Class I Histone Deacetylase Inhibition by Tianeptinaline Modulates Neuroplasticity and Enhances Memory

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

| Citation          | Zhao, Wen-Ning et al. "Class I Histone Deacetylase Inhibition by Tianeptinaline Modulates Neuroplasticity and Enhances Memory." ACS Chemical Neuroscience 9, 9 (June 2018): 2262–2273 © 2018 American Chemical Society |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| As Published      | http://dx.doi.org/10.1021/acchemneuro.8b00116                                                                                                                                                               |
| Publisher         | American Chemical Society (ACS)                                                                                                                                                                             |
| Version           | Author’s final manuscript                                                                                                                                                                                   |
| Citable link      | https://hdl.handle.net/1721.1/126372                                                                                                                                                                        |
| Terms of Use      | Article is made available in accordance with the publisher’s policy and may be subject to US copyright law. Please refer to the publisher’s site for terms of use.                                                 |
Class I Histone Deacetylase Inhibition by Tianeptinaline Modulates Neuroplasticity and Enhances Memory

Wen-Ning Zhao,†,‡,§,∥ Balaram Ghosh,†,‡,∥ Marshall Tyler,†,‡,∥ Jasmin Lalonde,†,‡,∥ Nadine F. Joseph,§,⊥ Nina Kosaric,†,‡,∥ Daniel M. Fass,†,‡ Li-Huei Tsai,§ Ralph Mazitschek,∥ and Stephen J. Haggarty,†,‡,∥

†Chemical Neurobiology Laboratory, Center for Genomic Medicine, Massachusetts General Hospital, 185 Cambridge Street, Boston, Massachusetts 02114, United States
‡Departments of Psychiatry & Neurology, Massachusetts General Hospital & Harvard Medical School, Boston, Massachusetts 02114, United States
§Department of Brain and Cognitive Sciences, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States
∥Center for Systems Biology, Massachusetts General Hospital, 185 Cambridge Street, Boston, Massachusetts 02114, United States

ABSTRACT: Through epigenetic and other regulatory functions, the histone deacetylase (HDAC) family of enzymes has emerged as a promising therapeutic target for central nervous system and other disorders. Here we report on the synthesis and functional characterization of new HDAC inhibitors based structurally on tianeptine, a drug used primarily to treat major depressive disorder (MDD) that has a poorly understood mechanism of action. Since the chemical structure of tianeptine resembles certain HDAC inhibitors, we profiled the in vitro HDAC inhibitory activity of tianeptine and demonstrated its ability to inhibit the lysine deacetylase activity of a subset of class I HDACs. Consistent with a model of active site Zn2+ chelation by the carboxylic acid present in tianeptine, newly synthesized analogues containing either a hydroxamic acid or ortho-aminoanilide exhibited increased potency and selectivity among the HDAC family. This in vitro potency translated to improved efficacy in a panel of high-content imaging assays designed to assess HDAC target engagement and functional effects on critical pathways involved in neuroplasticity in both primary mouse neurons and, for the first time, human neurons differentiated from pluripotent stem cells. Most notably, tianeptinaline, a class I HDAC-selective analogue of tianeptine, not only tianeptine itself, increased histone acetylation, and enhanced CREB-mediated transcription and the expression of Arc (activity-regulated cytoskeleton-associated protein). Systemic administration of tianeptinaline to mice confirmed its brain penetration and was found to enhance contextual fear conditioning, a behavioral test of hippocampal-dependent memory. Tianeptinaline and its derivatives provide new pharmacological tools to dissect chromatin-mediated neuroplasticity underlying memory and other epigenetically related processes implicated in health and disease.

KEYWORDS: Cognitive enhancer, neuroplasticity, Arc, CREB, histone deacetylases, epigenetic, chromatin, acetylation, neuroepigenetics, human stem cells, neuropharmacology

INTRODUCTION

Aberrant chromatin-mediated neuroplasticity has been implicated in cognitive and mood disorders. The reversible post-translational modification of histones represents a highly dynamic epigenetic regulation mechanism. Of the various histone modifications that have been characterized in the context of the nervous system, the acetylation and deacetylation of ε-amino groups of lysine side chains within the N-terminal tails of histones have emerged as important regulators of neuronal gene expression and neuroplasticity. Histone deacetylation is achieved by histone deacetylases (HDACs), which have been grouped into four classes based on structural homology and catalytic mechanism: class I (HDACs 1–3, 8), class II (HDACs 4–7, 9–10), class III (SIRT1–7), and class IV (HDAC 11). Class I, II, and IV HDACs rely on a zinc ion (Zn2+) in the active site for enzymatic activity, whereas class III HDACs are the NAD+-dependent family of sirtuins (SIRT1–7). Accumulating evidence indicates that HDACs can have both facilitating and suppressive influences on cognitive processes. Modulation of HDAC activities by small-molecule inhibitors holds promise as a new mechanistic

Received: March 8, 2018
Accepted: June 6, 2018

DOI: 10.1021/acschemneuro.8b00116
ACS Chem. Neurosci. XXXX, XXX, XXX–XXX
class of therapeutics to treat a range of central and peripheral nervous system disorders.\(^1\)\(^-\)\(^{3,8-14}\)

Although not approved by the United States Food and Drug Administration, outside of the U.S.A., tianeptine is used primarily for the treatment of major depressive disorder (MDD) with additional beneficial effects for anxiety, asthma, and irritable bowel syndrome.\(^15,16\) Clinical studies have confirmed tianeptine’s efficacy and safety with a low rate of adverse effects such as sleep disturbances, body weight changes, and cardiovascular dysfunction.\(^17-19\) Structurally, tianeptine is a tricyclic antidepressant (TCA), but functionally it contrasts with typical TCAs in that it enhances, rather than inhibits, serotonin (5-hydroxytryptamine; 5-HT) uptake in the brain.\(^16,20\) While recent findings suggest that tianeptine induces antidepressant effects by indirect alteration of glutamate receptor activity and reduction of stress-induced atrophy of neuronal dendrites,\(^19\) the precise nature of the mechanisms of neuroplasticity involved remains poorly understood.

