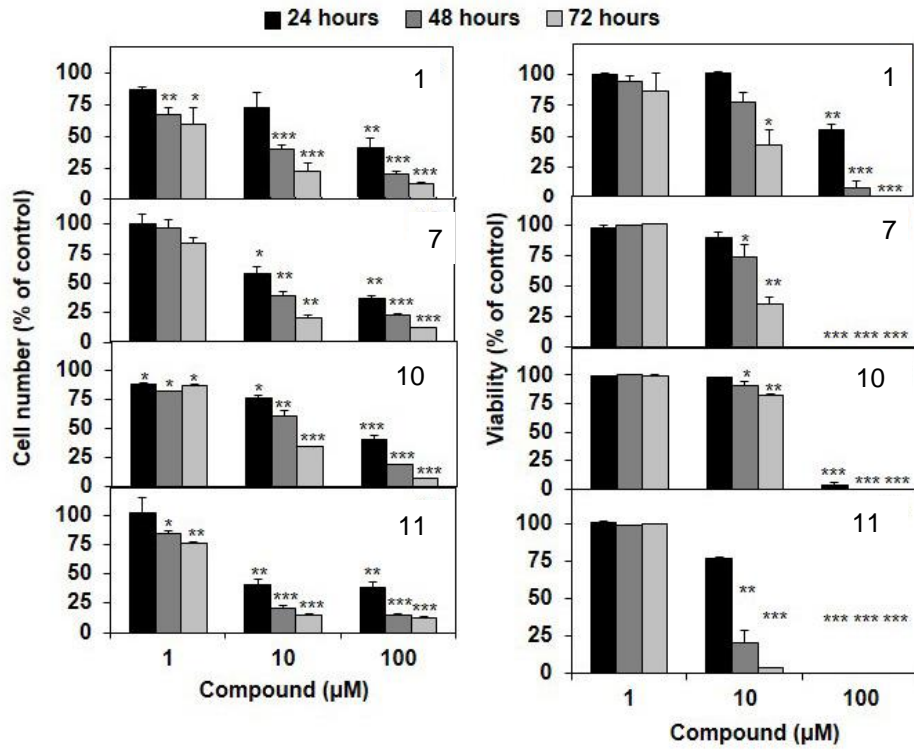


B Reactivation fold (fold-induction) of luciferase expression of the CMV-luc construct in KG-1 cells.^a

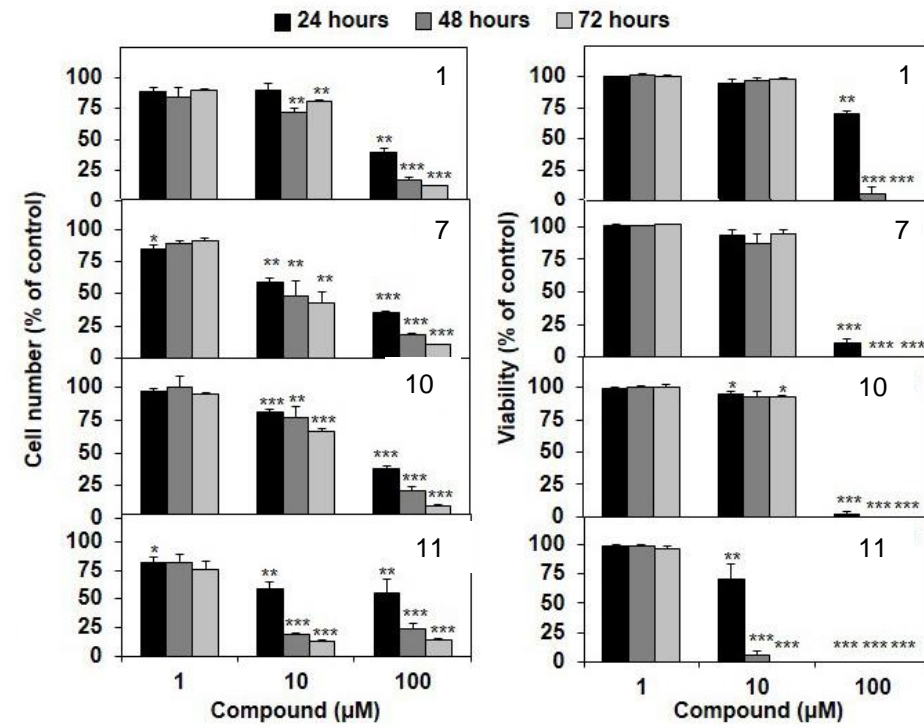
compound	fold-induction				
	0.5 μ M	1 μ M	5 μ M	10 μ M	25 μ M
1	1.0 \pm 0.1	1.5 \pm 0.0	1.3 \pm 0.0	2.8 \pm 0.1	12.5 \pm 0.8
7	0.9 \pm 0.0	1.0 \pm 0.0	2.1 \pm 0.1	3.4 \pm 0.1	0.6 \pm 0.0 ^b
10	0.9 \pm 0.0	1.2 \pm 0.1	2.5 \pm 0.0	2.8 \pm 0.0	0 ^b
11	1.0 \pm 0.1	1.0 \pm 0.0	2.4 \pm 0.1	4.1 \pm 0.1	7.5 \pm 0.6
SGI-1027	1.1 \pm 0.2	1.5 \pm 0.5	17.1 \pm 5.9	10.2 \pm 7.6^b	0 ^b

^aValues are means of at least two experiments. ^bDecrease in reactivation fold due to the toxicity of the compounds at high concentration.

A Histiocytic lymphoma U-937 cells



B Burkitt's lymphoma RAJI cells



C Effect of selected quinazolinones 1, 7, 10 and 11 on human lymphoma U-937 and RAJI cell viability at 48 h.^a

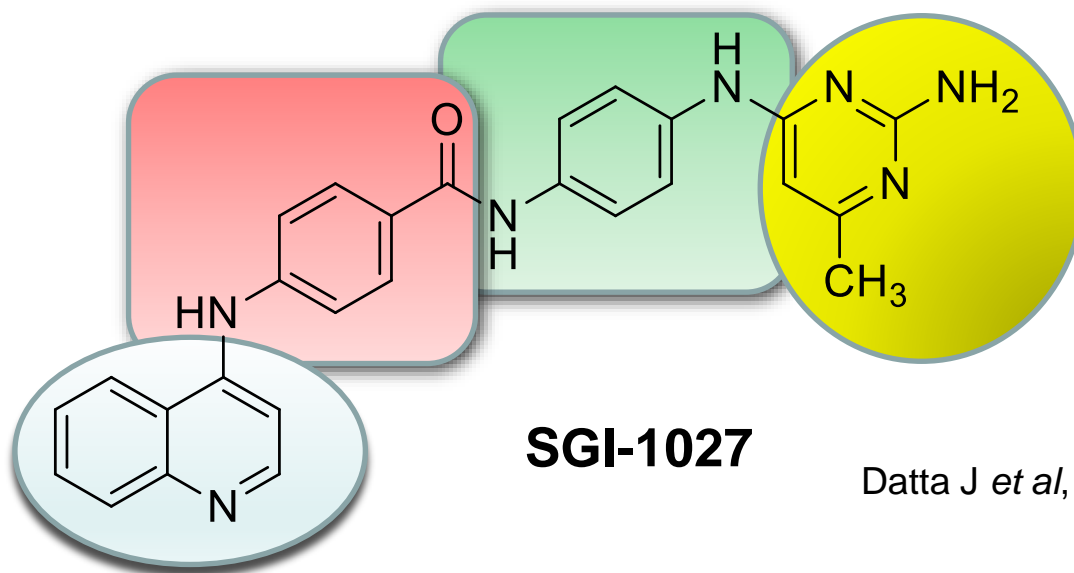
compd	IC ₅₀ (mean ± SD, μM)	
	U-937	RAJI
1	16.6 ± 3.8	21.1 ± 2.5
7	14.7 ± 3.8	18.7 ± 4.3
10	18.8 ± 1.2	19.7 ± 1.4
11	4.4 ± 1.4	3.4 ± 0.3
SGI	1.7 ± 1.1	9.1 ± 0.8

Quinazolinones DNMT3A inhibitors: effects in cancer cells

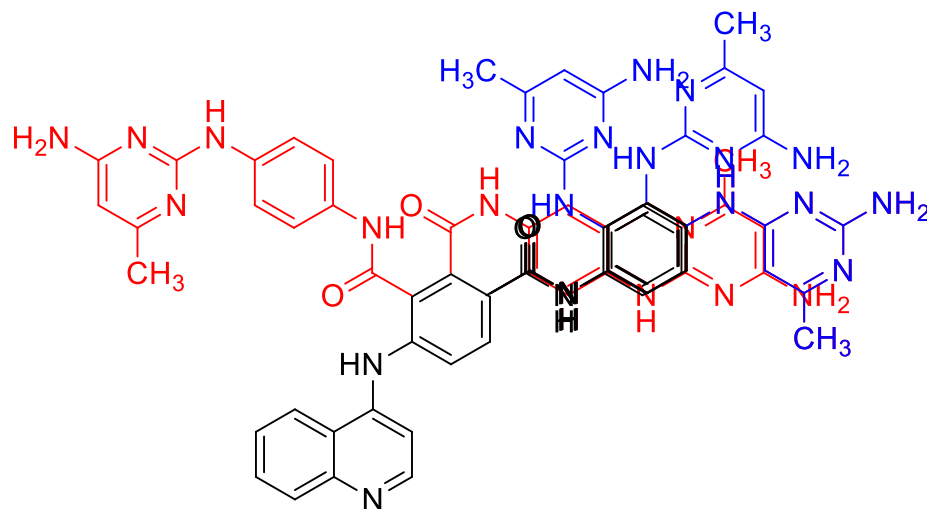
M Diederich's lab

^aData represent the mean (± SD) of at least three independent experiments.

SGI-1027: an Attractive Molecule



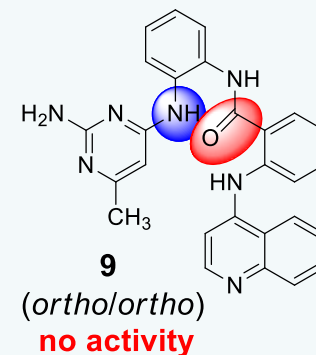
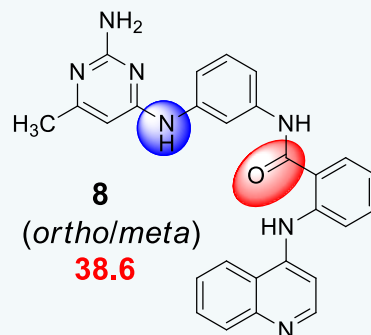
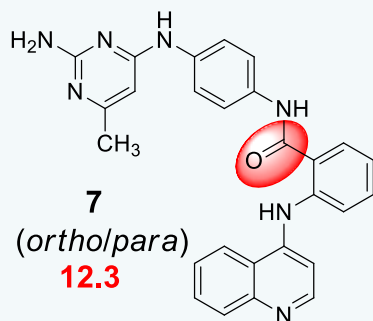
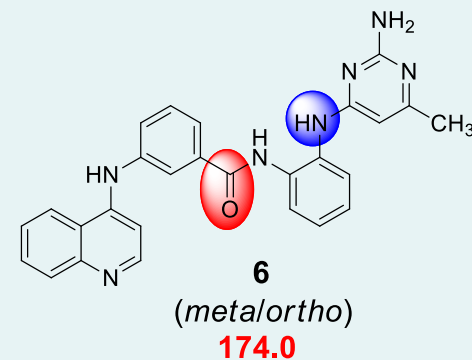
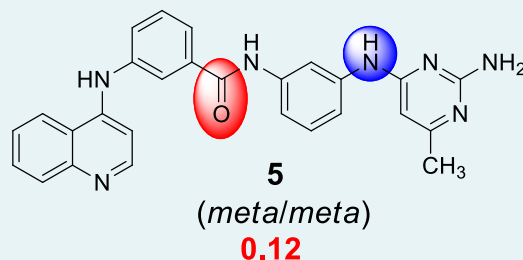
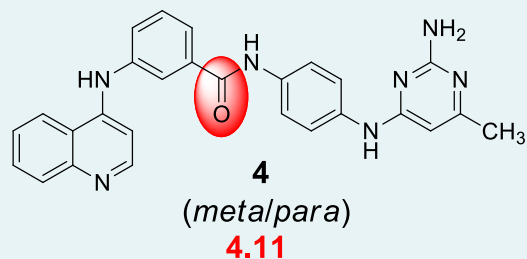
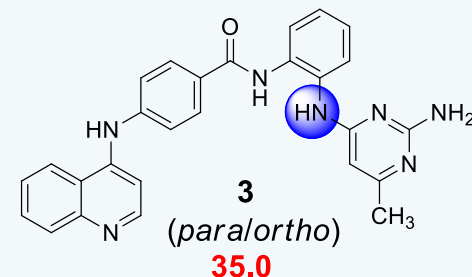
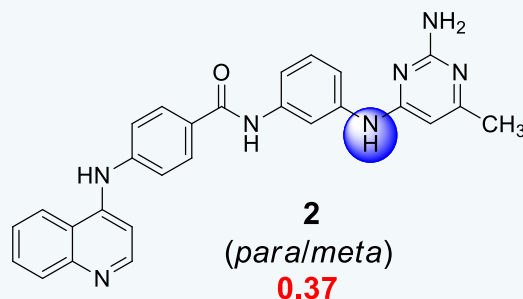
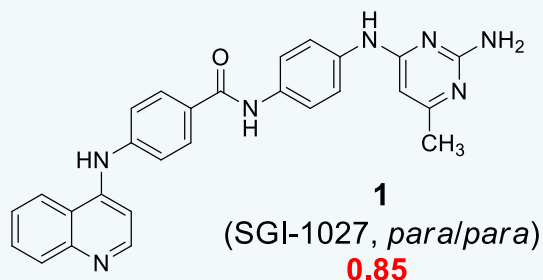
Datta J *et al*, Cancer Res 2009



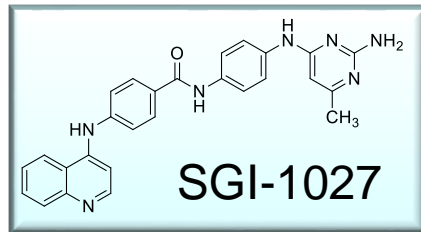
Valente S *et al*, J Med Chem 2014, 57, 701-13

SGI-1027 Regioisomers: Nanoscale HTS vs DNMT1

References:
SAH = 0.28 μM
sinefungin = 7.4 μM



SGI analogs: nanoscale HTS



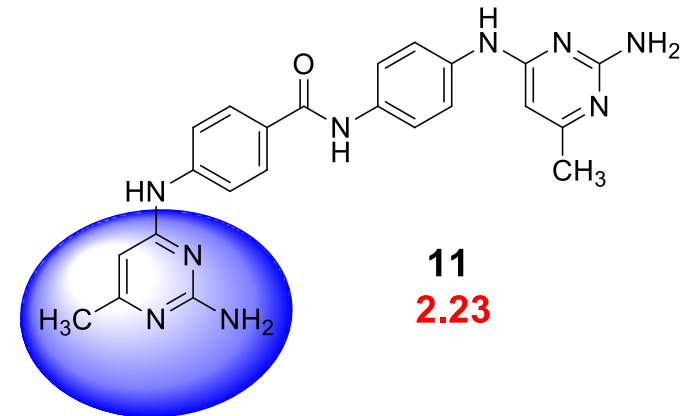
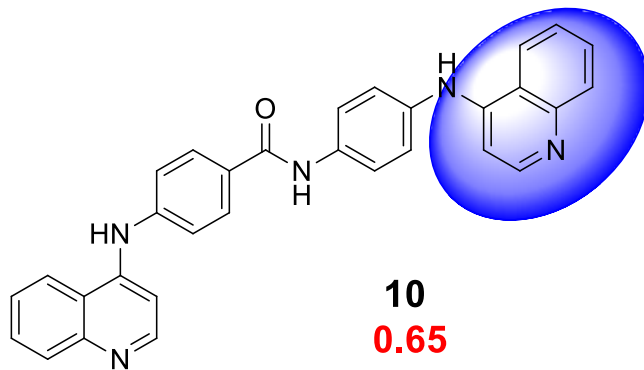
References:

SGI-1027 = 0.85 μM

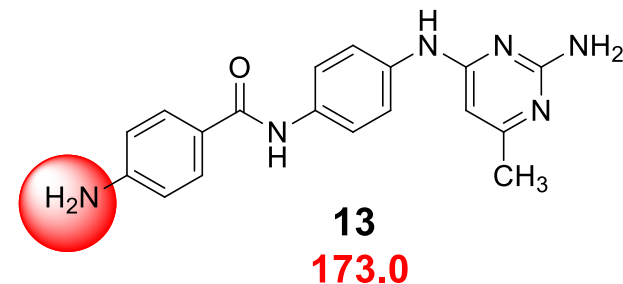
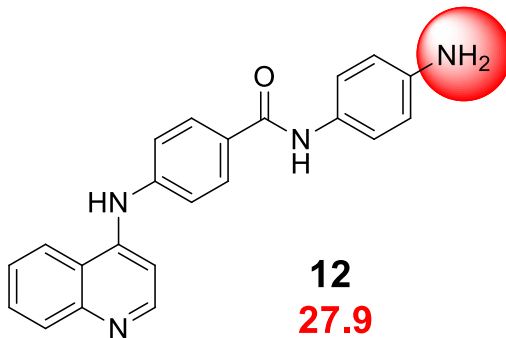
SAH = 0.28 μM

sinefungin = 7.4 μM

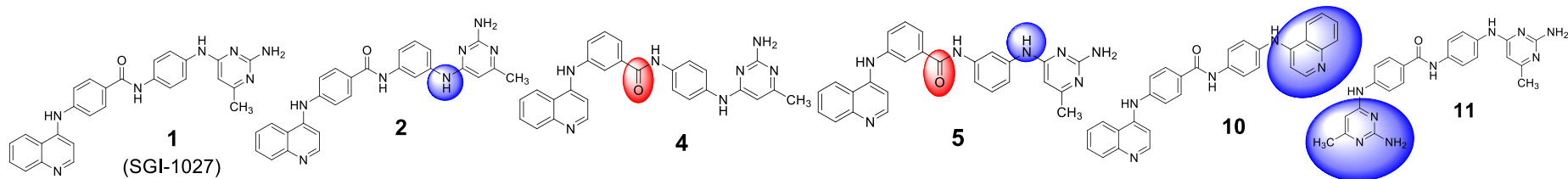
symmetric analogues



truncated analogues



Selected SGI Analogs: Human DNMT1 and DNMT3A/3L Assays



IC₅₀=35 ± 3 μM

IC₅₀=52 ± 1 μM

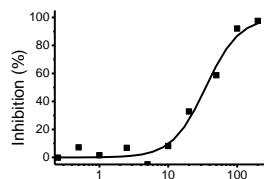
IC₅₀=265 ± 5 μM

IC₅₀=9 ± 1 μM

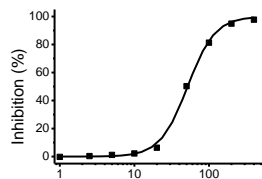
IC₅₀=33 ± 1 μM

IC₅₀=68 ± 4 μM

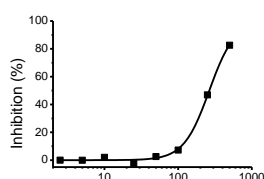
DNMT1



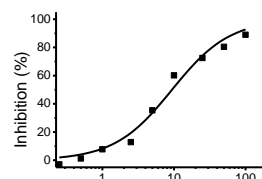
1 (μM)



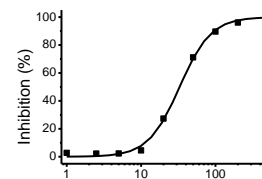
2 (μM)



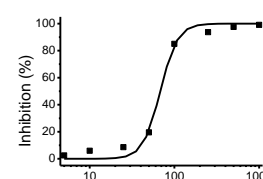
4 (μM)



5 (μM)



10 (μM)



11 (μM)

DNMT3A/3L

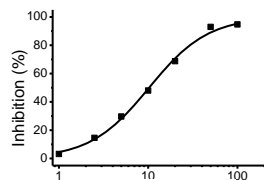
IC₅₀=10 ± 1 μM

IC₅₀=21 ± 3 μM

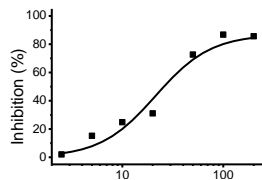
IC₅₀=42 ± 4 μM

IC₅₀=2.8 ± 0.2 μM

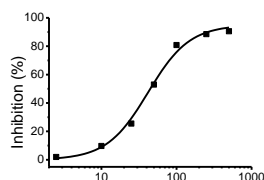
IC₅₀=27 ± 5 μM **IC₅₀=18 ± 2 μM**



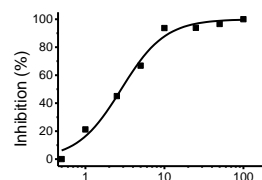
1 (μM)



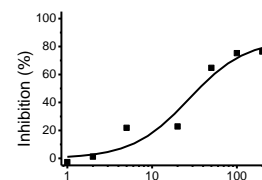
2 (μM)



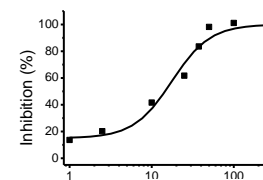
4 (μM)



5 (μM)

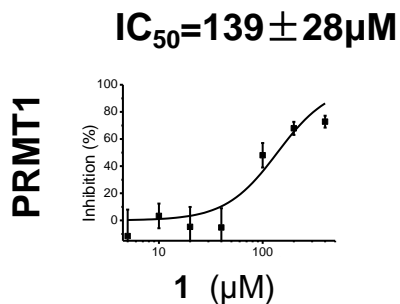


10 (μM)

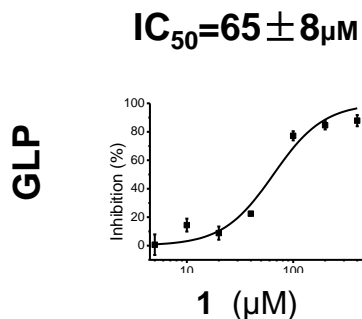


11 (μM)

Selectivity towards PRMT1 and GLP

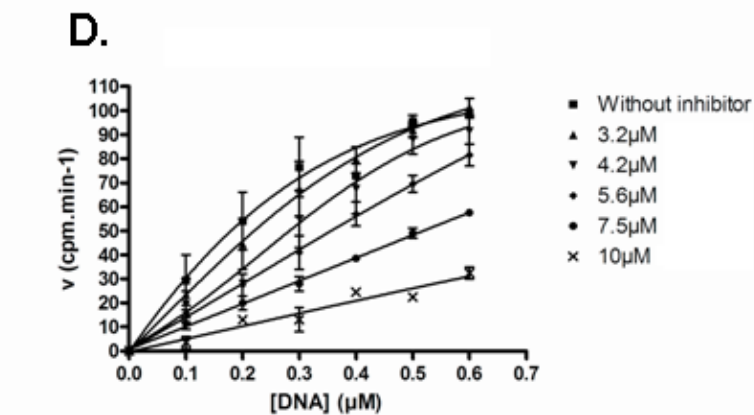
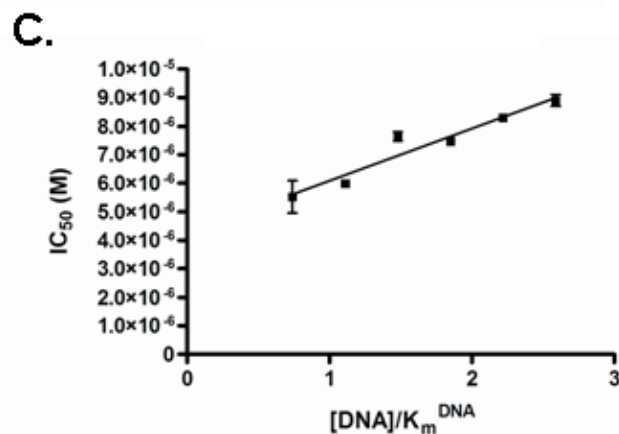
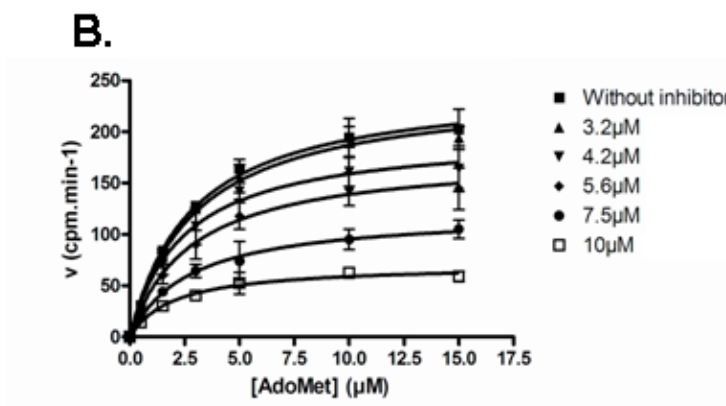
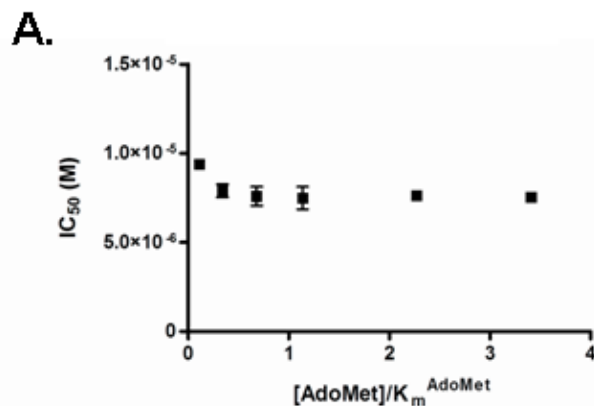
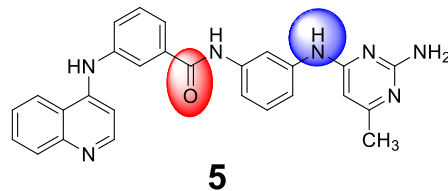


	$IC_{50}, \mu M$	DNMT1-selectivity
1 (SGI)	139	4.0
2	300	5.8
4	300	1.1
5	300	33.3
10	100	3.0
11	>1000	>14.7



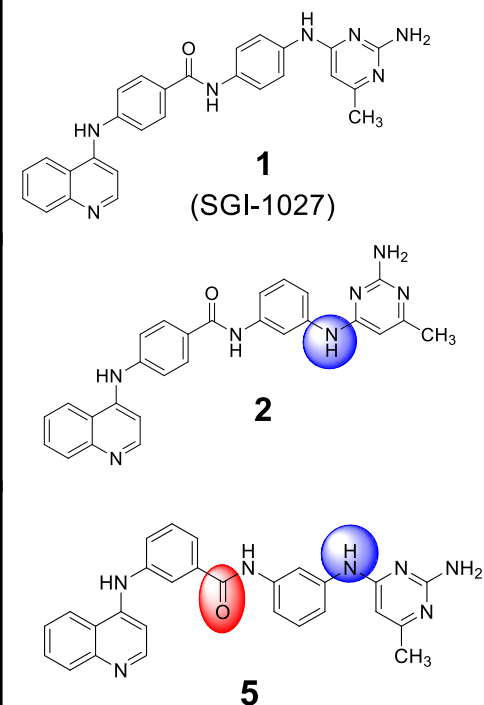
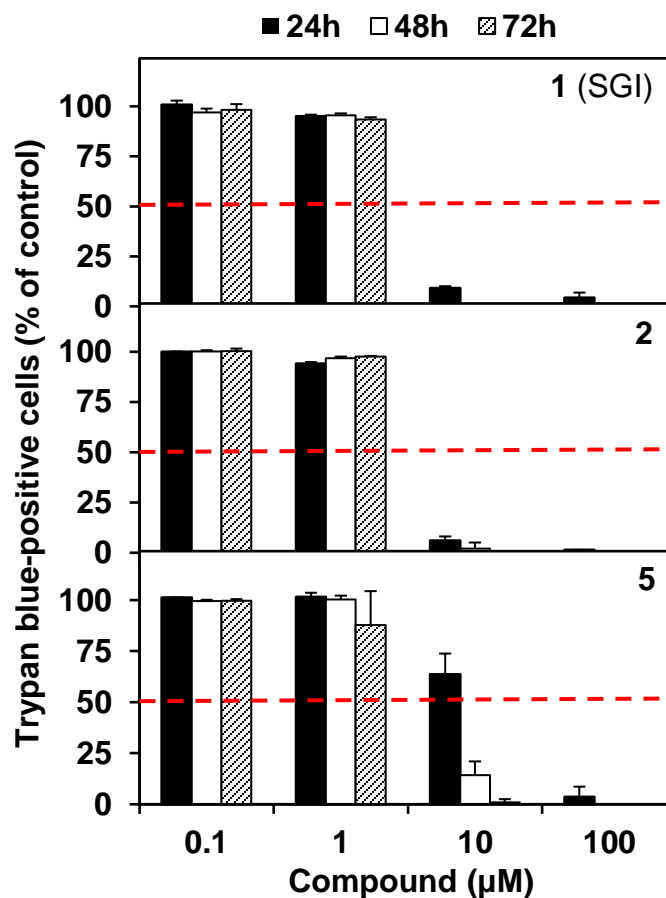
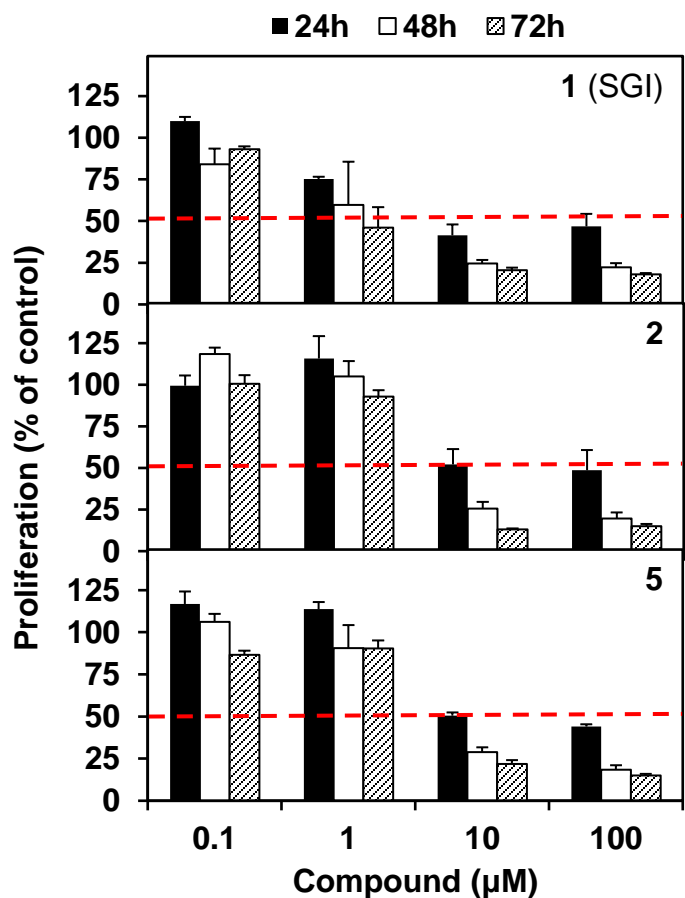
	$IC_{50}, \mu M$	DNMT1-selectivity
1 (SGI)	65	1.9
2	600	11.5
4	400	1.5
5	100	11.1
10	100	3.0
11	500	7.3

Compound 5: mechanism of action



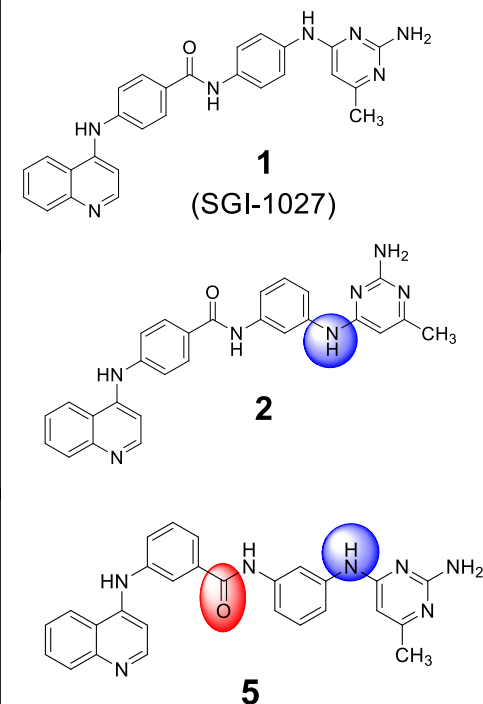
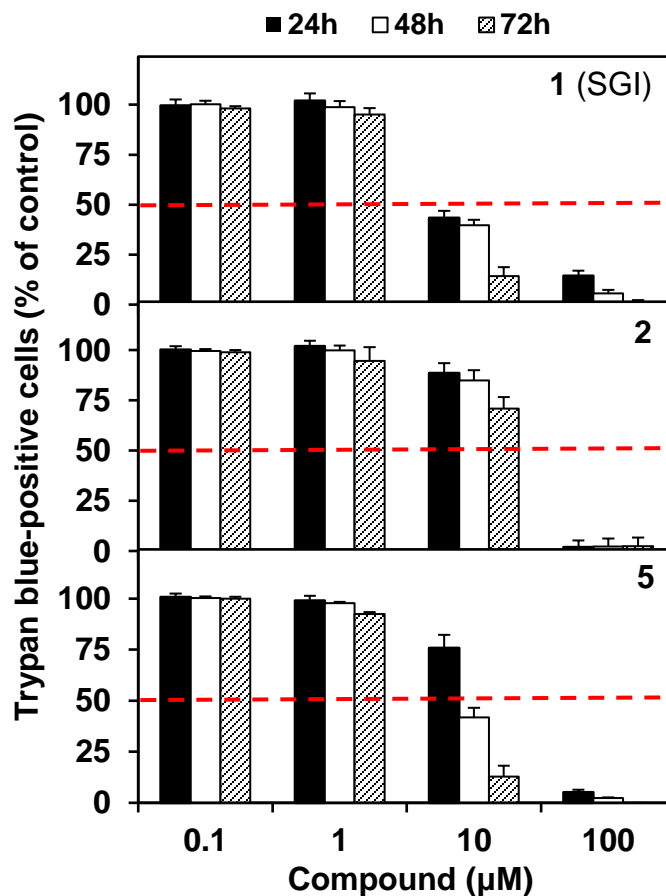
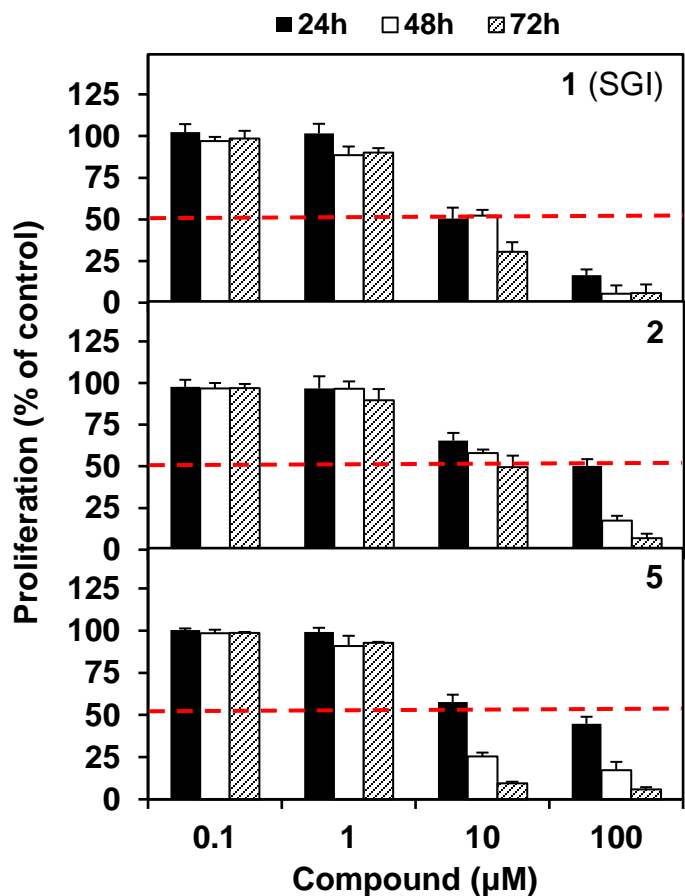
SGI Analogs: Cancer Cell Evaluation/1

Histiocytic lymphoma U-937 cells



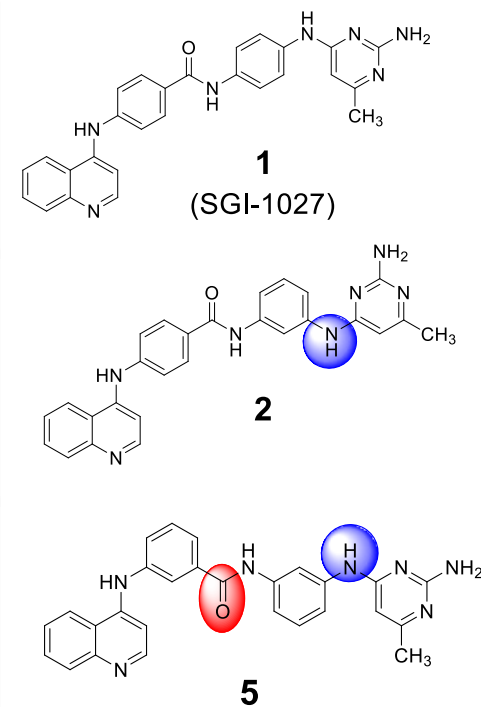
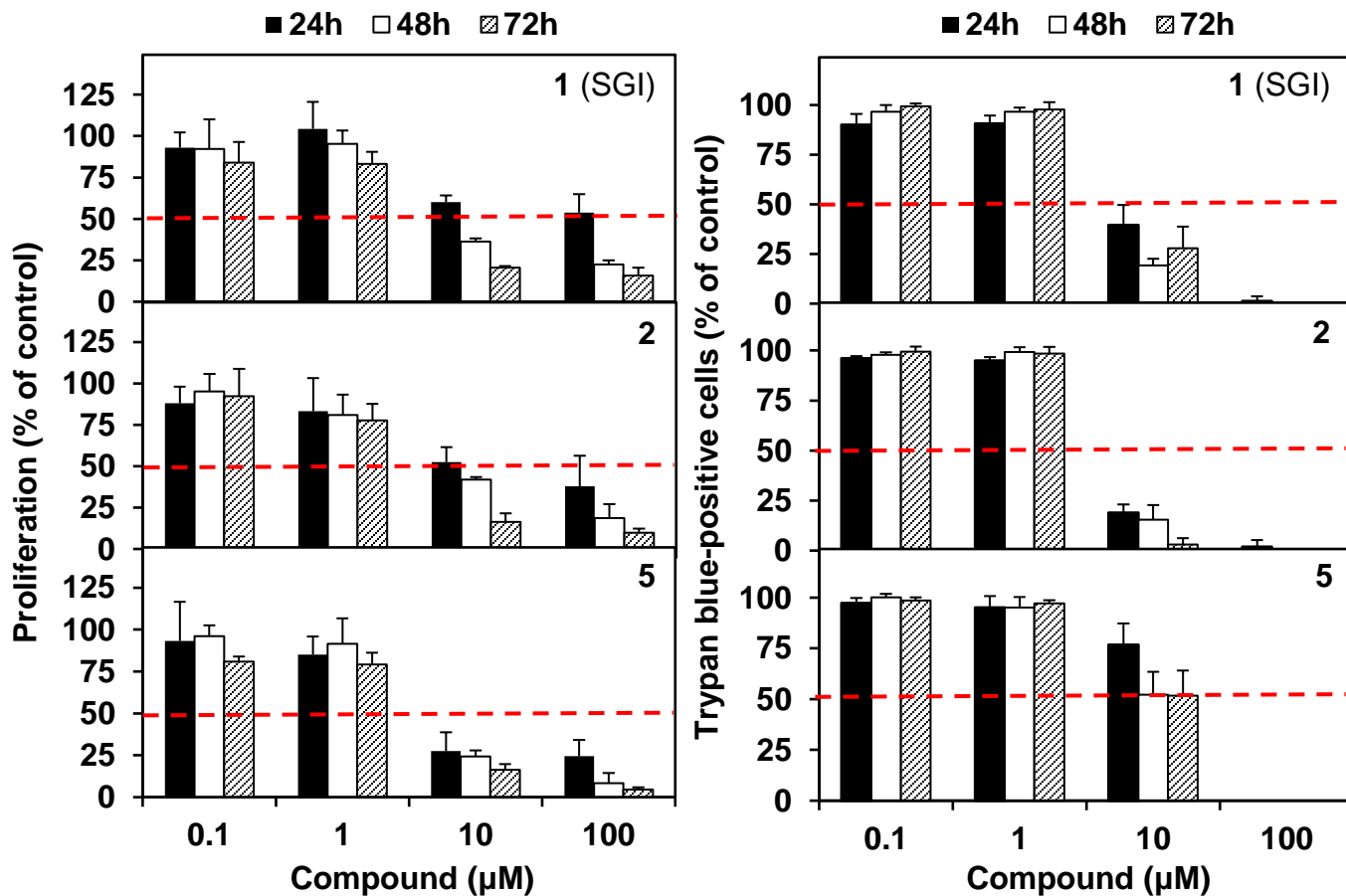
SGI Analogs: Cancer Cell Evaluation/2

Burkitt's lymphoma RAJI cells



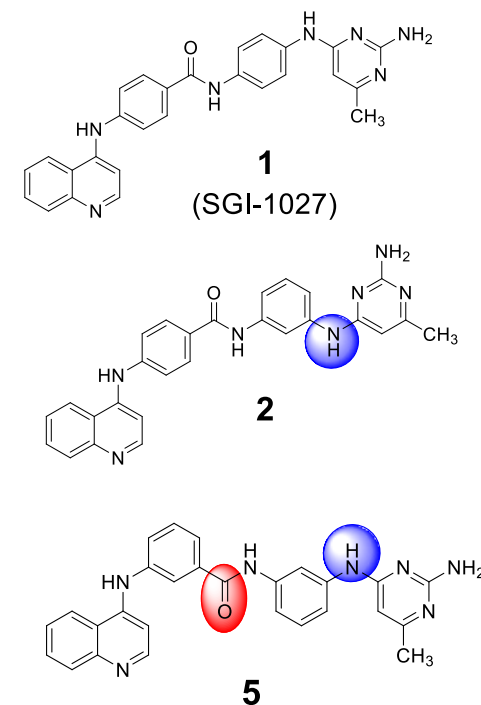
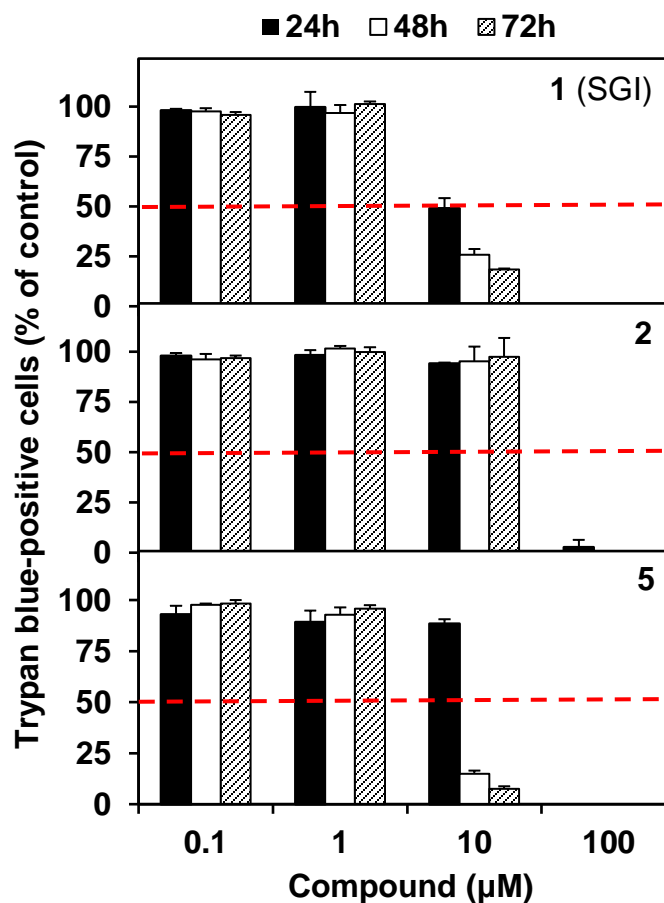
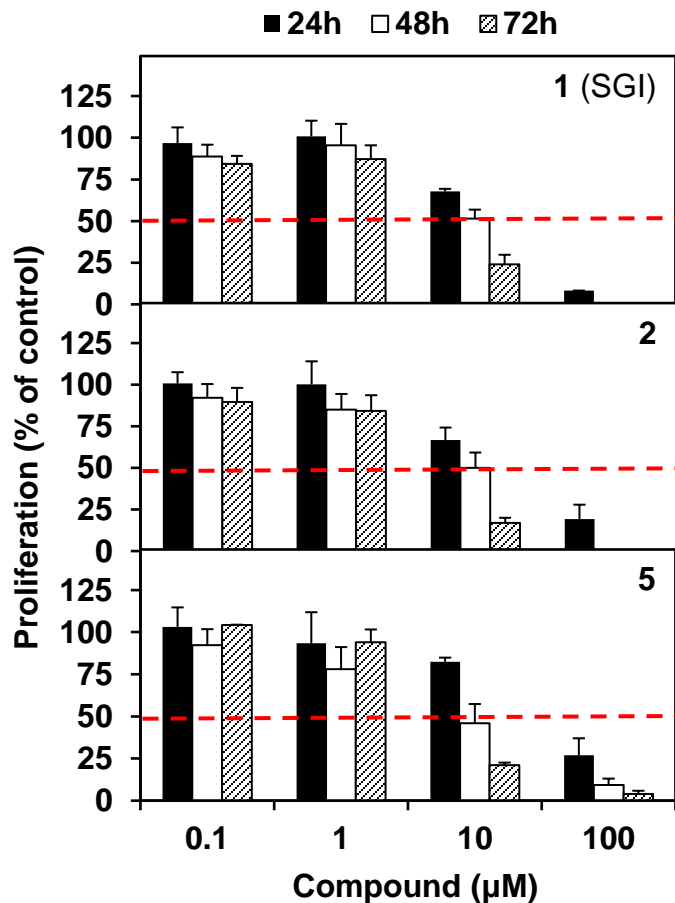
SGI Analogs: Cancer Cell Evaluation/3

Breast cancer MDA-MB-231 cells



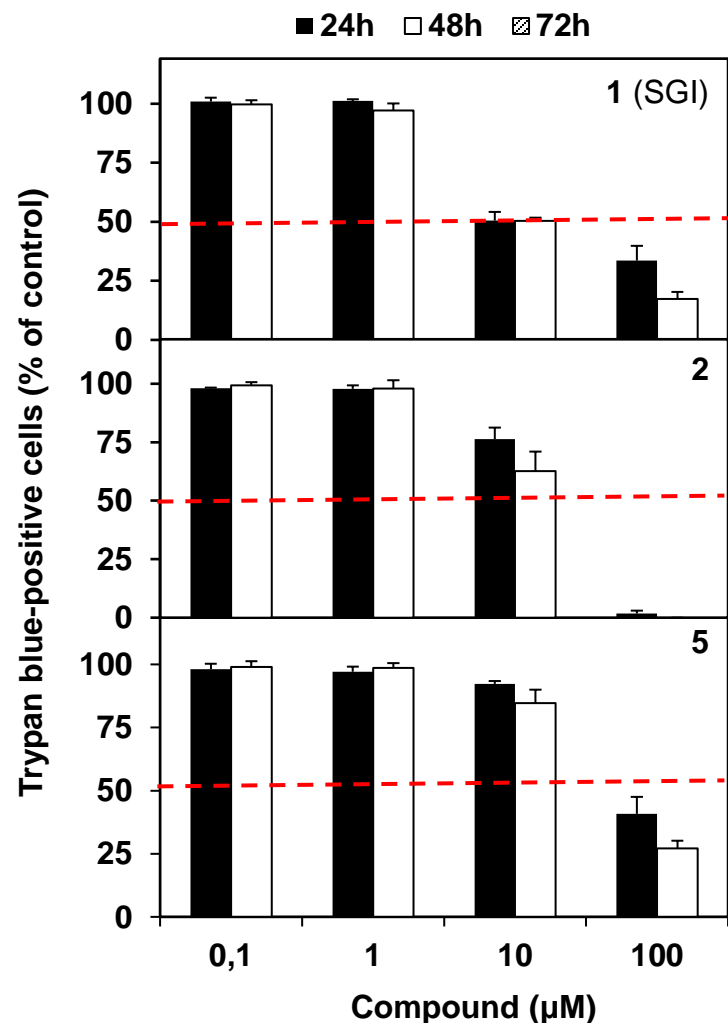
SGI Analogs: Cancer Cell Evaluation/4

Prostate cancer PC-3 cells



Toxicity and Selectivity

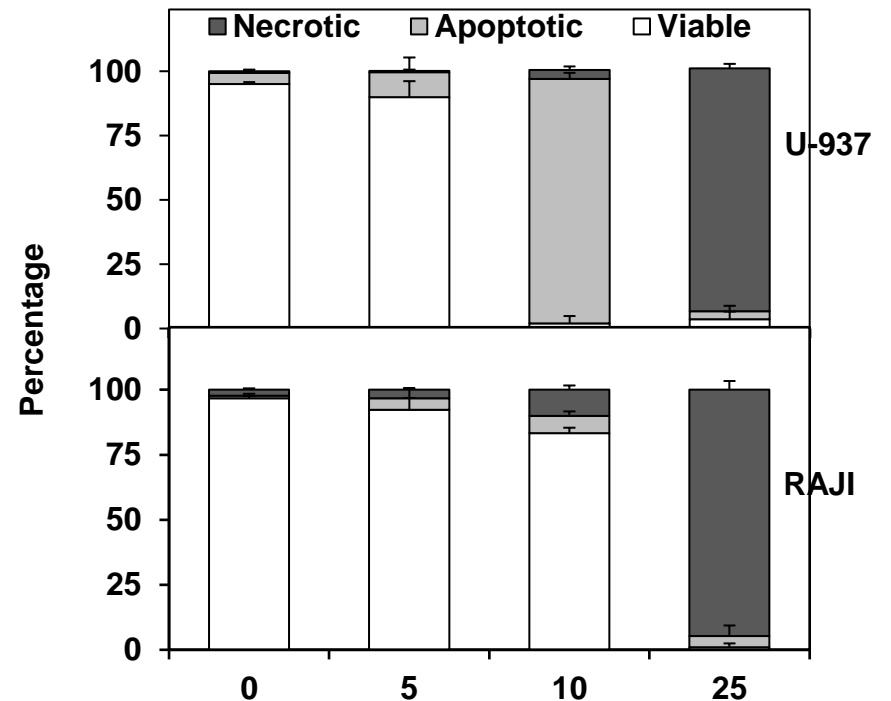
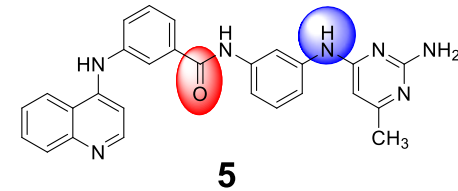
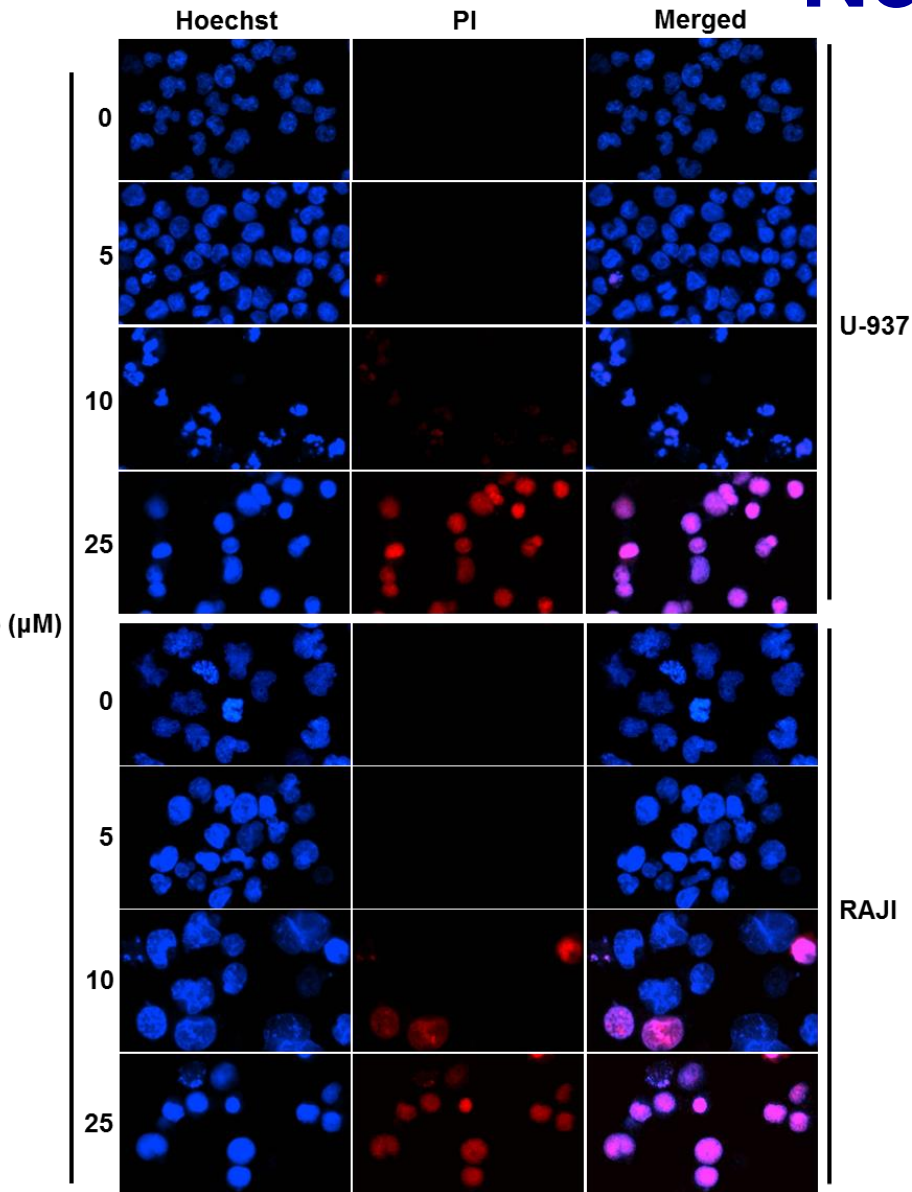
Periferal Blood Mononuclear Cells



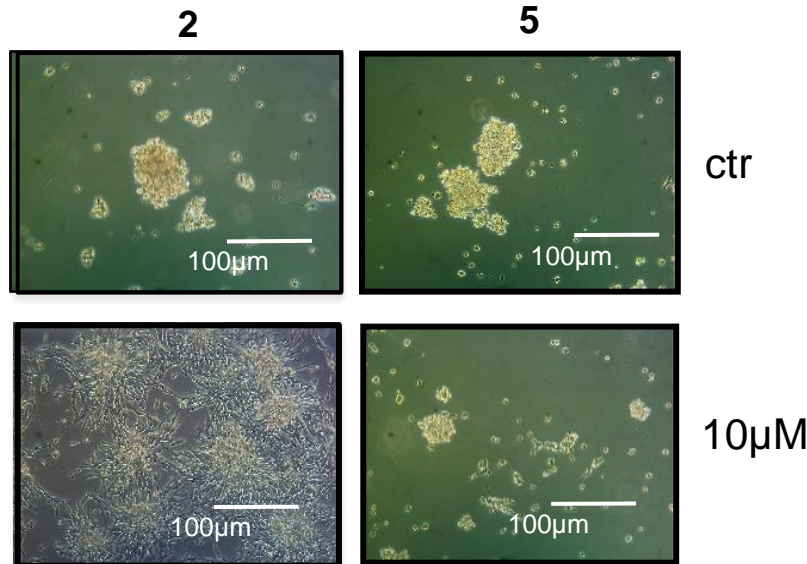
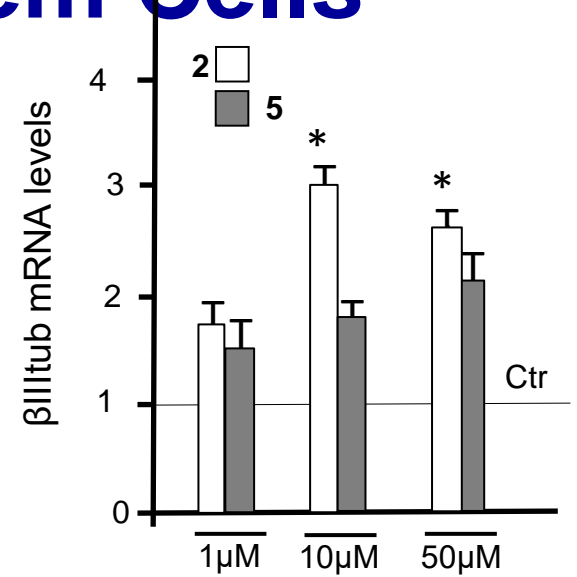
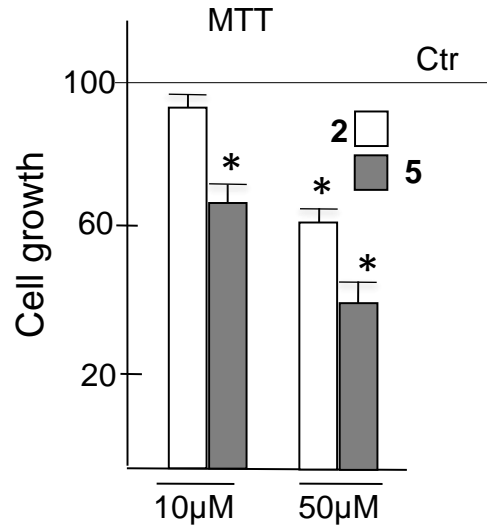
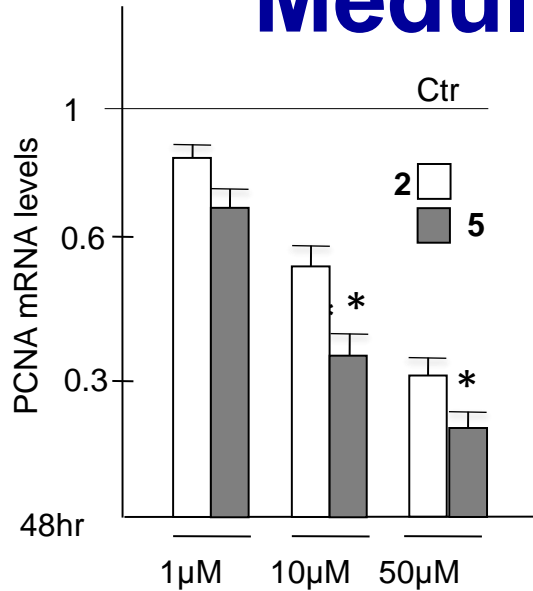
cpd	IC_{50} , μM (<i>fold selectivity</i>)				
	PBMCs	U-937	RAJI	PC-3	MDA-MB-231
1 (SGI)	23.8 ± 8.4	1.7 ± 1.1 (14)	9.1 ± 0.8 (2.6)	6.5 ± 0.6 (3.7)	4.8 ± 1.6 (5)
2	15.2 ± 2.0	2.7 ± 0.9 (5.6)	26.5 ± 3.4 (0.6)	29.8 ± 1.8 (0.5)	7.4 ± 3.0 (2)
5	57.4 ± 9.3	4.3 ± 1.7 (13.3)	8.8 ± 0.4 (6.5)	6.6 ± 2.9 (8.7)	9.7 ± 1.5 (6)

