Design and Synthesis of Mercaptoacetamides as Potent, Selective, and Brain Permeable Histone Deacetylase 6 Inhibitors

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ACS Med. Chem. Lett. **2017**, DOI: 10.1021/acsmedchemlett.7b00012

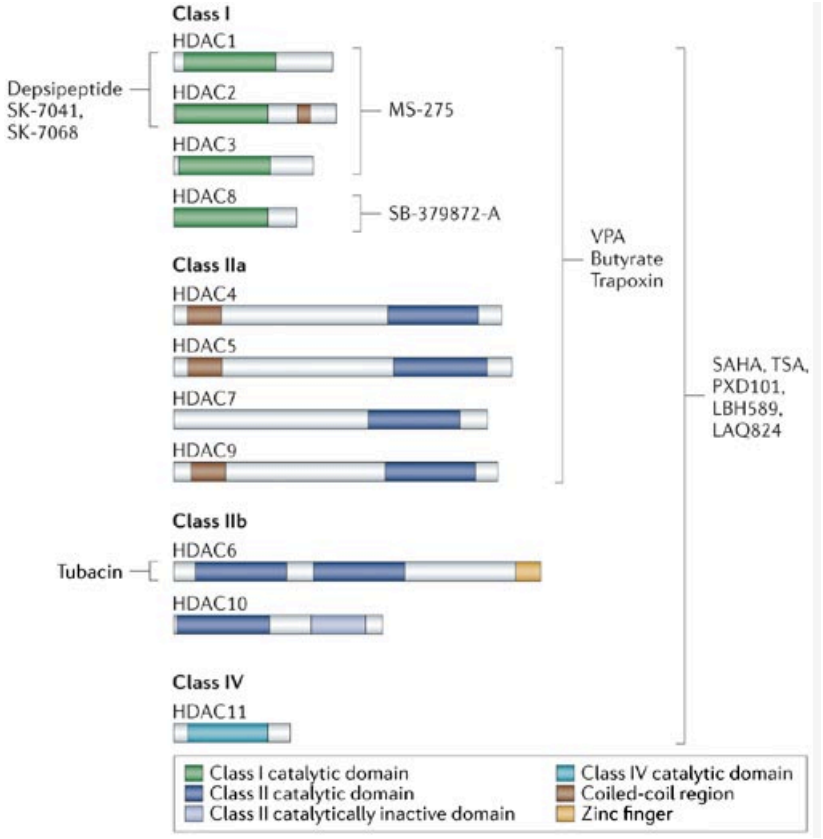
Wipf Group Current Literature

Chaemin Lim

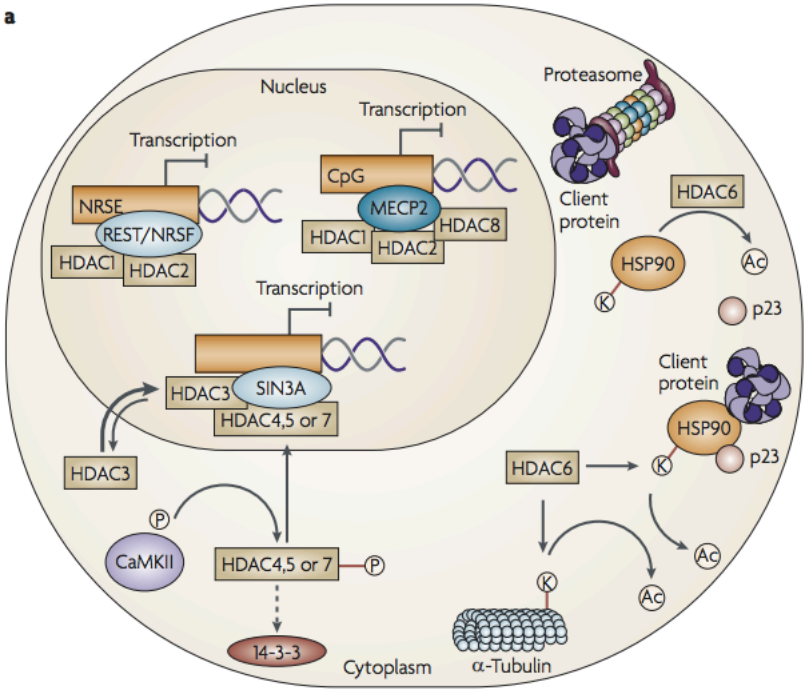
05/06/2017

Histone deacetylases (HDACs)

- A family of zinc-dependent enzymes that specifically remove the acetyl groups from lysine residues on target proteins, including nuclear histones, transcription factors, HSP90, cortactin, and α-tubulin.
- Important role in epigenetic regulation – therapeutic targets for the treatment of a wide range of diseases, including cancer, neurodegenerative diseases, arthritis, and others.

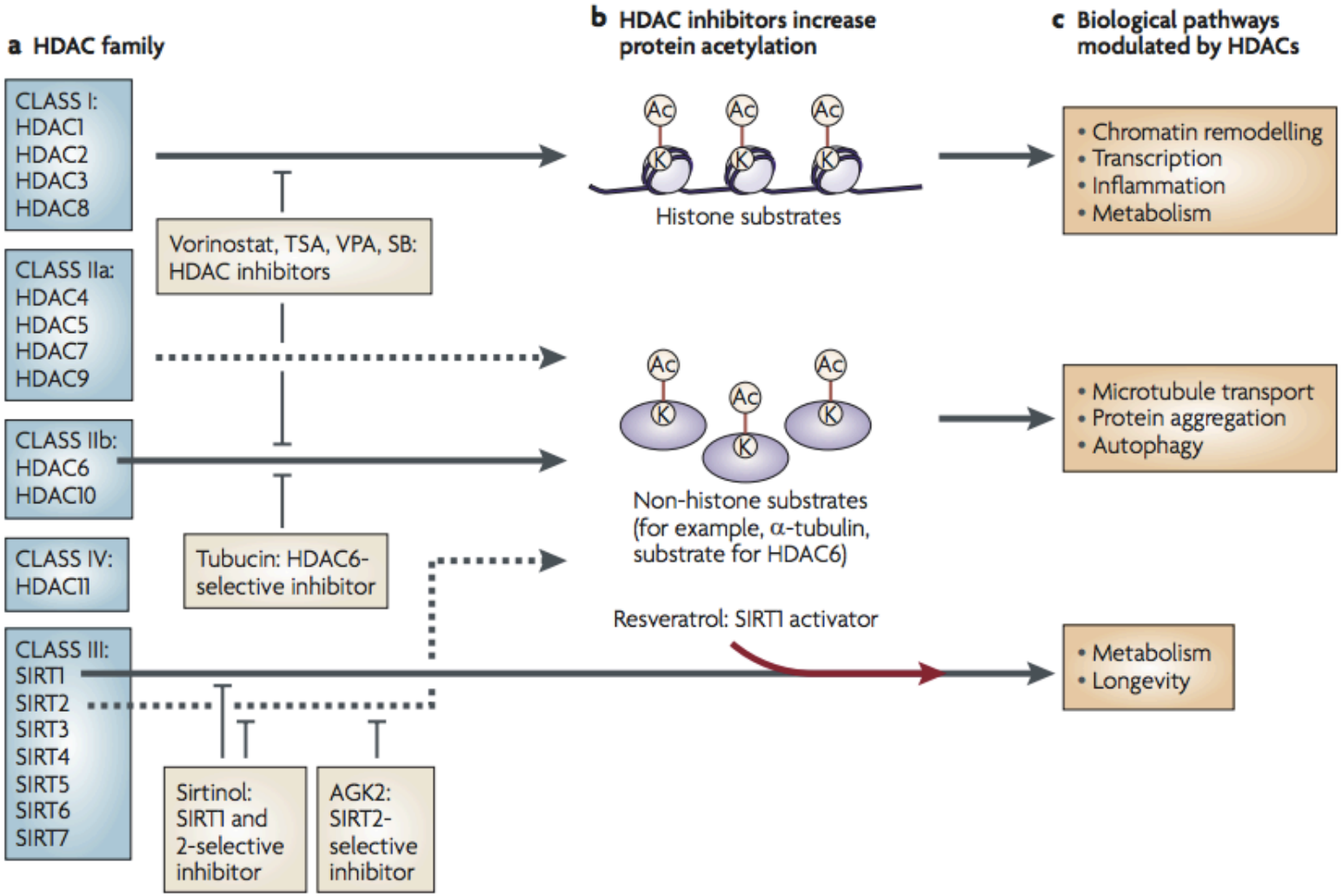


Nat. Rev. Drug Discovery **2006**, 5, 769-784.



