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Synthesis and Biological Evaluation of *ortho*-Aryl *N*-Hydroxycinnamides as Potent Histone Deacetylase (HDAC) 8 Isoform-Selective Inhibitors

Wei-Jan Huang,*[a] Yi-Ching Wang,[b] Shi-Wei Chao,[a, c] Chen-Yui Yang,[d] Liang-Chieh Chen,[a] Mei-Hsiang Lin,[c] Wen-Chi Hou,[a] Mei-Yu Chen,[b] Tai-Lin Lee,[a] Ping Yang,[a] and Chung-I Chang*[d, e]

Histone deacetylases (HDACs) are a family of enzymes that play a crucial role in biological process and diseases. In contrast to other isozymes, HDAC8 is uniquely incapable of histone acetylation. In order to delineate its physiological function, we developed HDAC8-selective inhibitors using knowledge-based design combined with structural modeling techniques. Enzyme inhibitory analysis demonstrated that some of the resulting compounds (**22b**, **22d**, **22f**, and **22g**) exhibited anti-HDAC8 activity superior to PCI34051, a known HDAC8-specific inhibitor, with IC_{50} values in the range of 5–50 nM. Among them, compound **22d** showed antiproliferative effects toward several human lung cancer cell lines (A549, H1299, and CL1-5); it exhibited cytotoxicity against human lung CL1-5 cells similar to that of SAHA yet without significant cytotoxicity for normal IMR-90 cells. Expression profiling of HDAC isoforms in three cancer cell lines indicated that the HDAC8 level in CL1-5 is higher than that in H1299 and CL1-1 cells, a result consistent with the differential cytotoxicity of compound **22d**. These results suggest the effectiveness of our design concept, which may lead to a tool compound for studying the specific role of HDAC8 in cellular biological processes.

Introduction

Histone deacetylases (HDAC) play a role in the reversible acetylation of histones, transcription factors, and other proteins, which are associated with chromatin remolding and regulation of gene expression.[1] HDAC up-regulation can cause inappropriate gene expression associated with the pathogenesis of many forms of malignancy. Studies revealed that HDAC inhibition can induce cancer cell growth arrest, differentiation, and apoptosis. Thus, it has been validated as a promising strategy in cancer therapy.

Mammalian HDACs can be divided into four classes based on their sequence homology, subcellular distribution, and catalytic activity. Class I (HDAC1, -2, -3, -8), class II (HDAC4, -5, -6, -7, -9, -10), and class IV (HDAC11) enzymes contain zinc as a cofactor in the catalytic site, whereas class III (sirtuins 1–7) require NAD^+ for activity. Moreover, class II HDACs are subdivided into class IIa (HDAC4, -5, -7, -9) and class IIb (HDAC6, -10).[2]

Although the cellular functions of individual HDAC isoforms are still unclear, an urgent need for a new strategy in cancer chemotherapy has accelerated progress toward a number of structurally diverse pan-HDAC inhibitors. For examples, hydroxamate-based compounds such as SAHA,[3] LBH-589,[4] SB-939,[5] PCI-24781,[6] JNJ26481585,[7] ITF-2357,[8] short-chain fatty acids such as butyrate and valproate,[9] cyclic tetrapeptides including FK-228,[10] trapoxin (TPX),[11] apicidin,[12] and 2-aminobenzamides including MS-275[13] and MGCD0103[14] are currently in clinical trials for solid and hematologic malignancies. Particularly, SAHA and FK-228 have been approved by FDA for treatment of refractory cutaneous T-cell leukemia (CTCL).[15]

The physiological role of HDAC isoforms were delineated using knockout and transgenic mice, as well as RNA interference (RNAi).[16] In contrast to other class I enzymes, HDAC8 is an isozyme unique for its inability to enhance acetylation of histone and α-tubulin.[17] Instead, it is known to associate with actin cytoskeleton and act as a cytosolic differentiation marker in smooth muscle.[18] In addition, HDAC8 siRNA against neuroblastoma cells can induce antiproliferation and differentiation.[19] Studies have also revealed that HDAC8 may be recognized as a therapeutic target in acute myeloid leukemia (AML).[20] Balasubramanian and co-workers reported that

[a] *Dr. W.-J. Huang, S.-W. Chao, L.-C. Chen, Dr. W.-C. Hou, T.-L. Lee, P. Yang*
Graduate Institute of Pharmacognosy, Taipei Medical University
250 Wu-Xing St., Taipei 110 (Taiwan, Republic of China)
E-mail: wjhuang@tmu.edu.tw

[b] *Dr. Y.-C. Wang, M.-Y. Chen*
Department of Pharmacology, National Cheng Kung University
1 University Rd., Tainan 701 (Taiwan, Republic of China)

[c] *S.-W. Chao, Dr. M.-H. Lin*
School of Pharmacy, Taipei Medical University
250 Wu-Xing St., Taipei 110 (Taiwan, Republic of China)

[d] *C.-Y. Yang, Dr. C.-I. Chang*
Institute of Biological Chemistry, Academia Sinica
128 Academia Rd., Nankang, Taipei 115 (Taiwan, Republic of China)
E-mail: chungi@gate.sinica.edu.tw

[e] *Dr. C.-I. Chang*
Institute of Biochemical Sciences, National Taiwan University
No. 1, Sec. 4, Roosevelt Rd., Taipei 106 (Taiwan, Republic of China)

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HDAC8 inhibitors induced apoptosis in a variety of T-cells through a PLCγ1-induced calcium mobilization mechanism.[17]