M Diederich's lab

Compd 5 in U-937 and RAJI Cells: Necrosis vs Apoptosis



Antiproliferative and Cytodifferentiating Effects in Medulloblastoma Stem Cells

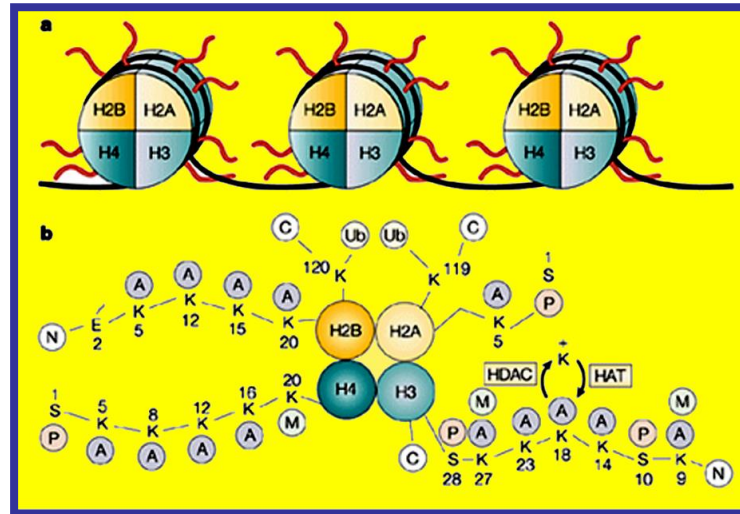


Histone covalent modifications

Acetylation/
deacetylation

Histone
methylation/
demethylation

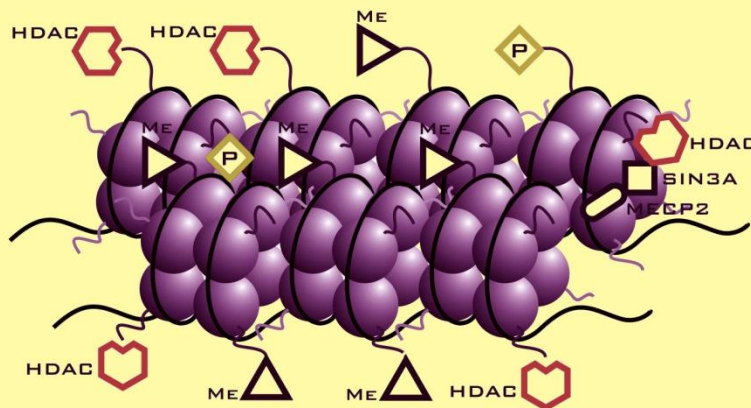
ADP-ribosylation



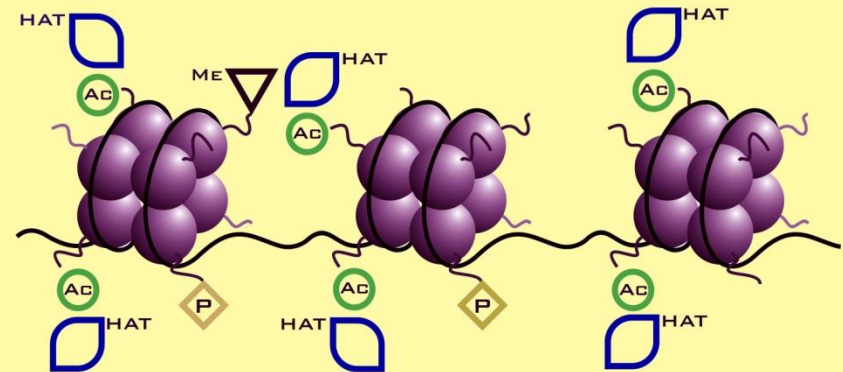
Ubiquitylation

Phosphorylation

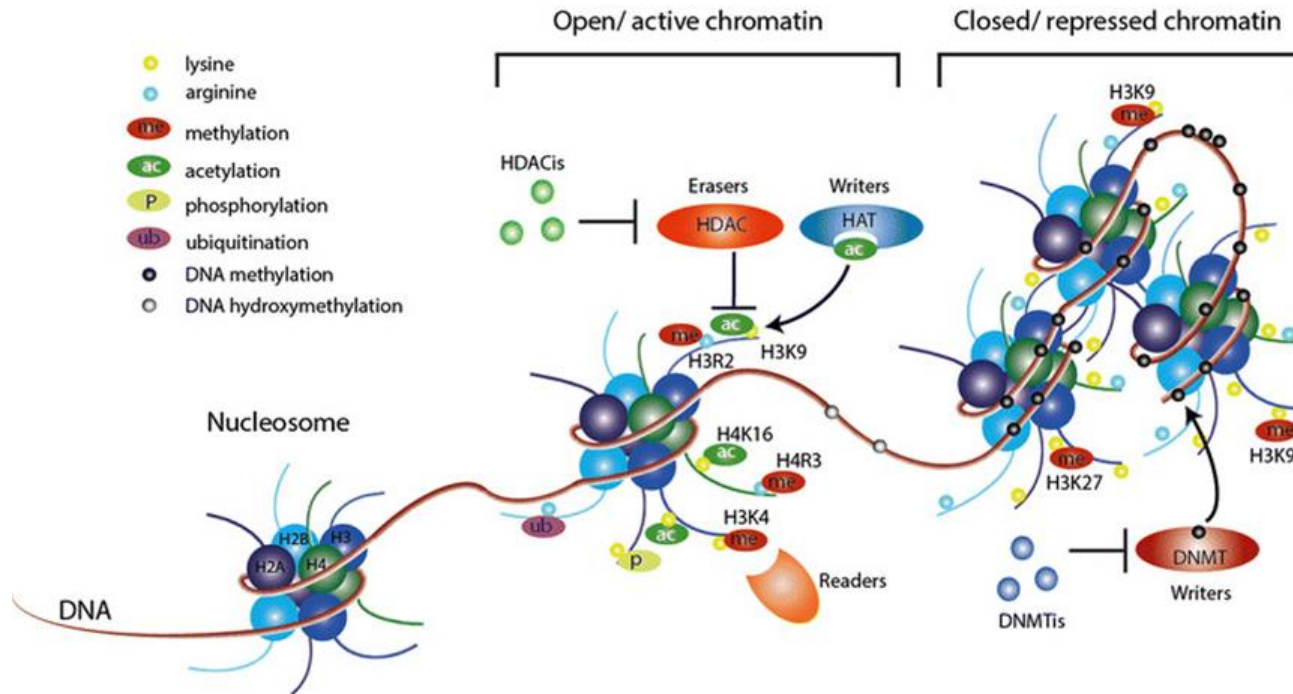
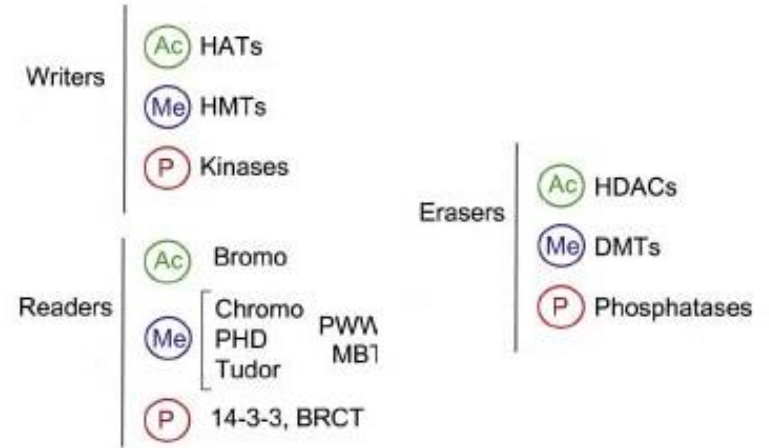
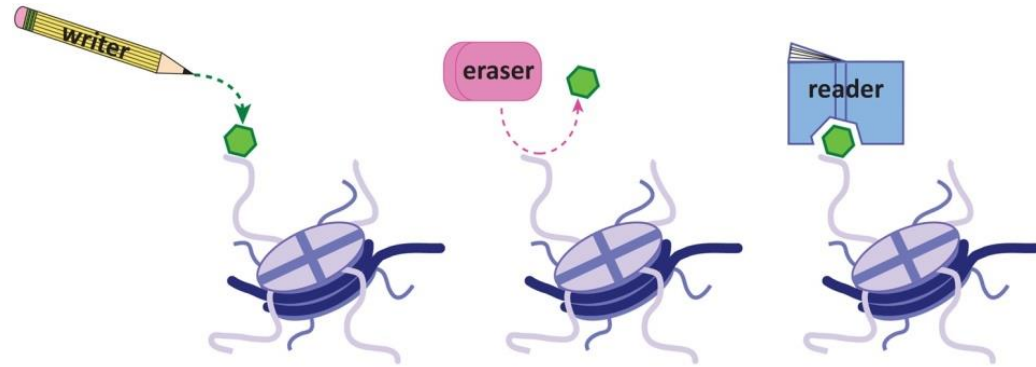
a Heterochromatin



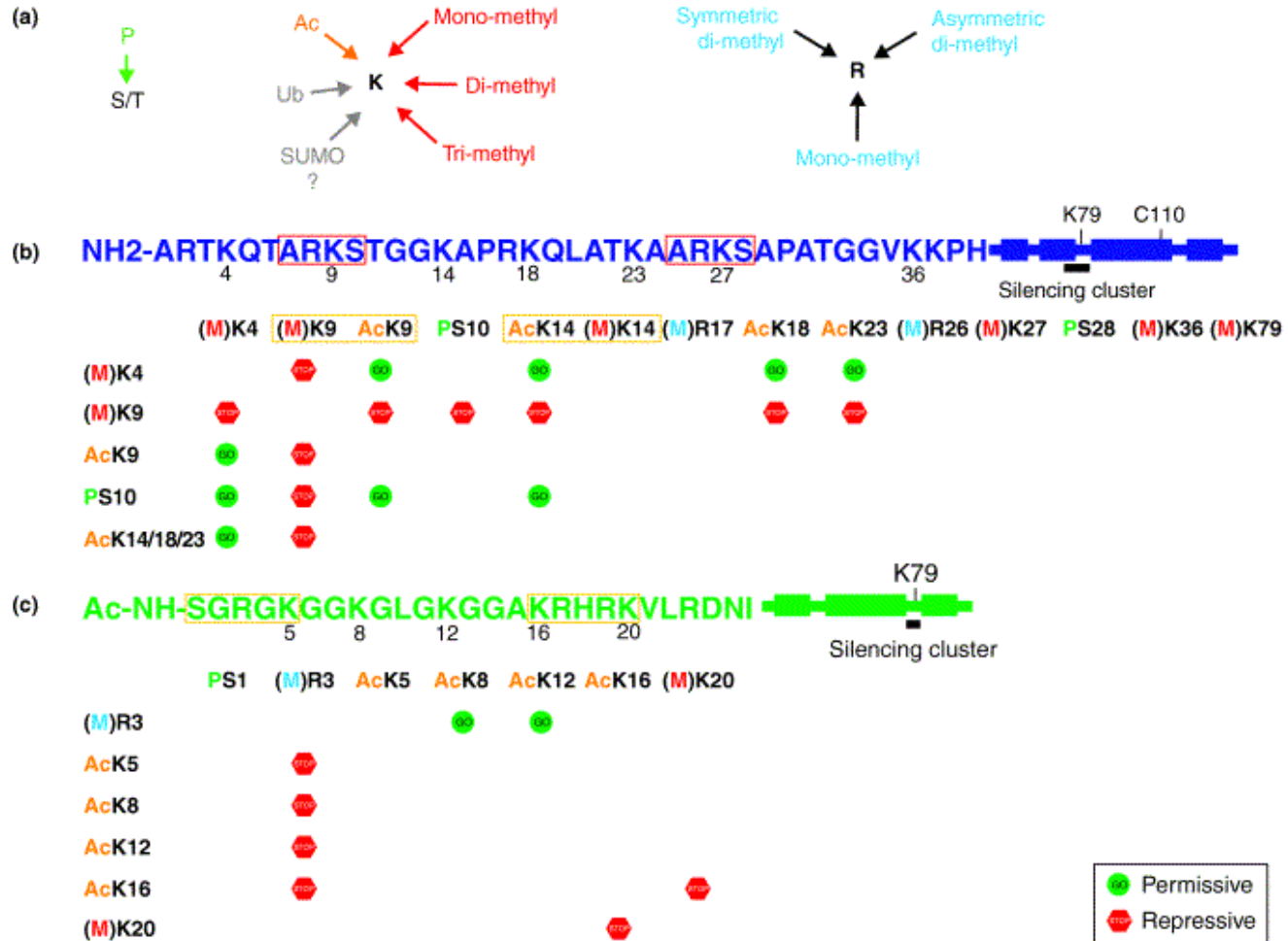
b Euchromatin



Main Actors in Epigenetics

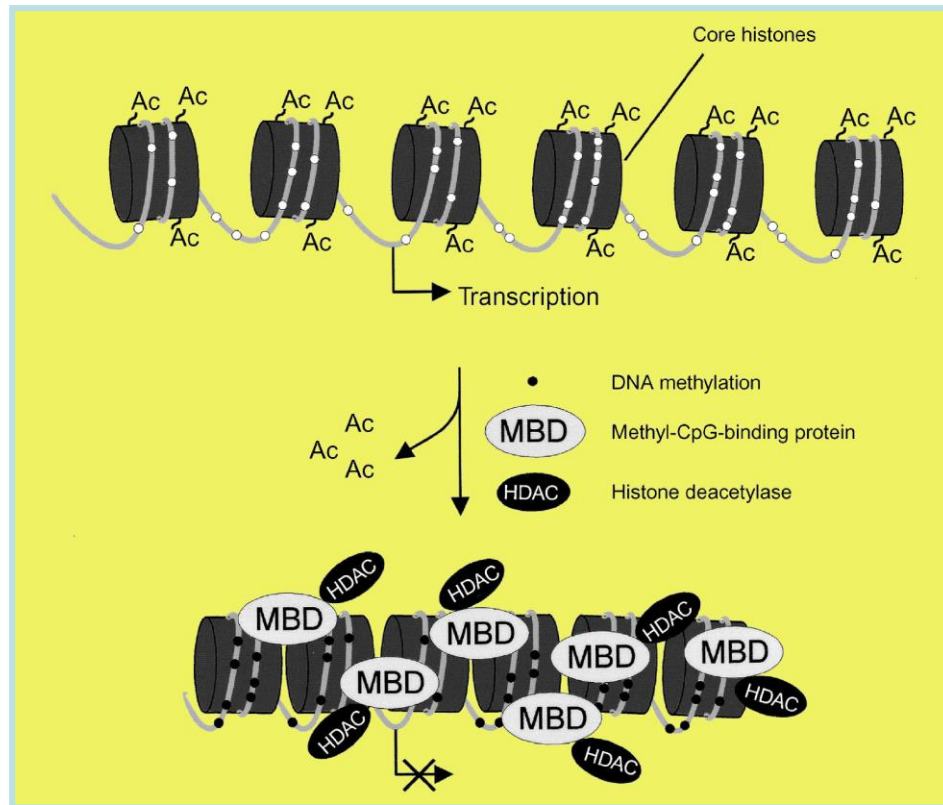


The histone code



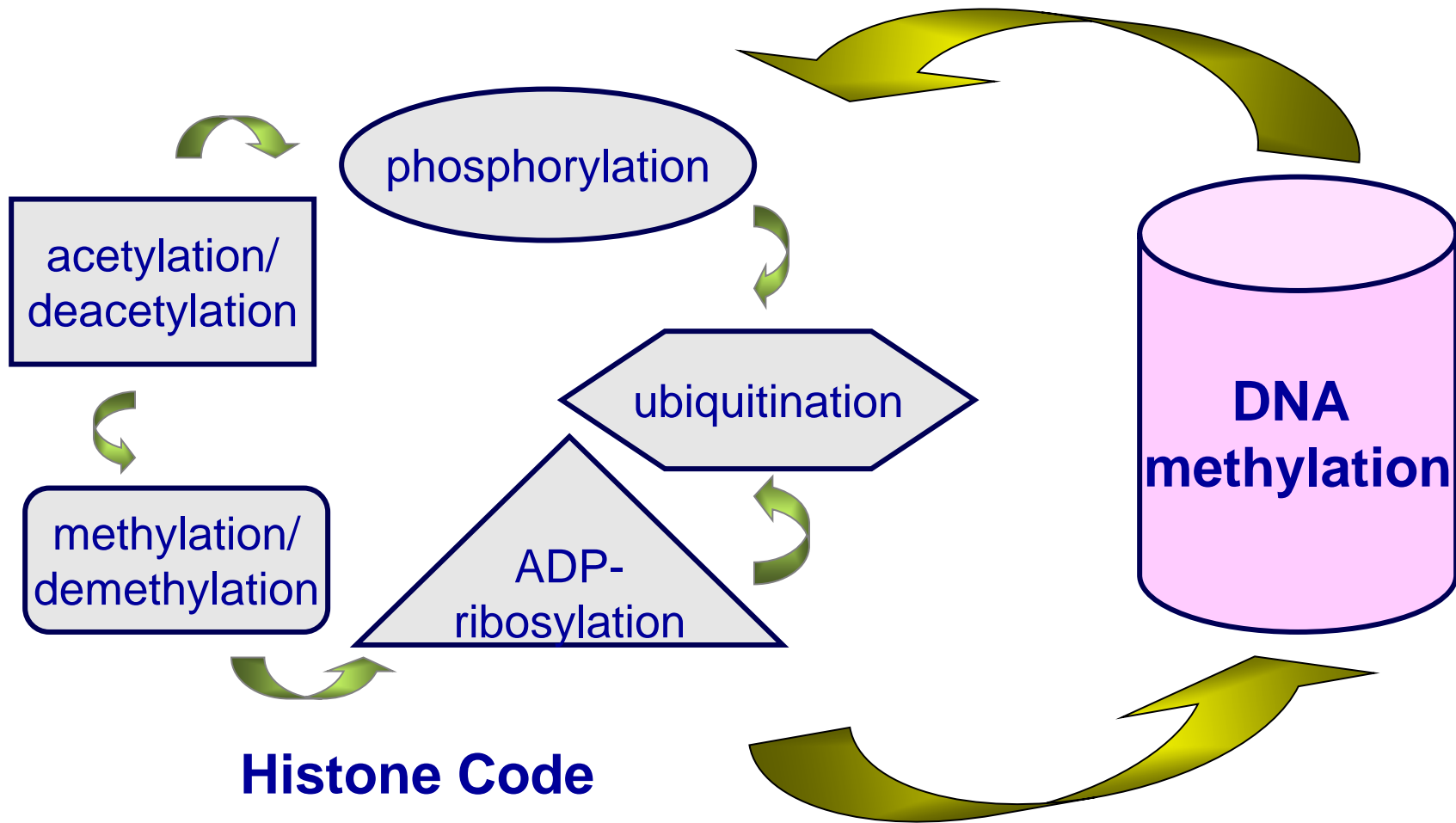
Fischle, W. *et al.*, *Curr. Opin. Cell Biol.* **2003**, *15*, 172-183.

A second cross-talk: DNA methylation and histone code



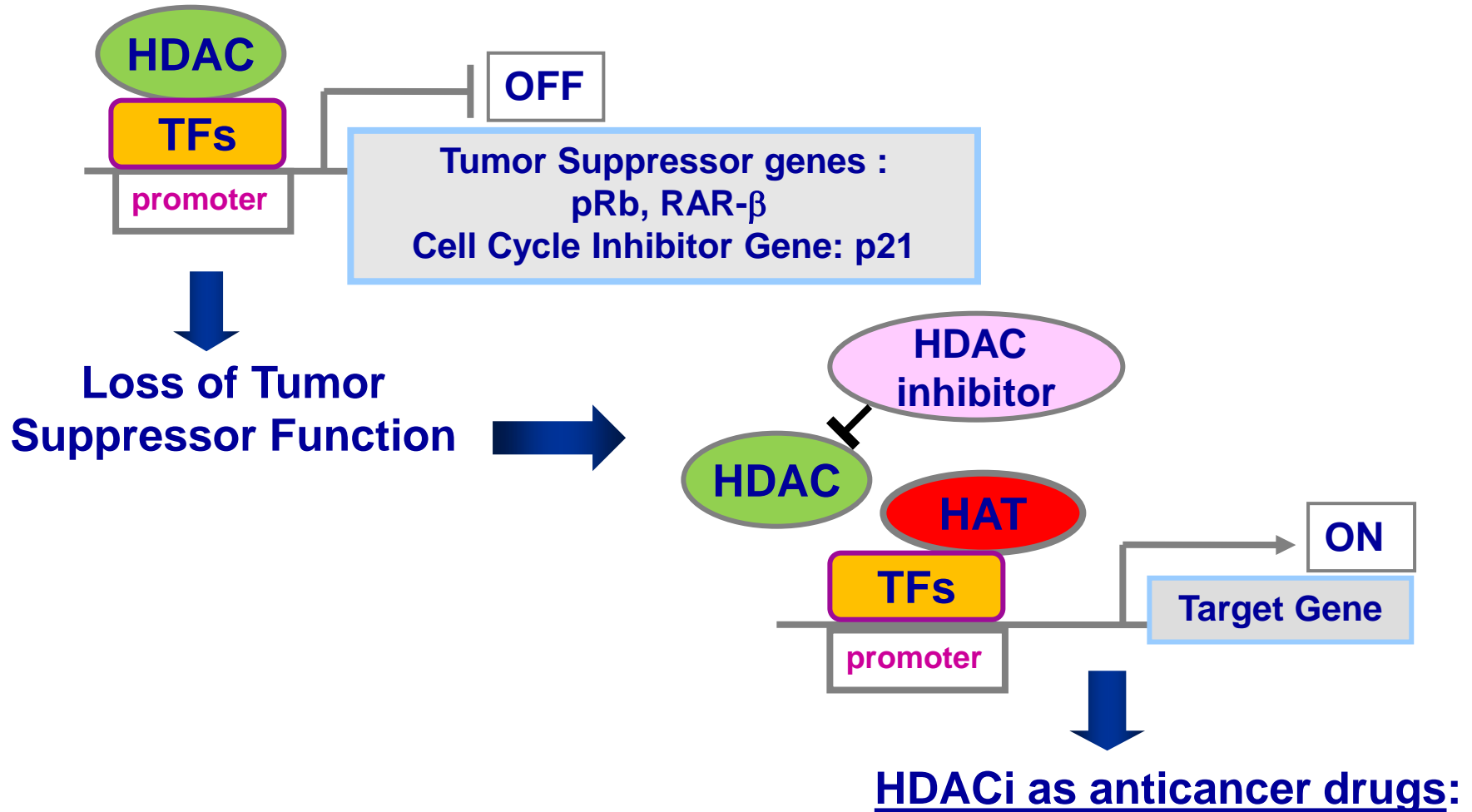
The attachment of the 5-methylcytosine binding proteins (MBDs) to a methylated promoter results in the recruitment of HDACs

A crowded network of signals...



EPIGENETICS

Epigenetic therapy: the case of HDACi

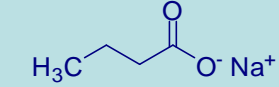
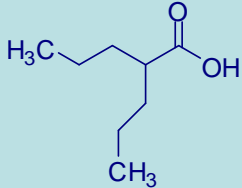
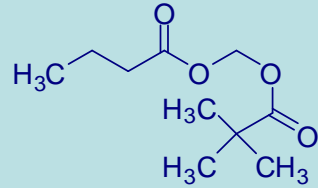


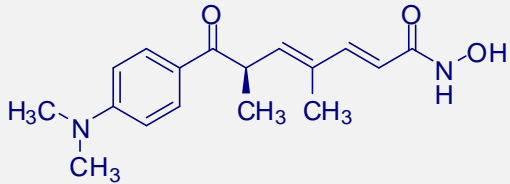
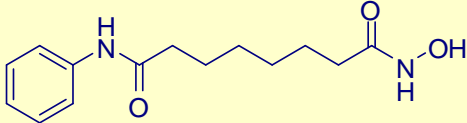
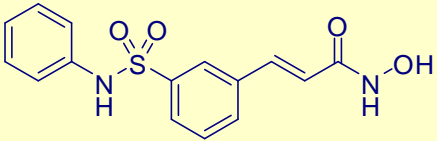
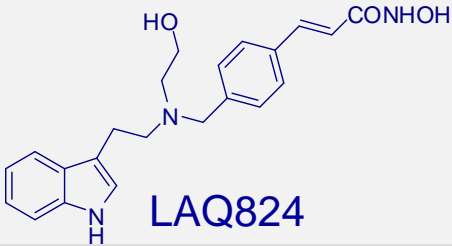
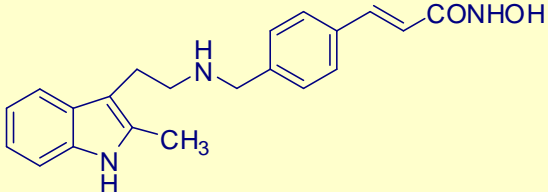
HDACi as anticancer drugs:
silenced genes can be reactivated by HDACi, re-establishing selected programs for cell cycle arrest, differentiation, & apoptosis

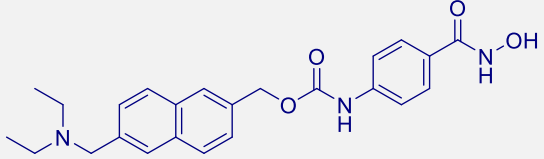
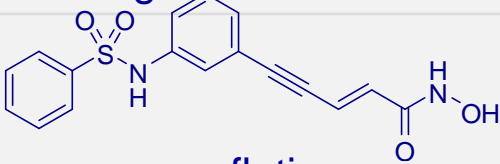
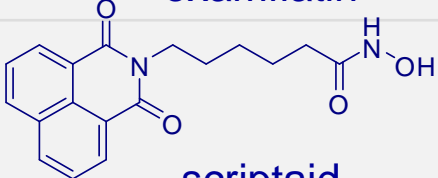
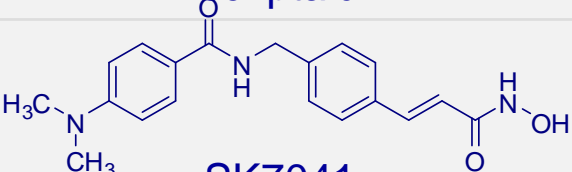
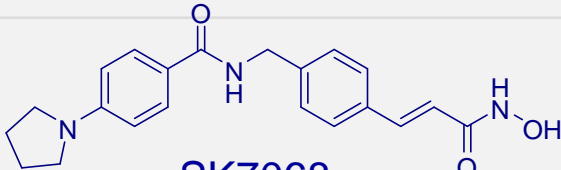
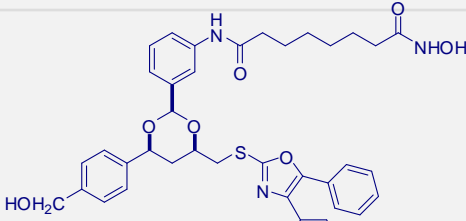
Classification of HDACs

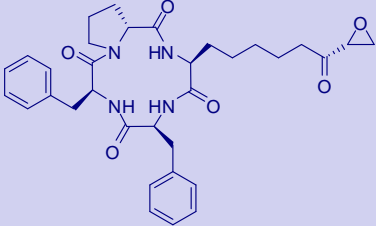
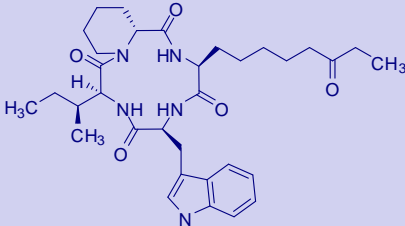
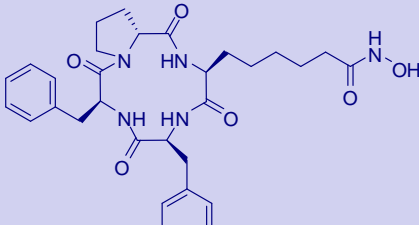
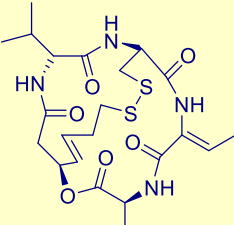
HDAC	cls	interactions	cellular localiz	tissue expression
HDAC1	I	DNMT1, ATM, BRCA1, MECP2, MYOD, p53, pRb, NF-κB	nuclear	ubiquit.
HDAC2	I	DNMT1, BRCA1, pRb, NF-κB, GATA2	nuclear	ubiquit.
HDAC3	I	pRb, NF-κB	nucl/cytopls	ubiquit.
HDAC8	I	α-SMA	nucl/cytopls	ubiquit.
HDAC11	IV	HDAC6	nuclear (?)	tissue specific (?)
HDAC4	Ila	14-3-3, MEF2, calmodulin	shuttling n/c	heart, muscle, brain
HDAC5	Ila	14-3-3, MEF2, calmodulin	shuttling n/c	heart, muscle, brain
HDAC6	Ilb	tubulin, PP1, dynactin, HDAC11	nucl/cytopls	heart,liver,kidney,pancreas
HDAC7	Ila	14-3-3, MEF2, calmodulin	shuttling n/c	placenta,pancreas,muscle
HDAC9	Ila	14-3-3, MEF2, calmodulin	shuttling n/c	heart, muscle, brain
HDAC10	Ilb	PP1, LcoR	nucl/cytopls	liver, spleen, kidney
SIRT1	III	p53, Ku70, PPARγ, PGC1-α, NF-κB, FOXO, H3K9, H4K16	nucleus	brain, skel muscle, heart, kidney
SIRT2	III	α-tubulin, H4K16	cytoplasm	brain, skeletal muscle
SIRT3	III	AceCS2	mitochondria	ND
SIRT4	III	glutamate dehydrogenase	mitochondria	ND
SIRT5	III	cytochrome c, CPS1	mitochondria	ND
SIRT6	III	DNA pol β	nucleus	ND
SIRT7	III	RNA polymerase I	nucleulus	ND

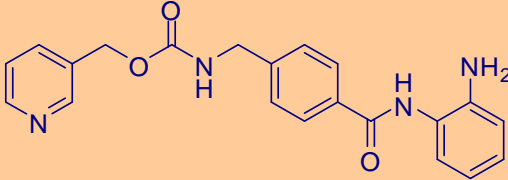
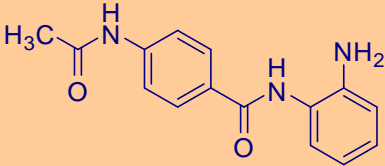
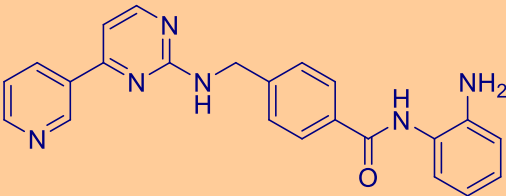

Class I/II HDACi: clinical trial status

class	compd	[range]	HDAC specificity	clinical trials
short-chain fatty acids	 sodium butyrate	mM	class I, IIa	Phase I, II (colorectal)
	 valproic acid	mM	class I, IIa	Phase I, II (AML, leukemias)
	 AN-9	μM	NA	Phase I, II

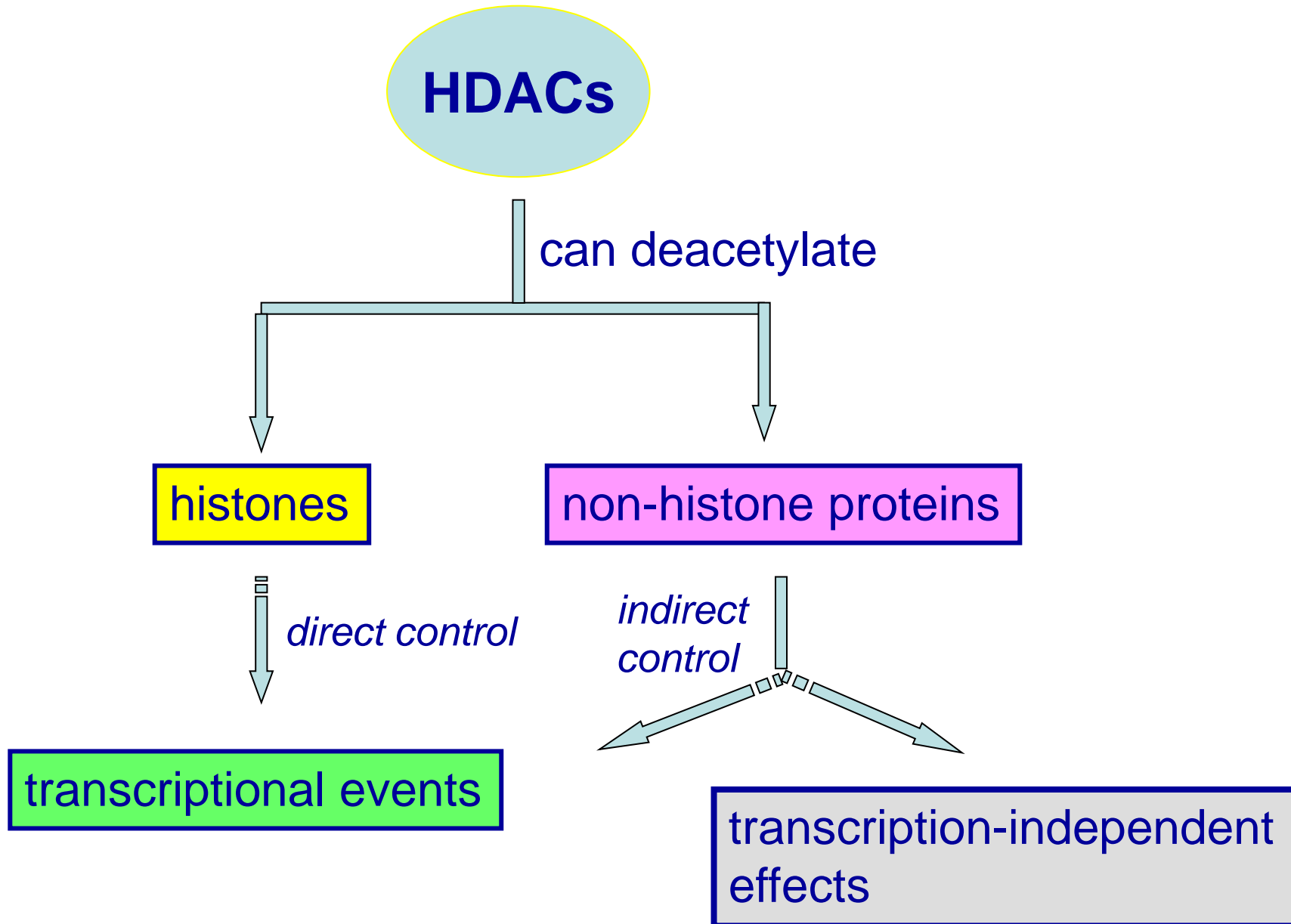
class	compd	[range]	HDAC specificity	clinical trials
hydroxamates	 <p>trichostatin A (TSA)</p>	nM	class I, II	preclinical
	 <p>suberoylanilide hydroxamic acid (SAHA, vorinostat)</p>	μM	class I, II	FDA approved (oct 2006) for CTCL. Phase I, II, III
	 <p>PXD101 (belinostat)</p>	μM	class I,II	FDA approved (Jul 2014) for PTCL. Phase I, II, III
	 <p>LAQ824</p>	nM	class I, II	Phase I
	 <p>LBH589 (panobinostat)</p>	nM	class I, II	FDA approved (Feb 2015) for Mult. Myeloma. Phase I, II, III

class	compd	[range]	HDAC specificity	clinical trials
hydroxamates	 <p data-bbox="639 315 826 354">givinostat</p>	μM	class I, II	Phase I/II. Orphan drug (arthritis, polycythaemia)
	 <p data-bbox="629 505 832 544">oxamflatin</p>	μM	NA	preclinical
	 <p data-bbox="649 696 813 735">scriptaid</p>	μM	NA	preclinical
	 <p data-bbox="653 886 803 925">SK7041</p>	nM	HDAC1,2	NA
	 <p data-bbox="653 1079 807 1118">SK7068</p>	nM	HDAC1,2	NA
	 <p data-bbox="658 1358 803 1396">tubacin</p>	μM	class IIb	NA

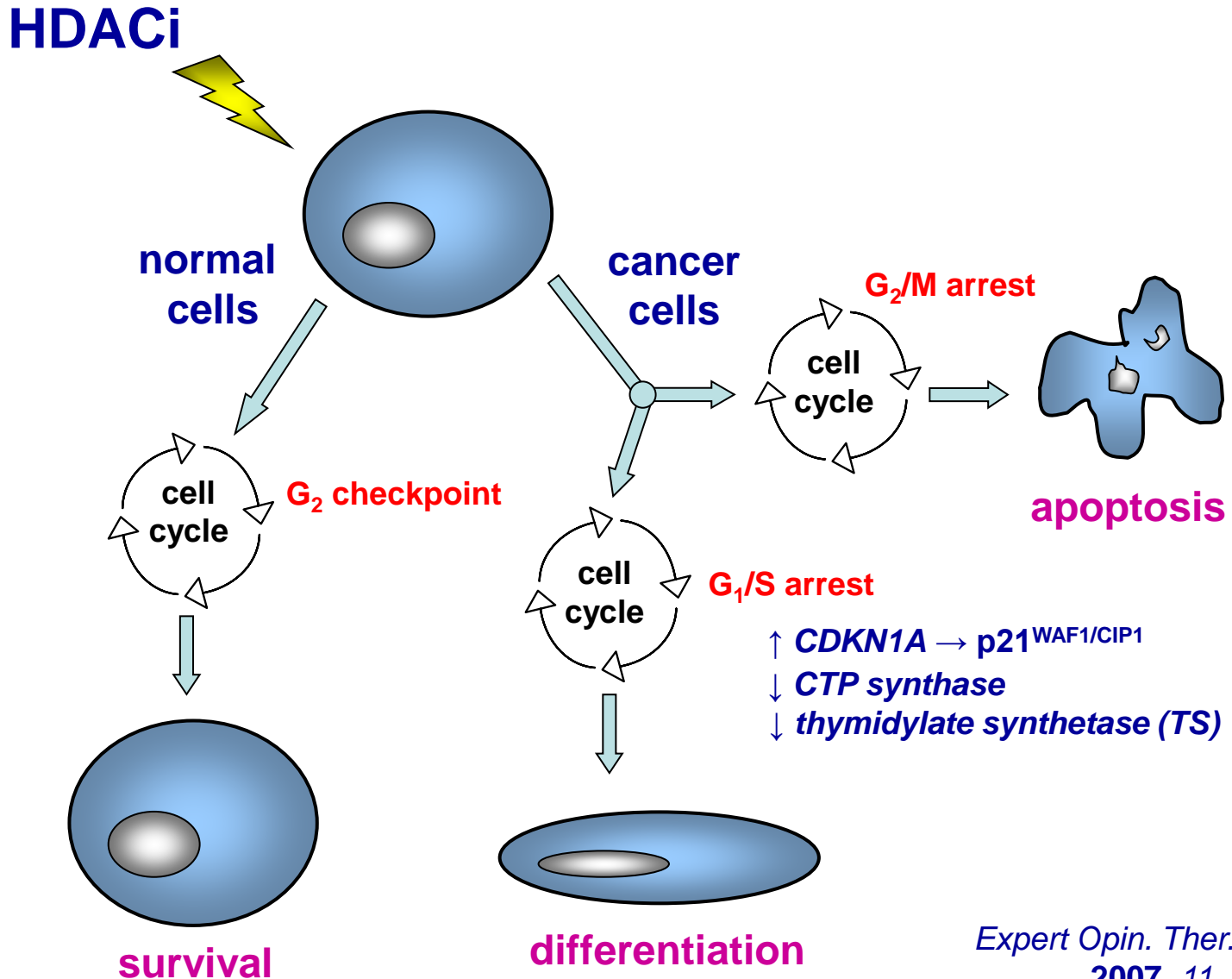
class	compd	[range]	HDAC specificity	clinical trials
cyclic tetrapeptides	 <p data-bbox="620 405 774 448">trapoxin</p>	nM	class I, IIa	preclinical
	 <p data-bbox="620 702 774 745">apicidin</p>	nM	HDAC1,3 not 8	preclinical
	 <p data-bbox="620 1016 774 1059">CHAP1</p>	nM	class I	preclinical
	 <p data-bbox="494 1330 919 1373">FK-228 (romidepsin)</p>	nM	class I	FDA approved (nov 2009) for CTCL. Phase I, II, III

class	compd	[range]	HDAC specificity	clinical trials
benzamides	 <p>MS275 (entinostat)</p>	μM	HDAC1,2,3 marginally 8	Phase I, II (solid tumors and lymphoma)
	 <p>CI994</p>	μM	NA	Phase I, II, III (solid tumors)
	 <p>MGCD101 (mocetinostat)</p>	nM	HDAC1,2, marginally 3	Phase I, II (solid tumors and lymphoma)
	 <p>chidamide</p>	μM	HDAC1,2,3 marginally 8	Approved in China (2014) for PTCL

Substrates and effects of HDAC inhibition



Cellular effects of HDACi



HDACi-induced biological effects

Apoptosis pathways

death receptor ptw

↑ TNF family ligands and receptors (ie, TRAIL, Fas)

mitochondrial death ptw

↑ Bim, Bmf, APAF1, caspase 9

↓ BCL2, BCL-X_L, MCL1, XIAP

↑ ROS production

indirect gene expression regulation

Ac E2F1, p53, STAT1, STAT3, NF-κB

transcription-independent effects

Ac Ku70

† HDAC6-PP1-α-tubulin complex → Ac α-tubulin

Ac HSP90 → degradation of client proteins

HDACi

Immunomodulatory effects

↑ MHCs, MICA, MICB

↑ CD40, CD80, CD86

↑ ICAM1

↓ TNFα

↓ IL1

↓ IFNγ

Anti-angiogenic effects

↓ VEGF

↓ bFGF

↓ HIF1α

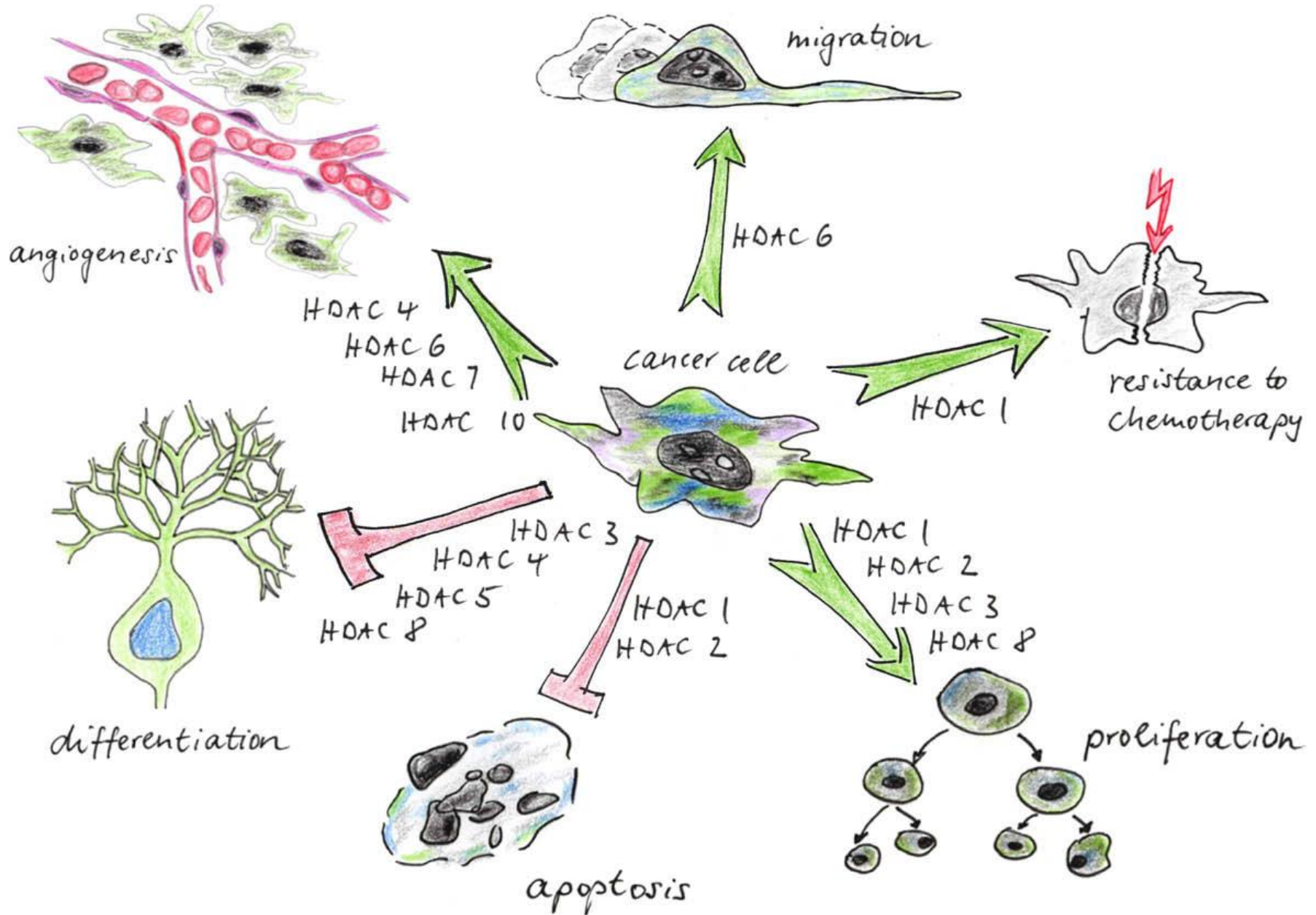
↓ angiopoietin

↓ TIE2

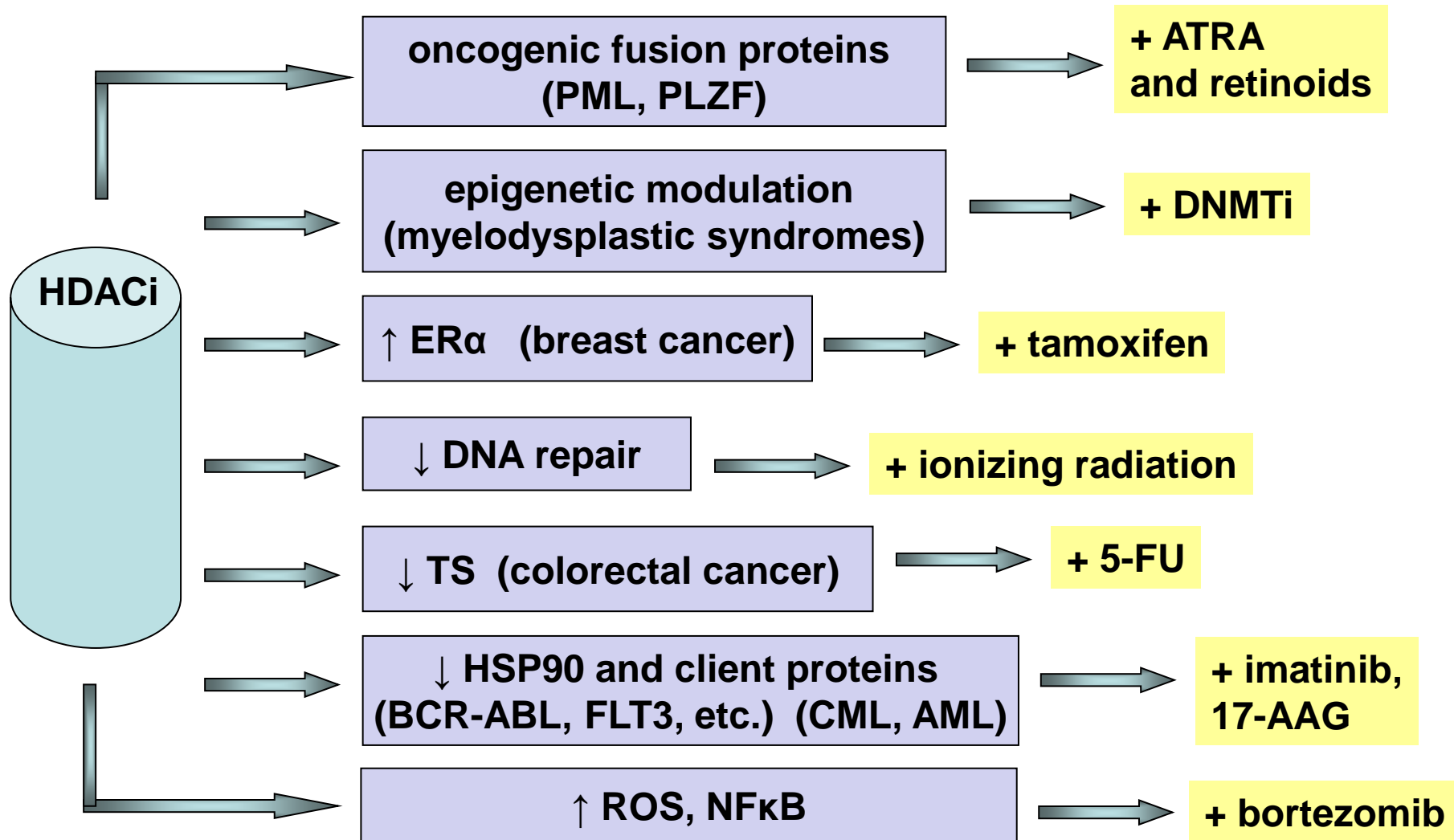
↓ eNOS

↓ CXCR4

HDAC and Cancer



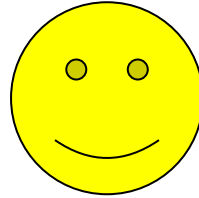
Mechanism-based utility of HDACi: combination treatment



HDACi: opened questions

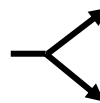
- ❖ Which is the **role** of different HDAC enzymes in different diseases (not only cancer) ?
- ❖ Is **class/subunit specificity** an advantage or will **pan-inhibitors** be more successful ?
- ❖ Is the **future** of HDACi in combination therapies ?

Sirtuins as potential therapeutic targets



- **Sensors** of cell metabolic state (NAD⁺, nicotinamide levels)
- Mediate **caloric restriction** (CR) health/survival beneficial effects also in mammals
- Delay aging and **prolong lifespan** in non-mammalian species
- In humans regulate:
 - cell survival under stress
 - neuro/cardioprotection
 - metabolism (↑ insulin secretion, ↓ adipogenesis, etc.)

Sirtuins' activators



Age-related diseases

(diabetes, neurodegeneration, heart failure, etc...)

Metabolic disorders

(obesity, atherosclerosis, etc...)

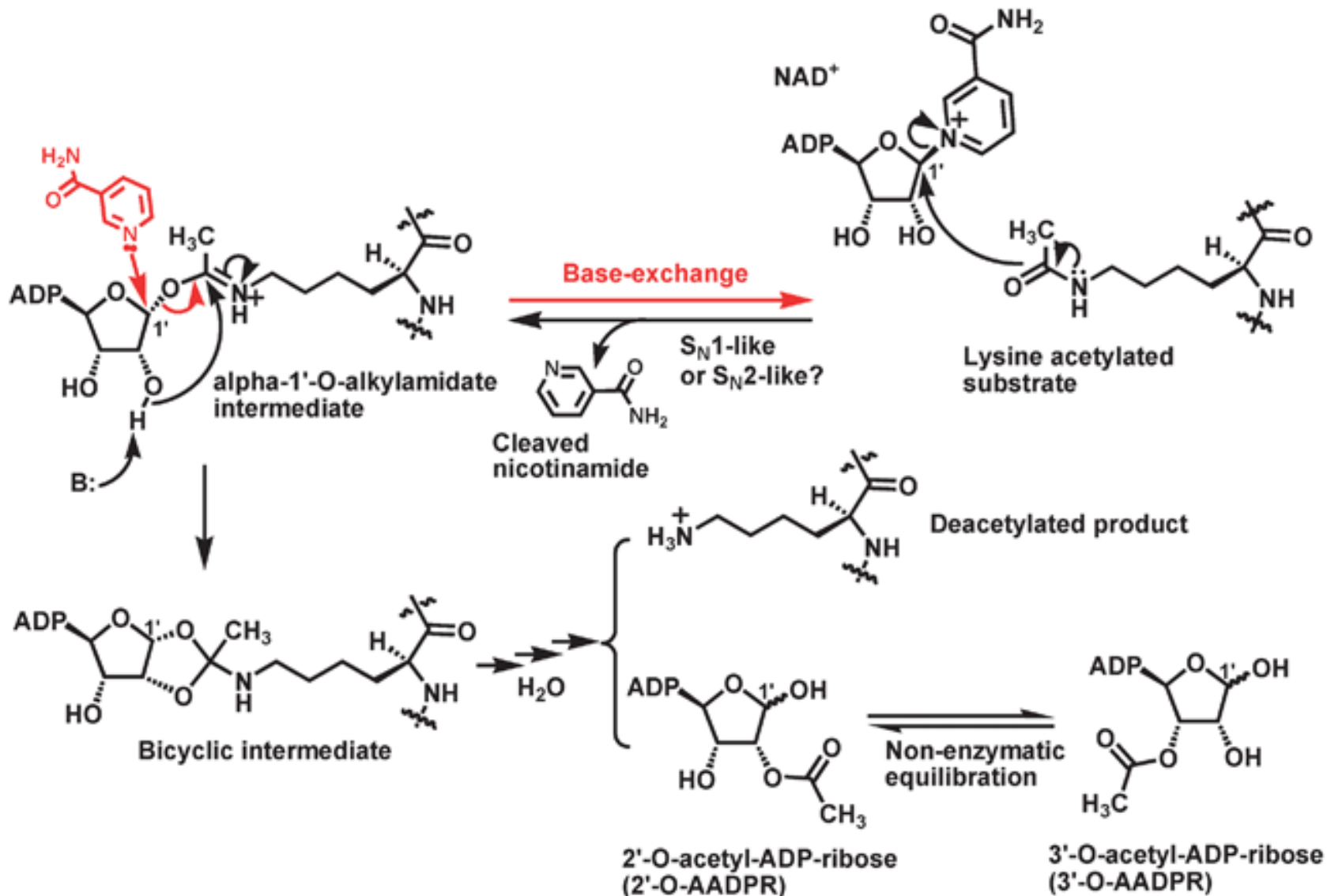


- **Upregulated** in some tumor types (SIRT1/3/7)
- **Tumor suppressor** proteins (p53, etc.) **inactivation**, **oncoprotein** (BCL6) **activation**
- **Anti-apoptotic** (E2F1, FOXO3a, Ku70, etc.), **anti-differentiation** (BCL6) activities
- Cell cycle progression, chromosomal stability, DNA repair (SIRT1/2/6)
- Skeletal muscle differentiation block (SIRT1)
- Tat-dependent HIV-1 transcription promotion (SIRT1)

Sirtuins' inhibitors

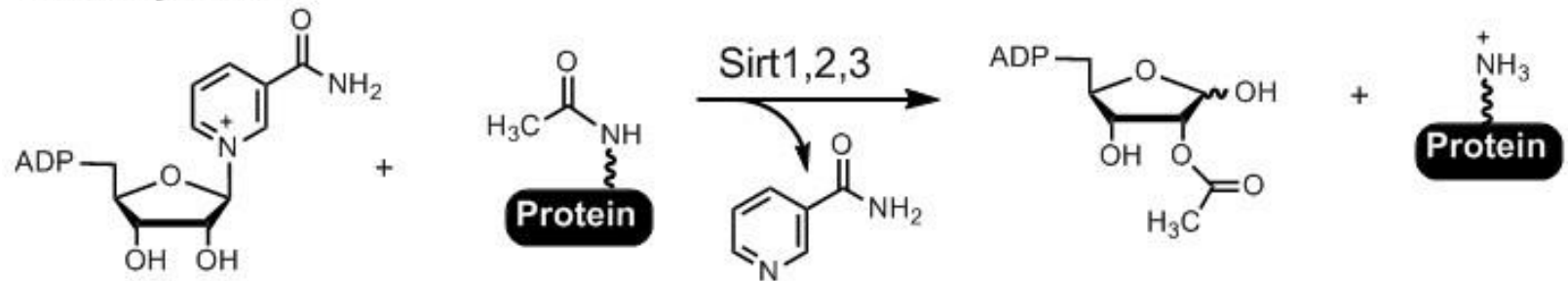


Mechanism for the Sirtuin-Catalysed Deacylation Reaction

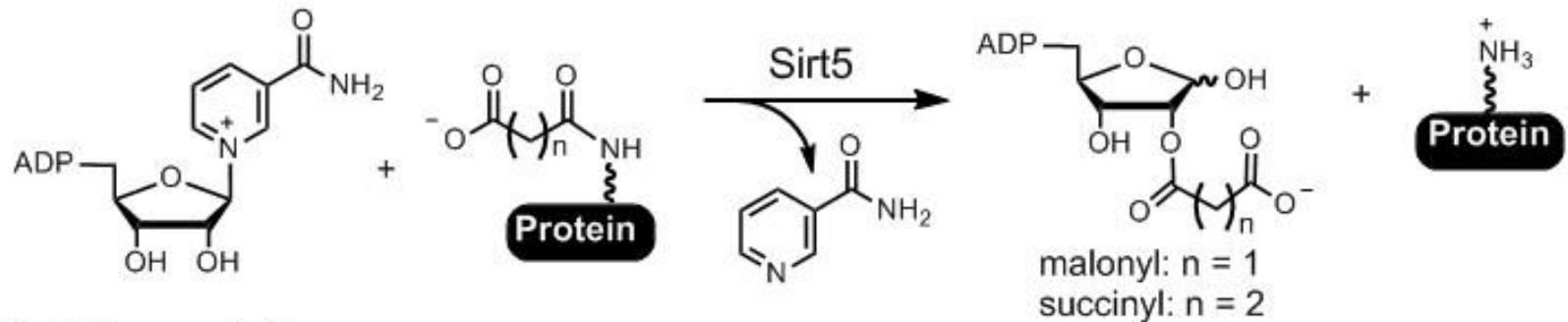


Sirtuins: Not Only Deacetylases

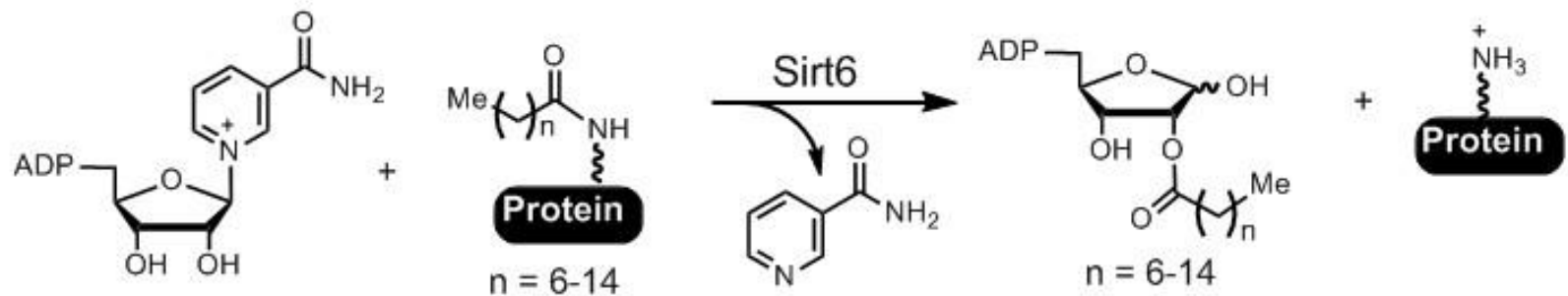
Deacetylation:



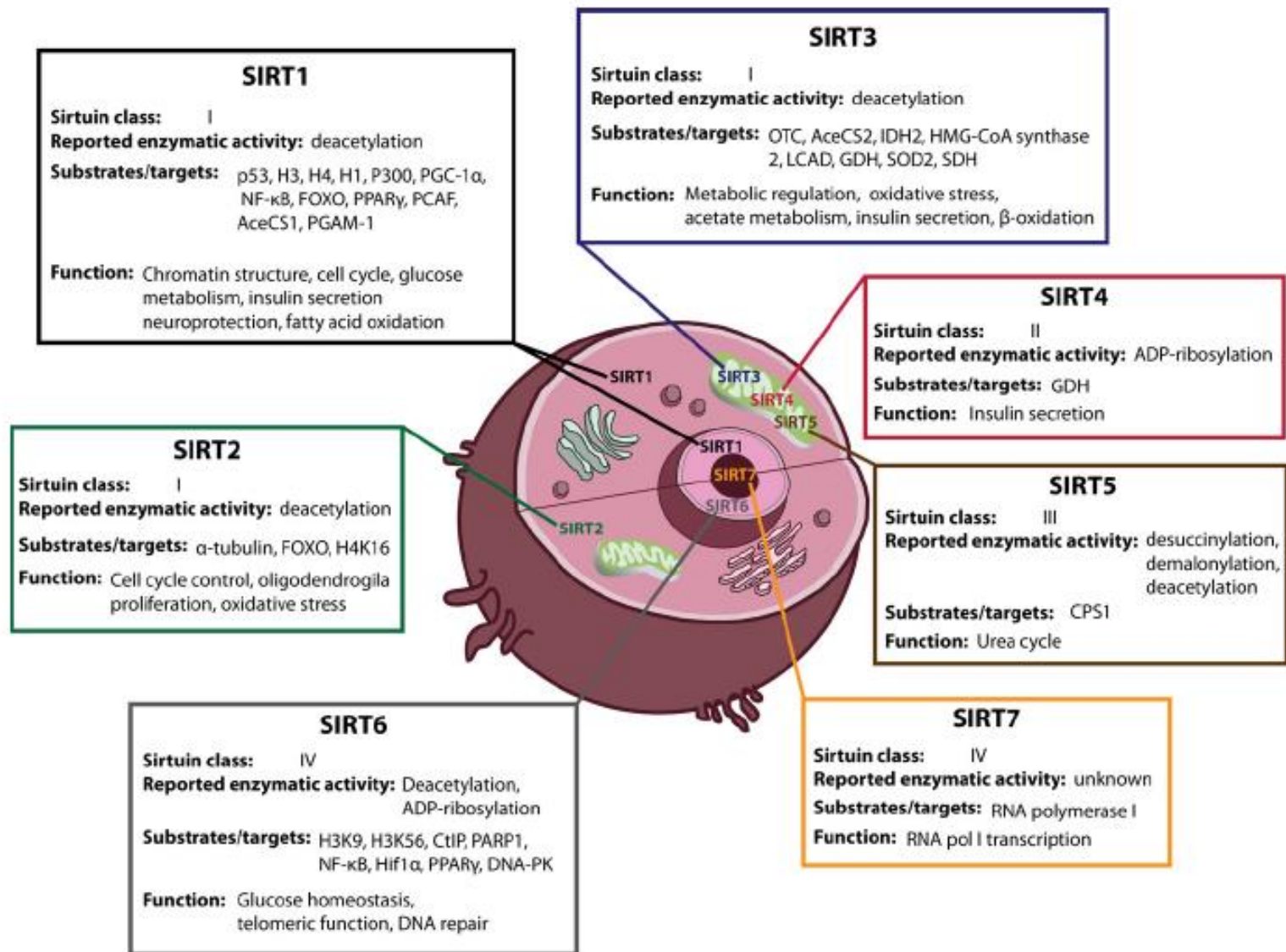
Demalonylation and desuccinylation:



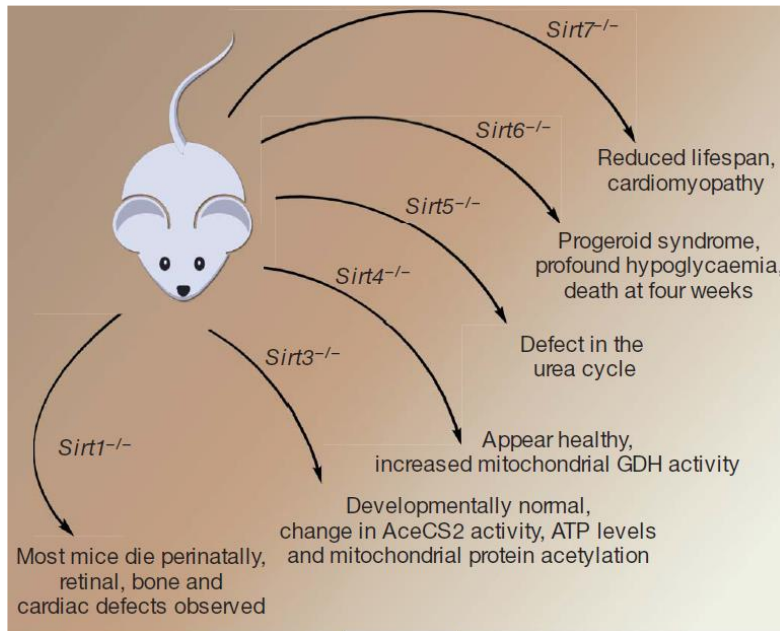
Defatty-acylation:



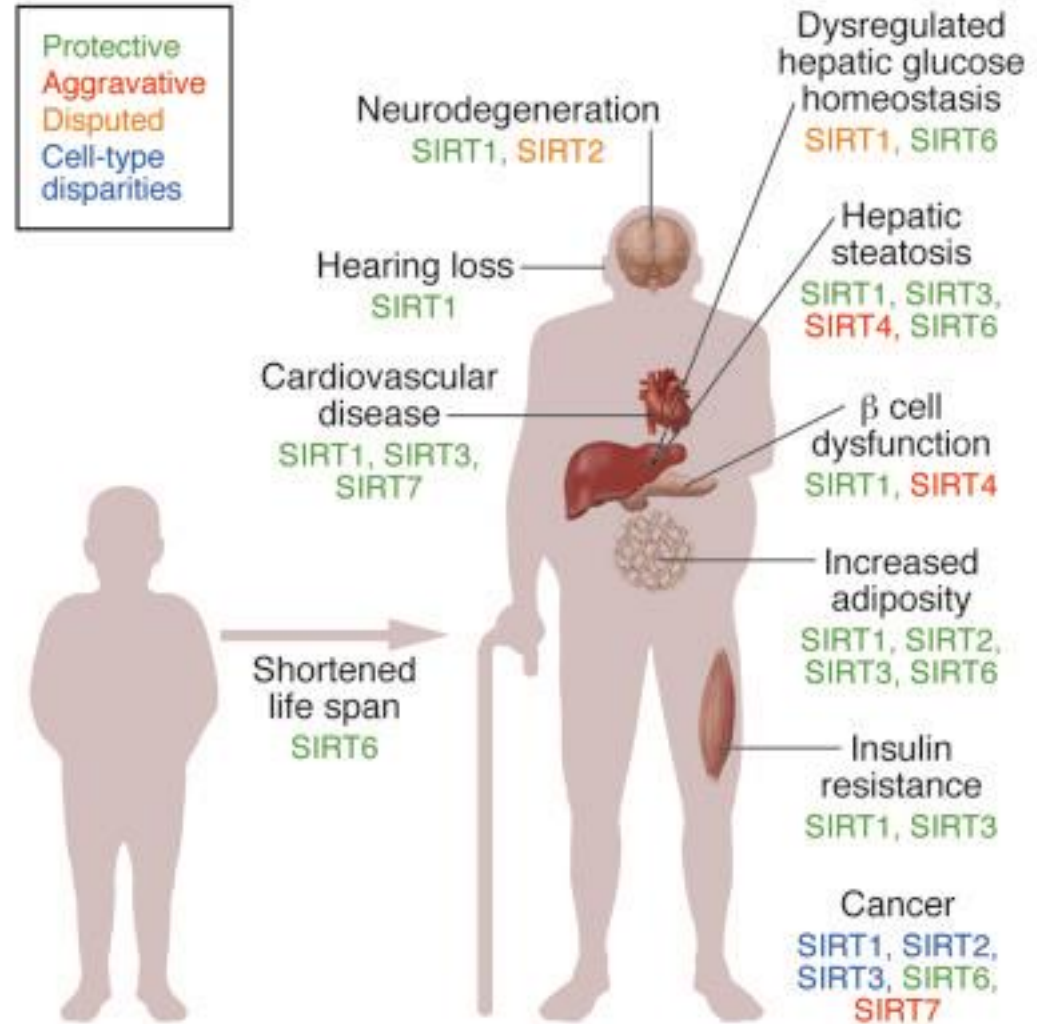
Sirtuins and Metabolic Diseases



Dissecting the Biological Roles of Sirtuins



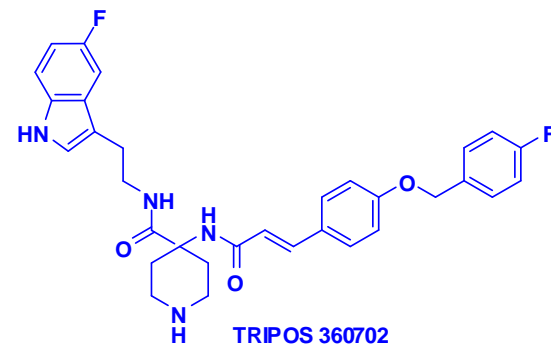
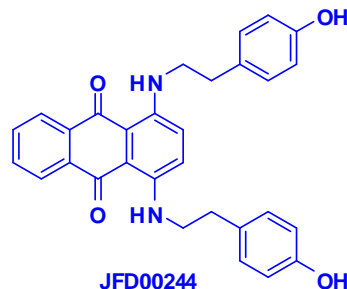
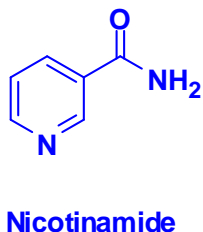
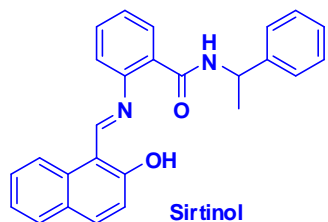
Finkel T et al, *Nature* 2009, 460, 587



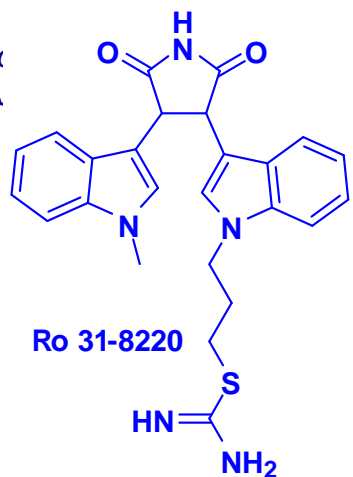
Hall JA et al, *J Clin Invest* 2013, 123, 973

Sirtuin Modulators

Sirtuin Inhibiting Compounds (STICs)

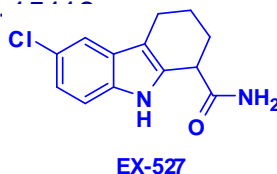


Grozier, C.M. *et al.*,
Redalov A *et al* *Proc*



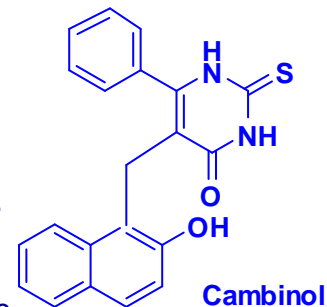
, 38837-38843.
J01, 98, 15113-
45099-45107.

