Discovery of the First Histone Deacetylase 6/8 Dual Inhibitor.

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · MAY 2013

Impact Factor: 5.45 · DOI: 10.1021/jm400390r · Source: PubMed

Journal of Medicinal Chemistry

Brief Article

pubs.acs.org/jmc

Discovery of the First Histone Deacetylase 6/8 Dual Inhibitors

David E. Olson, Florence F. Wagner, Taner Kaya, Jennifer P. Gale, Nadia Aidoud, Emeline L. Davoine, Fanny Lazzaro, Michel Weïwer, Yan-Ling Zhang, and Edward B. Holson*

Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, Massachusetts 02142, United States

Supporting Information

ABSTRACT: We disclose the first small molecule histone deacetylase (HDAC) inhibitor (**3**, BRD73954) capable of potently and selectively inhibiting both HDAC6 and HDAC8 despite the fact that these isoforms belong to distinct phylogenetic classes within the HDAC family of enzymes. Our data demonstrate that meta substituents of phenyl hydroxamic acids are readily accommodated upon binding to HDAC6 and, furthermore, are necessary for the potent inhibition of HDAC8.

INTRODUCTION

Histone deacetylases (HDACs) are enzymes that catalyze the removal of acetyl groups from the ϵ-nitrogen of lysine residues on histone as well as nonhistone proteins.[1] Such posttranslational modifications can regulate numerous cellular processes, including gene expression,[2] making these enzymes attractive targets for the treatment of cancer[3] as well as psychiatric,[4] metabolic,[5] and infectious diseases.[6] These enzymes can be divided into the NAD^+-dependent sirtuins (class III) and the Zn-dependent HDACs. The latter can be divided into three classes, one of which (class II) is further subdivided into two subclasses (Figure 1): class I (HDACs 1, 2, 3, and 8), class IIa (HDACs 4, 5, 7, and 9), class IIb (HDACs 6 and 10), and class IV (HDAC11).[7]

Currently, many of the clinically relevant HDAC inhibitors (e.g., LBH-589 (Panobinostat), SAHA (Vorinostat)) are neither class nor isoform selective. LBH-589 (a hydroxamic acid) is a prototypical example of a multi-isoform inhibitor demonstrating potent inhibition across HDAC classes I, IIa and IIb (Figure 1). In contrast, MS-275 (Entinostat, an *ortho*-aminoanilide) is an example of a subclass I selective inhibitor with potent activity toward HDACs 1, 2, and 3 only. While these agents demonstrate clinical efficacy toward select neoplasms, all exhibit dose-dependent toxicity such as nausea, fatigue, and thrombocytopenia.[8] Furthermore, it has been demonstrated that the concurrent inhibition of HDACs 1 and 2 contributes to myelosuppression via a mechanism based toxicity.[9] Selective inhibition of only the desired HDAC isoforms has been hypothesized to yield drugs that elicit fewer side effects and are better tolerated.[10] Until now, medicinal chemists have focused on developing compounds selective for either a few isoforms within the same class (e.g., HDACs 1, 2, and 3) or a single isoform (e.g., HDAC6 or HDAC8).[11] A representative set of inhibitors selective for different combinations of HDAC isoforms is shown in Figure 1. Ideally, the development of a "toolkit" of inhibitors encompassing the various permutations of selectivities within and across the HDAC classes would: (1) refine our structural understanding of the similarities and differences between these enzymes, (2) further biological investigations into the functions of individual isoforms, and (3) potentially provide better tolerated therapeutic agents.

Herein, we report our efforts toward this endeavor and disclose that it is possible to achieve simultaneous inhibition of