To date, several HDAC8 selective inhibitors have been reported. These include aryl hydroxamate (**1**),[21] azetidinone (**2**),[22] the pyridine-based thiourea SB-379278A,[23] the 12-membered macrocycle-based phenyl hydroxamate A8B4,[24] and indole-based PCI34051[17] (Figure 1). Of these, compounds **1**, **2**, and SB-379278A had moderate HDAC8-specific inhibitory activity within micromolar range. A8B4 was reported with double-digit nanomolar affinity, but its cell activity was not known. PCI34051 showed excellent potency against HDAC8 (IC_{50} = 56 nM); however, it exhibited limited cellular activity only in T-cell leukemia cells and not in other tumor cells. We therefore sought to develop potent HDAC8-selective inhibitors with better cellular effects. Analysis of the PCI34051 structure showed that the benzyl group might access the secondary surface pocket of HDAC8, which is hydrophobic in nature and has shown various conformations in different co-crystal structures.[25] Thus, we incorporated the PCI34051 benzyl moiety into LBH-589 core *N*-hydroxycinnamides of different chain lengths and modified extensively with different substitutions. The resulting series were assayed against HDAC8, which allowed us to identify phenyl *N*-hydroxycinnamide **22a** as a lead compound with a potency similar to that of PCI34051 (Scheme 1). Further structure optimization, assisted by molecular docking analysis in the HDAC8 crystal structure with compound **22a**, yielded several compounds (**22b**, **22d**, **22f**, and **22g**) with excellent anti-HDAC8 potency (IC_{50} = 5–50 nM). The selected compounds were evaluated for antiproliferative effect on four human lung cancer cells (A549, H1299, CL1-1, and CL1-5).

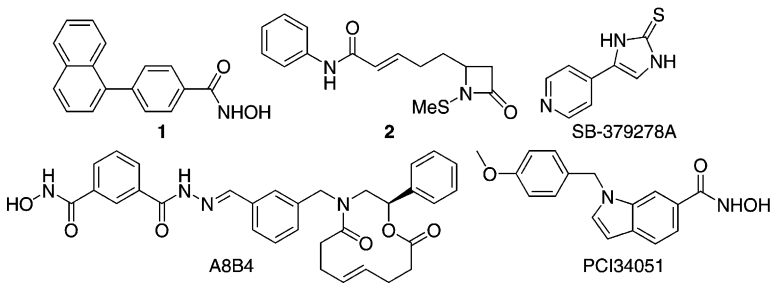
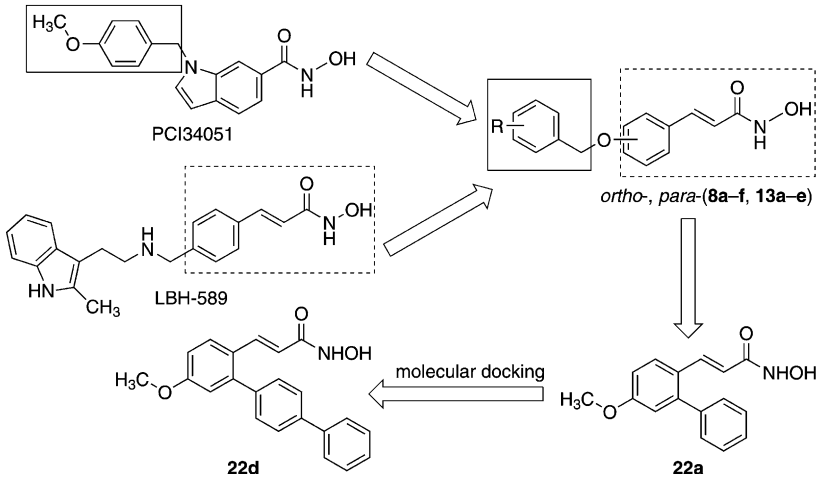


Figure 1. Example of HDAC8-selective inhibitors.

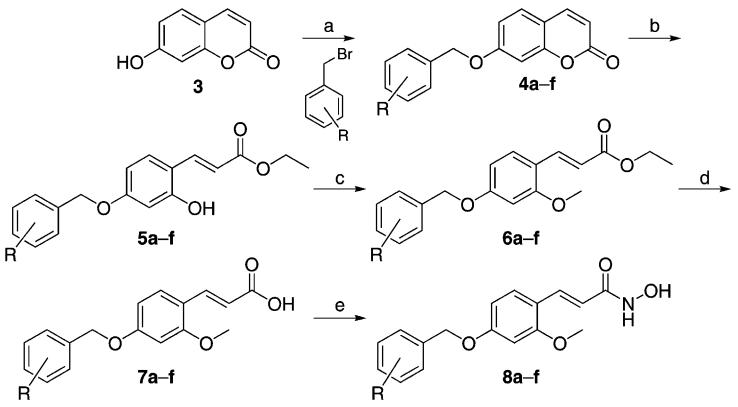


Scheme 1. Design of potent HDAC8 inhibitor **22d**.

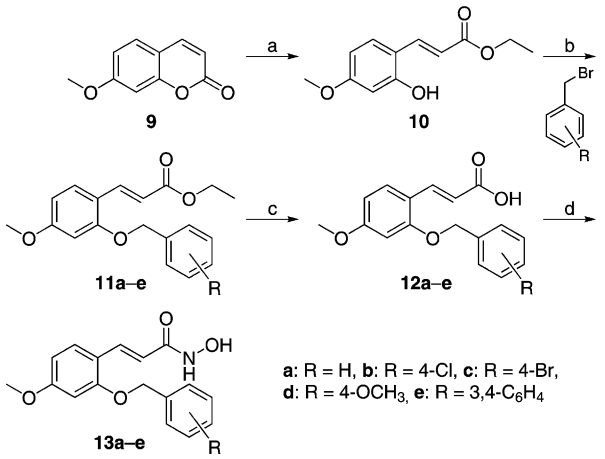
Results and Discussion

Chemistry

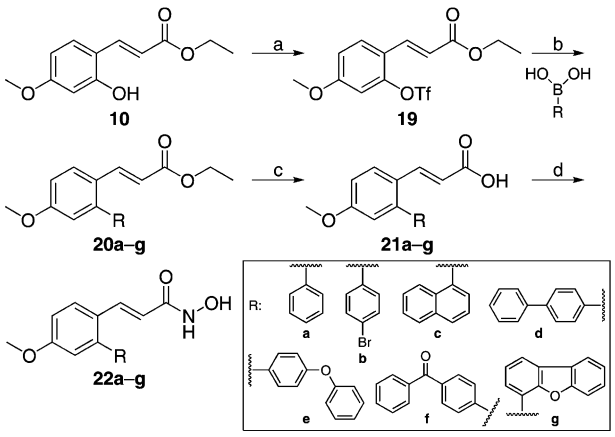
The synthesis of *N*-hydroxycinnamides **8a–f** is illustrated in Scheme 2. 7-Hydroxycoumarin **3** reacted with the appropriate benzyl bromides gave corresponding coumarins **4a–f**. Ethanolysis of compounds **4a–f** using sodium ethoxide under anhydrous conditions provided (*E*)-ethyl cinnamates **5a–f**,[26] respectively. Methylation of compounds **5a–f** reacted with DMS gave corresponding cinnamic esters **6a–f**. Saponification of compounds **6a–f** in the presence of LiOH gave corresponding cinnamic acids **7a–f** in quantitative yields. Compounds **7a–f** reacted with ethyl chloroformate to produce the corresponding activated