Tervo, A. J., *et al.*, *J. Med. Chem.* **2004**, 47, 6292-6298..

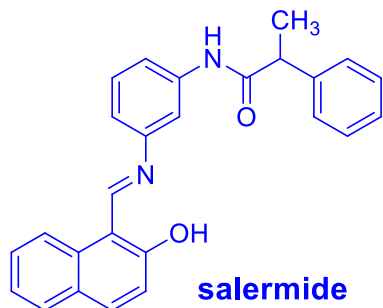
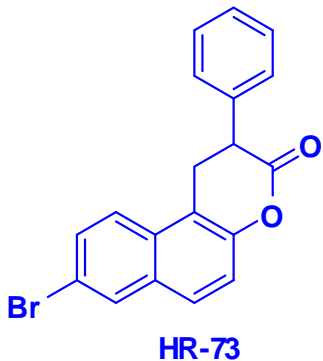


Pagans, S. *et al.*, *PLoS Biol.* **2005**, 3, e41.

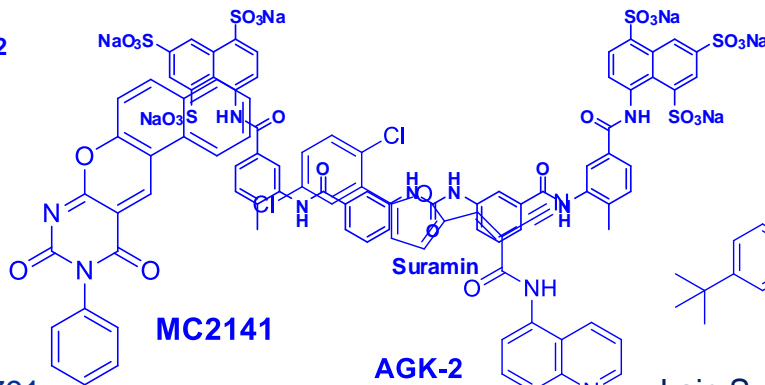
Trapp, J. *et al.*, *J. Med. Chem.* **2006**, 49, 7307-7316.



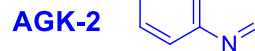
Napper, A. D. *et al.*, *J. Med. Chem.* **2005**, 48, 8045-8054.



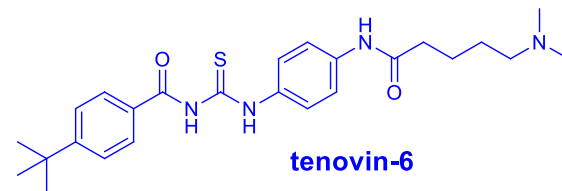
Lara E. *et al*, *Oncogene* 2009, 28, 781-791



Rotili, D. *et al.*, *ChemMedChem* 2010, 5, 604-617



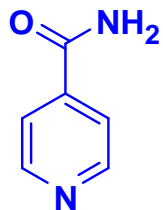
Hetweg, B. *et al.*,
Cancer Res. **2006**, 66, 4368-4377.



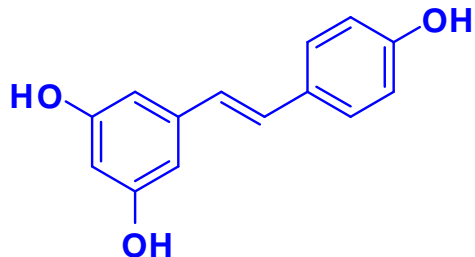
Lain S *et al*, *Cancer Cell* **2008**, 13, 454-463

TF *et al*, *Science* 2007, 317, 516-519

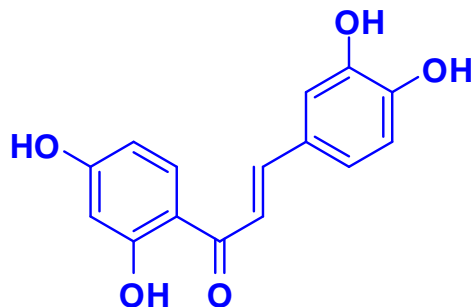
Sirtuin Activating Compounds (STACs)



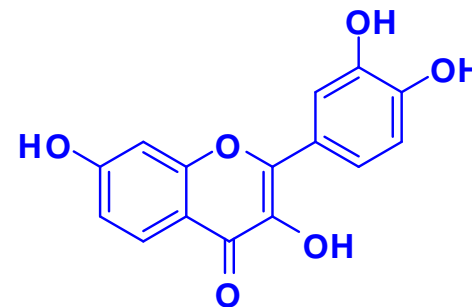
Isonicotinamide



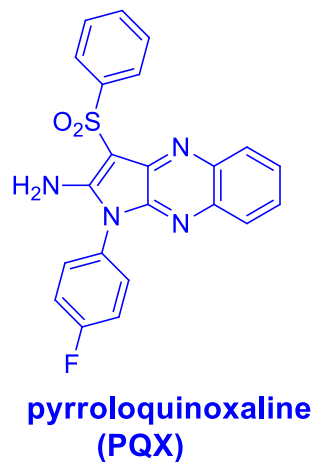
Resveratrol



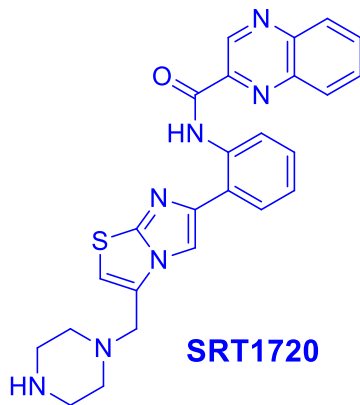
Butein



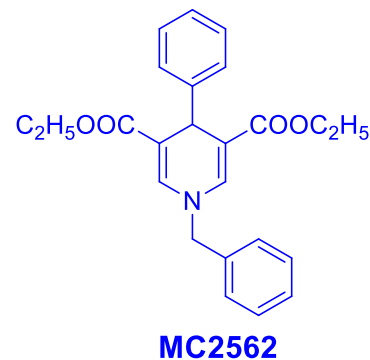
Fisetin



pyrroloquinoxaline
(PQX)



SRT1720



MC2562

Sauve AA *et al*, *Mol Cell* **2005**, 17, 595-601

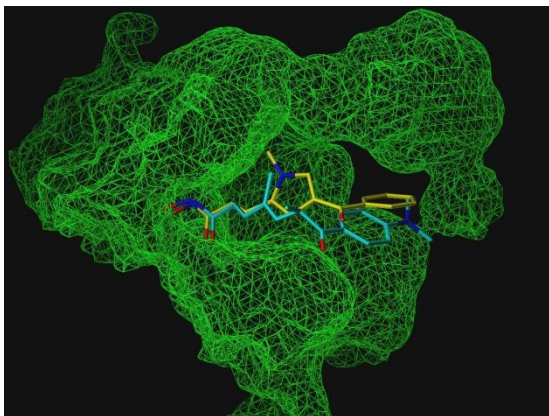
Baur JA *et al*, *Nature* **2006**, 444, 337-342

Milne JC *et al*, *Nature* **2007**, 450, 712-716

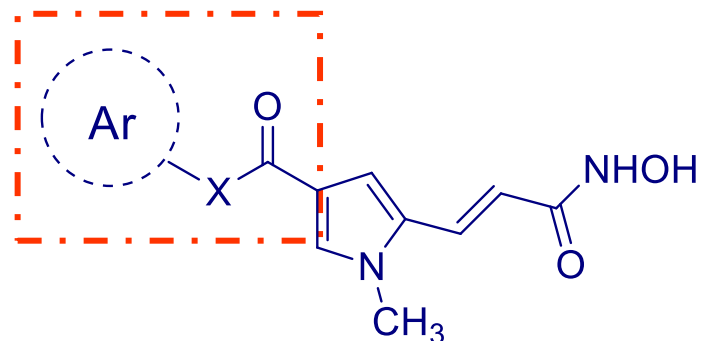
Nayagam VM *et al*, *J Biomol Screening* **2006**, 11, 959-967

Mai A *et al*, *J Med Chem* **2009**, 52, 5496-5504

HDAC inhibitors: our experience



Finnin *et al*, *Nature* 1999, 401, 188-193



APHAs

IC₅₀s: 3.8 to 0.04 μM

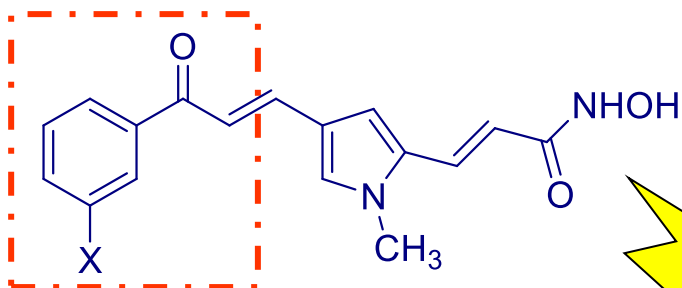
J Med Chem 2001-2004

ChemMedChem 2006

J Chem Inf Model 2006

Int J Biochem Cell Biol 2007, 2009

Mol Pharmacol 2007



MC1568 (X = F)

MC1575 (X = Cl)

J Med Chem 2003, 2005

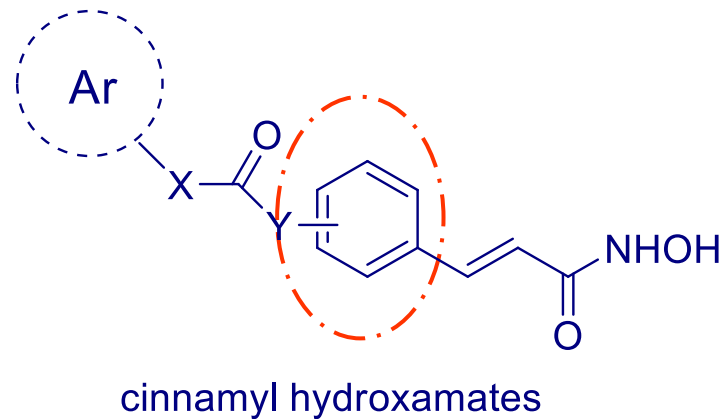
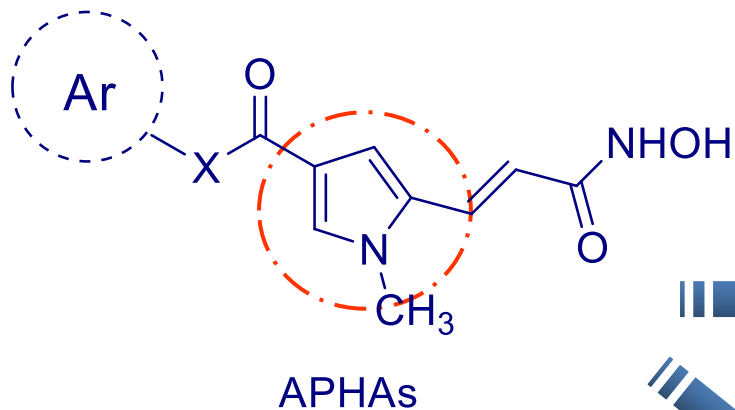
Cancer Res 2006

Circ Res 2008

EMBO Rep 2009

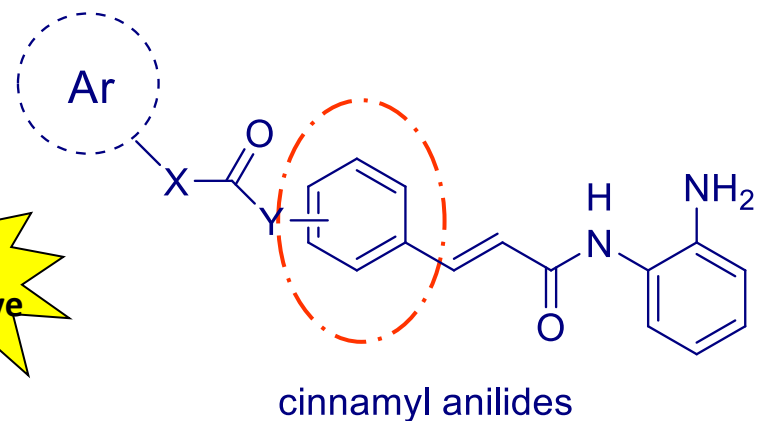


chemical
manipulations



IC₅₀s: low nM range
apoptosis: >60% (U937 cells)

compd	IC ₅₀ , μM		
	HCT116	A549	K562
MC2578	0.26	0.59	0.18
MC2590	0.07	0.32	0.05
MC2602	0.21	0.60	0.13
MC2625	0.07	0.22	0.04
MC2664	0.43	1.28	0.37
MC2651	0.56	1.35	0.54
MC2666	0.32	1.51	0.33



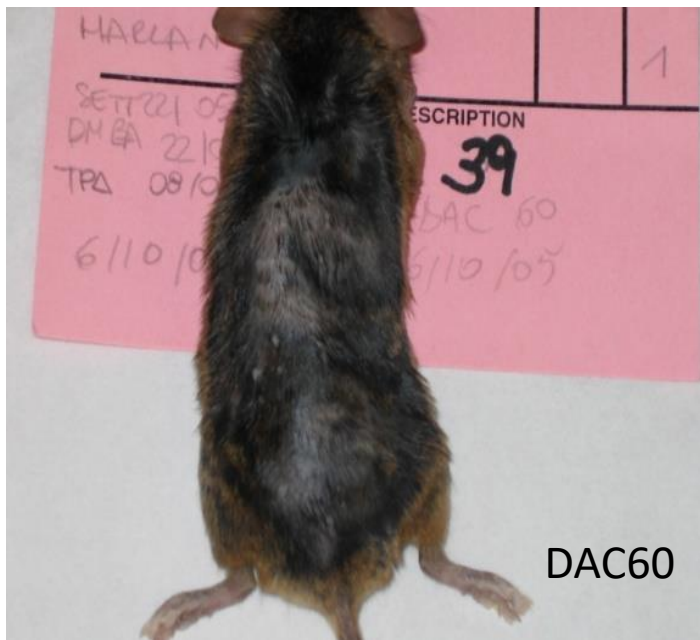
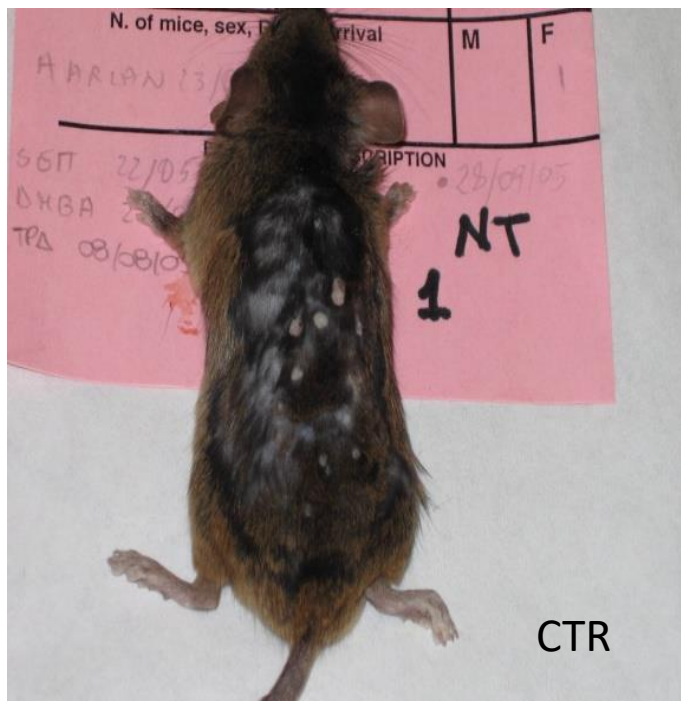
IC₅₀s: sub-μM/nM range
differentiation: >80% (U937 cells)

Med Chem 2005

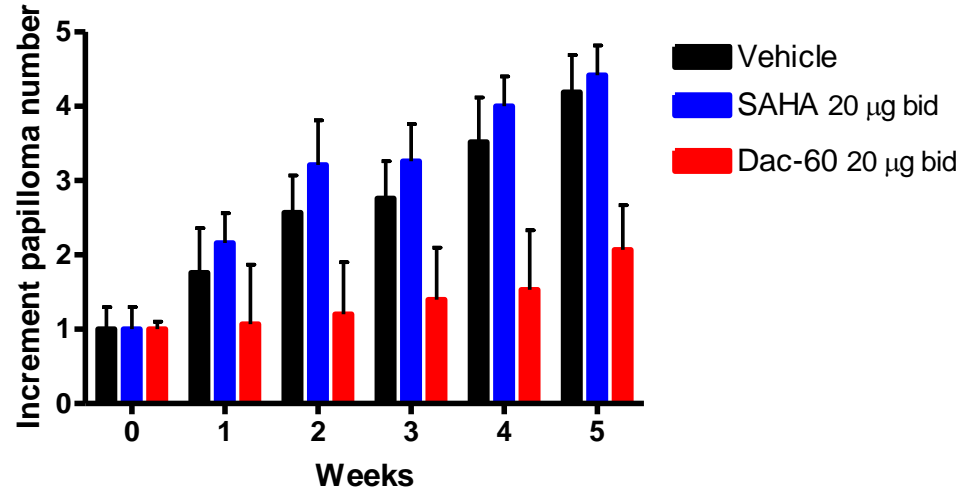
Minucci S *et al*, PCT Int. Appl. 2006, WO 2006037761

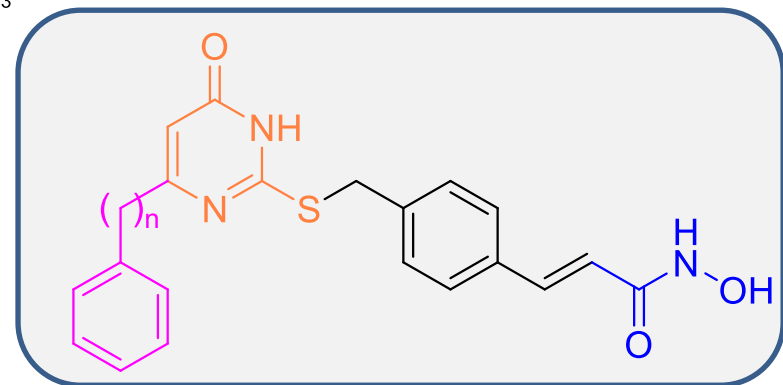
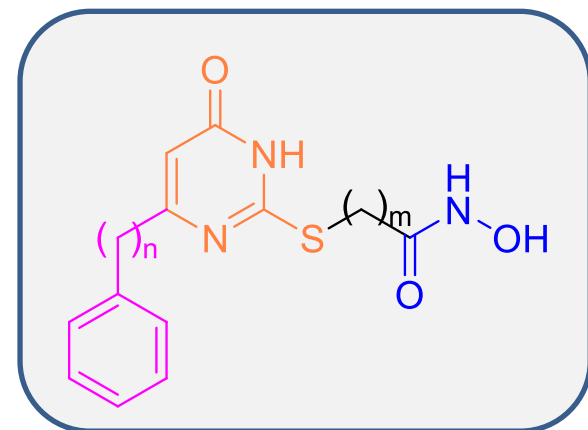
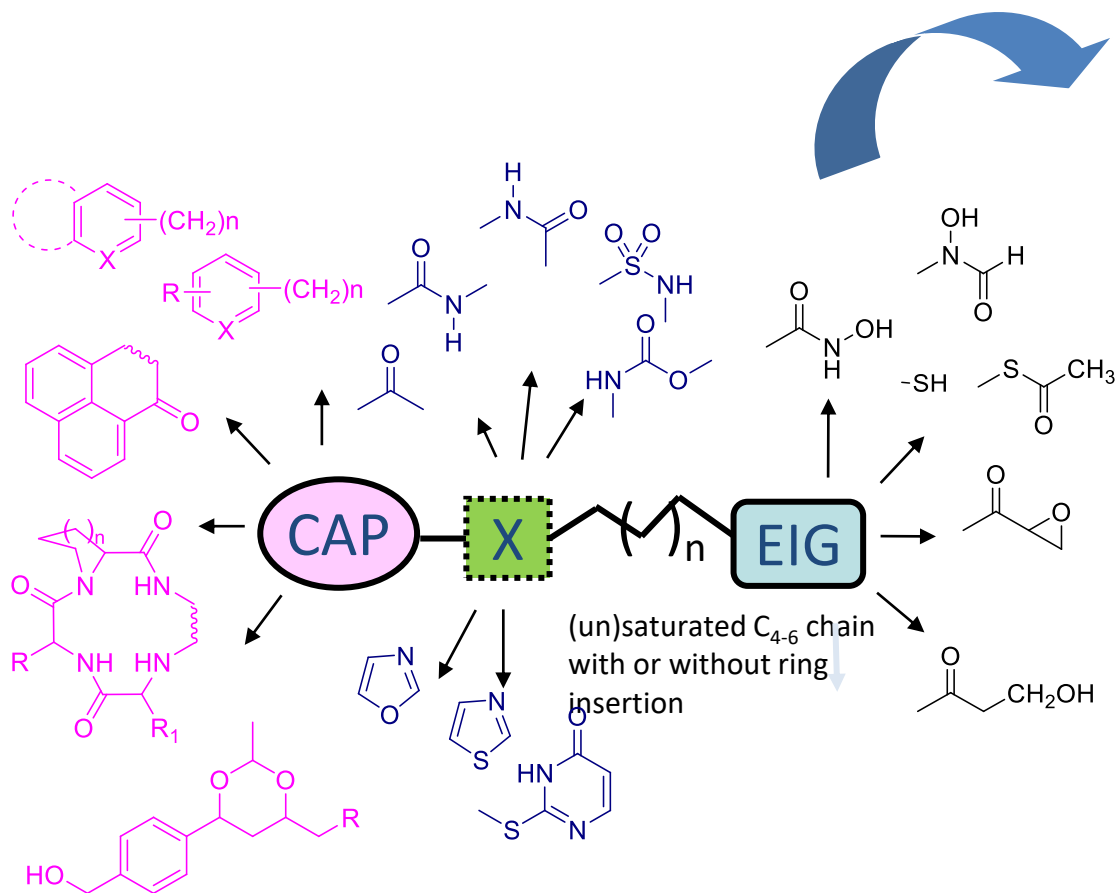
Mai A *et al*, PCT Int. Appl. 2007, WO 2007113249

J Med Chem 2010



In vivo efficacy in papilloma induced mouse model





UBHAs

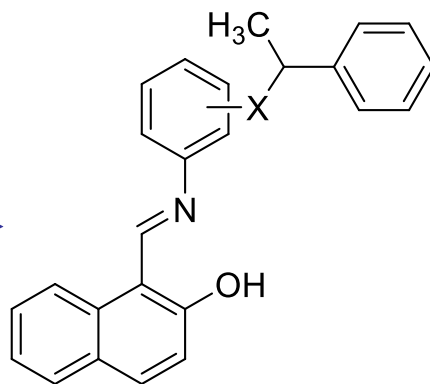
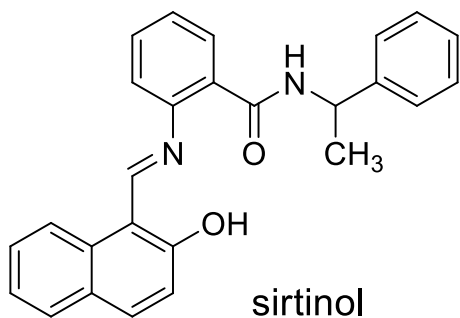
IC₅₀s = low nM range
 apoptosis: >80% (U937 cells)
 differentiation: >40% (U937 cells)

Pharmacophore model for HDACi

Miller TA et al, *J Med Chem* 2003, 46, 5097-116
 Mai A et al, *Med Res Rev* 2005, 25, 261-309
 Mai A, *Expert Opin Ther Targets* 2007, 11, 835-51
 Paris et al, *J Med Chem* 2008, 51, 1024-40

Bioorg Med Chem Lett 2005, 2007, 2008
J Med Chem 2006
FEMS Yeast Res 2007

SIRT inhibitors: our experience



sirtinol analogues

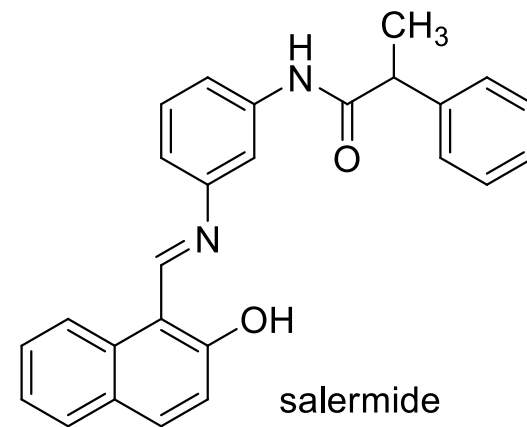
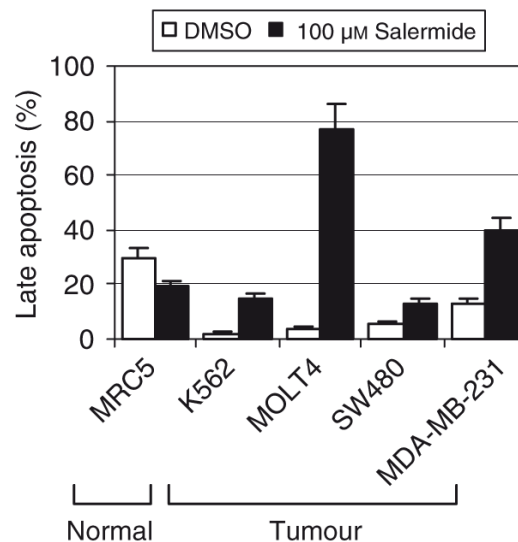
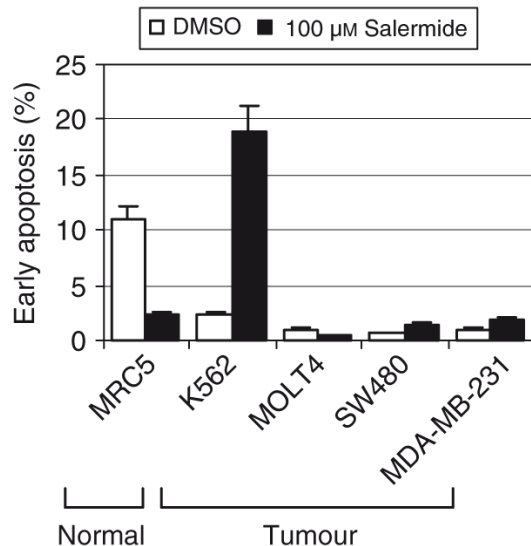
X = CONH, NHCO, SO₂NH, SO₂CH₂, SCH₂

IC₅₀s = low μM range

apoptosis: >70% (U937 cells)

differentiation: >50% (U937 cells)

Grozinger CM *et al*, *J Biol Chem*
2001, 276, 38837-43

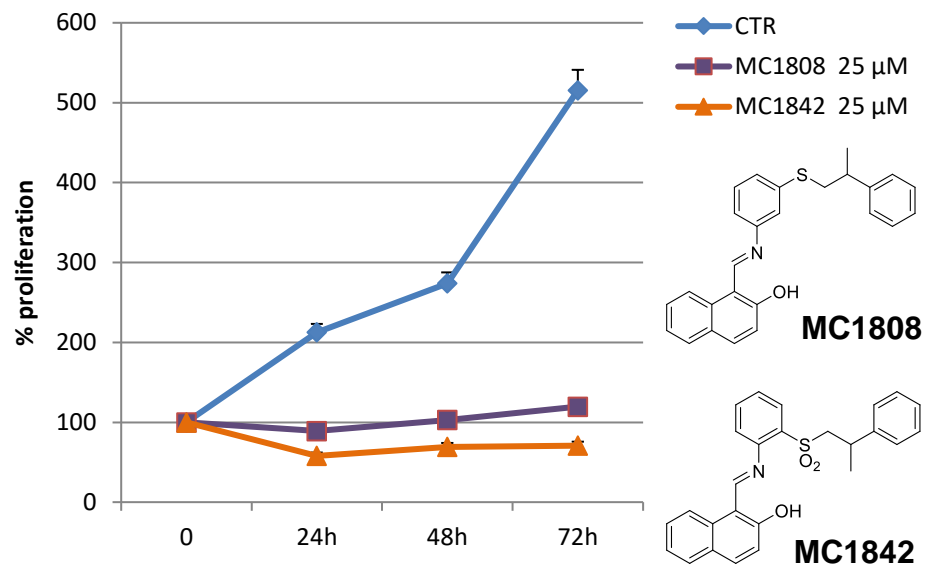
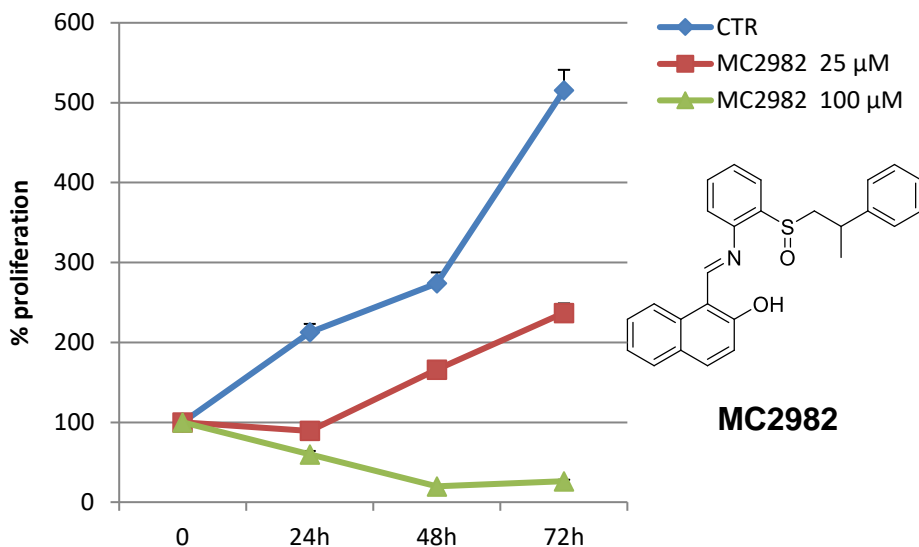
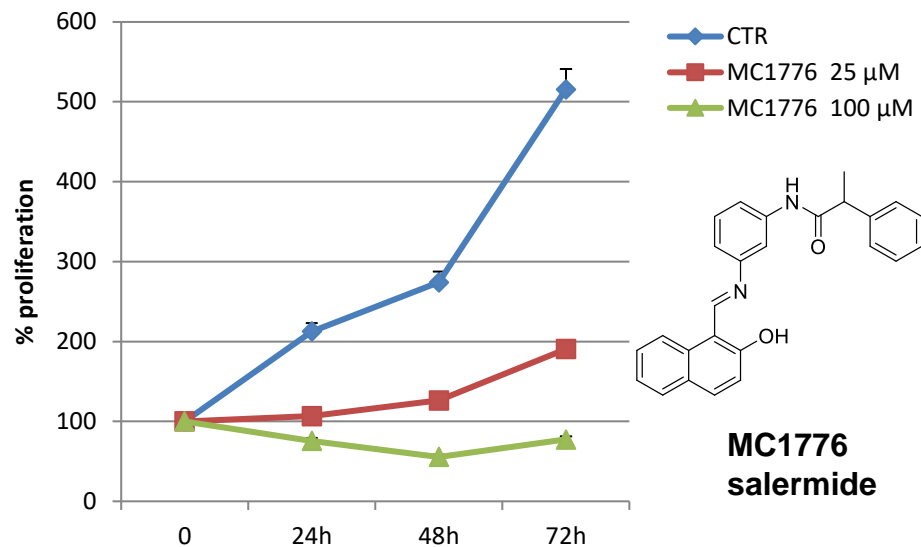
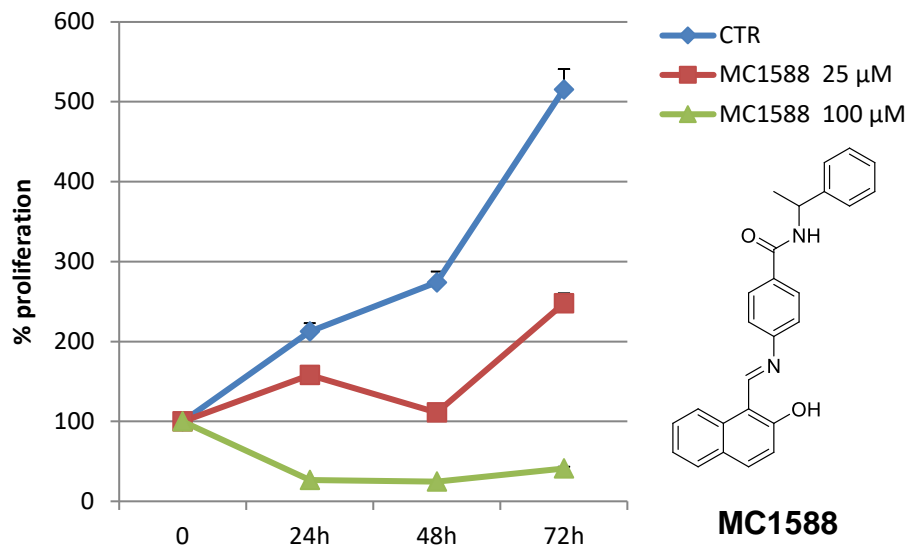


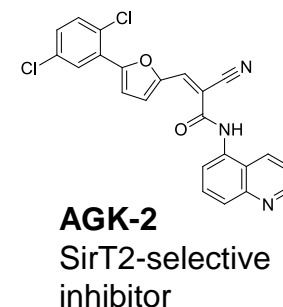
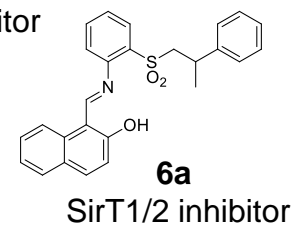
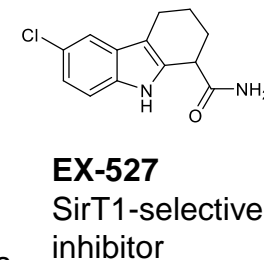
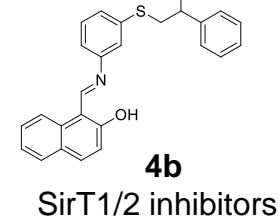
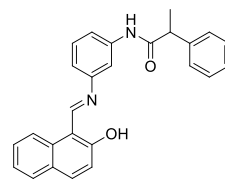
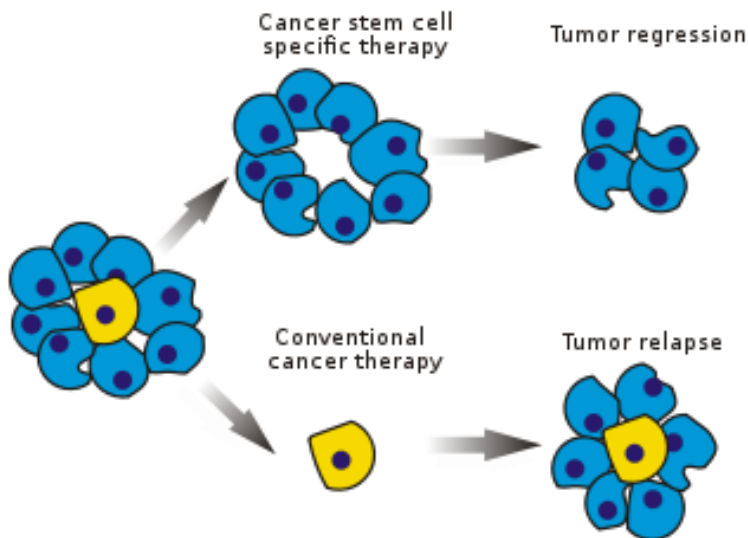
Mai A *et al*, *J Med Chem* 2005, 48, 7789-95

Sinclair DA and Mai A, PCT (2007) WO-2007084162

Fraga MF *et al*, *Oncogene* 2009, 28, 781-791

MOLT-4 Antiproliferative Assays

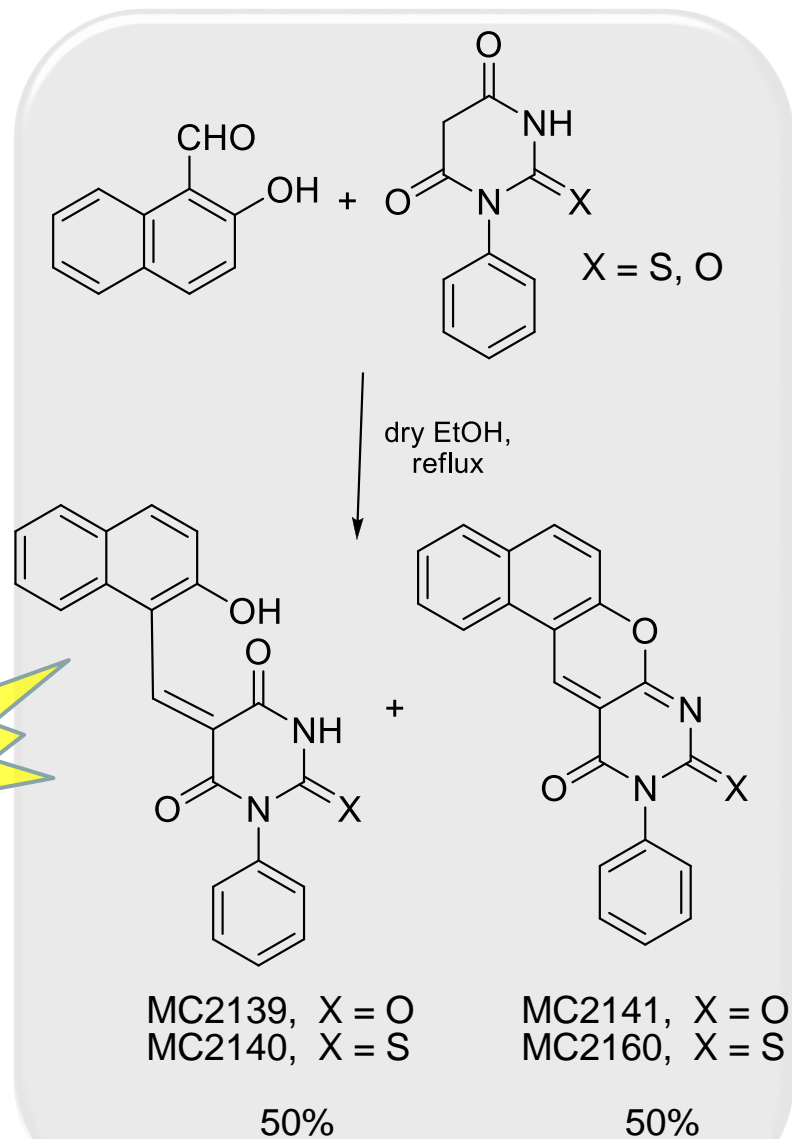
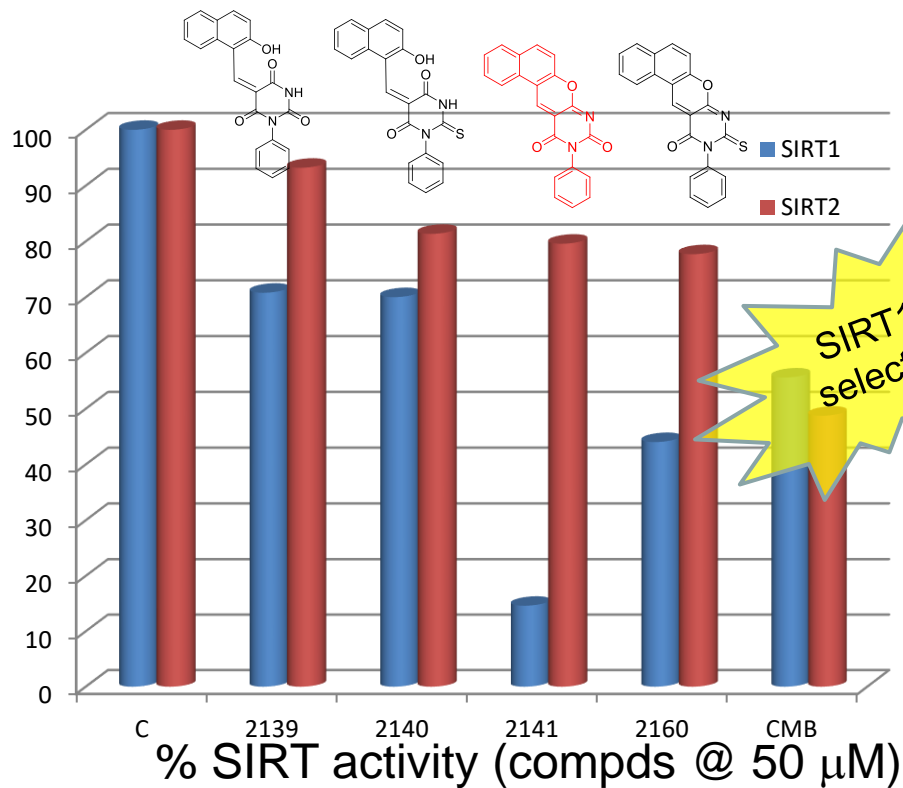
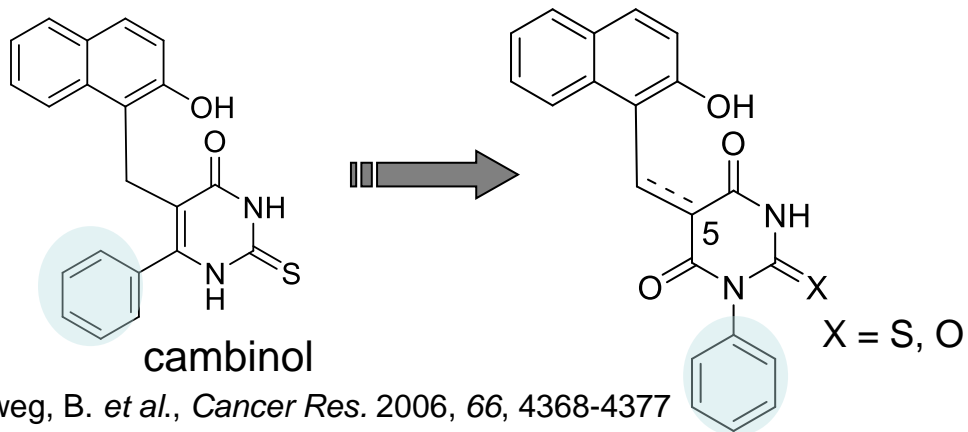


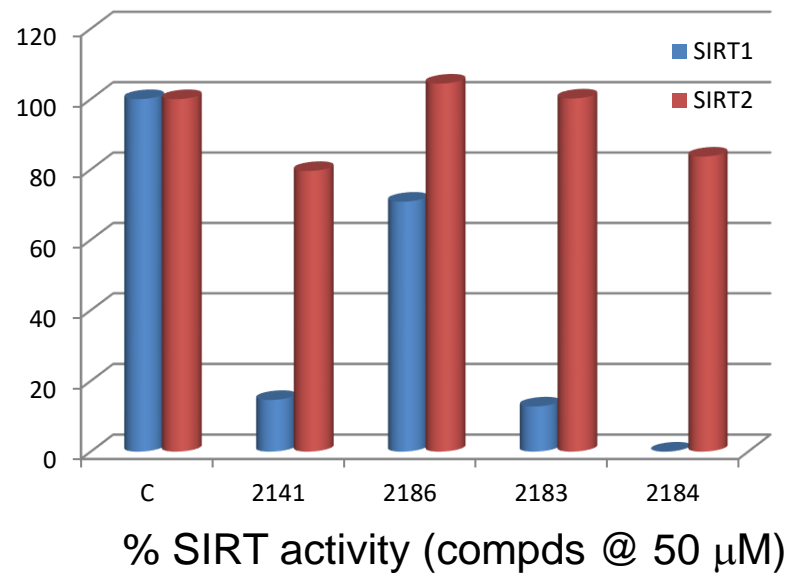
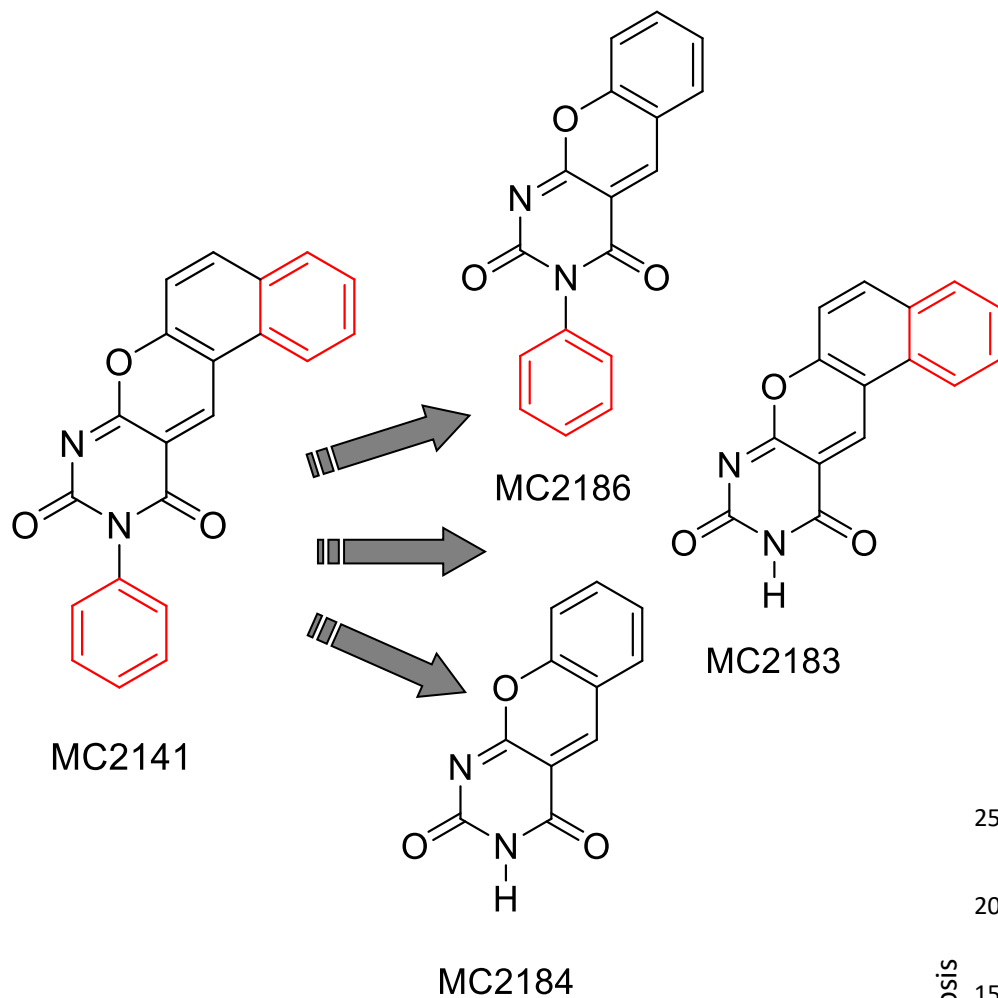


compd	CC ₅₀ , μM			
	CRC CSCs		GBM CSCs	
	CRO	1.1	30P	30PT
2b	6.7 ± 1.2	9.7 ± 3.1	41.0 ± 5.2	36.5 ± 2.5
4b	14.5 ± 1.2	20.0 ± 2.8	15.5 ± 1.9	15.6 ± 3.6
5b	ND ^c	23.4 ± 0.6	35.0 ± 1.9	ND
6a	15.7 ± 1.0	19.5 ± 0.7	17.4 ± 1.7	15.3 ± 3.1
6c	ND	34.0 ± 2.2	30.2 ± 1.8	ND
EX-527	20% inhibition ^b	no inhibition ^b	10% inhibition ^b	20% inhibition ^b
AGK-2	50% inhibition ^b	50% inhibition ^b	12.5 ± 0.5	9.6 ± 1.0

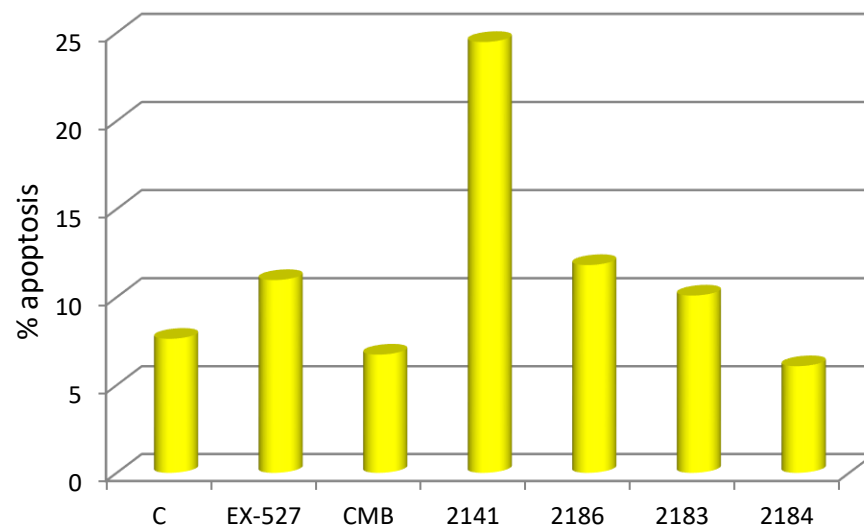
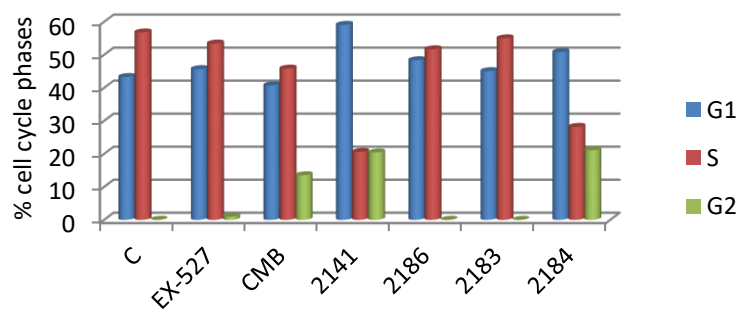
^aValues are means of three experiments. ^bAt 50 μM. ^cND, not detected.

Benzooxadeazaflavins (BDF4s)

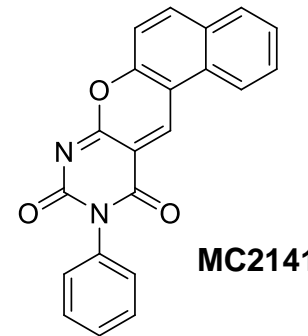




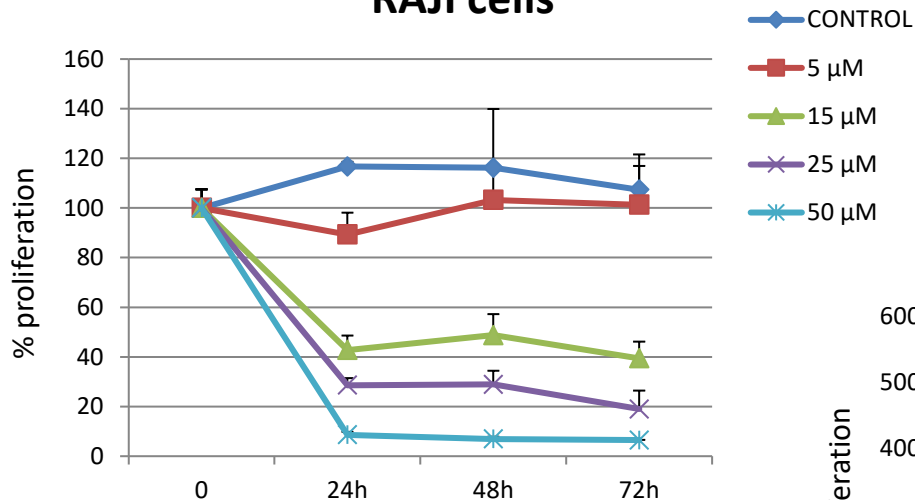
U937 cells, 50 μM, 50 h



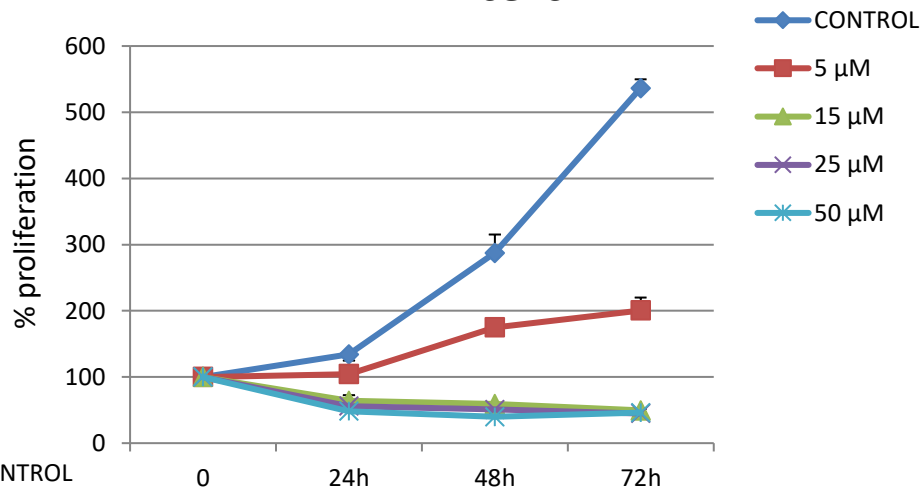
Antiproliferative Activities of MC2141



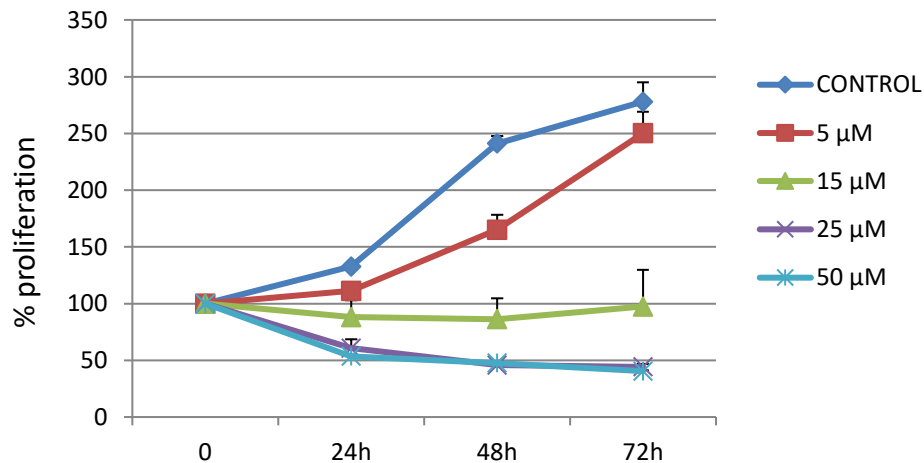
RAJI cells



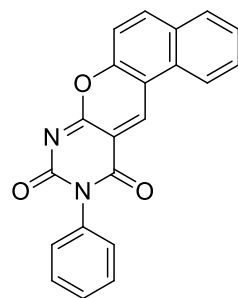
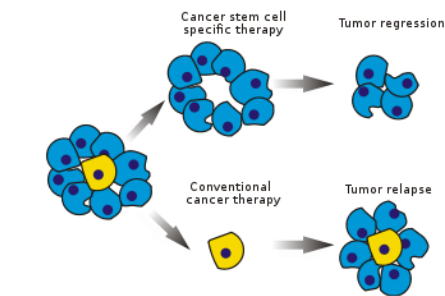
DLD1 cells



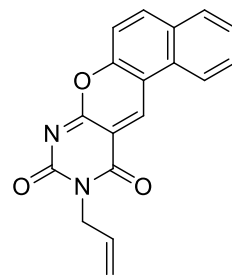
HeLa cells



M. Fraga's lab



1, MC2141

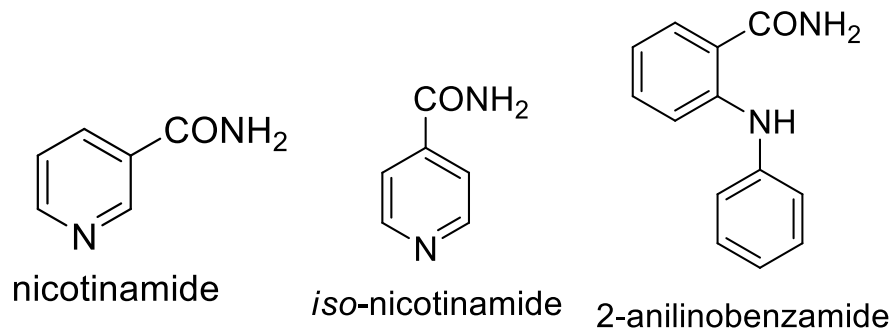


2d

compd	CC ₅₀ , μM			
	CRC CSCs		GBM CSCs	
	CRO	1.1	30P	30PT
1	7.0 ± 0.6	5.5 ± 0.7	4.9 ± 0.2	3.9 ± 0.7
2a	75.4 ± 11.9	33.5 ± 10.7	34.2 ± 4.6	33.4 ± 2.8
2b	23.6 ± 2.6	14.9 ± 2.1	15 ± 1.7	15.6 ± 6.0
2d	9.6 ± 0.5	8.5 ± 0.4	6.6 ± 1.2	5.0 ± 3.8
EX-527	20% ^a	NI ^{a,b}	10% ^a	20% ^a
AGK-2	50% ^a	50% ^a	12.5 ± 0.5	9.6 ± 1.0

^aInhibition at 50 μM; ^bNI, no inhibition

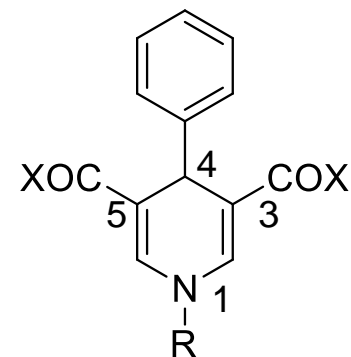
1,4-Dihydropyridines (DHPs)



Bitterman, K. J. *et al.*, *J. Biol. Chem.* 2002, 277, 45099-107

Sauve, A. A. *et al.*, *Mol. Cell* 2005, 17, 595-601

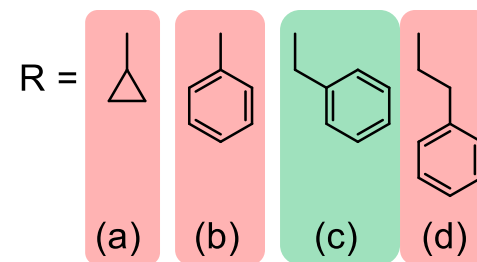
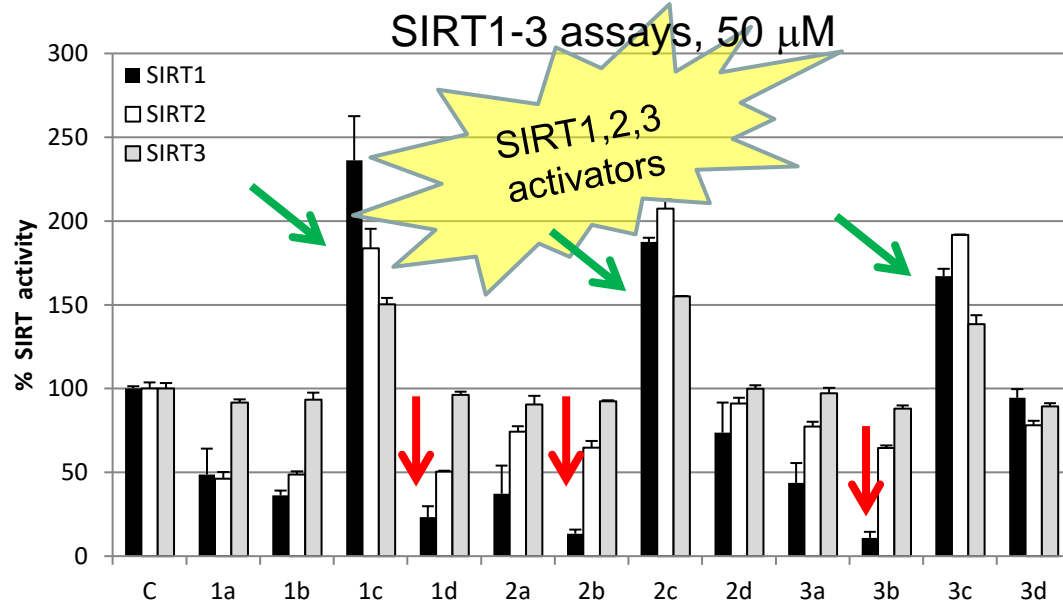
Suzuki, T. *et al.*, *ChemMedChem* 2006, 1, 1059-62



