

# **Correlation Between the Isoform Specificity of HDAC Inhibitors and Their Ability to Reactivate Latent HIV-1 Infection**

**Nicolas Sluis-Cremer**

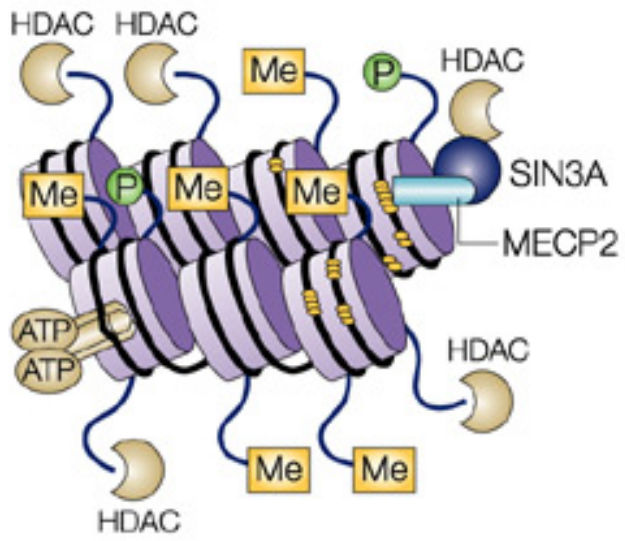
*Department of Medicine, Division of Infectious Diseases,  
University of Pittsburgh*



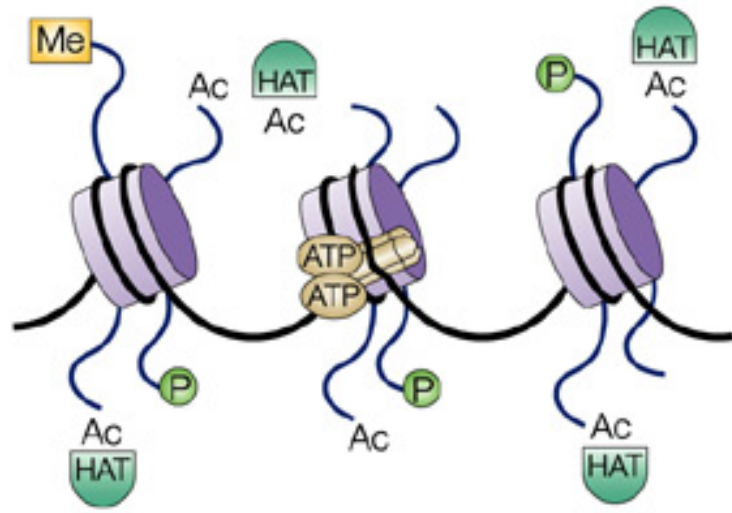
# Nucleosome Regulation

**Closed Chromatin:**  
**Transcriptional**  
**Repression**

**Open Chromatin:**  
**Transcriptional**  
**Activation**



**HAT**  
→  
←  
**HDACs**



# Histone Deacetylases (HDACs)

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## Class 1

- Include HDAC 1, 2, 3, 8
- Classified based on their sequence homology to yeast RPD3
- 350-500 amino acids in length
- HDAC 1, 2 found in nucleus
- HDAC 3 found in nucleus and cytoplasm

## Class 2

- Include HDAC 4, 5, 6, 7, 9, 10
- Classified based on their sequence homology to yeast HDA1
- ~1000 amino acids in length
- Generally cytosolic but can be chaperoned into the nucleus

# HIV Latency and HDACs

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- HDAC 1 found at the HIV-1 LTR  
(Coull et al., J Virol. 2000; Williams et al., EMBO J. 2006; Jiang et al., J Virol. 2007)
- HDAC 1 & 2 found at the HIV-1 LTR  
(Marban et al., EMBO J. 2009)
- HDAC 1, 2 & 3 found at the HIV-1 LTR  
(Keedy et al., J Virol. 2009)
- Inhibitors selective for class I HDACs (1, 2, 3, 8) are more efficient activators of the HIV-1 LTR than inhibitors selective for class 2 HDACs (4, 5, 6, 7, 9, 10)  
(Archin et al., AIDS, 2009)
- Potent activators of HIV-1 expression are found among non-class selective and class 1 selective HDAC inhibitors  
(Savarino et al., Retrovirology, 2009)