Scheme 2. Synthesis of **8a–f**. *Reagents and conditions*: a) K_2CO_3, acetone, N_2, 12 h, 75–85%; b) NaOEt, EtOH, N_2, 6 h, 72–79%; c) DMS, K_2CO_3, acetone, N_2, 12 h, 80–88%; d) LiOH, MeOH, 12 h, 92–96%; e) 1. $ClCO_2Et$, Et_3N, THF, RT, 1 h; 2. $NH_2OH{\cdot}HCl$, KOH, MeOH, RT, 3 h, 51–58%.

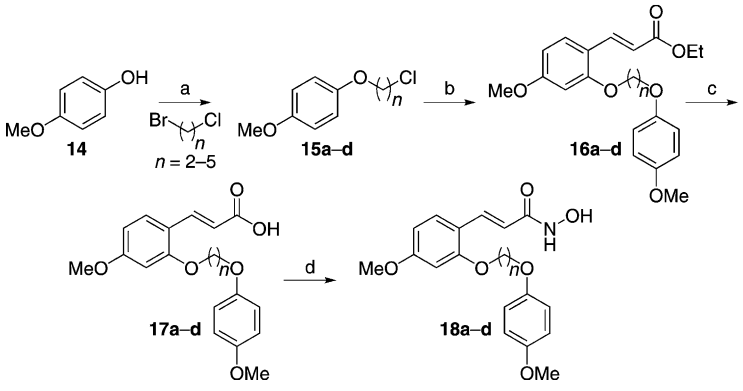


Scheme 3. Synthesis of **13a–e**. *Reagents and conditions*: a) NaOEt, EtOH, N_2, 6 h, 80%; b) K_2CO_3, acetone, N_2, 12 h, 74–77%; c) LiOH, MeOH, 12 h, 93–97%; d) 1. $ClCO_2Et$, Et_3N, THF, RT, 1 h; 2. $NH_2OH{\cdot}HCl$, KOH, MeOH, RT, 3 h, 40–47%.



Scheme 5. Synthesis of **22a–g**. *Reagents and conditions*: a) Tf_2O, py, CH_2Cl_2, RT, 2 h, 62%; b) $Pd(PPh_3)_4$, K_2CO_3, DMF, 90 °C, 12 h, 38–99%; c) LiOH, MeOH, 89–99%; d) 1. $ClCO_2Et$, Et_3N, THF, RT, 1 h; 2. $NH_2OH{\cdot}HCl$, KOH, MeOH, RT, 3 h, 39–59%.

mixed anhydride in situ, and subsequent treatment of the prepared hydroxylamine gave *N*-hydroxycinnamides **8a–f**, respectively. The synthesis of *N*-hydroxycinnamides **13a–e** is described in Scheme 3. Ethanolysis of 7-methoxycoumarin **9** gave (*E*)-ethyl cinnamate **10**. Reaction of (*E*)-ethyl cinnamate **10** with the appropriate benzyl bromides provided cinnamic esters **11a–e**, respectively. *N*-Hydroxycinnamides **13a–e** were achieved starting from compounds **11a–e** through saponification followed by reaction with ethyl chloroformate and hydroxylamine according to the synthetic approach for **8a–f**. The synthesis of *N*-hydroxycinnamides **18a–d** is shown in Scheme 4.

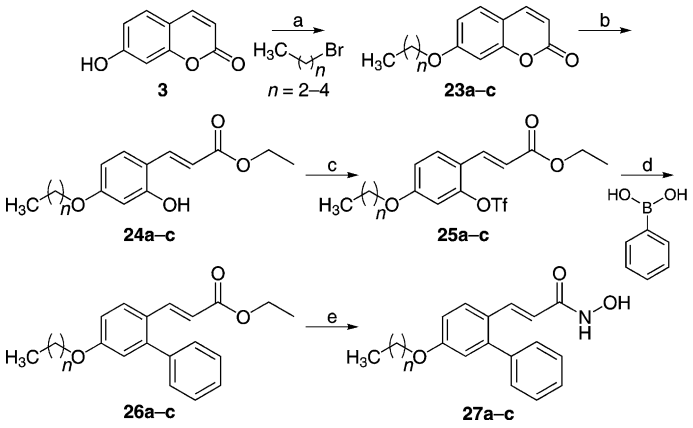


Scheme 4. Synthesis of **18–d**. *Reagents and conditions*: a) K_2CO_3, MeCN, N_2, 12 h, 82–91%; b) **10**, K_2CO_3, DMF, N_2, RT, 12 h, 43–54%; c) LiOH, MeOH, 12 h, 91–96%; d) 1. $ClCO_2Et$, Et_3N, THF, RT, 1 h; 2. $NH_2OH{\cdot}HCl$, KOH, MeOH, RT, 3 h, 53–64%.