Nat. Rev. Drug Discovery **2008**, 7, 854-868

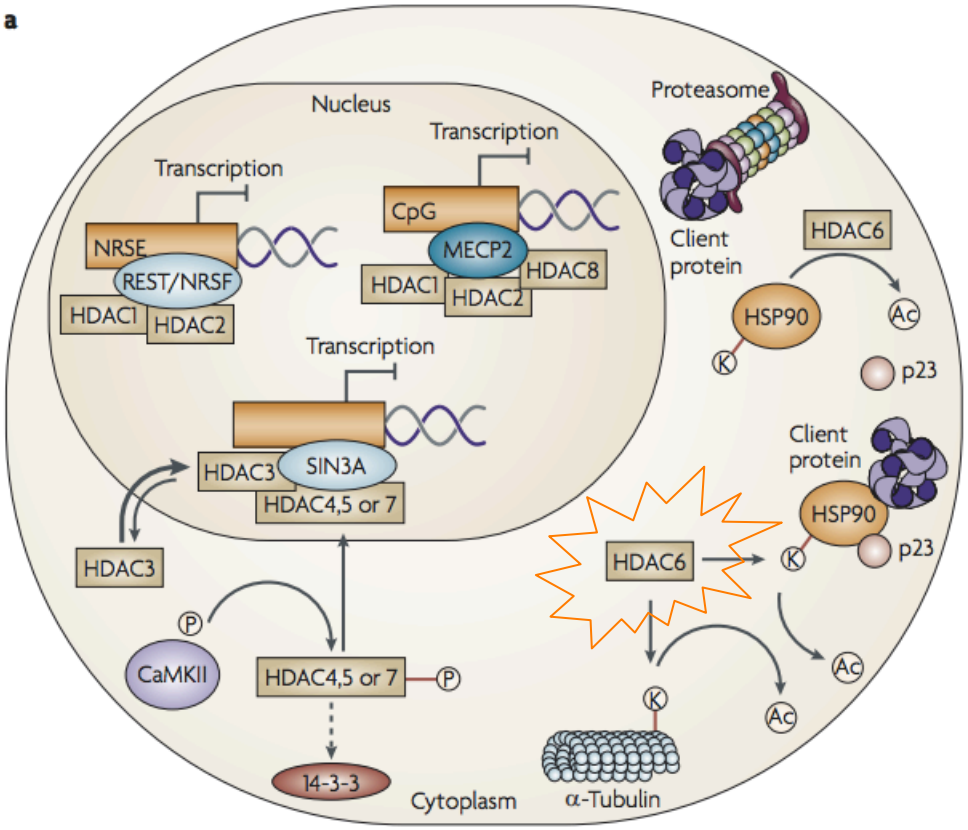
Targeting HDACs



Nat. Rev. Drug Discovery **2008**, 7, 854-868

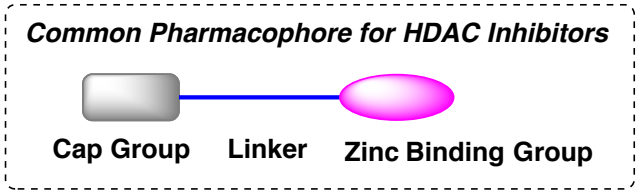
HDAC6 Selective Inhibitors

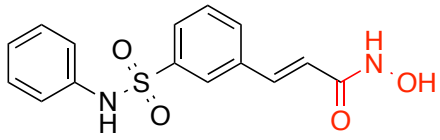
- Unlike other HDAC family members, HDAC6 primarily resides within the cytosol and mainly targets non-histone substrates such as α-tubulin, HSP90, cortactin, etc.
- HDAC6 plays a critical role in cell motility, microtubule stability and function as well as aggreome formation → potential role in cancer and neurodegenerative disorders.
- HDAC6 is relatively highly expressed in the central nervous system (CNS), and aberrant expression of HDAC6 has been linked to the pathological development of a host of CNS disorders.



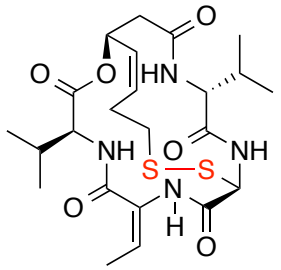
Nat. Rev. Drug Discovery **2008**, 7, 854-868

Current HDAC Inhibitors

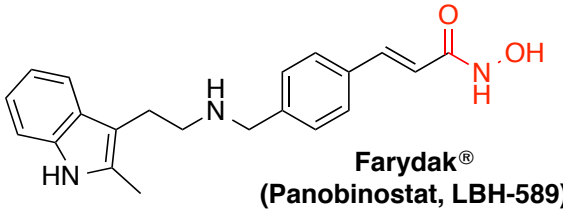




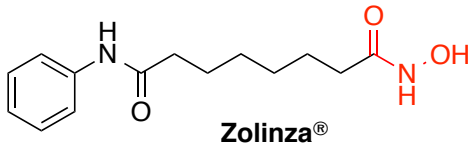
Beleodaq®
(Bellinostat, PXD101)



Istodax®
(Romidepsin, FK228)



Farydak®
(Panobinostat, LBH-589)

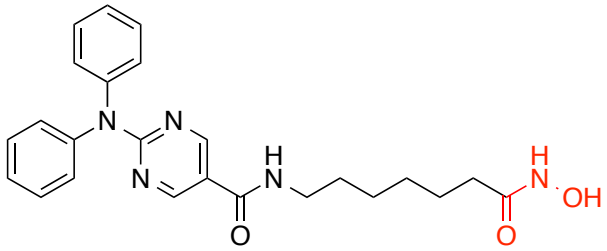


Zolinza®
(Vorinostat, SAHA)

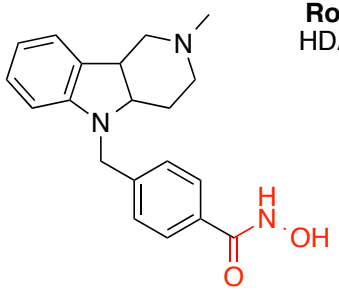
Table 2 | **Isoform-selective HDAC inhibitors**

HDAC inhibitor	Specificity	Stage	Diseases	Refs
Compound 60	HDAC1, HDAC2	Preclinical	Neurology	112
MRLB-223	HDAC1, HDAC2	Preclinical	Cancer	111
RG2833; 109	HDAC3	Phase I trial	Friedreich's ataxia	105,106
RGFP966	HDAC3	Preclinical	Cancer, neurology	107,217
BG45	HDAC3	Preclinical	Cancer	108
Rocilinostat (ACY-1215)	HDAC6	Phase IIa trial	Cancer	*
ACY-738, ACY-775	HDAC6	Preclinical	Neurology	218
Tubacin	HDAC6	Preclinical	Cancer	219,220
Tubastatin A (tubastatin)	HDAC6	Preclinical	Inflammation, neurodegeneration	178,221
C1A	HDAC6	Preclinical	Cancer	222
HPOB	HDAC6	Preclinical	Cancer	223
Quinazolin-4-one derivatives	HDAC6	Preclinical	Alzheimer's disease	224
PCI-34051	HDAC8	Preclinical	Cancer	225
C149	HDAC8	Preclinical	Cancer	226
Jδ	HDAC8	Preclinical	NA	227
BRD73954	HDAC6, HDAC8	Preclinical	NA	228

HDAC, histone deacetylase; NA, not applicable. *See ClinicalTrials.gov identifiers NCT01323751, NCT01997840 and NCT01583283 for further information.