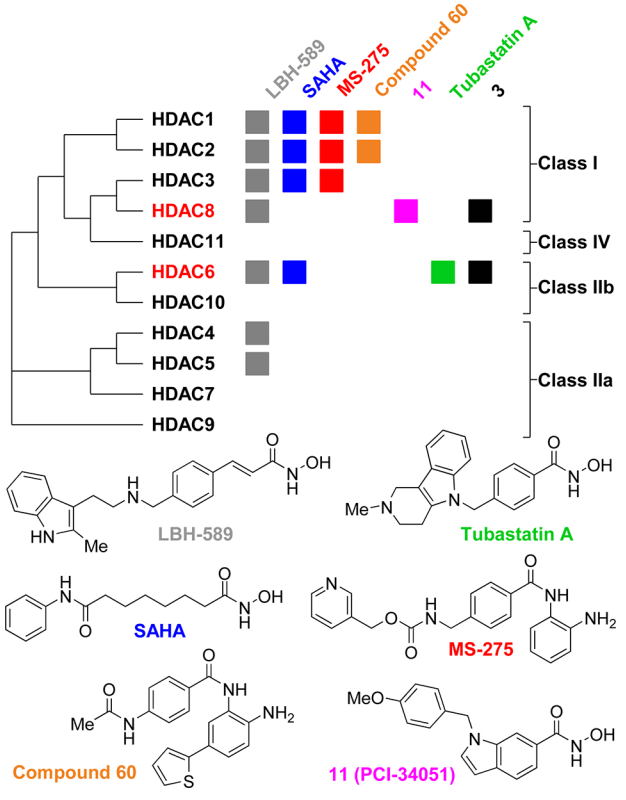


Figure 1. Binding profiles and chemical structures of HDAC inhibitors. Colored blocks denote IC_{50} values <300 nM. Evolutionary relationships between the various Zn-dependent HDACs are shown. The lengths of the branches are not proportional to evolutionary distance (adapted from ref 2b).

Received: March 16, 2013
Published: May 14, 2013

HDACs 6 and 8, two isoforms with disparate sequence homology belonging to distinct classes. Dual inhibition of these two enzymes might prove beneficial in a number of indications. For instance, both HDAC6 and HDAC8 have been implicated in breast cancer metastasis,[3a] and we speculate that inhibition of both isoforms could potentially abrogate any functional redundancy exhibited by these enzymes. Furthermore, inhibition of HDAC8 has shown promise as an effective strategy for treating neuroblastoma,[12] while inhibition of HDAC6 has proven useful for treating a variety of cancers due to its effects on the ubiquitin pathway as a single agent and in combination therapies.[13] Dual inhibition of these two isoforms could provide a larger therapeutic window and have beneficial additive or synergistic effects in neuroblastoma and/or related neoplastic disorders.

Recently, we reported our efforts toward developing ligand-efficient selective inhibitors of HDAC6 such as **1**.[14] We discovered that small capless phenyl hydroxamic acids were potent and selective inhibitors of HDAC6. Furthermore, we observed that a variety of extended para substituents such as the phenethyl carboxamide of compound **2** were not only tolerated but also did not reduce the selectivity of these inhibitors for HDAC6 (>100-fold versus HDACs 2, 4, and 8). While exploring positional and structural modifications to these phenyl hydroxamic acids, we discovered that transposing the phenethyl carboxamide substituent from the para (**2**) to the meta (**3**) position not only retained HDAC6 inhibitory activity (IC_{50} = 0.036 μM) but resulted in a 10-fold increase in potency for HDAC8 (IC_{50} = 0.12 μM) with a concomitant reduction in potency for HDAC2 (Table 1). To the best of our knowledge, compound **3** (BRD73954) represents the first HDAC inhibitor capable of selectively and potently inhibiting HDAC6 (class IIb) and HDAC8 (class I) simultaneously.[15]

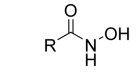
Next, to understand this unique *interclass* structure–activity relationship, we probed the effect of different meta substitutions on potency and selectivity. We synthesized a series of compounds by varying the linking motifs at this position as well as the physicochemical and steric properties of these molecules (Table 1).[16] A comparison between the phenethyl carboxamide **3** and the 3-pyridylethyl carboxamide **4** suggests the importance of the substitutent's hydrophobicity for achieving potent inhibition of HDAC8, as the less hydrophobic compound **4** exhibited reduced potency for HDAC8 (IC_{50} = 0.42 μM) but not for HDAC6 (IC_{50} = 0.059 μM).

