ACS Medicinal Chemistry Letters

Letter

Design, Synthesis and Blood-Brain Barrier Transport Study of Pyrilamine Derivatives as Histone Deacetylase Inhibitors

Seiya Hiranaka, Yuma Tega, Kei Higuchi, Toshiki Kurosawa, Yoshiharu Deguchi, Mayumi Arata, Akihiro Ito, Minoru Yoshida, Yasuo Nagaoka, and Takaaki Sumiyoshi

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.8b00099 • Publication Date (Web): 23 Aug 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Design, Synthesis and Blood-Brain Barrier Transport Study of Pyrilamine Derivatives as Histone Deacetylase Inhibitors

Seiya Hiranaka,[†] Yuma Tega,[‡] Kei Higuchi,[‡] Toshiki Kurosawa,[‡] Yoshiharu Deguchi,[‡] Mayumi Arata,[‖] Akihiro Ito,[□,§] Minoru Yoshida,[·,‖] Yasuo Nagaoka,[†] and Takaaki Sumiyoshi[*,†]

[†] Department of Life Science and Biotechnology, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Yamate-cho 3-3-35, Suita, Osaka, 564-8680, Japan

[‡] Faculty of Pharma-Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

[·] Chemical Genomics Research Group, RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

[‖] Seed Compounds Exploratory Unit for Drug Discovery Platform, RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

[§] School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

KEYWORDS: Histone deacetylase, HDAC inhibitor, Blood brain barrier, Pyrilamine sensitive proton coupled organic cation antiporter, Selective inhibitor

ABSTRACT: We designed and synthesized a pyrilamine derivative **1** as a selective class I HDAC inhibitor that targets pyrilamine sensitive organic cation antiporter (PYSOCA) at the blood brain barrier (BBB). Introduction of pyrilamine moiety to benzamide type HDAC inhibitors kept selective class I HDAC inhibitory activity and increased BBB permeability. Our BBB transport study showed that compound **1** is a substrate of PYSOCA. Thus, our findings suggest that the hybrid method of HDAC inhibitor and substrate of PYSOCA such as pyrilamine is useful for development of HDAC inhibitors with increased BBB permeability.

Histone deacetylases (HDACs) are a family of enzymes that repress gene expression by catalyzing the post translational hydrolytic cleavage of acetyl groups present on lysine residues of nuclear histones.[1,2] Excluding the non-zinc-dependent HDACs, HDACs consist of eleven isoforms categorized into four classes as follows: class I (HDAC1, 2, 3, 8), class IIa (HDAC4, 5, 7, 9), class IIb (HDAC6, 10) and class IV (HDAC11).[3] Since the discovery that transcription is activated by the HDAC inhibitor trichostatin A (TSA)[4], medicinal chemists have discovered a variety of HDAC inhibitors (Figure S1, see Supporting Information). Among them, romidepsin (FK228), vorinostat (SAHA), and panovinostat have been approved as anti-cancer drugs by the United States Food and Drug Administration (Figure S1). Also, HDACs are the attractive drug targets in many central nervous system (CNS) diseases.[5] In fact, HDAC inhibitors showed disease-modifying effects in CNS disease models.[6] For example, class I HDAC inhibitors and HDAC6 inhibitors improved disease-like behaviors in Alzheimer's disease.[7-10] In addition, HDAC inhibitor improved disease phenotype in Huntington's disease model.[11-14] Moreover, CI-994 was exposed to brain and improved post-traumatic stress disorder (PTSD)-like behavior in rats.[15] Although a variety of CNS-penetrant HDAC inhibitors have been identified (Figure S2, see Supporting Information), there is no HDAC inhibitors approved as CNS drugs. One of the hurdles of applying HDAC inhibitors to CNS drugs is that they generally show poor blood-brain barrier (BBB) permeability due to the presence of polar hydroxamic acid or anilinic amino group as zinc-binding region (ZBN). In fact, positron emission tomography (PET) studies using radiolabeled HDAC inhibitors indicate that brain penetration of MS-275, butyric acid, 4-phenyl butyric acid, valproic acid and SAHA were poor.[16-20] Efforts to discover HDAC inhibitors with improved BBB permeability have mainly focused on increasing the lipophilicity. For example, Hooker and co-workers reported that some HDAC inhibitors showed good CNS distribution, based on PET studies.[21-25] However, increasing the lipophilicity generally leads to metabolic instability and increases affinities to other off-target proteins and protein binding.[26] Therefore, a new strategy is needed for delivery of HDAC inhibitors to the CNS.