Rocilinostat, ACY-1215
HDAC6 selective inhibitor



Tubastatin A
HDAC6 selective inhibitor

Nat. Rev. Drug Discovery **2014**, 13, 673–691.

Non-hydroxamate HDAC Inhibitors

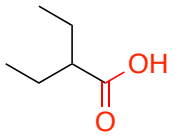
- Limitations of the hydroxamic acid group

: Genotoxicity (long-term treatment), off-target effects, including interaction with the hERG cardiac potassium channel

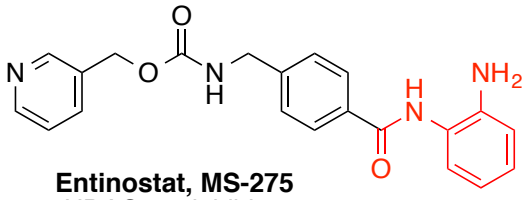
: Low bioavailability, short intravenous half-life, easily hydrolysed into carboxylic acid and metabolized

- Efforts on Zinc Binding Group (ZBG) modification

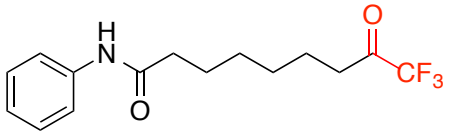
: Small fatty acids, epoxyketones, benzamides, electrophilic ketones (trifluoromethyl ketones, α-ketoamides, heterocyclic ketones), N-formylhydroxylamine, thiols, mercaptoamides, etc…

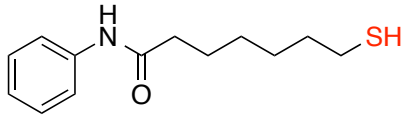


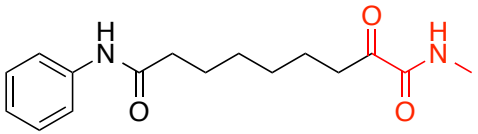
Valproic acid

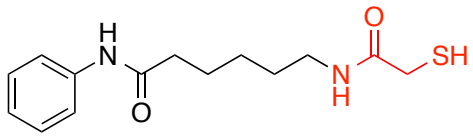


Entinostat, MS-275
HDAC1, 3 inhibitor

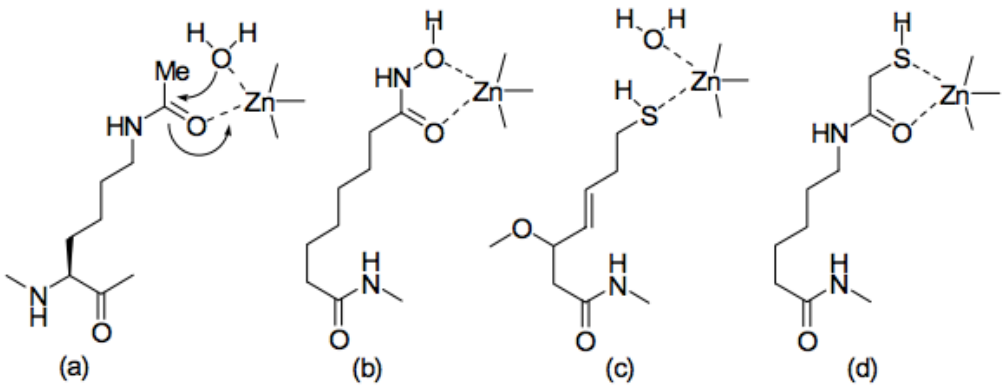






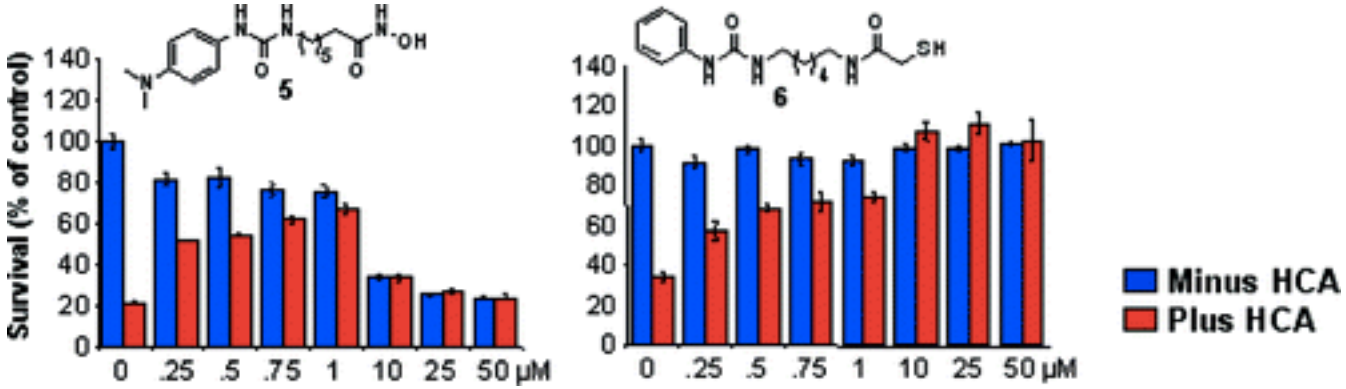


Mercaptoacetamide-based HDACIs



- Possible mode of interaction of the zinc ion of HDAC with
(a) acetylated lysine moiety of histone
(b) hydroxamate group of HDACI
(c) thiol group from FK228 + DTT
(d) designed mercaptoacetamide-based inhibitors.

