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

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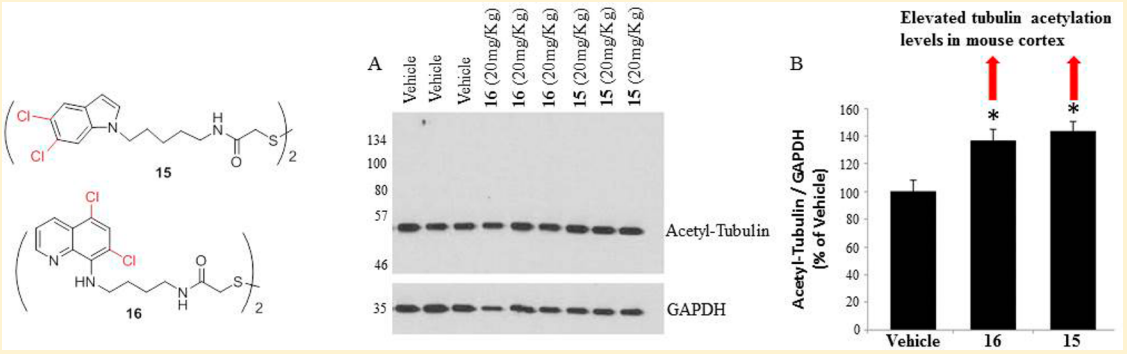
Supporting Information



ABSTRACT: A series of nonhydroxamate HDAC6 inhibitors were prepared in our effort to develop potent and selective compounds for possible use in central nervous system (CNS) disorders, thus obviating the genotoxicity often associated with the hydroxamates. Halogens are incorporated in the cap groups of the designed mercaptoacetamides in order to increase brain accessibility. The indole analogue **7e** and quinoline analogue **13a** displayed potent HDAC6 inhibitory activity (IC_{50}, 11 and 2.8 nM) and excellent selectivity against HDAC1. Both **7e** and **13a** together with their ester prodrug **14** and disulfide prodrugs **15** and **16** were found to be effective in promoting tubulin acetylation in HEK cells. The disulfide prodrugs **15** and **16** also released a stable concentration of **7e** and **13a** upon microsomal incubation. Administration of **15** and **16** *in vivo* was found to trigger an increase of tubulin acetylation in mouse cortex. These results suggest that further exploration of these compounds for the treatment of CNS disorders is warranted.

KEYWORDS: *HDAC6 inhibitors, mercaptoacetamides, brain permeable, CNS disorders*

Histone deacetylases (HDACs) are 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.[1,2] The HDACs together with the histone acetyltransferases (HATs) regulate the acetylation state of lysine residues present in the histone tails and thus the condensation status of chromatin, therefore modulating patterns of gene expression.[3] Because of their important role in epigenetic regulation, HDACs have been identified as therapeutic targets for the treatment of a wide range of diseases, including cancer, neurodegenerative diseases, arthritis, and others.[4,5]

The HDAC family includes 11 zinc-containing members, which are classified into four groups, class I (including HDACs 1, 2, 3, and 8), class IIa (including HDACs 4, 5, 7, and 9), class IIb (including HDACs 6 and 10), and class IV (including HDAC 11).[6−8] In these isozymes, HDAC6 has emerged as an attractive target for drug development due to its unique biology. Unlike other HDAC family members, HDAC6 primarily resides within the cytosol and is responsible for regulating the acetylation status of specific cytosolic proteins, such as α-tubulin, HSP90, cortactin, etc.[9] 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.[10] Selective inhibition of HDAC6 by small molecules promotes survival and regrowth of neurons following injury, and HDAC6 inhibition thus holds promise for the treatment of spinal cord injury, depression, and neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases.[11,12] Moreover, selective inhibition of HDAC6 does not lead to serious

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toxicity as has been observed with inhibition of the class I isoforms, making it a presumably a safer drug target.[13]