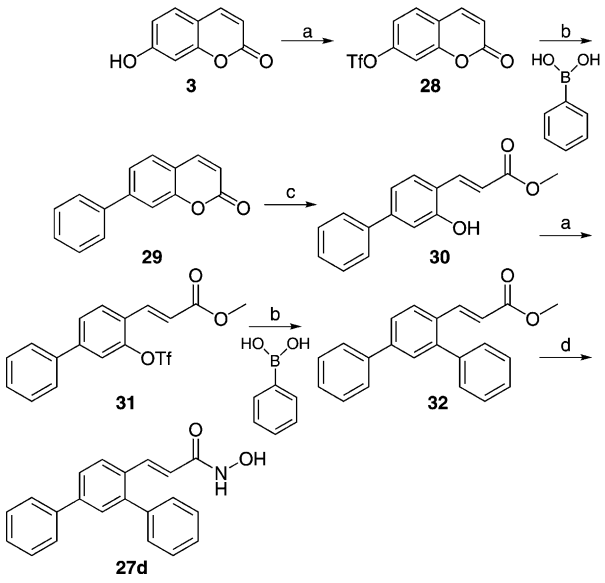
Reaction of 4-methoxyphenol **14** with the appropriate chloro-substituted alkyl bromides with chain lengths of two to five carbons yielded corresponding compounds **15a–d**. Coupling of ethyl cinnamate **10** with **15a–d** gave compounds **16a–d**, respectively. Saponification of compounds **16a–d** and subsequent reaction with ethyl chloroformate and hydroxylamine gave corresponding *N*-hydroxycinnamides **18a–d**. The synthesis of *ortho*-aryl *N*-hydroxycinnamides **22a–g** is described in Scheme 5. Reaction of ethyl cinnamate **10** with trifluoromethanesulfonyl (triflic) anhydride in the presence of pyridine gave compound **19**. Suzuki coupling[27] of **19** with the appropriate aryl borates using catalytic tetrakis(triphenylphosphine) palladium yielded compounds **20a–g**, respectively. Using compound **20a–g** as the starting material, saponification followed by reaction with ethyl chloroformate and hydroxylamine gave corresponding the *N*-hydroxycinnamides **22a–g**. The synthesis of *ortho*-phenyl *N*-hydroxycinnamides **27a–c** with various chain lengths at the *para* position is achieved as described in Scheme 6. Reaction of 7-hydroxycoumarin **3** with the appropriate alkyl bromides with a three- to five-carbon chain length gave **23a–c**, respectively. Ethanolysis of compounds **23a–c** yielded corresponding (*E*)-ethyl cinnamates **24a–c**. Reaction of compounds **24a–c** with triflic anhydride provided **25a–c**, respectively. Suzuki coupling of compounds **25a–c** with phenyl borate gave corresponding compounds **26a–c**. Ethyl cinnamates **26a–c** was reacted directly with hydroxylamine in the presence of NaOH to yield *N*-hydroxycinnamides **27a–c**, respectively. The synthesis of *N*-hydroxycinnamide **27d** with a phenyl group at the *ortho* and *para* position was achieved as illustrated in Scheme 7. Reaction of 7-hydroxycoumarin **3** with triflic anhydride gave **28**. Suzuki coupling of compound **28** with phenyl borate yielded phenyl coumarin **29**. Methanolysis of compound **29** provided (*E*)-methyl cinnamate **30**. Trifluoromethanesulfonylation of compound **30** and subsequent Suzuki coupling was repeated to give **32**. Compound **32** was converted into *N*-hydroxycinnamide **27d** using hydroxylamine and NaOH. Details on the synthesis, isolation, and characterization of reaction intermediates can be found in the Supporting Information.

Biology

The *para*-benzyl *N*-hydroxycinnamides **8a–f** and *ortho*-benzyl *N*-hydroxycinnamides **13a–e** were initially screened for inhibi-



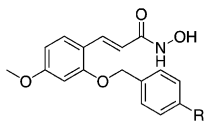
Scheme 6. Synthesis of **27a–c**. *Reagents and conditions*: a) K_2CO_3, acetone, N_2, 12 h, 89–93%; b) NaOEt, EtOH, N_2, 6 h, 31–42%; c) Tf_2O, py, CH_2Cl_2, RT, 2 h, 62–92%; d) $Pd(PPh_3)_4$, K_2CO_3, DMF, 90 °C, 12 h, 84–91%; e) 50% NH_2OH, NaOH, MeOH/THF, RT, 3 h, 51–57%.



Scheme 7. Synthesis of **27d**. *Reagents and conditions*: a) Tf_2O, py, CH_2Cl_2, RT, 2 h, **27**: 80%, **30**: 68%; b) $Pd(PPh_3)_4$, K_2CO_3, DMF, 90 °C, 12 h, **28**: 75%, **31**: 44%; c) NaOMe, MeOH, N_2, 6 h, 35%; d) 50% NH_2OH, NaOH, MeOH/THF, RT, 3 h, 44%.

tory activity against HDAC8 at a concentration of 1 μM, using SAHA as a reference compound (Figure 2). Interestingly, *ortho*-substituted series **13a–e** showed higher potency than *para*-substituted **8a–f**. Compounds **13a**, **13c**, and **13d** were further evaluated for IC_{50} values against HDAC8, as well as against HeLa nuclear extract that contained mainly HDACs1–3, to analyze isoform selectivity (Table 1). Most of these compounds preferred HDAC8 (IC_{50} = 205–613 nM) over other class I isoforms (IC_{50} > 10000 nM), suggesting that the *ortho*-oriented benzyl group may exploit the secondary hydrophobic surface pocket of HDAC8. Next, we synthesized *N*-hydroxycinnamides **18a–d** with various linker chains added to the *ortho*-aryl groups, as well as *ortho*-phenyl *N*-hydroxycinnamide **22a** with a shortened linker chain (Table 2, Table 3). Although the enzyme inhibitory activity of compounds **18a–d** (IC_{50} = 112–191 nM) were all substantially increased relative to the original benzyl-substituted series, compound **22a** with no flexible linker showed the best HDAC8 inhibitory activity (IC_{50} = 72 nM),

Table 1. Inhibition of HDAC8 and HeLa nuclear HDAC by compounds **13a**, **13c**, and **13d**.



Compd	R	IC_{50} [nM][a] HDAC8	HeLa HDAC
13a	H	206.5 ± 13.4	> 10000
13c	Br	613.5 ± 60.8	> 10000
13d	OCH_3	397.2 ± 12.7	> 10000
SAHA	–	1855.1 ± 0.1	41.7 ± 3.2

[a] Data are expressed as the mean ± SD of three determinations.