Given the desirable properties of tianeptine and its enigmatic mechanism of action, we reasoned that a more complete understanding of its cellular targets might help advance the development of a new class of CNS therapeutic agents. In this context, we hypothesized that tianeptine may function as an HDAC inhibitor based on the chemical structure analysis and functional similarities to the known effects of HDAC-selective inhibitors on neuroplasticity and affective behavior.\(^21\) Thus, in order to explicitly test this hypothesis and continue our efforts to develop HDAC inhibitors with improved physiochemical and pharmacological properties, we synthesized several tianeptine analogues incorporating HDAC-biasing elements and evaluated their HDAC inhibitory activities in comparison to tianeptine itself. We also implemented a panel of newly developed, high-throughput neuronal assays to evaluate these potential HDAC inhibitors for their biological activities in the context of human or mouse neurons. We first examined their abilities to increase histone acetylation in human induced pluripotent stem cell (iPSC)-derived neurons. Second, we examined induction of CREB-mediated transcription in human iPSC-derived neural progenitor cells (NPCs),\(^22-24\) a pathway that plays important roles in neuroplasticity and is critical to learning and memory. Finally, we evaluated these potential HDAC inhibitors for their abilities to enhance neuroplasticity in mouse cortical neuronal cultures by examining nuclear Arc (activity-regulated cytoskeleton-associated protein) expression.\(^25\) Taken as a whole, our data provide evidence that tianeptine can indeed inhibit class I human HDACs using in

Figure 1. Tianeptine and its analogues of HDAC inhibitors. (A) Structural resemblance of tianeptine to carboxylic acid HDAC inhibitors. (B) In vitro inhibitory activities of tianeptine toward the deacetylase activity of recombinant human HDACs 1–3. Asterisk (*) indicates data generated in the same assays published in Fass et al., 2010. (C) Synthesized tianeptine analogues with Zn\(^{2+}\)-chelating elements taken from reference HDAC inhibitors. (D) Structure of pimelic diphenylamide 106. (E) Illustration of Zn\(^{2+}\)-binding moiety and internal cavity motif of three OAA HDAC inhibitors.
vitro biochemical assays with a potency similar to other carboxylic acid HDAC inhibitors (butyric acid, valproic acid), but that it lacks detectable functional effects on histone acetylation in cells. Among the tianeptine analogues synthesized and characterized we highlight tianeptinaline, which we demonstrate has robust effects on cellular histone acetylation, is active in a panel of functional assays reporting on chromatin-mediated neuroplasticity, and is capable of crossing the blood-brain barrier when administered systemically and can enhance hippocampal-dependent memory in mice.

Our studies presented here advance the field of neuroepigenetics by describing two newly developed, human neuronal cell-based, high-throughput assays for assessment of the effects of epigenetic and other small-molecule probes of neuroplasticity: (1) an acetyl-histone high-content image-based assay and (2) a CREB signaling reporter assay. The Arc imaging assay using mouse cortical neurons is foreseeably adapted to human iPSC-derived neurons to enable a comprehensive investigation of the epigenetic regulation of human Arc expression and post-translational control of its stability. To our knowledge, our effort is the first to use human neurons derived from stem cells in assays of the pharmacological effects of HDAC inhibitors. Combined with human iPSC technology, it is feasible now to examine the effect of HDAC inhibitors and other small-molecule probes of epigenetic mechanisms on mechanisms of chromatin-mediated neuroplasticity in the context of patient-specific genetic backgrounds, therefore potentially revealing differential response of patients and advancing the chemical neuroscience of human disease.

### RESULTS AND DISCUSSION

Tianeptine’s chemical structure consists of three main components: a tricyclic moiety, an aliphatic linker, and a carboxylic acid (Figure 1A). The molecule resembles HDAC inhibitors in two respects. First, the carboxylic acid is shared by HDAC inhibitors such as butyric acid and valproic acid (VPA).26 Second, the three-component feature of tianeptine shares the “cap-linker-chelator” pharmacophore model of hydroxamate (HA) HDAC inhibitors (e.g., SAHA, TSA) and some ortho-aminoanilide (OAA) HDAC inhibitors (e.g., CI-994 (tacedinaline), MS-275 (entinostat), RGFP136).27−29 The structural resemblance prompted us to probe tianeptine for HDAC inhibitory activities. As shown in Figure 1B, micromolar inhibitory activities were detected toward HDAC1 (IC50: 82.2 μM) and HDAC2 (IC50: 25.4 μM) but HDAC3 was refractory (IC 50: >2 mM). The measured HDAC2 inhibitory activity is only modestly higher than that of VPA (HDAC1: 39 μM; HDAC2: 62 μM; HDAC3: 161 μM) tested in the same assay format with recombinant human HDACs and class-specific substrates.26

Since tianeptine is a clinically used drug that possesses favorable pharmacological and pharmacokinetic (PK) properties,15,16,19 and given the need to still identify brain-penetrant, selective class I HDAC inhibitors that are capable of beneficially impacting neuroplasticity, we reasoned combining tricyclic and aliphatic linker components of tianeptine with structural moieties from existing HDAC inhibitors might yield HDAC inhibitors with desirable pharmacological and pharmacokinetic profiles. By replacing the carboxylic acid end, a weak Zn2+ chelator, with substantially more potent HA, Table 1. In Vitro Biochemical Profiling of Tianeptine Analogues against the Deacetylase Activity of Recombinant Human HDACs and Tabulated Neuronal Assay Results

"Asterisk (*) indicates data generated in the same assays published in Fass et al., 2010. N/A = not assessed.
or potent and selective OAA, we hypothesized that the new HDAC inhibitors would have improved activities and isoform selectivity. Four reference HDAC inhibitors were chosen for making tianeptine analogues: SAHA (vorinostat), a hydroxamate HDAC class I/Ilb inhibitor; CI-994 (tacedinealine) and Cpd-60, class I HDAC inhibitors; and BRD3308, a fluorinated version of CI-994 that in vitro biochemical assays with recombinant HDAC catalytic domains is selective for HDAC3 over HDAC1/2. The corresponding hybrid compounds were synthesized (Supporting Information: Chemical synthesis, Schemes 1–4), and their chemical structures are shown in Figure 1C.

The carboxylic acid of tianeptine was replaced by the OAA moiety from CI-994 structurally resembles pimelic diphenylamide (Figure 1D), a reported slow, tight-binding inhibitor of class I HDACs with isoform selectivity and binding kinetics similar to those of CI-994. The carboxylic acid of tianeptine was replaced by the OAA moiety from CI-994, structurally resembles pimelic diphenylamide (Figure 1D), a reported slow, tight-binding inhibitor of class I HDACs with isoform selectivity and binding kinetics similar to those of CI-994. The carboxylic acid of tianeptine was replaced by the OAA moiety from CI-994, structurally resembles pimelic diphenylamide (Figure 1D), a reported slow, tight-binding inhibitor of class I HDACs with isoform selectivity and binding kinetics similar to those of CI-994. The carboxylic acid of tianeptine was replaced by the OAA moiety from CI-994, structurally resembles pimelic diphenylamide (Figure 1D), a reported slow, tight-binding inhibitor of class I HDACs with isoform selectivity and binding kinetics similar to those of CI-994.