Currently, four HDACIs have been approved by the FDA, including suberoylanilide hydroxamic acid (Vorinostat), FK228 (Romidepsin), LBH-589 (Panobinostat), and PXD101 (Belinostat).[14] All of these compounds are pan-inhibitors (low HDAC isoform selectivity), and they are used exclusively for the treatment of various types of cancer. Many HDAC6 selective inhibitors have been developed, including ACY-1215, Tubacin, Tubastatin A, etc., and some of them are being evaluated in clinical trials (ACY-1215 and ACY-241).[14−16] However, a majority of currently reported HDAC6 selective inhibitors bear a hydroxamic acid moiety as the zinc binding group (ZBG). The hydroxamic acid group has in many cases proven to be genotoxic, thus leading to chromosomal aberrations, and as such represents a major hindrance for clinic development beyond oncology, especially for long-term treatments.[17] To identify more promising HDAC6 inhibitors, our group has initiated studies to develop HDAC6 selective inhibitors with a mercaptoacetamide group as the ZBG. Our previous efforts led to the discovery of MF-2−30, which is a potent and selective HDAC6 inhibitor (Figure 1).[18] Compared to the hydroxamic

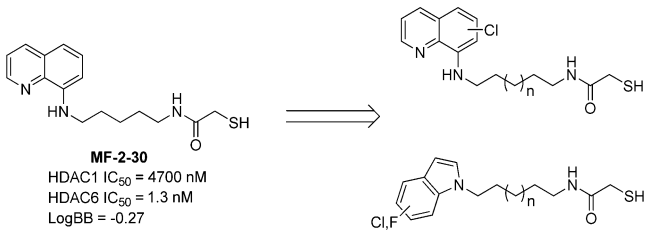


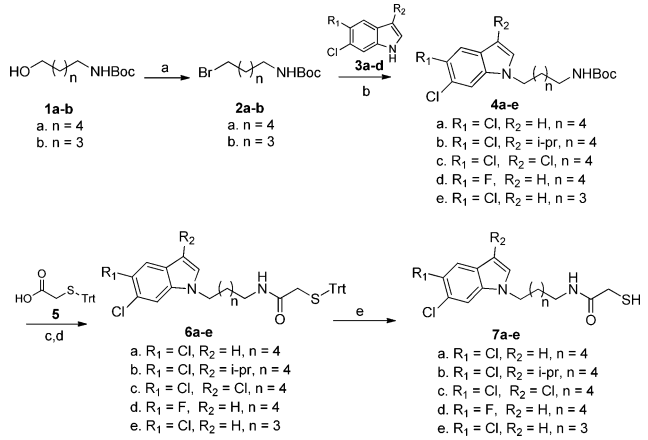
Figure 1. Structure of MF-2−30 and strategy of optimization.

acid inhibitors, the mercaptoacetamides are less likely to be genotoxic. Moreover, the mercaptoacetamides have also been reported to exhibit superior neuroprotective effects in cortical neurons compared to hydroxamates.[19] For these reasons, the mercaptoacetamides may represent possible candidates in the quest for HDAC6 inhibitors for treatment of neurological disorders.

MF-2−30 has a negative LogBB value (−0.27), and as such, this compound is predicted to have poor brain penetration properties, thus limiting its use in the treatment of CNS diseases. In this letter, we explore modifications of the quinoline cap group of MF-2−30 by incorporating halogen atoms to increase the compounds' lipophilicity and thus brain accessibility. We have also designed a series of halogenated-indole based mercaptoacetamides, which have been little explored in the prior literature. The results of these studies will facilitate the development of potent, selective HDAC6 inhibitors with appropriate physicochemical properties for treatment of CNS diseases.