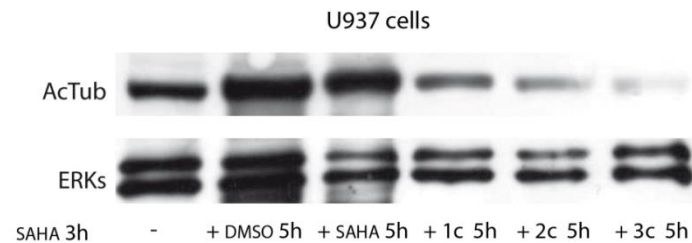
1a-d X = OC₂H₅

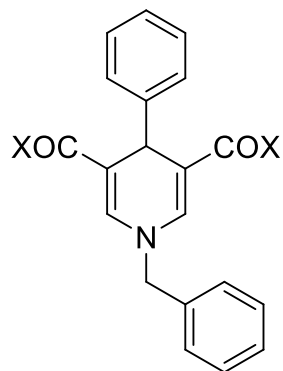
2a-d X = OH

3a-d X = NH₂



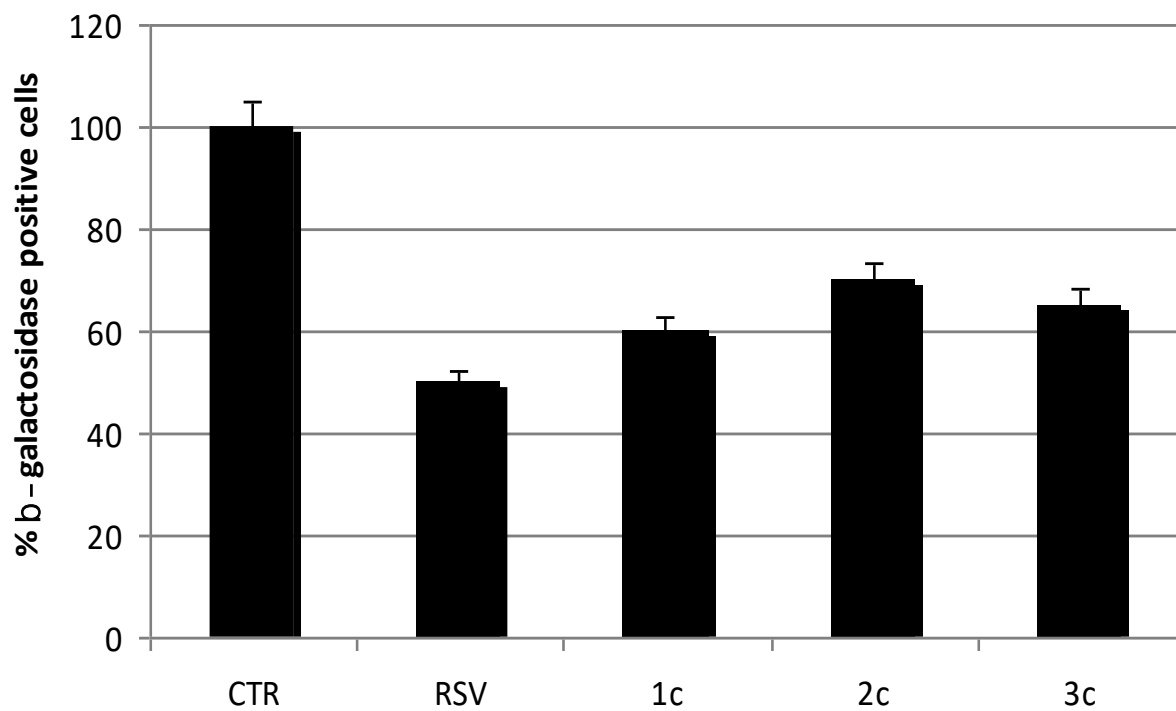
Mai, A. *et al.*, *J. Med. Chem.* 2009, 52, 5496-5504



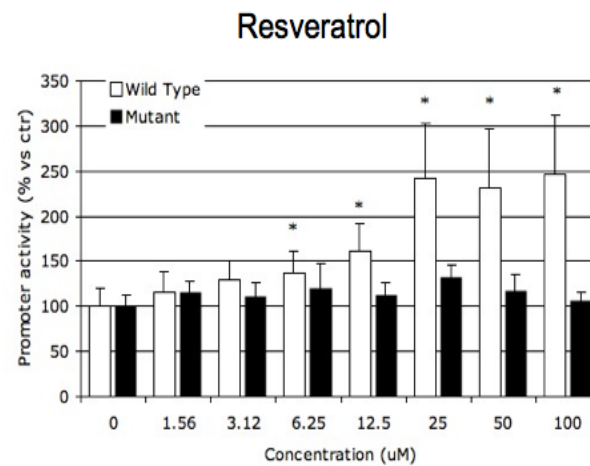
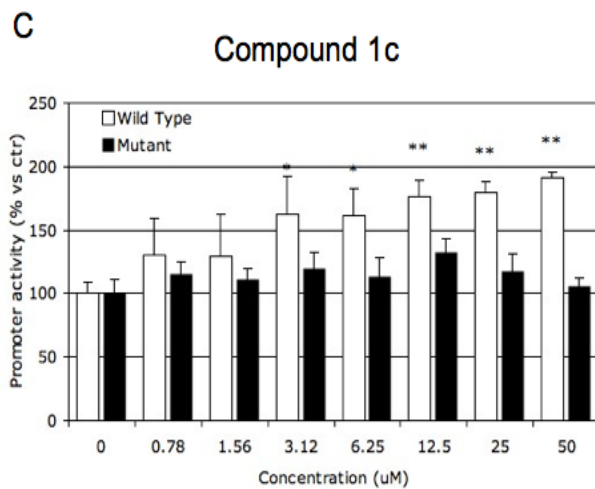
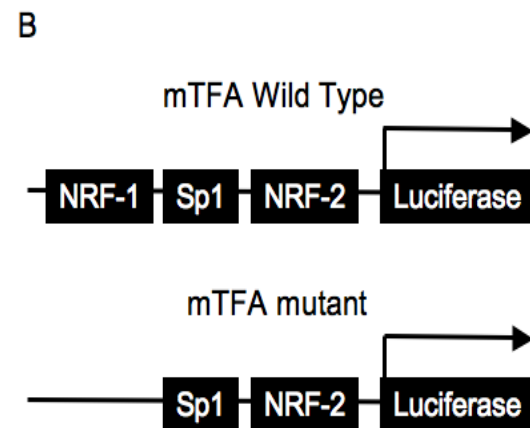
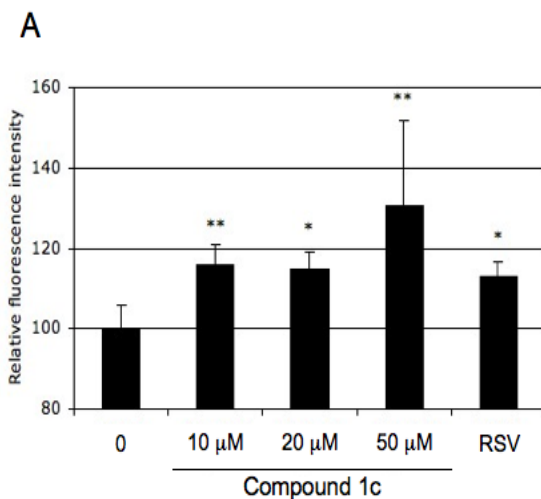
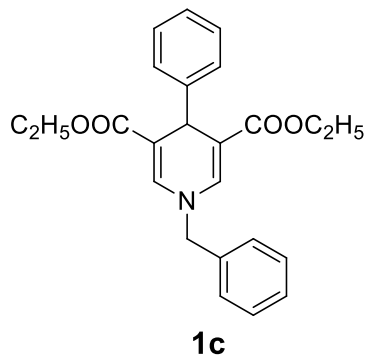


- 1c** X = OC₂H₅
2c X = OH
3c X = NH₂

hMSC, 50 μ M, 48 h

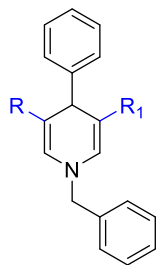


mouse C2C12 myoblasts, 16 h



E. De Fabiani's lab

SIRT activators

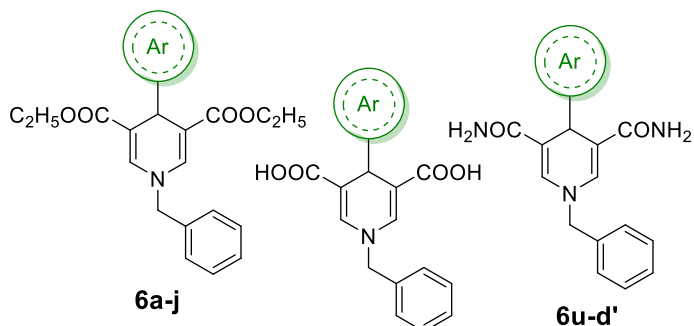


5a R = COOC₂H₅, R₁ = COOH

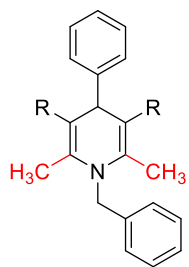
5b R = COOC₂H₅, R₁ = CONH₂

5c R = COOH, R₁ = CONH₂

5d R = R₁ = CN



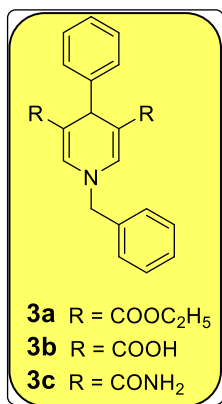
Ar = 2-furyl, 2- and 3-thienyl, 2- and 5-thiazolyl, 4-biphenyl, 1- and 2-naphthyl, benzo[b]thiophen-2-yl, 3-quinolinyl



4a R = COOC₂H₅

4b R = COOH

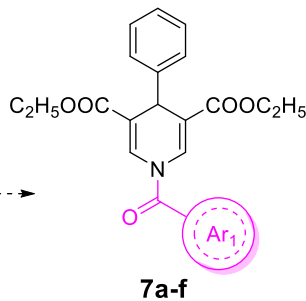
4c R = CONH₂



3a R = COOC₂H₅

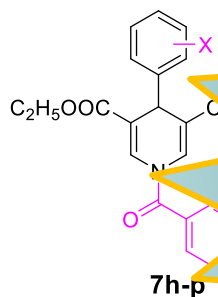
3b R = COOH

3c R = CONH₂



7a-f

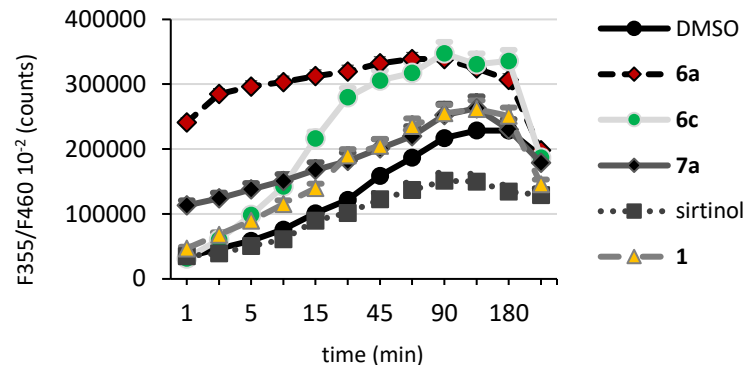
Ar₁ = phenyl, O-benzyl, 2-pyrazinyl, 1- and 2-naphthyl, 2-quinoxalyl



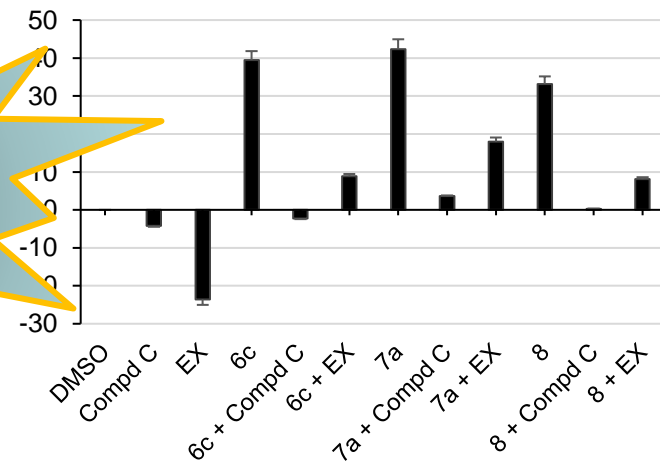
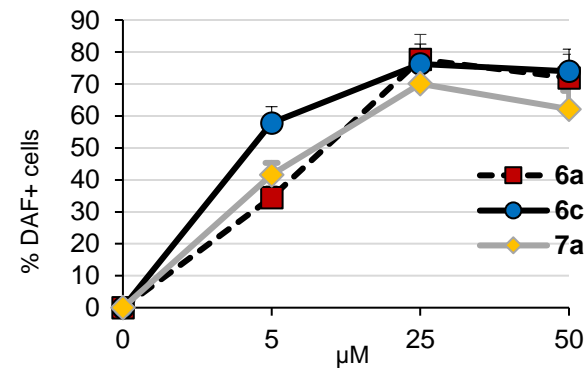
7h-p

X = 2-, 3- and 4-Me, 2-, 3- and 4-Cl, 2-, 3- and 4-OMe

SirT1 activity (HaCat cells)



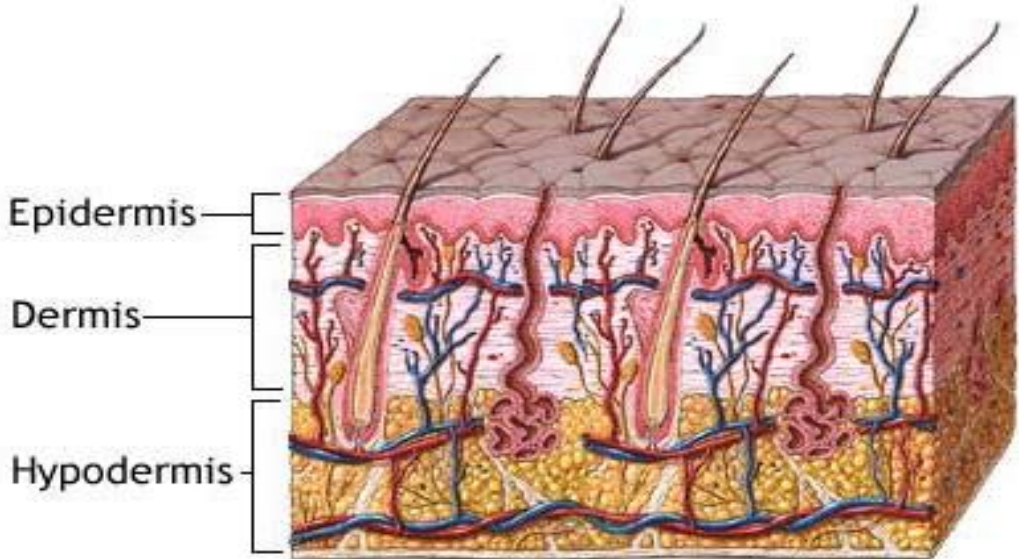
NO release (HaCat cells)



Involvement of the AMPK/SirT1 axis

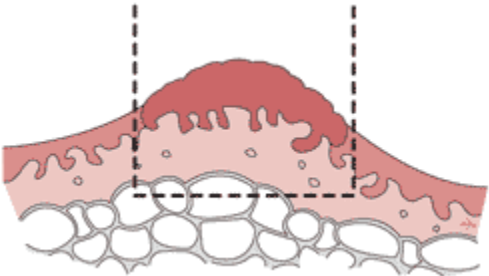
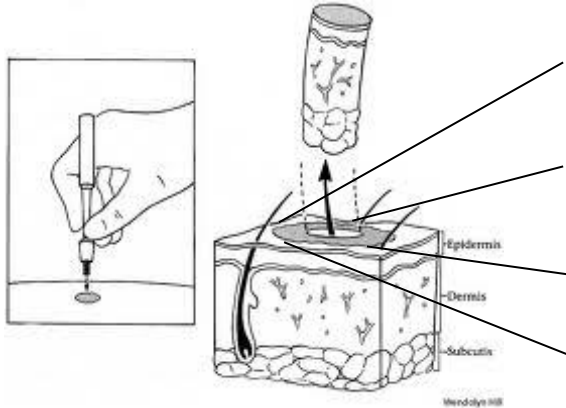
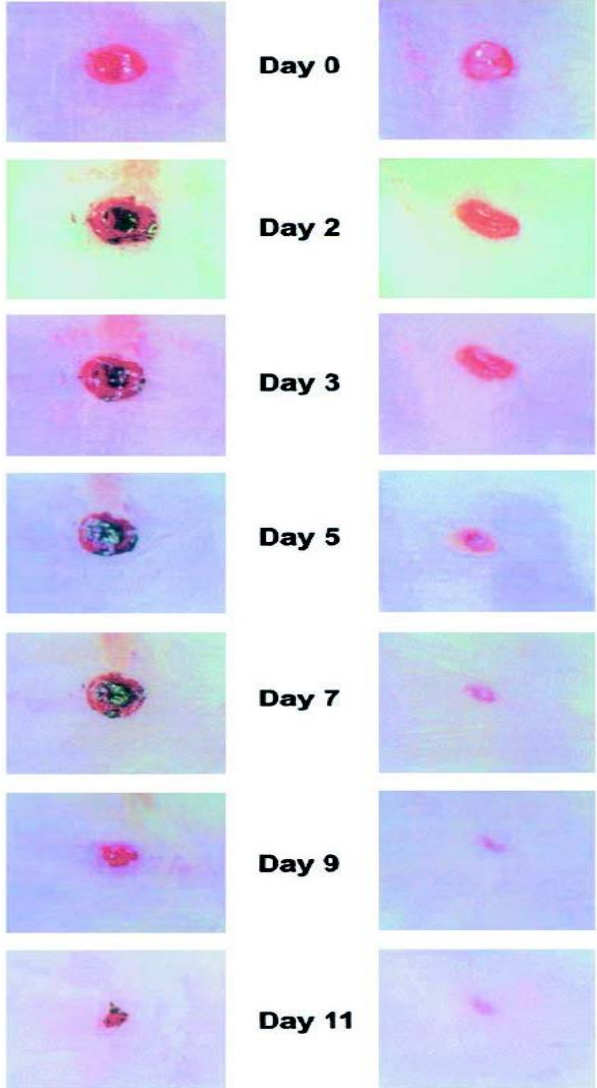
Normal and Pathological Wound Healing

Normal skin



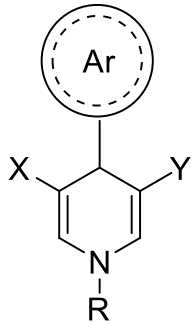
Diabetic

Normal



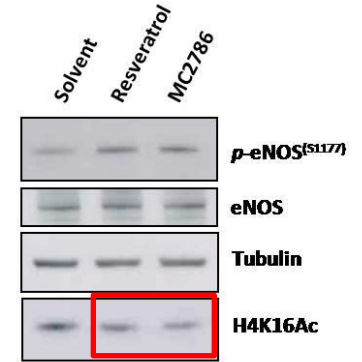
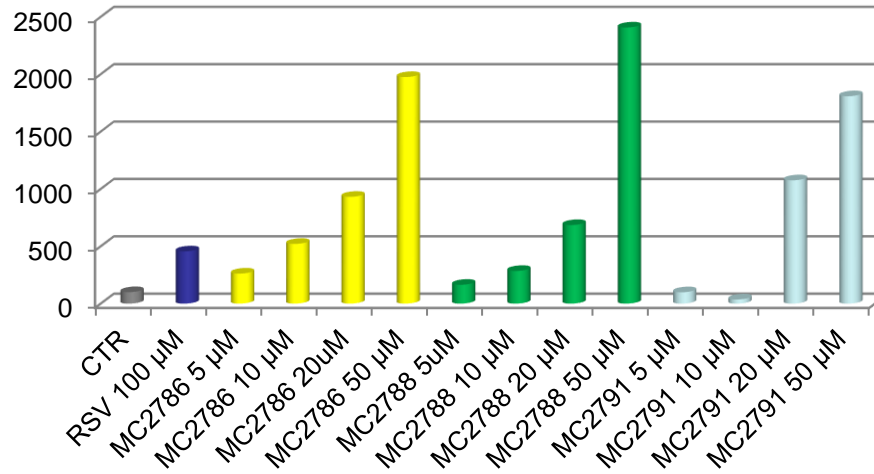
Punch Biopsy

MOUSE

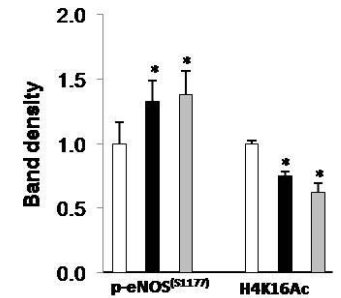
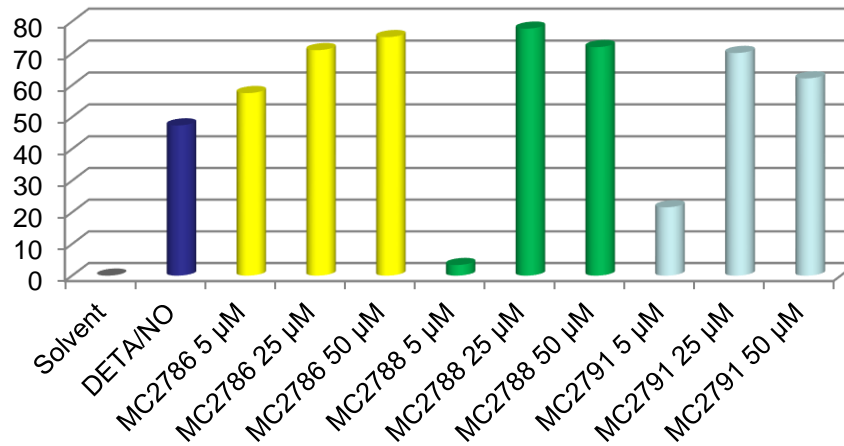


MC2786
MC2788
MC2791

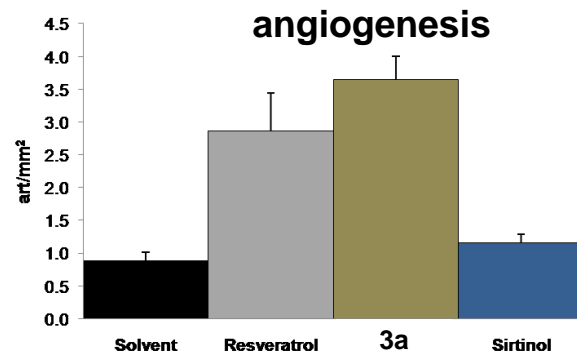
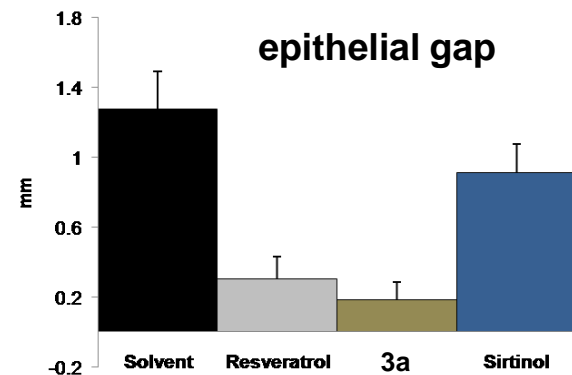
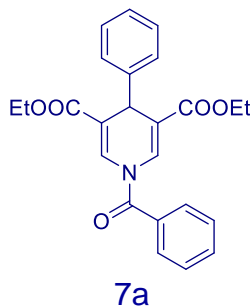
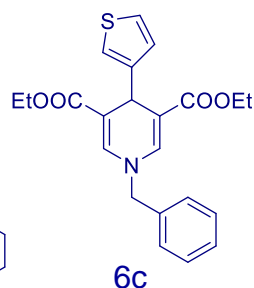
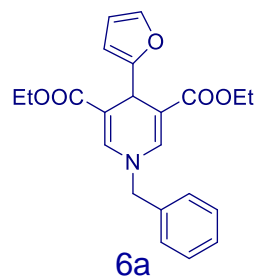
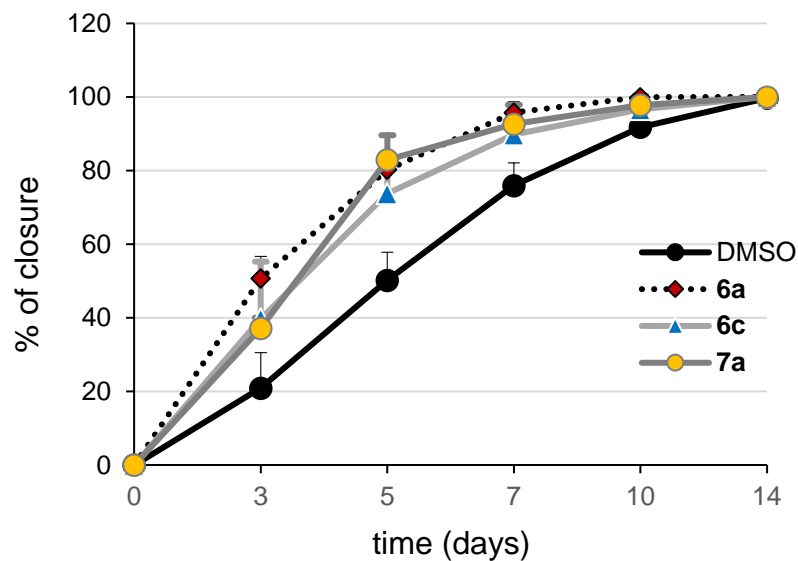
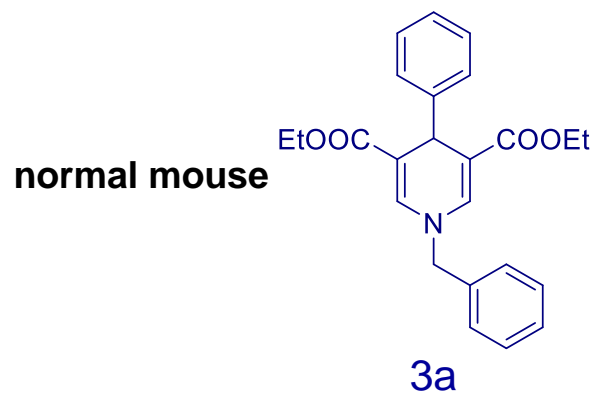
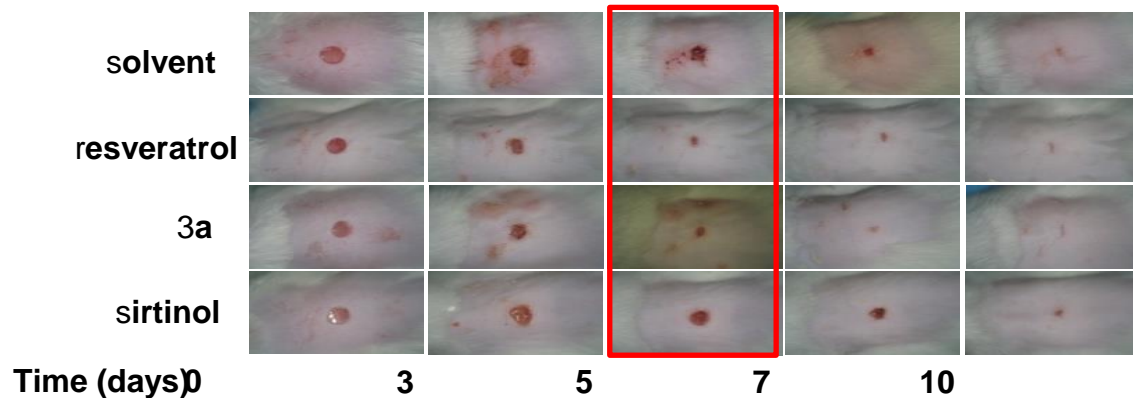
% SirT1 activity



% NO release (HaCat cells, 1h)



SirT1 Activation Promotes Skin Repair

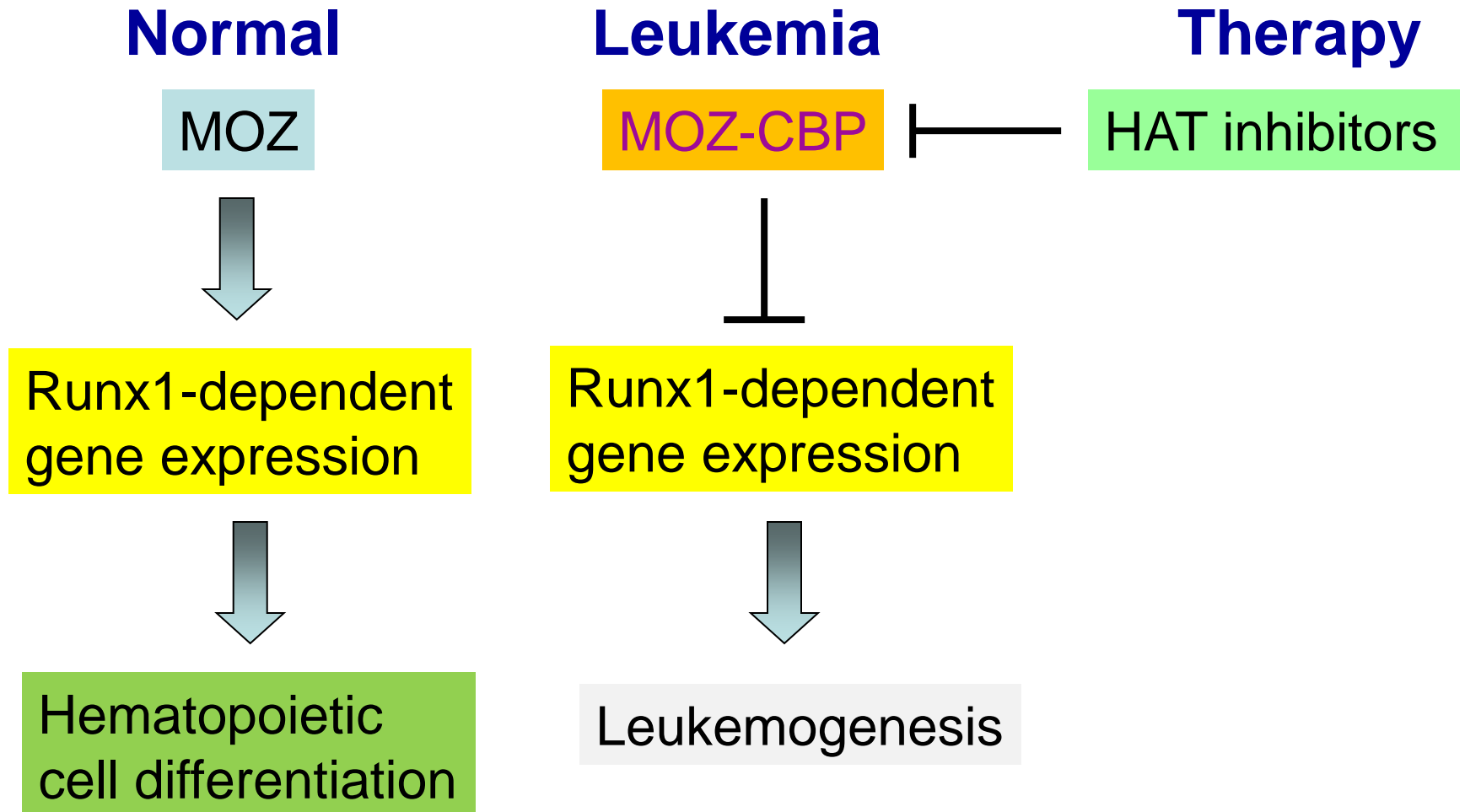


C. Gaetano's lab

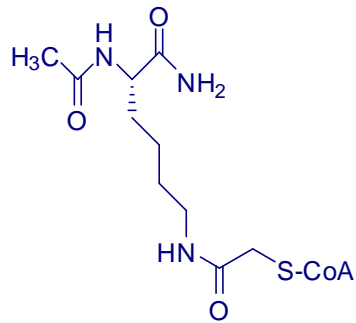
HATs and cancer: a potential role

HAT	family	target	involvement in cancer
GCN5	GCN5/PCAF	H2B, H4, cMyc	Critical regulator of cell cycle and cMyc
PCAF	GCN5/PCAF	H3, H4, cMyc, p53, MyoD, E2F	Critical regulator of cell cycle, p53, E2F, and cMyc
CBP, p300	p300/CBP	H2A, H2B, H3, H4, pRb, E2F, p53, c-Myb, MyoD, AR, FoxO	<i>Translocation:</i> MOZ/MORF/MLL-p300/CBP fusions <i>Mutation:</i> biallelic mutation p300 epithelial cancer <i>Inactivation:</i> hematological malignancy
TIP60	MYST	H2A, H3, H4, cMyc, AR	Association with AR in prostate cancer
MOZ	MYST	H3, H4	Fusions with p300/CBP and TIF2
MORF	MYST	H3, H4	Fusions with p300/CBP
ACTR	SRC	H3, H4	Upregulation in breast cancer correlates with resistance to tamoxifen

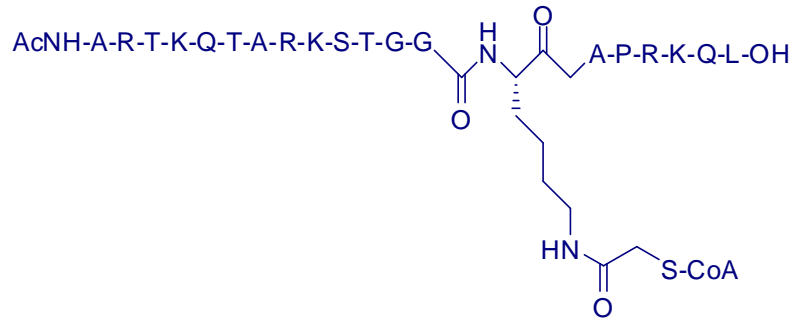
Aberrant HATs and leukemogenesis: an example



HAT inhibitors



Lys-CoA

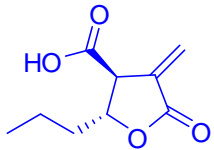


H3-CoA-20

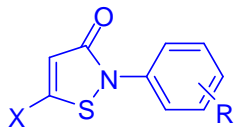


anacardic acid

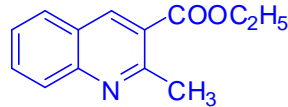
Lau, O. D. *et al.*, *Mol. Cell* **2000**, 5, 589-595.



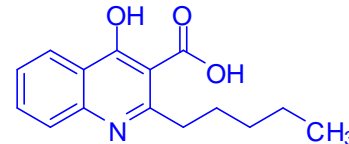
MB-3



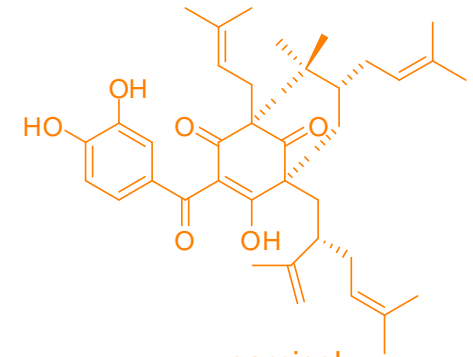
isothiazolones



MC1626



MC1823



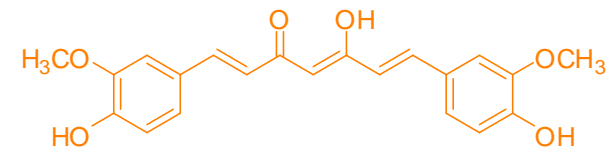
garcinol

Biel, M. *et al.*, *Angew. Chem. Int. Ed.* **2004**, 43, 3974-3976.

Stimson, M. *et al.*, *Mol. Cancer Ther.* **2005**, 4, 1521-1532.

Ornaghi, P. *et al.*, *Biochem. Pharmacol.* **2005**, 70, 911-917.

Mai, A. *et al.*, *J. Med. Chem.* **2006**, 49, 6897-6907.



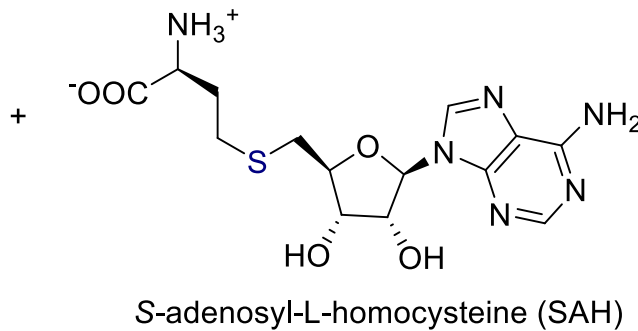
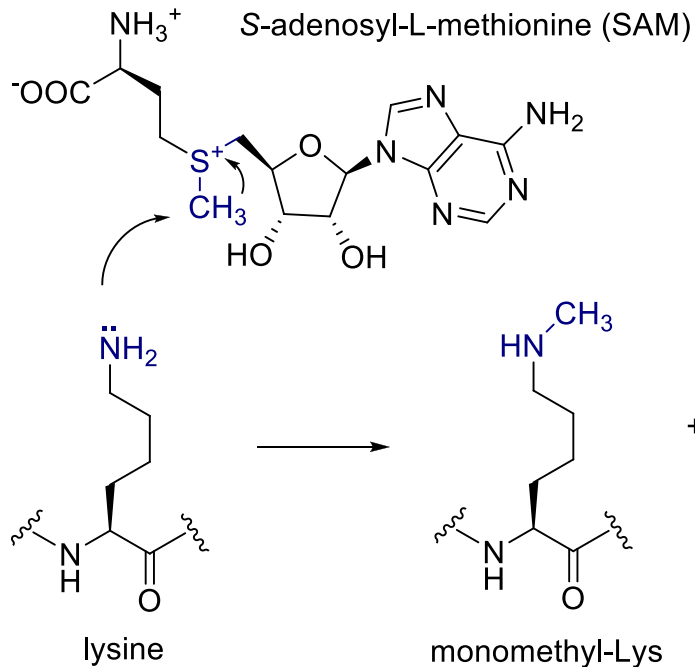
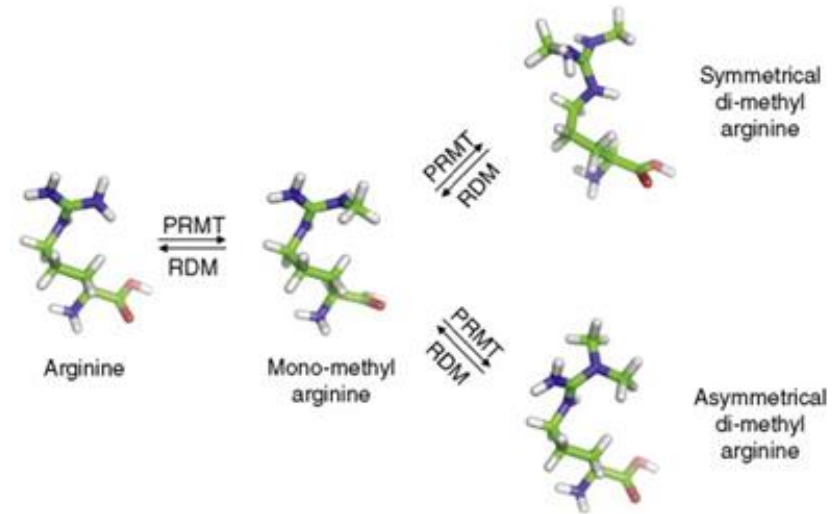
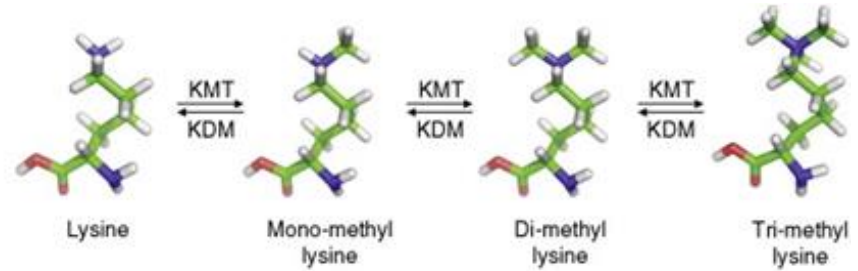
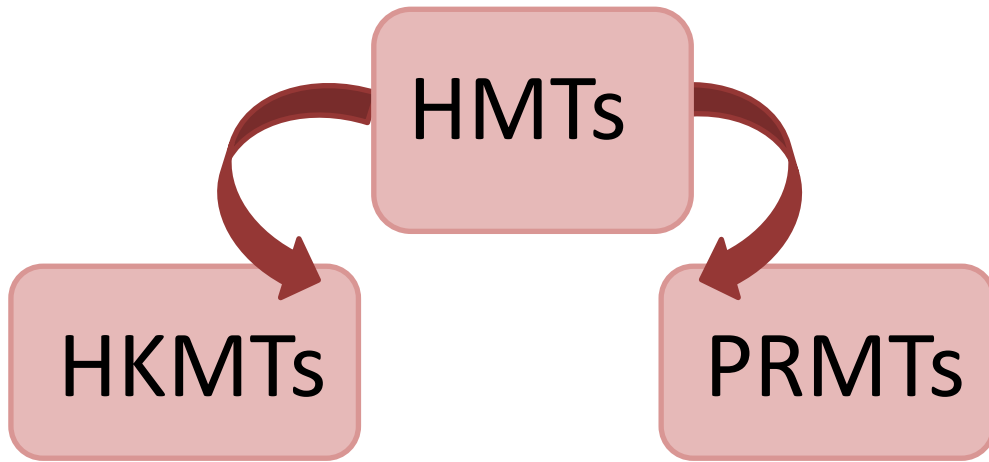
curcumin

Balasubramanyam, K. *et al.*, *J. Biol. Chem.* **2003**, 278, 19134-19140.

Balasubramanyam, K. *et al.*, *J. Biol. Chem.* **2004**, 279, 33716-33726.

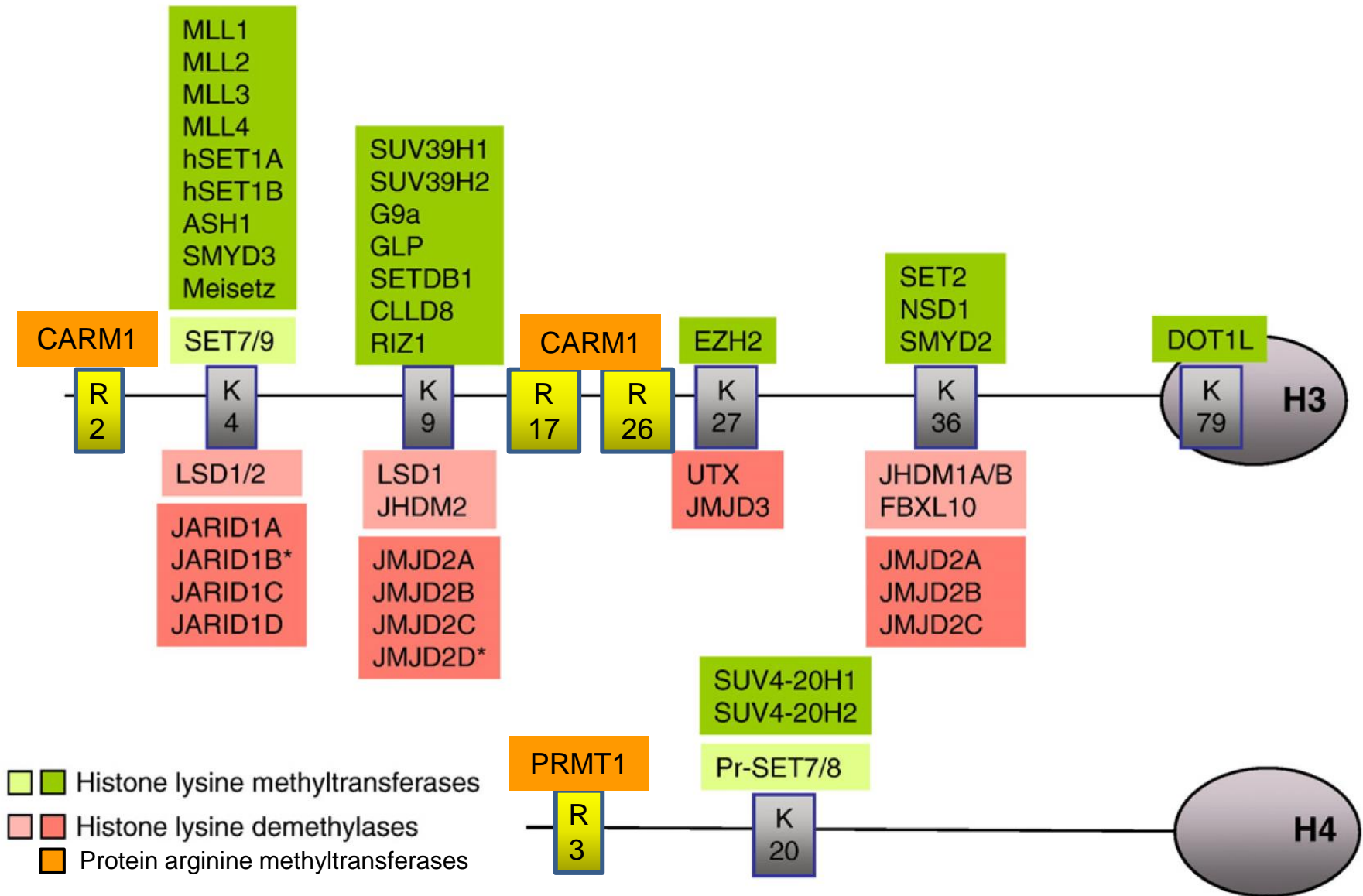
Balasubramanyam, K. *et al.*, *J. Biol. Chem.* **2004**, 279, 51163-51171.

Histone Methyltransferases



S-adenosyl-L-homocysteine (SAH)

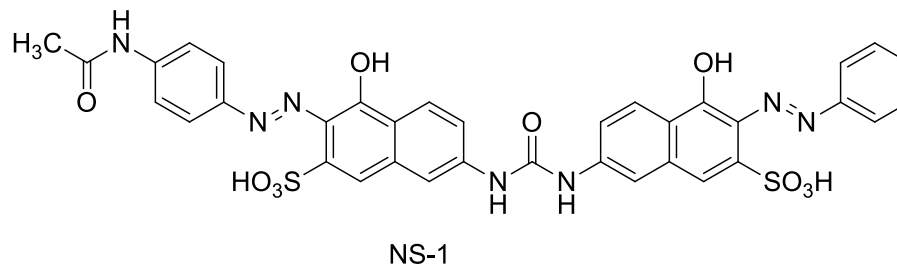
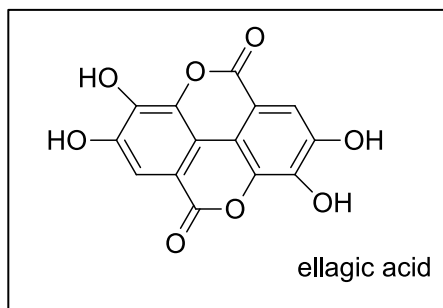
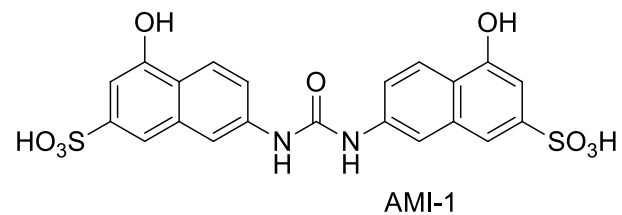
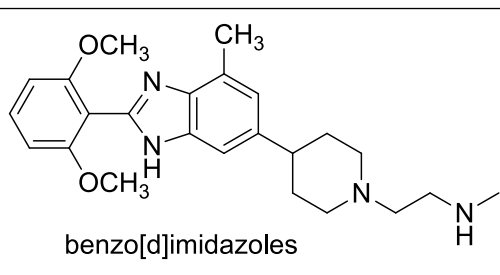
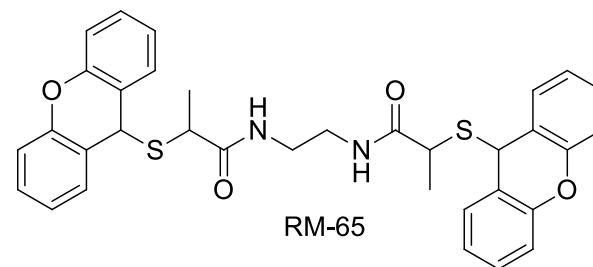
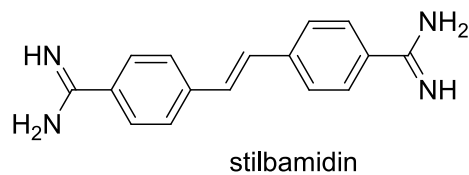
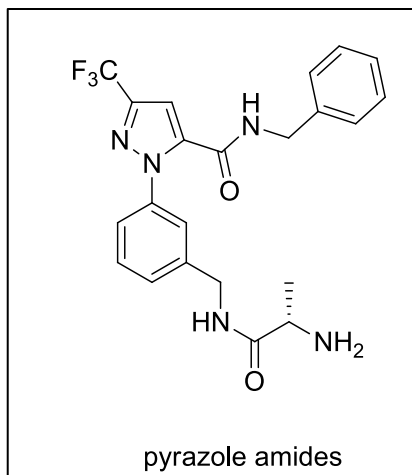
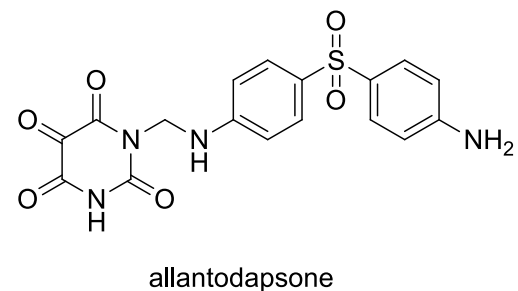
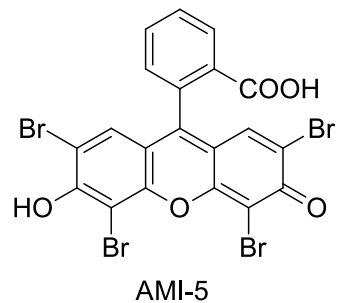
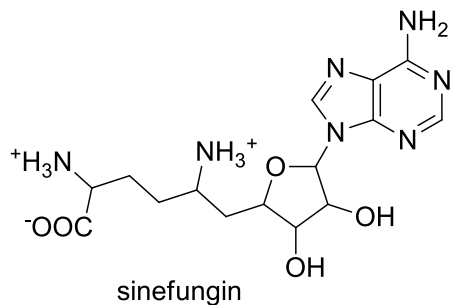
Methylation vs Demethylation



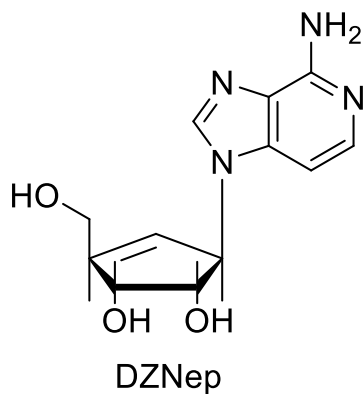
HMTs and Cancer

HMT	Target	Involvement in Cancer
MLL	H3K4	Tumor promotion Translocation, amplification, tandem duplication >50 different MLL fusions
EZH2	H3K27	Tumor promotion. Cell cycle defects Overexpression in breast, prostate, gastric, bladder, endometrial cancer, CRC, HCC, lymphoma, melanoma
SMYD3	H3K4	Tumor promotion. Cell cycle defects Overexpression in breast cancer, CRC, HCC
hDOT1L	H3K79	Tumor promotion. Transcription regulation Leukemogenesis in association with AF10 fusion proteins
SUV39H1	H3K9; HP1, pRb, E2F, HDAC	Tumor promotion. Cell cycle defects Overexpression in colon cancer. B cell lymphoma in KO mice
RIZ1	H3K9; pRb	Tumor suppression Mutated/downregulated in liver, breast and gastric cancer
PRMT1	H4R3; STAT1	Acts with p300 in activation of NR transcription; increases the response to IFN
PRMT4/ CARM1	H3R2; H3R17; H3R26	Cooperates with p300 in activation of NR transcription
PRMT5	H2A, H4, cyclin E	Negatively regulates proliferation

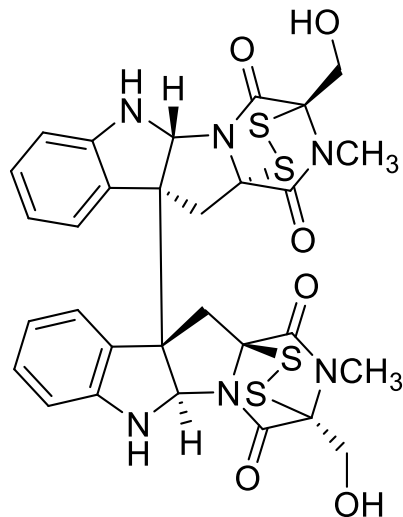
PRMT inhibitors



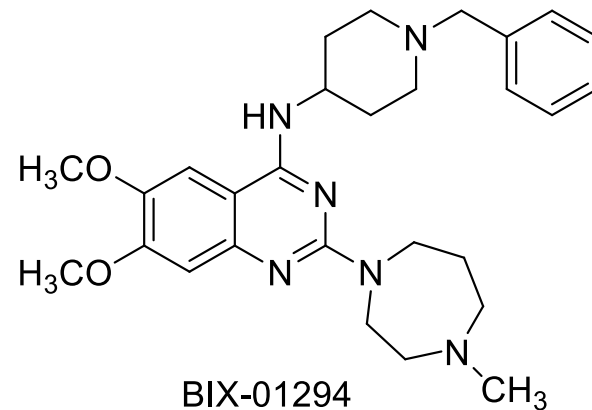
HKMT inhibitors



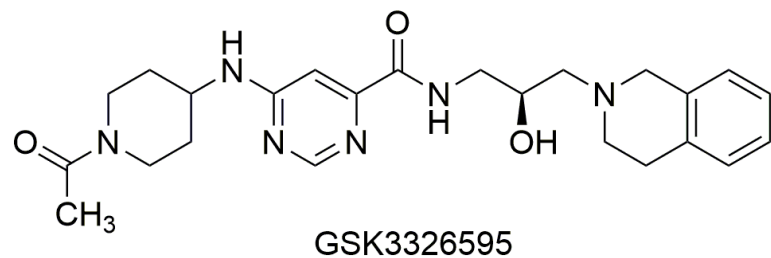
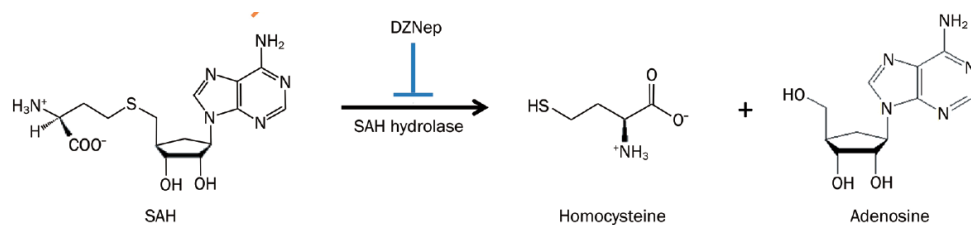
SAH hydrolase inhibitor
EZH2 inhibitor



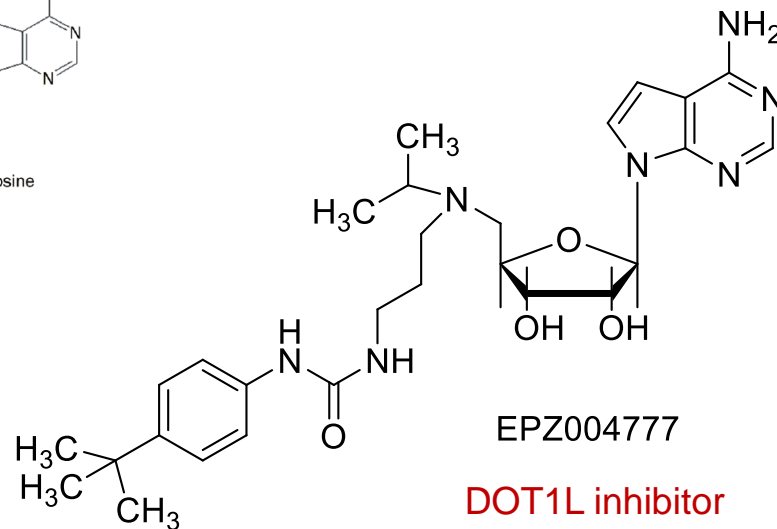
chaetocin
SUV39H1 inhibitor



G9a inhibitor

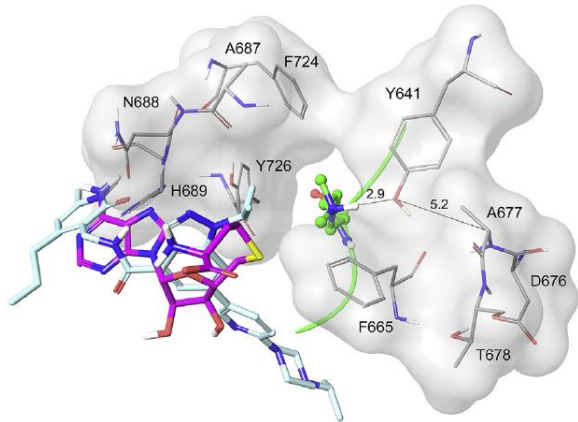


PRMT5 inhibitor



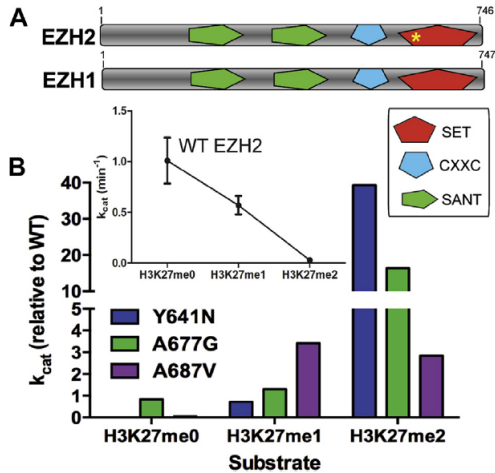
DOT1L inhibitor

EZH2 - Enhancer of Zeste Homolog 2

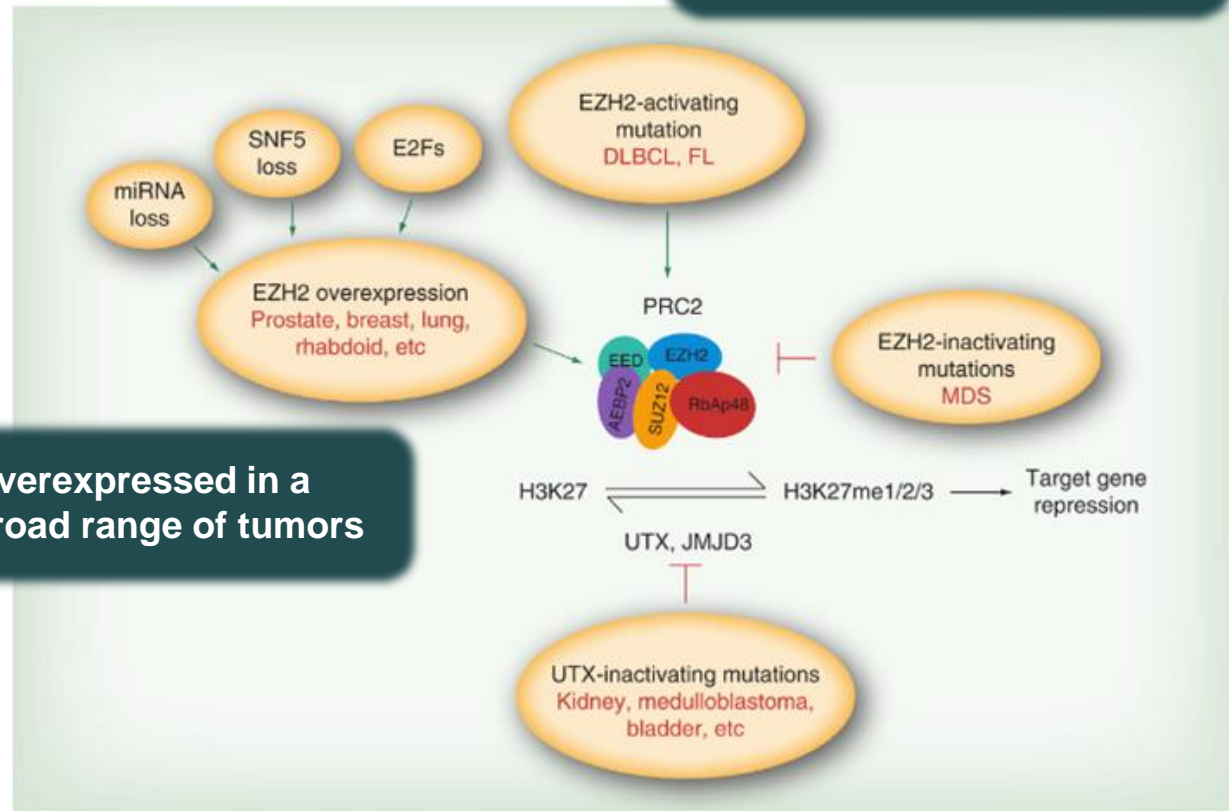


Homology model of EZH2 active site

Hot spot mutations (Y641X, A677G, A687V) identified in lymphomas



Gain-of-function mutations



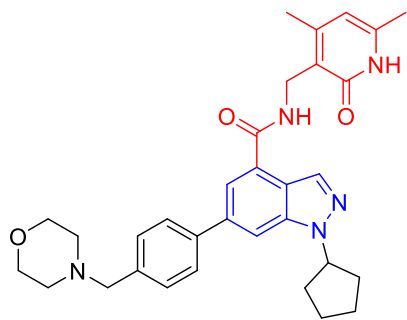
Overexpressed in a broad range of tumors

Figure 1. Deregulation of H3K27 methylation in cancer.

DLBCL: Diffuse large B-cell lymphoma; FL: Follicular lymphoma; MDS: Myelodysplastic syndrome.