# Study Objective

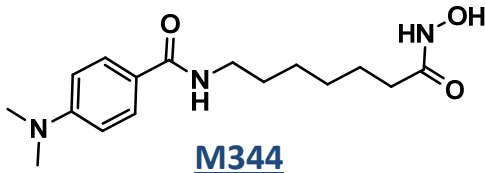
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Use a multidisciplinary approach (biochemistry, virology, genetics) to identify the HDAC isoforms responsible for HIV-1 latency

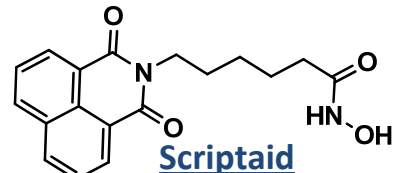
-Use J89GFP cells as a model for HIV-1 latency (Jurkat cell line latently infected with NL4-3 HIV-1 engineered to express EGFP)

(Kutsch et al., J Virol. 2002)

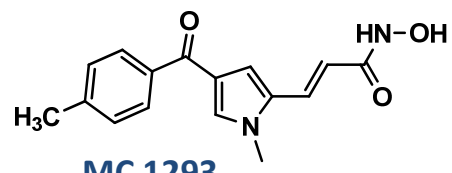
# Chemical Structures of HDACi



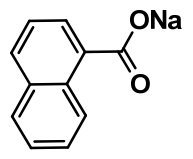
4-Dimethylamino-N-(6-hydroxycarbonylhexyl)-benzamide



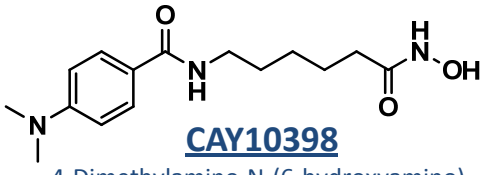
6-(1,3-Dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-N-hydroxyhexanamide



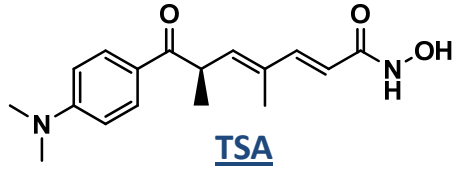
3-(4-Toluoyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide



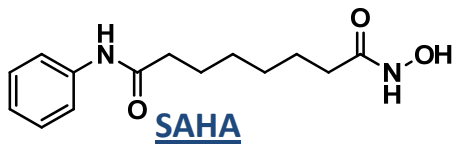
sodium 1-naphthoate



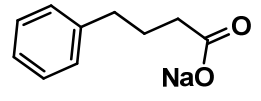
4-Dimethylamino-N-(6-hydroxyamino)-6-(oxohexyl)-benzamide



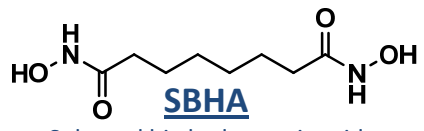
[R-(E,E)-7-[4-(Dimethylamino)phenyl]-N-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamamide]



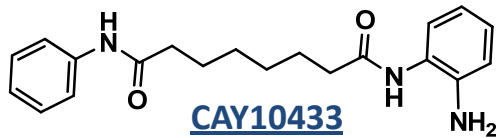
Suberoylanilide hydroxamic acid



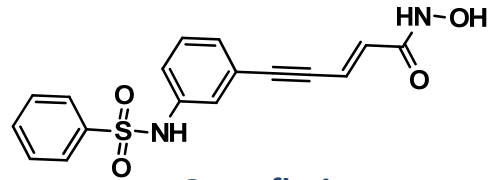
Sodium 4-phenylbutyrate



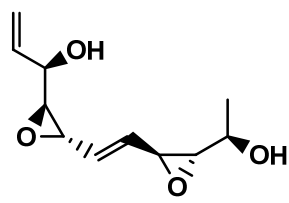
Suberoyl bis-hydroxamic acid



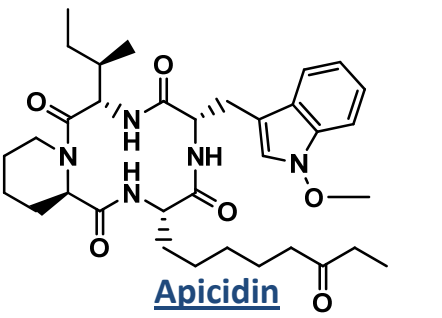
N-phenyl-N'-(2-Aminophenyl) hexamethylenediamide