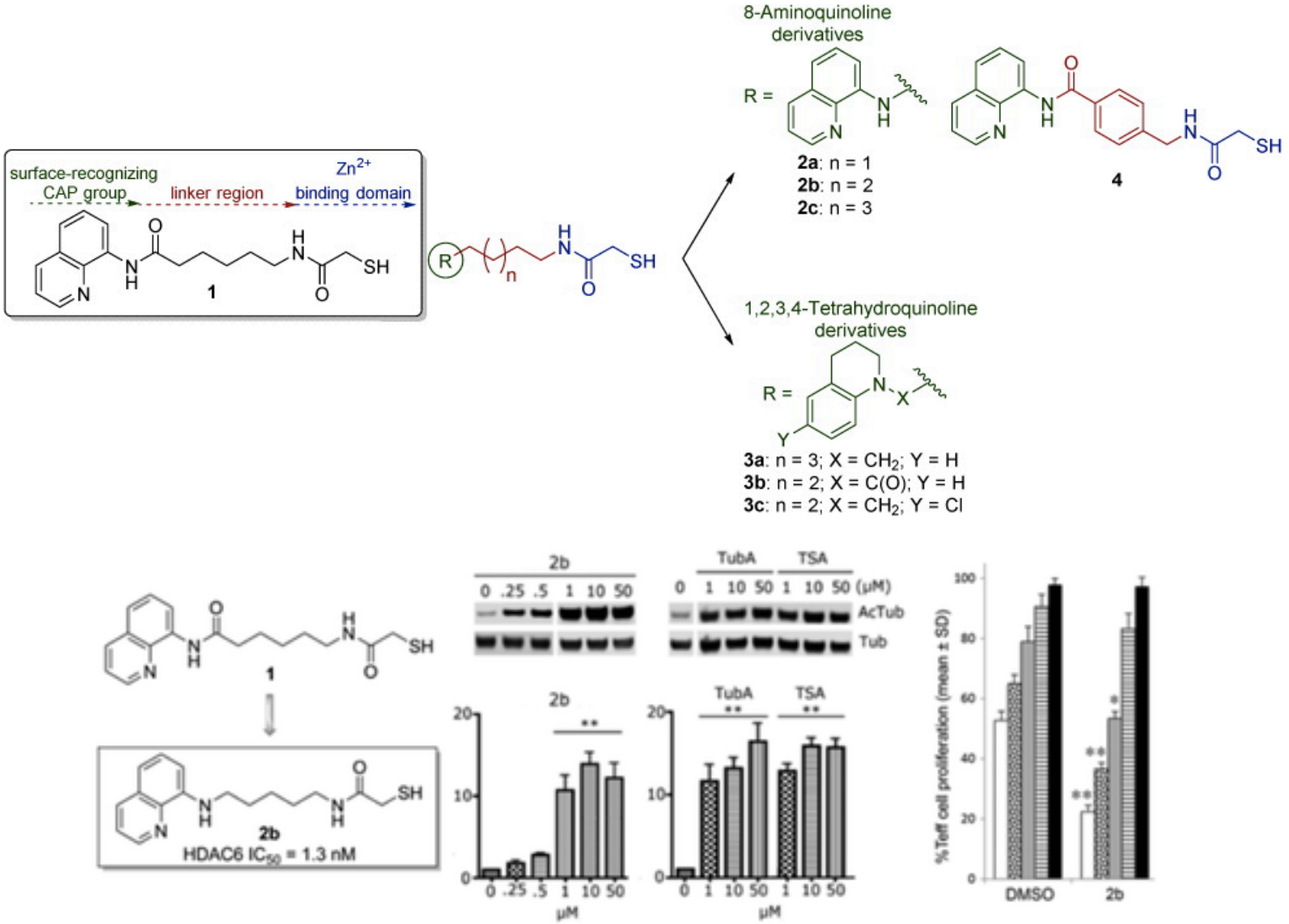
B. Chen; A. P. Kozikovski* et al. *Bioorg. Med. Chem. Lett.* **2005**, 15, 1389-1392.



- This study reveals that some of the mercaptoacetamide-based HDAC inhibitors are fully neuroprotective, whereas the hydroxamates show toxicity at higher concentrations.

A. P. Kozikowski* et al. *J. Med. Chem.* **2007**, 50, 3054–3061.

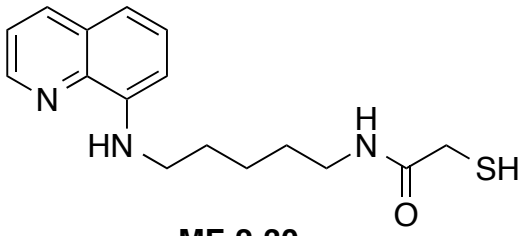
Mercaptoacetamide-based HDACIs



M. C. F. Segretti; A. P. Kozikowski* et al. *ACS Med. Chem. Lett*. **2015**, 6, 1156–1161.

In this Study

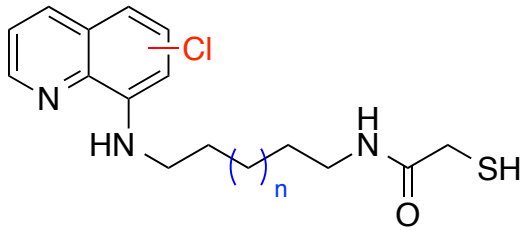
- Optimization of previous hit MF-2-30



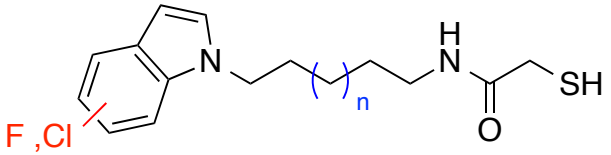
MF-2-30

HDAC1 IC_{50} = 4700 nM
HDAC6 IC_{50} = 1.3 nM
LogBB = -0.27

Quinoline-Based Mercaptoacetamides



Indole-Based Mercaptoacetamides



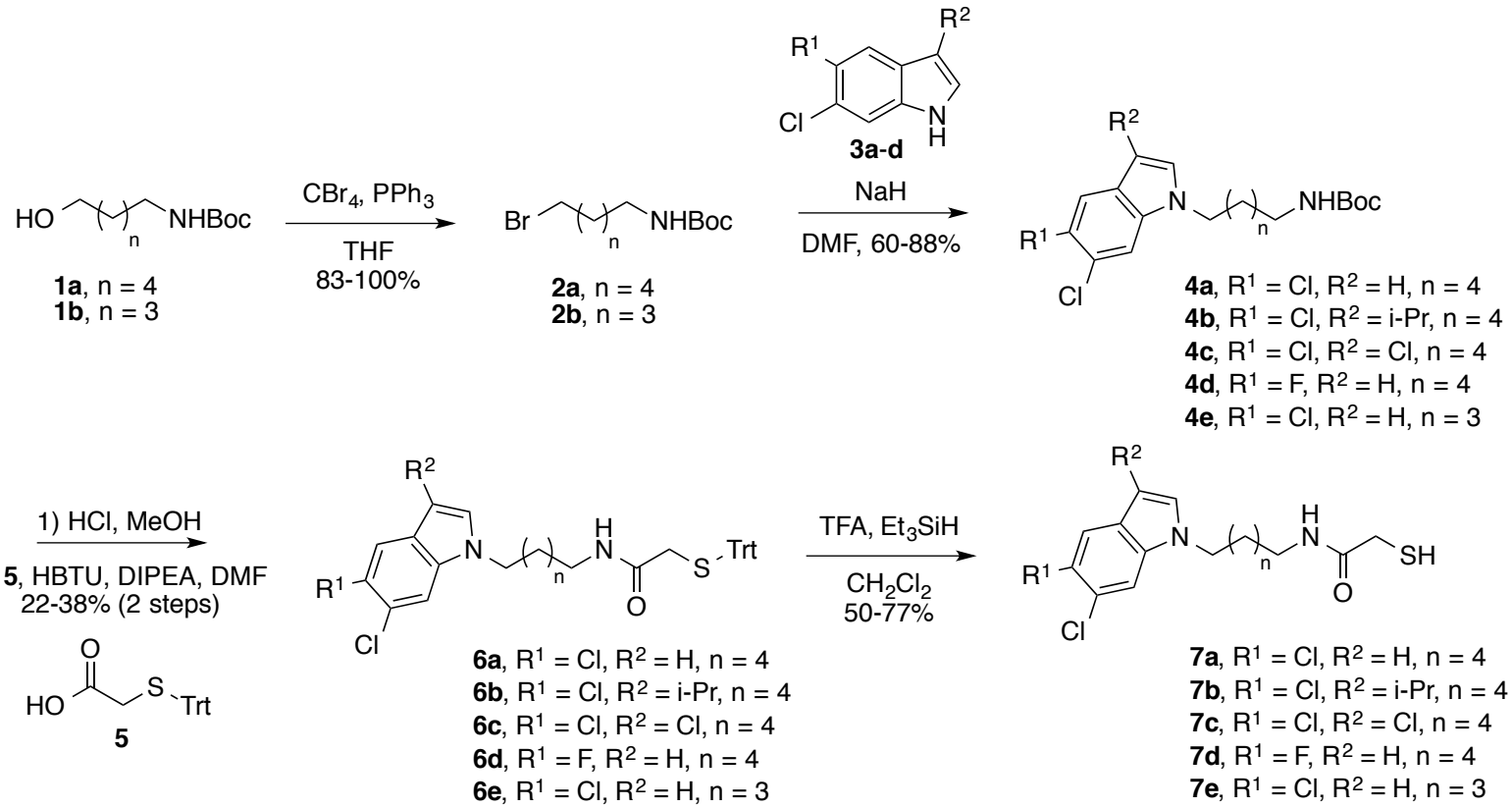
Incorporation of halogen atoms to increase the compounds' lipophilicity