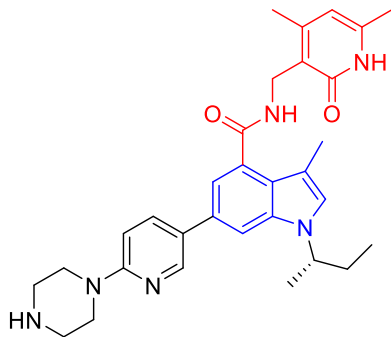
Tan Jin-Zhi et al. *Acad Pharm Sin* 2014, 35, 161-174
 McCabe MT., Creasy C.L. *Epigenomics* 2014, 6, 341-351
 Yap, D. B. et al. *Blood* 2011, 117, 2451-2459
 Bowen, X. et al. *Experim Hematol* 2015, 43, 698-712

Catalytic Inhibitors of EZH2



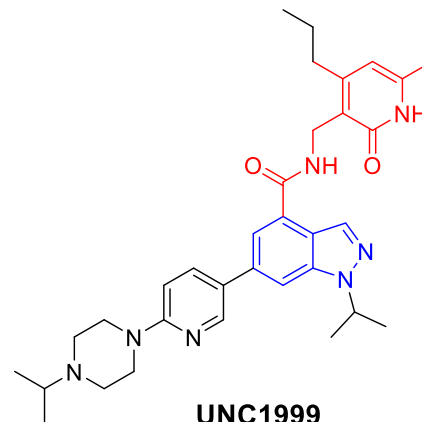
EPZ005687

Nat Chem Biol 2012



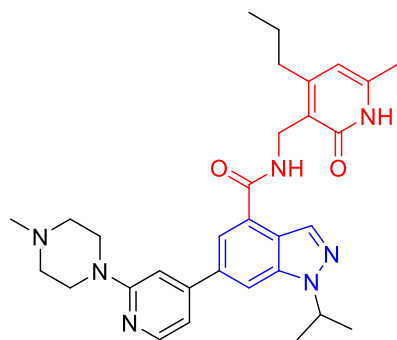
GSK126

Nature 2012



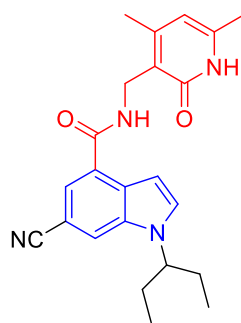
UNC1999

ACS Chem Biol 2013



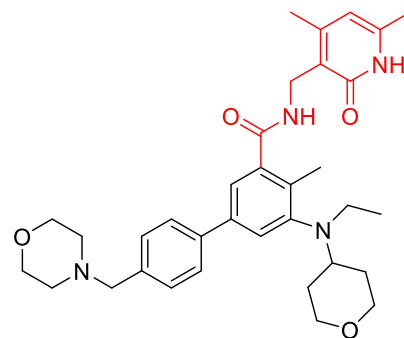
GSK343

ACS Med Chem Lett 2012



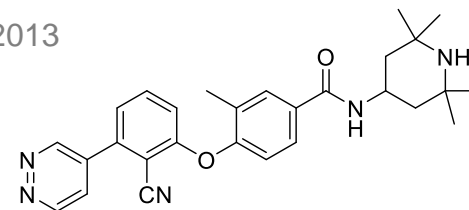
E1

PNAS USA 2012



EPZ6438

tazemetostat
PNAS USA 2013



Compound 3

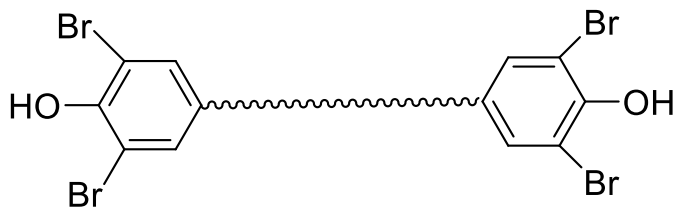
Chem Biol 2013

2012

2013

HMT inhibitors: our experience

Epigenetic Multiple Ligands (*Epi-MLs*)

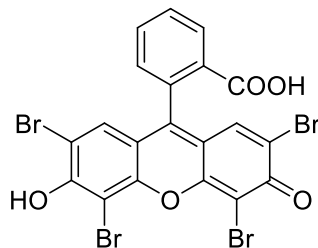


PRMT inhibition: 60-80% at 50 μM

HKMT inhibition: 60-80% at 25 μM

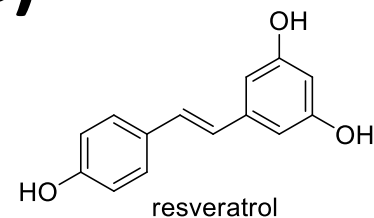
HAT inhibition: 70-100% at 50 μM

SIRT inhibition: 60-100% at 25 μM



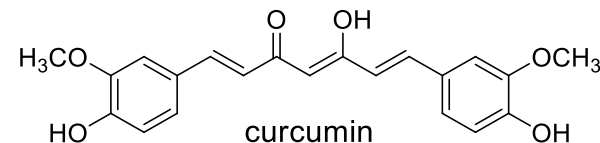
AMI-5

HMT inhibitor



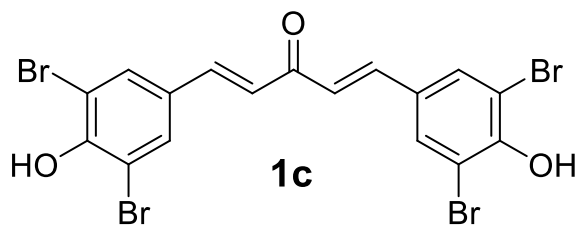
resveratrol

SIRT modulators

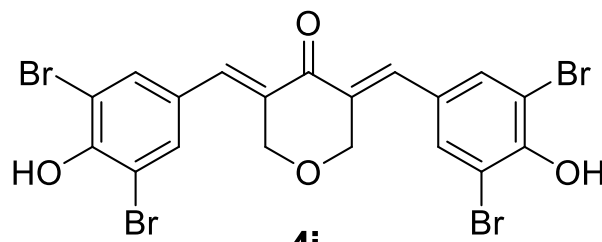


curcumin

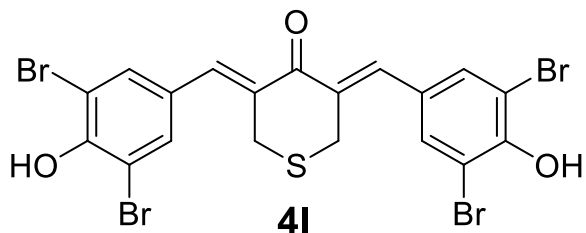
HAT inhibitor



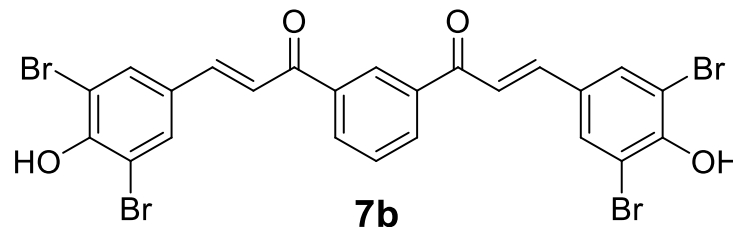
1c



4j



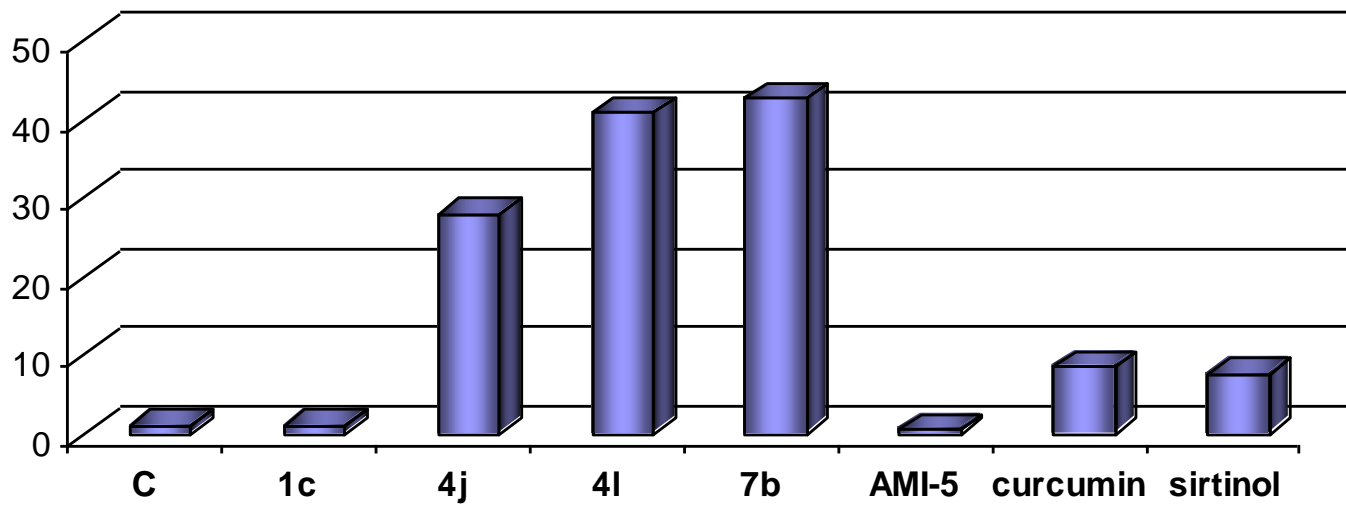
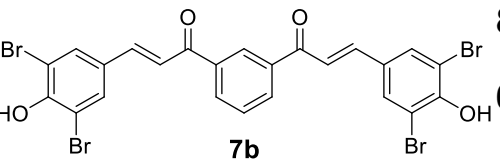
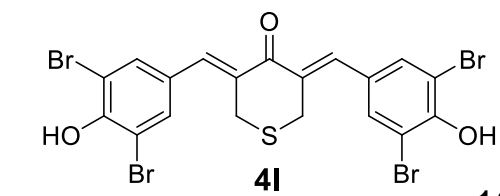
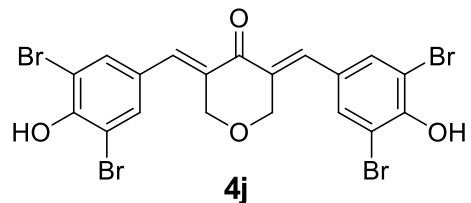
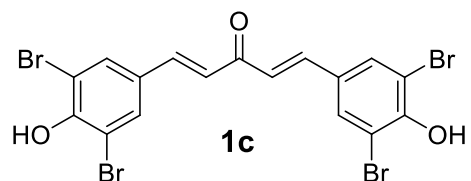
4l



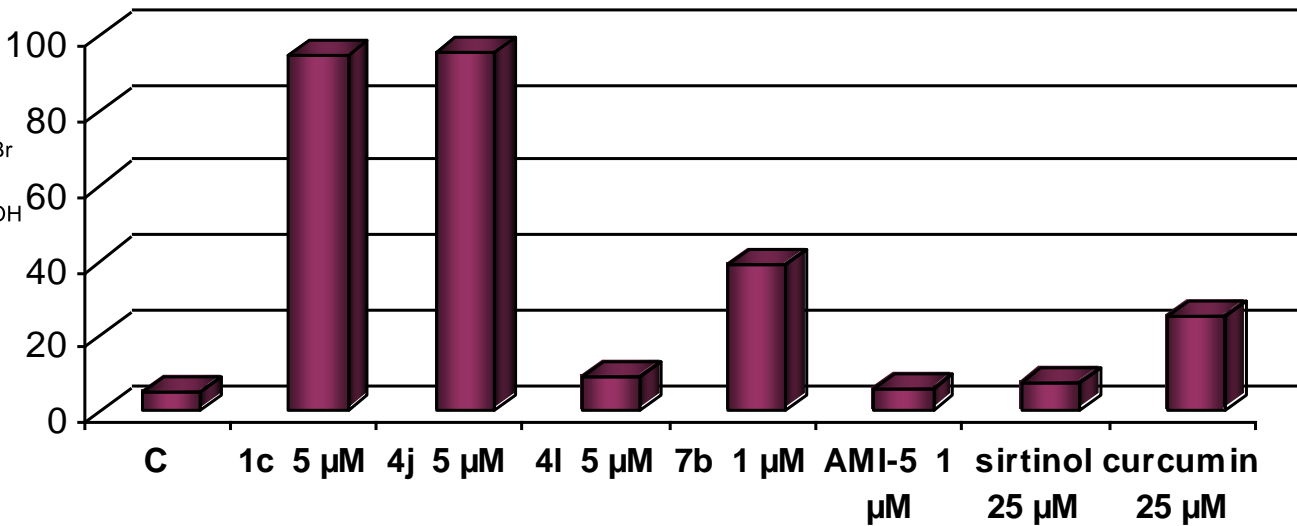
7b

U937 cells, 30h

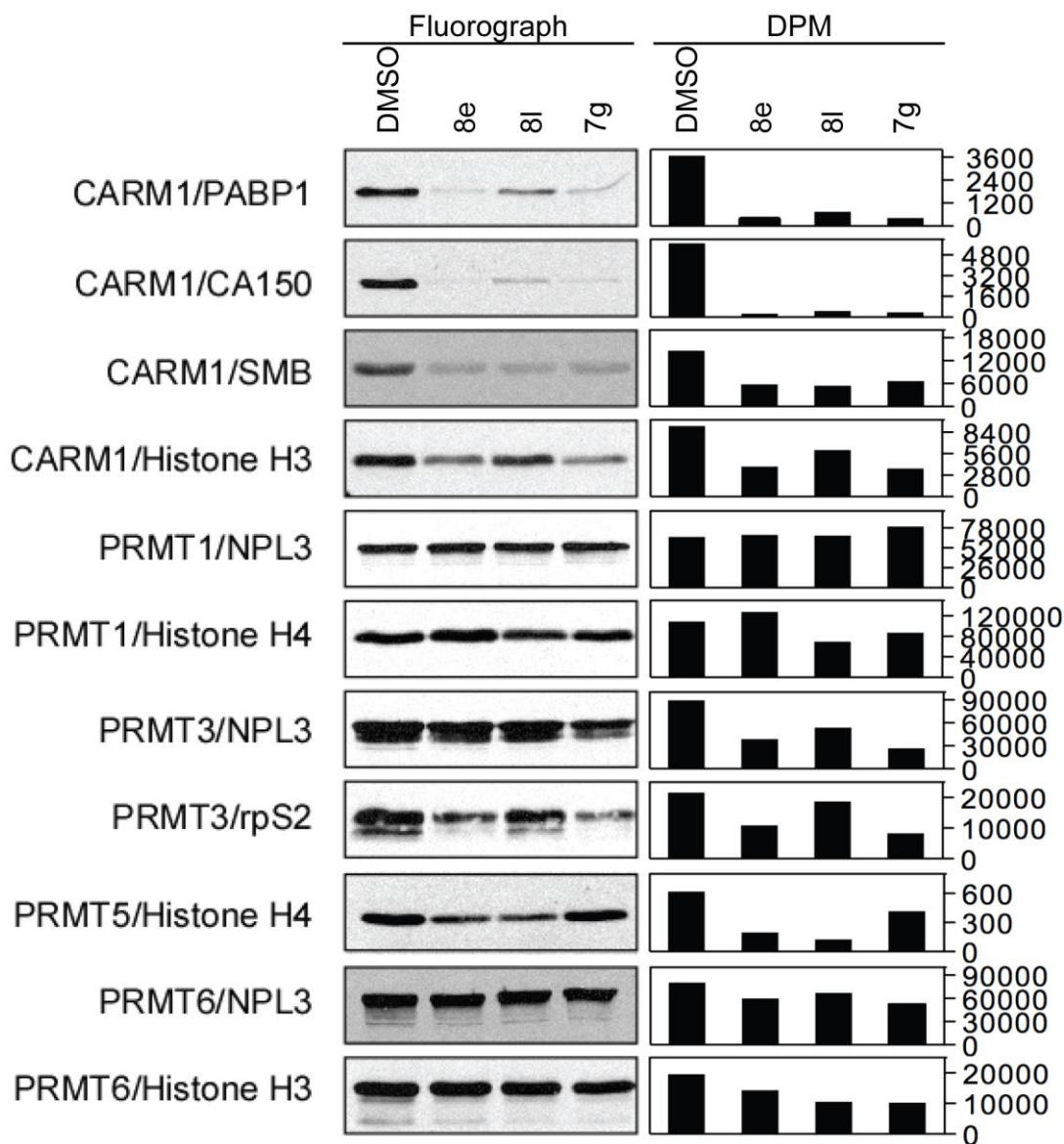
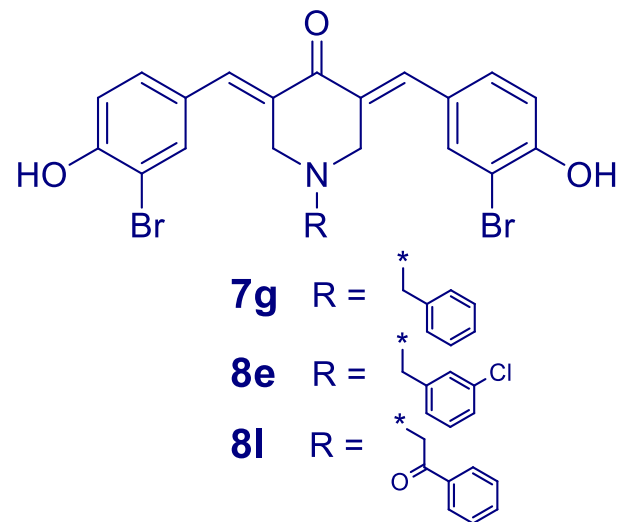
% apoptosis (25 μ M)



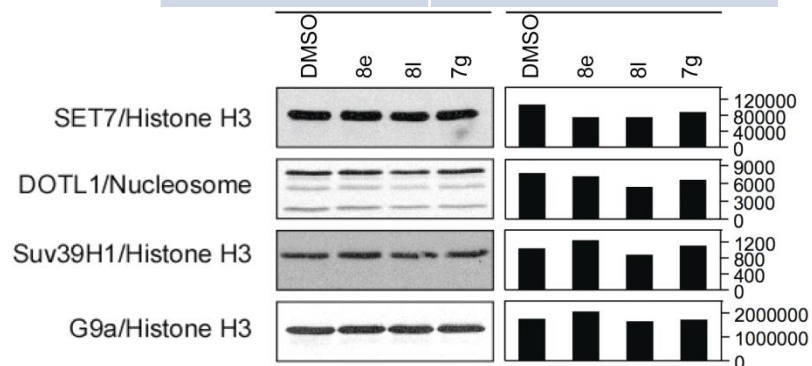
% CD11c/PI- cells



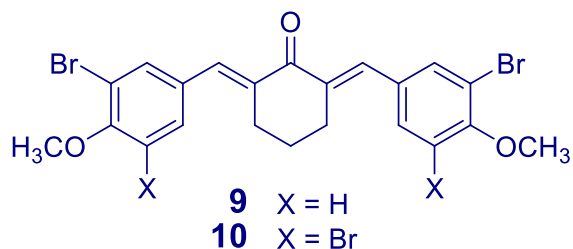
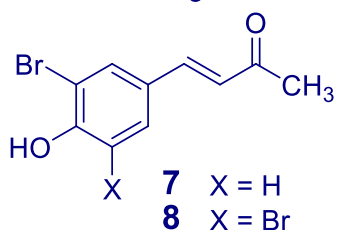
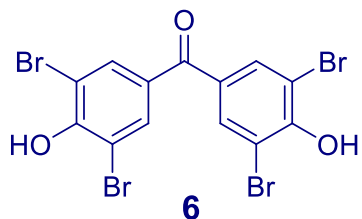
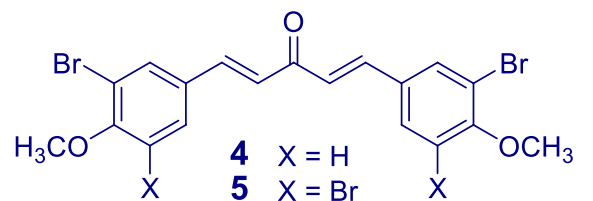
CARM1-selective inhibitors



compd	CARM1/PABP1 IC ₅₀ , μM
7g (MC2055)	8.6
8e (MC2326)	8.1
8l (MC2298)	14.4

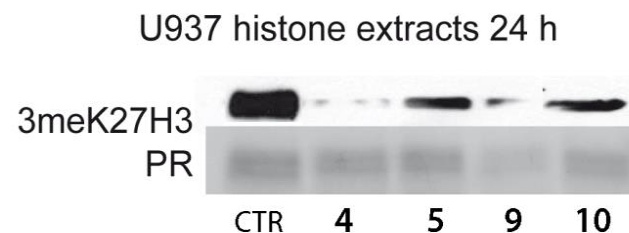


Against a panel of HKMTs...



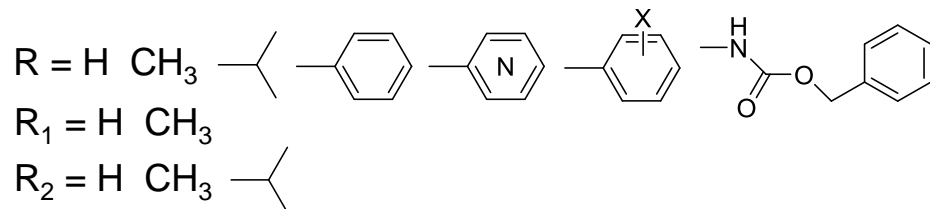
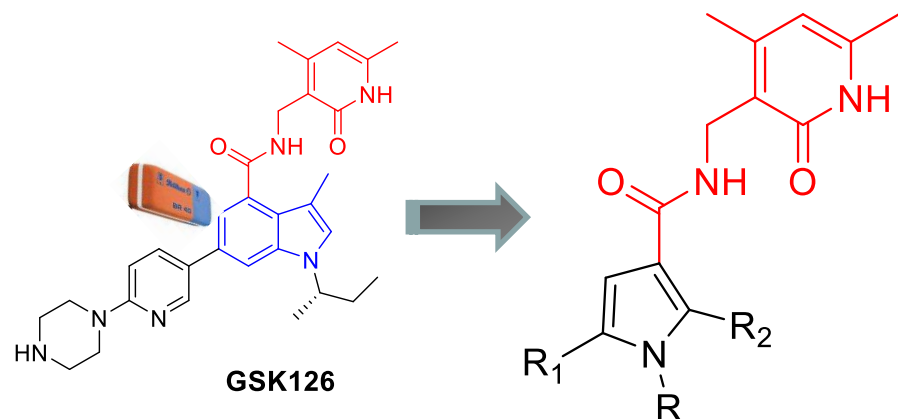
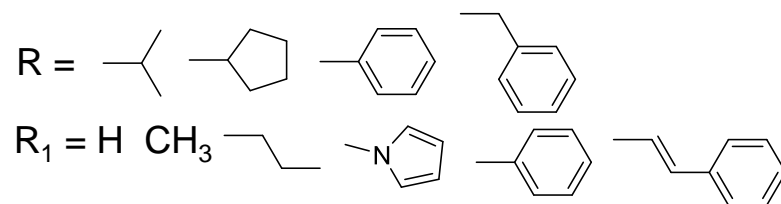
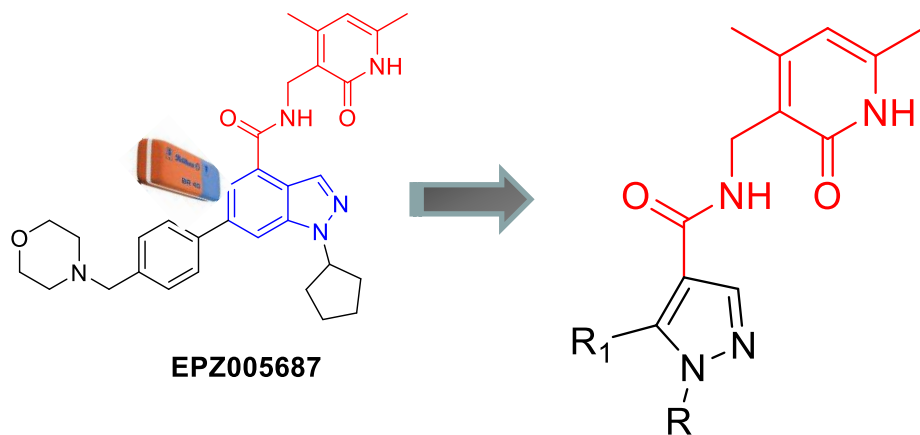
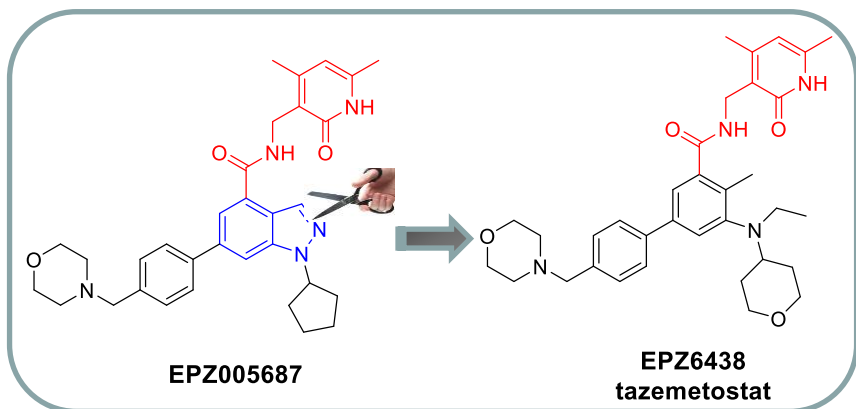
compd	IC ₅₀ , μM		
	PR-SET7	G9a	SET7/9
4	9.0	>250	>250
5	3.3	>250	>250
6	38.8	>250	>250
7	>250	>250	>250
8	>250	>250	>250
9	10.2	>250	>250
10	2.6	>250	164.4

compd	EZH2, IC ₅₀ μM
4	74.9
5	8.7% @ 75 μM
9	313.8
10	6.2% @ 75 μM
SAH	66.8

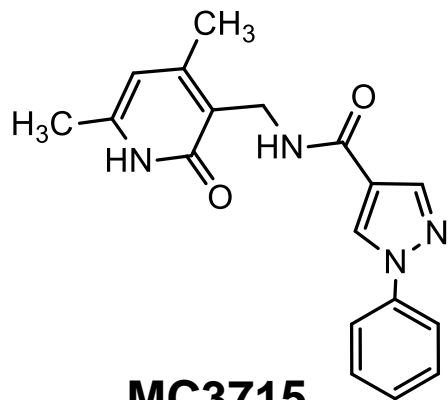


Palacios D *et al*, *Stem Cell Stem* **2010**
Valente S *et al*, *Biochimie*, **2012**

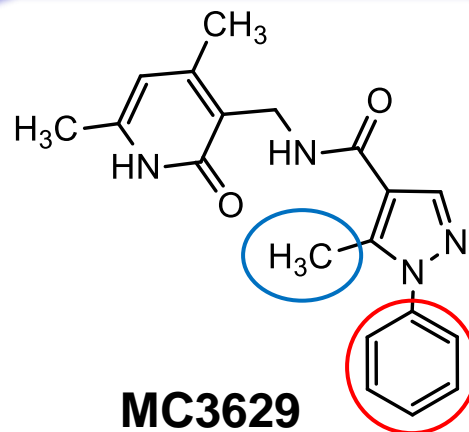
Pyrazole and Pyrrole Analogues as EZH2 Inhibitors



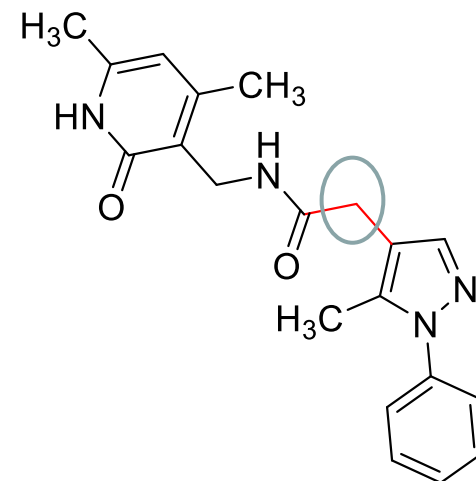
Pyrazole-based EZH2 Inhibitors: SAR Study



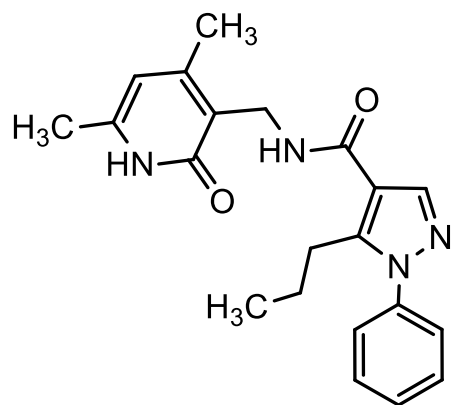
MC3715
IC₅₀ 98.8 μM



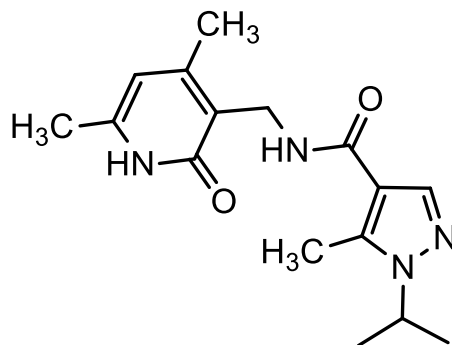
MC3629
IC₅₀ 15.4 μM



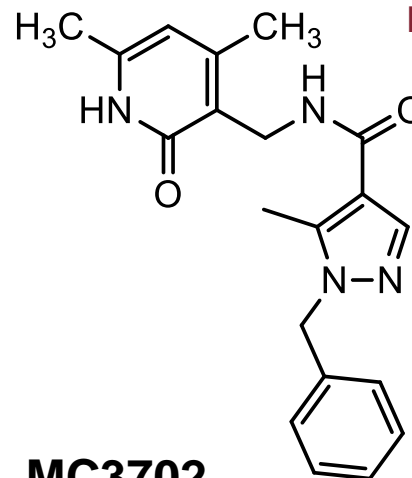
MC3710
no inhibition



MC3623
IC₅₀ 86 μM

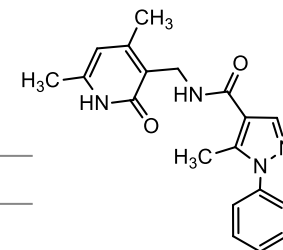


MC3690
IC₅₀ 343 μM

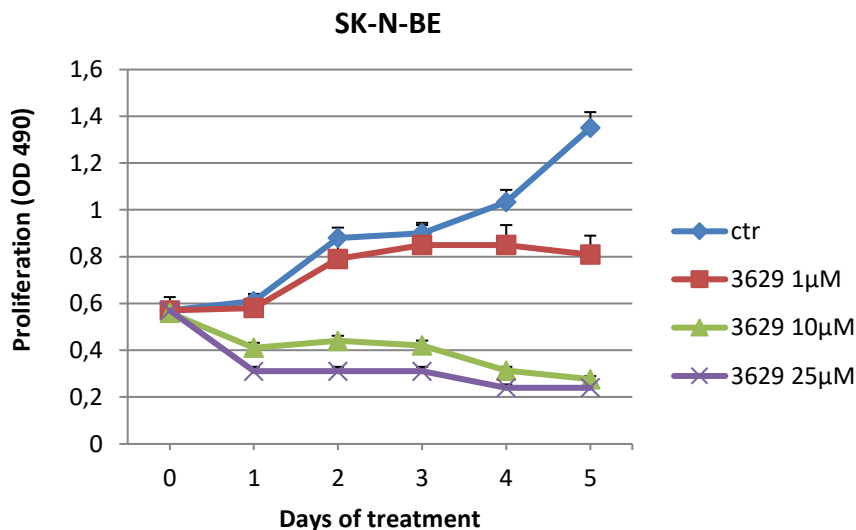
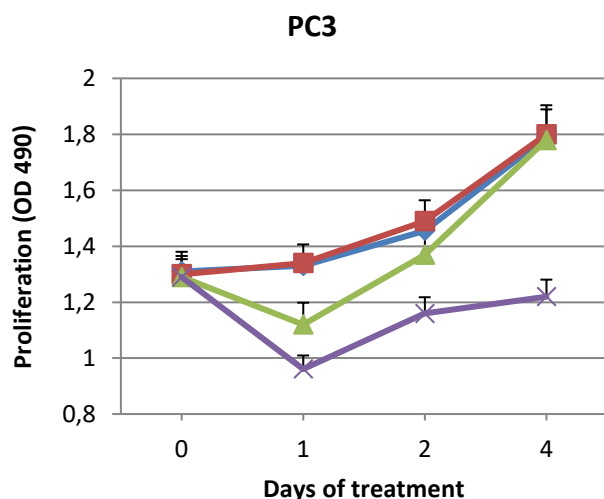
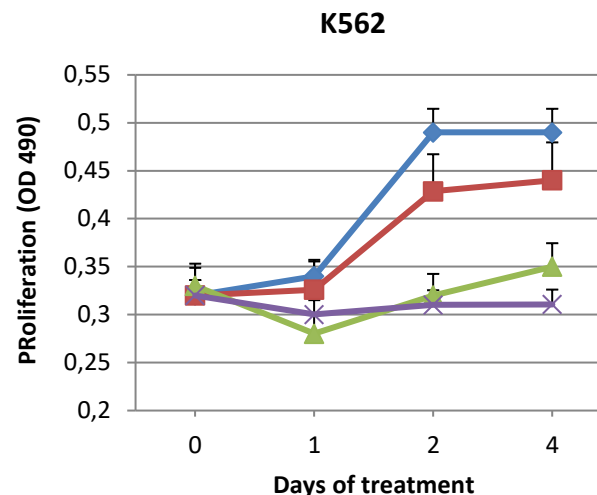
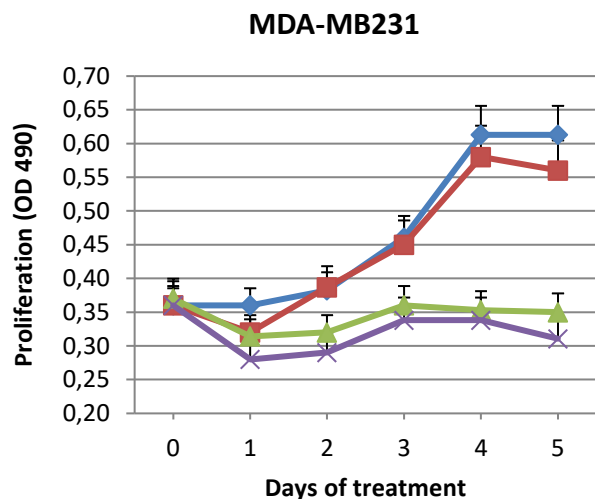


MC3702
IC₅₀ 19.6 μM

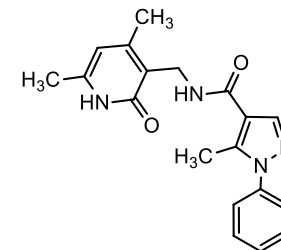
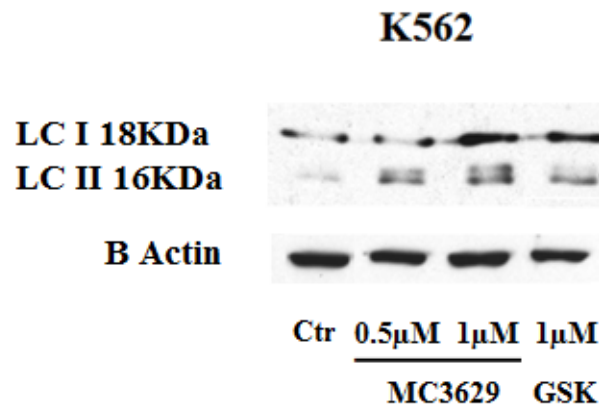
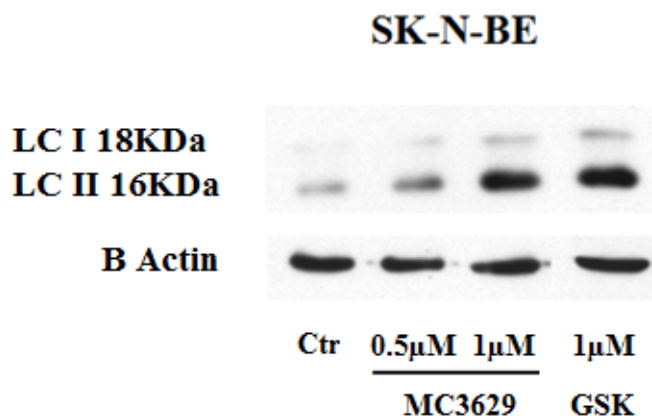
MC3629: Antiproliferative Activity (MTT) in Cancer Cells



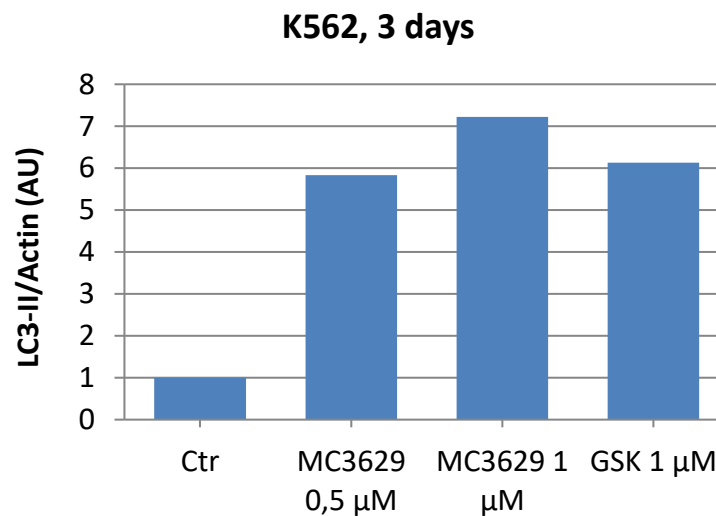
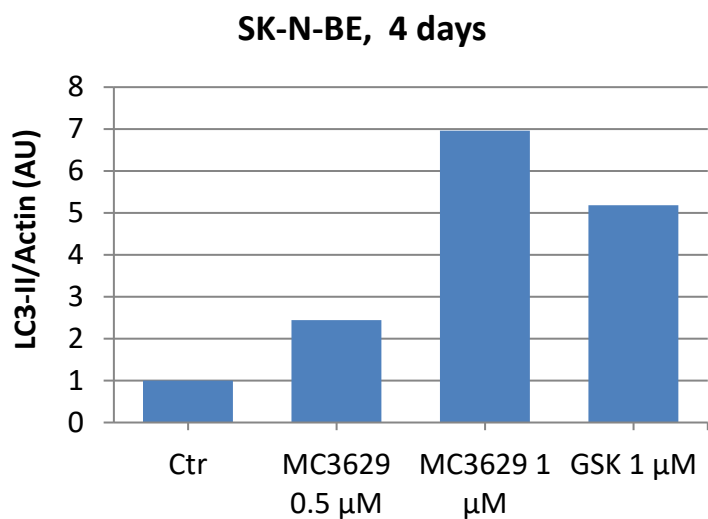
MC3629



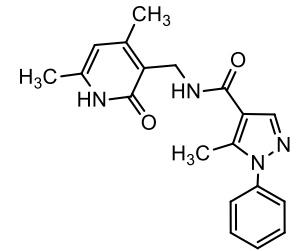
MC3629: Autophagy Induction



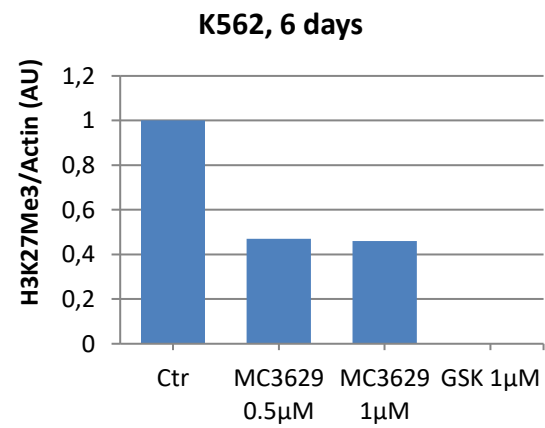
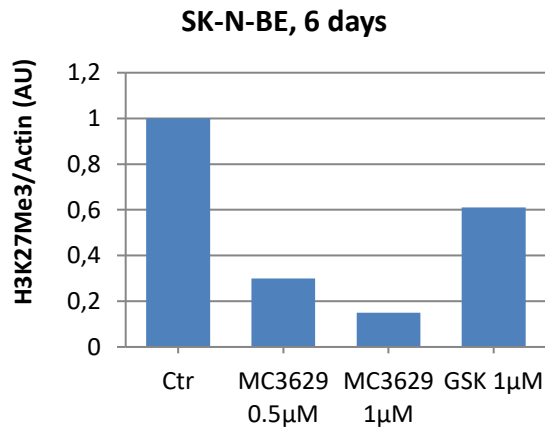
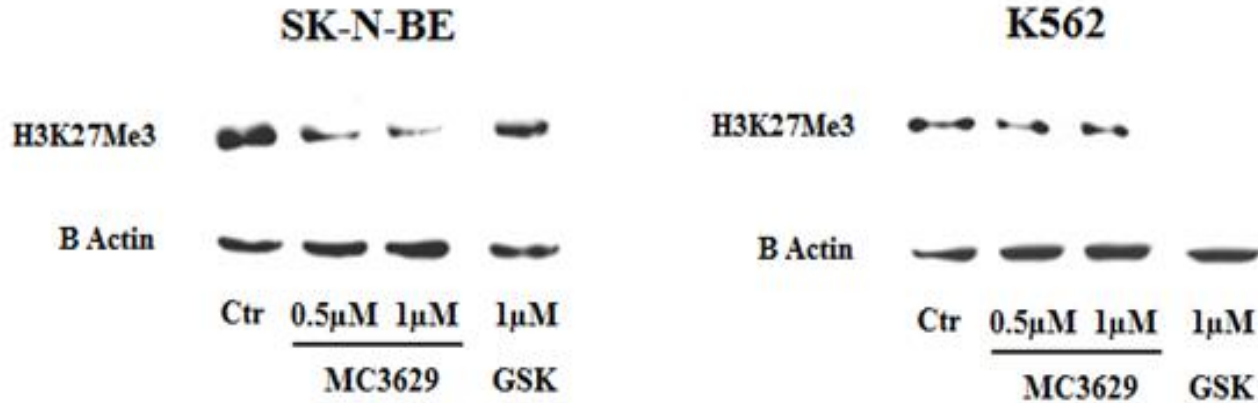
MC3629



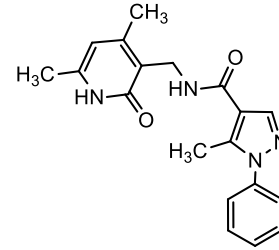
MC3629: Target Modulation



MC3629

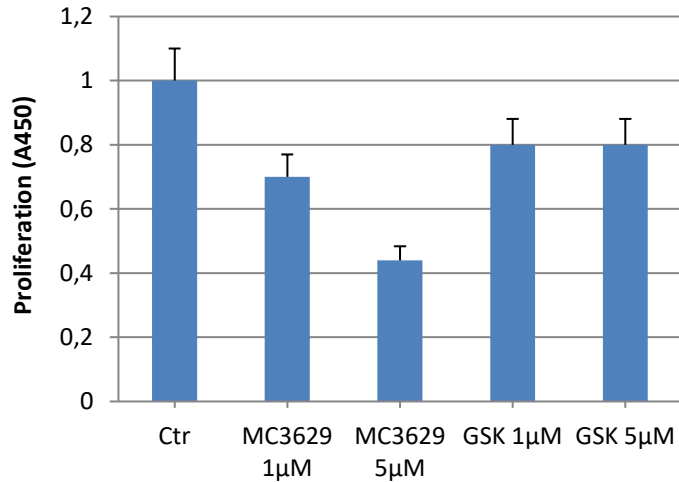


MC3629: Antiproliferative Activity (MTT & PCNA) in mMB10 Cancer Stem Cells

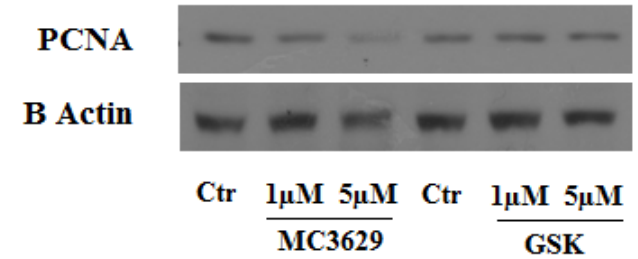


MC3629

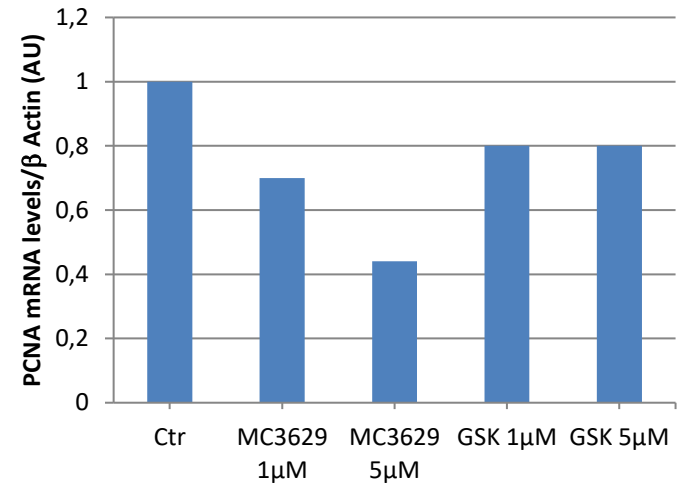
mMB10, MTT assay, 48h



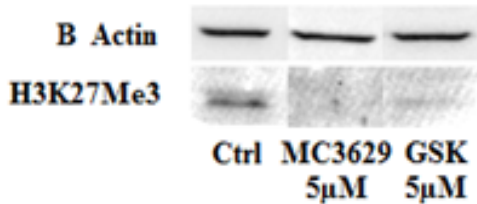
PCNA mRNA levels



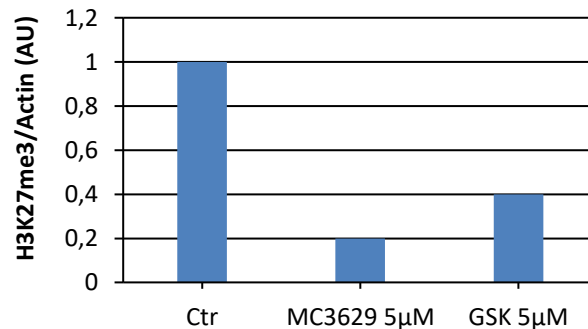
PCNA, 48h



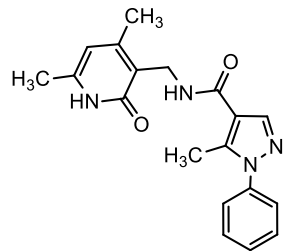
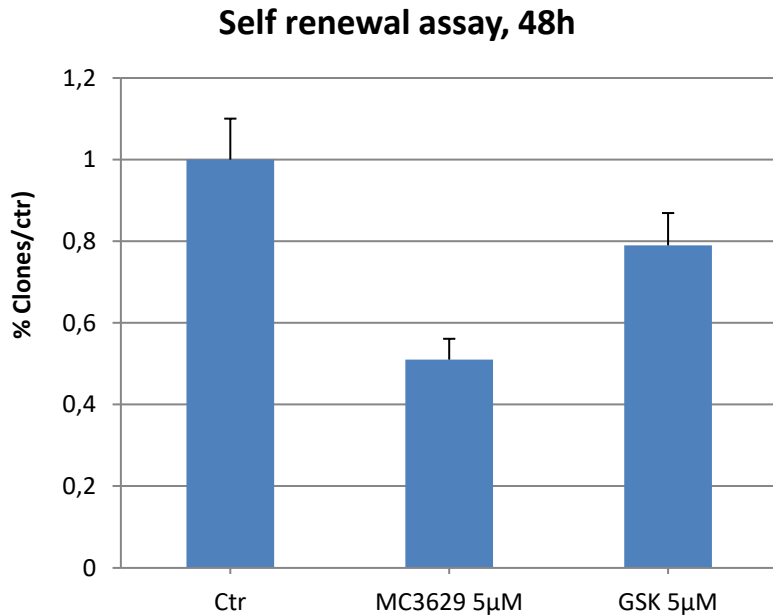
mMB10



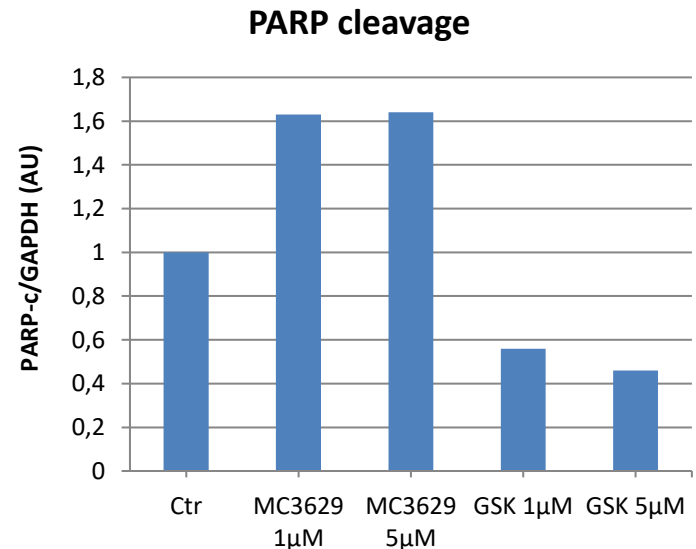
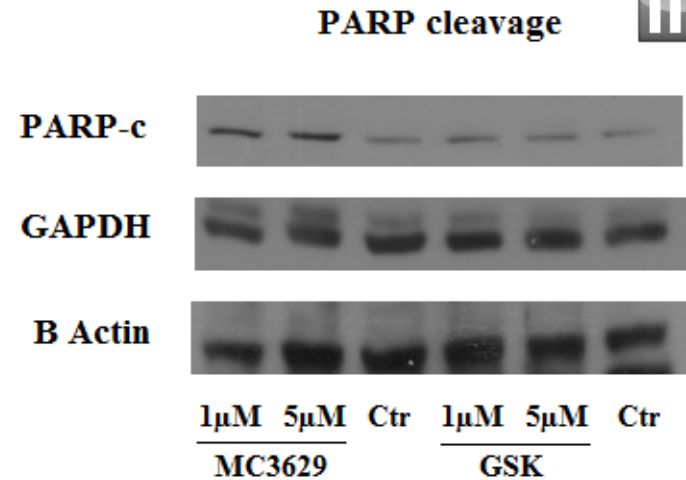
mMB10, 48h



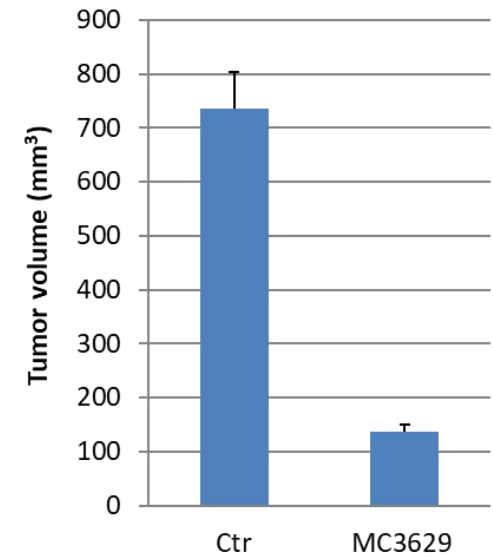
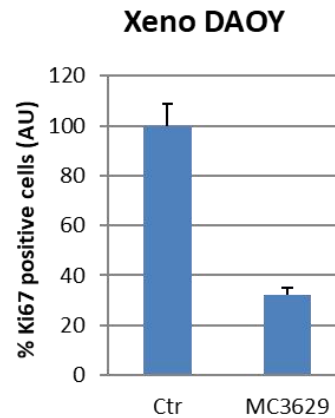
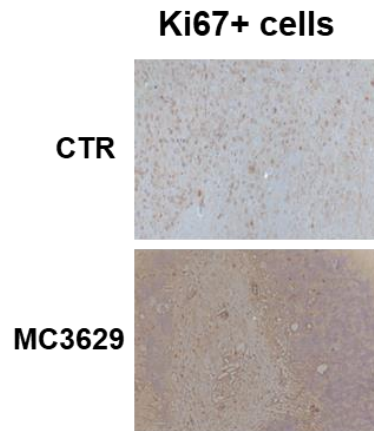
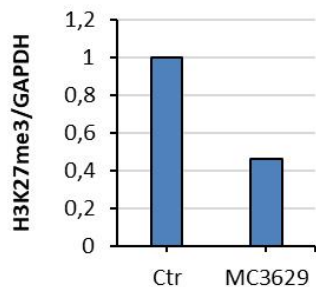
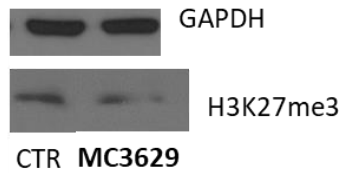
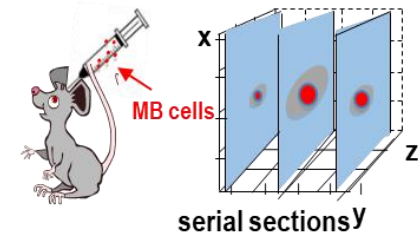
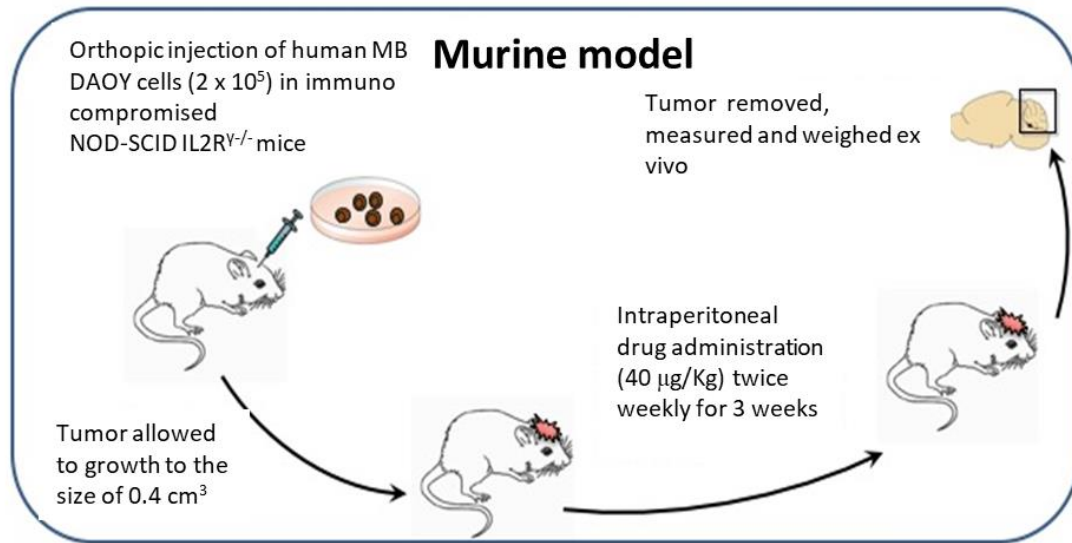
Effects of MC3629 on PARP Cleavage and Clonogenic Activity in mMB10 Cancer Stem Cells



MC3629



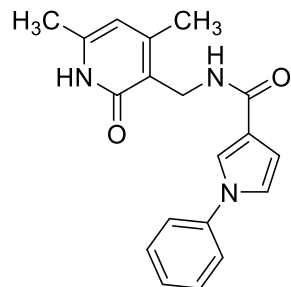
Effect of MC3629 in the DAOY MB-SHH Mouse Model



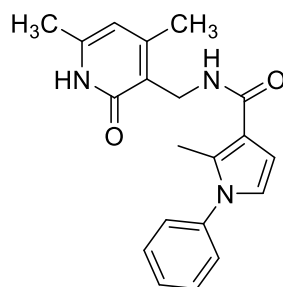
Ferretti's lab

MC3629

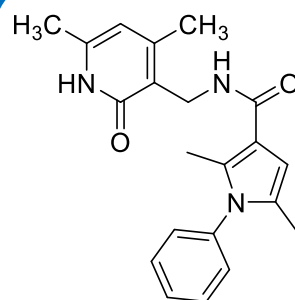
Pyrrole-based EZH2 Inhibitors



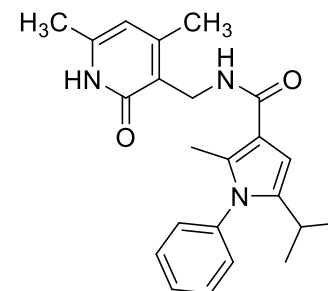
MC3689
37.8 μM



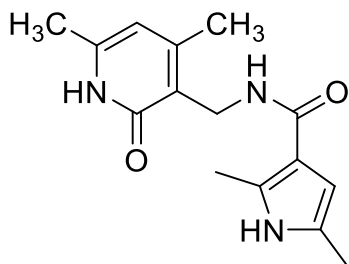
MC3706
8.51 μM



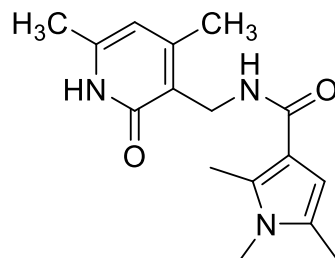
MC3707
5.78 μM



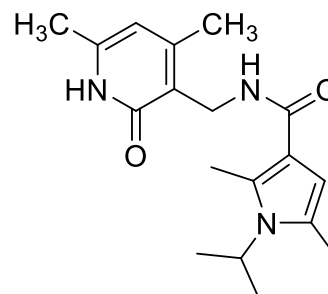
MC3800
22.4 μM



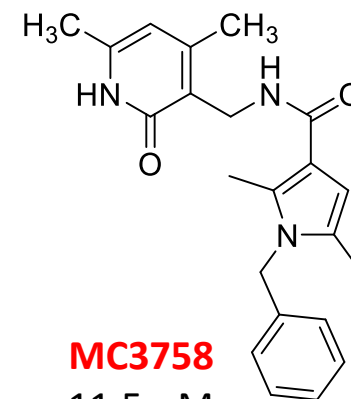
MC3776
12.29%
inhibition @100 μM



MC3771
19.22%
inhibition @100 μM

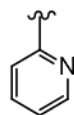
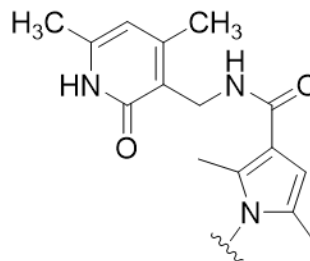
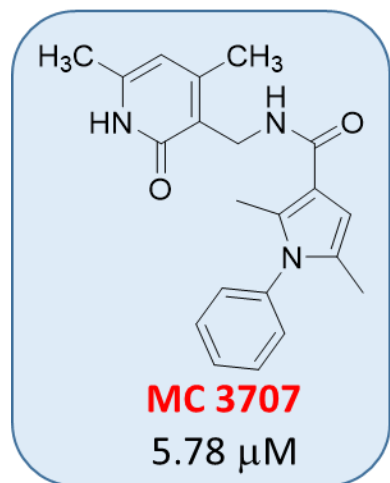


MC3761
38.5 μM

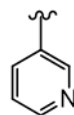


MC3758
11.5 μM

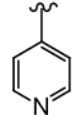
Pyrrole-based Series: SAR Studies



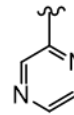
60.8



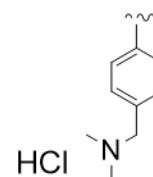
9.6



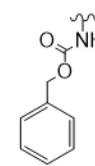
8.1



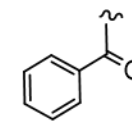
43.7



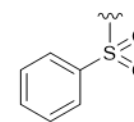
53.6



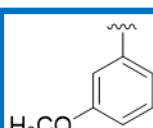
110



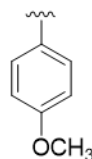
103



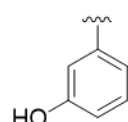
15.7



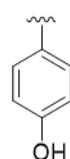
1.05



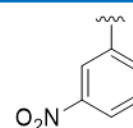
6.87



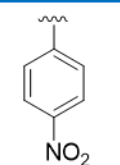
7.74



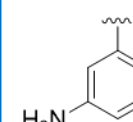
12.2



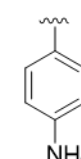
0.80



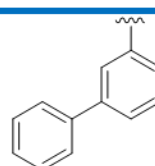
1.62



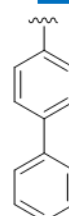
3.03



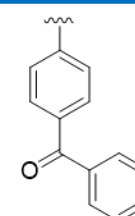
42.3



0.33



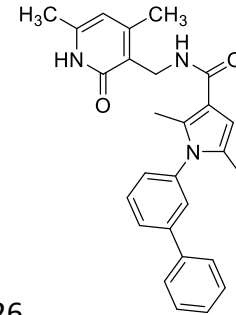
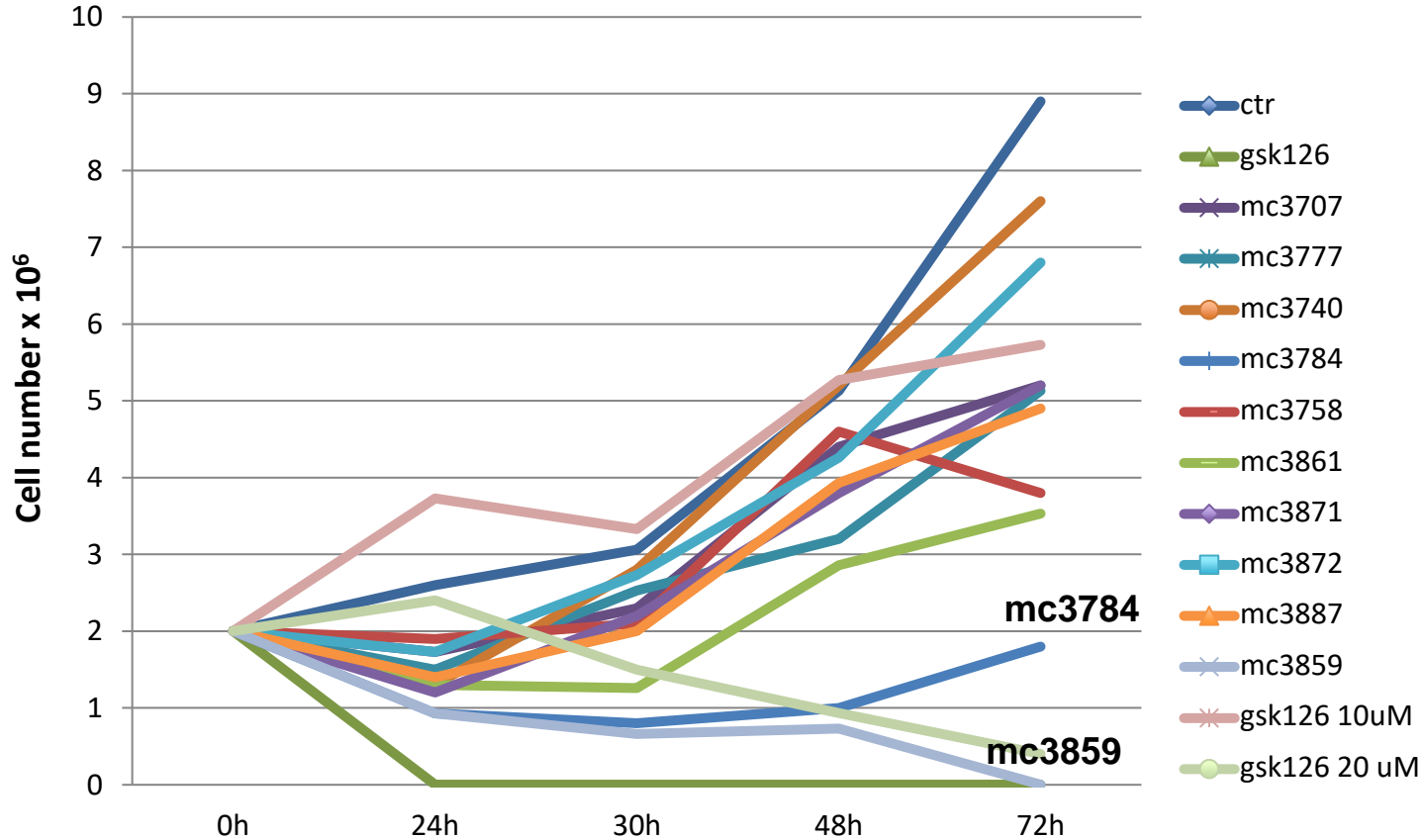
7.07