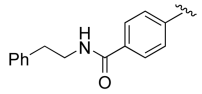
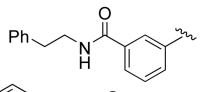
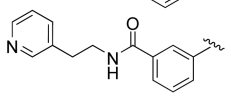
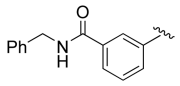
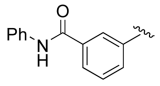
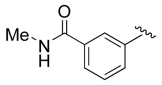
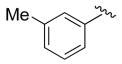
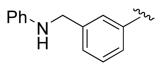
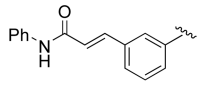
Next, we varied the linker length to examine the effects on HDAC6 and 8 inhibition as well as to define the minimal pharmacophoric elements necessary for dual inhibition of these isoforms. Utilizing an amide linkage, we shortened the meta substituent of compound **3** by one (benzyl carboxamide, **5**) or two carbons (phenyl carboxamide, **6**) and observed little effect on potency for HDAC6 (IC_{50} = 0.034 and 0.057 μM, respectively) or HDAC8 (IC_{50} = 0.21 and 0.11 μM, respectively). Moreover, both compound **5** and **6** remained highly selective, with a >50-fold preference for HDAC6 and 8 as compared to HDACs 2 and 4. Further truncation of this series to the simple methyl carboxamide **7** resulted in a significant loss in potency for HDAC6 (IC_{50} = 2.5 μM) and HDAC8 (IC_{50} = 14 μM). However, the simple meta methyl substituted compound **8** displayed increased potency and selectivity for HDAC6 (IC_{50} = 0.65 μM) resembling the overall binding profile of the phenyl hydroxamic acid **1**.[14]

Finally, after observing the surprisingly poor inhibition displayed by the methyl carboxamide **7** toward HDACs 6 and 8, we turned our attention to alternate meta linking motifs. Removal of the carbonyl group in **6** provided the methylene linked aniline **9**, which displayed a significant loss in potency and selectivity. Ultimately, we replaced the four-atom spacer in **3** with the highly rigid meta cinnamide linkage to provide compound **10**. Compound **10** displayed excellent potency for HDAC6 (IC_{50} = 0.021 μM) and HDAC8 (IC_{50} = 0.037 μM) as well as greater than 130-fold selectivity versus HDACs 2 and 4.

To ascertain the selectivities of **3** and **10** across the broader family of HDAC isoforms, we profiled these compounds against HDACs 1–9[16] (Table 2). Compounds **3** and **10** maintained excellent selectivity toward HDAC6 and HDAC8 as compared to the other class I and II HDACs tested, as these compounds were 75- and 130-fold less potent for the next closest isoforms, respectively. These selectivities are comparable to those exhibited by tubastatin A[11e] and **11** (PCI-34051),[11f] state-of-the-art HDAC6- and HDAC8-selective inhibitors, respectively. Although structurally simple, compounds **3** and **10** exhibit remarkable selectivity within this family of closely related zinc hydrolases, and this selectivity may translate to more distantly related metalloenzymes. Compound **10**

Table 1. IC_{50} Values for HDACs 2, 4, 6, and 8



Compound	R Group	HDAC Isoform Inhibition IC50 (μM)[a] 2	4	6	8
1[b]		7.9 ± 0.10	>33	0.12 ± 0.013	1.9 ± 0.36
2[b]		0.61 ± 0.035	>33	0.004 ± 0.0001	1.2 ± 0.14
3		9.0 ± 6.6	>33	0.036 ± 0.018	0.12 ± 0.064
4		20 ± 4.3	>33	0.059 ± 0.028	0.42 ± 0.18
5		11 ± 1.6	>33	0.034 ± 0.014	0.21 ± 0.11
6		30 ± 11	>33	0.057 ± 0.021	0.11 ± 0.047
7		>33	>33	2.5 ± 1.6	14 ± 10
8		>33	>33	0.65 ± 0.52	3.3 ± 1.7
9		>33	>33	1.3 ± 0.61	1.7 ± 0.87
10		4.8 ± 1.7	14 ± 0.71	0.021 ± 0.002	0.037 ± 0.012

[a]Values are the average of at least two experiments. Data are shown as IC_{50} values in μM ± standard deviation. Compounds were tested using a 12-point dose curve with 3-fold serial dilution starting from 33 μM.[17] [b]Data were taken from ref 14. Data for additional compounds are shown in the Supporting Information.

dx.doi.org/10.1021/jm400390r | J. Med. Chem. 2013, 56, 4816–4820

Table 2. IC_{50} Values for HDACs 1−9

	HDAC Isoform Inhibition, IC_{50} (μM)[a]								
Compound	HDAC1	HDAC2	HDAC3	HDAC4	HDAC5	HDAC6	HDAC7	HDAC8	HDAC9
SAHA	0.005 ± 0.002	0.018 ± 0.009	0.004 ± 0.002	>33	11 ± 4.6	0.002 ± 0.001	>33	1.0 ± 0.70	>33
3	12 ± 2.0	9.0 ± 6.6	23 ± 10	>33	>33	0.036 ± 0.018	13 ± 1.7	0.12 ± 0.064	>33
10	6.1 ± 0.33	4.8 ± 1.7	18 ± 0.35	14 ± 0.71	26 ± 5.2	0.021 ± 0.002	8.4 ± 1.0	0.037 ± 0.012	12 ± 6.7