→ *Increase brain accessibility*

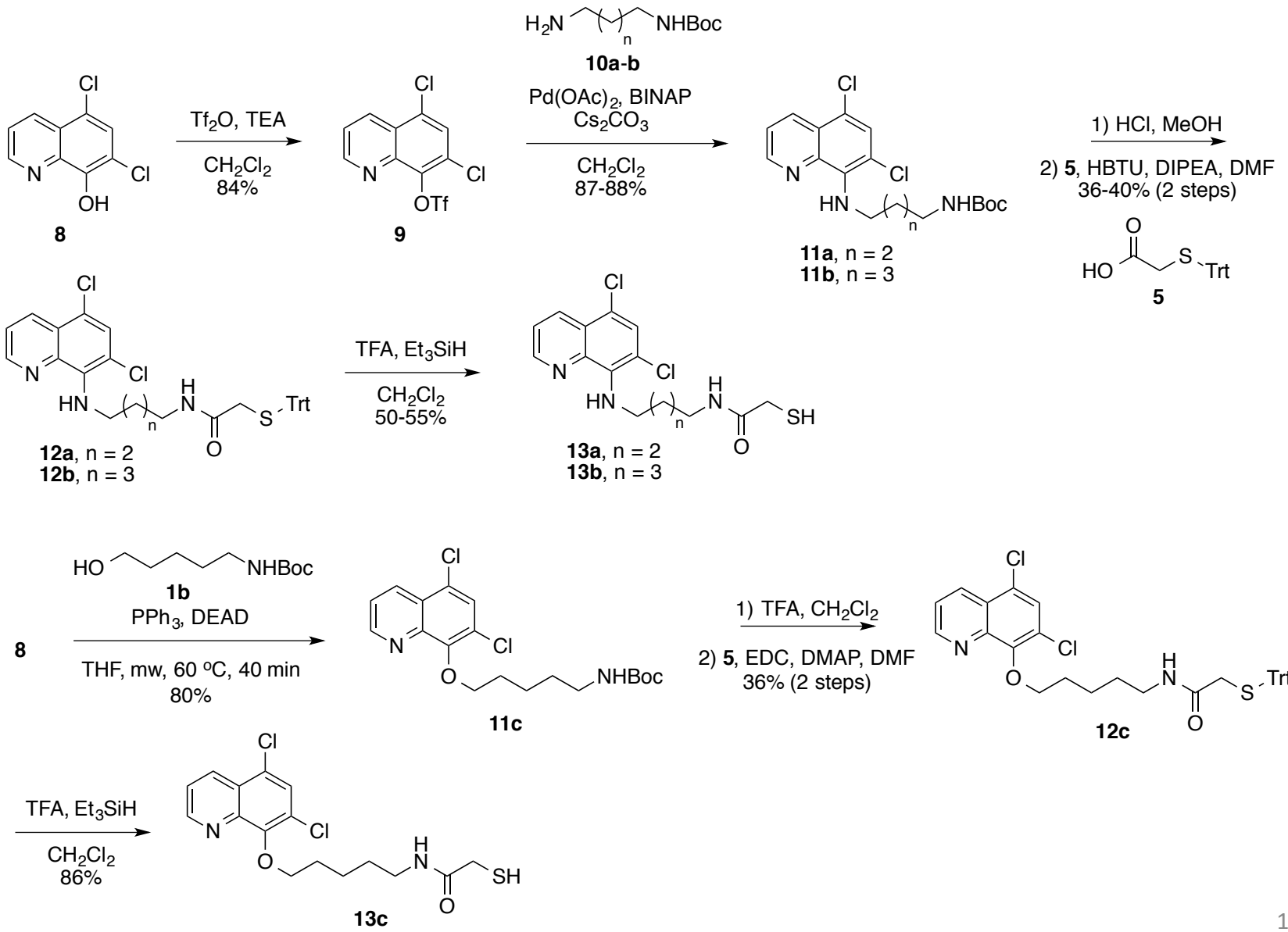
→ *Development of potent, selective HDAC6 inhibitors for treatment of CNS diseases*