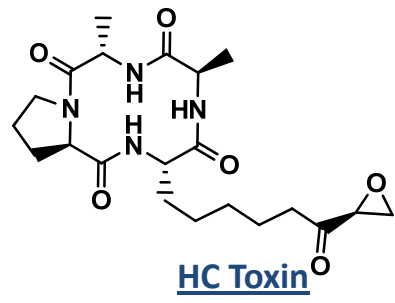
(2E)-5-[3-(Phenylsulfonamino)phenyl]pent-2-en-4-ynohydroxamic acid



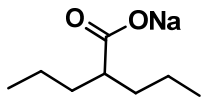
4,5,8,9-Dianhydro-1,2,6,7,11-pentadeoxy-D-threo-D-ido-undeca-1,6-dienitol



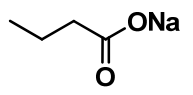
Cyclo[(2S)-2-amino-8-oxodecanoyl-1-methoxy-L-tryptophyl-L-isoleucyl-(2R)-2-piperidinexcarbonyl]



Cyclo-(D-Pro-L-Ala-D-Ala-L-2-amino-8-oxo-9,10-epoxydecanoic acid)



Sodium 2-propylpentanoate



Sodium butyrate

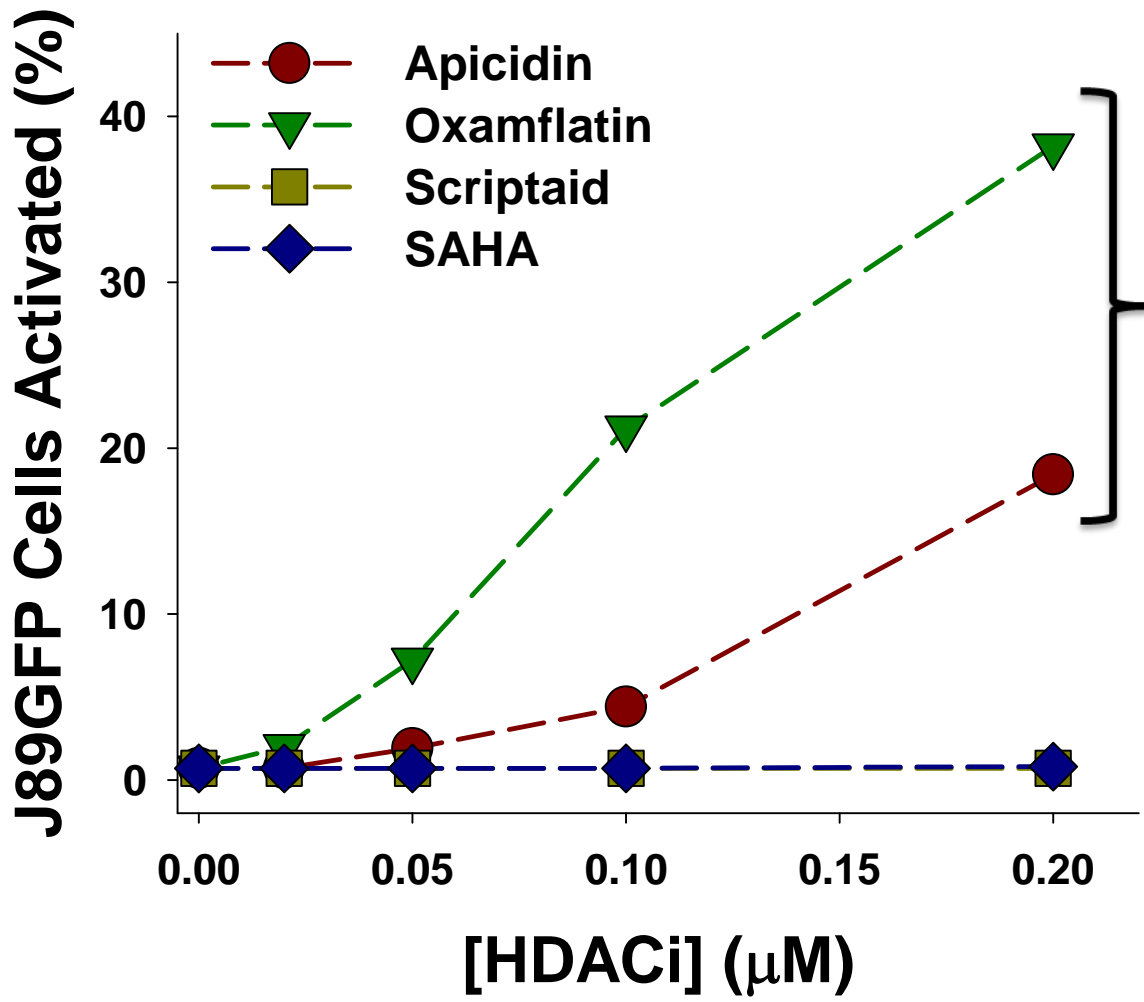
# HDACi: Inhibition of HDAC1, Toxicity, and Reactivation of Latent HIV-1