**Tianeptine Analogues Possess HDAC Inhibitory Activities.** After synthesizing the four tianeptine analogues, we determined their IC_{50} values for class I HDACs 1–3, the class IIa HDAC5, and the class III HDAC6 (Table 1). As expected, CI-994 is a selective inhibitor of the class I HDACs 1–3, whereas SAHA has broader activities, additionally inhibiting the class IIb HDAC6. Tianeptinostat, the hydroxamate derivative, exhibited a similar HDAC inhibitory profile to SAHA’s, although its inhibitory potencies were decreased by 2.5 to 4-fold for HDACs 1–3 (IC_{50} values for HDAC1, 2, 3: SAHA: 0.007, 0.010, 0.021 μM; tianeptinostat: 0.028, 0.033, 0.051 μM). They have comparable inhibitory potency toward HDAC6 (SAHA: 0.0012 μM; tianeptinostat: 0.0016 μM). Performing similarly to CI-994, tianeptine inhibited HDACs 1–3 with similar IC_{50} values for HDAC2 and 3 (CI-994: 0.115, 0.124 μM; tianeptine: 0.120, 0.155 μM) and a slight increase for HDAC1 (CI-994: 0.145 μM; tianeptine: 0.240 μM). Compared to tianeptinostat, tianeptinamine was less potent toward inhibiting HDACs 1–3, but more specific in that tianeptinamine did not inhibit HDAC6.

Compounds 3 and 4, the derivative of Cpd-60, was the most HDAC subtype-selective among the four tianeptine analogues as it inhibited HDACs 1 and 2, but not HDAC3. Compound 3 maintained the HDAC1/2 selectivity from its related analogue Cpd-60 (HDAC1: 0.001; HDAC2: 0.008; HDAC3: 0.458). Compound 4, the tianeptine derivative of the HDAC3-selective inhibitor BRD3308, was a less potent inhibitor of HDACs 1–3, but showed preferential inhibition among these isoforms toward HDAC3, consistent with the original design of BRD3308 for HDAC3 selectivity. Additionally, to explore if chirality conveys different activities to tianeptinamine, we synthesized compound 5, the (−)-enantiomer of tianeptinamine (Figure 2A; Supporting Information: Chemical synthesis, Scheme 5). No significant difference was observed between compound 5 and tianeptinamine in their in vitro HDAC inhibitory activities (Figure 2B).

These studies demonstrated that, consistent with the structural properties of the “cap-linker-chelator” pharmacophore for HDAC inhibitors and the known inhibitory activity of carboxylic acid based HDAC inhibitors, tianeptine exhibits subclass 1 (i.e., HDAC1/2) deacetylase inhibitory activity of a magnitude similar to that of VPA. While tianeptine plasma levels in humans after multiple dosing have been measured to be ∼1 μM, the precise concentration of tianeptine in brain with chronic administration, and if it accumulates with time, is not known. Assuming this is lower than the maximum plasma levels, since this concentration is significantly lower (∼80X) than that at which we observed inhibition of class I HDACs, the relevance of our findings for tianeptine’s therapeutic mechanism of action remains unclear. Additionally, two major in vivo tianeptine metabolites, MCS and MC3, have been identified and differ from tianeptine in the aliphatic linker length with 5-carbon or 3-carbon chain, respectively. Thus, in order to further rule out or provide other potential evidence for the relevance of HDACs as an in vivo target of tianeptine, and because the aliphatic linker length has been shown critical for extending the terminal hydroxamic acid moiety into the catalytic site for the “cap-linker-zinc chelator” pharmacophore model of HDAC inhibitors, it would be of interest in the future to test these MCS and MC3, as well as other possible metabolites, for HDAC inhibitory activity. To further answer these questions in an in vivo context, the recent development of the HDAC positron emission tomography (PET) imaging tracer [11C]Martinostat could afford the opportunity in the future to determine if tianeptine engages class I HDACs in vivo in human brain and other tissues. Further challenging the hypothesis that the observed in vitro HDAC inhibition by tianeptine is relevant to its in vivo therapeutic effects, tianeptine has recently been reported to be a full agonist of mu-opioid receptor (MOR) and this activation has been proposed to mediate its acute and chronic antidepressant-like effect.

This notion is further supported by the binding of partial agonism of MOR by buprenorphine, which also has an antidepressant-like effect. Thus, it would be of interest in the future to examine if any of our tianeptine derivatives also bind to the MOR and to determine if they possess an antidepressant-like effect that can be dissociated from MOR binding.

These outstanding issues concerning tianeptine’s in vivo therapeutic mechanism aside, inspired by our data demonstrating that tianeptine was a subclass 1 HDAC-selective inhibitor, we proceeded to synthesize a panel of new tianeptine analogues with HDAC inhibitor biasing motifs and characterized their HDAC inhibitory activity. The structure–activity relationship we observed for the panel of tianeptine analogues was consistent with the notion that the Zn^{2+}-binding moiety and the additional internal cavity motif contribute greatly to...
Figure 3. High-content imaging assay of tianeptine analogues on histone acetylation in human iPSC-derived neuronal cultures. (A) 4 week differentiated human neuronal cultures in a 96-well plate format immunostained for neuronal dendritic marker MAP2. (B) Representative images of H3K9ac immunostaining of human neuronal cultures showing brighter nuclei in 5.56 μM CI-994-treated culture. H3K9ac or H4K12ac was detected in cellular nuclei, intensities of which were quantified. (C) Cellular activities of tianeptine analogues and reference HDAC inhibitors in induction of H3K9ac. (D) Cellular activities of tianeptine analogues and reference HDAC inhibitors in induction of H4K12ac. Compounds were assayed in an 8-point dose response with the highest concentration of 50 μM and 3-fold serial dilution. Experiments were repeated three times. Each data point represents the mean of quadruplicate measurements from one biological replicate. Error bars display standard error of the mean (SEM).
the efficacy and selectivity of HDAC inhibitors (Figure 1E). In particular, when we substituted the weak Zn$^{2+}$-binding carboxylic in tianeptine with either a HA or OAA moiety, the resulting HDAC inhibitors showed much more potent inhibitory activities than observed for tianeptine alone. HA and OAA substitutions also maintained the potency and selectivity of the parent HDAC inhibitor with HA HDAC inhibitors being more potent and less selective among isoforms and OAA HDAC inhibitors being less potent but more selective among isoforms. More interestingly, among the three OAA substitutions, the additional internal cavity motifs transferred the HDAC1/2 subtype selectivity to new HDAC inhibitors compound 3 and compound 4.