ACS Med. Chem. Lett. **2017**, DOI: 10.1021/acsmedchemlett.7b00012

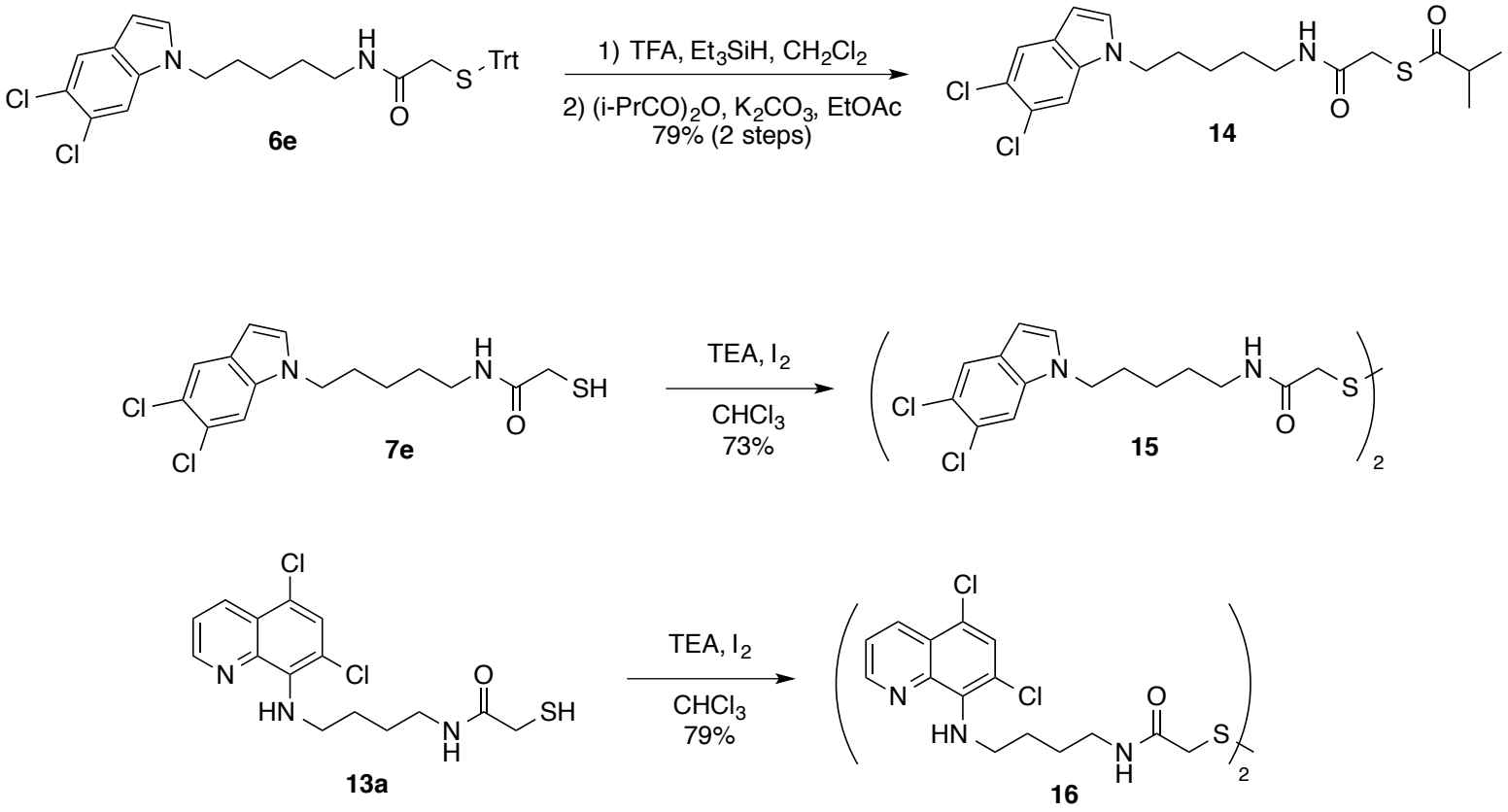
Synthesis: Indole analogs



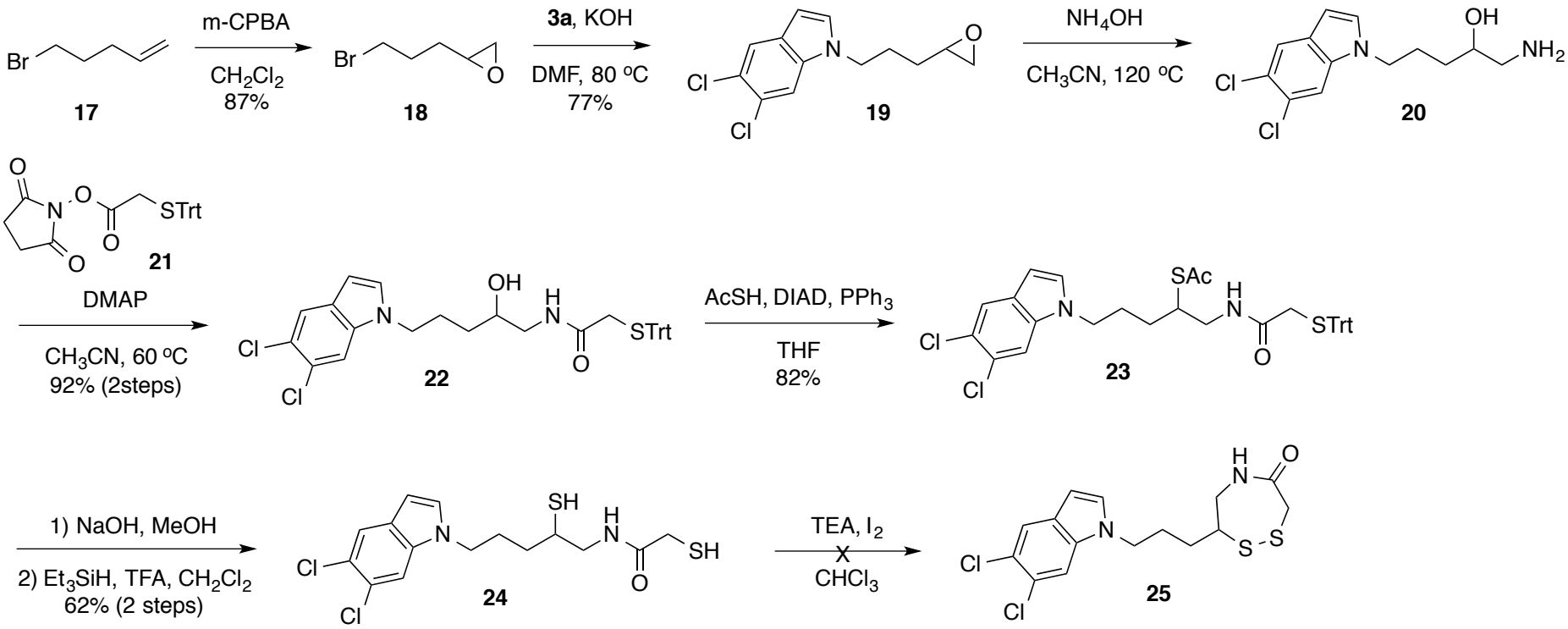
Synthesis: Quinoline analogs



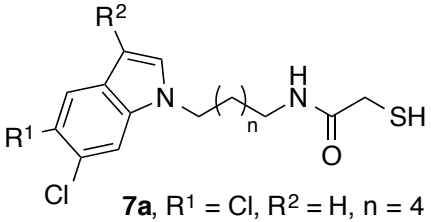
Synthesis: Thioester 14 & Disulfide Prodrugs 15 and 16



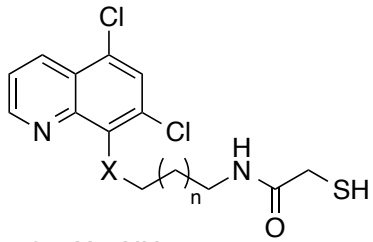
Synthesis: Dithiol analog



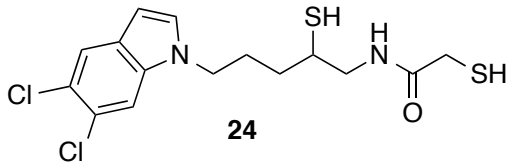
HDAC1 and -6 Inhibitory Activities of the analogs



7a, R^1 = Cl, R^2 = H, n = 4
7b, R^1 = Cl, R^2 = i-Pr, n = 4
7c, R^1 = Cl, R^2 = Cl, n = 4
7d, R^1 = F, R^2 = H, n = 4
7e, R^1 = Cl, R^2 = H, n = 3



13a, X = NH, n = 2
13b, X = NH, n = 3
13c, X = O, n = 3

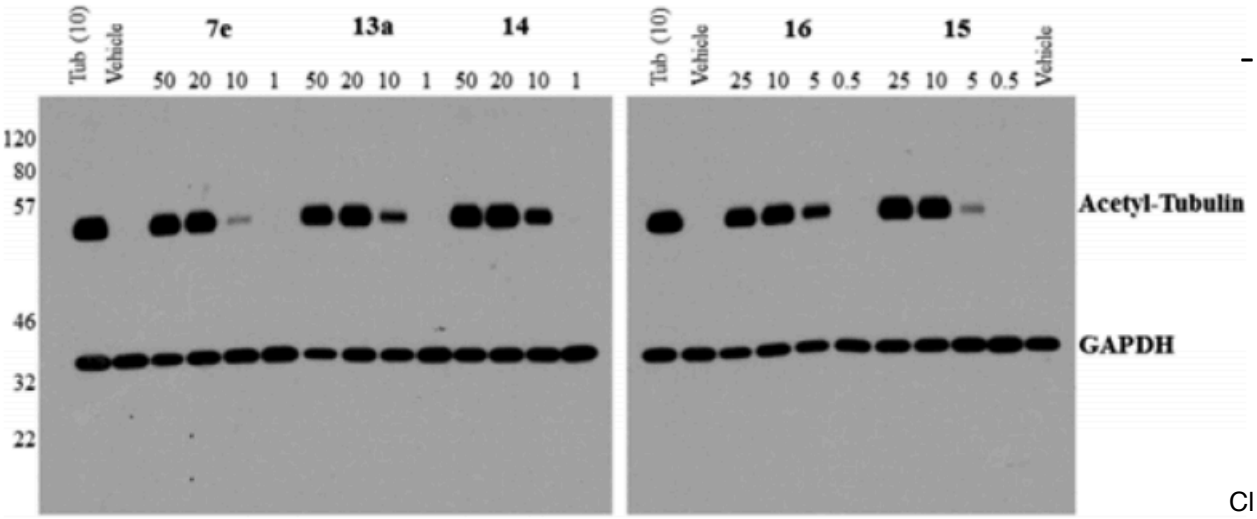


24

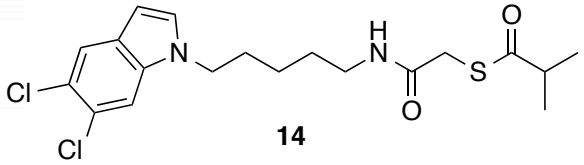
compd	LogBB[b]	HDAC1 (IC_{50}, nM)	HDAC6 (IC_{50} nM)	selectivity HDAC1/ HDAC6
trichostatin A	N.T.[c]	7.7 ± 1.3	2.3 ± 1.2	3.3
7a	0.49	>30000	63.9 ± 8.0	>470
7b	0.66	28700 ± 1900	1570 ± 42	18
7c	0.50	>30000	241 ± 81	>124
7d	0.30	29300 ± 1100	65.1 ± 6.9	450
7e	0.37	7490 ± 318	11.4 ± 0.9	657
13a	0.17	6880 ± 650	2.79 ± 0.12	2470
13b	0.38	6570 ± 820	14.8 ± 5.2	444
13c	0.16	>30000	33.3 ± 2.5	>901
24	0.20	N.T.[c]	534 ± 3.5	N.T.[c]