The indole-based mercaptoacetamides (**7a−e**) were synthesized starting from *N*-Boc protected amino alcohols with varying chain lengths (**1a−d**, Scheme 1). The alcohols **1a−d** were first converted to the bromides **2a−d** with carbon tetrabromide and triphenylphosphine in good yields. Then, the bromides **2a−d** were used to alkylate various substituted indoles **3a−d** in the presence of sodium hydride to afford **4a−e**. The Boc protecting groups of **4a−e** were subsequently cleaved under acidic conditions, and the products were reacted with 2-(tritylthio)acetic acid (**5**) with the aid of 2-(1*H*-benzotriazol-1-

Scheme 1. Synthesis of Indole-Based Mercaptoacetamides 7a−e[a]

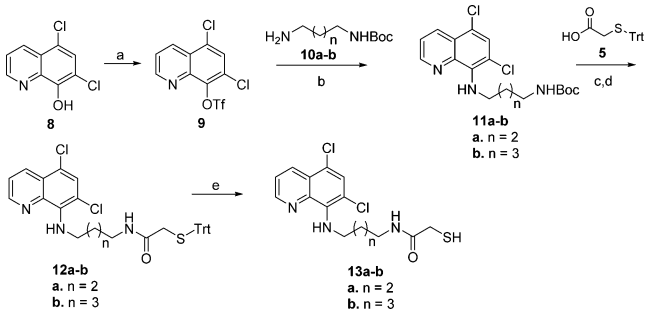


[a]Reagents and conditions: (a) CBr_4, PPh_3, THF, 83−100%; (b) **3a−d**, NaH, DMF, 60−88%; (c) HCl, MeOH; (d) **5**, HBTU, DIPEA, DMF, 22−38% over two steps; (e) TFA, Et_3SiH, DCM, 50−77%.

yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) to provide **6a−e**. Finally, the trityl groups of **6a−e** were removed with trifluoroacetic acid and triethylsilane to afford the final products **7a−e**.

The quinoline-based mercaptoacetamides **13a−b** were prepared from the hydroxyquinoline **8**, which was first treated with trifluoromethanesulfonic anhydride in the presence of triethylamine to afford the triflate **9** (Scheme 2). Then, the

Scheme 2. Synthesis of Quinoline-Based Mercaptoacetamides 13a−b[a]



[a]Reagents and conditions: (a) Tf_2O, TEA, DCM, 84%; (b) $Pd(OAc)_2$, BINAP, Cs_2CO_3, DCM, 87−88%; (c) MeOH, HCl; (d) **5**, HBTU, DIPEA, DMF, 36−40%; (e) TFA, Et_3SiH, DCM, 50−55%.

triflate **9** was subjected to a Buchwald−Hartwig cross coupling reaction with the amines **10a−b** to provide **11a−b** in good yield. The Boc protecting groups of **11a−b** were cleaved under acidic conditions followed by coupling with 2-(tritylthio)acetic acid (**5**) in the presence of HBTU to afford **12a−b**. Finally, the trityl groups were removed to provide the final products **13a−b**.

To synthesize the quinoline-based mercaptoacetamides **13c**, the hydroxyquinoline **8** was first reacted with the alcohol **1b** under Mitsunobu conditions to afford **11c** (Scheme 3). Then, the Boc group of **11c** was cleaved under acidic conditions followed by coupling with 2-(tritylthio)acetic acid (**5**) to afford **12c**. Finally, the trityl protecting group of **12c** was hydrolyzed to afford the product **13c** in good yield.

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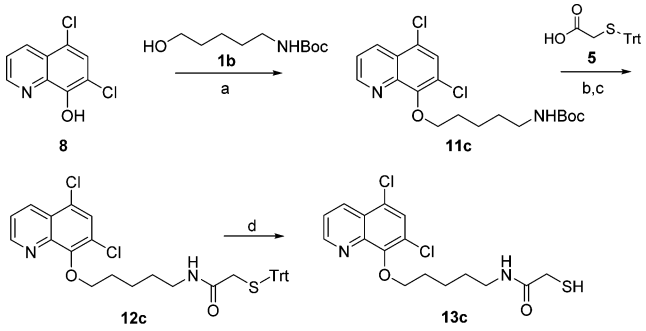
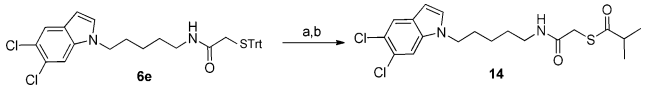
Scheme 3. Synthesis of Quinoline-Based Mercaptoacetamides 13c[a]