**Tianeptine Analogues Induce Histone Acetylation in Human iPSC-Derived Neurons.** Cellular levels of H3K9 (histone 3 lysine 9) and H4K12 (histone 4 lysine 12) acetylation are associated with learning and memory enhancements in normal and aged mice. Inhibition of class I HDAC activities leads to elevated H3K9ac and H4K12ac. Previously, we established mouse primary neuronal culture high-content imaging assays to evaluate cellular activities of HDAC inhibitors by measuring the acetylation levels of H3K9 and H4K12 in neuronal nuclei. While these cellular assays provide powerful functional readouts of HDAC target engagement in a relevant, postmitotic cell type, one limitation of the use of rodent neurons is the potential for species differences in the HDACs themselves or the HDAC complexes they form that may alter the effectiveness or selectivity of compounds. To address this limitation, we adapted our high-content histone imaging assay to work with human iPSC-derived neurons in a format compatible with high-throughput screening for the first time. To accomplish this, we differ-

---

**Figure 4.** Functional profiling of tianeptine analogues for CREB signaling in human iPSC-derived neural progenitor cells. (A) Schematic of CREB reporter system in human iPSC-derived neural progenitor cells. (B) Responsiveness of the CREB reporter system to forskolin or crebinostat. Crebinostat induced CREB reporter activities in the forskolin-dependent manner. (C) CREB reporter activities measured for reference HDAC inhibitors. (D) Cellular activities of tianeptine analogues in induction of CREB signaling reporter. Dose response tests (10 points) were conducted in the presence of 2.5 μM forskolin. Experiments were repeated three times. Each data point represents the mean of quadruplicate measurements in one biological replicate. Error bars display standard error of the mean (SEM).
entiated human iPSC-derived neural progenitor cells (NPCs), which can be expanded on a sufficiently large enough scale to allow routine compound testing, for 4 weeks to produce 96-well plates of postmitotic neurons.1,45 We then treated the neurons with eight-point 3-fold dilution doses of reference and the newly synthesized HDAC inhibitors. After 24-h compound treatments, neuronal cultures were fixed and immunostained for histone acetylation marks H3K9ac and H4K12ac. Figure 3A shows a representative image of human neuronal cultural immunostained for MAP2, the dendritic marker of postmitotic neurons. Shown in Figure 3B are representative images of H3K9ac staining of cultures treated with DMSO or CI-994 (5.56 μM), with brighter nuclei detected with CI-994 treatment. Intensities of H3K9ac and H4K12ac in neuronal nuclei were quantified by high-content image analysis, and the resulting dose response curves are shown in Figure 3C and D, respectively, with EC_{50} values reported. For reference compounds, sigmoid-shaped dose response curves were obtained for SAHA and CI-994 with similar EC_{50} values in induction of H3K9ac (SAHA: 0.35 μM; CI-994:0.56 μM) and H4K12ac (SAHA: 0.54 μM; CI-994:0.53 μM). Cpd-60 exhibited a more linear increase in induction of H3K9ac and H4K12ac. Sigmoid-shaped dose response curves were also obtained for tianeptinostat and tianeptinoline in induction of H3K9ac and H4K12ac, though the maximum inductions (plateaus) were slightly reduced compared to SAHA and CI-994. In H3K9ac assays, tianeptinostat and tianeptinoline had EC_{50} values of 1.72 μM and 3.98 μM, respectively. In H4K12ac assays, tianeptinoline exhibited half-maximal inhibition at 0.67 μM, which was lower than that observed for tianeptinostat (7.66 μM). In comparison, compound 3 and compound 4 had low activities in induction of H3K9ac and H4K12ac, which is consistent with their greater selectivity for a subset of HDACs and therefore reduced global effects on histone acetylation. Plotting the EC_{50} values of H3K9 acetylation against the IC_{50} values of HDAC1/2/3 inhibition for tianeptin analogues, as well as SAHA and CI-994, revealed a strong correlation between the ex vivo neuronal activities and the in vitro inhibitory activities (Supporting Information Figure S1). The calculated correlation value r for HDAC1/2/3 is 0.95, 0.93, and 0.89, respectively. Taken together, these histone acetylation data in human iPSC-derived neuronal cultures indicate that tianeptinostat and tianeptinoline are able to permeate cell membranes and engage with endogenous HDAC complexes to modulate histone acetylation.

As part of our ex vivo assay platform for characterizing HDAC inhibitors, we introduced here, for the first time, the use of a high-content image-based assay to quantify changes in histone acetylation in human iPSC-derived neuronal cultures. The activity of both tianeptinostat and tianeptinoline, as well as reference HDAC inhibitors, in this assay provides a direct measure of their ability to engage endogenous HDAC complexes. As expected, based upon the in vitro potency toward recombinant human HDACs, we found the hydroxamate tianeptinostat the most potent of the series of tianeptine analogues tested. While the activities we observed for H3K9ac and H4K12ac were generally well correlated to each other, future adaptation of this assay to include an extended panel of histone modification antibodies and consideration of markers enabling the separate measure of excitatory and inhibitor neurons will enable the generation of multidimensional epigenetic signatures to aid in the discovery and optimization of next-generation neuroepigenetic probes.

### Tianeptinostat and Tianeptinoline Activate CREB Signaling in Human iPSC-Derived Neuronal Cells.

Beyond changes in histone acetylation in human neurons, we sought next to determine whether tianeptine, and its analogues that we found to be more potent inducers of histone acetylation, could have a functional impact on a transcription. For this purpose, we turned to the transcription factor CREB (cAMP response element-binding protein), which has been shown to play a key role in the signaling pathways regulating neuroplasticity underlying learning and memory.22–24 The CREB signaling pathway is known to be activated by elevated cellular cAMP, leading to activation of protein kinase A which phosphorylates CREB, followed by the binding of the coactivator CREB binding protein (CBP, a histone acetyltransferase) to phospho-CREB53 (Figure 4A). These events lead to expression of the corresponding genes. HDAC inhibitors have been shown to regulate CREB-dependent transcription,54 and a potent HDAC inhibitor with cognition-enhancing properties in mice, crebinostat, was identified by screening for enhancers of CREB-mediated transcription.42,50 To facilitate identification of CREB signaling enhancers in the context of human neuronal cells, we generated a luciferase-based reporter system in the human iPSC-derived NPCs (Figure 4A). We validated this CREB signaling reporter system as responsive to forskolin, a natural product stimulator of adenyl cyclase which raises cAMP levels, and forskolin-dependent induction of reporter activity by crebinostat was detected (Figure 4B). With this system, we then tested reference and newly synthesized HDAC inhibitors in a 10-point dose response (0.1−50 μM) in the presence of 2.5 μM forskolin (Figure 4C and D). We observed that CREB reporter activity was increased in a dose-dependent manner by SAHA, CI-994, and Cpd-60, with maximum ~5-fold induction observed for SAHA, 2.5-fold for CI-994, and 2-fold for Cpd-60. Tianeptinostat induced an ~8-fold increase in CREB reporter activity at 62.5 μM, the highest concentration tested while the highest induction of ~2.5-fold was observed for tianeptinoline at 6.25 μM followed by a decrease in reporter activity at higher concentrations. Additional testing of compound 5, the (−)-enantiomer of tianeptinoline, revealed a 1.5-fold induction of CREB signaling reporter activity at a concentration of 50 μM, which was approximately half of the maximum induction observed for tianeptinostat (Supporting Information Figure S2). Finally, compounds 3 and 4 produced modest increases in CREB reporter activity, with a maximum 1.5-fold increase activity, that were not statistically significant, and substantially lower than the level of activation observed with tianeptinostat and tianeptinoline.