[a]Values are the average of at least two experiments. Data are shown as IC_{50} values in μM ± standard deviation. Compounds were tested using a 12-point dose curve with 3-fold serial dilution starting from 33.3 μM.[17]

represents the most potent and selective dual inhibitor of HDACs 6 and 8 reported to date.

Intrigued by the ability of a small molecule ligand to preferentially bind phylogenetically dissimilar isoforms, we performed molecular docking simulations of compound **3** into model structures of HDAC6 and HDAC8. We chose compound **3** with the meta amide linkage to rationalize the observed structure−activity relationships of a larger subset of compounds (see compounds **3**−**6**). For HDAC6, we generated a homology model using a multiple mapping method with multiple templates (HDACs 4 and 7) as described by the Fiser group through an automated web server (M4T Server ver 3.0).[17] For HDAC8, we used the crystal structure reported by Somoza and colleagues in 2004 (see Supporting Information for details, PDB 1VKG.A).[18] For both enzymes, docking runs using induced-fit models were performed with Glide XP[19] followed by ligand minimization in MOE (Chemical Computing Group, Inc.).

We performed molecular docking simulations with compound **3** in HDAC6, and the results are shown in Figure 2A,C. Compound **3** adopts an optimal binding pose in the catalytic domain and forms key H-bonds with His130, His131, and Tyr302. These residues are critical as they stabilize the inhibitor in a binding conformation that allows for efficient zinc atom chelation. This coordination complex is further stabilized by hydrophobic interactions between the phenyl linkage of compound **3** and residues Phe140, Phe200, and Leu269 (Figure 2C). Additionally, the meta linked phenethyl carboxamide of **3** extends from this shallow binding domain into solvent space devoid of close contacts with the protein surface. This is consistent with the observed SAR of compounds **3**−**6**, which demonstrate no significant change in HDAC6 potency. In addition, the shallow and more accessible binding domain in HDAC6 is reminiscent of other computational reports on this isoform.[11e,20]

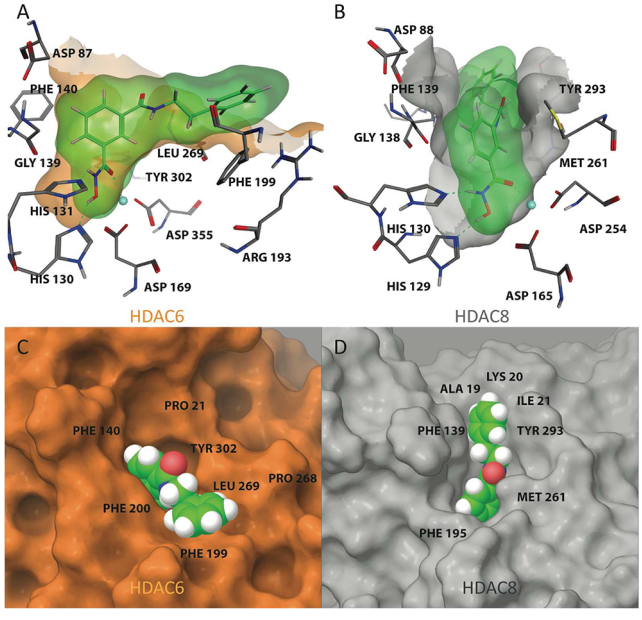


Figure 2. Compound **3** docked into HDACs 6 (A,C) and 8 (B,D). The enzymes in (C) and (D) are aligned, demonstrating that **3** occupies distinct subpockets in HDAC6 and HDAC8.