HDACi	Inhibition of HDAC1 IC <sub>50</sub> (nM)	Toxicity in Jurkat Cells CC <sub>50</sub> (μM)	Maximal Sub-Cytotoxic Dose (μM)	J89GFP Cells Expressing HIV-1 after 24 or 48 h Exposure to HDACi (%)
Apicidin	0.005	0.5-1.0	0.1	20 / 20.7
HC Toxin	1.0	0.05	0.005	1.3 / 1
M344	423	0.5	0.1	3.1 / 3.1
MC1293	1 944	10.0	5	2.7 / 5
Scriptaid	0.66	1.0-5.0	0.5	24.8 / 22.9
Oxamflatin	0.01	1.0-5.0	0.5	82.2 / 57.5
SB	>50 000	> 10 000	1000	66.4 / 56.2
SBP	>50 000	1 000 - 10 000	1000	2.5 / 1.7
TSA	3.3	0.1	0.05	17.2 / 15.4
Valproic Acid	> 50 000	10 000	1000	4.3 / 8.5
SAHA	0.36	0.5	0.5	1.6 / 11.2
CAY10398	70.1	1.0	0.5	0.9 / 1
CAY10433	1 045	1 000	10	5.2 / 11.8
Depudecin	6 027	5.0	1	1.3 / 0.9
SBHA	7 844	100	100	57.5 / 35.6
SN	>50 000	10	10	1 / 0.7