Since these CREB reporter gene assays were performed in proliferative NPCs, we reasoned that the inability of tianeptinostate to further activate CREB signaling at higher concentrations could possibly be a result of an antiproliferative effects that HDAC inhibitors are known to have. Indeed, as shown in Supporting Information Figure S3, both tianeptinostat and tianeptinoline, as well as compound 5, the (−)-enantiomer of tianeptinoline, reduced the number of viable NPCs over a 24 h treatment similar to the effects of potent HDAC inhibitors like SAHA in the same assay. Since at the same concentrations (0.1−50 μM) tested for the analogues of tianeptine we did not observe an induction of the CREB reporter by the parent tianeptine (data not shown), we increased the highest concentration tested to 250 μM. Unlike the hydroxamic and OAA analogues, these higher doses
of tianeptine were well tolerated by the neuronal cells (Supporting Information Figure S4A). At these higher concentrations, we were able to observe a modest (~2-fold) induction of CREB reporter activity in a dose-dependent manner (Supporting Information Figure S4B). These observations are consistent with a weak HDAC inhibitory activity of tianeptine, but we cannot rule out contributions of additional targets given the high doses used.

**Tianeptinostat and Tianeptinaline Effectively Increase Arc Levels in Mouse Cortical Neurons.** Brain-derived neurotrophic factor (BDNF) is a critical regulator of activity-induced gene expression and neuroplasticity.\(^{55}\) Activity-regulated cytoskeleton-associated protein (Arc), an immediate-early gene whose expression is essential for synaptic plasticity, is upregulated by BDNF and is a CREB target gene.\(^{56,57}\) Recent work in our laboratory has established that HDAC inhibitors potentiate BDNF-induced nuclear Arc expression in mouse primary cortical neurons through a mechanism that includes regulation of Arc acetylation and blocking ubiquitin-dependent degradation.\(^{25}\) Thus, to complement our human neuronal assays and determine the applicability of tianeptinostat and tianeptinaline as probes of HDAC function in the rodent nervous system, we determined whether they were capable of affecting histone acetylation and Arc expression levels in our primary mouse cortical neuron high-content imaging assay. For these experiments, we treated mouse primary cortical cultures for 6 h with 100 ng/mL BDNF either alone or with compounds (1, 5, or 10 \(\mu\)M) and measured nuclear Arc expression in neurons (Figure 5A). Figure 5C shows representative images of DIV14 mouse cortical neuronal cultures treated for 6 h with 100 ng/mL BDNF alone or following a 15 min pretreatment with 10 \(\mu\)M tianeptinaline. Cells were immunostained for neuronal dendritic marker MAP2 (red), histone acetylation on H3K9 (blue), and Arc protein (green).

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Functional assay of tianeptine analogues examining nuclear expression of Arc protein and H3K9 acetylation in mouse cortical neurons. (A) Quantification of nuclear Arc protein expression levels based on mean pixel intensity normalized to BDNF-treatment. (B) Quantification of H3K9 acetylation levels based on mean pixel intensity normalized to BDNF-treatment. E16 DIV14 cortical neurons were treated for 6 h with 100 ng/mL BDNF alone or following a 15 min pretreatment with 1, 5, or 10 \(\mu\)M of compound. Error bars represent standard deviation based on \(N = 3\) biological replicates per treatment. Mean pixel intensities were collected from approximately 300 neurons per biological replicate. (C) Representative images of mouse cortical neuronal cultures treated for 6 h with 100 ng/mL BDNF alone or following a 15 min pretreatment with 10 \(\mu\)M tianeptinaline. Cells were immunostained for neuronal dendritic marker MAP2 (red), histone acetylation on H3K9 (blue), and Arc protein (green).
In the Arc expression assay using mouse cortical cultures, H3K9ac was used to both identify neuronal nuclei and quantify acetylation of H3K9. Consistent with the results reported in iPSC-derived human neurons, both SAHA and CI-994 effectively elevated acetylation of H3K9 (Figure 5B), with saturating effects observed for SAHA and dose-dependent effects for CI-994. Tianeptinostat and tianeptinaline increased H3K9ac in a dose dependent manner and to a slightly lesser degree compared to their corresponding analogues SAHA and CI-994, also in agreement with the human neuronal data. Minimal changes in H3K9ac were observed for compounds 3 and 4 in mouse cortical neurons, in agreement with the minimal effect of these compounds on Arc levels. Collectively, these data demonstrate that both tianeptinostat and tianeptinaline have the capability of engaging HDACs and modulating neuroplasticity in both human and mouse neurons and further implicate Arc regulation as a target of class I HDACs.

Tianeptinaline Enhances Associative Memory in Mice. Given the selective nature of tianeptinaline for class I HDACs and its effectiveness in the human and mouse neuronal assays, we chose tianeptinaline for further in vivo studies with the dual objective of: 1) determining if it was capable of penetrating brain tissue when administered systemically; and 2) if it possesses cognitive enhancing properties. To assess the pharmacokinetic profile in brain of adult mice, tianeptinaline was administered via a single intraperitoneal (i.p.) dose of 25 mg/kg. Total tianeptinaline concentration (not necessarily free fraction) peaked at 0.25 h in the brain with a Cmax of 690 nM and clearance T1/2 of 1.68 h (Figure 6A). These data indicate that with i.p. administration tianeptinaline is rapidly distributed to and cleared from the brain and therefore is expected to cause pulsatile inhibition of HDAC targets. The pulsatile profile of brain concentrations follows a similar profile measured for the total tianeptinaline in plasma for which Cmax of 2.57 μM was observed with a similar clearance T1/2 of 1.85 h (Supporting Information Figure S5A, B). The brain to plasma Cmax ratio was 0.27 with the overall exposure (AUClast) ratio of 0.33 indicating a greater peripheral than CNS exposure as summarized in Supporting Information Figure S5C.