[a]Reagents and conditions: (a) Ph_3P, DEAD, THF, mw, 60 °C, 40 min, 80%; (b) TFA, DCM; (c) EDC, DMAP, TEA, DMF, 36% in two steps; (d) TFA, Et_3SiH, DCM, 86%.

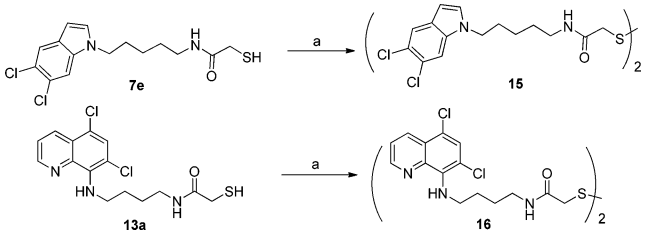
The sulfhydryl group of mercaptoacetamide is known to be unstable and can easily be oxidized. Thioester and disulfide prodrugs, in which the sulfhydryl group was transiently masked, are reported to have improved activity in cells and also *in vivo* studies.[20−22] Thus, prodrugs of **7e** and **13a** were prepared. For preparation of the indole bearing thioester **14**, compound **6e** was treated with trifluoroacetic acid and triethylsilane to cleave the trityl protecting group, followed by reaction with isobutyric anhydride in the presence of potassium carbonate to provide the ester **14** in good yield (Scheme 4). The disulfides **15**−**16** were prepared by oxidizing the corresponding thiols (**7e** and **13a**) with iodine in the presence of triethylamine (Scheme 5).

Scheme 4. Synthesis of Thiol Ester Prodrug 14[a]



[a]Reagents and conditions: (a) TFA, Et_3SiH, DCM; (d) $(i\text{-PrCO})_2O$, K_2CO_3, EtOAc, 79% over two steps.

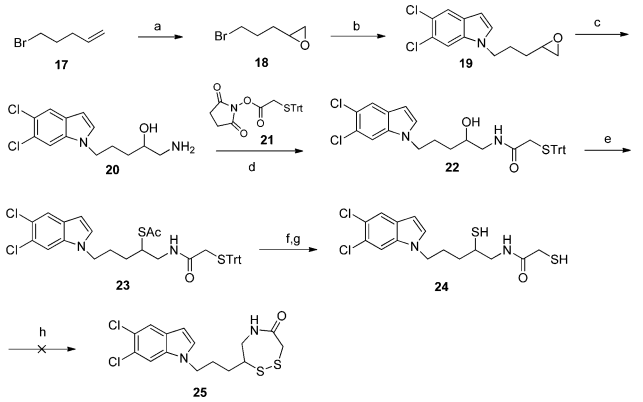
Scheme 5. Synthesis of Disulfide Prodrugs 15-16[a]



[a]Reagents and conditions: (a) TEA, I_2, $CHCl_3$, 73−79%.