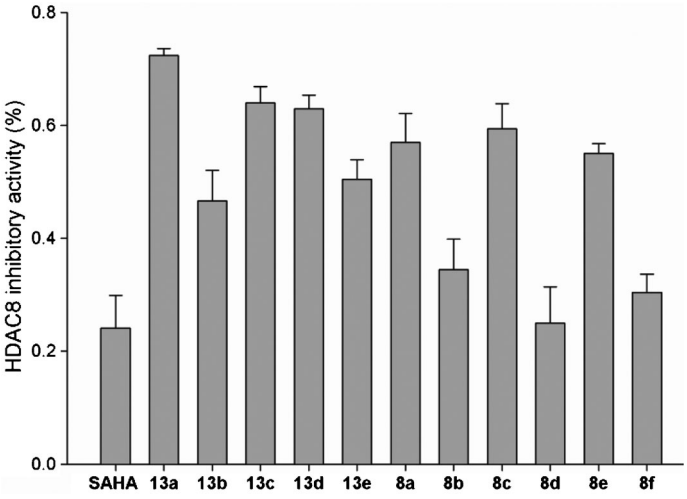
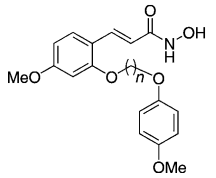


Figure 2. Inhibition of HDAC8 by compounds **8a–f**, **13a–e**, and SAHA. All compounds were tested at a concentration of 1 μM.

which was compatible to PCI34051 (IC_{50} = 56 nM). We further performed molecular docking for HDAC8 crystal structure with compound **22a** and PCI34051 (Figure 3), which showed that further hydrophobic incorporation into the *ortho*-phenyl moiety of compound **22a** may potentially make additional contacts to the active site of HDAC8. To test the molecular modeling results, we synthesized compounds **22b–g** and **27a–d** and examined the resulting compounds for enzyme inhibitory activity. As shown in Table 3, compounds **22b**, **22d**, **22f**, and **22g** showed inhibitory activities superior to PCI34051 for HDAC8; in particular, **22b** and **22d** were around ten- and twofold more potent, respectively. Compound **22b** was 13-fold more potent than **22a**, suggesting that introduction of a *para*-bromo group results in a significant increase in binding affinity. Compounds **22d** and **22f–g** were two- to threefold more potent than **22a**,

Table 2. Inhibition of HDAC8 and HeLa nuclear HDAC by compounds with varying chain length (*n*).

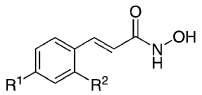


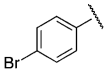
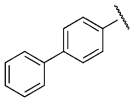
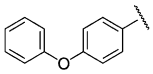
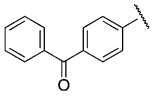
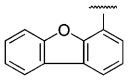
Compd	*n*	IC_{50} [nM][a] HDAC8	HeLa HDAC
18a	2	122.5 ± 3.5	> 10000
18b	3	191.3 ± 2.2	> 10000
18c	4	112.1 ± 2.2	> 10000
18d	5	200.3 ± 6.5	> 10000
PCI45051	–	55.7 ± 0.7	> 10000

[a] Data are expressed as the mean ± SD of three determinations.

indicating that the introduction of additional coplanar phenyl group leads to an increase in activity. By contrast, compound **22e** was threefold less potent than **22a**, suggesting that the dramatic loss of activity is perhaps due to the twisted phenyl conformation adopted in ether moiety. The *para*-alkyl-substituted phenyl *N*-hydroxycinnamides **27a–d** were approximately two- to fourfold less potent than **22a**, suggesting that increasing the carbon chain length attached to the *para*-oxygen position weakens binding affinity. We evaluated the potent HDAC8 inhibitors **22b**, **22d**, **22f**, and **22g** for antiproliferative activity in human lung cancer cell lines, including A549 cells, H1299 cells, CL1-1 cells, and CL1-5 cells using SAHA and PCI34051 as reference compounds (Table 4). Compound **22b** exhibited low cytotoxicity in all four cancer cell lines despite its potent HDAC8 inhibition. We suspect that it may have poor solubility, which was also observed in enzymatic assays. In addition, compounds **22d** and **22g** showed higher cytotoxicity than PCI34051 in three cancer cell lines. Although compound **22d** showed moderate antiproliferative effects in human lung cancer A549 and H1299 cells, it exhibited activity similar to that of SAHA in CL1-5 cells with no significant cytotoxicity in normal IMR-90 cells (Figure 4). Interestingly, the HDAC8 level in CL1-5 is higher than that in H1299 and A549 (Figure 5). To verify whether these compounds were HDAC8-selective, we tested inhibitory activities of compounds **22b** and **22d** against a panel of purified HDACs, including class I (HDAC1, 2, 3),[28] class II (HDAC4, 6, 10)[29] and class IV (HDAC11)[30] enzymes. Table 5 shows that these compounds were inactive toward most other HDACs and had limited activity against HDAC1 and 3.

Table 3. Inhibition of HDAC8 and HeLa nuclear HDAC by compounds **22a–g** and **27a–d**.



Compd	R^1	R^2	IC_{50} [nM][a] HDAC8	HeLa HDAC
22a	OCH_3		72.4 ± 0.1	> 10000
22b	OCH_3		5.7 ± 0.1	> 10000
22c	OCH_3		78.0 ± 4.3	> 10000
22d	OCH_3		27.2 ± 3.1	> 10000
22e	OCH_3		173.8 ± 5.9	> 10000
22f	OCH_3		41.8 ± 3.3	> 10000
22g	OCH_3		47.7 ± 0.7	> 10000
27a	OC_3H_7		132.6 ± 12.6	> 10000
27b	OC_4H_9		289.2 ± 30.1	> 10000
27c	OC_5H_{11}		186.4 ± 59.8	> 10000
27d	C_6H_5		174.9 ± 0.2	> 10000
PCI34051	–	–	55.7 ± 0.7	> 10000

[a] Data are expressed as the mean ± SD of three determinations.