One approach would be targeting compounds to transporters expressed in brain capillary endothelial cells, which form the BBB. Polt and co-workers designed prodrugs targeting glucose transporter 1.[27] In addition, large amino acid transporter 1[28] and vitamin C transporter 2[29] have been used to deliver compounds to the brain. On the other hand, Wang and co-workers focused on pyrilamine-sensitive proton-coupled organic cation (H^+/OC) antiporter (PYSOCA); they designed conjugates of naproxen and cyclic tertiary amines, and confirmed that they were distributed to CNS.[30] Nevertheless, no BBB permeable shuttle moiety is yet available for targeting HDAC inhibitors to transporters at the BBB. Here, we describe the design, synthesis and pharmacokinetic evaluations of compound **1** as a BBB permeable HDAC inhibitor targeting PYSOCA. A transport study using human immortalized brain capillary endothelial cells (hCMEC/D3) confirmed that compound **1** is recognized by PYSOCA. In addition, we confirmed that compound **1** showed both HDAC-inhibitory activity *in vitro* and increased BBB permeability in rats.

We focused on pyrilamine as a shuttle moiety to increase BBB permeability because PYSOCA recognizes a variety of centrally acting drugs.[31,32] Indeed, Yamazaki and co-workers

have reported that PYSOCA mediates BBB penetration and brain accumulation of some pyrilamine-containing histamine H_1 receptor antagonists,[33,34] including compounds containing a polar moiety. Thus, we adopted a hybrid strategy based on linking benzamide-type HDAC inhibitors with substrates of PYSOCA. Class I selective HDAC inhibitors generally include *N*-(2-aminophenyl)pyridylamide or *N*-(2-aminophenyl)benzamide as the ZBN (Figure 1). Since the 2-aminopyridine structure is a known linker moiety,[35] we designed the pyrilamine-based hybrid compound **1** (Figure 1).

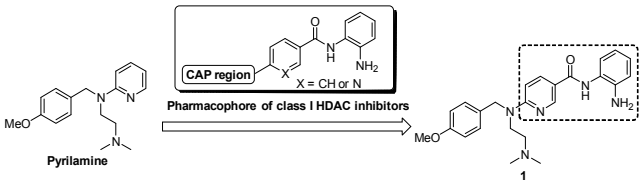
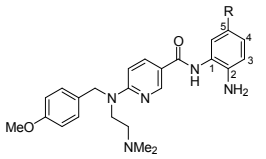


Figure 1. Hybrid design strategy for compound **1**.

Table 1. Structure-activity relationship study of compound 1 and its derivatives.



entry	compound	R	HDAC inhibition (%, 100 μM) HDAC1	HDAC6
1	**1**	H	61	11
2	**2**	Cl	62	7
3	**3**	Br	52	21
4	**4**	I	45	16
5	**5**	thiophen-2-yl	64	15
6	**6**	phenyl	62	14
7	**7**	furan-2-yl	64	15
8	CI-994		67	18
9	TSA		100	98

The SAR study of compound **1** and its derivatives are summarized in Table 1. Compound **1** inhibited HDAC1 (61 % at 100 μM), but did not inhibit HDAC6 (11% at 100 μM). The inhibitory activities against HDACs are similar to those of CI-994 (entries 1 and 9). Replacement of hydrogen atom at 5-position of benzene ring by chloride kept HDAC1 inhibitory activity (entry 2). On the other hand, introduction of bromide or iodide at 5-position decreased HDAC1 inhibitory activity (entries 3 and 4). The compounds with thiophen-2-yl, furan-2-yl or phenyl group at 5-position of benzene ring inhibited HDAC1 at the same content (entries 5-7). These results indicate that introduction of internal cavity motif does not significantly increase HDAC1 inhibitory activity in spite of the increase of molecular weight. Based on our criteria that molecular weight is less than 430 which is generally advantageous for CNS drugs,[36] we picked up compound **1** for further evaluation. After that, we evaluated IC_{50} values and isozyme selectivity to clarify pharmacological potential of compound **1**. As a result, compound **1** showed selectivity for class I HDACs over other HDAC isozymes (HDAC1 IC_{50} = 1.5 μM, HDAC2 IC_{50} = 5.5 μM, HDAC3 IC_{50} = 12.0 μM, HDAC10 IC_{50} = 15.5 μM, other HDACs: < 10% inhibition at 30 μM, Tables S1 and S2). These results indicate that pyrilamine moiety is acceptable as a CAP region.

Next, we evaluated functional cell-based assay for HDAC inhibitory activity (Figure S3, see Supporting Information) by monitoring acetylated histone protein. Evaluation of time-dependent inhibition is necessary to clarify the potency of compound **1** because benzamide-type HDAC inhibitors show slow and tight binding.[37] As a result, both compound **1** and CI-994 increased acetylated histone H3K9 in HeLaS3 cells at 10 μM (Figure S3A). In addition, *in vitro* kinetic analysis suggest that compound **1** inhibited HDAC time-dependently (Figure S3B). Treatment of both compound **1** and CI-994 increased acetylated histone H3K9 from six hours after the addition of the compounds. On the other hand, TSA increased acetylated histone immediately (Figure S3B). Especially, compound **1** showed more continuous HDAC inhibitory activity than CI-994 and TSA (Figures S3C, S3D and S3E). These results indicate that compound **1** and CI-994 inhibited HDAC at the same content in HeLaK3 cells.