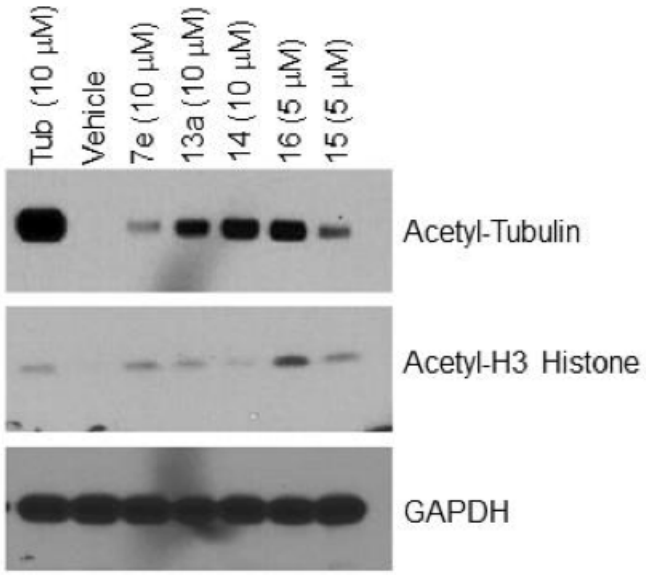
[a]Results were determined by Reaction Biology Corp. (Malvern, PA, USA); IC_{50} values displayed are the mean of two experiments. [b]LogBB values were calculated using ACD software. [c]Not tested.

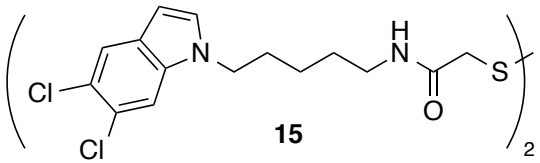
Cellular Activities of 7e and 13a

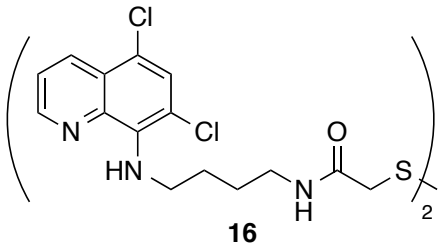


- Western blots showing acetylated tubulin levels in HEK-293 cells following 24 h treatment with **7e**, **13a**, **14**, **15**, and **16** at the indicated concentrations.









- Western blots showing acetylated tubulin and acetyl-H3 histone levels in HEK-293 cells following 24 h treatment with **7e**, **13a**, **14**, **15**, and **16** at the indicated concentrations.

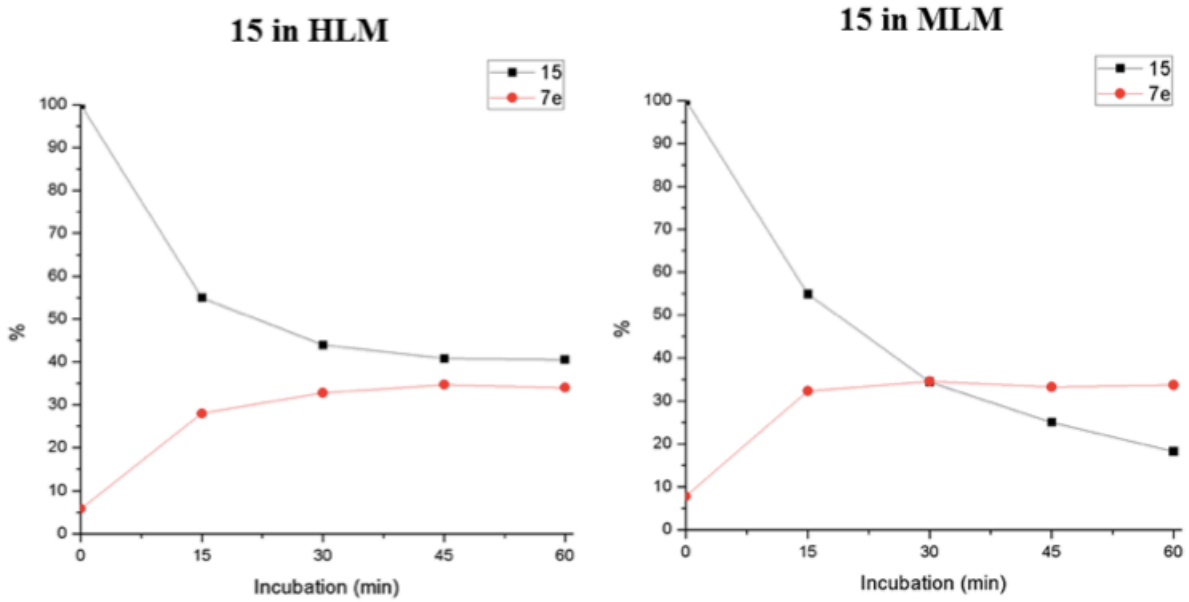
Metabolic Stabilities of Selected Analogs

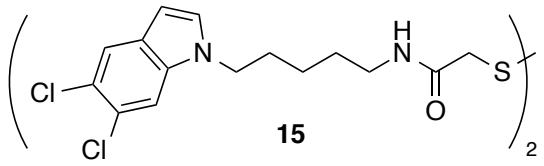
- Metabolic stabilities of mercaptoacetamides 7b, 7e, 13a, and 13b, and prodrugs 14-16 in pooled human and mouse liver microsomes.[a]

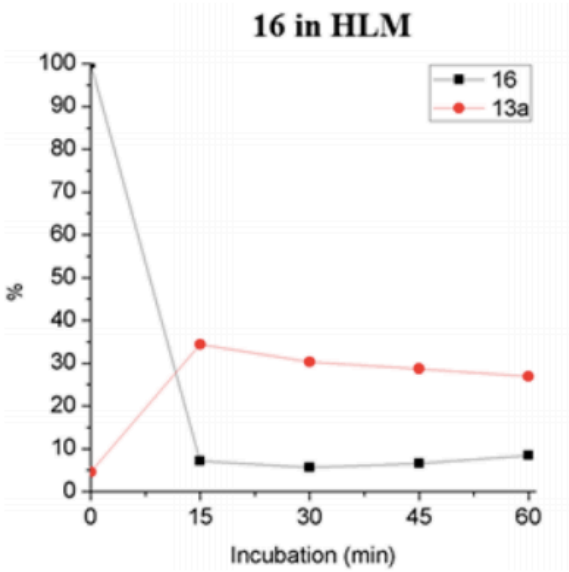
	human liver microsomes		male mouse liver microsomes	
compd	% remaining at 60 min	$t_{1/2}$ (min)	% remaining at 60 min	$t_{1/2}$ (min)
7b	33.3	48.8	44.3	70.0
7e	25.3	35.2	29.5	41.3
13a	22.3	32.2	61.7	91.2
13b	32.1	42.5	39.6	51.0
14	<1	<1	<1	<1
15	40.5	49.5	18.2	24.8
16	8.5	20.7	18.2	25.9

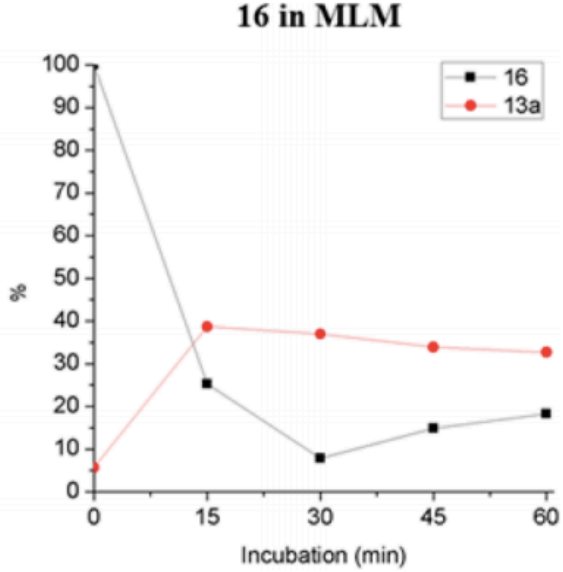
[a]All tests were performed in duplicate with NADPH.