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<b>Apicidin</b>	<b>0.005</b>	<b>0.5-1.0</b>	<b>0.1</b>	<b>20 / 20.7</b>
HC Toxin	1.0	0.05	0.005	1.3 / 1
M344	423	0.5	0.1	3.1 / 3.1
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<b>Scriptaid</b>	<b>0.66</b>	<b>1.0-5.0</b>	<b>0.5</b>	<b>24.8 / 22.9</b>
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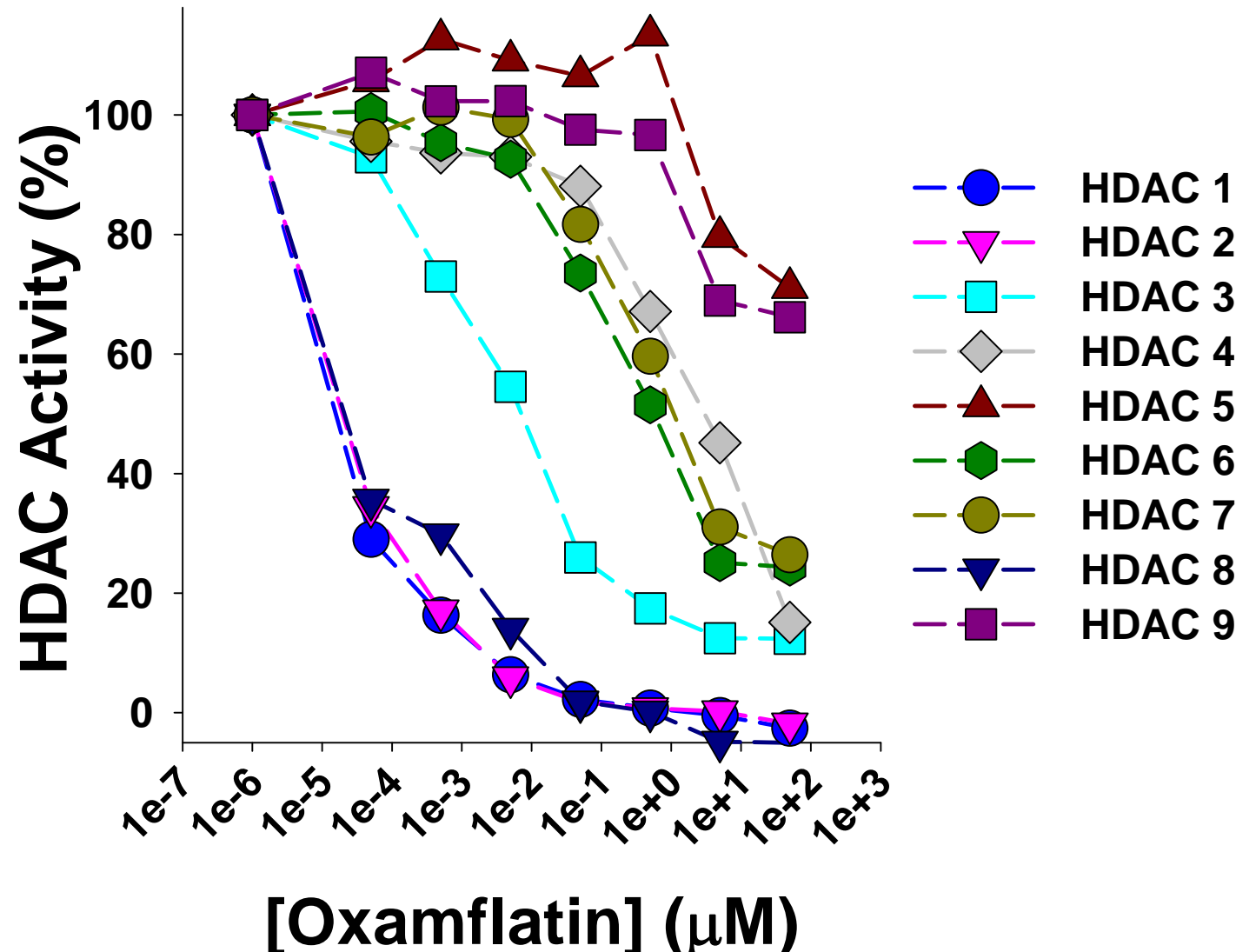
# Reactivation of Latent HIV-1 Expression by HDACi's



**DNA Microarray Analyses**  
7-9% of genes significantly up- or down-regulated by Apicidin & Oxamflatin  
Expression of HIV proteins contributes to this!

**DNA Microarray Analyses**  
2-4% of genes significantly up- or down-regulated by Scriptaid & SAHA

# HDACi Exhibit a Wide Range of Activity Against Different HDAC Isoforms



# HDACi Activity Against Class 2 HDACs

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HDAC	IC <sub>50</sub> (nM)			
	Oxamflatin	Apicidin	Scriptaid	SAHA
HDAC 4	3800	>50,000	14000	>50,000
HDAC 5	>50,000	>50,000	>50,000	>50,000
HDAC 6	390	>50,000	34	5500
HDAC 7	840	>50,000	2200	>50,000
HDAC 9	>50,000	>50,000	>50,000	>50,000