In an effort to synthesize the cyclic disulfide **25**, the starting material, 5-bromo-1-pentene (**17**), was first oxidized with *m*-CPBA to afford the oxirane (**18**, Scheme 6). Without purification, the crude product of **18** was reacted with 5,6-dichloroindole (**3a**) in the presence potassium hydroxide to provide **19** in good yield. The oxirane ring was opened by heating **19** with ammonium hydroxide in a sealed tube at 120 °C, and the product **20** was reacted with **21** in hot acetonitrile to give compound **22** in excellent yield. Compound **22** underwent Mitsunobu reaction with thioacetic acid to afford the thioester **23**, which was hydrolyzed with sodium hydroxide and subsequently treated with TFA/triethylsilane to provide

Scheme 6. Synthesis of Dithiol 24[a]



[a]Reagents and conditions: (a) *m*-CPBA, DCM, 87%; (b) **3a**, KOH, DMF, 80 °C, 77%; (c) NH_4OH, CH_3CN, 120 °C; (d) **21**, DMAP, CH_3CN, 60 °C, 92% over two steps; (e) AcSH, DIAD, PPh_3, THF, 82%; (f) NaOH, MeOH; (g) Et_3SiH, TFA, DCM, 62% over two steps; (h) I_2, TEA, $CHCl_3$.

the dithiol **24**. In the last step, the cyclization of **24** to form **25** could not be achieved. Most likely, this is due to conformational reasons since cyclization requires the amide bond of **24** to adopt a cis conformation, which is known to exist in relatively low abundance (0.07−0.12%) in typical secondary amides.[23] Nonetheless, this attempted synthesis provided a unique bis-thiol for the HDAC6 assay.

The HDAC1 and -6 inhibitory activities of mercaptoacetamides **7a**−**e**, **13a**−**c**, and **24** are summarized in Table 1.

Table 1. HDAC Inhibitory Activity of Mercaptoacetamides 7a−e, 13a−c, and 24[a]

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.

Incorporation of two chlorine atoms into the quinoline ring (compound **13b**) decreased HDAC6 inhibition (IC_{50} 14.8 nM) when compared with MF-2−30 (1.3 nM). Replacing the secondary amine linker with an ether (compound **13c**) further reduced the HDAC6 inhibitory activity to an IC_{50} of 33.3 nM. Interestingly, shortening the linker length (from five carbons to four carbons) boosted the HDAC6 inhibitory activity, and the resulting compound **13a** displayed excellent potency (IC_{50}, 2.79 nM) and selectivity (2470-fold over HDAC1) toward HDAC6. This SAR diverges from our previous observations on quinoline based mercaptoacetamides in which analogues containing five-

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carbon linkers proved more potent than those with four-carbon linkers.[6]

Similar to quinoline, the indole caps also afforded potent and selective HDAC6 inhibitors, namely **7a**, **7d**, and **7e**. Generally, the SAR indicates that substituents (isopropyl, chlorine) at the 3-position of the indole ring are not favorable for HDAC6 inhibition, as compounds **7b** and **7c** were much less potent than **7a**. Replacing the chlorine atom in the indole's 5-position with fluorine (**7d**) had little effect on HDAC6 inhibition. In line with our observations for the quinoline analogues, shortening the linker from six carbon atoms to five carbon atoms increased HDAC6 inhibitory activities (**7a** vs **7e**). Introduction of the second thiol group in the linker region (compound **24**) resulted in a compound with relatively poor HDAC6 inhibition.

Among the quinoline and indole based mercaptoacetamides, compounds **7e** and **13a** are the most potent HDAC6 inhibitors in each series. Both **7e** and **13a** displayed excellent potency and selectivity for HDAC6 against HDAC1 and HDAC8 (data in Table S2). Moreover, both compounds also have significantly better LogBB values (0.37 and 0.17, respectively) than MF-2−30 (LogBB −0.27). Thus, these two compounds **7e** and **13a** represent candidates for further investigation.