We next examined if tianeptinaline treatment affected associative memory using the contextual fear-conditioning paradigm (Figure 6B). Mice were divided into three groups receiving vehicle, 25 mg/kg tianeptinaline, or 5 mg/kg CI-994. After 10 days of daily intraperitoneal injections, mice received foot shocks on the 11th day given in a specific context followed by an additional injection. Mice were tested for context-dependent freezing behavior on the following day. As shown in Figure 6C, i.p. administration of tianeptinaline (25 mg/kg) produced ∼25% increase in freezing time, while CI-994 (5 mg/kg) produced ∼40% increase, indicating that like CI-994, tianeptinaline effectively enhanced associative learning. No differential exploratory behaviors were observed among the three animal groups immediately prior to foot shocks (data not shown) indicating that none of the treatments affected baseline motor behavior.

Collectively, our exploration of the structure–activity relationship of tianeptine derivatives led to the synthesis and in-depth functional characterization of tianeptinaline as an HDAC inhibitor with structural components shared between tianeptine and OAA HDAC inhibitors like CI-994. Our ex vivo assays for neuroplasticity coupled with in vivo behavioral experiments demonstrate the cognitive enhancing properties of this small molecule. Having demonstrated its brain penetration...
and provided initial data supporting tianeptineline’s effect on behavior, it will be of interest to determine if neuroplasticity-related gene expression (e.g., Arc) can be measured in the hippocampus or amygdala in mice exposed to tianeptineline and to more broadly explore its effect in HDAC-regulated behavioral paradigms.10,25,58,59

While epigenetic modulation holds promise for the development of a new mechanistic class of treatments for neurodegenerative and neuropsychiatric disorders, there is still limited structural diversity among existing small molecules of this class with demonstrated potential to affect behavior in vivo. In particular, to the best of our knowledge, CI-994 is the only other OAA-based, class I HDAC-selective inhibitor that has been shown to enhance fear-conditioning memory in wild-type mice (i.e., not restoration of memory in mutant neurodegenerative disease model mice) when administered systemically. Thus, our fusion of structural properties of known HDAC inhibitor with a CNS-permeable, antidepressant harboring favorable pharmacological properties represents an expansion of known chemical structures that behave as HDAC inhibitors in vivo in a manner capable of enhancing memory. It would be of interest in the future to test tianeptineline in the mutant neurodegenerative disease model mice for restoration of memory or in mood-related behavior mouse models for potential antidepressant-like effect.

In summary, the demonstration of the in vivo memory enhancing effect of the tianeptineline analogue tianeptineline expands the pharmacological toolkit of epigenetic regulators that can be used to probe mechanisms of HDAC-mediated neuroplasticity in health and disease. One important direction not yet addressed by our studies on tianeptineline is the determination of whether this compound selectively engages a subset of the multiprotein class I HDAC complexes in brain tissue, including the NURD, COREST, and SIN3A complexes.3 Such intra-HDAC complex selectivity has been reported for compounds such as CI-994 that lack the ability to effectively inhibit HDAC2-SIN3A complexes but do inhibit HDAC2-NURD and HDAC2-COREST complexes.60 If tianeptineline also shows selectivity for a subset of HDAC complexes, such findings will help better define which HDAC complexes are sufficient to inhibit in order to have the desired effect of enhancing hippocampal-dependent memory. This may also provide a strategy to overcome the undesired toxic effects of class I HDAC inhibitors and may also help point to additional therapeutic targets that are components of class I HDAC complexes.3

Furthermore, given recent studies that have revealed varying on/off rates of HDAC inhibitors,21,36–38,61 in particular compounds like tianeptineline that contain an OAA moiety, it would be of interest to characterize the full kinetic properties of this series of tianeptineline analogues. Presently, the structural elements that contribute to varying target residence time remain incompletely understood and it is likely that a continuum exists between compounds with fast versus slow kinetic properties. In the case of tianeptineline and the other OAA analogues of tianeptineline, it is possible that the observed in vitro potency toward HDACs will increase with further incubation due to their likely slow-on rate and that once bound there will be long off rates in comparison to the hydroxamic acid tianeptinostat or the tianeptine itself as the carboxylic acid. If this is the case, as recent studies on epigenetic regulation of both the frataxin (FXN) and granulin (GRN) genes highlight as possible,12,13 an emerging concept is that it will be possible to tune the kinetic properties of next-generation HDAC inhibitors to achieve genomic locus-selective control of gene expression. In particular, these studies revealed reciprocal regulation of FXN and GRN in human cells by slow-binding and fast-binding HDAC inhibitors, respectively. These observations, along with growing evidence for feasibility of developing HDAC subcomplex-selective inhibition26,61 (SJH, manuscript in preparation), suggest that this may be feasible to develop next-generation epigenetic probes that exploit the particular structural properties and functional requirements of chromatin within a given locus in the genome. Since epigenetic states of chromatin can vary between cell types, tissues, and disease status,52 being able to target the unique combination of epigenetic regulatory features for a particular genomic locus could conceivably be leveraged to provide a functional selectivity that enhances the efficacy and reduces undesired side effects of HDAC inhibitors and other epigenetic therapeutics. In conclusion, besides the new chemical probes synthesized and characterized here, the human iPSC-derived neuronal assays that have been implemented here for the first time open up new avenues for advancing the neuropharmacology of the chromatin modifying and remodeling complexes involved in regulating the human epigenome.

## METHODS

More detailed description of experimental procedures is included in the Supporting Information.

### In Vitro HDAC Enzymatic Assays

HDAC activities were measured in vitro using recombinant human HDACs 1–3, 6 (BPS Bioscience). The trypsin-coupled enzymatic assays for HDAC activity were performed as described in previous publications26,39

### Histone Acetylation Assays Using iPSC-Derived Human Neuronal Cultures

Human neuronal cultures were generated from iPSC-derived neural progenitor cells (NPCs). Four week differentiated neuronal cultures in 96-well plates were treated 1 μM compounds for 24 h followed by fixation and immunostaining for histone acetylation marks H3K9ac and H4K12ac (Millipore, #07-352), as well as the neuronal dendritic marker MAP2 (EnCor Biotechnology, CPCA-MAP2). Immunofluorescent intensities of H3K9ac or H4K12ac in nuclei were quantified by high-content image analysis (IN Cell Analyzer Workstation 3.7.2, GE Healthcare). HDAC inhibitory activities were reported as increased H3K9ac and H4K12ac intensities normalized to DMSO-treated samples.