# HDACi Activity Against Class 1 HDACs

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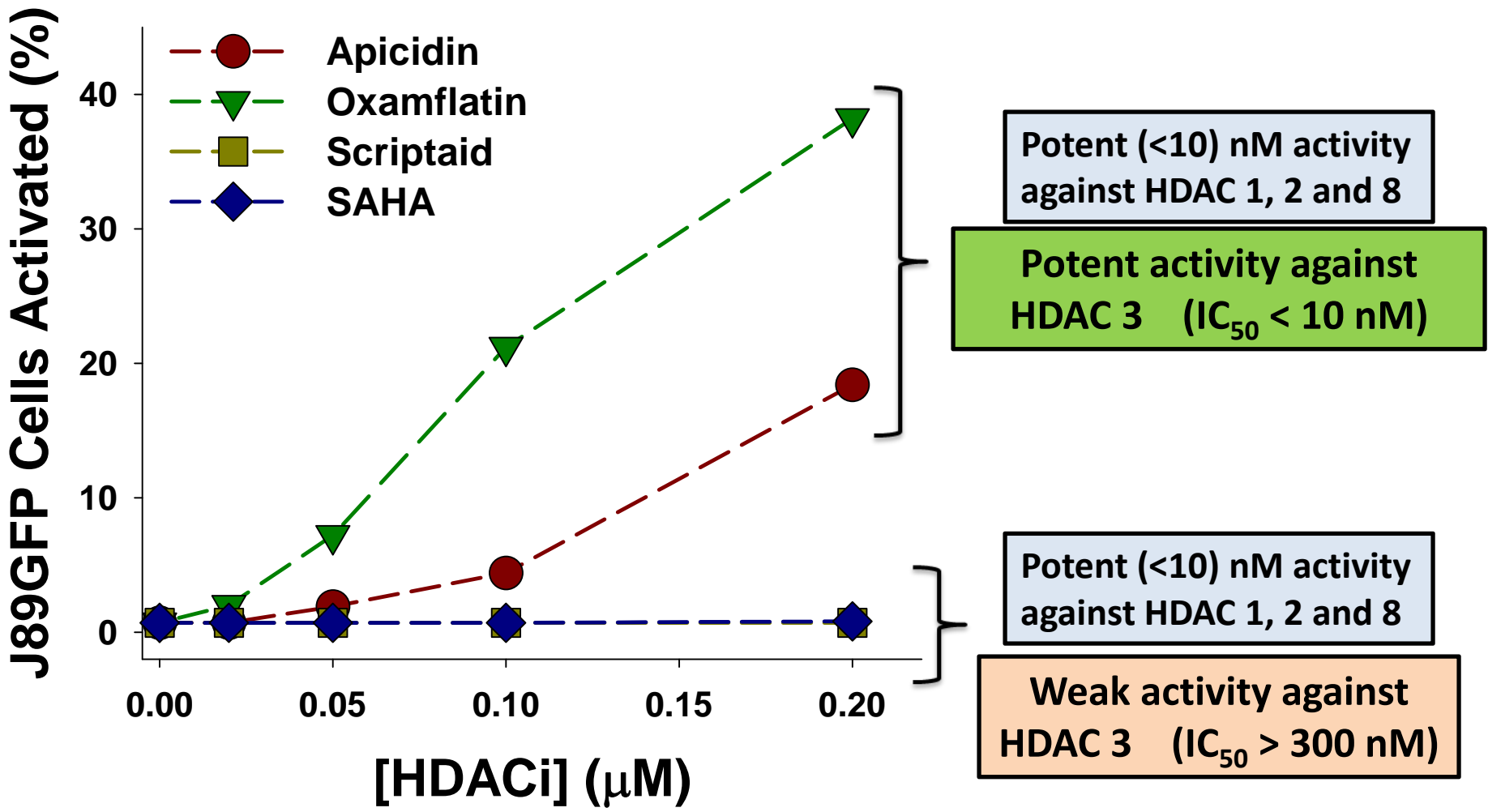
HDAC	IC <sub>50</sub> (nM)			
	Oxamflatin	Apicidin	Scriptaid	SAHA
HDAC 1	0.010	0.0052	0.66	0.36
HDAC 2	0.016	0.012	1.4	0.62
HDAC 3	4.3	6.5	320	600
HDAC 8	0.023	0.012	9.0	2.1