To assess the cellular activities of these two HDAC6 inhibitors, HEK 293 cells were treated with mercaptoacetamides **7e** and **13a**, the ester prodrug **14**, and disulfide prodrugs **15** and **16**, and the acetylation of tubulin (the major substrate of HDAC6) and H3 (the substrate of HDAC1) was monitored (Figure 2 and Figure S1). The results indicate that both **7e** and

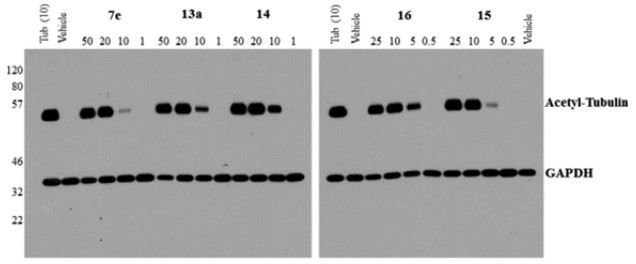

Figure 2. Western blots showing acetylated tubulin levels in HEK-293 cells following 24 h treatment with selected mercaptoacetamides at the indicated concentrations. Tubastatin at 10 μM is included on each blot as a positive control. The negative control lane on each blot is DMSO (left blot) or medium (right blot). GAPDH immunoreactivity is shown for each blot to normalize for lane loading, and no significant differences in unmodified total tubulin were observed between samples (data not shown).

13a induce tubulin acetylation in a dose-dependent manner. Compound **13a** appears to be more potent than **7e** in cells, as a clear induction of tubulin acetylation was observed with 10 μM of **13a**. This is consistent with their HDAC6 inhibitory activities (IC_{50} 2.8 nM compared to 11 nM). Interestingly, the ester prodrug **14** showed improved cellular activity compared to **7e**, possibly due to its increased cell penetration. In addition, both disulfide prodrugs **15** and **16** displayed comparable efficacy to the corresponding mercaptoacetamides **7e** and **13a**. Consistent with HDAC6 selective inhibition, **7e**, **13a**, **14**, and **15** displayed only a modest increase in H3 acetylation, which was similar in magnitude to that seen with Tubastatin A (Figure S1). The disulfide prodrug **16** did display a moderate effect on H3 acetylation, but this effect remained well below what was observed for acetyl-tubulin at the same dose (Figure S1).

To investigate the compounds' metabolic stabilities *in vivo*, microsomal stabilities were measured for selected mercaptoacetamides and also the prodrugs, and the results are summarized in Table 2. The indole **7e** showed limited stability

Table 2. 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.

in both human and mouse liver microsomes ($t_{1/2}$, 35.2 and 41.3 min, respectively). Compound **7b** offered slightly improved metabolic stability compared to **7e**, likely due to the incorporation of an isopropyl group in the metabolically active site of the indole ring. The quinoline **13a** showed good stability in mouse liver microsomes, but the half-life in human liver microsomes is short. Compound **13b** has a half-life comparable to that of **7e** in both human and mouse liver microsomes.

The ester prodrug **14** was found to be very unstable toward hydrolysis and almost quantitatively converted to **7e** when exposed to microsomes. This result is consistent with the previous reports that thioesters are usually metabolically unstable in microsomes ($t_{1/2}$, <3 min).[22] The disulfide prodrugs **15** and **16** afforded compounds with better stability than the ester, having half-lives (20.7−49.5 min) slightly shorter than or comparable to those of the corresponding mercaptoacetamides. In fact, we observed that the disulfides **15** and **16** are readily converted to the corresponding mercaptoacetamides in microsomes. As shown in Figure 3 and Figure S2, the incubation of disulfide **15** or **16** with both human and mouse liver microsomes resulted in the release of **7e** or **13a**, which reached a stable concentration after 15 min.

Since both disulfide prodrugs **15** and **16** effectively induce tubulin acetylation in cells and are capable of releasing a stable

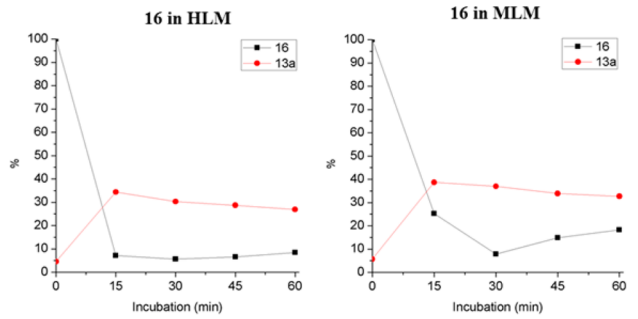

Figure 3. Metabolism of disulfide prodrug **16** in pooled human (HLM) and mouse liver microsomes (MLM), with the release of mercaptoacetamides **13a**. The percentage of released **13a** was calculated as (actual concentration of **13a**)/(2 × initial concentration of **16**).