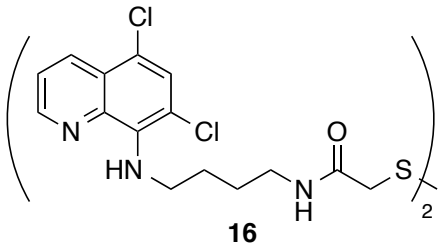
Metabolism of disulfide prodrugs 15 and 16





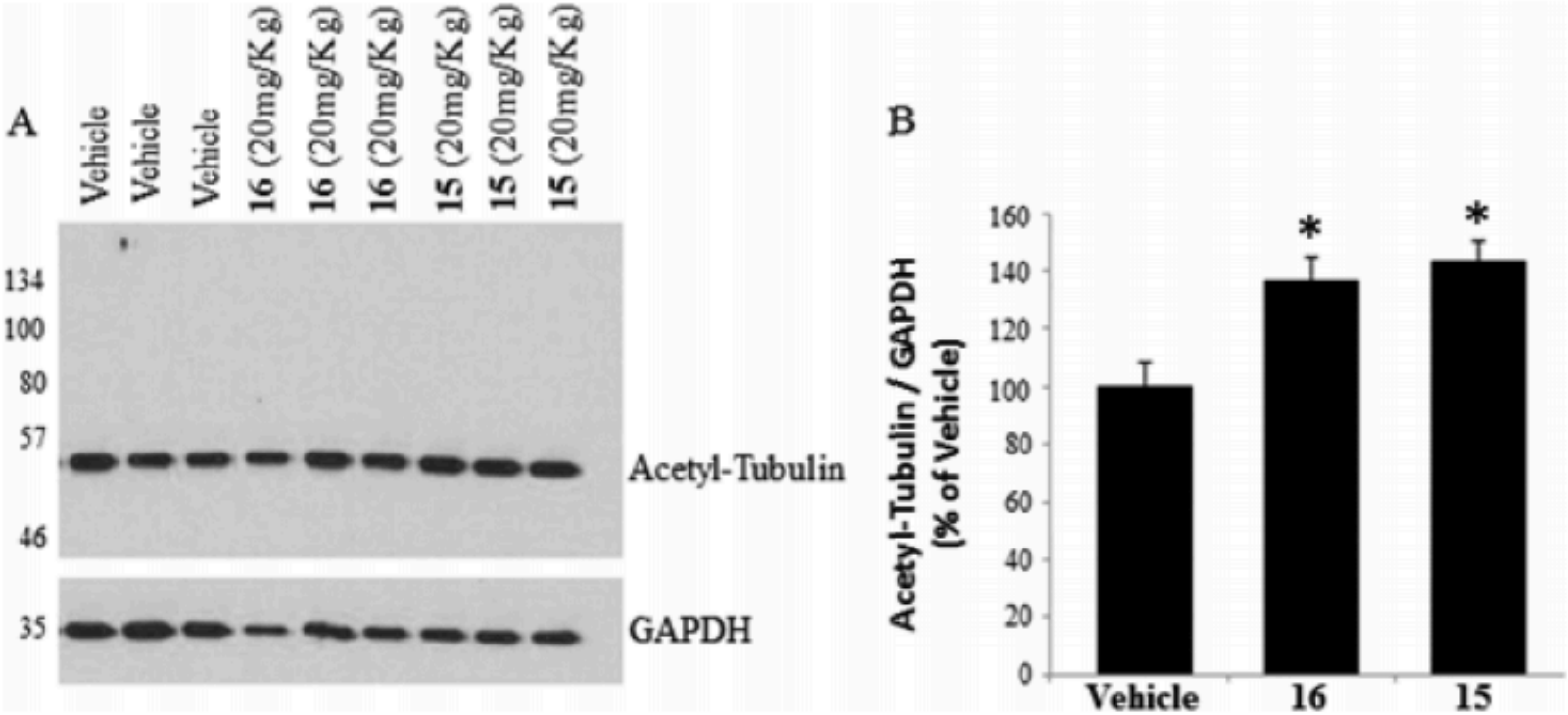






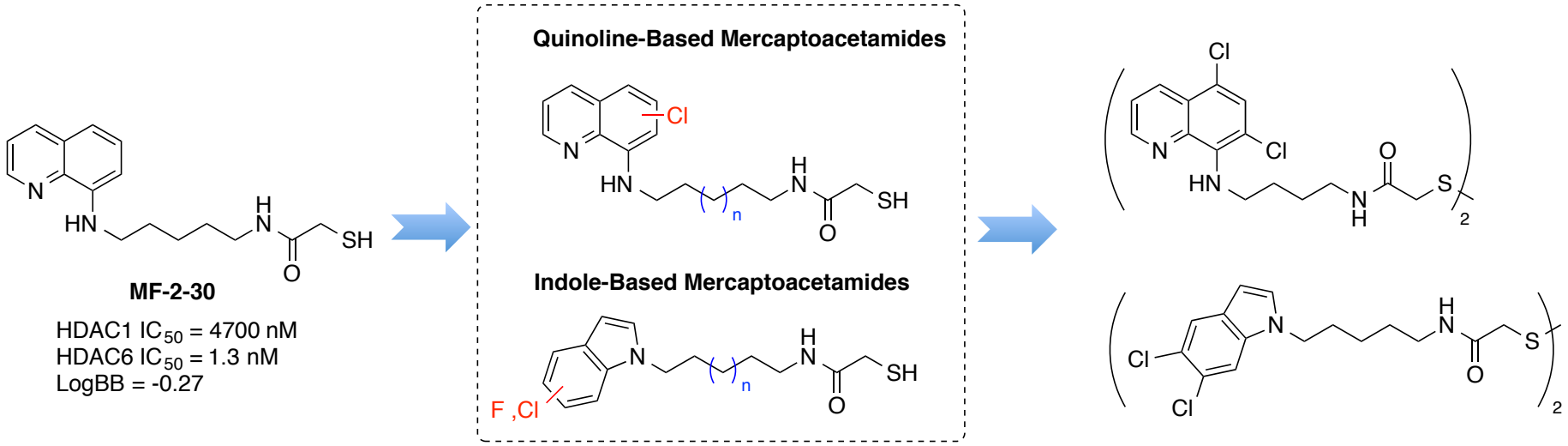
Further Evaluation of disulfide prodrugs 15 and 16

- Mice were treated with **15** and **16** at doses of 20 mg/kg, and the relative tubulin acetylation levels in the cerebral cortex were monitored.



- Both **15** and **16** increased tubulin acetylation levels in the cortex.
→ 40% increase for **15** vs 30% for **16**

Summary



- Developed potent HDAC6 inhibitors with excellent selectivity against HDAC1.
- New analogs have improved properties for brain penetration.
- The disulfide prodrugs for selected compounds showed *in vitro* (in HEK293 cells) and *in vivo* activities (in mouse cortex).
- These mercaptoacetamides are less likely to be burdened with the genotoxicity associated with hydroxamates.
- Possible candidates for further studies in animal models of CNS disorders.
- Selectivity against other isoforms?