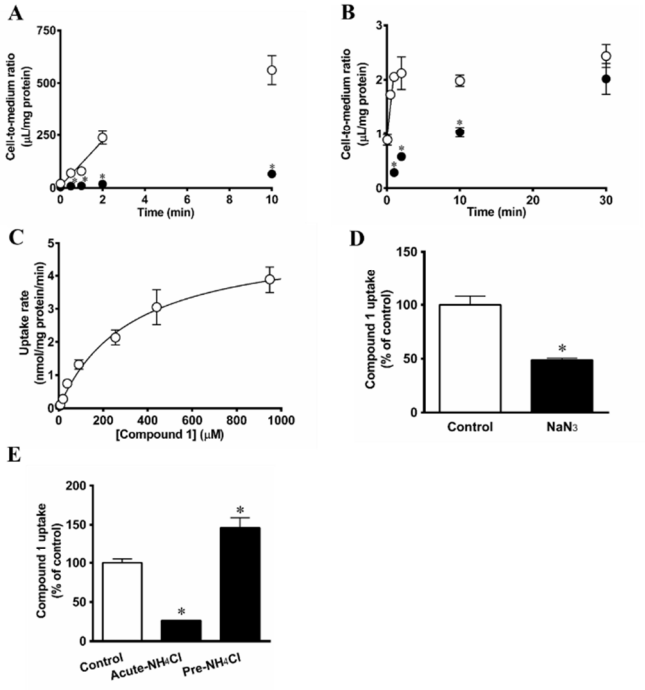


Figure 2. Time-courses of uptake of (A) compound **1** and (B) CI-994 by hCMEC/D3 cells. Uptake were measured at 37 °C (open circles) or 4 °C (closed squares) for the indicated times. Each point represents the mean ± S.E. (n = 4). Where the S.E. is not shown, it is smaller than the symbol. Uptake of 1 and CI-994 by hCMEC/D3 cells increased linearly (black lines) for at least 2 min and 1 min, respectively. *$p < 0.01$, significantly different from the uptake at 4 °C at the corresponding time. (C) Concentration-dependency of the uptake of compound **1** by hCMEC/D3 cells. The concentration-dependency of the uptake of compound **1** was examined at 37 °C for 2 min. Kinetic parameters obtained from Eq. 1 were K_m 326 ± 72 μM and V_{max} 5.17 ± 0.86 nmol/mg protein/min. The solid line was calculated according to Eq. 1 with these parameters. Each point represents the mean ± S.E. (n = 4). Where the S.E. is not shown, it is smaller than the symbol. (D), (E) Dependency of the uptake of compound **1** by hCMEC/D3 cells on metabolic energy (D) and intracellular pH (E). (D) hCMEC/D3 cells were pretreated with NaN_3 (0.1%) for 20 min to deplete cellular ATP, and uptake of compound **1** was measured at 37 °C for 2 min. (E) hCMEC/D3 cells were pretreated with transport buffer in the absence (Control and Acute) or presence (Pre) of 30 mM NH_4Cl, and then uptake of compound **1** was measured in the absence (Control and Pre) or presence (Acute) of

30 mM NH_4Cl at 37 °C for 1 min. Each column represents the mean ± S.E. (n = 4). *p < 0.01, significantly different from control.

To examine whether compound **1** and CI-994 are taken up via PYSOCA, we first evaluated their uptake by hCMEC/D3 cells at 37°C or 4 °C (Figures 2A and 2B). The uptake clearance of compound **1**, which was calculated from the initial slope, was estimated as 107 μL/min/mg protein. This clearance was 85-fold greater than that of CI-994 (1.26 μL/min/mg protein). The uptake of compound **1** was significantly lower at 4 °C for up to 10 min, while CI-994 uptake at 4 °C slowly increased and reached a similar cell-to-medium ratio to that at 37 °C by 30 min. Uptake of compound **1** was concentration-dependent with a K_m of 326 ± 72 μM and a V_{max} of 5.17 ± 0.86 nmol/mg protein/min (Figure 2C). These results suggest that compound **1** is taken up by hCMEC/D3 cells via a carrier-mediated transport process.