Following the same docking procedures in HDAC8, compound **3** achieves optimal chelation geometry and forms key H-bonds with His129 and His130 (Figure 2B). In contrast to the binding mode observed in HDAC6, the *meta*-phenethyl carboxamide in **3** resides in a deep secondary pocket whose boundaries are defined by Tyr293, Phe139, and Lys20 (Figure 2D). This secondary hydrophobic pocket (formed via a conformational change in Phe139) has been previously described to accommodate similar "L-shaped" ligands such as **11**,[11f] a potent and selective inhibitor of HDAC8, as well as other meta-substituted hydroxamic acids.[21] The role of this secondary binding pocket in HDAC8 affinity is evident when comparing the binding affinity of compound **1** (HDAC8, IC_{50} = 1.9 μM) versus compound **3** (HDAC8, IC_{50} = 0.12 μM), which extends into this space (Figure 2B,D). In HDAC8, the meta-substituent forms key hydrophobic interactions and extends into a well-defined secondary pocket leading to increased potency (cf. compound **1** vs **3**), whereas in HDAC6, this substituent is exposed to solvent, a finding that is consistent with the observed SAR.

Next, we attempted to validate the observed biochemical potencies and selectivities of these HDAC6/8-selective inhibitors in a cellular context by examining their HDAC6 activity.[22] HeLa cells were treated with inhibitors for 48 h at 10 μM, and the resulting acetylation changes in α-tubulin (a known substrate for HDAC6)[23] and histone H3 (a known substrate for HDACs 1, 2, and 3) were assessed (Figure 3).[1b] While treatment with compounds **3** and **10** resulted in a robust increase in α-tubulin acetylation, no change in the acetylation state of H3 was observed, which is consistent with the ability of these compounds to inhibit HDAC6 but not HDACs 1, 2, or 3 in the biochemical assay. In contrast, increases in the acetylation of α-tubulin as well as H3 were observed when the cells were treated with the nonselective inhibitor SAHA.

dx.doi.org/10.1021/jm400390r | J. Med. Chem. 2013, 56, 4816−4820

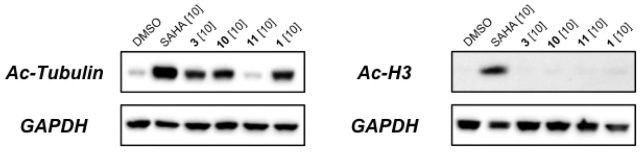


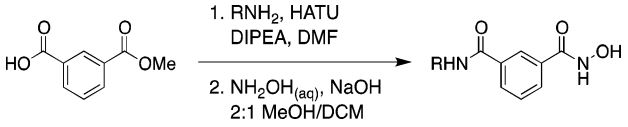
Figure 3. Treatment of HeLa cells for 48 h with compounds **3** and **10** increased Ac-tubulin but not Ac-H3. Concentrations in μM are shown in brackets. GAPDH was used as a loading control.

CONCLUSIONS

We have discovered a set of small molecules that are selective for only two HDAC isoforms belonging to distinct phylogenetic classes. Our biochemical and computational data provide evidence that evolutionary relationships between HDACs cannot always predict molecular recognition or ligand binding similarities. Potency and selectivity for HDAC6 seems to be driven by close contacts between the linking phenyl motif and an optimal hydroxamic acid chelating geometry at the zinc coordination center. The meta-substituents are primarily oriented toward solvent and play a marginal role in binding. However, for HDAC8, potency and selectivity seem to be strongly dependent on the presence of a hydrophobic meta-substituent binding in a well-defined secondary pocket. These dual HDAC6/8-selective inhibitors are active in cells, and the results reported here will help guide future efforts toward developing novel HDAC inhibitors with optimized selectivity profiles.

EXPERIMENTAL SECTION

Representative procedure for the synthesis of **3**–**10**:



To a solution of 3-(methoxycarbonyl)benzoic acid (1.1 equiv) in DMF (0.1 M) was sequentially added 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) (1.5 equiv), *N*,*N*-diisopropylethylamine (3.0 equiv), and amine (1.0 equiv). The reaction was monitored by LCMS. After completion, the reaction was quenched with saturated $NaHCO_{3(aq)}$ and extracted with EtOAc. The organic extracts were washed with water and then brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The resulting solid was purified by flash chromatography on silica gel. Next, the purified material was dissolved in a 1:2 mixture of DCM/MeOH (0.1 M). The resulting solution was cooled to 0 °C before the addition of 50 wt % aqueous hydroxylamine (30 equiv) followed by 1 M $NaOH_{(aq)}$ (10 equiv). The reaction was monitored by LCMS. After completion, the solvent was removed under reduced pressure and the resulting solid was dissolved in water. The pH was adjusted to 7 with 1N $HCl_{(aq)}$ and the product precipitated. Typically, no further purification was necessary; however, preparatory HPLC was used in select cases.