# HDACi Activity Against Class 1 HDACs

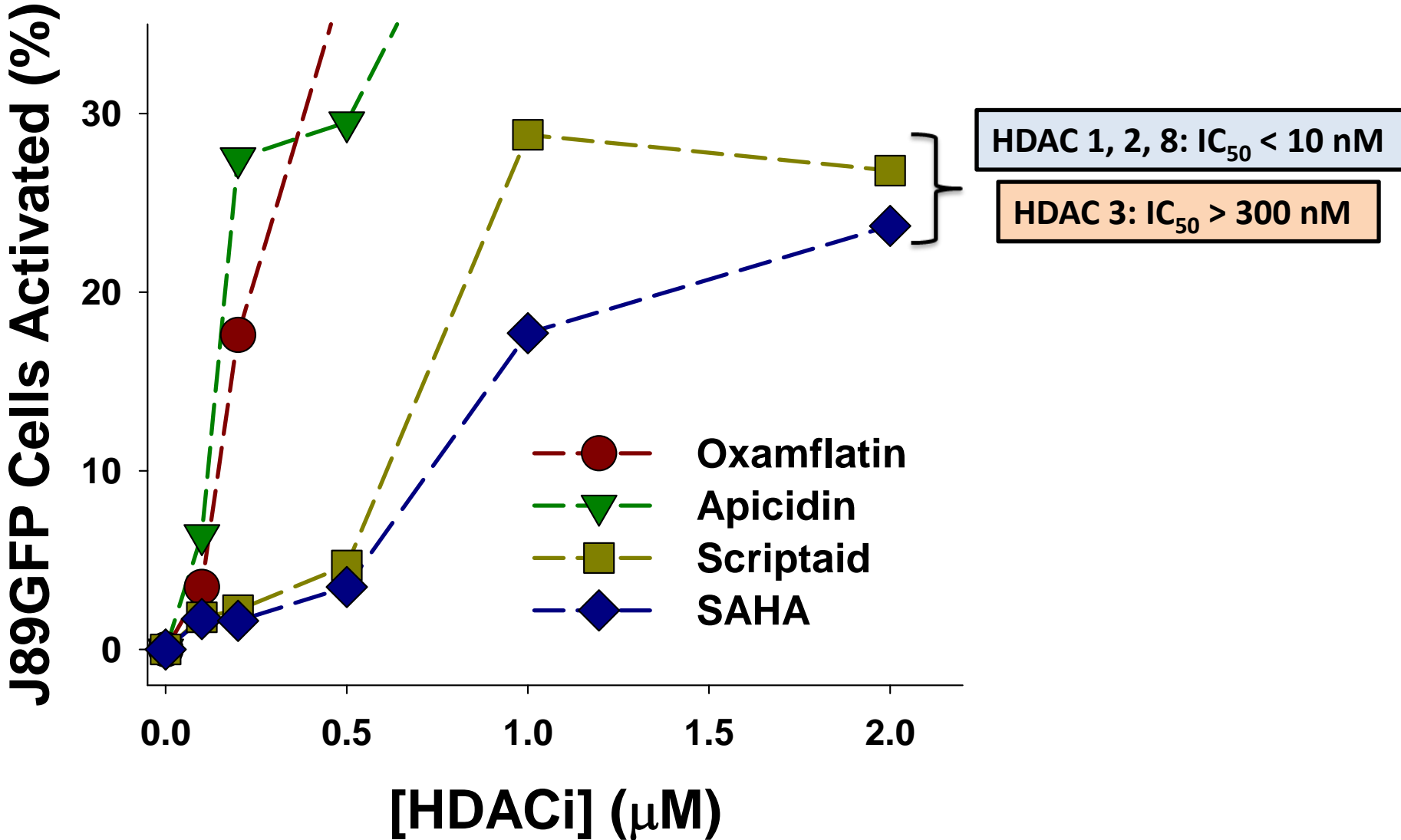
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# Reactivation of Latent HIV-1 Expression