### CREB Signaling Reporter Assay

Activities of CREB signaling stimulation were measured using a CREB reporter gene stable NPC line. Cells in 384-well plates were treated with compounds in the absence or presence of 2.5 μM forskolin for 6 h followed by lysing with SteadyGlo reagent (Promega) and read for luminescence on an EnVision plate reader. Activities of CREB signaling stimulation are expressed as fold change over DMSO-treated samples.

### Nuclear Arc Expression and Acetylation of H3K9 in Mouse Cortical Neurons

All procedures with mice performed in accordance with the Massachusetts General Hospital Subcommittee on Research Animal Care and with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals: Eighth Edition. Primary cortical neuron cultures were prepared from forebrains of E16.5 C57BL/6 mouse embryos as described in our previous study.25 Compounds and BDNF (100 ng/mL; Millipore Corp.) were added directly to the culture medium at DIV14 for 6 h. Cells were fixed, permeabilized, and immunostained for MAP2 (Millipore, AB5543), Arc (Synaptic Systems, #156005), and H3K9ac (Millipore, #07-352). Images were taken on an IN Cell 6000 (GE Healthcare Life Sciences) and analyzed with CellProfiler for automated pixel intensity measurement of nuclear Arc and H3K9ac immunofluorescence signals.

### In Vivo Pharmacokinetic Study

The in vivo pharmacokinetic profile of tianeptineline was evaluated in mice (Sai Life Sciences...
Limited). Twenty-seven male C57BL/6 mice were administered intraperitoneally a single 25 mg/kg dose. Blood samples and brains were collected from a set of three mice at nine time points (predose, 0.08, 0.25, 0.5, 1, 2, 4, 8, and 24 h). The plasma and brain concentrations—time data for tianeptine were used for the pharmacokinetic analysis.

Mouse Contextual Fear Conditioning Test. C57BL/6 mice were housed and cared for following standard procedures in accordance with the Committee for Animal Care of the Division of Comparative Medicine at the Massachusetts Institute of Technology and with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals: Eighth Edition. Mice of 9–10 weeks of age were injected daily intraperitoneally with either vehicle alone (5% DMSO, 30% Cremophor, 65% saline) or test compound dissolved in vehicle for 10 consecutive days, followed by a training day and a test day. Three groups of mice (vehicle, 25 mg/kg tianeptine, or 5 mg/kg CI-994) with a total of 22 mice in each group were tested over two trials, and the data from both trials were combined for graphing and statistical analyses.

### ASSOCIATED CONTENT

#### Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.8b00116

Supplemental figures, detailed experimental procedures including chemical synthesis schemes, and supplemental references (PDF)

#### AUTHOR INFORMATION

**Corresponding Author**
*E-mail: shaggarty@mgh.harvard.edu.

**ORCID**

Wen-Ning Zhao: 0000-0002-5824-2032
Balaram Ghosh: 0000-0002-3425-2439
Marshall Tyler: 0000-0001-5401-3104
Stephen J. Haggarty: 0000-0002-7872-168X

**Present Addresses**

1 B.G.: Department of Pharmacy, Birla Institute of Technology and Sciences at Hyderabad Campus, India 500 078.
2 J.L.: Department of Molecular and Cellular Biology, University of Guelph, 50 Stone Road, East, Guelph, ON, Canada N1G 2W1.
V N.F.J.: PhD program, The Scripps Research Institute.
O N.K.: Stem Cell Biology and Regenerative Medicine PhD Program, Stanford University School of Medicine, Stanford, CA 94305.

**Author Contributions**

S.J.H., R.M. and W.-N.Z. conceptualized the study. B.G., R.M., and S.J.H. designed and synthesized chemical compounds.
W.Z. performed CREB reporter assay. N.K. contributed to the design and synthesis of chemical compounds. S.J.H., R.M. and W.-N.Z. conceptualized the study. B.G., R.M., W.-N.Z. and S.J.H. wrote original draft. B.G. contributed text on compound assays. N.F.J. conducted mouse behavior test. D.M.F. coordinated the PK study. L.-H.T. directed animal work. W.-N.Z. wrote original draft. B.G. written revised manuscript. All authors have read and commented on the manuscript.

**Funding**

S.J.H. was supported through funding from the NIH (R01DA028301, R01DA030321), the Bluefield Project to Cure FTD, and the MGH Research Scholars Program. R.M. was supported through funding from the NIH (P50CA086355).

**Notes**

The authors declare the following competing financial interest(s): R.M. has financial interests in SHAPE Pharmaceuticals and Acetylon Pharmaceuticals. He is also the inventor on IP licensed to these two entities that is unrelated to this study. S.J.H. has financial interest in Rodin Therapeutics and is an inventor on IP licensed to this entity that is unrelated to this study.

**ACKNOWLEDGMENTS**

We would like to thank members of the Haggarty Laboratory, Mazitschek Laboratory, and Tsai laboratory for helpful discussion. We also acknowledge Dr. Surya A. Reis for providing primary mouse cortical cultures used in these studies.