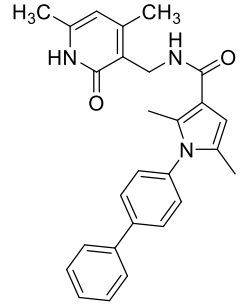
4.46

Pyrrole-based Series: Antiproliferative Activities in Leukemias

U937 cells, 50 μ M

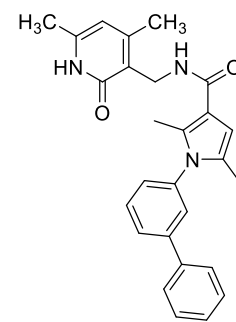
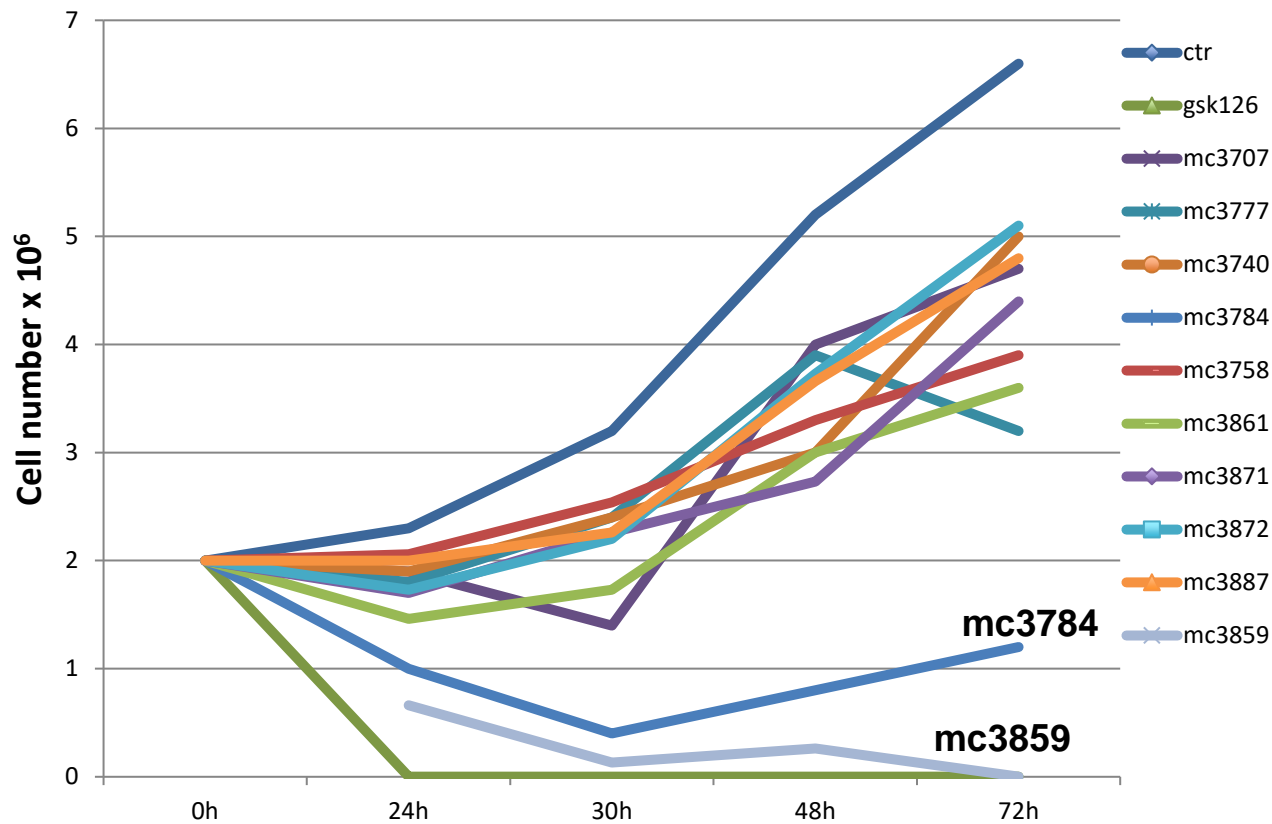


MC3859
(0.33 μ M)

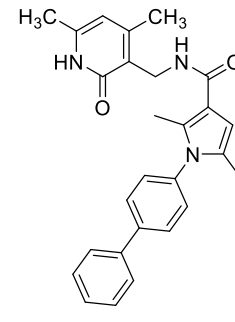


MC3784
(7.07 μ M)

NB4 cells, 50 μ M

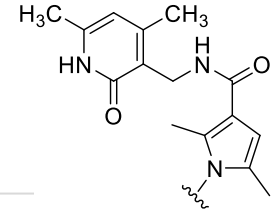


MC3859
(0.33 μ M)

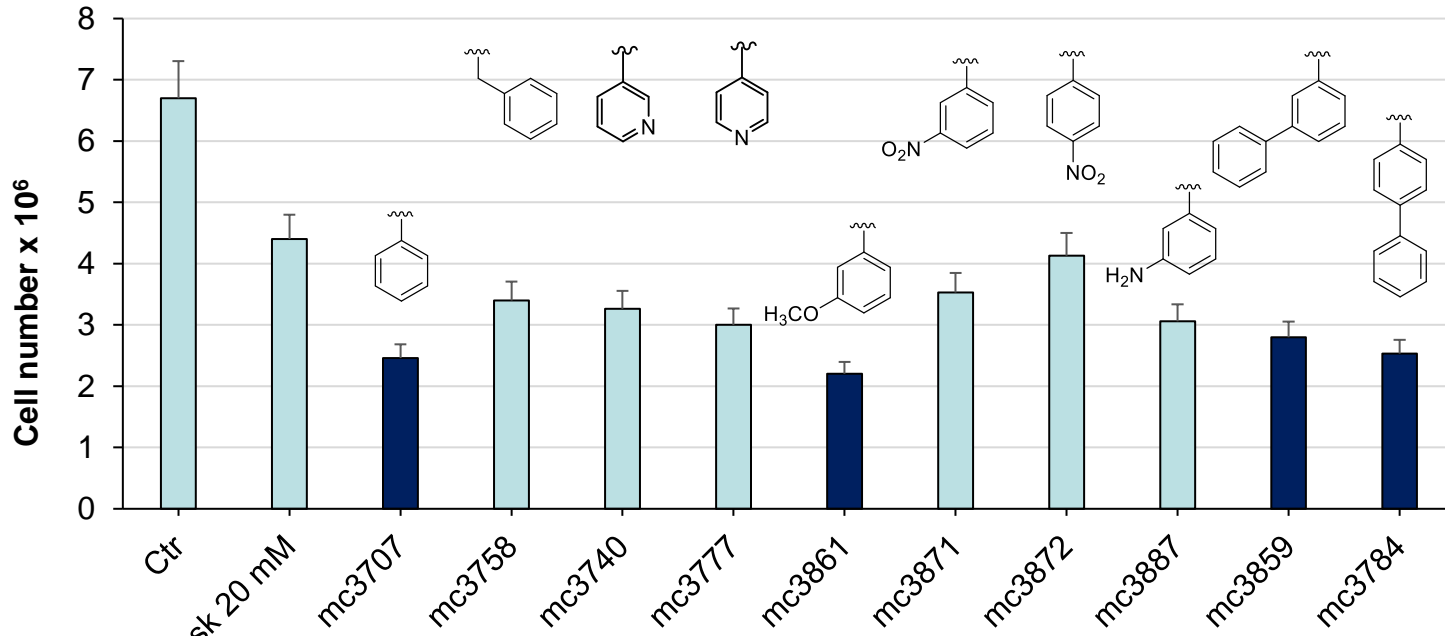


MC3784
(7.07 μ M)

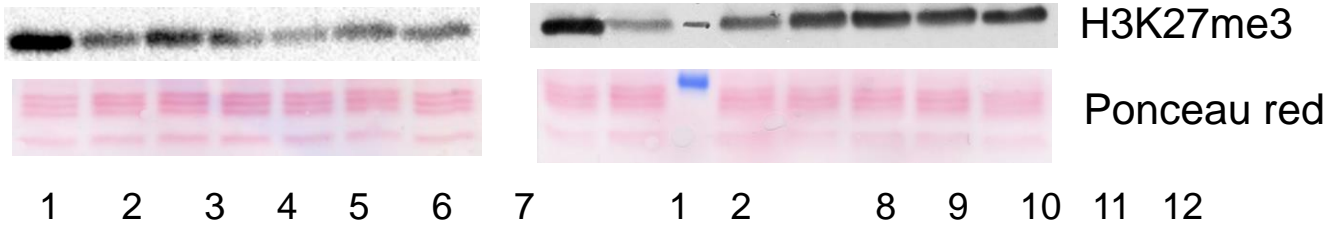
Pyrrole-based Series: Antiproliferative Activity in Breast Cancer



MDA-MB231, 50 μ M, 72h

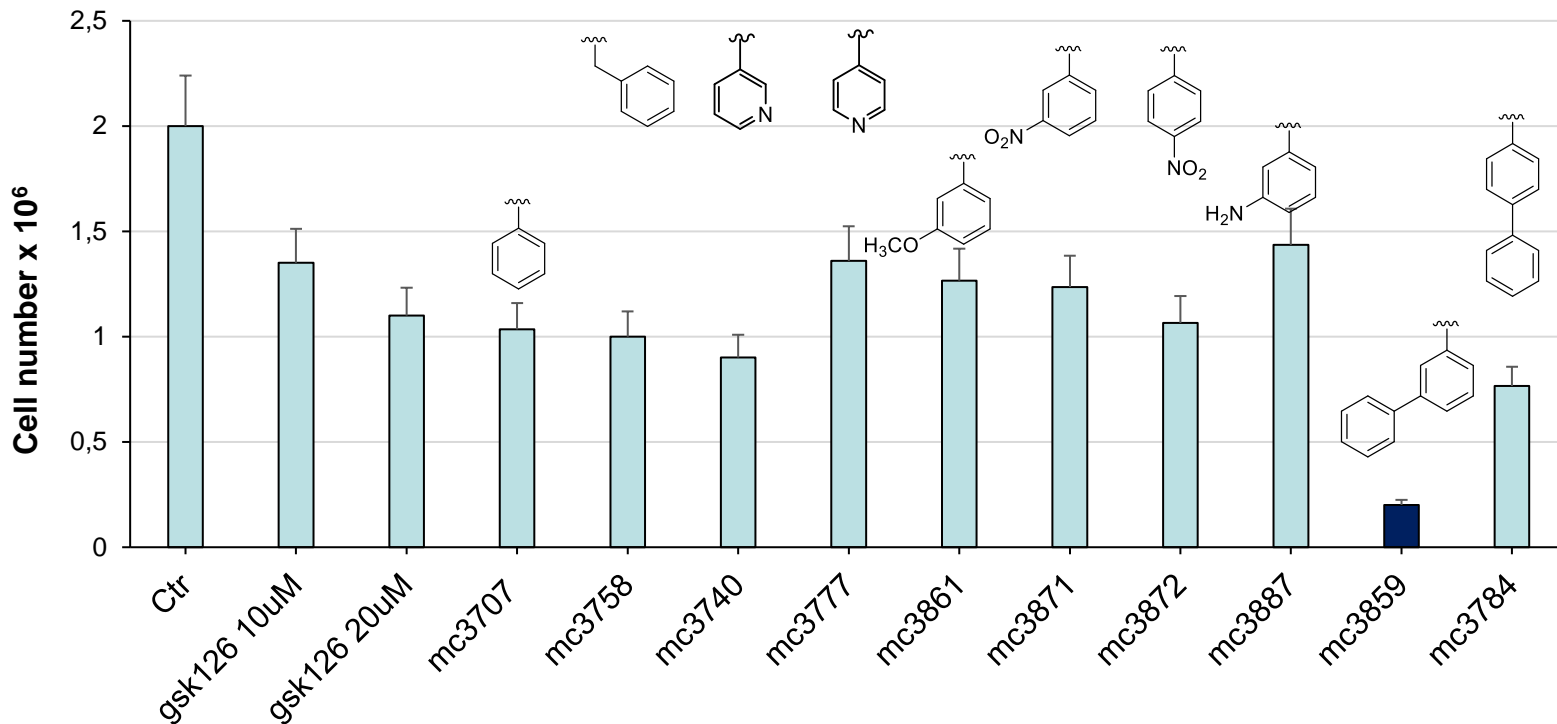
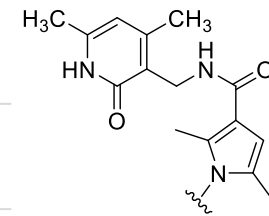


- Legend
- 1= ctr
 - 2= gsk126 20 μ M
 - 3= mc3707
 - 4= mc3777
 - 5= mc3740
 - 6= mc3784
 - 7= mc3758
 - 8= mc3861
 - 9= mc3871
 - 10= mc3872
 - 11= mc3887
 - 12= mc3859

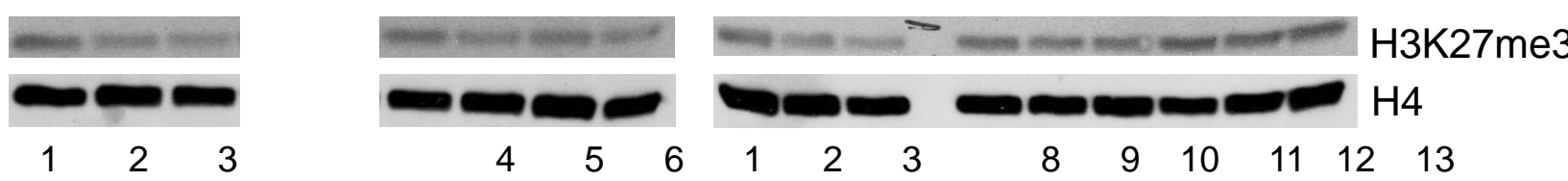


Pyrrole-based Series: Antiproliferative Activity in Kelly Neuroblastoma Cells

KELLY, 50 μ M, 72h

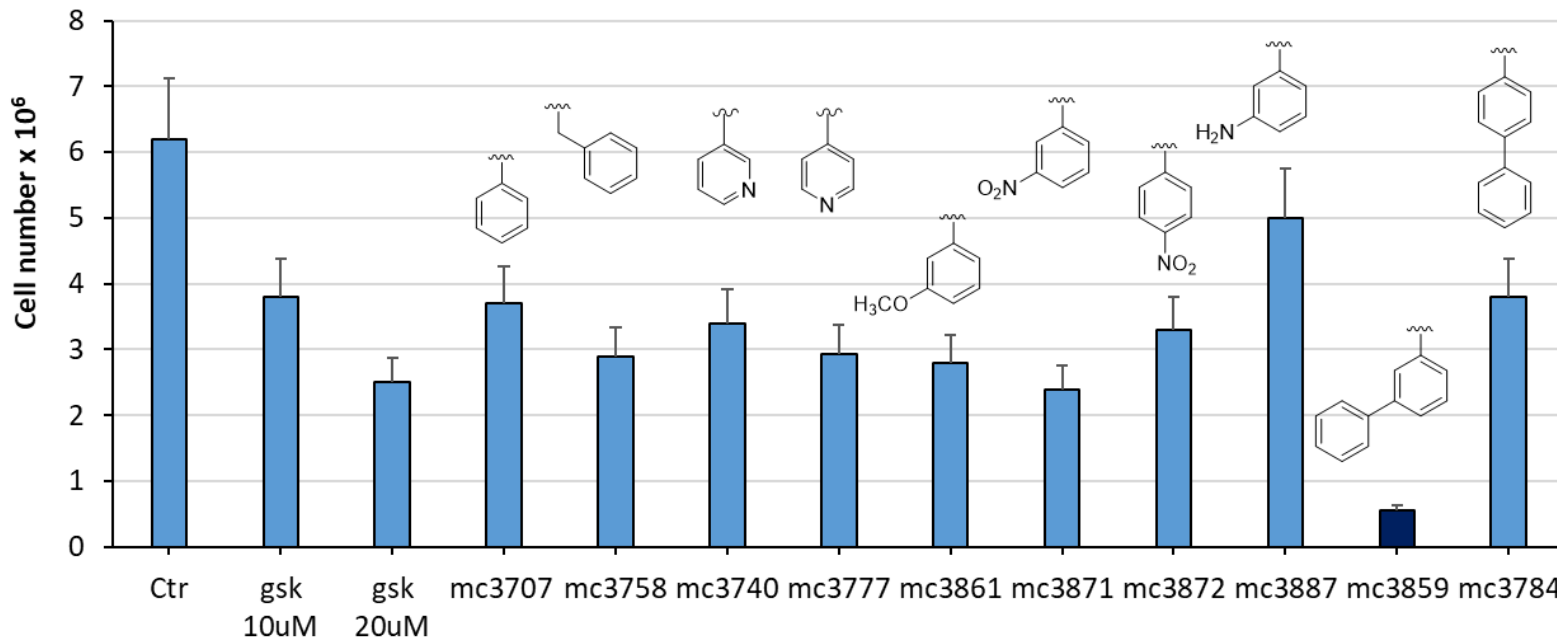
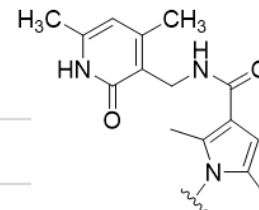


- Legend
- 1= ctr
 - 2= gsk 10 μ M
 - 3= gsk 20 μ M
 - 4= mc3707
 - 5= mc3777
 - 6= mc3740
 - 7= mc3784
 - 8= mc3758
 - 9= mc3861
 - 10= mc3871
 - 11= m3872
 - 12= mc3887
 - 13= mc3859

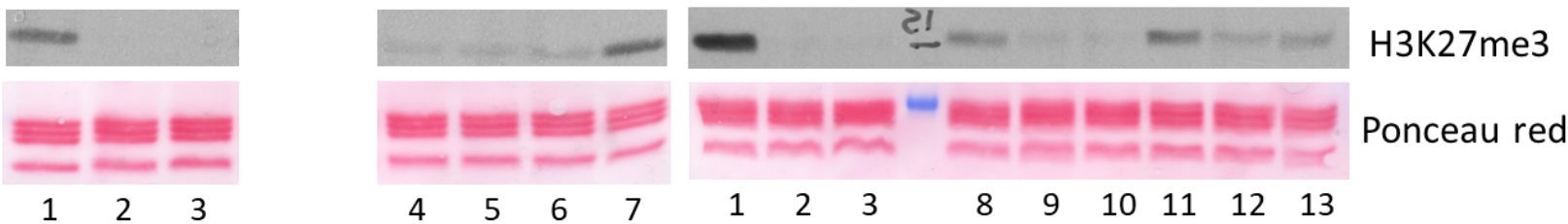


Pyrrole-based Series: Antiproliferative Activity in SH-SY5Y Neuroblastoma Cells

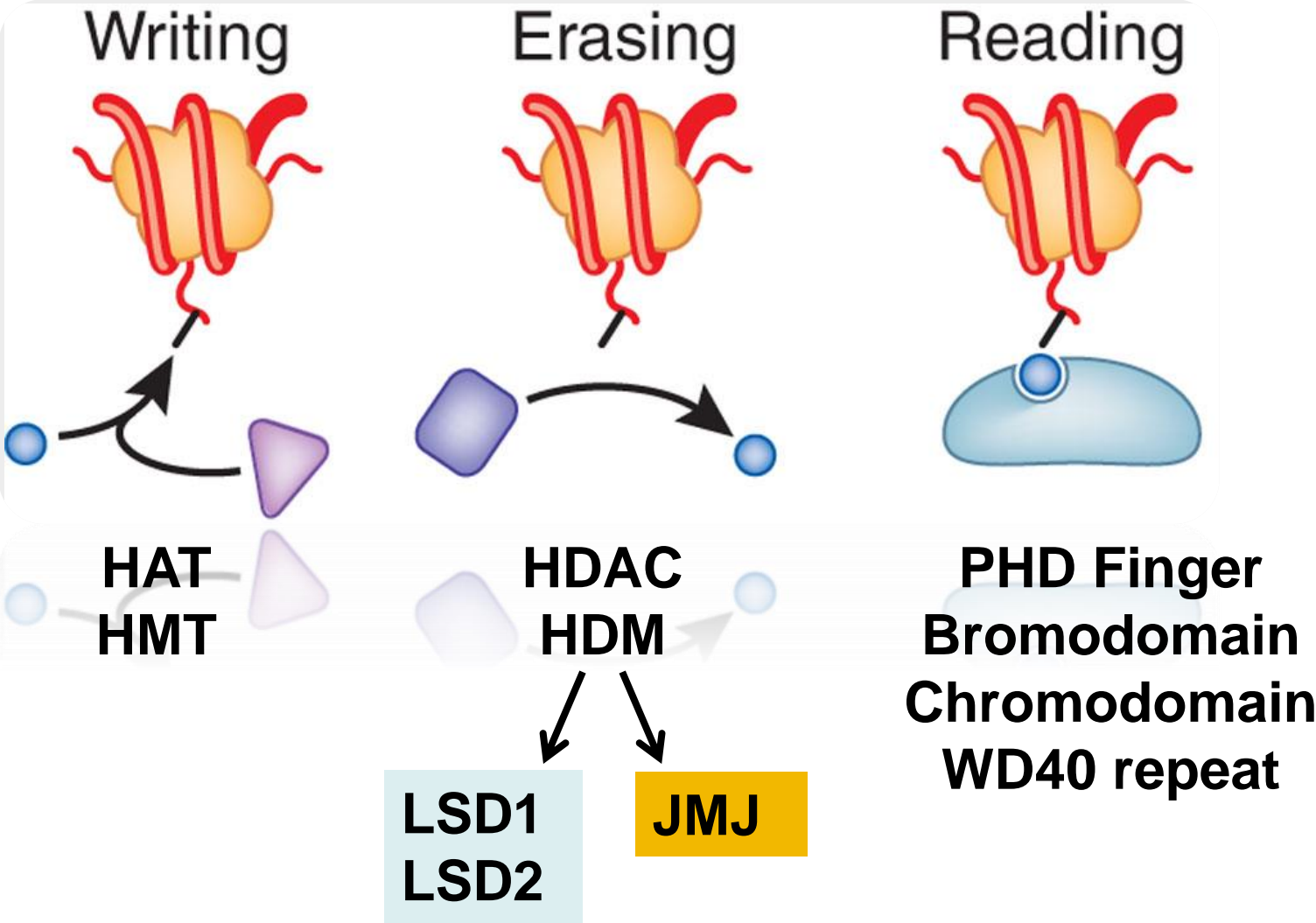
SHSY5Y, 50 μ M, 72h

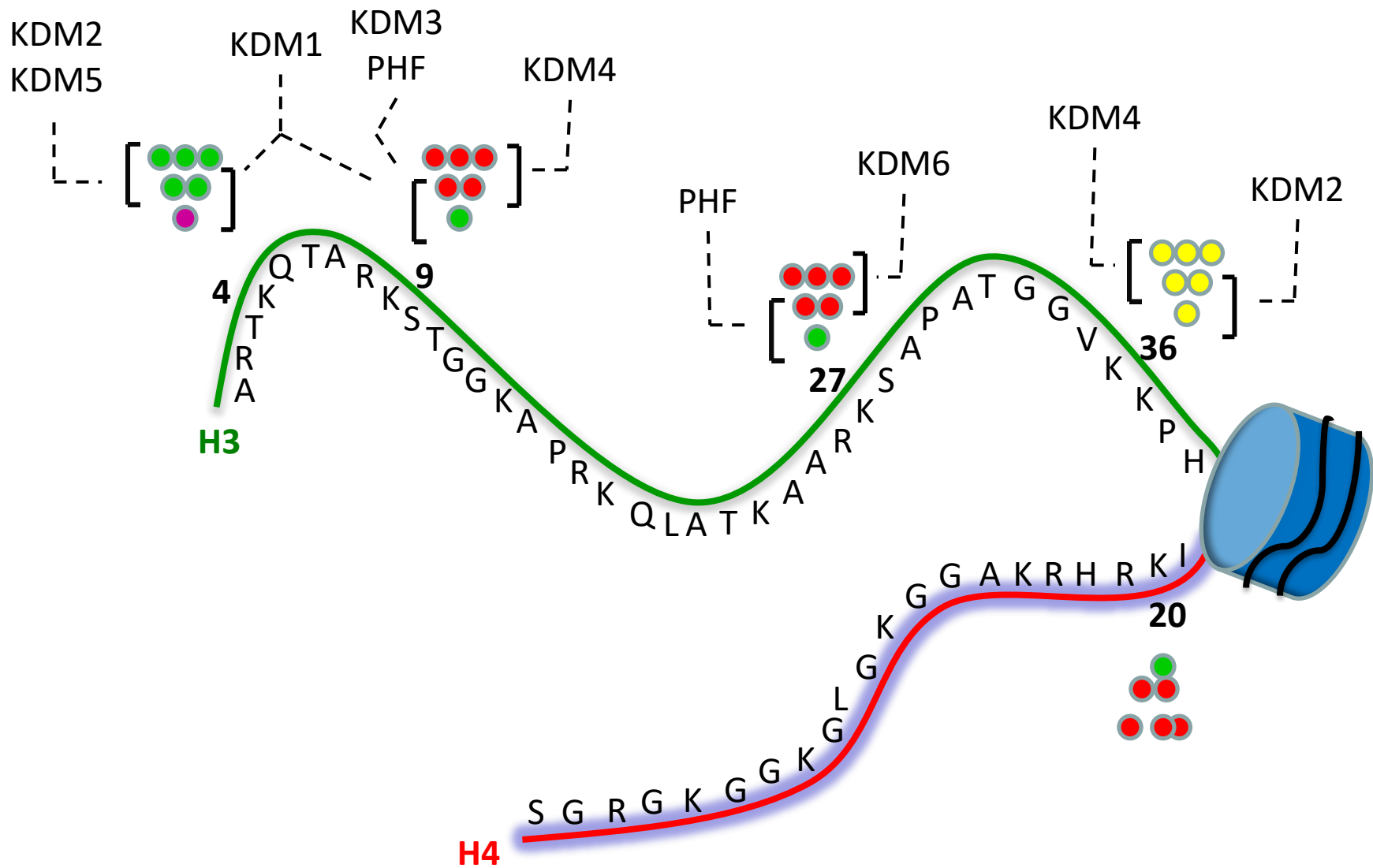


- Legend
- 1= ctr
 - 2= gsk 10 μ M
 - 3= gsk 20 μ M
 - 4= mc3707
 - 5= mc3777
 - 6= mc3740
 - 7= mc3784
 - 8= mc3758
 - 9= mc3861
 - 10= mc3871
 - 11= m3872
 - 12= mc3887
 - 13= mc3859



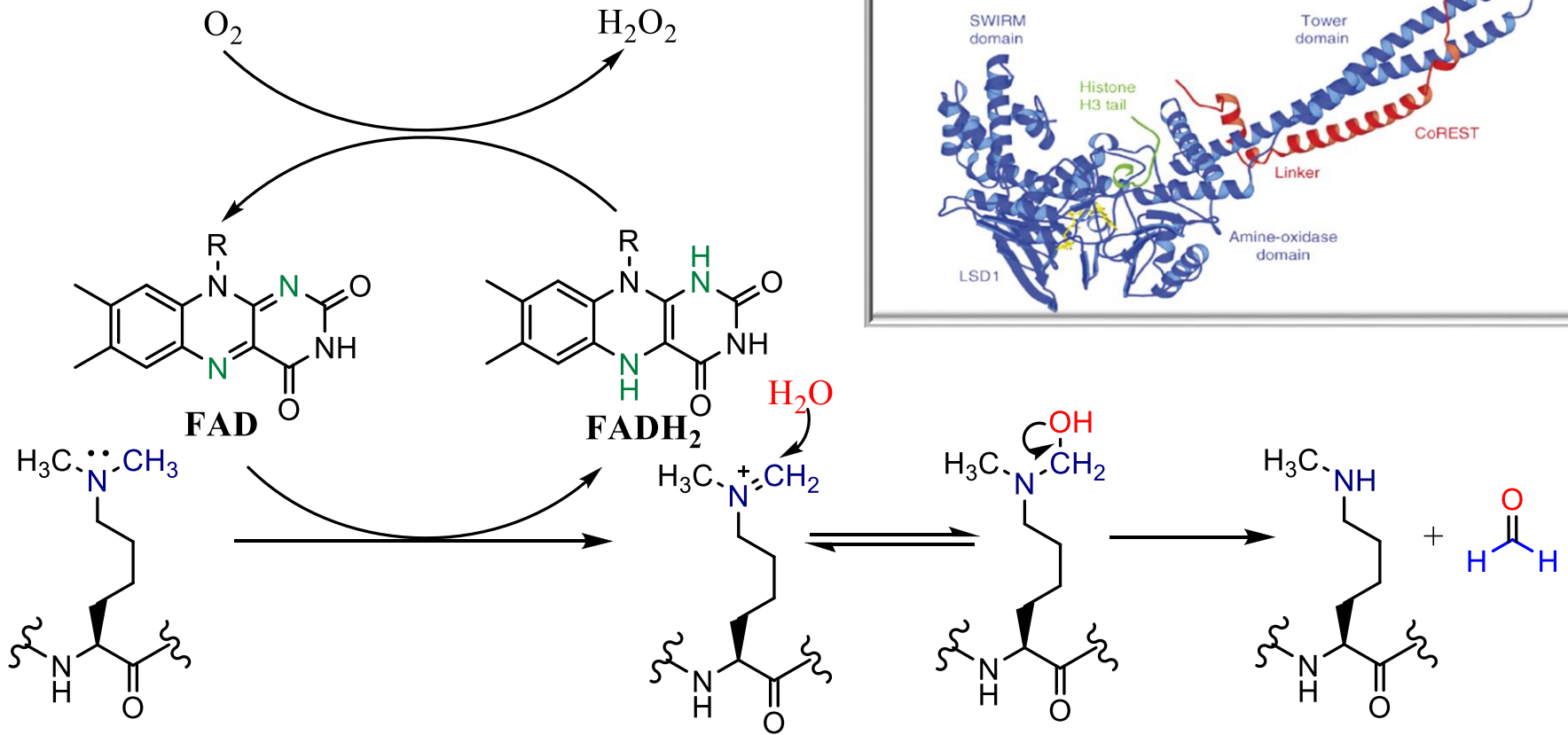
Histone Demethylases





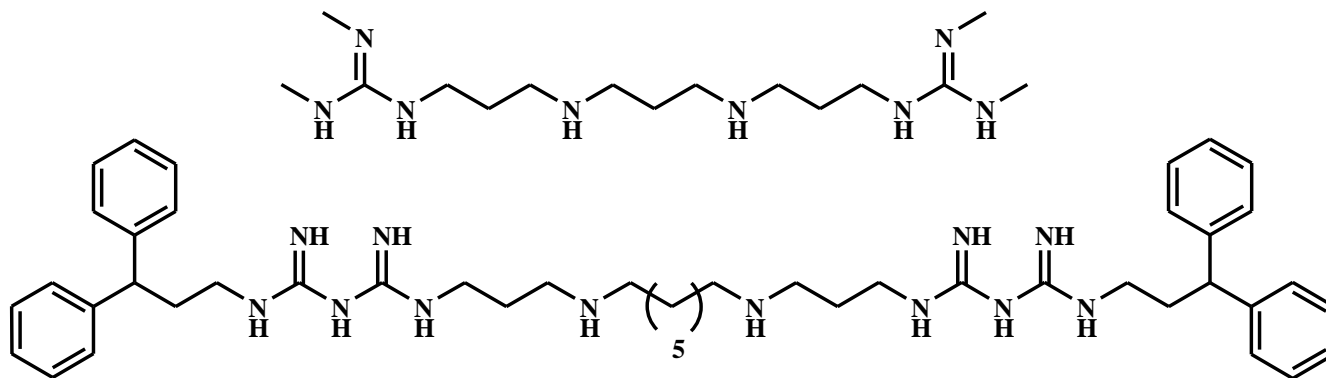
- Promoters, active genes
- Heterochromatin, repressed genes
- Enhancer
- Elongation

Lysine Specific Histone Demethylase 1 (LSD1) and -2 (LSD2)



Shi Y et al. Cell 2004, 119, 941-53. Karytinovs A et al. J Biol Chem. 2009, 284, 17775-82.

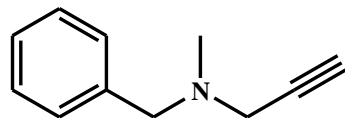
LSD1 Inhibitors



← Poliamines derivatives

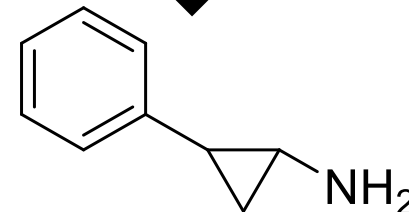
Huang Y, et al. *Proc. Natl. Acad. Sci.* 2007, 104, 8023-8028.

Pargiline

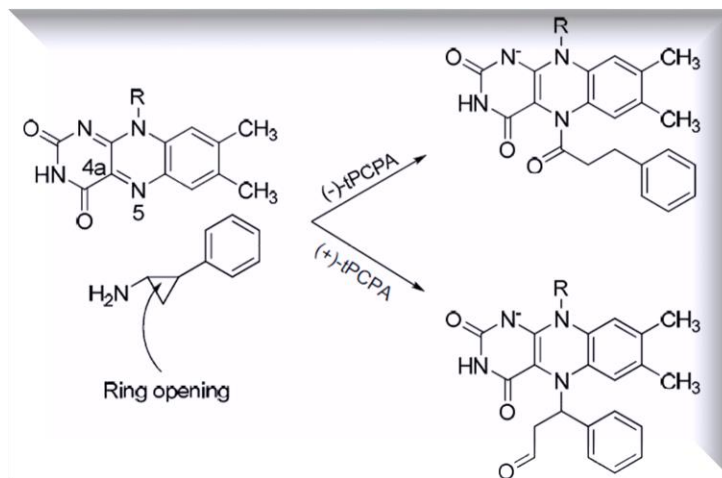


Kahl P, et al. *Cancer Res.* 2006,66,11341-11347.

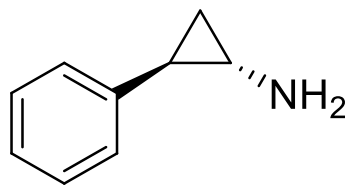
Tranylcpromine derivatives



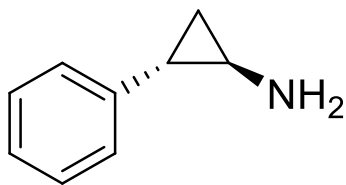
tranylcpromine



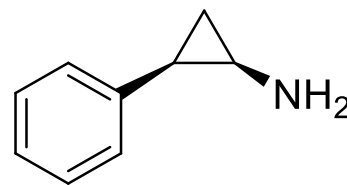
LSD1 inhibitors



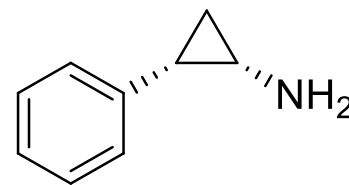
(*R,S*)-(-)-PCPA



(*S,R*)-(+)-PCPA

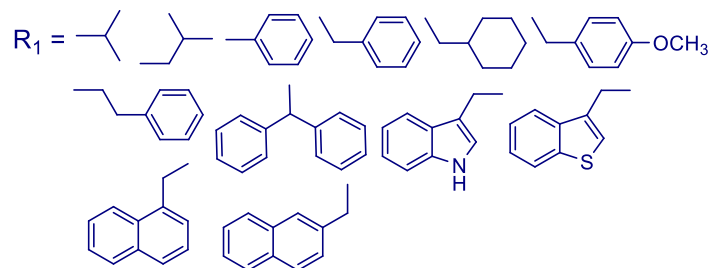
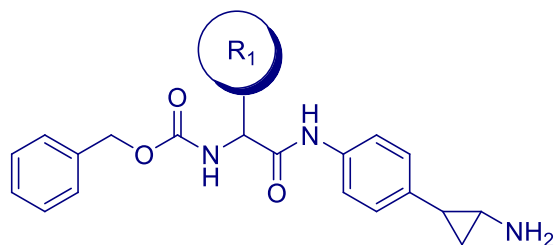
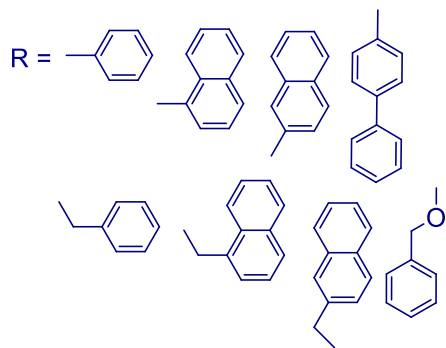
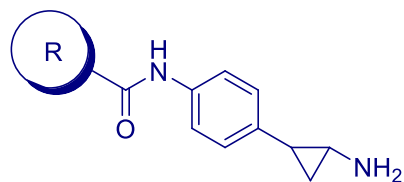


(*R,R*)-(-)-PCPA

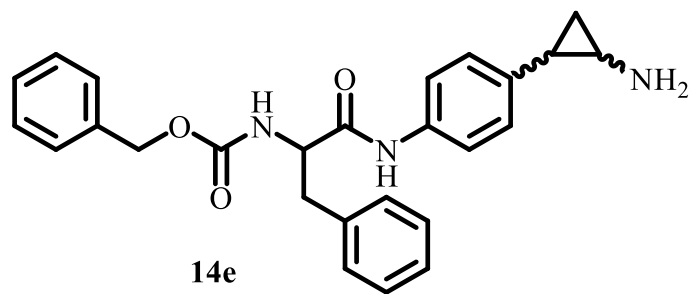
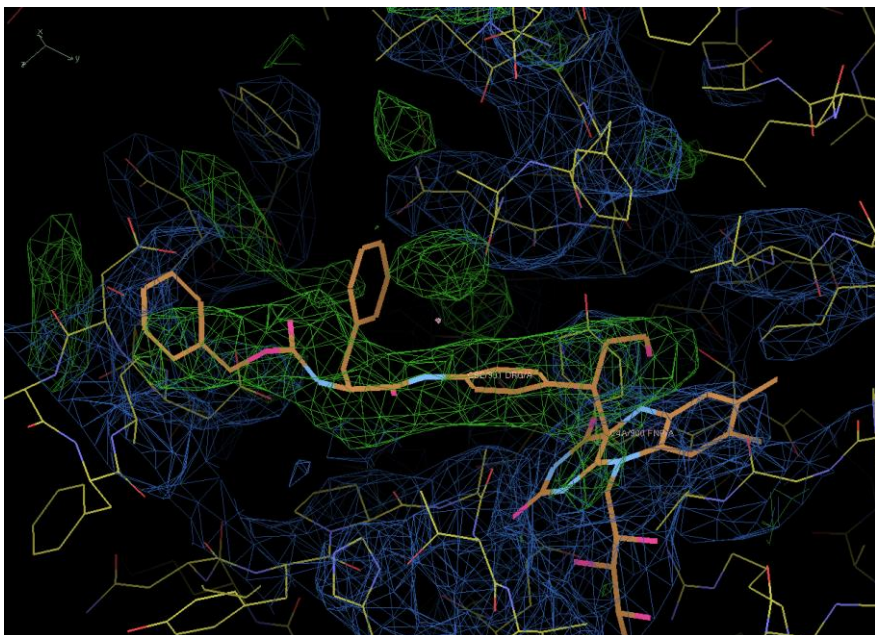


(*S,S*)-(+)-PCPA

compd	K_i , μM			
	LSD1	LSD2	MAO-A	MAO-B
(\pm)-tPCPA	271	186	19	16
(+)-tPCPA	284	137	ND	4.4
(-)-tPCPA	168	127	ND	89
(+)-cPCPA	364	131	ND	39
(-)-cPCPA	506	68	ND	50
(+)-Br-tPCPA	58	66	ND	0.4
(-)-Br-tPCPA	28	82	ND	2.3
(+)-Br-cPCPA	23	61	ND	0.7
(-)-Br-cPCPA	44	21	ND	1.9



compd	K _i , μM			
	LSD1	LSD2	MAO-A	MAO-B
 13a	1.9	20	0.5	7.4
 13b	1.1	61	2.3	3.5
 14e	1.3	38	11.2	NI
 14l	40	12	49	NI
(±)-tPCPA	271	186	19	16

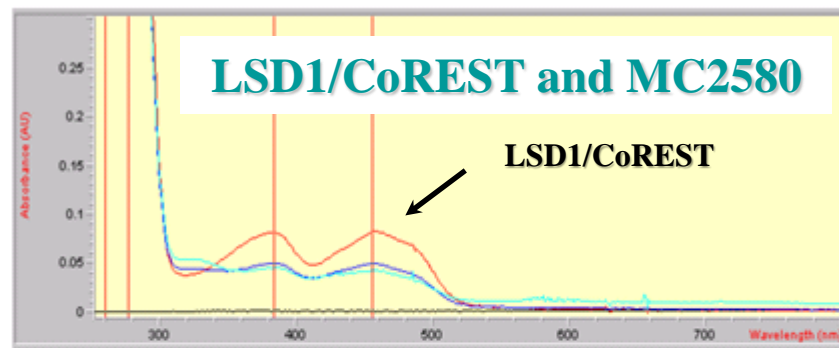


14e
MC2580

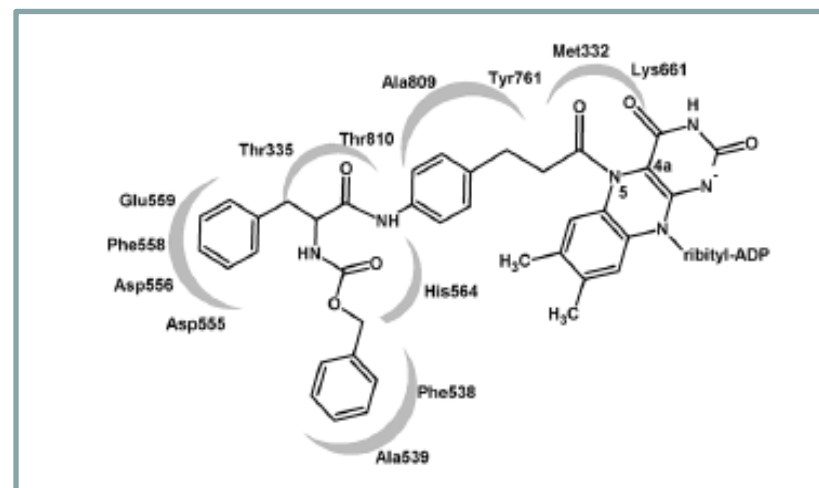
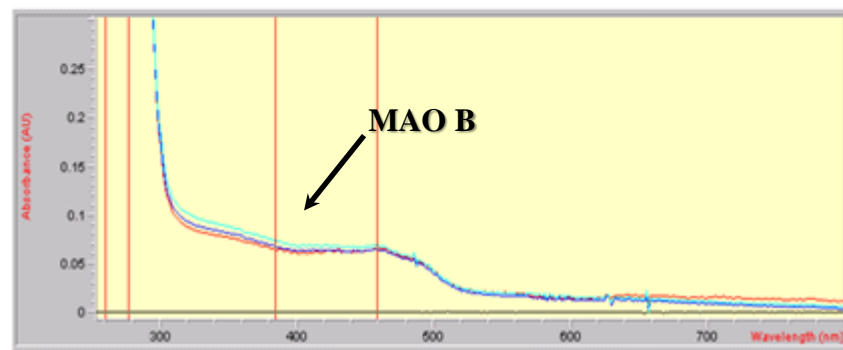
Binda C. *et al.*, *J. Am. Chem. Soc.* **2010**, *132*, 6827-6833.

LSD1-CoREST + 14e

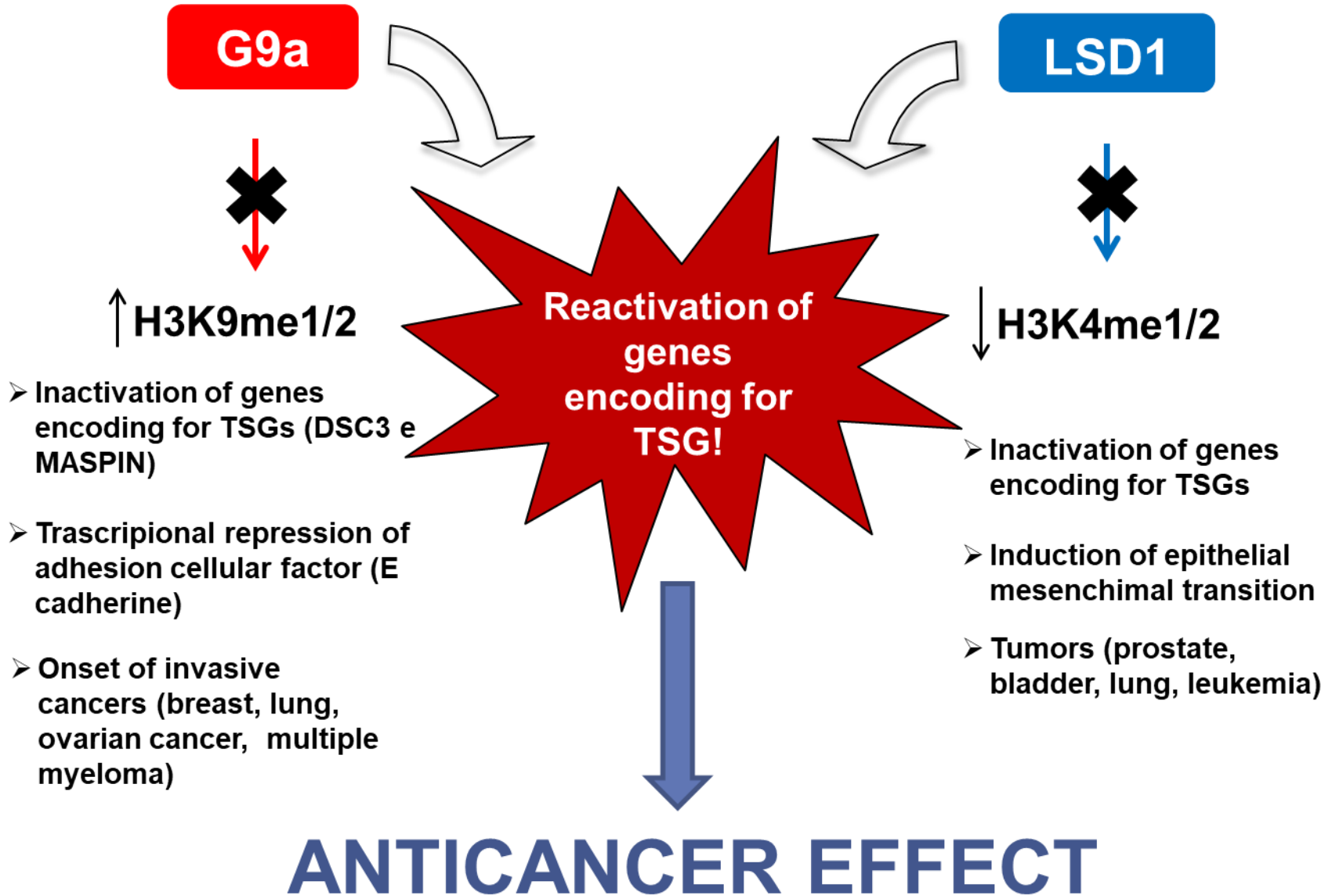
K_i: 1.3 μM



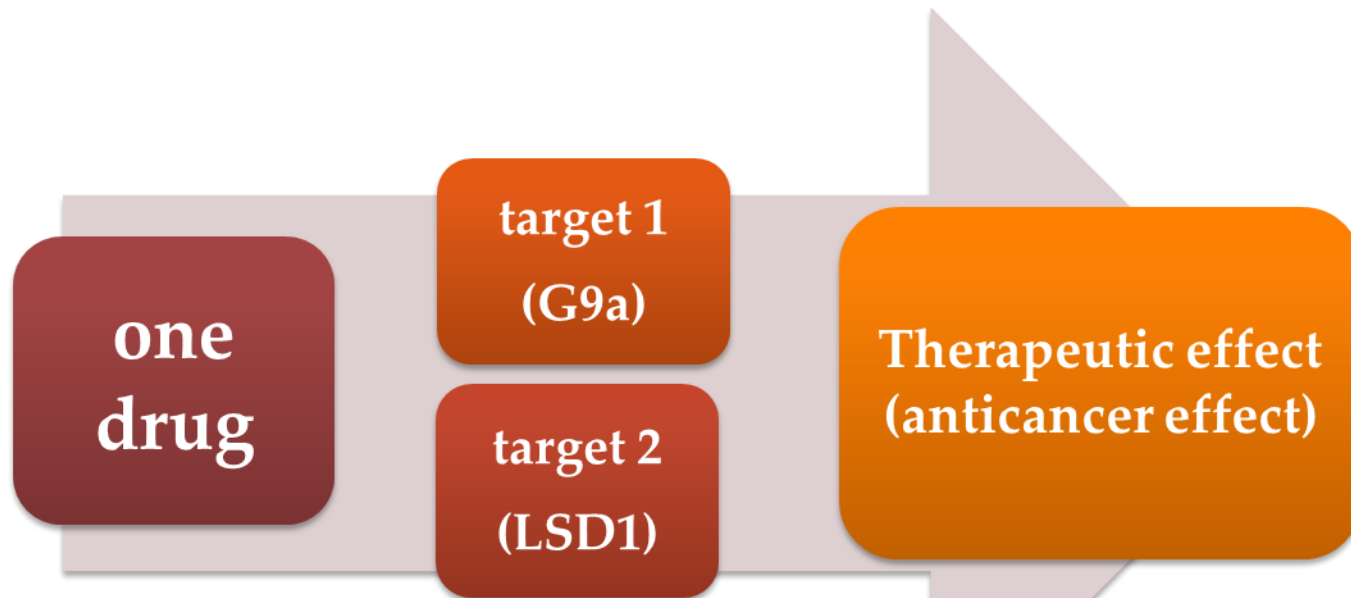
MAOB + 14e



G9a and LSD1



Polypharmacology Approach

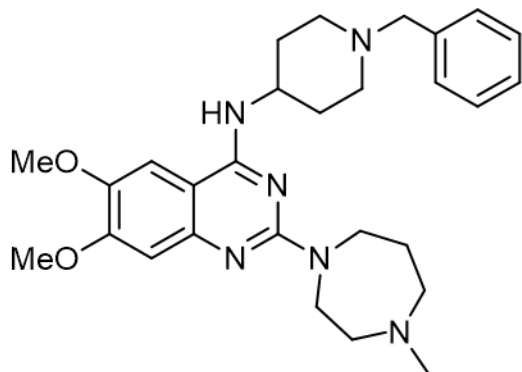
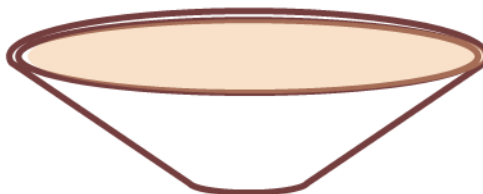


For the treatment of multifactorial disease, modulation of multiple targets may result in a synergistic effect.

A single molecule with dual activity may have a superior pharmacokinetic and safety profile compared to multiple molecules administered in combination.

Rational Project

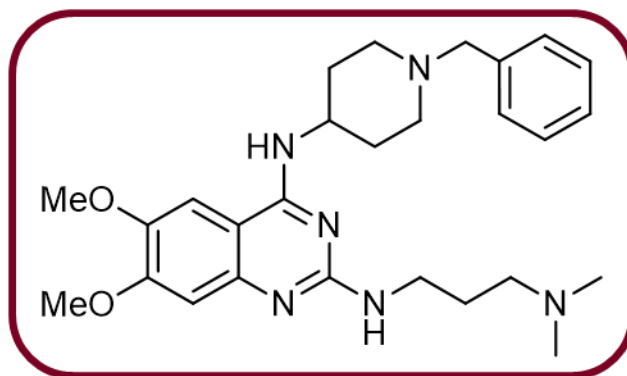
The binding of LSD1 with the substrate is based on electrostatic interactions due to the presence of negatively charged residues into the active site. We selected quinazoline based derivatives, previously discovered as G9a/GLP inhibitors, equipped with positively charged substituents.



BIX-01294

IC_{50} G9a= 1.7 μ M

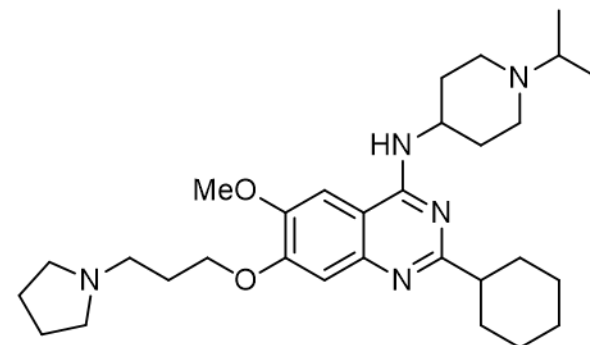
ΔT_m LSD1 = +1.5° C
 K_d LSD1 = 1.93 μ M
 K_i LSD1 = >15 μ M



MC3774

IC_{50} G9a= 0.78 μ M

ΔT_m LSD1 = +6.5° C
 K_d LSD1 = 0.24 μ M
 K_i LSD1 = 0.44 μ M



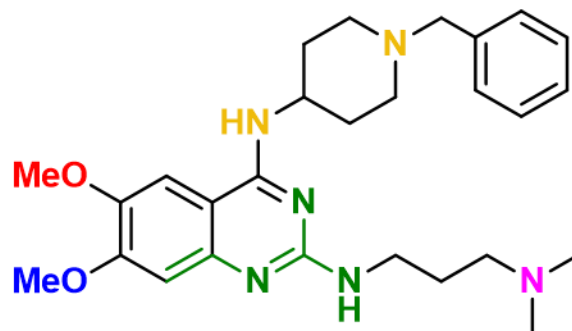
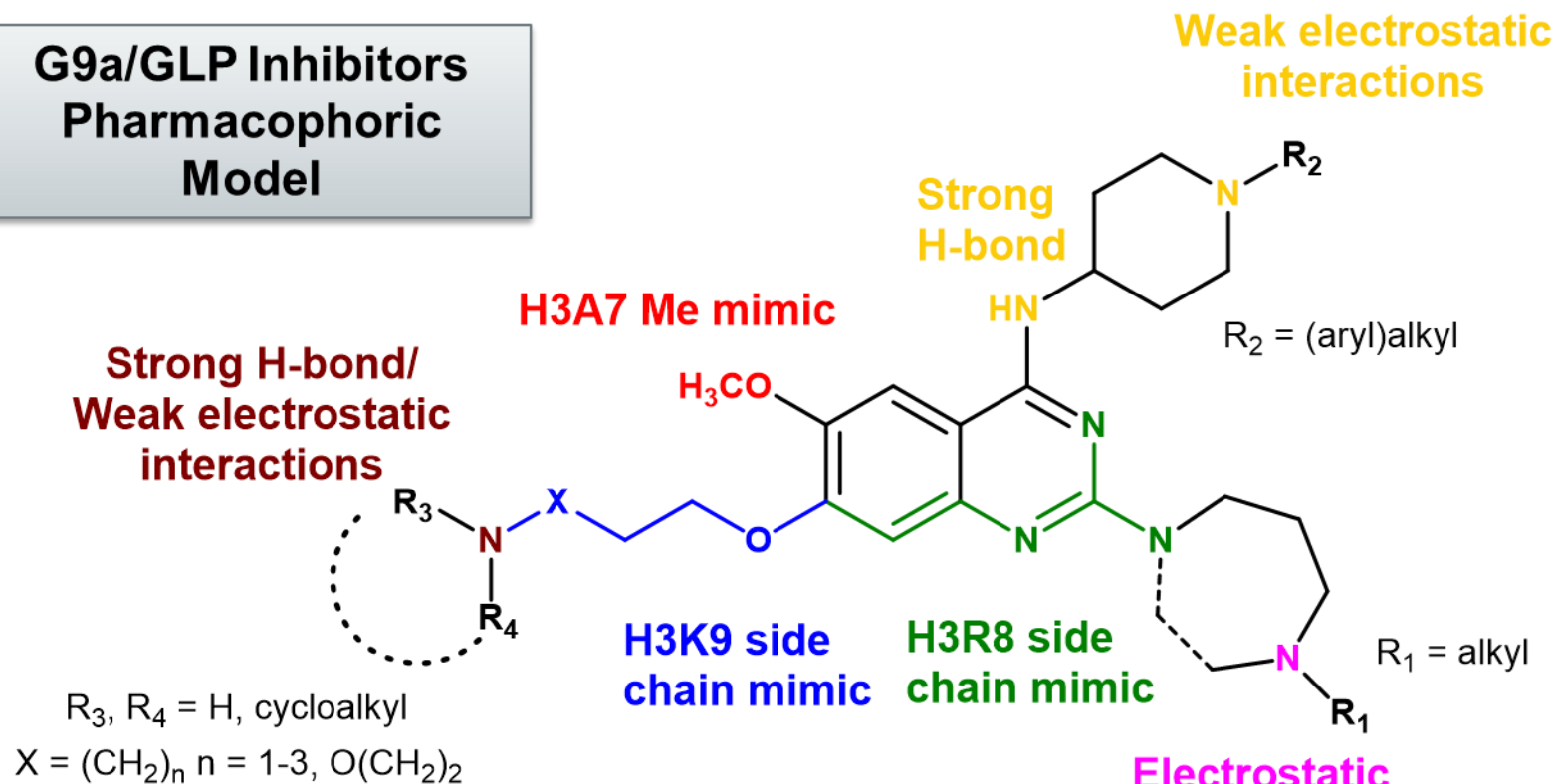
UNC0638