ASSOCIATED CONTENT

Supporting Information

Analytical data for all final compounds as well as procedures for the HDAC inhibition assay, cell culture experiments, and computational chemistry and PDB files. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: 617-714-7454. E-mail: edholson@broadinstitute.org.

Author Contributions

The manuscript was written through contributions of all authors. All authors have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Stephen Johnston for analytical/purification support and John McGrath for compound management support. We also thank Josh Ketterman for help with cell culture experiments as well as Katherine Foley and Anna McGlashen for assisting in synthetic chemistry efforts. Funding was provided by the Stanley Medical Research Institute.

ABBREVIATIONS USED

HDACs, histone deacetylases; SAHA, suberoylanilide hydroxamic acid; H3, histone H3

REFERENCES

(1) (a) Haberland, M.; Montgomery, R. L.; Olson, E. N. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nature Rev. Genet.* **2009**, *10*, 32–42. (b) Fass, D. M.; Kemp, M. M.; Schroeder, F. A.; Wagner, F. F.; Wang, Q.; Holson, E. B. Histone acetylation and deacetylation. In *Epigenetic Regulation and Epigenomics: Advances in Molecular Biology and Medicine*; Wiley-VCH Verlag & Co. KGaA: Weinheim, Germany, 2012; pp 515–561.

(2) (a) Marks, P. A.; Richon, V. M.; Miller, T.; Kelly, W. K. Histone deacetylase inhibitors. *Adv. Cancer Res.* **2004**, *91*, 137–168. (b) de Ruijter, A. J. M.; Van Gennip, A. H.; Caron, H. N.; Kemp, S.; Van Kuilenburg, A. B. P. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* **2003**, *370*, 737–749. (c) Glaser, K. B.; Staver, M. J.; Waring, J. F.; Stender, J.; Ulrich, R. G.; Davidsen, S. K. Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. *Mol. Cancer Ther.* **2003**, *2*, 151–163. (d) Spange, S.; Wagner, T.; Heinzel, T.; Krämer, O. H. Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *Int. J. Biochem. Cell Biol.* **2009**, *41*, 185–198.

(3) (a) Park, S. Y.; Jun, J. A.; Jeong, K. J.; Heo, H. J.; Sohn, J. S.; Lee, H. Y.; Park, C. G.; Kang, J. Histone deacetylases 1, 6 and 8 are critical for invasion in breast cancer. *Oncol. Rep.* **2011**, *25*, 1677–1681. (b) Acharya, M. R.; Sparreboom, A.; Venitz, J.; Figg, W. D. Rational development of histone deacetylase inhibitors as anticancer agents: a review. *Mol. Pharmacol.* **2005**, *68*, 917–932.

(4) (a) Gray, S. G. Epigenetic treatment of neurological disease. *Epigenomics* **2011**, *3*, 431–450. (c) Graff, J.; Tsai, L.-H. The potential of HDAC inhibitors as cognitive enhancers. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 311–330.

(5) (a) Gluckman, P. D.; Hanson, M. A.; Buklijas, T.; Low, F. M.; Beedle, A. S. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nature Rev. Endocrinol.* **2009**, *5*, 401–408. (b) Mihaylova, M. M.; Vasquez, D. S.; Ravnskjaer, K.; Denechaud, P.-D.; Yu, R. T.; Alvarez, J. G.; Downes, M.; Evans, R. M.; Montminy, M.; Shaw, R. J. Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. *Cell* **2011**, *145*, 607–621.

(6) (a) Rotilli, D.; Simonetti, G.; Savarino, A.; Palamara, A. T.; Migliaccio, A. R.; Mai, A. Non-cancer uses of histone deacetylase inhibitors: effects on infectious diseases and beta-hemoglobinopathies. *Curr. Top. Med. Chem.* **2009**, *9*, 272–291. (b) Andrews, K. T.; Haque,