Next, we investigated whether the above transport properties are consistent with those previously reported for PYSOCA. To examine the effect of ATP depletion, hCMEC/D3 cells were pretreated with NaN_3 as a metabolic inhibitor. The uptake of compound **1** was significantly inhibited by NaN_3 (Figure 2D). On the other hand, uptake was increased by intracellular acidification (Figure 2E, pre-treatment with NH_4Cl), and decreased by intracellular alkalization (Figure 2E, acute treatment with NH_4Cl). These results suggest that the uptake is dependent on metabolic energy and an outward H^+-gradient. These properties are consistent with those of PYSOCA reported previously.[34,38]

Third, we determined the effect of several known substrates of PYSOCA on uptake of compound **1** and CI-994 by hCMEC/D3 cells. Indeed, uptake of compound **1** was significantly inhibited by pyrilamine[33], diphenhydramine[34], memantine[39], tramadol[32], clonidine[40] and varenicline[31]. In contrast, TEA, MPP^+, decynium-22, L-carnitine, and choline, which are typical substrates of OCTs, OCTNs, and choline transporter, had little effect on the uptake of compound **1** (Table S4, see Supporting Information). On the other hand, CI-994 uptake was not significantly decreased by these inhibitors (Table S4).

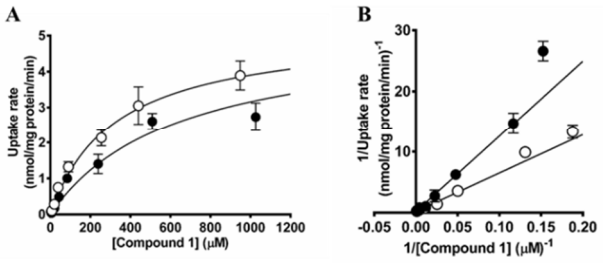


Figure 3. Inhibition by diphenhydramine of the uptake of compound **1** into hCMEC/D3 cells. Uptake of compound **1** at various concentrations was measured at 37 °C for 2 min in the absence (open circles) or presence (closed circles) of diphenhydramine (100 μM). Where the S.E. is not shown, it is smaller than the symbol. Data were subjected to Michaelis-Menten plot (A) and Lineweaver-Burk plot (B) analysis. Kinetic parameters were obtained by nonlinear least-squares regression according to Eqs. 1 and 2 (see Supporting Information). The straight lines were drawn using the kinetic parameters obtained. Each point shows the mean ± S.E. (n = 4).

The above results suggest that organic cation transport via PYSOCA is primarily involved in the uptake of compound **1** by hCMEC/D3 cells, but not in CI-994. Diphenhydramine is a known substrate of PYSOCA.[34] The uptake of compound **1** was competitively inhibited by diphenhydramine with a K_i value of 104 ± 28 μM, again suggesting that PYSOCA is involved in the uptake of compound **1** by hCMEC/D3 cells (Figure 3).

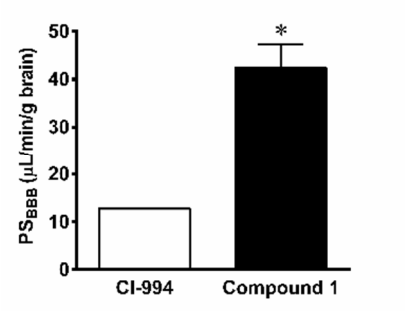
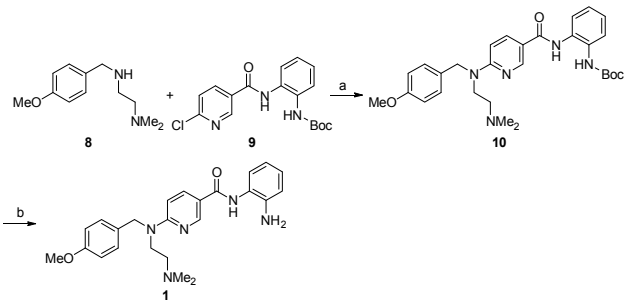


Figure 4. BBB permeability of compound **1** and CI-994 across the rat BBB. The right cerebral hemisphere of the rat was perfused with buffer containing compound **1** or CI-994 (20 μM) at a rate 4.9 mL/min for 1 min. Each column represents the mean ± S.E. (n = 3). *p < 0.01, significantly different from the PS_{BBB} of CI-994.

Finally, *in situ* brain perfusion was performed to measure blood-to-brain transport of compound **1** and CI-994 across the BBB. The results are shown in Figure 4. The BBB permeability (PS_{BBB}) of compound **1** was determined to be 42.4 ± 4.9 μL/min/g brain, which is 3.3-fold greater than that of CI-994 (12.7 ± 0.1 μL/min/g brain). Discrepancy in the BBB permeability between *in vitro* and *in vivo* may be due to species difference of efflux transporter activity such as P-gp. In fact, it has been reported that the protein expression level of P-gp (mdra1) in rodent animal microvessels is significantly higher than that in humans.[41]

Preparation of compound **1** is shown in Scheme 1. Reaction of compound **9**[42] with commercially available compound **8** under microwave irradiation afforded compound **10** in 61% yield, and then deprotection of the Boc group afforded **1** in 90% yield. Synthesis of compounds **2**–**7** are provided in Supporting Information (Schemes S2, S3 and S4, see Supporting Information).