IC_{50} G9a= < 0.03 μ M

ΔT_m LSD1 = <0.5° C
 K_d LSD1 = 8.33 μ M

SAR for G9a Inhibition

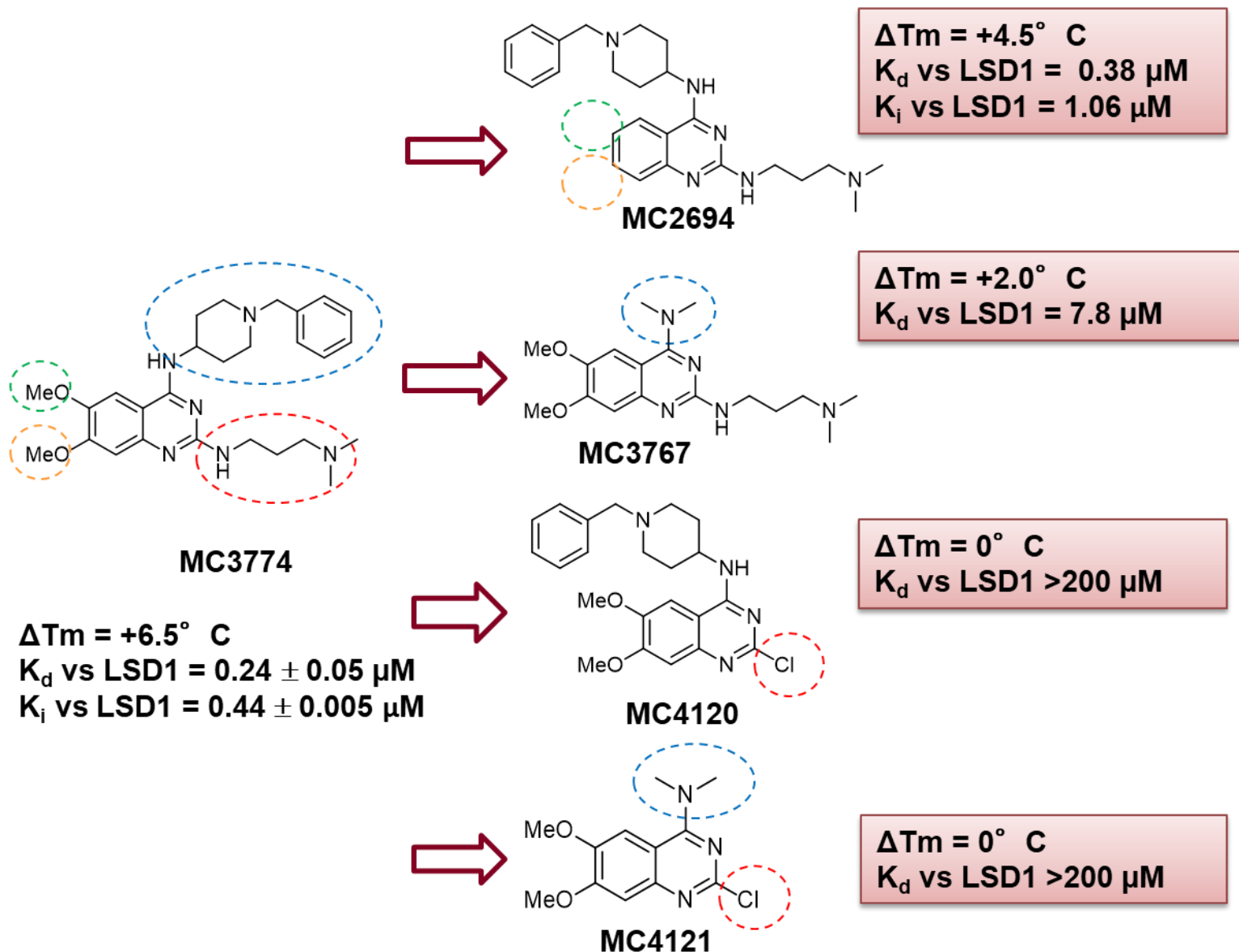
G9a/GLP Inhibitors Pharmacophoric Model



MC3774

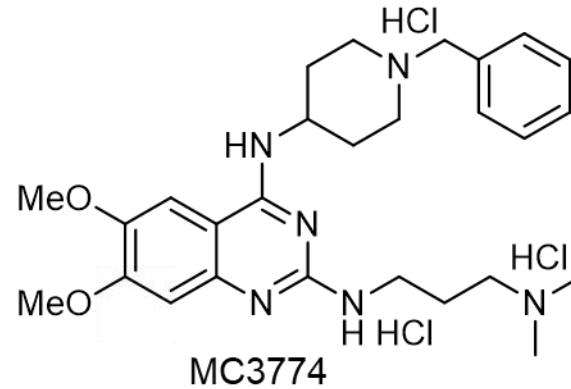
IC₅₀ G9a = 0.78 μM

Preliminary SAR for LSD1 Inhibition

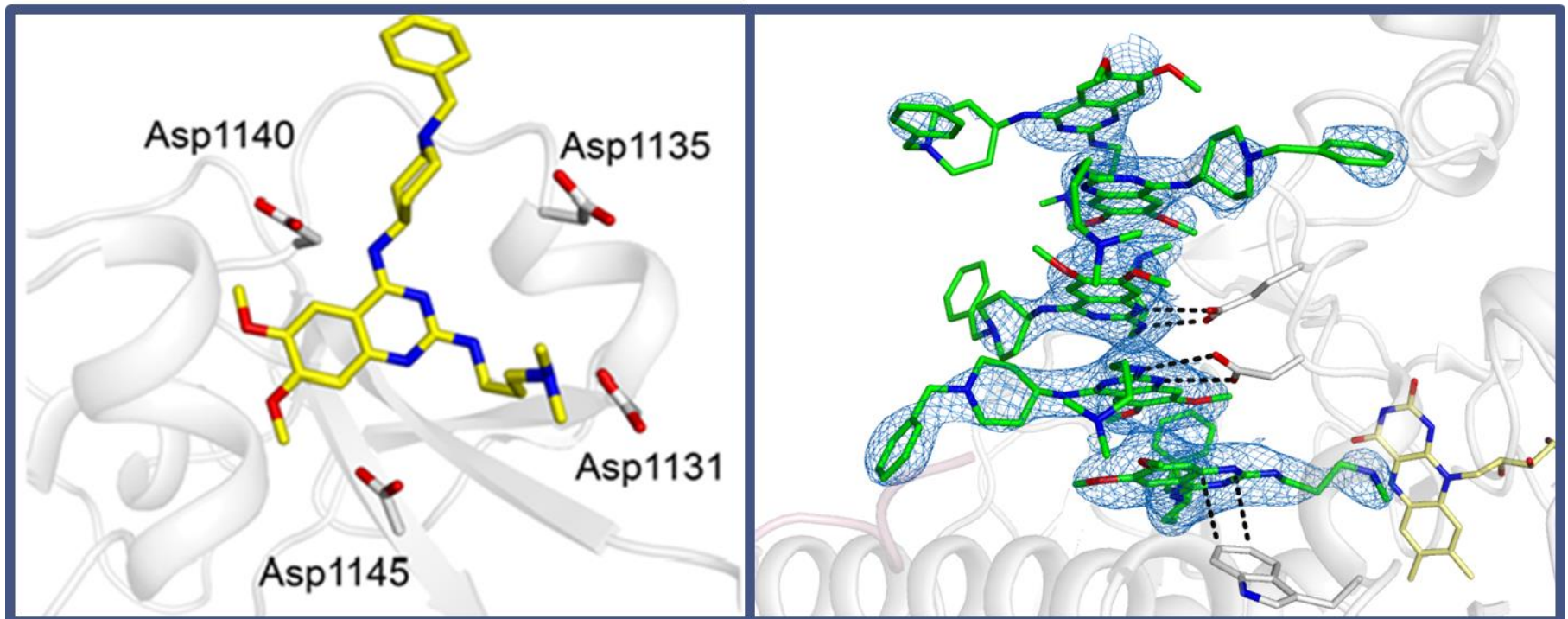


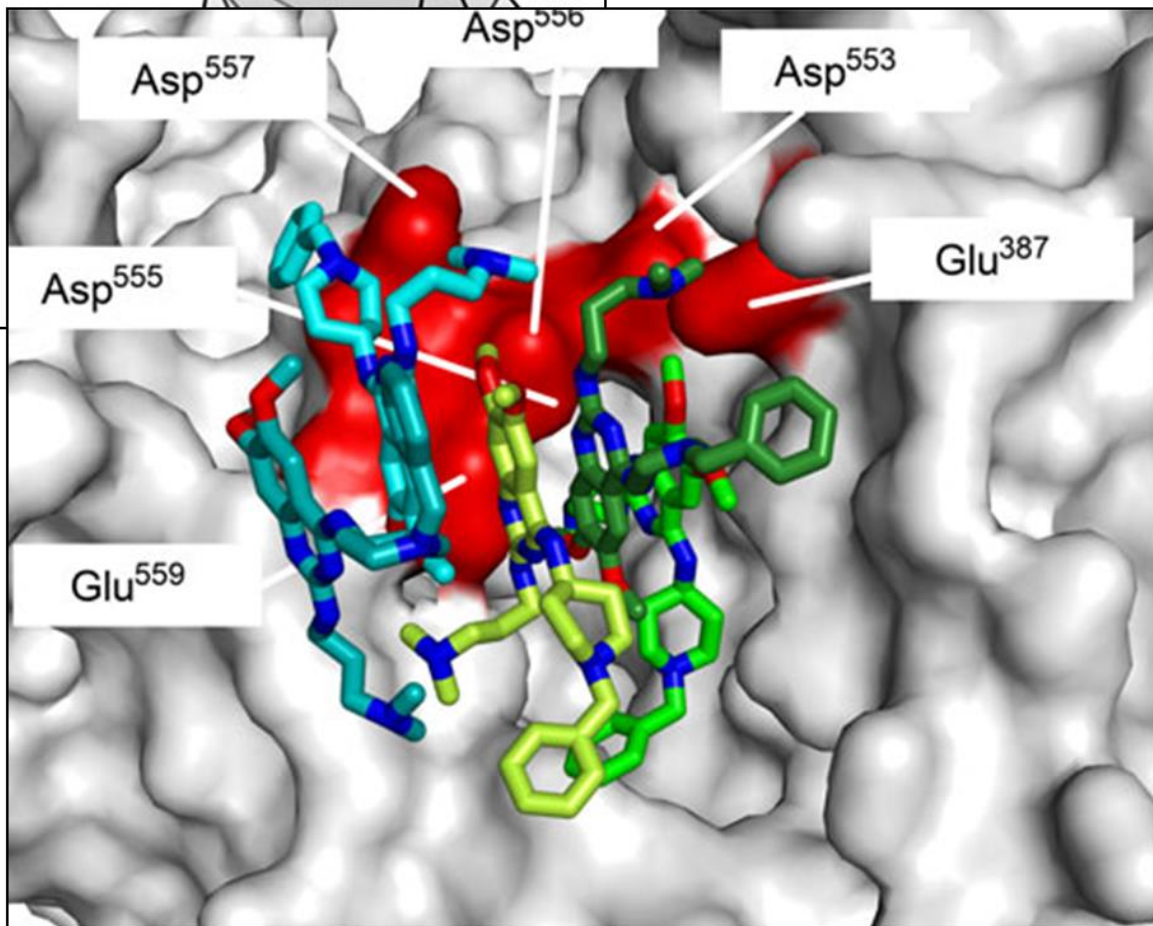
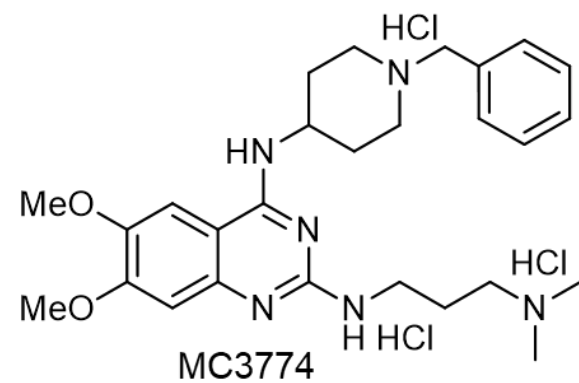
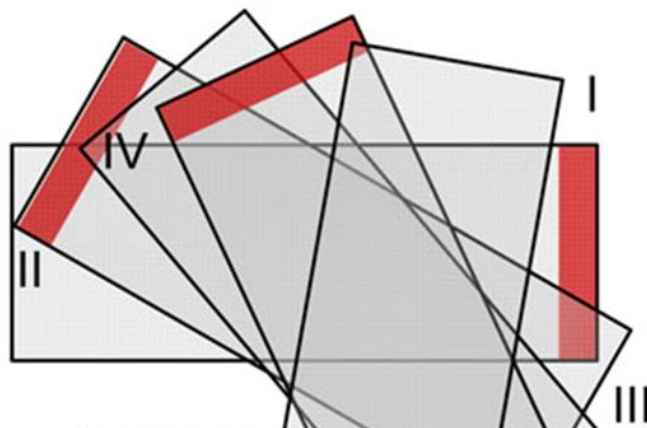
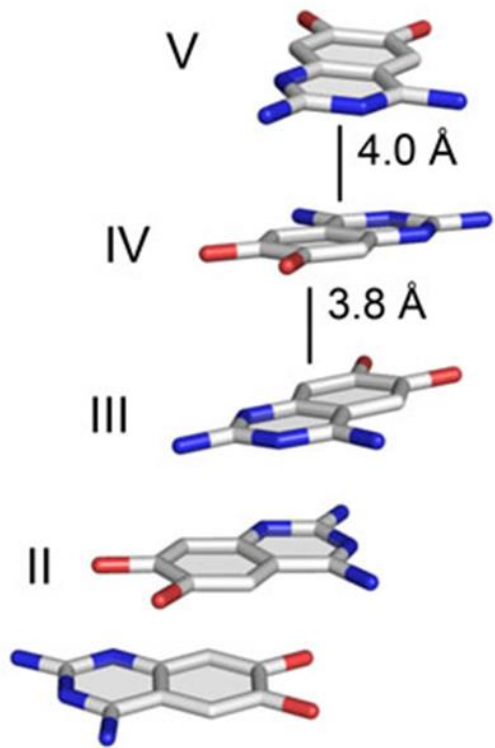
MC3774: Binding Modes

G9a

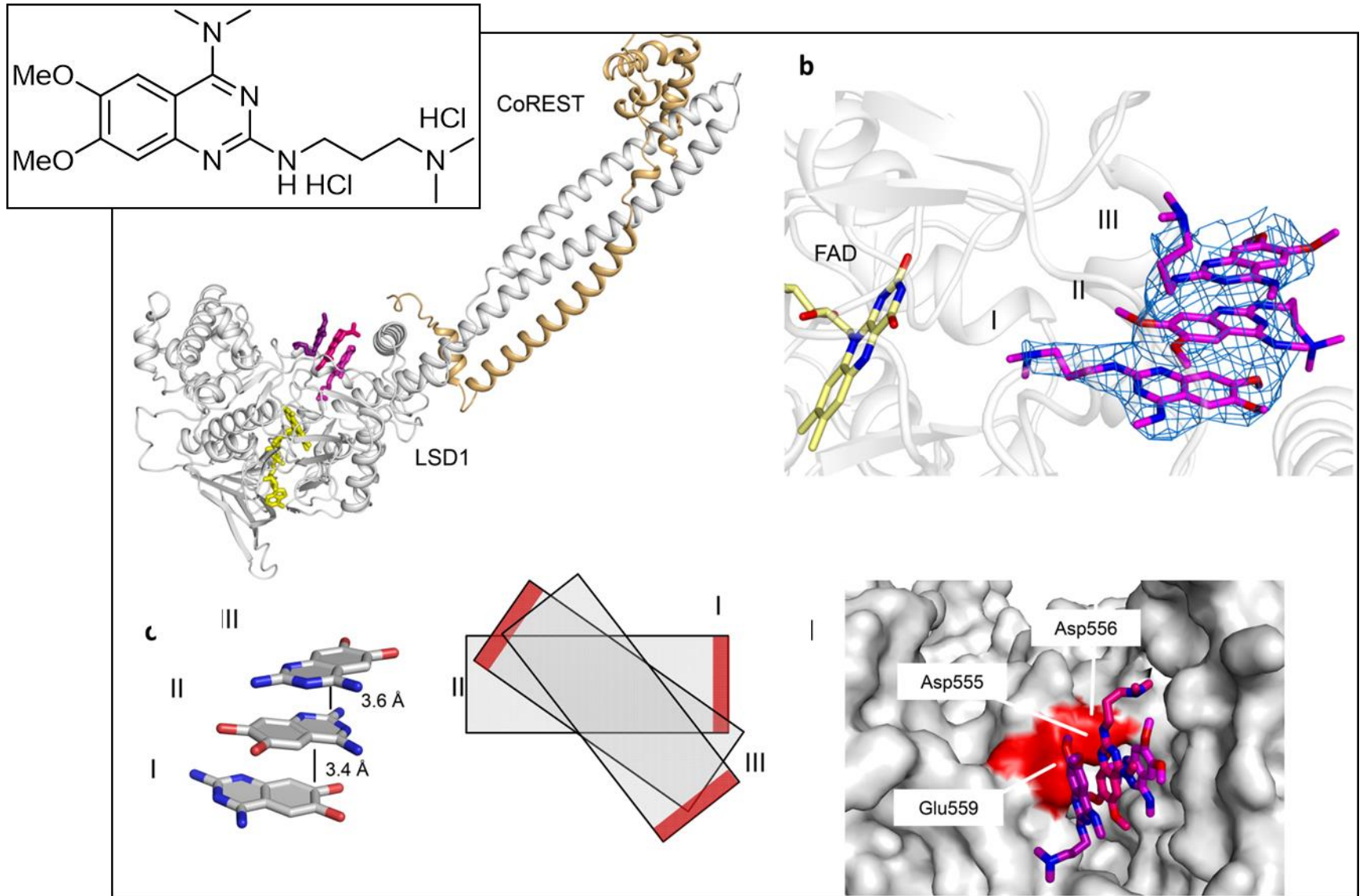


LSD1

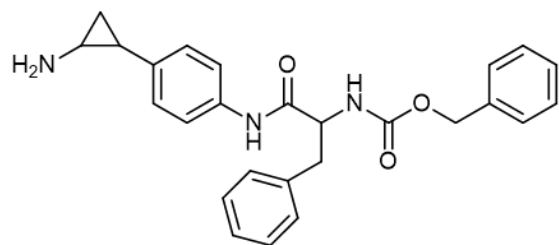
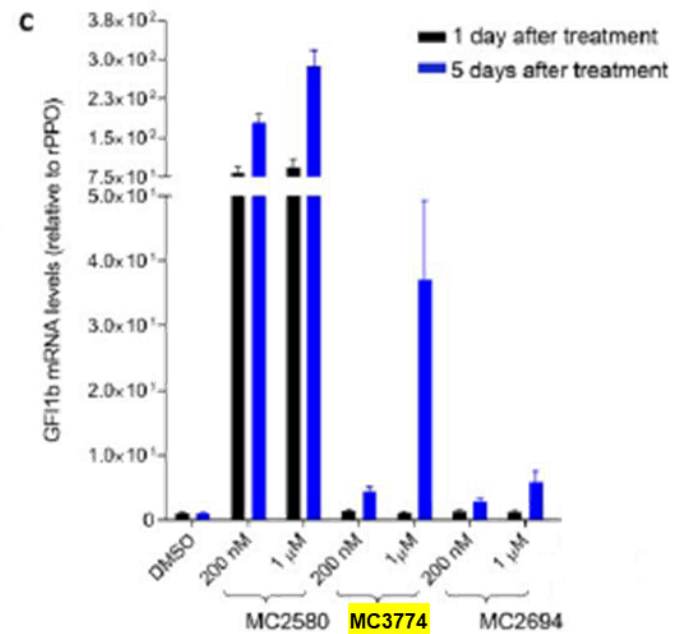
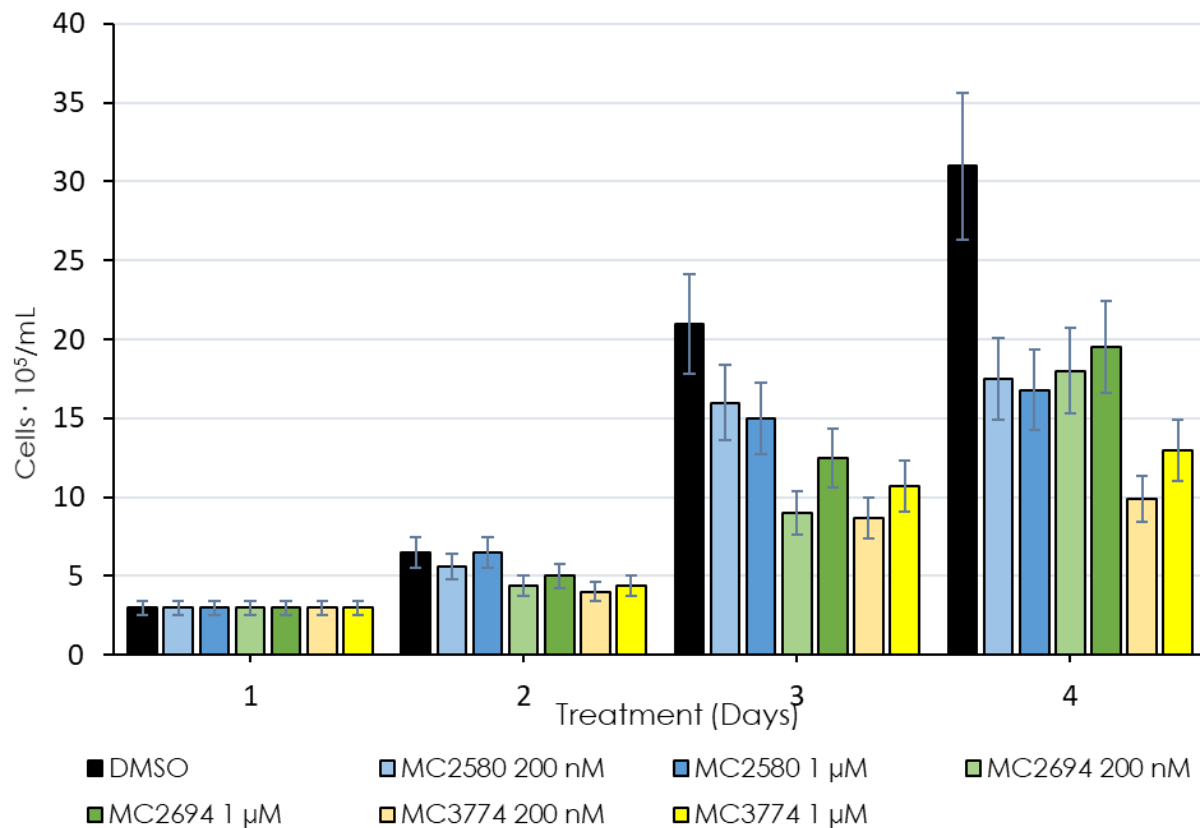




MC3767: Binding Mode

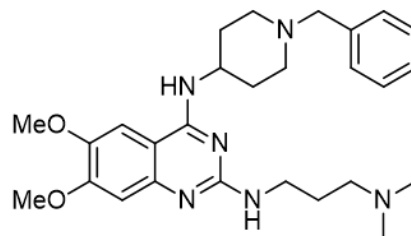


Biological data of MC3774 in MV4-11 cell line



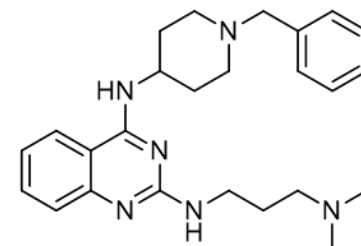
MC2580

irreversible
IC₅₀ = 0.1 μM



MC3774

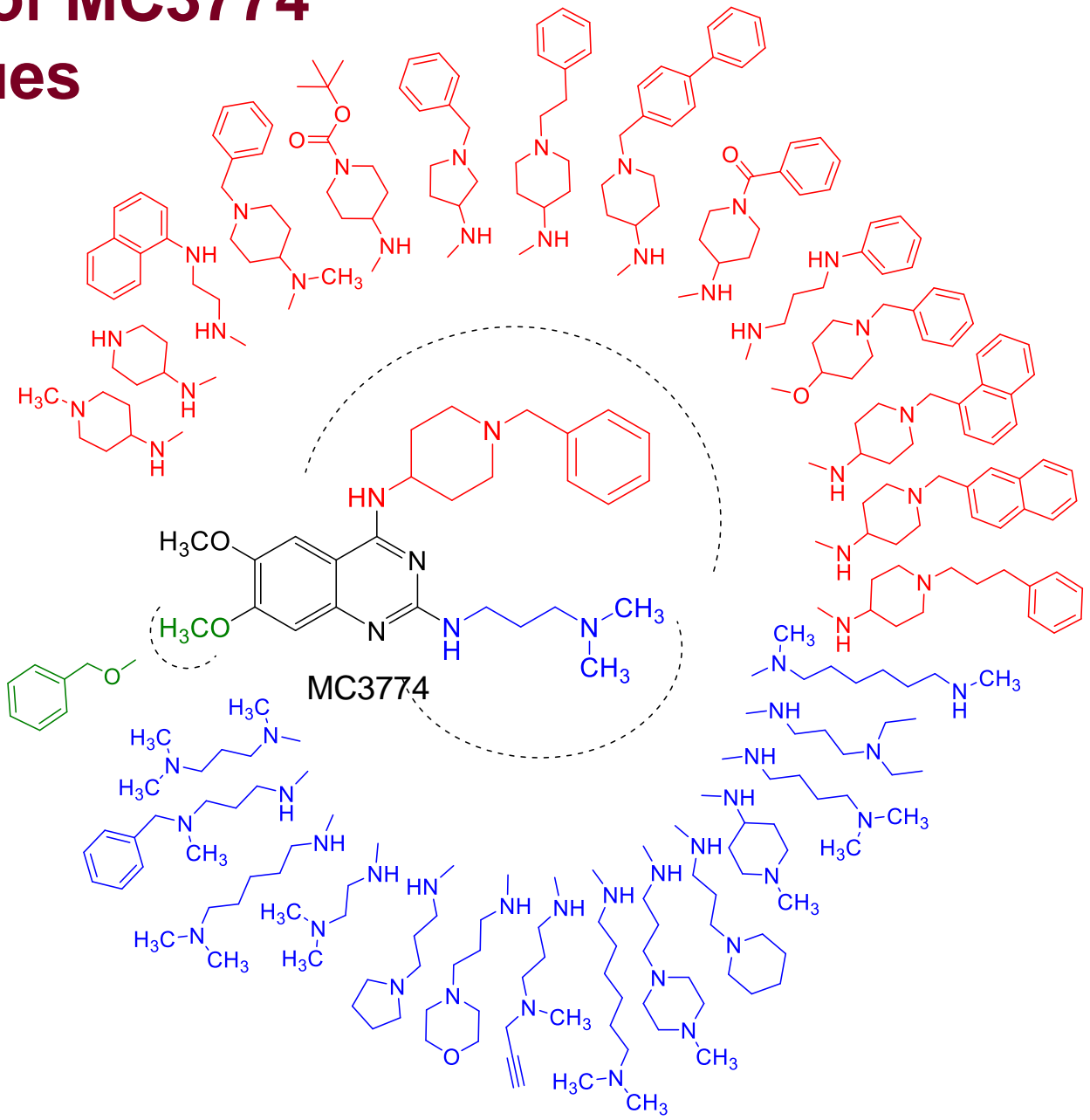
reversible
K_i = 0.44 μM



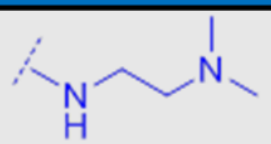
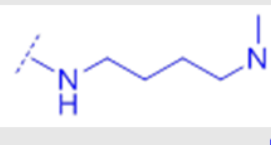
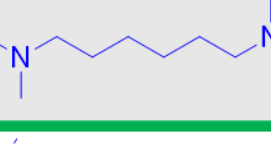
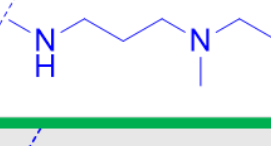
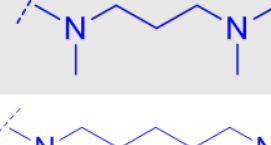
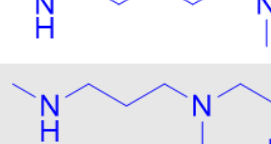
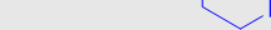
MC2694

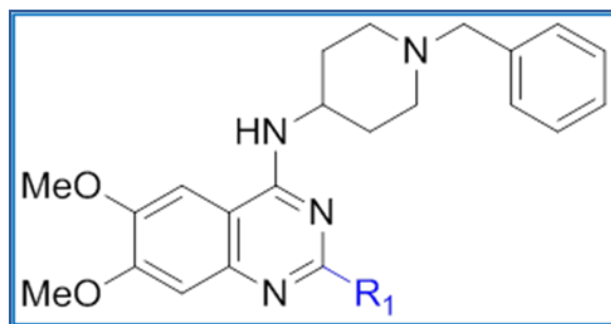
reversible
K_i = 1.06 μM

Design of MC3774 Analogues



LSD1 and G9a Enzymatic Assays: Effect of C2-substitution (1)

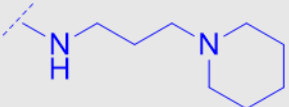
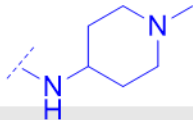
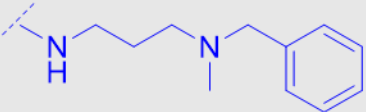
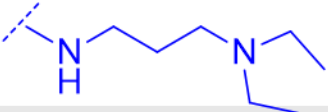
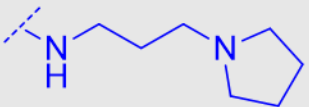
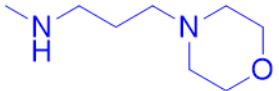
Cpd	R ₁	K _d LSD1 (μM)	IC ₅₀ G9a (μM)
MC3992		0.24 ± 0.03	6.26
MC3999		0.11 ± 0.01	4.72
MC4009		0.69 ± 0.08	3.65
MC4016		0.06 ± 0.006	30.1
MC4017		1.30 ± 0.14	6.42
MC4005		0.13 ± 0.01	3.11
MC4083		0.19 ± 0.03	15.3

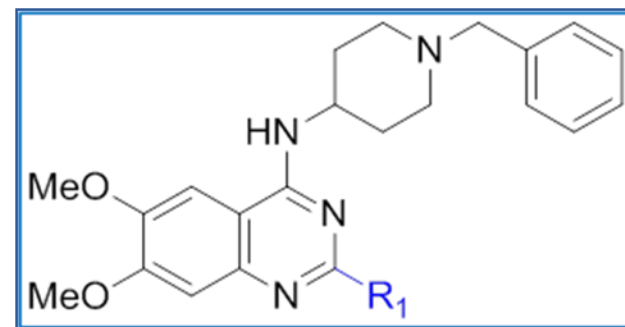


MC3774: K_d vs LSD1 = 0.24 ± 0.05

IC₅₀ vs G9a = 0.78 μM

LSD1 and G9a Enzymatic Assays: Effect of C2-substitution (2)

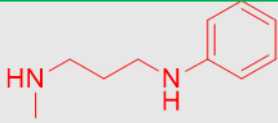
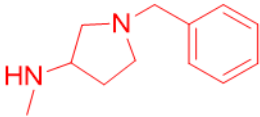
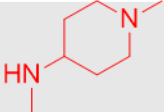
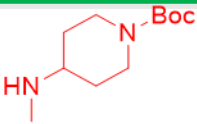
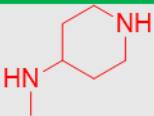
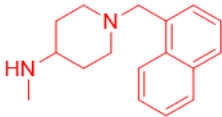
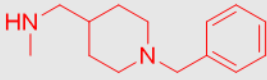
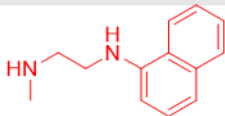
Cpd	R ₁	K _d LSD1 (μM)	IC ₅₀ G9a (μM)
MC4072		0.07 ± 0.01	3.66
MC4038		0.08 ± 0.01	7.03
MC4011		0.16 ± 0.02	10.7
MC4026		0.09 ± 0.01	4.38
MC4035		0.08 ± 0.01	4.51
MC4081		0.37 ± 0.07	26.6

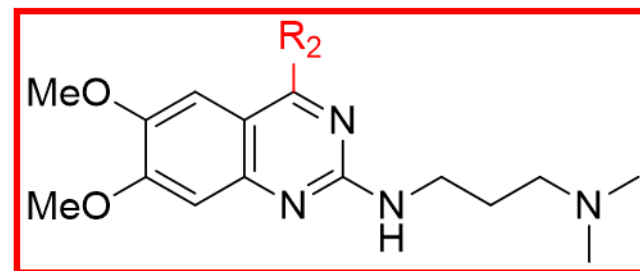


MC3774: K_d vs LSD1 = 0.24 ± 0.05

IC₅₀ vs G9a = 0.78 μM

LSD1 and G9a Enzymatic Assays: C4-substitution (1)

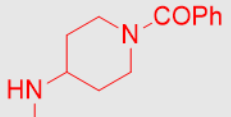
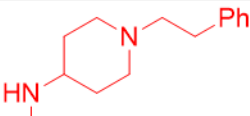
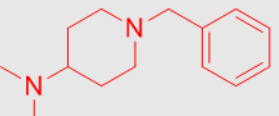
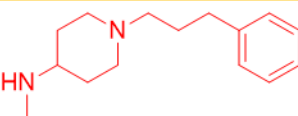
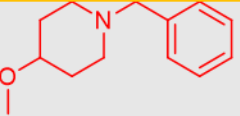
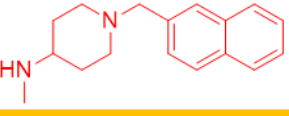
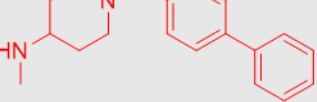
Cpd	R ₂	K _d LSD1 (μM)	IC ₅₀ G9a (μM)
MC4059		0.07 ± 0.006	56.7
MC4060		0.25 ± 0.03	9.13
MC4027		1.49 ± 0.16	4.90
MC3997		0.08 ± 0.01	52.9
MC4004		1.56 ± 0.14	5.69
MC4103		0.08 ± 0.01	3.73
MC4118		0.11 ± 0.02	14.1
MC4129		0.23 ± 0.03	ND

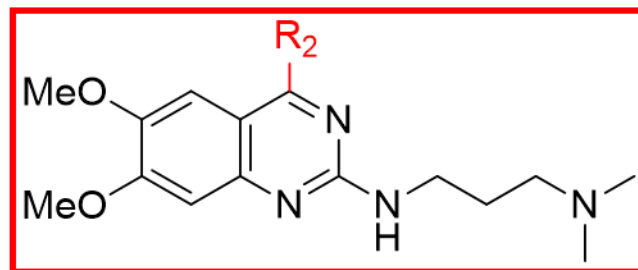


MC3774: K_d vs LSD1 = 0.24 ± 0.05

IC₅₀ vs G9a = 0.78 μM

LSD1 and G9a Enzymatic Assays: C4-substitution (2)

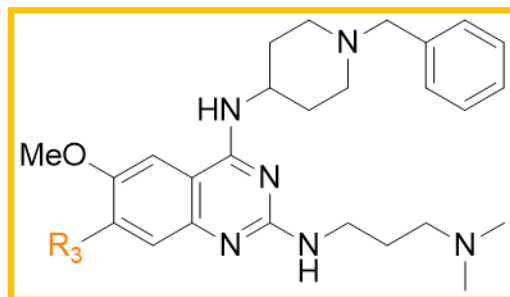
Cpd	R ₂	K _d LSD1 (μM)	IC ₅₀ G9a (μM)
MC4012		0.97 ± 0.09	37.9
MC4007		0.15 ± 0.02	3.63
MC4053		0.36 ± 0.03	>100
MC4013		0.08 ± 0.01	0.73
MC4048		2.85 ± 0.32	No inh.
MC4106		0.07 ± 0.01	1.83
MC4114		0.08 ± 0.01	1.25



MC3774: K_d vs LSD1 = 0.24 ± 0.05

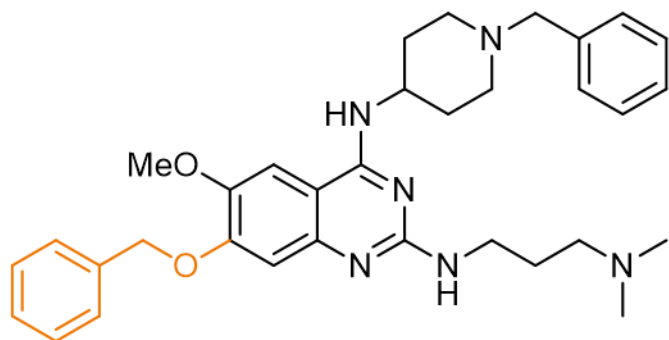
IC₅₀ vs G9a = 0.78 μM

LSD1 and G9a Enzymatic Assays: C7-substitution



MC3774: K_d vs LSD1 = 0.24 ± 0.05

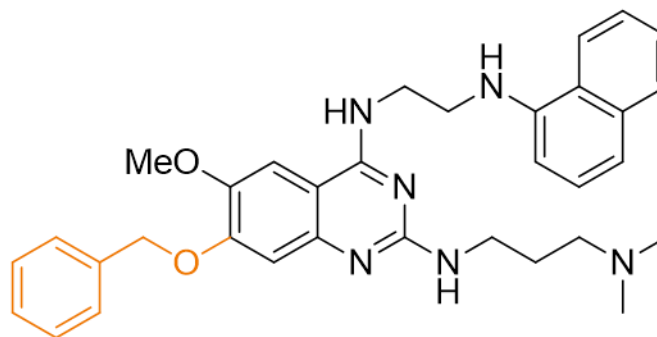
IC_{50} vs G9a = $0.78 \mu\text{M}$



MC4057

K_d vs LSD1 = 0.07 ± 0.006

IC_{50} vs G9a = $43.4 \mu\text{M}$



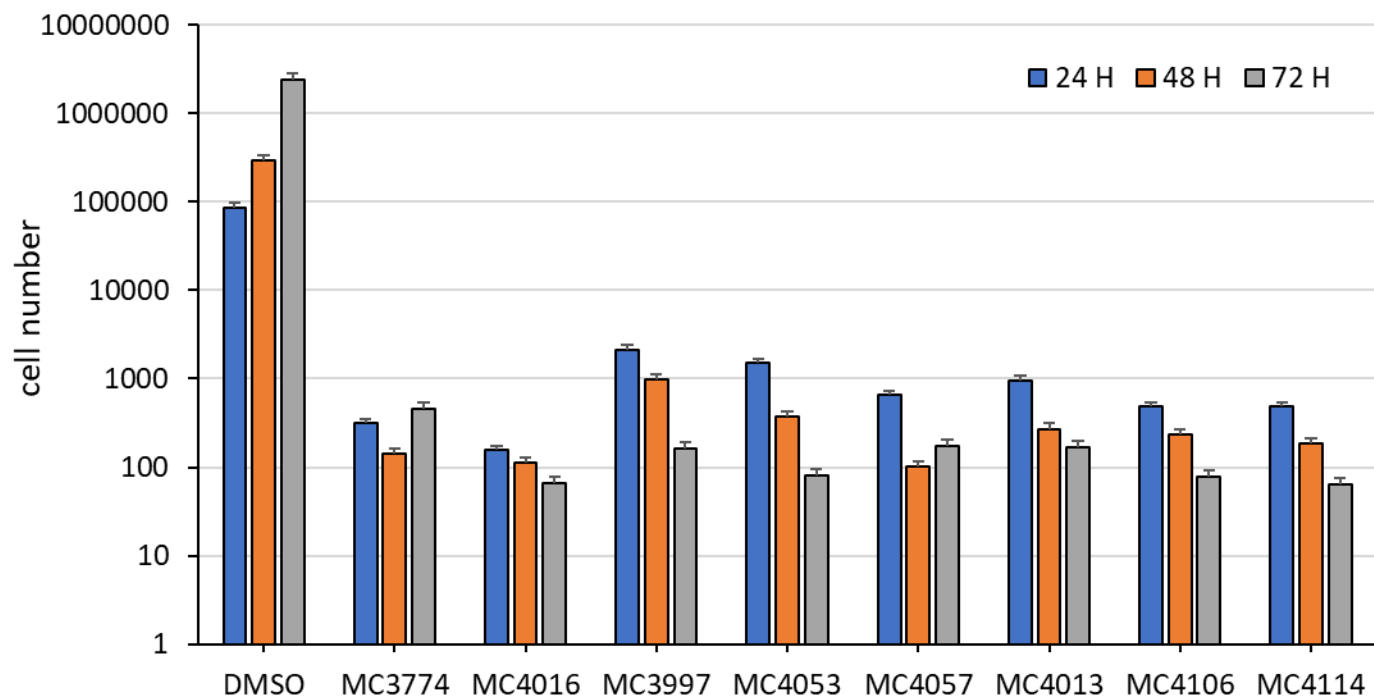
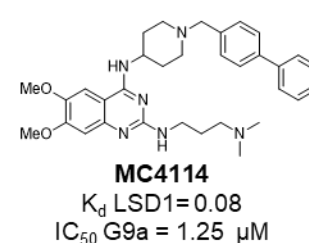
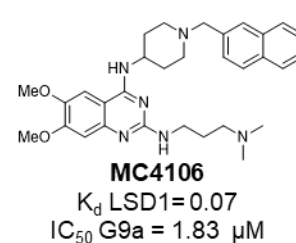
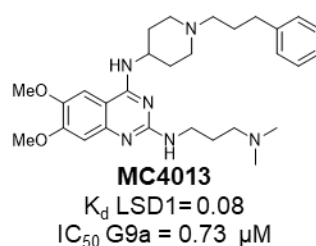
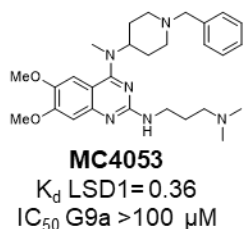
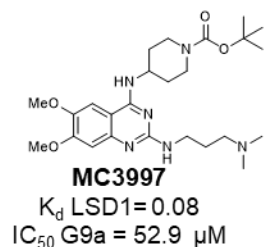
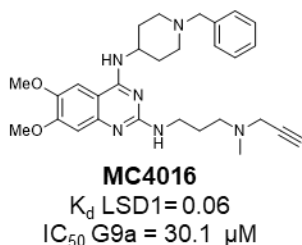
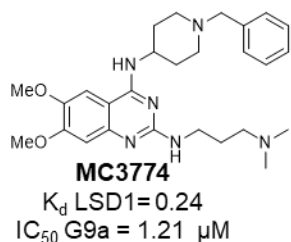
MC4125

K_d vs LSD1 = 0.13 ± 0.02

IC_{50} vs G9a = $23.6 \mu\text{M}$

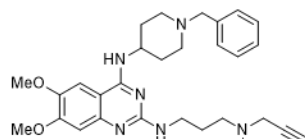
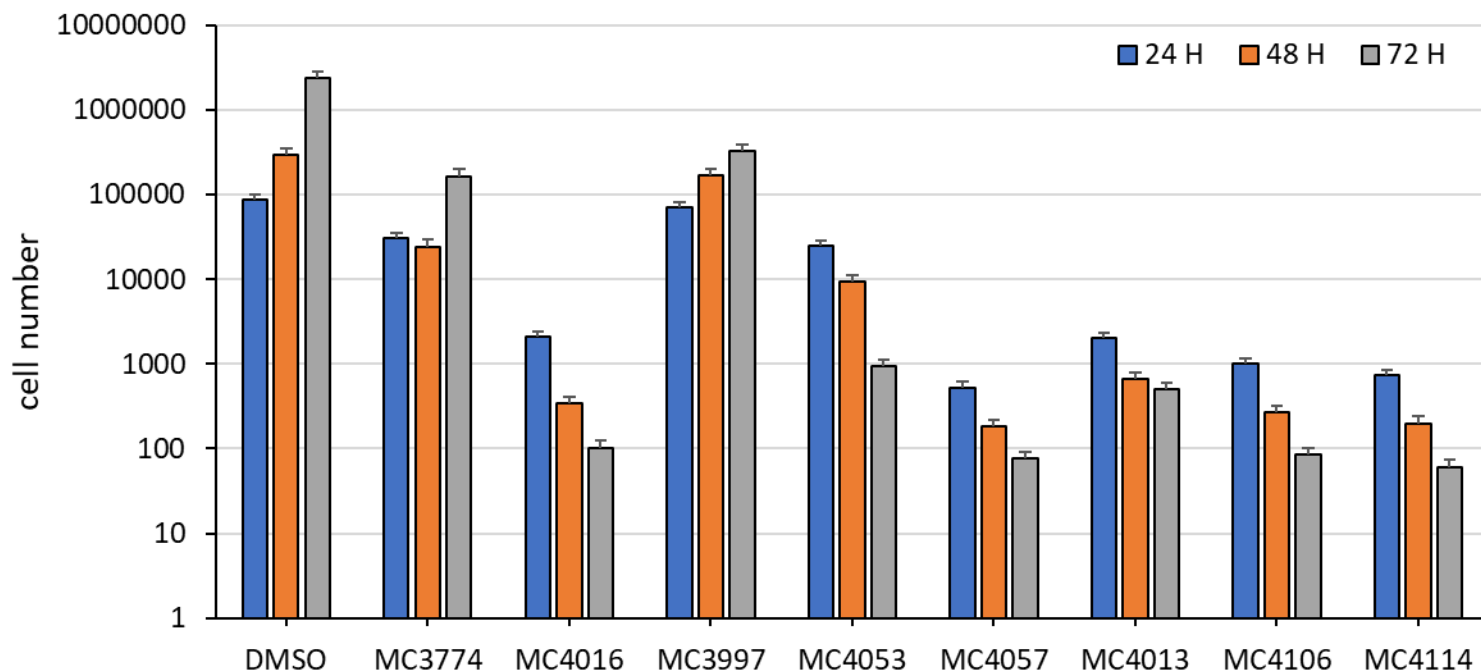
Antiproliferative Effect in Leukemia Cells (1)

THP-1 cells, 5 μM

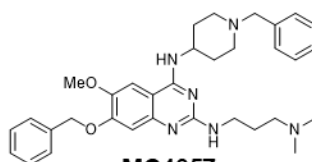


Antiproliferative Effect in Leukemia Cells (2)

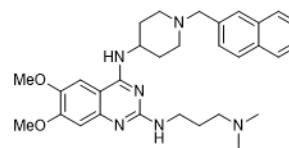
THP-1 cells, 1 μM



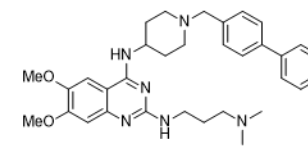
MC4016
 K_d LSD1=0.06
 IC_{50} G9a = 30.1 μM



MC4057
 K_d LSD1=0.07
 IC_{50} G9a = 43.4 μM



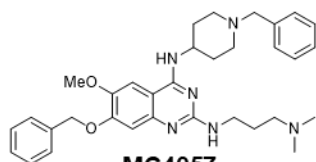
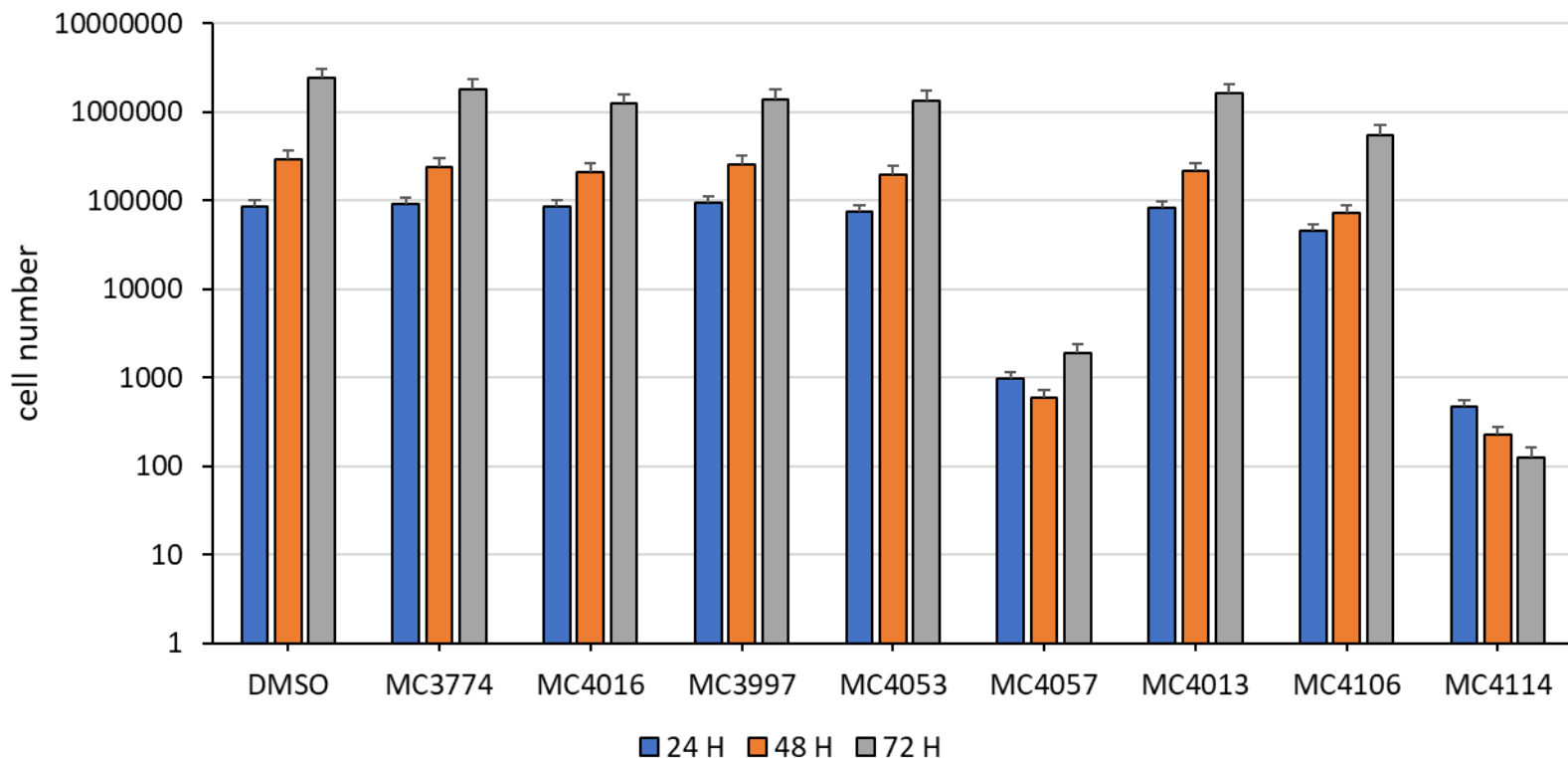
MC4106
 K_d LSD1=0.07
 IC_{50} G9a = 1.83 μM



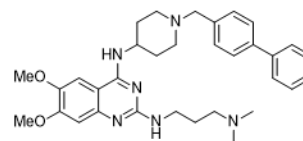
MC4114
 K_d LSD1=0.08
 IC_{50} G9a = 1.25 μM

Antiproliferative Effect in Leukemia Cells (3)

THP-1 cells, 0.2 μM



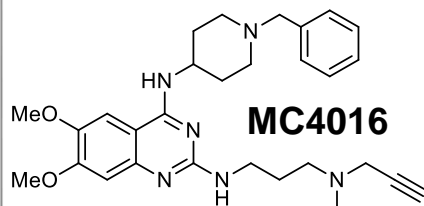
K_d LSD1 = 0.07
 IC_{50} G9a = 43.4 μM



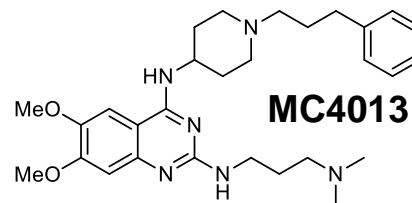
K_d LSD1 = 0.08
 IC_{50} G9a = 1.25 μM

Conclusions

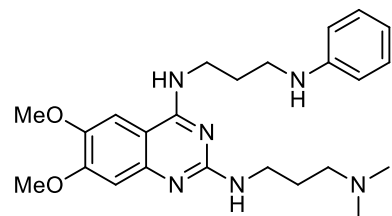
Compounds that Showed **LSD1 Selective** Inhibitory Activity



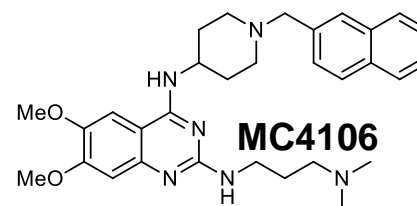
K_d LSD1= 0.06
 IC_{50} G9a= 30.1 μ M



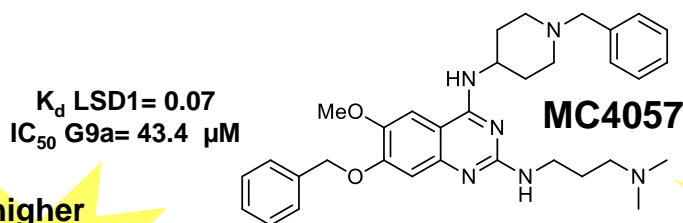
K_d LSD1= 0.08
 IC_{50} G9a= 0.73 μ M



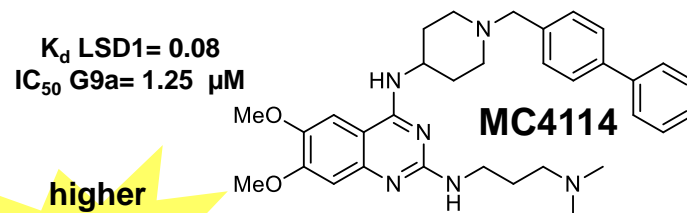
K_d LSD1= 0.07
 IC_{50} G9a= 56.7 μ M



K_d LSD1= 0.07
 IC_{50} G9a= 1.83 μ M



K_d LSD1= 0.07
 IC_{50} G9a= 43.4 μ M



K_d LSD1= 0.08
 IC_{50} G9a= 1.25 μ M

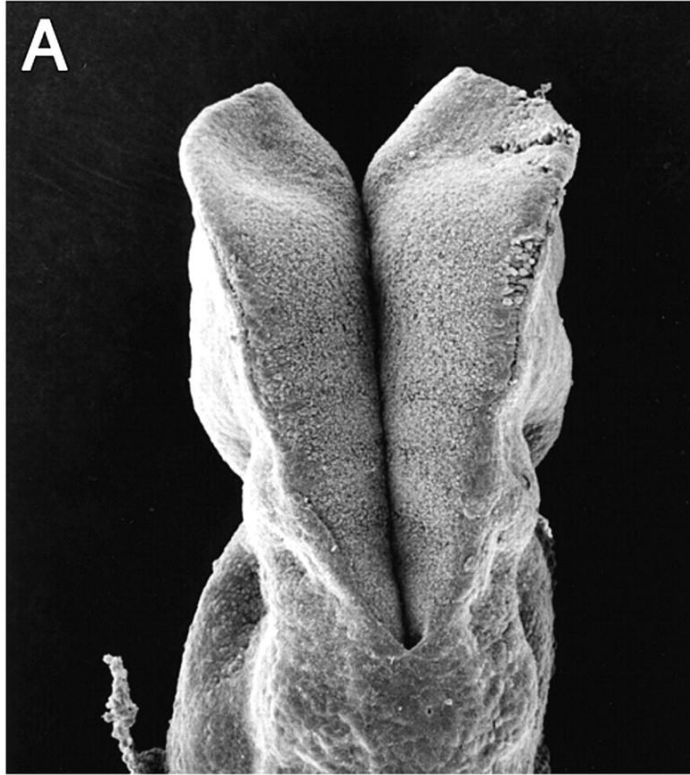
higher
potency in
THP-1
cells

higher
potency in
THP-1
cells

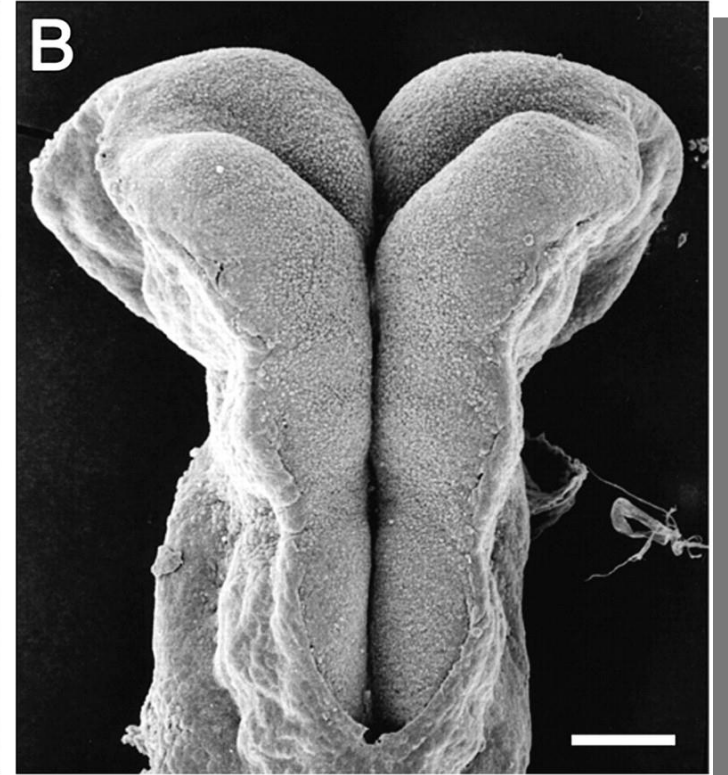
Compounds that Showed **LSD1/G9a Dual** Inhibitory Activity

Jumonji = Cruciforme

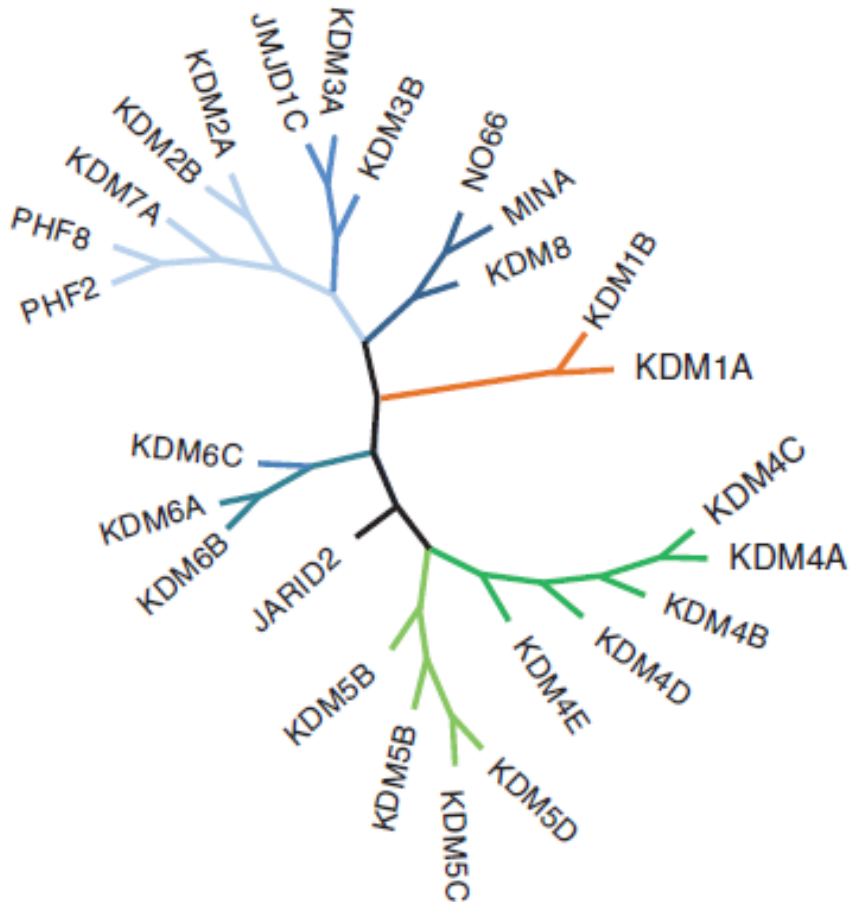
Wild-Type



Jumonji-Mutant



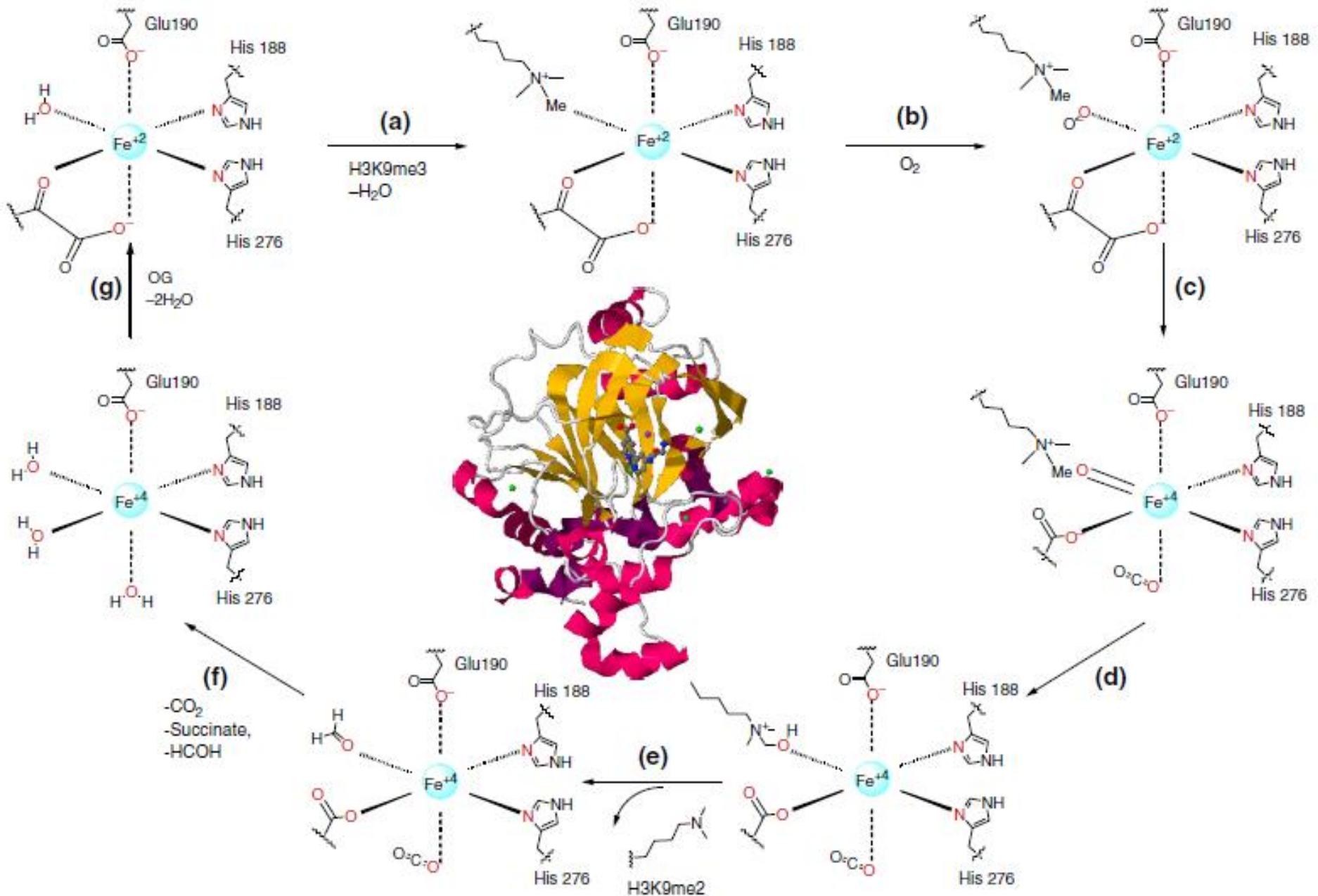
The Jumonji Family



			H3K4			H3K9			H3K27			H3K36			H4K20		
			me1	me2	me3	me1	me2	me3	me1	me2	me3	me1	me2	me3	me1	me2	me3
KDM1A	LSD1	AOF2															
KDM1B	LSD2	AOF1															
KDM2A	FBXL11	JHDM1A															
KDM2B	FBXL10	JHDM1B															
PHF2		JHDM1E															
PHF8		JHDM1F															
KDM3A	JMJD1A	JHDM2A															
KDM3B	JMJD1B	JHDM2B															
	JMJD1C	JHDM2C															
KDM4A	JMJD2A	JHDM3A															
KDM4B	JMJD2B	JHDM3B															
KDM4C	JMJD2C	JHDM3C															
KDM4D	JMJD2D	JHDM3D															
KDM4E	JMJD2E																
KDM5A	JARID1A	RBBP2															
KDM5B	JARID1B	PLU-1															
KDM5C	JARID1C	SMCX															
KDM5D	JARID1D	SMCY															
KDM6A	UTX																
KDM6B	JMJD3																
KDM6C	UTY																
KDM7A		JHDM1D															
KDM8	JMJD5																

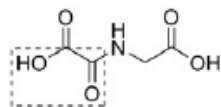
- effective substrate replicated in vitro using histone tail peptide
- not replicated in vitro with histone tail peptide, only detected in cells
- binds this residue, may provoke a switch in substrate specificity
- effective substrate replicated in vitro only when using intact nucleosomes
- weak substrate affinity replicated in vitro using histone tail peptide

Catalytic mechanism of Jumonji enzymes

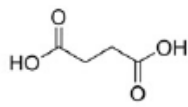


JmjC Inhibitors

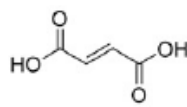
A TCA Cycle Intermediates and 2OG Mimetics



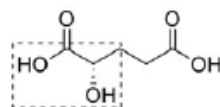
NOG, **32**



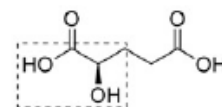
Succinate, **33**



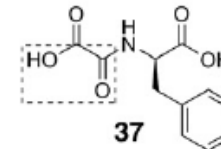
Fumarate, **34**



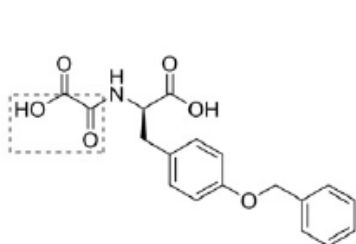
(*S*)-2HG, **35**



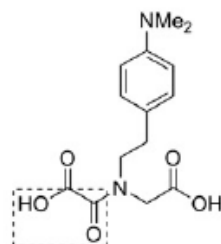
(*R*)-2HG, **36**



37

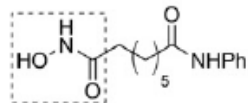


38

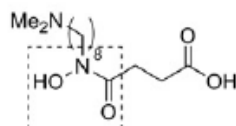


39

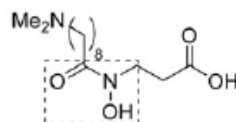
B Hydroxamic Acids and Daminozide



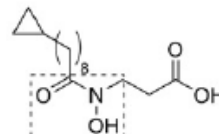
SAHA, **40**



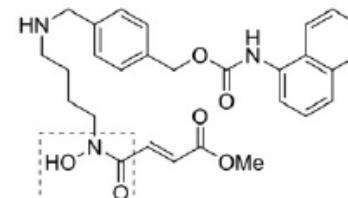
41



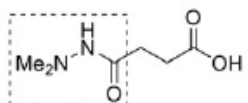
42



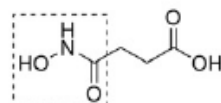
43



Methylstat, **44**

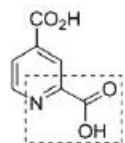


Daminozide, **45**

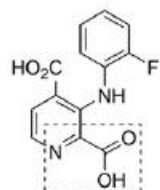


46

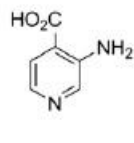
C Pyridine Derivatives



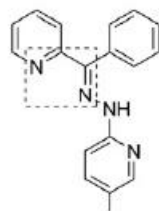
2,4-PDCA, 47



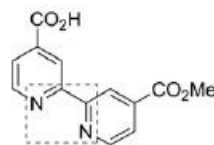
48



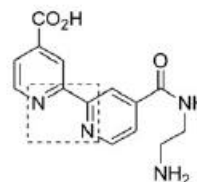
49



50

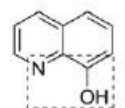


51

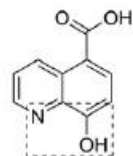


52

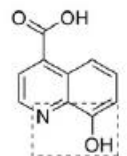
D 8-Hydroxyquinoline Derivatives



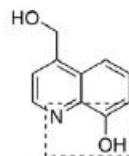
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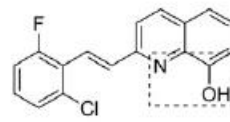
IOX1, 54



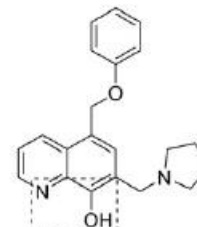
4C8HQ, 55



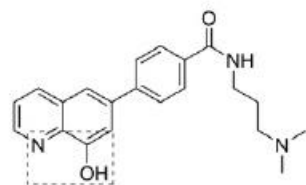
56



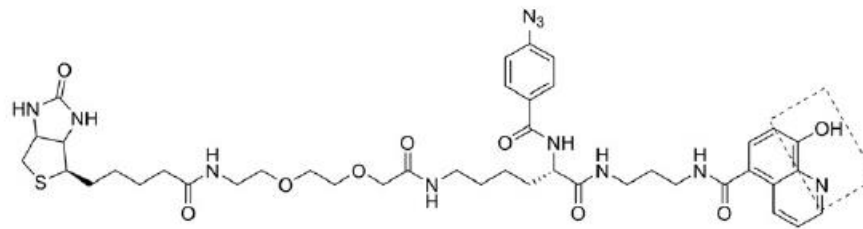
57



58

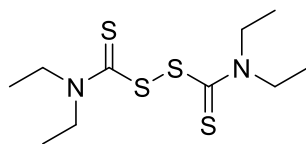


59

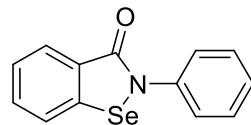


60

E Zinc ion extruders



disulfiram



ebselen

Pan-KDM inhibitors

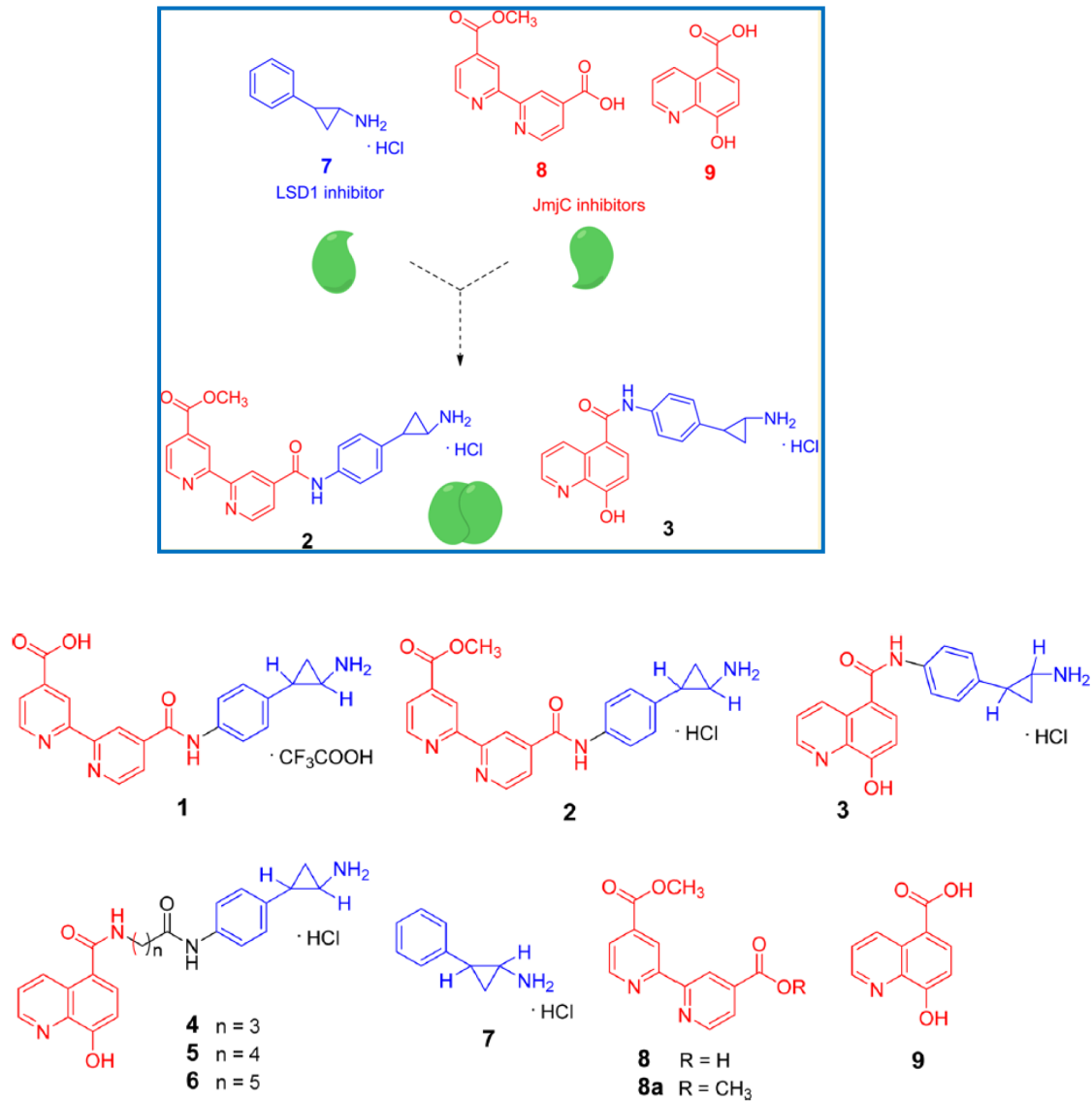
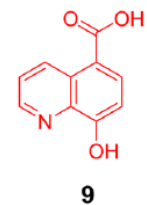
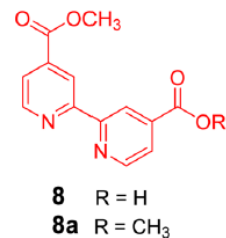
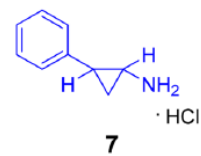
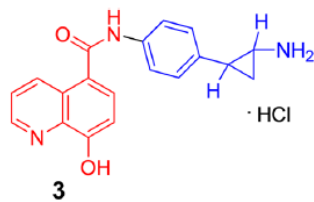
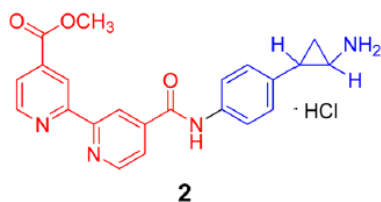


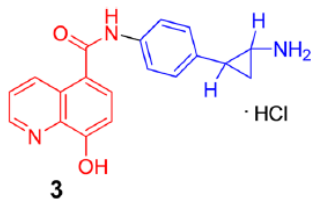
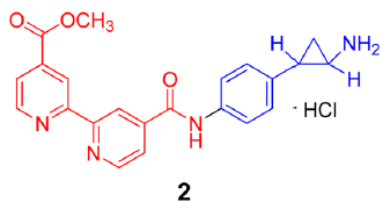
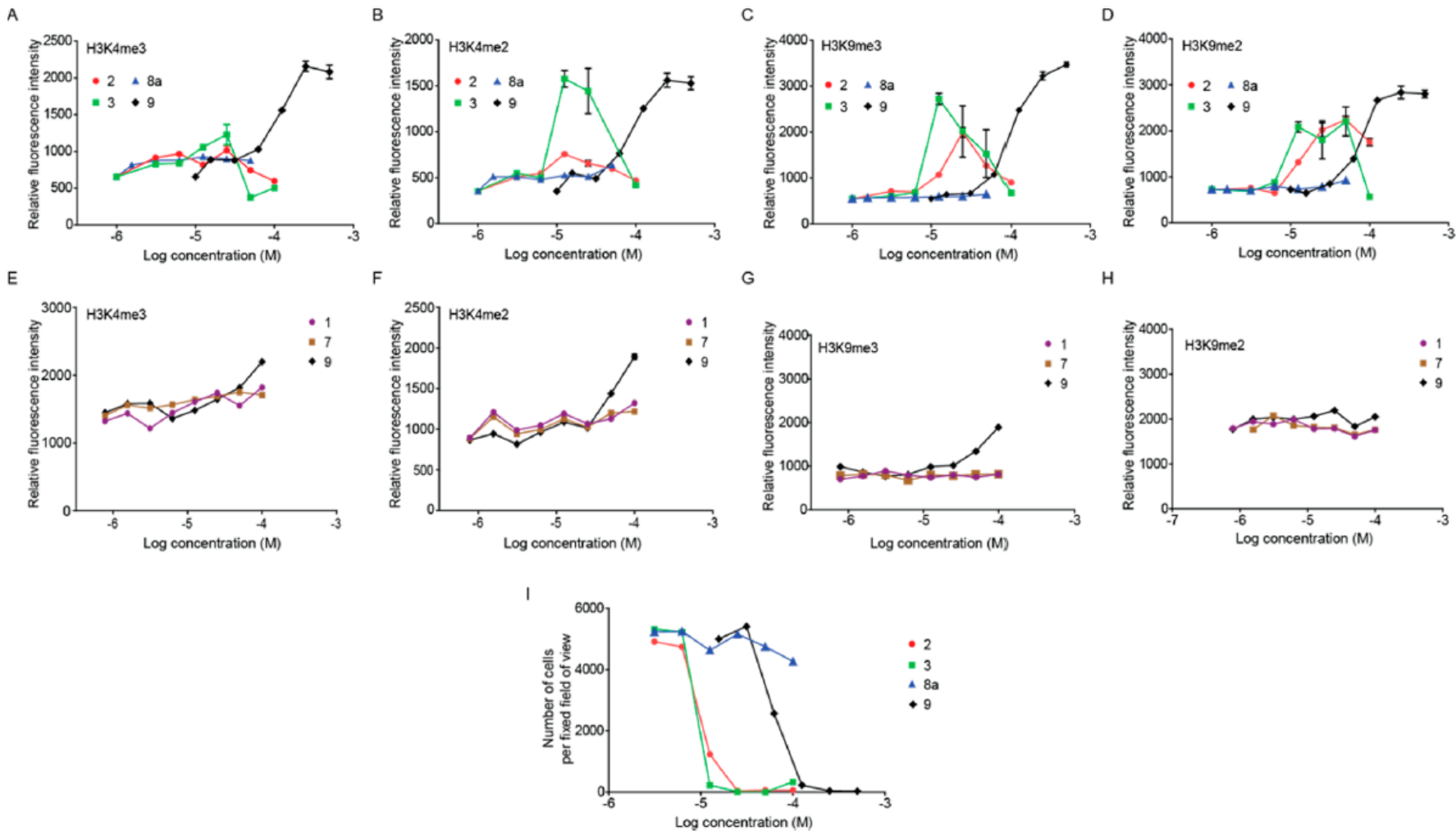
Figure 1. Structures of pan-demethylase inhibitors 1–6 described here and single-family target inhibitors 7–9.