Conclusions

In conclusion, we developed an *ortho*-aryl *N*-hydroxycinnamide series using knowledge-based design combined with molecular modeling techniques and showed their biological evaluation for enzyme inhibitory activity as well as antiproliferative effects on cancer cells. Four compounds—**22b**, **22d**, **22f**, and **22g**—showed potent HDAC8 inhibition over other isoforms, demonstrating the effectiveness of our design concept. Particularly, compound **22d** exhibited higher cellular effects than PCI34051 in several human lung cancer cell lines as well as activity similar to SAHA for one cancer cell line, while showing no cytotoxicity in normal cells.

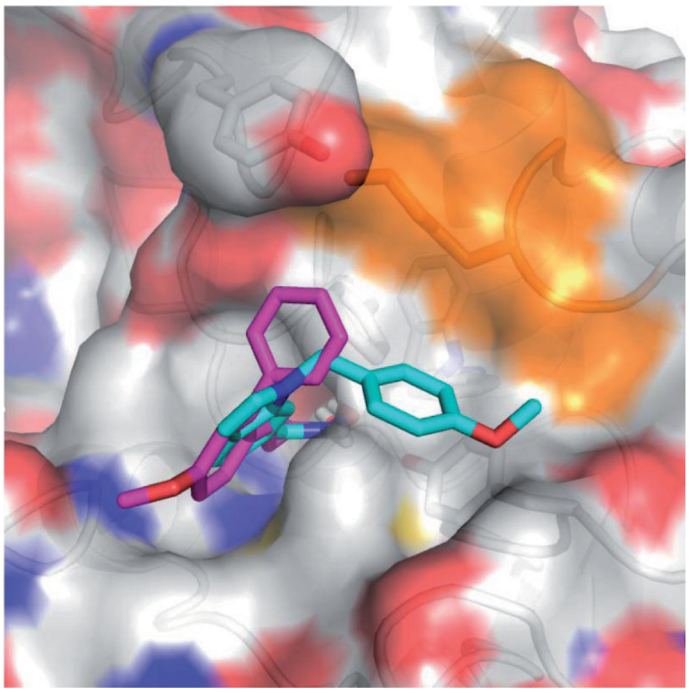


Figure 3. Molecular modeling results of compound **22a** (magenta sticks) and PCI34051 (cyan sticks) docked to HDAC8 (PDB code 1T69; in surface representation) with the highlighted malleable loop L1 (orange), the conformational change in which would expose the secondary surface pocket.

The results suggest that this compound may be used as a tool to probe the physiological role of HDAC8. Further work is underway to identify target genes of HDAC8 using the present compound.

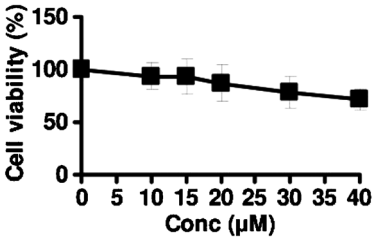


Figure 4. Effect of compound **22d** on cell viability in normal human lung IMR90 cells. Cells were treated with 10–40 μM of compound **22d** for 48 h.

Table 4. Cytotoxicity of compounds **22b**, **22d**, **22f**, and **22g** against various lung cancer cell lines.

Compd	IC_{50} [μM][a] A549	H1299	CL1-1	CL1-5
22b	>10	>10	>10	>10
22d	7.9±1.5	7.2±0.7	>10	7.0±1.5
22f	>10	8.4±0.2	>10	>10
22g	>10	6.6±0.7	8.5±1.2	8.7±0.1
SAHA	1.5±0.7	4.9±0.3	2.9±0.5	6.2±0.6
PCI34051	>10	>10	>10	>10

[a] Data are expressed as the mean ±SD of three determinations.

Experimental Section

Chemistry

General: ^{1}H NMR spectrum was obtained on a Bruker AV400 or AV500 spectrometer using standard pulse programs. Melting point was recorded on a Fisher–Johns apparatus (uncorrected). MS data were measured on a JEOL JMX-HX110 mass spectrometer (HREIMS and HRFABMS), a JMS-SX102A mass spectrometer (EIMS and FABMS), or a Finnigan Mat 95S mass spectrometer (HRESIMS and ESIMS). TLC analyses were carried out on silica gel plates (KG60-F_{254}, Merck). The microplate spectrophotometer Victor 2X (PerkinElmer, Fremont, CA, USA) was used for fluorometric analysis. Unless otherwise mentioned, all chemicals and materials were used as received from commercial suppliers without further purification. CH_2Cl_2 was distilled from CaH under N_2. THF was distilled from sodium and benzophenone under N_2. All test compounds were estimated to be at least 98% pure as judged by HPLC analysis, which was performed on an Ascentis C_{18} column (150×4.6 mm) using an L-2130 pump (Hitachi) and a UV/Vis L-2420 detector (Hitachi) with UV detection at 250 nm.

(*E*)-*N*-Hydroxy-4-benzyloxy-2-methxycinnamide (8a): KOH (1.23 g, 22.00 mmol) was added to a solution of NH_2OH (1.53 g, 22.00 mmol) in MeOH (20 mL). The resulting solution was stirred in an ice bath for 1 h. Filtration

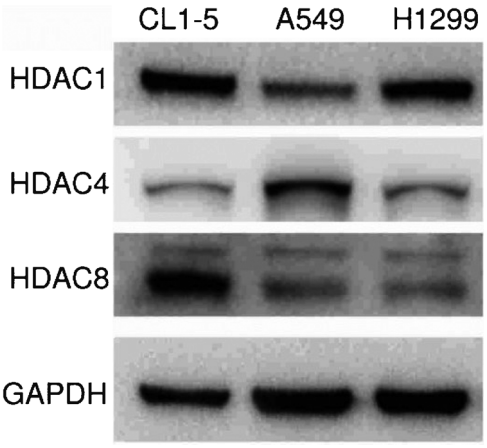


Figure 5. Basal expression levels of HDAC1, HDAC4, and HDAC8 proteins in CL1-5, A549, and H1299 lung cancer cell lines. Cell lysates of CL1-5, A549, and H1299 cells were subjected to immunoblotting using antibodies against HDAC1, HDAC4, HDAC8, and GAPDH proteins. GAPDH served as an internal control. Immunoblots shown are representative of three independent experiments.