Scheme 1. Synthesis of the hybrid compound 1[a]



[a]Reagents and conditions: (a) pyridine, DMSO, 110 °C, 4.5 h, microwave, 61%; (b) TFA, 0.5 h, 90%.

In summary, to overcome the low BBB permeability of existing HDAC inhibitors, we designed compound **1** using a pyrilamine moiety as a shuttle targeting PYSOCA for drug delivery to the CNS. The synthesized hybrid compound **1** showed selective class I HDAC inhibitory activity. Transport studies using hCMEC/D3 cells confirmed that compound **1** is

a substrate of PYSOCA, and *in situ* brain perfusion confirmed increased BBB permeability of compound **1** in the brain compared with CI-994. This design strategy appears to promising for development of potent BBB-permeable HDAC inhibitors to treat CNS diseases.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the

ACS Publications website at DOI:

Experimental procedures for the biological experiments and synthetic procedures and characterization data for the compounds.

AUTHOR INFORMATION

Corresponding Author

* Tel: +81-6-6368-1773. Fax: +81-6-6388-8609.
E-mail: t-sumiyo@kansai-u.ac.jp

Author Contributions

T.S. and S.H. conceived and directed medicinal chemistry. M.Y. and A.I. directed pharmacology. M.A. contributed histone acetylation assay. Y.D. directed pharmacokinetic study. Y.T., K.H. and T.K. contributed PK studies. The manuscript was written through contributions of all authors.

Funding Sources

Part of this work was supported by the Kansai University Subsidy for Supporting Young Scholars, 2015 "Development of macrocyclization reaction via a photoaffinity reaction", JSPS KAKENHI Grant Number 15K18903, Grant-in-Aid for Young Scientists (B) – Japan and MEXT – Supported Program for the Strategic Research Foundation at Private Universities (2013–2017) – Japan.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We thank Dr. Pierre-Olivier Couraud (Institut Cochin, Paris, France) for supplying hCMEC/D3 cells under license from INSERM. The authors also gratitude to Dr. Teruki Honma for calculations of physicochemical properties and Dr. Shinichi Uesato for useful suggestions.

ABBREVIATIONS

HDAC, histone deacetylase; BBB, blood brain barrier; CNS, central nervous system; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A; PET, positron emission tomography; PYSOCA, pyrilamine sensitive proton coupled organic cation antiporter; hCMEC, human cardiac microvascular endothelial cells; ATP, adenosine triphosphate; DMSO, dimethyl sulfoxide; TFA, trifluoroacetic acid; TEA, tetraethylammonium; MPP^+, 1-methyl-4-phenylpyridinium; OCTs, organic cation transporters; OSTNs, organic cation/carnitine transporters

REFERENCES

(1) Walsh, C. T.; Garneau-Tsodikova, S.; Gatto, G. J. Protein posttranslational modifications: the chemistry of proteome diversifications. *Angew. Chem., Int. Ed.* **2005**, *44*, 7342−7372.

(2) Bradner, J. E.; West, N.; Grachan, M. L.; Greenberg, E. F.; Haggarty, S. J.; Warnow, T.; Mazitschek, R.; Chemical phylogenetics of histone deacetylases. *Nat. Chem. Biol.* **2010**, *6*, 238–243.

(3) 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.

(4) Yoshida. M.; Kijima, M.; Akita, M.; Beppu, T.; Potent and Specific Inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J. Biol. Chem.* **1990**, *265*, 17174–17179.

(5) Kazantsev, A. G.; Thompson, L. M. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. *Nat. Rev. Drug Discov.*, **2008**, *7*, 854–868.

(6) Didonna, A.; Opal, P. The promise and perils of HDAC inhibitors in neurodegeneration. *Ann. Clin. Transl. Neurol.*, **2015**, *2*, 79–101.

(7) Kilgore, M.; Miller, C. A.; Fass, D. M.; Hennig, K. M.; Haggarty, S. J.; Sweatt, J. D.; Rumbaugh, G. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology*, **2010**, *35*, 870–880.

(8) Rumbaugh, G.; Sillivan, S. E.; Ozkan, E. D.; Rojas, C. S.; Hubbs, C. R.; Aceti, M.; Kilgore, M.; Kudugunti, S.; Puthanveettil, S. V.; Sweatt, J. D.; Rusche, J.; Miller, C. A. Pharmacological selectivity within class I histone deacetylases predicts effects on synaptic function and memory rescue. *Neuropsychopharmacology*, **2015**, *40*, 2307–2316.