A.; Jones, M. K. HDAC Inhibitors in Parasitic Diseases. *Immunol. Cell Biol.* **2012**, *90*, 66−77.
(7) Gregoretti, I. V.; Lee, Y.-M.; Goodson, H. V. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J. Mol. Biol.* **2004**, *338*, 17−31.
(8) Fraczek, J.; Vanhaecke, T.; Rogiers, V. Toxicological and metabolic considerations for histone deacetylase inhibitors. *Expert Opin. Drug Metab. Toxicol.* **2013**, *9*, 441−457.
(9) Wilting, R. H.; Yanover, E.; Heideman, M. R.; Jacobs, H.; Horner, J.; van der Torre, J.; DePihnho, R. A.; Dannenberg, J.-H. Overlapping functions of HDAC1 and HDAC2 in cell cycle regulation and haematopoiesis. *EMBO J.* **2010**, *29*, 2586−2597.
(10) (a) Ononye, S. N.; van Heyst, M.; Falcone, E. M.; Anderson, A. C.; Wright, D. L. Toward isozyme-selective inhibitors of histone deacetylase as therapeutic agents for the treatment of cancer. *Pharm. Pat. Analyst* **2012**, *1*, 207−221. (b) Balasubramanian, S.; Verner, E. V.; Buggy, J. J. Isoform-specific histone deacetylase inhibitors: the next step? *Cancer Lett.* **2009**, *280*, 211.
(11) (a) Bieliauskas, A. V.; Pflum, M. K. H. Isoform-selective histone deacetylase inhibitors. *Chem. Soc. Rev.* **2008**, *37*, 1402−1413. (b) Bradner, J. E.; West, N.; Grachan, M. L.; Greenberg, E. F.; Haggarty, S. J.; Warnow, T.; Mazitschek, R. Chemical phylogenetics of histone deacetylases. *Nature Chem. Biol.* **2010**, *6*, 238−243. (c) Methot, J. L.; Chakravarty, P. K.; Chenard, M.; Close, J.; Cruz, J. C.; Dahlberg, W. K.; Fleming, J.; Hamblett, J. E.; Hamill, J. E.; Harrington, P.; Harsch, A.; Heidebrecht, R.; Hughes, B.; Jung, J.; Kenific, C. M.; Kral, A. M.; Meinke, P. T.; Middleton, R. E.; Ozerova, N.; Sloman, D. L.; Stanton, M. G.; Szewczak, A. A.; Tyagarajan, S.; Witter, D. J.; Secrist, J. P.; Miller, T. A. Exploration of the internal cavity of histone deacetylase (HDAC) with selective HDAC1/HDAC2 inhibitors (SHI-1:2). *Bioorg. Med. Chem. Lett.* **2008**, *18*, 973−978. (d) Moradei, O. M.; Mallais, T. C.; Frechette, S.; Paquin, I.; Tessier, P. E.; Leit, S. M.; Fournel, M.; Bonfils, C.; Trachy-Bourget, M.-C.; Liu, J.; Yan, T. P.; Lu, A.-H.; Rahil, J.; Wang, J.; Lefebvre, S.; Li, Z.; Vaisburg, A. F.; Besterman, J. M. novel aminophenyl benzamide-type histone deacetylase inhibitors with enhanced potency and selectivity. *J. Med. Chem.* **2007**, *50*, 5543−5546. (e) Butler, K. V.; Kalin, J.; Brochier, C.; Vistoli, G.; Langley, B.; Kozikowski, A. P. Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin A. *J. Am. Chem. Soc.* **2010**, *132*, 10842−10846. (f) Balasubramanian, A.; Ramos, J.; Luo, W.; Sirisawad, M.; Verner, E.; Buggy, J. J. A novel histone deacetylase 8 (HDAC8)-specific inhibitor PCI-34051 induces apoptosis in T-cell lymphomas. *Leukemia* **2008**, *22*, 1026.
(12) Oehme, I.; Deubzer, H. E.; Wegener, D.; Pickert, D.; Linke, J. P.; Hero, B.; Kopp-Schneider, A.; Westermann, F.; Ulrich, S. M.; von Deimling, A.; Fischer, M.; Witt, O. Histone deacetylase 8 in neuroblastoma tumorigenesis. *Clin. Cancer Res.* **2009**, *15*, 91−99.
(13) (a) Lee, J.-Y.; Koga, H.; Kawaguchi, Y.; Tang, W.; Wong, E.; Gao, Y.-S.; Pandey, U. B.; Lu, J.; Taylor, J. P.; Cuervo, A. M.; Yao, T.-P. HDAC6 controls autophagosome maturation essential for ubiquitin-selective quality-control autophagy. *EMBO J.* **2010**, *29*, 969−980. (b) Namdar, M.; Perez, G.; Ngo, L.; Marks, P. A. Selective inhibition of histone deacetylase 6 (HDAC6) induces DNA damage and sensitizes transformed cells to anticancer agents. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 20003−20008.