Table 5. Inhibition of class I (1, 2, 3), II (4, 6, 10), and IV (11) HDACs by compounds **22b**, **22d**, and PCI34051.

Compd	IC_{50} [μM][a] 1	2	3	4	6	10	11
22b	4.5±0.1	>20	4.8±0.5	>20	>20	>20	>20
22d	3.0±0.2	>20	3.0±0.1	>20	>20	>20	>20
PCI34051	7.5±0.4	>20	>20	>20	>20	>20	>20

[a] Data are expressed as the mean ±SD of three determinations.

to remove the white salt gave a solution of NH_2OH in MeOH. A solution of **7a** (3.12 g, 11.00 mmol) in freshly distilled THF (30 mL) was treated with ethyl chloroformate (1.58 mL, 16.50 mmol) and Et_3N (3.06 mL, 22.00 mmol) and was stirred at room temperature for 1 h. The prepared free NH_2OH solution was then added to the reaction, and stirring was continued for 3 h. The reaction was diluted with distilled water (100 mL), acidified with 1 N $HCl_{(aq)}$ to pH 2–3, and extracted with EtOAc (3×50 mL). The organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo. The residue was purified by silica gel chromatography (EtOAc/*n*-hexane, 1:2–1:1) to give **8a** (1.81 g, 55%) as a white solid: mp: 128–135 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.60 (s, 1H), 8.89 (s, 1H), 7.58 (d, *J* = 15.9 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.33 (d, *J* = 7.4 Hz, 1H), 6.70 (d, *J* = 1.9 Hz, 1H), 6.64 (dd, *J* = 1.9, 8.5 Hz, 1H), 6.37 (d, *J* = 15.9 Hz, 1H), 5.14 (s, 2H), 3.83 ppm (s, 3H); ^{13}C NMR (125 MHZ, [D$_6$]DMSO): δ = 161.6, 160.0, 137.2, 134.9, 129.6, 128.4, 127.9, 127.7, 116.9, 106.6, 99.2, 69.8, 55.2 ppm; HRMS-EI: *m/z* $[M]^+$ calcd for $C_{17}H_{17}NO_4$: 299.1157, found: 299.1160.

(*E*)-*N*-Hydroxy-4-(4-chlorobenzyloxy)-2-methoxycinnamide (8b): Following the procedure as described for **8a**, reaction of **7b** (3.50 g, 11.00 mmol) in THF (40 mL) with ethyl chloroformate (1.54 mL, 16.50 mmol) and Et_3N (2.31 mL, 16.50 mmol) gave **8b** (1.94 g, 53%) as a white solid: mp: 167–172 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.57 (d, *J* = 15.8 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.8 Hz, 1H), 6.68 (s, 1H), 6.62 (d, *J* = 8.8 Hz, 1H), 6.36 (d, *J* = 15.8 Hz, 1H), 5.14 (s, 2H), 3.82 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.1, 161.0, 159.4, 136.3, 133.0, 130.1, 129.7, 128.9, 117.4, 116.9, 107.1, 99.9, 69.1, 56.2 ppm; HRMS-EI: *m/z* $[M-H]^-$ calcd for $C_{17}H_{15}NO_4Cl$: 332.0690, found: 332.0693.

(*E*)-*N*-Hydroxy-4-(4-bromobenzyloxy)-2-methoxycinnamide (8c): Following the procedure as described for **8a**, reaction of **7c** (3.80 g, 10.50 mmol) in THF (40 mL) with ethyl chloroformate (1.47 mL, 15.75 mmol) and Et_3N (2.21 mL, 15.75 mmol) gave **8c** (2.26 g, 57%) as a white solid: mp: 165–170 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.60 (s, 1H), 8.89 (s, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 16.0 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 3H), 6.69 (d, *J* = 2.1 Hz, 1H), 6.63 (dd, *J* = 2.1, 8.6 Hz, 1H), 6.37 (d, *J* = 16.0 Hz, 1H), 5.13 (s, 2H), 3.84 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.1, 161.0, 159.4, 136.7, 133.7, 131.9, 130.4, 129.7, 121.5, 117.4, 116.9, 107.1, 99.9, 69.1, 56.2 ppm; HRMS-EI: *m/z* $[M]^+$ calcd for $C_{17}H_{16}BrNO_4$: 377.0263, found: 377.0259.

(*E*)-*N*-Hydroxy-4-(4-methoxybenzyloxy)-2-methoxycinnamide (8d): Following the procedure as described for **8a**, reaction of **7d** (3.50 g, 11.15 mmol) in THF (35 mL) with ethyl chloroformate (1.56 mL, 16.73 mmol) and Et_3N (2.35 mL, 16.73 mmol) gave **8d** (2.02 g, 55%) as a white solid: mp: 140–150 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.60 (s, 1H), 8.89 (s, 1H), 7.58 (d, *J* = 15.9 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 2H), 6.94 (d, *J* = 8.3 Hz, 2H), 6.67 (s, 1H), 6.63 (d, *J* = 8.5 Hz, 1H), 6.37 (d, *J* = 15.9 Hz, 1H), 5.05 (s, 2H), 3.83 (s, 3H), 3.75 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.2, 161.3, 159.6, 159.4, 133.8, 130.1, 129.6, 129.1, 117.2, 116.7, 114.3, 107.2, 99.8, 69.7, 56.1, 55.6 ppm; HRMS-EI: *m/z* $[M]^+$ calcd for $C_{18}H_{19}NO_5$:329.1263, found: 329.1259.

(*E*)-*N*-Hydroxy-4-[(naphthalen-4-yl)methoxy]-2-methoxycinnamide (8e): Following the procedure as described for **8a**, reaction of **7e** (3.15 g, 9.43 mmol) in THF (35 mL) with ethyl chloroformate (1.32 mL, 14.15 mmol) and Et_3N (1.99 mL, 14.15 mmol) gave **8e** (1.67 g, 51%) as a white solid: mp: 150–158 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.61 (s, 1H), 8.91 (s, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.61 (d, *J* = 9.1 Hz, 1H), 7.56 (m, 3H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.46 (d, *J* = 15.9 Hz, 1H), 6.78 (s, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.39 (d, *J* = 15.9 Hz, 1H), 5.59 (s, 2H), 3.84 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.2, 161.4, 159.4, 133.8, 132.7, 131.6, 129.7, 129.3, 129.0, 127.4, 127.0, 126.5, 125.9, 124.4, 117.3, 116.9, 107.3, 99.8, 68.5, 56.2 ppm; HRMS-ESI *m/z* $[M-H]^-$calcd for $C_{21}H_{18}NO_4Cl$: 348.1236, found: 348.1235.