(9) Majid, T.; Griffin, D.; Criss II, Z.; Jarpe, M.; Pautler, R. G. Pharmacologic treatment with histone deacetylase 6 inhibitor (ACY-738) recovers Alzheimer's disease phenotype in amyloid precursor protein/presenilin 1 (APP/PS1) mice. *Alzheimer's Dement.*, **2015**, *1*, 170–181.

(10) Zhang, L.; Liu, C.; Wu, J.; Tao, J. –J.; Sui, X. -L.; Yao, Z. –G.; Xu, Y. –F. Huang, L.; Zhu, H.; Sheng, S. –L.; Qin, C. Tubastatin A/ACY-1215 improves cognition in Alzheimer's disease transgenic mice. *J. Alzheimer's Dis.*, **2014**, *41*, 1193–1205.

(11) Jia, H.; Kast, R. J.; Steffan. J. S.; Thomas, E. A. Selective histone deacetylase (HDAC) inhibition imparts beneficial effects in Huntington's disease mice: implications for the ubiquitin–proteasomal and autophagy systems. *Hum. Mol. Genet.* **2012**, *21*, 5280–5293.

(12) Thomas, E. A.; Coppola, G.; Desplats, P. A.; Tang, B.; Soragni, E.; Burnett, R.; Gao, F.; Fitzgerald, K. M.; Borok, J. F.; Herman, D.; Geschwind, D. H.; Gottesfeld, J. M. The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington's disease transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.*, **2008**, *105*, 15564–15569.

(13) Jia, H.; Morris, C. D.; Williams, R. M.; Loring, J. F.; Thomas, E. A. HDAC inhibition imparts beneficial transgenerational effects in Huntington's disease mice via altered DNA and histone methylation. *Proc. Natl. Acad. Sci. U.S.A.*, **2014**, E56–E64.

(14) Jia, H.; Wang, Y.; Morris, C. D.; Jacques, V.; Gottesfeld, J. M.; Rusche, J. R.; Thomas, E. A. The effects of pharmacological inhibition of histone deacetylase 3 (HDAC3) in Huntington's disease mice. *PLOS One*, **2016**, *11*, e0152498.

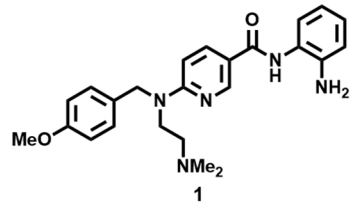
(15) Gräff, J.; Joseph, N. F.; Horn, M. E.; Samiei, A.; Meng, J.; Seo, J.; Rei, D.; Bero, A. W.; Phan, T. X.; Wagner, F.; Holson, E.; Xu, J.; Sun J.; Neve, R. L.; March, R. H.; Haggarty, S. J.; Tsai, L.-H. Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. *Cell*, **2014**, *156*, 261–276.

(16) Hooker, J. M.; Kim, S. W.; Alexoff, D.; Xu, Y.; Shea, C.; Reid, A.; Volkow, N.; Fowler, J. S. Histone deacetylase inhibitor MS-275 exhibits poor brain penetration: pharmacokinetic studies of [^{11}C]MS-275 using positron emission tomography. *ACS Chem. Neurosci*., **2010**, 1, 65–73.

(17) Hendricks, J. A.; Keliher, E. J.; Mrinelli, B.; Reiner, T.; Weissleder, R.; Mazitschek, R. In vivo PET imaging of histone deacetylases by ^{18}F-suberoylanilide hydroxamic acid (^{18}F-SAHA). *J. Med. Chem.*, **2011**, *54*, 5576–5582.

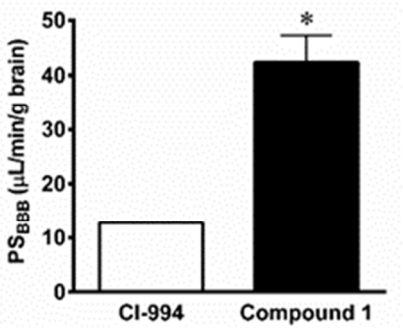
(18) Kim, S. W.; Hooker, J. M.; Otto, N.; Win, K.; Muench, I.; Shea. C.; Carter, P.; King, P.; Reid, A. E.; Volkow, N. D.; Fowler, J. S. Whole-body pharmacokinetics of HDAC inhibitor drugs,