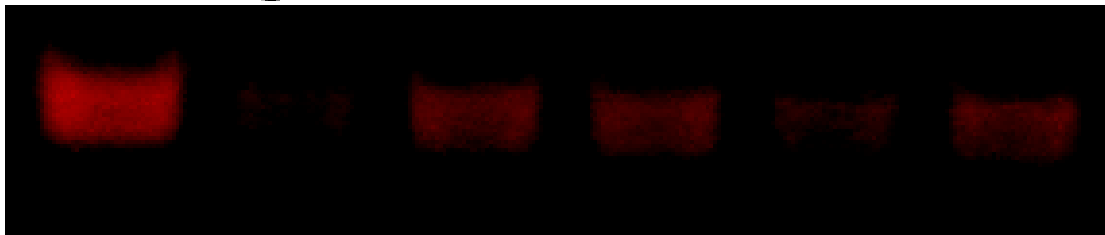
# Reactivation of Latent HIV-1 Expression



# HDAC 1 & 3 are Both Present at the HIV-1 LTR

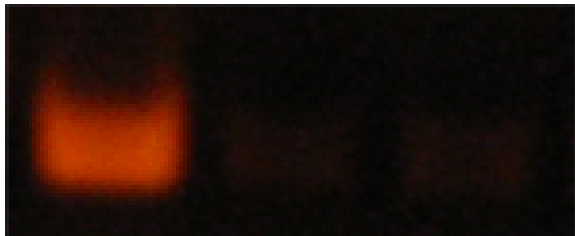
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Input    Rabbit    HDAC1    HDAC1    HDAC2    HDAC3  
          IgG



HIV LTR promoter  
-363 to -173  
(includes NF-kB binding sites)

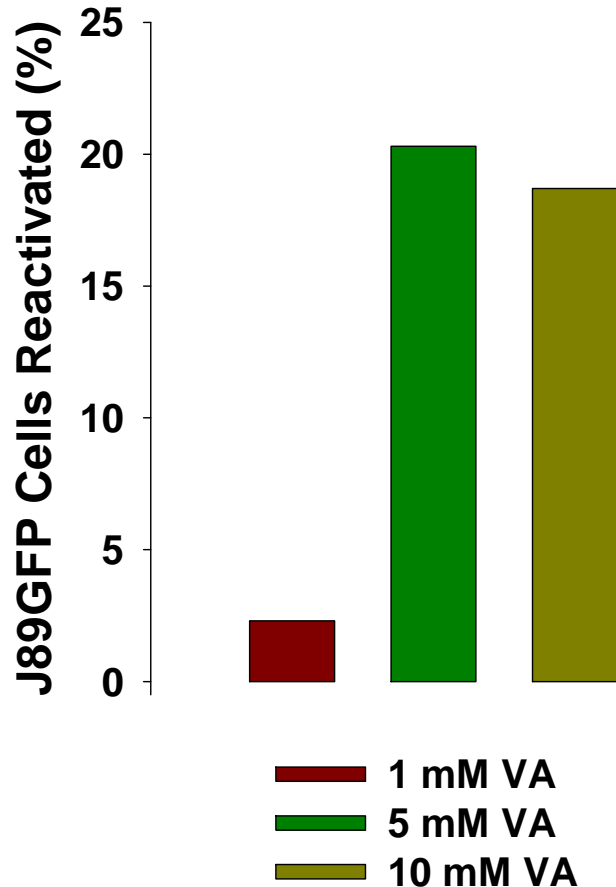
Input    Rabbit    HDAC8  
          IgG



HIV LTR promoter  
-363 to -173  
(includes NF-kB binding sites)

# Valproic Acid, HDAC 3 & Decay of Latent Reservoirs in HIV-1 Infected Patients

$IC_{50}$  for inhibition of  
HDAC 1: 170 $\mu$ M  
HDAC 3: 5.5mM



## TOTAL Therapeutic Concentration of Valproic Acid

*Trough: 40  $\mu$ g/mL (270  $\mu$ M)*

*Peak: 100  $\mu$ g/mL (690  $\mu$ M)*

## FREE Therapeutic Concentration of Valproic Acid

*4-15  $\mu$ g/mL (27 – 100  $\mu$ M)*

# Conclusions

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This study suggests that inhibition of HDAC 3 may be important to reactivate HIV-1 gene expression in latently infected cells.

# Acknowledgements

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Kelly Huber

Joseph Plaks

Geneviève Doyon

Zandrea Ambrose