(14) Wagner, F. F.; Olson, D. E.; Gale, J. P.; Kaya, T.; Weïwer, M.; Aidoud, N.; Thomas, M.; Davoine, E. L.; Lemercier, B.; Zhang, Y.-L.; Holson, E. B. Potent and selective inhibition of HDAC6 does not require a surface-binding motif. *J. Med. Chem.* **2013**, *56*, 1772−1776.
(15) There have been limited reports of selective HDAC6 or HDAC8 inhibitors that exhibit modest potency for HDAC8 or HDAC6, respectively. See ref 11e as well as (a) Galletti, P.; Quintavalla, A.; Ventrici, C.; Giannini, G.; Cabri, W.; Penco, S.; Gallo, G.; Vincenti, S.; Giacomini, D. Azetidinones as zinc-binding groups to design selective HDAC8 inhibitors. *ChemMedChem.* **2009**, *4*, 1991−2001. Additionally, HDAC3/6 dual inhibitors have been reported (b) Kozikowski, A. P.; Tapadar, S.; Luchini, D. N.; Kim, K. H.; Billadeau, D. D. Use of nitrile oxide cycloaddition (NOC) reaction for molecular probe generation: a new class of enzyme selective histone deacetylase inhibitors (HDACIs) showing picomolar activity at HDAC6. *J. Med. Chem.* **2008**, *51*, 4370−4373.
(16) We chose to measure inhibition of HDAC8 along with HDACs 2, 4, and 6 as representatives for classes I, IIa, and IIb, respectively. We chose HDACs 2 and 4 due to the availability of crystal structures for these isoforms. Inhibition of HDAC10 and 11 was not measured due to either low purity of the available recombinant HDAC enzyme preparations and/or lack of activity of the enzymes and low substrate conversion. See , Holson, E.; Wagner, F.; Weïwer, M.; Zhang, Y. L.; Haggarty, S. H.; Tsai, L. H. Inhibitors of histone deacetylases. WO2012149540 (A1), Nov 1, 2012.
(17) Fernandez-Fuentes, N.; Rai, B. K.; Madrid-Aliste, C. J.; Fajardo, J. E.; Fiser, A. Comparative protein structure modeling by combining multiple templates and optimizing sequence-to-structure alignments. *Bioinformatics* **2007**, *23*, 2558−2565, http://manaslu.aecom.yu.edu/M4T/.
(18) Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B. C.; Verner, E.; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. *Structure* **2004**, *12*, 1325−1334.
(19) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: a new approach for rapid, accurate docking and scoring. 1. method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1739−1749.
(20) (a) Estiu, G.; Greenberg, E.; Harrison, C. B.; Kwiatkowski, N. P.; Mazitschek, R.; Bradner, J. E.; Wiest, O. Structural origin of selectivity in class II-selective histone deacetylase inhibitors. *J. Med. Chem.* **2008**, *51*, 2898−2906.
(21) (a) Estiu, G.; West, N.; Mazitschek, R.; Greenberg, E.; Bradner, J. E.; Wiest, O. On the inhibition of histone deacetylase 8. *Biorg. Med. Chem.* **2010**, *18*, 4103−4110. (b) Suzuki, T.; Ota, Y.; Ri, M.; Bando, M.; Gotoh, A.; Itoh, Y.; Tsumoto, H.; Tatum, P. R.; Mizukami, T.; Nakagawa, H.; Iida, S.; Ueda, R.; Shirahige, K.; Miyata, N. Rapid discovery of highly potent and selective inhibitors of histone deacetylase 8 using click chemistry to generate candidate libraries. *J. Med. Chem.* **2012**, *55*, 9562−9575.
(22) Identifying a reliable cellular substrate for HDAC8 remains a challenge. See (a) Wolfson, N. A.; Pitcairn, C. A.; Fierke, C. A. HDAC8 substrates: histones and beyond. *Biopolymers* **2012**, *99*, 112−126.
(23) Hubbert, C.; Guardiola, A.; Shao, R.; Kawaguchi, Y.; Ito, A.; Nixon, A.; Yoshida, M.; Wang, X.-F.; Yao, T.-P. HDAC6 is a microtubule-associated deacetylase. *Nature* **2002**, *417*, 455−458.