butyric acid, valproic acid and 4-phenylbutyric acid and measured with carbon-11 labeled analogs by PET. *Nucl. Med. Biol.,* **2013**, *40*, 912–918.
(19) Seo, Y. J.; Muench, L.; Reid, A.; Chen, J.; Kang, Y.; Hooker, J. M.; Volkow, N. D.; Fowler, J. S.; Kim, S. W. Radionuclide labeling and evaluation of candidate radioligands for PET imaging of histone deacetylase in the brain. *Bioorg. Med. Chem. Lett*., **2013**, *23*, 6700–6705.
(20) Strebl, M. G.; Campbell, A. J.; Zhao. N. W.; Schroeder, F. A.; Riley, M. M.; Chindavong, P. S.; Morin, T. M.; Haggarty, S. J.; Wagner, F. F.; Ritter, T.; Hooker, J. M. HDAC6 brain mapping with [^{18}F]bavarostat enabled by a Ru-mediated deoxyfluorination. *ACS Cent. Sci.,* **2017**, *3*, 1006–1014.
(21) Wang, C.; Schroeder, A; Wey, H. Y.; Borra, R.; Wagner, F. F.; Reis, S.; Kim, W. S.; Holson, B. E.; Haggarty, J. S.; Hooker, M. J. In vivo imaging of histone deacetylases (HDACs) in the central nervous system and major peripheral organs. *J. Med. Chem.*, **2014**, *57*, 7999–8009.
(22) Reid, A. E.; Hooker, J.; Shumay, E.; Logan, J.; Shea, C.; Kim, S. W.; Collins, S.; Xu, Y.; Volkow, N.; Fowler, J. S. Evaluation of 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide for PET imaging of histone deacetylase in the baboon brain. *Nucl. Med. Biol.,* **2009**, *36*, 247–258.
(23) Wang. C.; Eessalu, T. E.; Barth, V. N.; Mitch, C. H.; Wagner, F. F.; Hong, Y.; Neelamegam, R.; Schroeder, F. A.; Holson, E. B.; Haggarty, S. J.; Hooker, J. M. Design, synthesis, and evaluation of hydroxamic acid-based molecular probes for in vivo imaging of histone deacetylase (HDAC) in brain. *Am. J. Nucl. Med. Mol. Imaging*, **2014**, *4*, 29–38.
(24) Schroeder, F. A.; Wang, S.; Van de Bittner, G. C.; Neelamegam, R.; Takakura, W. R.; Karunakaran, A.; Wey, H. Y.; Reis, S. A.; Gale, J.; Zhang, Y. L.; Holson E. B.; Haggarty, S. J.; Hooker, J. M. PET imaging demonstrates histone deacetylase target engagement and clarifies brain penetrance of known and novel small molecule inhibitors in rat. *ACS Chem. Neurosci.*, **2014**, *5*, 1055–1062.
(25) Seo, Y. J.; Kang, Y.; Muench, L.; Reid, A.; Caesar, S.; Jean, L.; Wagner, F.; Holson, E.; Haggarty, S. J.; Weiss, P.; King. P.; Carter, P.; Volkow, N. D.; Fowler, J. S.; Hooker, J. M.; Kim. W. S. Image-guided synthesis reveals potent blood-brain barrier permeable histone deacetylase inhibitors. *ACS Chem. Neurosci.*, **2014**, *5*, 588–596.
(26) Di, L.; Rong, H.; Feng, B. Demystifying brain penetration in central nervous system drug discovery. *J, Med. Chem.*, **2013**, *56*, 2–12.
(27) (a) Bilsky, E. J.; Egleton, R. D.; Mitchell, S. A.; Palian, M. M.; Davis, P.; Huber, J. D.; Jones, H.; Yamamura, H. I.; Janders, J.; Davis, T. P.; Porreca, F.; Hruby, V. J.; Polt, R. Enkephalin glycopeptide analogues produce analgesia with reduced dependence liability. *J. Med. Chem.*, **2000**, *43*, 2586–2590. (b) Gynther, M.; Ropponen, J.; Laine, K.; Leppänen, J.; Haapakoski, P.; Peura, L.; Järvinen, T.; Rautio, J. Glucose promoiety enables glucose transporter mediated brain uptake of ketoprofen and indomethacin prodrugs in rats. *J. Med. Chem*., **2009**, *52*, 3348–3353.
(28) Peura, L.; Malmioja, K.; Huttunen, K.; Leppänen, J.; Hämäläinen, M.; Forsberg, M. M.; Rautio, J.; Laine, K. Design, synthesis and brain uptake of LAT1-targeted amino acid prodrugs of dopamine. *Pharm. Res*., **2013**, *30*, 2523–2537.
(29) Zhao, Y.; Qu, B.; Wu, X.; Li, X.; Liu, O.; Jin, X.; Guo, L.; Hai. L.; Wu, Y. Design, synthesis and biological evaluation of brain targeting L-ascorbic acid prodrugs of ibuprofen with "lock-in" function. *Eur. J. Med. Chem.*, **2014**, *82*, 314–323.