**REFERENCES**

1 (1) Nott, A., et al. (2012) HDAC Inhibitors as Novel Therapeutics in Aging and Alzheimer’s Disease. Epigenetic Regulation in the Nervous System, Chapter 8, Elsevier, Academic Press.
2 (2) Nestler, E. J. (2009) Epigenetic mechanisms in psychiatry. Biol. Psychiatry 65 (3), 189–90.
3 (3) Fass, D. M., et al. (2014) Epigenetic mechanisms in mood disorders: targeting neuroplasticity. Neuroscience 262, 112–30.
4 (4) Berndsen, C. E., and Denu, J. M. (2008) Catalysis and substrate selection by histone/protein lysine acetyltransferases.Curr. Opin. Struct. Biol. 18 (6), 682–9.
5 (5) Selvi, B. R., et al. (2010) Tuning acetylation levels with HAT activators: therapeutic strategy in neurodegenerative diseases. Biochim. Biophys. Acta, Gene Regul. Mech. 1799 (10–12), 840–53.
6 (6) Millard, C. J., et al. (2017) Targeting Class I Histone Deacetylases in a "Complex" Environment. Trends Pharmacol. Sci. 38 (4), 363–377.
7 (7) Graff, J., and Tsai, L. H. (2013) The potential of HDAC inhibitors as cognitive enhancers. Annu. Rev. Pharmacol. Toxicol. 53, 311–30.
8 (8) Graff, J., et al. (2012) An epigenetic blockade of cognitive functions in the neurodegenerating brain. Nature 483 (7388), 222–6.
9 (9) Guan, J. S., et al. (2009) HDAC3 is a critical negative regulator of long-term memory formation. J. Neurosci. 31 (2), 764–74.
10 (10) Alam, M. S., Getz, M., and Haldar, K. (2016) Chronic administration of an HDAC inhibitor treats both neurological and systemic Niemann-Pick type C disease in a mouse model. Sci. Trans. Med. 8 (326), 326e23.
11 (11) Sorgagni, E., and Gottesfeld, J. M. (2016) Translating HDAC inhibitors in Friedman’s axiata. Expert Opin. Orphan Drugs 4 (9), 961–970.
12 (12) She, A., et al. (2017) Selectivity and Kinetic Requirements of HDAC Inhibitors as Progranulin Enhancers for Treating Frontotemporal Dementia. Cell Chem. Biol. 24 (7), 892–906 e5.
13 (13) Haggarty, S. J., and Tsai, L. H. (2011) Probing the role of HDACs and mechanisms of chromatin-mediated neuroplasticity. Neurobiol. Learn. Mem. 96 (1), 41–52.
14 (14) Zolada, P. R., et al. (2008) Tianeptine: an antidepressant with memory-protective properties. Curr. Neuropharmacol 6 (4), 311–21.
15 (15) Wagstaff, A. J., Ormrod, D., and Spencer, C. M. (2001) Tianeptine: a review of its use in depressive disorders. CNS Drugs 15 (3), 231–59.
16 (16) Loo, H., et al. (1990) [Tolerability of tianeptine in 170 patients with depression treated during one year]. Encephale 16 (6), 445–52.
(18) Guelfi, J. D., et al. (2004) Clinical safety and efficacy of tianeptine in 1,858 depressed patients treated in general practice. *Neuropsychobiology* 25 (3), 140–8.

(19) Wilde, M. I., and Benfield, P. (1995) Tianeptine. A review. Its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depression and coexisting anxiety and depression. *Drugs* 49 (5), 411–39.

(20) Labrid, C., Mocaer, E., and Kamoun, A. (1992) Neurochemical and pharmacological properties of tianeptine, a novel antidepressant. *Br J. Psychiatry Suppl.* No. 15, 56–60.

(21) Schroeder, F. A., et al. (2013) A selective HDAC 1/2 inhibitor modulates chromatin and gene expression in brain and alters mouse behavior in two mood-related tests. *PLoS One* 8 (8), e71323.

(22) Silva, A. J., et al. (1998) CREB and memory. *Annu. Rev. Neurosci.* 21, 127–48.

(23) Benito, E., and Barco, A. (2010) CREB’s control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. *Trends Neurosci.* 33 (5), 230–40.

(24) Sakamoto, K., Karelina, K., and Obrietan, K. (2011) CREB: a multifaceted regulator of neuronal plasticity and protection. *J. Neurochem.* 116 (1), 1–9.

(25) Lalonde, J., et al. (2017) Chemogenomic analysis reveals key role for lysine acetylation in regulating Arc stability. *Nat. Commun.* 8 (1), 1659.

(26) Fass, D. M., et al. (2011) Effect of Inhibiting Histone Deacetylase with Short-Chain Carboxylic Acids and Their Hydroxamic Acid Analogs on Vertebrate Development and Neuronal Chromatin. *ACS Med. Chem. Lett.* 2 (1), 39–42.

(27) Bielauskas, A. V., and Pflum, M. K. (2008) Isoform-selective histone deacetylase inhibitors. *Chem. Soc. Rev.* 37 (7), 1402–13.

(28) Finnin, M. S., et al. (1999) Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* 401 (6749), 188–93.

(29) Vannini, A., et al. (2004) Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* 101 (42), 15064–9.

(30) Witter, D. J., et al. (2008) Optimization of biaryl Selective HDAC1/2 Inhibitors (SHI-1-2). *Bioorg. Med. Chem. Lett.* 18 (2), 726–31.

(31) Wagner, F. F., et al. (2016) An Isochemogenic Set of Inhibitors of class I histone deacetylases. *ACS Chem. Biol.* 11 (5), 726–8.

(32) Prakash, S., et al. (2001) Chronic oral administration of CI-1127 of benzydime- type histone deacetylase inhibitors with enhanced potency and selectivity. *J. Med. Chem.* 50 (23), 5543–6.

(33) Bartok, K. M., et al. (2014) Selective HDAC inhibition for the disruption of latent HIV-1 infection. *PLoS One* 9 (8), e102684.

(34) Chou, C. J., Herman, D., and Gottesfeld, J. M. (2008) Pimelic diphenylamide 106 is a slow, tight-binding inhibitor of class I histone deacetylases. *J. Biol. Chem.* 283 (51), 35402–9.

(35) Methot, J. L., et al. (2008) Exploration of the internal cavity of histone deacetylase (HDAC) with selective HDAC1/HDAC2 inhibitors (SHI-1-2). *Bioorg. Med. Chem. Lett.* 18 (3), 973–8.

(36) Moradei, O. M., et al. (2007) Novel aminophenyl benzamide type histone deacetylase inhibitors with enhanced potency and selectivity. *J. Med. Chem.* 50 (23), 5543–6.

(37) Todorov, L., et al. (2015) HDAC1/HDAC2 inhibitors: Brain penetrant HDAC inhibitors for neuroepigenetic regulation. *Bioorg. Med. Chem. Lett.* 26 (4), 1265–71.

(38) Wey, H. Y., et al. (2016) Insights into neuroepigenetics through human histone deacetylase PET imaging. *Sci. Transl. Med.* 8 (351), 351ra106.

(39) Gassaway, M. M., et al. (2014) The atypical antidepressant and neurorestorative agent tianeptine is a mu-opioid receptor agonist. *Transl. Psychiatry* 4, e411.

(40) Nair, M. B., et al. (1998) Photochromically induced fluorometric detection of tianeptine and some of its metabolites. *Analyst* 123 (11), 2267–70.

(41) Ghosh, B., et al. (2016) Dissecting structure-activity-relationships of crebinostat: Brain penetrant HDAC inhibitors for neuroepigenetic regulation. *Bioorg. Med. Chem. Lett.* 26 (4), 1265–71.

(42) Prakash, S., et al. (2001) Chronic oral administration of CI-1127 of benzydime- type histone deacetylase inhibitors with enhanced potency and selectivity. *J. Med. Chem.* 50 (23), 5543–6.