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concentration of corresponding mercaptoacetamides (**7e** and **13a**) in microsomes, they were selected for further studies *in vivo*. Mice were treated with **15** or **16** at doses of 20 mg/kg, and the relative tubulin acetylation levels in the cerebral cortex were monitored (Figure 4). Both **15** and **16** significantly increased

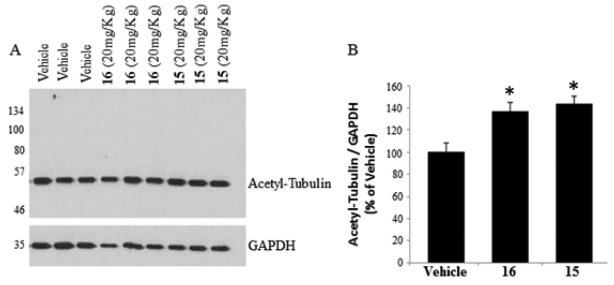

Figure 4. Left: Western blot illustrating tubulin acetylation levels in mouse cortex following treatment with **15** and **16**. For this assay, mice (n = 3) were injected at 18:00 with 20 mg/kg drug IP, then given a second 20 mg/kg dose in the morning at 10:00. Mice were sacrificed and tissues harvested 6 h later. GAPDH immunoreactivity is shown to normalize for protein loading per lane. Right: histogram showing the cumulative results expressed as the densitometric ratio of acetyltubulin to GAPDH per lane, normalized as a percentage of vehicle treated mice. * denotes a significant difference from vehicle levels ($p < 0.05$; one-way ANOVA with Bonferroni posthoc test).

tubulin acetylation levels in the cortex (40% increase for **15** vs 30% for **16**). These results demonstrate that administration of either disulfide prodrug effectively delivers the corresponding mercaptoacetamide to the brain at concentrations sufficient to elicit changes in acetyl-tubulin levels. Whether these changes capture the peak effect in the brain or their respective duration of effect *in vivo* will require further investigation.

In this report, we have identified several improved mercaptoacetamides that were designed for possible use in the treatment of CNS disorders. Halogens were incorporated into the cap group in order to increase lipophilicity and brain accessibility. Several of the prepared analogues, in particular **7e** and **13a**, displayed potent HDAC6 inhibitory activity and excellent selectivity against HDAC1. Prodrugs (an ester and disulfides) were also prepared from **7e** and **13a**. The disulfide prodrugs **15** and **16** were found to release a stable concentration of the active mercaptoacetamides **7e** and **13a** upon incubation with microsomes. Both mercaptoacetamides **7e** and **13a** and their prodrugs **14**, **15**, and **16**, induced a dose-dependent increase in acetylated tubulin in HEK293 cells *in vitro*. Administration of the disulfide prodrugs **15** and **16** *in vivo* also triggered a clear increase of acetylated tubulin in mouse cortex. Since these mercaptoacetamides are less likely to be burdened with the genotoxicity associated with hydroxamates (a consequence of their ability to undergo the Lossen rearrangement to afford an electrophilic isocyanate), the present results suggest that these compounds are possible candidates for further studies in animal models of CNS disorders.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00012.

Experimental details for chemistry and biological assays (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CNS, central nervous system; HDAC, histone deacetylase; HAT, histone acetyltransferase; HLM, human liver microsome; MLM, mouse liver microsome

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