(30) Wang, X.; Qi, B.; Su, H.; Li, J.; Sun, X.; He, Q.; Fu, Y.; Zhang, Z. Pyrilamine-sensitive proton-coupled organic cation (H^+/OC) antiporter for brain-specific drug delivery. *J. Control. Release*, **2017**, *254*, 34–43.
(31) Kurosawa, T.; Higuchi, K.; Okura, T.; Kobayashi, K.; Kusuhara, H.; Deguchi, Y. Involvement of proton-coupled organic cation antiporter in varenicline transport at blood-brain barrier of rats and in human brain capillary Endothelial Cells. *J. Pharm. Sci.*, **2017**, *106*, 2576–2582.
(32) Kitamura, A.; Higuchi, K.; Okura, T.; Deguchi, Y. Cocktail-dosing microdialysis study to simultaneously assess delivery of multiple organic-cationic drugs to the brain. *J. Pharm. Sci*., **2016**, *105*, 935–940.
(33) Yamazaki, M.; Terasaki, T.; Yoshioka, K.; Nagata, O.; Kato, H.; Ito, Y.; Tsuji, A. Carrier-mediated transport of H_1-antagonist at the blood-brain barrier: Mepyramine uptake into bovine brain capillary endothelial cells in primary monolayer cultures. *Pharm. Res*., **1994**, *11*, 975–978.
(34) Shimomura, K.; Okura, T.; Kato, S.; Couraud, P. O.; Schermann, J. M.; Terasaki, T.; Deguchi, Y. Functional expression of a proton-coupled organic cation (H^+/OC) antiporter in human brain. *Fluids and Barriers of CNS*., **2013**, *10*, 1–10.
(35) Newbold, A.; Matthews, G. M.; Bots, M.; Cluse, L. A.; Clarke, C. J. P.; Banks, K. M.; Cullinane, C.; Bolden, J. E.; Christiansen, A. J.; Dickins, R. A.; Miccolo, C.; Chiocca, S.; Kral, A. M.; Ozerova, N. D.; Miller, T. A.; Methot, J. L.; Richon, V. M.; Secrist, J. P.; Minucci, S.; Johnstone, R. W. Molecular and biologic analysis of histone deacetylase inhibitors with diverse specificities. *Mol. Cancer Ther.* **2013**, *12*(12), 2709–2721.
(36) Rankovic, Z. CNS physicochemical property space shaped by a diverse set of molecules with experimentally determined exposure in the mouse brain. *J. Med. Chem.*, **2017**, *60*, 5943–5954.
(37) Chou, C. J.; Herman, D.; Gottesfeld, J. M.; Pimelic diphenylamide 106 is a slow, tight-binding inhibitor of class I histone deacetylases. *J. Biol. Chem.*, **2008**, *283*, 35402–35409.
(38) Okura, T.; Hattori, A.; Takano, Y.; Sato, T.; Hammarlund-Udenaes, M.; Terasaki, T.; Deguchi Y. Involvement of the pyrilamine transporter, a putative organic cation transporter, in blood-brain barrier transport of oxycodone. *Drug Metab. Dispos.* **2008**, *36*, 2005–2013.
(39) Higuchi, K.; Kitamura, A.; Okura, T.; Deguchi, Y. Memantine transport by a proton-coupled organic cation antiporter in hCMEC/D3 cells, an in vitro human blood-brain barrier model. *Drug Metab. Pharmacokinet.,* **2015**, *30*, 182–187.
(40) Chapy, H.; Andre, P.; Decleves, X.; Scherrmann, J. M.; Cisternino, S. A. polyspecific drug/proton antiporter mediates diphenhydramine and clonidine transport at the mouse blood-brain barrier. *Br. J. Pharmacol.*, **2015**, *172*, 4714–4725.
(41) Uchida, Y.; Ohtsuki, S.; Katsukura, Y.; Ideda, C.; Suzuki, T.; Kamiie, J.; Terasaki, T. Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *J. Neurochem*, **2011**, *117*, 335–345.
(42) Hamblett, C. L.; Methot, J. L.; Mampreian, D. M.; Sloman, D. L.; Stanton, M. G.; Kral, A. M.; Fleming, J. C.; Cruz, J. C.; Chenard, M.; Ozerova, N.; Hitz, A. M.; Wang, H.; Deshmukh, S. V.; Nazef, N.; Harsch, A.; Hughes, B.; Dahlberg, W. K.; Szewczak, A. A; Middleton, R. E.; Mosley, R. T.; Secrist, J. P.; Millera, T. A. The discovery of 6-amino nicotinamides as potent and selective histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett.,* **2007**, *17*, 5300–5309.



1

HDAC1 IC_{50} = 1.5 μM
HDAC2 IC_{50} = 5.5 μM
HDAC3 IC_{50} = 12.0 μM
HDAC10 IC_{50} = 15.5 μM
HDAC4–9 and 11 < 10% inhibition at 30 μM



Insert Table of Contents artwork here