Table 1. Inhibition of Lysine-Specific Demethylase 1, Monoamine Oxidases, Jumonji C, and Other 2-Oxoglutarate-Dependent Enzymes by Pan-Demethylase Inhibitors 1–6^a

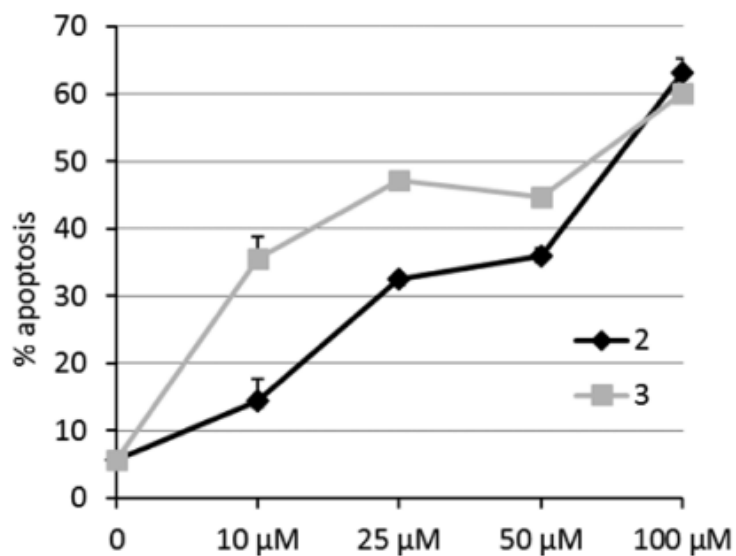
compd	IC ₅₀ , μM										
	LSD1 (KDM1)	MAO-A	MAO-B	FBXL11 (KDM 2/7)	JMJD1A (KDM3)	JMJD2C (KDM4)	JMJD2E (KDM4)	JARID1C (KDM5)	JMJD3 (KDM6)	FIH	PHD2
1	2.2	35.4	47.0	0.22	0.14	0.07	0.42	0.19	2.7	>100	278
2	<1	<1	43.3	12.2	37	2.7	16	8.5	76		
3	<1	8.9	81.0	7.8	31	1.2	3.9	26	27	25	8.5
4	<1			12	12	4.5	5.5	35	18		
5	1.6			8.2	9.7	3.1	3.5	21	14		
6	1.0			12	9.1	2.5	5.1	37	16		
7	2.1	4.5	2.5	>100	>100	>100	>100	>100	>100		
8	>100			4.8	1.1	3.5	5.0	0.03	11.2		
9	ND ^b			15	0.17	0.6	0.3	25	0.14		

^aThe KDM subfamily of each demethylase enzyme is shown in parentheses. Family-specific target inhibitors 7–9 were used as reference compounds. Inhibition assays were performed in duplicate. The errors in determinations of IC₅₀ are within ±10% of their values. ^bND, not detectable. Compound 9 interferes with the peroxidase used in the coupled enzymatic assay and the inhibition could not be reliably measured.

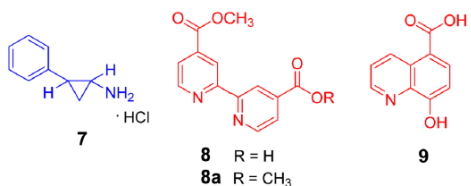
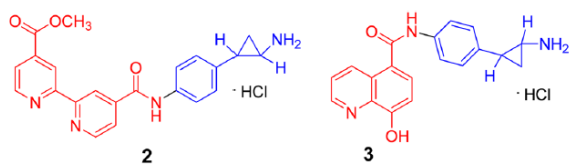
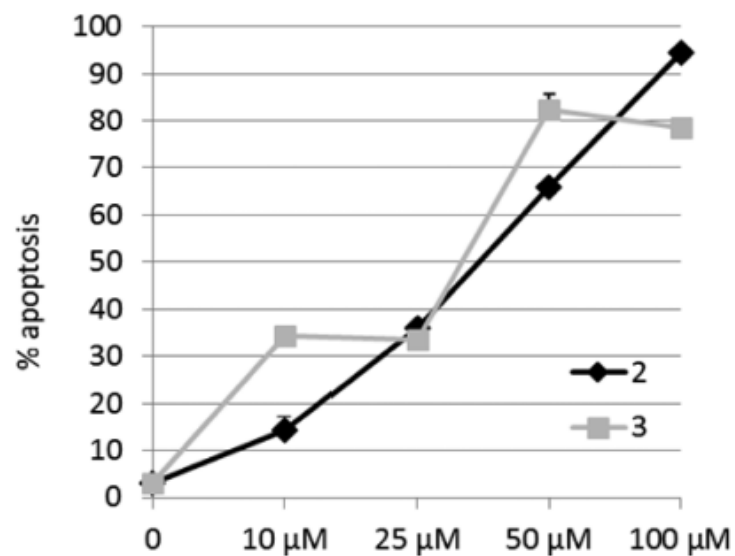




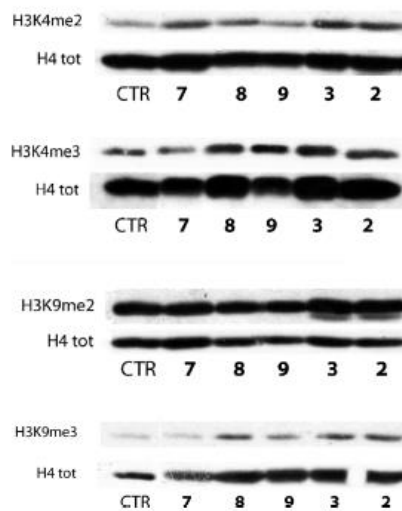
LNCaP cells, 48h



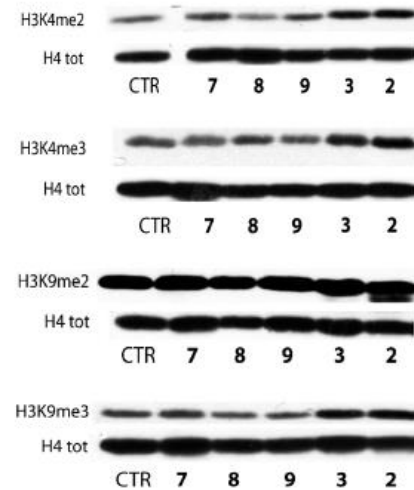
HCT116 cells, 48h

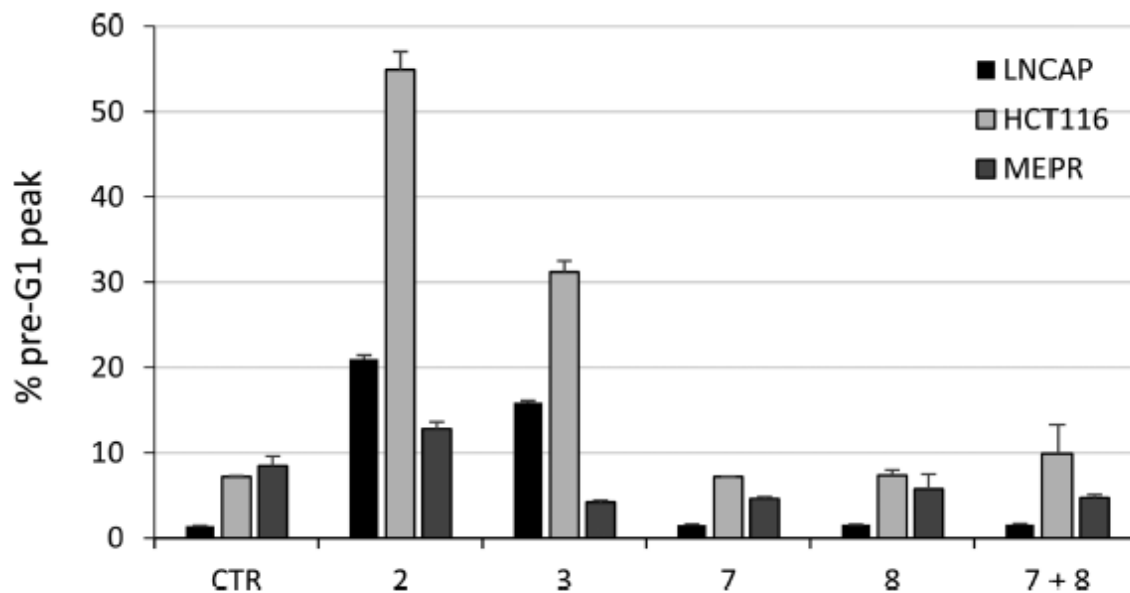
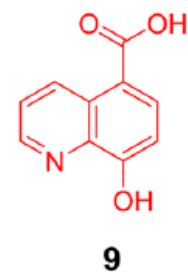
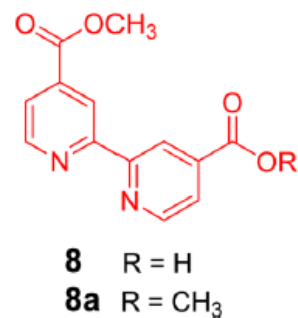
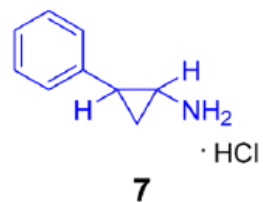
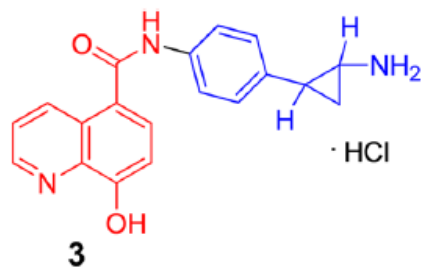
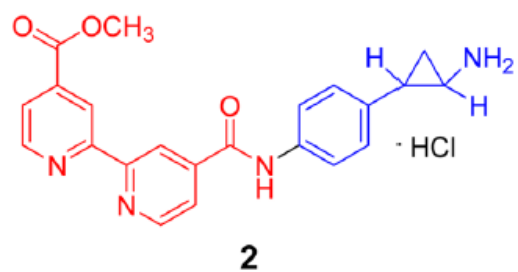


LNCaP cells



HCT116 cells





***Epi*-drugs in non-cancer diseases**

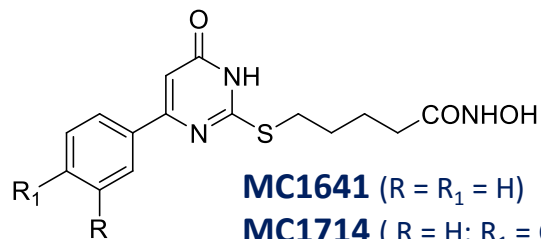
HDACi in *C. albicans* infection

HDACi in HIV-1 infection

HDACi in *P. falciparum* infection

Epi-drugs in neurodegenerative diseases

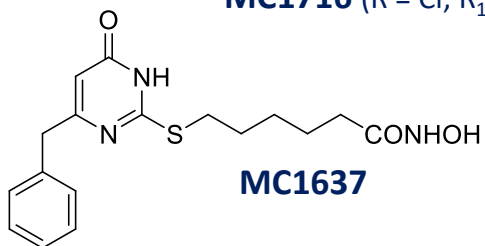
HDACi in *C. albicans* infection



MC1641 (R = R₁ = H)

MC1714 (R = H; R₁ = Cl)

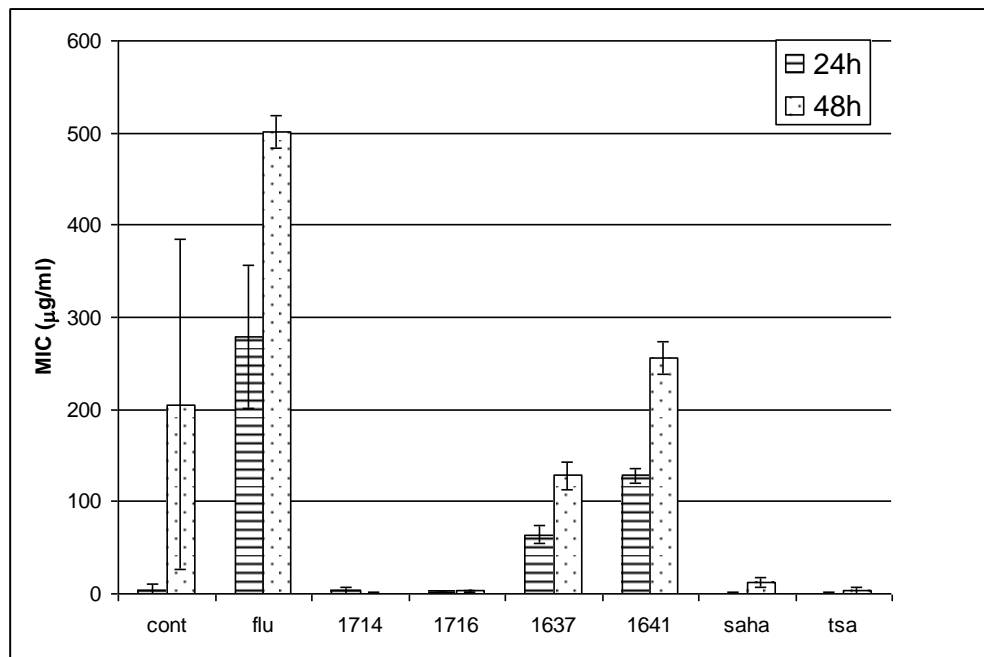
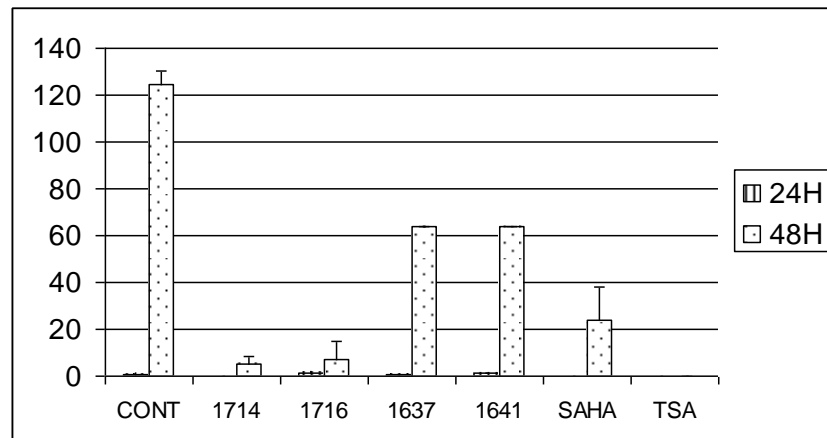
MC1716 (R = Cl; R₁ = H)

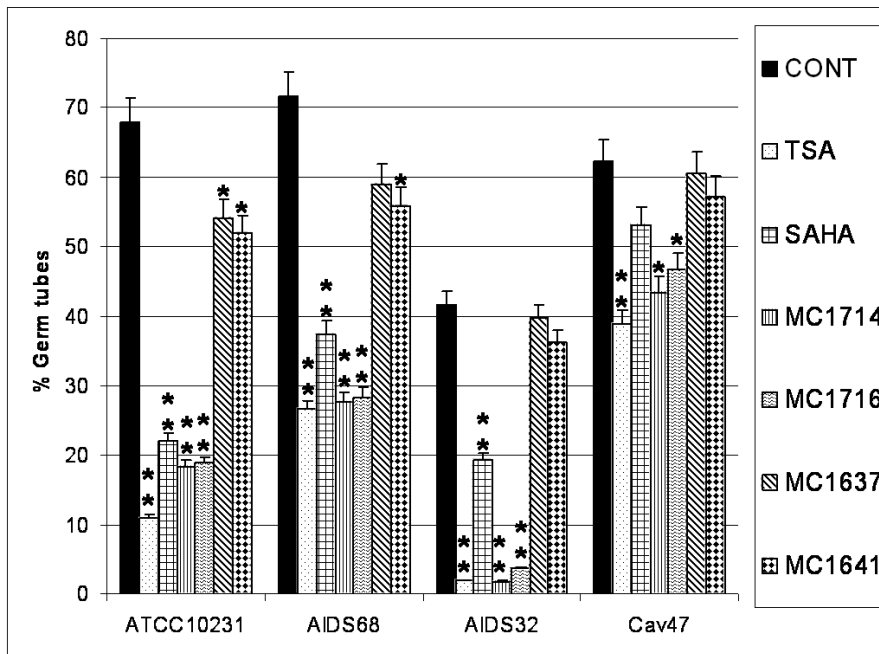


MC1637

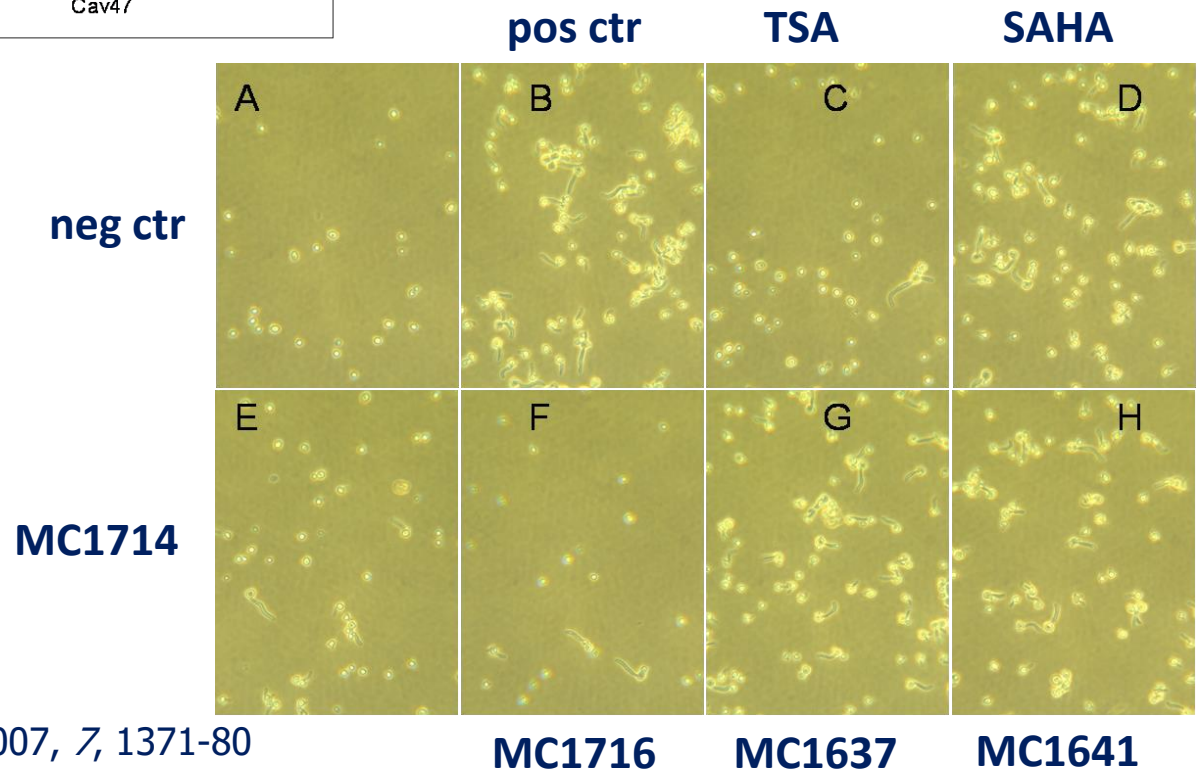
Effect on fluconazole-induced resistance in *C. albicans*

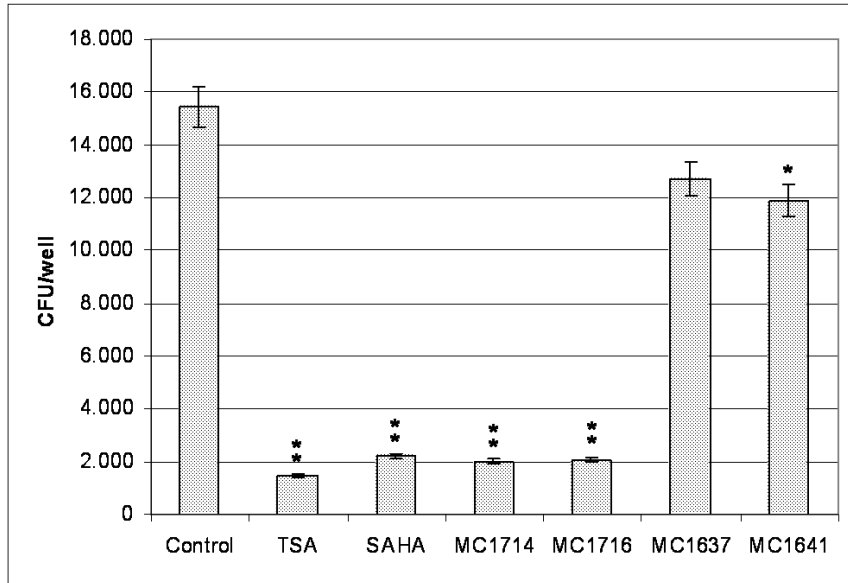
Effect on trailing growth in *C. albicans* strains



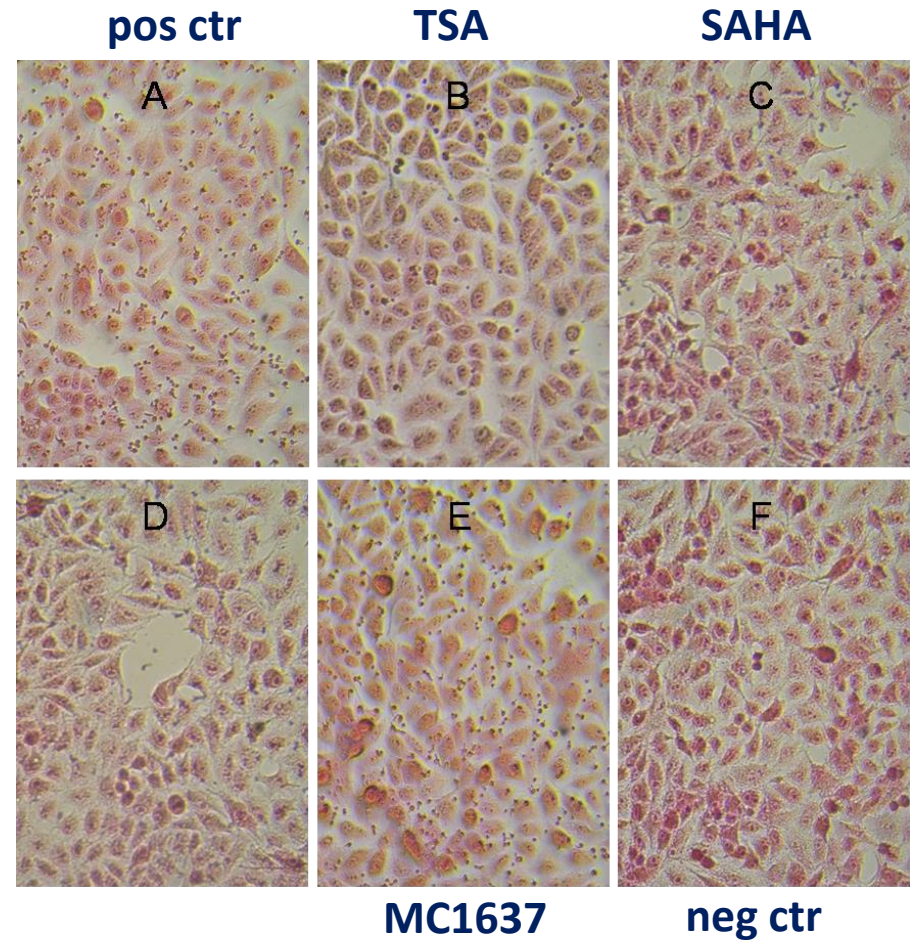


Inhibition of germ tube formation on *C. albicans* strains

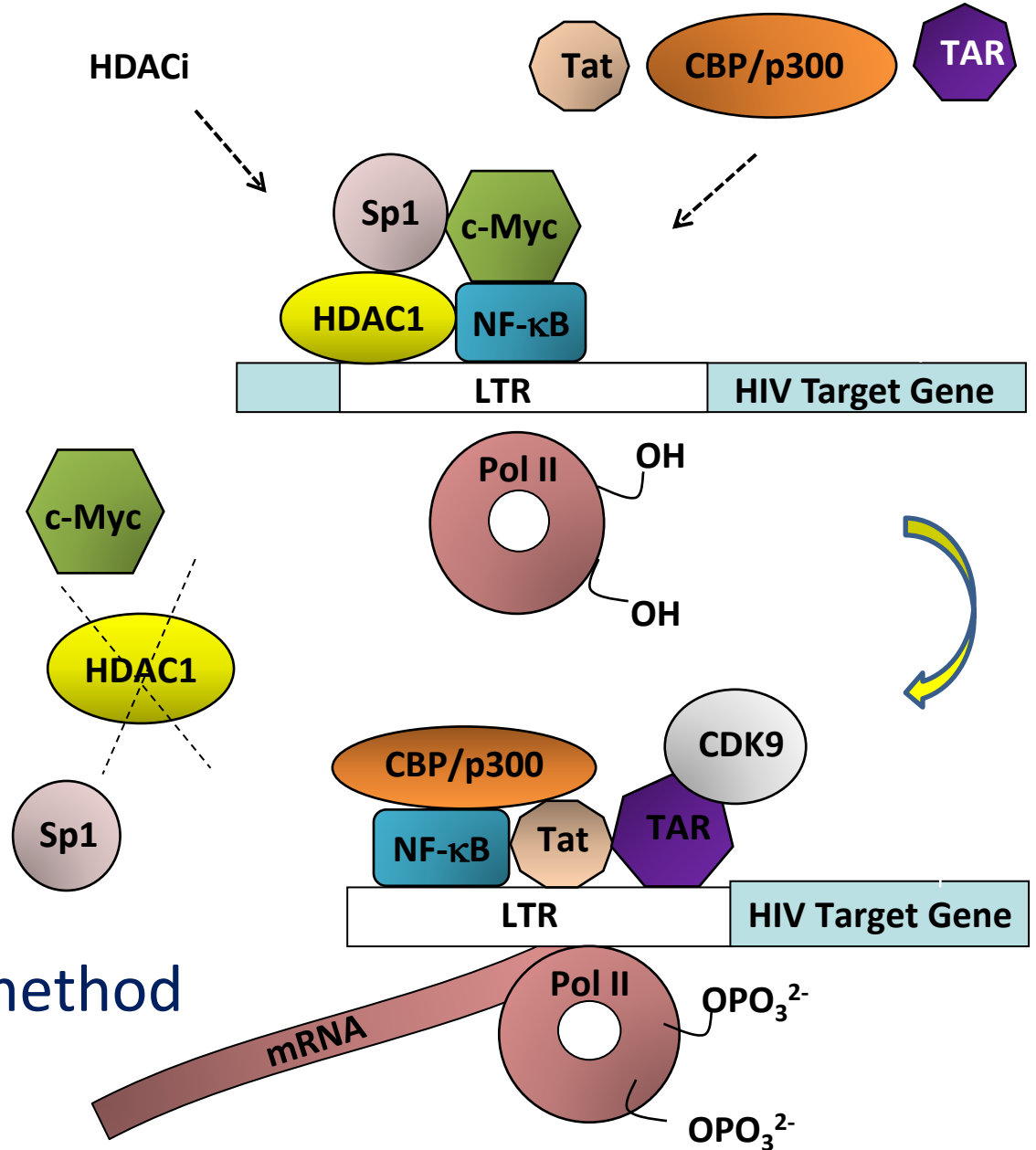
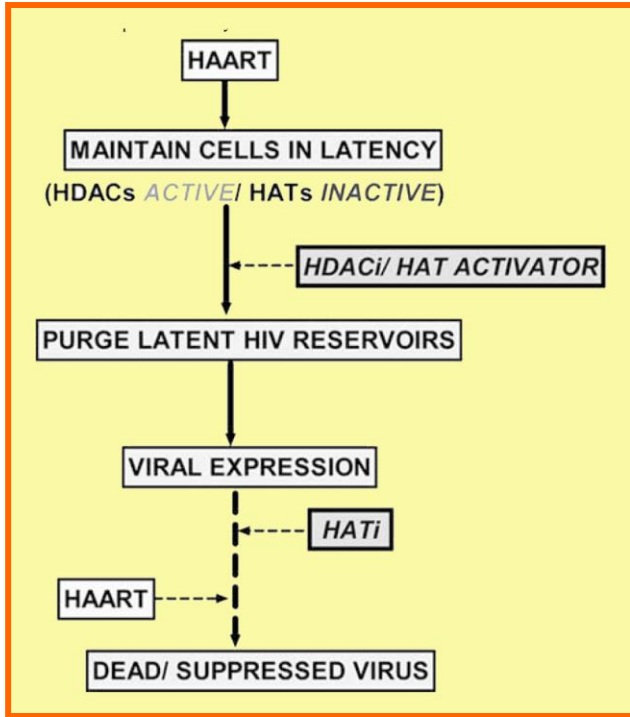




Inhibition of *C. albicans* (ATCC 10231 strain) adhesion to human cultured pneumocytes (A459 cells).

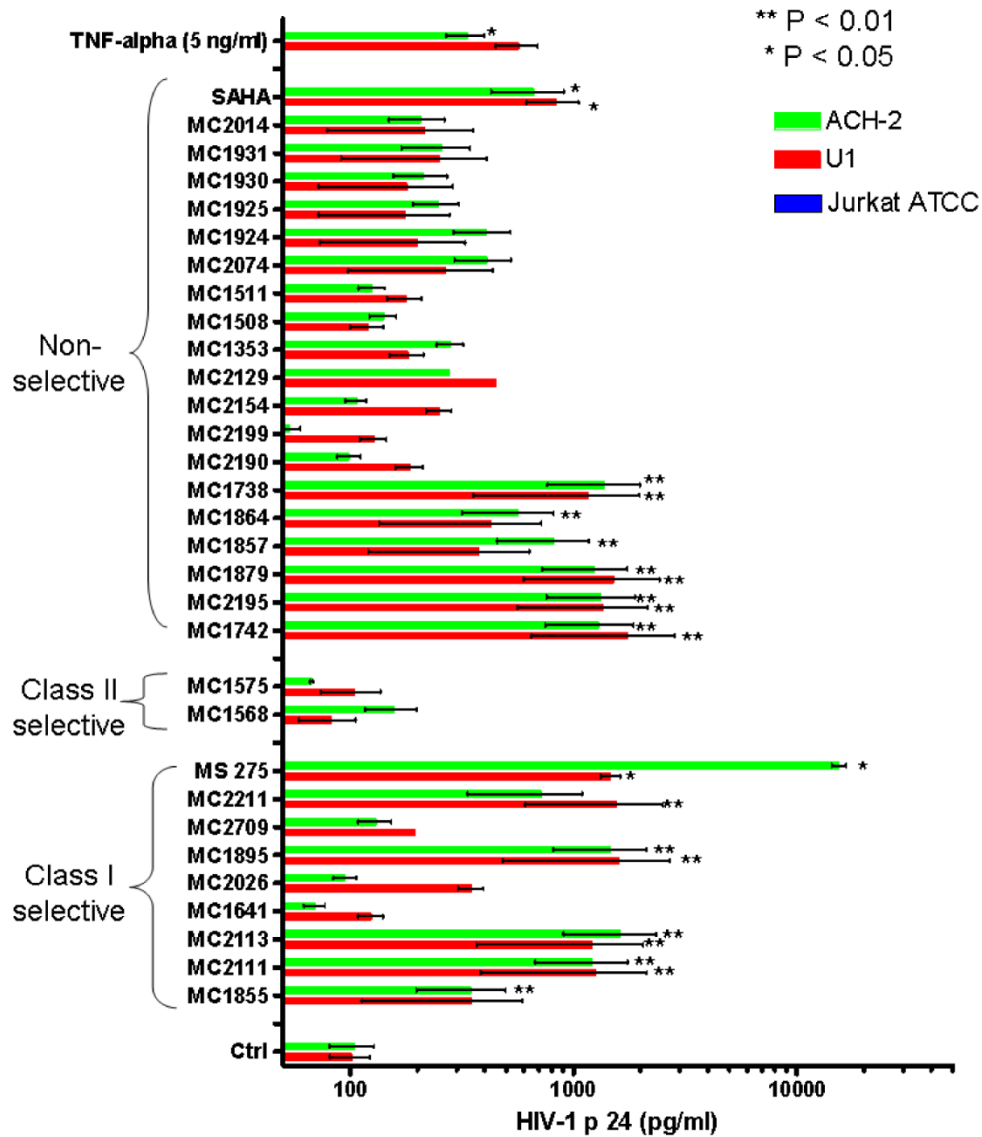


HDACi in HIV-1 infection

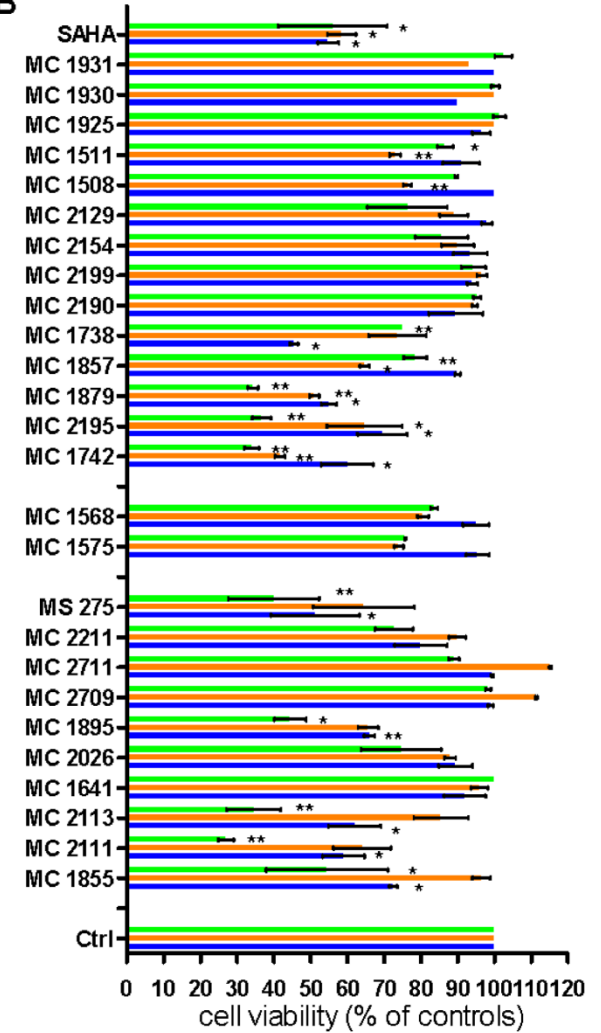


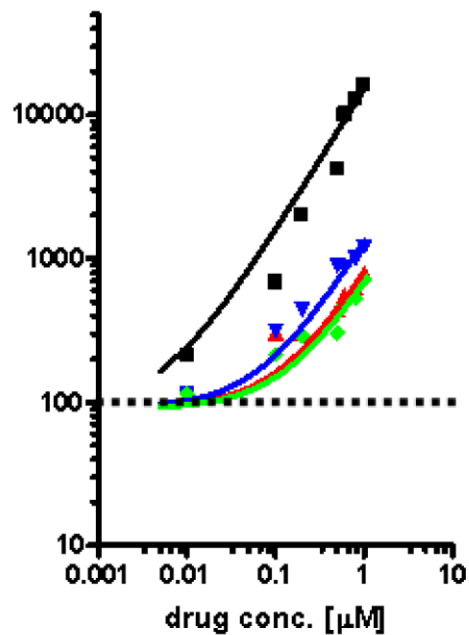
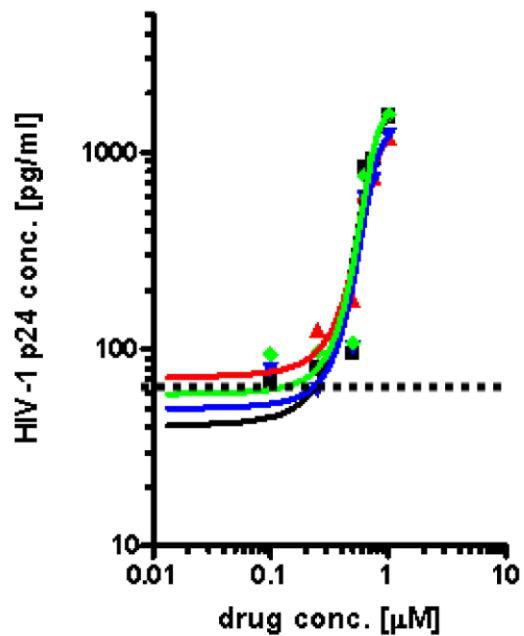
The "shock and kill" method

A

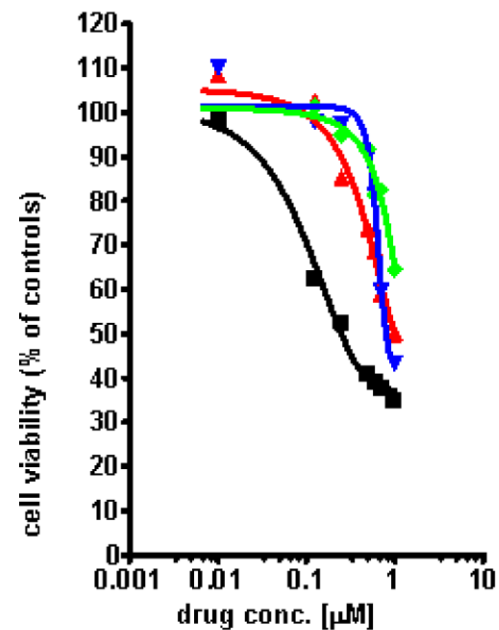


B

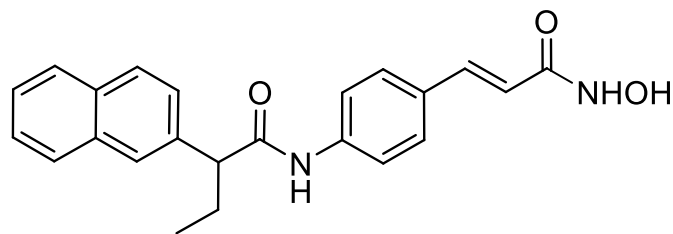




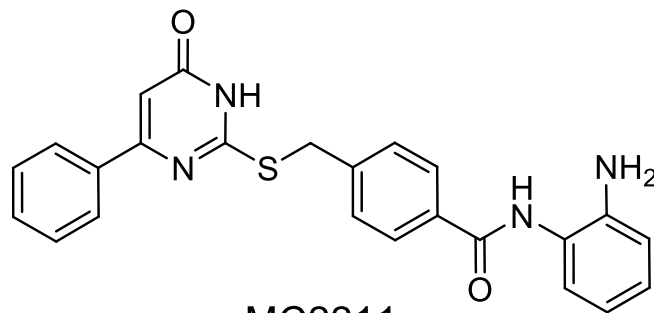
■ MS-275
 ▲ SAHA
 ▼ MC 2113
 ◆ MC2211



compd	EC ₅₀ , μM		CC ₅₀ , μM	
	U1	ACH-2	U1	ACH-2
MS-275	0.53	0.10	>1	0.25
SAHA	0.55	0.65	>1	0.99
MC2113	0.57	0.31	>1	0.80
MC2211	0.54	0.80	>1	1.19

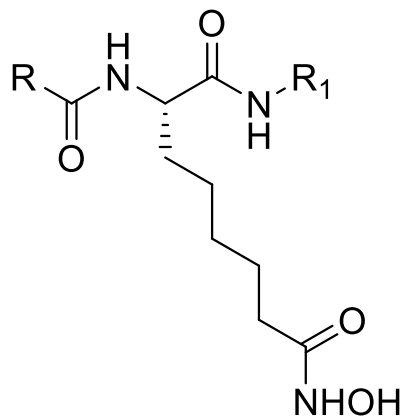


MC2113



MC2211

HDACi in *P. falciparum* infection

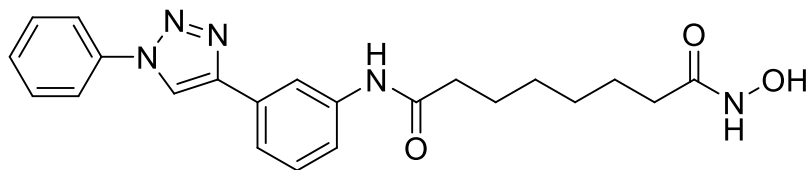


2-aminosuberic acid
(AS)-based compounds

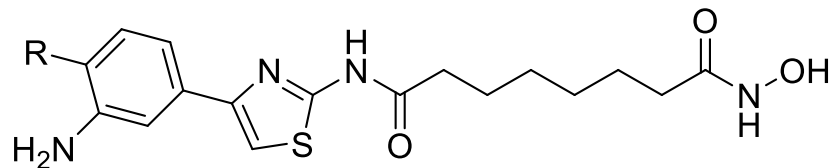
- 7** (R = BzO, R₁ = 4-biPh)
8 (R = 4-Br-Ph, R₁ = 4-biPh)
9 (R = Ph-CH=CH, R₁ = 8-quinolinylyl)
11 (R = PhCH=CH, R₁ = Bz)
12 (R = 2-indolyl, R₁ = Bz)
13 (R = 4-NMe₂-Ph, R₁ = 8-quinolinylyl)
14 (R = 2-indolyl, R₁ = 8-quinolinylyl)

Compound	<i>P. falciparum</i> IC ₅₀ , μM		mammalian cell activity IC ₅₀ , μM	selectivity index ^{a)}	
	CQ- sensitive	CQ- resistant		CQ- sensitive	CQ- resistant
AS-based (7)	0.029^{b)}	0.105^{c)}	2.2^{d)}	76	21
AS-based (8)	0.022^{b)}	0.041^{c)}	0.19^{d)}	9	5
AS-based (9)	0.015^{b)}	0.039^{c)}	1.24^{d)}	83	32
AS-based (11)	0.034^{b)}	0.102^{c)}	4^{d)}	118	39
AS-based (12)	0.034^{b)}	0.063^{c)}	1.26^{d)}	37	20
AS-based (13)	0.019^{b)}	0.071^{c)}	0.57^{d)}	30	8
AS-based (14)	0.013^{b)}	0.033^{c)}	0.337^{d)}	26	10

^{a)}Calculated as IC₅₀ of mammalian cells/IC₅₀ of *P. falciparum* ratio. ^{b)}3D7 strain. ^{c)}Dd2 strain. ^{d)}NFF cell line.



10c



WR compounds

WR301801 (R = H)

WR308298 (R = N(Me)CH₂CH₂OH)

WR308291 (R = OCH₃)

compound	<i>P. falciparum</i> IC ₅₀ , μM		mammalian cell activity IC ₅₀ , μM	selectivity index ^{a)}	
	CQ-sensitive	CQ-resistant		CQ-sensitive	CQ-resistant
10c	0.017^{b)}	0.032^{c)}	0.8^{d)}	47	25
WR301801	0.0008^{b)}	0.0016^{c)}	0.6^{e)}	750	375
WR308298	0.0008^{b)}	0.001^{c)}	0.54^{e)}	675	540
WR308291	0.0009^{b)}	0.0012^{c)}	3.2^{e)}	3556	2667

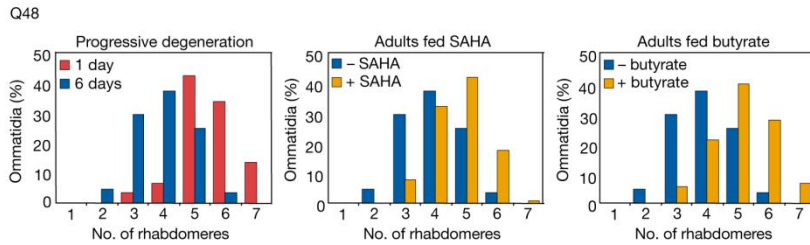
^{a)}Calculated as IC₅₀ of mammalian cells/IC₅₀ of *P. falciparum* ratio. ^{b)}D6 strain. ^{c)}W2 strain. ^{d)}SU 86.86 strain. ^{e)}RAW strain.

Chen Y *et al*, *J Med Chem* 2008, 51, 3437-3448.

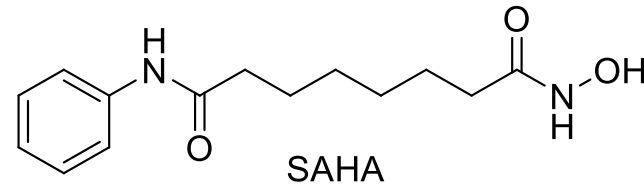
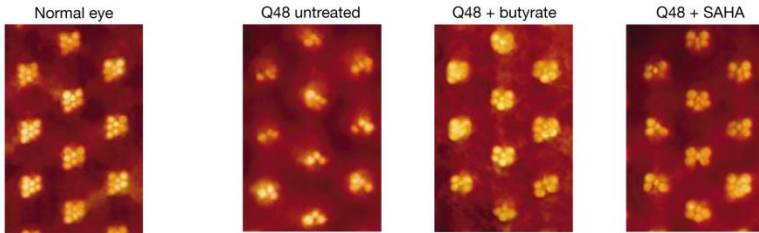
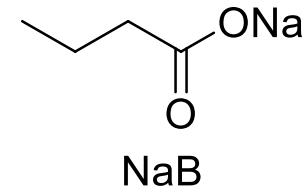
Dow GS *et al*, *Antimicrob Agents Chemother* 2008, 52, 3467-3477.

HDACi in neurodegenerative diseases

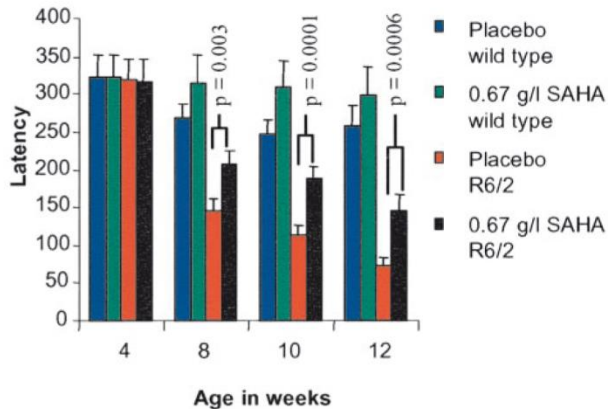
1. Huntington Disease



Q48 transgenic flies
 SAHA = 2 μ M
 NaB = 100 mM

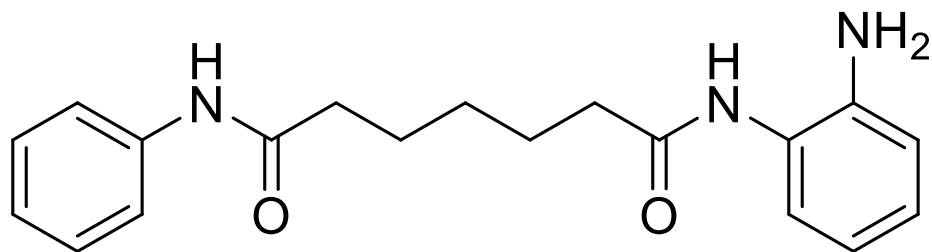
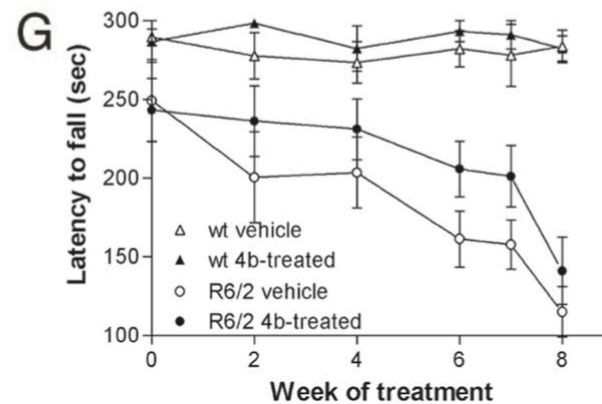
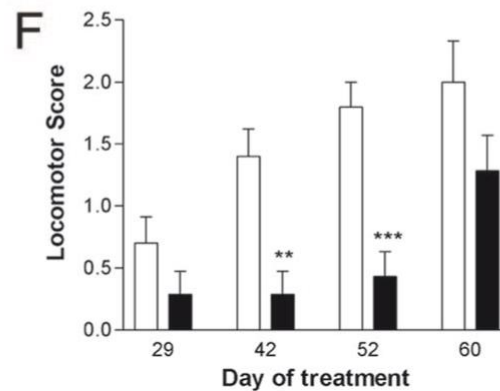
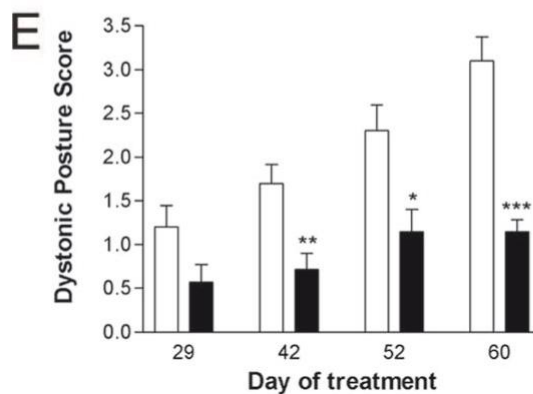
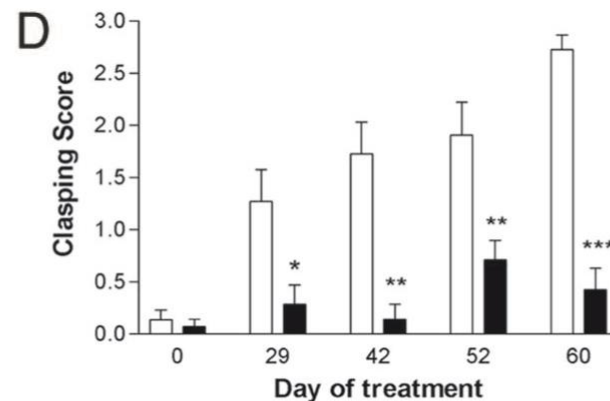
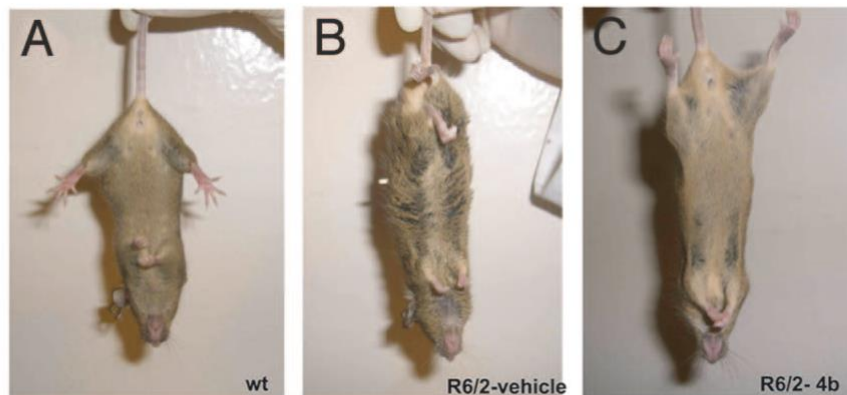


Steffan JS *et al*, *Nature* 2001, 413, 739-743.



Transgenic R6/2 mice
 RotaRod latency assay

Hockly E *et al*, *PNAS USA* 2003, 100, 2041-2046.

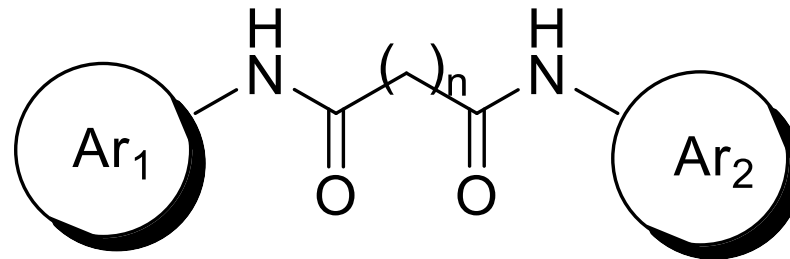


4b

(PAOA)

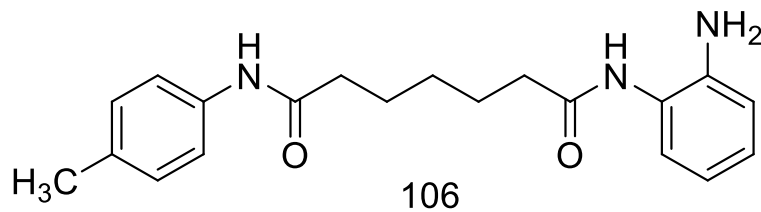
Thomas EA *et al*, *PNAS USA* 2008, *105*, 15564-15569.
 Wong JC *et al*, *JACS* 2003, *125*, 5586-7.
 Mai A *et al*, *Bioorg Med Chem Lett* 2008, *18*, 2530-5.

2. Friedreich's ataxia



HDAC inhibition (IC_{50} , HeLa extract): 14 to $>500 \mu\text{M}$
Fold change in *FXN* mRNA: 1.4 to 3.1

Herman D *et al*, *Nat Chem Biol* 2006, 2, 551-558.



HDAC inhibition

IC_{50} , μM after 3h incubation:

0.15 (HDAC1); 0.76 (HDAC2); 0.37 (HDAC3);

5.0 (HDAC8); >180 (HDAC4,5,7)

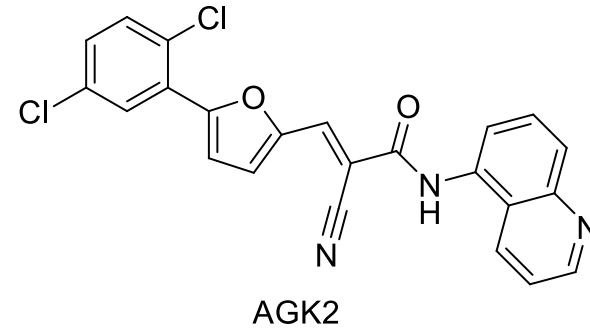
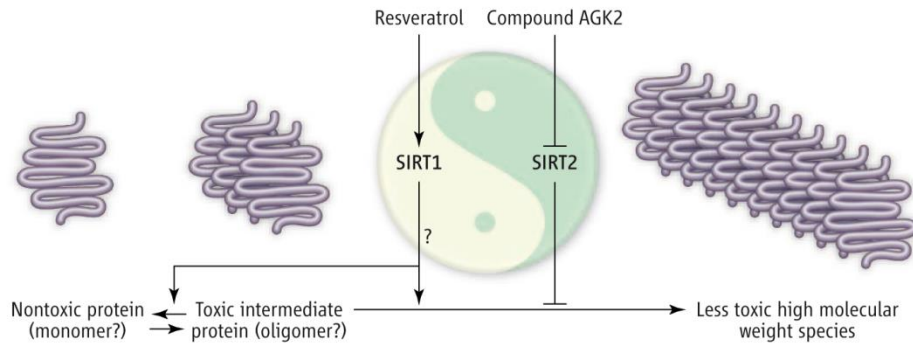
Chou CJ *et al*, *J Biol Chem* 2008, 283, 35402-9.
Xu C *et al*, *Chem Biol* 2009, 16, 980-9.

Fold change in *FXN* mRNA:

2 (after 24 h)

>5 (1-2 h after removal)

3. Parkinson Disease



IC₅₀s: >50 μM (SIRT1); 3.5 μM (SIRT2); >50 μM (SIRT3)