(*E*)-*N*-Hydroxy-4-(trifluoromethoxy)-2-methoxycinnamide (8f): Following the procedure as described for **8a**, reaction of **7f** (4.50 g, 12.23 mmol) in THF (45 mL) with ethyl chloroformate (1.71 mL, 18.35 mmol) and Et_3N (2.58 mL, 18.35 mmol) gave **8f** (2.72 g, 58%) as a white solid: mp: 155–160 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.58 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 16.6 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 1.9 Hz, 1H), 6.64 (d, *J* = 8.5 Hz, 1H), 6.37 (d, *J* = 16.6 Hz, 1H), 5.17 (s, 2H), 3.82 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 161.0, 159.4, 148.4, 136.8, 133.7, 130.1, 129.7, 121.5, 117.4, 117.0, 107.1, 99.9, 69.0, 56.2 ppm; HRMS-EI: *m/z* $[M]^+$ calcd for $C_{18}H_{16}F_3NO_5$: 383.0980, found: 383.0981.

(*E*)-*N*-Hydroxy-2-benzyloxy-4-methoxycinnamide (13a): KOH (1.46 g, 26.00 mmol) to a solution of $NH_2OH{\cdot}HCl$ (1.81 g, 26.00 mmol) in MeOH (20 mL). The resulting mixture was stirred in an ice bath for 1 h. Filtration to remove the white salt gave a solution of NH_2OH in MeOH. A solution of **12a** (3.69 g, 13.00 mmol) in freshly distilled THF (45 mL) was treated with ethyl chloroformate (1.29 mL, 20.97 mmol), Et_3N (3.61 mL, 26.00 mmol) and the resulting solution was stirred at room temperature for 1 h. The prepared free NH_2OH solution was then added to the reaction and stirring was continued for 3 h. The reaction was diluted with distilled water (100 mL), acidified with 1 N $HCl_{(aq)}$ to pH 2–3, and extracted with EtOAc (3×50 mL). The combined organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo. The residue was purified by silica gel chromatography (EtOAc/*n*-hexane, 1:1) to give **13a** (1.55 g, 40%) as a white solid: mp: 134–140 °C; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 8.02 (d, *J* = 15.8 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 3H) 7.52 (t, *J* = 7.2 Hz, 2H), 7.45 (t, *J* = 7.3 Hz, 1H), 6.80 (d, *J* = 2.2 Hz, 1H), 6.68 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.66 (d, *J* = 8.6 Hz, 1H), 6.52 (d, *J* = 15.8 Hz, 1H), 5.36 (s, 2H), 3.92 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.1, 162.1, 158.1, 137.3, 133.5, 129.1, 129.0, 128.4, 128.0, 117.2, 117.0, 106.7, 100.4, 70.1, 55.9 ppm; HRMS-FAB: *m/z* $[M+H]^+$ calcd for $C_{17}H_{18}NO_4$: 300.1235, found: 300.1234.

(*E*)-*N*-Hydroxy-2-(4-chlorobenzyloxy)-4-methoxycinnamide (13b): Following the procedure as described for **13a**, reaction of **12b** (2.53 g, 7.86 mmol) in THF (40 mL) with ethyl chloroformate (1.10 mL, 11.79 mmol) and Et_3N (1.65 mL, 11.79 mmol) gave **13b** (1.10 g, 42%) as a white solid: mp: 160–170 °C; ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ = 11.04 (s, 1H), 9.69 (s, 1H), 8.44 (d, *J* = 15.9 Hz, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.25 (br s, 4H), 7.48 (d, *J* = 1.9 Hz, 1H), 7.39 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.16 (d, *J* = 15.9 Hz, 1H), 6.01 (s, 2H), 4.57 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 164.1, 162.0, 157.9, 136.3, 133.5, 133.0, 129.9, 129.3, 129.1, 117.3, 117.0, 106.8, 100.4, 69.3, 55.9 ppm; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{17}H_{17}ClNO_4$: 334.0846, found: 334.0843.

(*E*)-*N*-Hydroxy-2-(4-bromobenzyloxy)-4-methoxycinnamide (13c): Following the procedure as described for **13a**, reaction of **12c** (3.50 g, 9.67 mmol) in THF (40 mL) with ethyl chloroformate (1.35 mL, 14.51 mmol) and Et_3N (2.03 mL, 14.51 mmol) gave **13c** (1.46 g, 40%) as a white solid: mp: 148–155 °C; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 10.17 (s, 1H), 7.18 (d, *J* = 16.1 Hz, 1H), 7.14 (d, *J* =

8.3 Hz, 2H), 7.00 (d, J=8.6 Hz, 1H), 6.96 (d, J=8.3 Hz, 2H), 6.21 (s, 1H), 6.13 (d, J=8.6 Hz, 1H), 5.90 (d, J=16.1 Hz, 1H), 4.73 (s, 2H), 3.32 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=164.1, 162.0, 157.9, 136.7, 133.5, 132.0, 130.2, 129.3, 121.6, 117.3, 117.0, 106.8, 100.4, 69.3, 55.9 ppm; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{17}H_{17}BrNO_4$: 378.0341, found: 378.0340.

(*E*)-*N*-Hydroxy-2-(4-methoxybenzyloxy)-4-methoxycinnamide (13d): Following the procedure as described for **13a**, reaction of **12d** (2.20 g, 7.00 mmol) in THF (40 mL) with ethyl chloroformate (1.86 mL, 10.51 mmol) and Et$_3$N (2.80 mL, 10.51 mmol) gave **13d** (1.04 g, 45%) as a white solid: mp: 145–150 °C; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ=7.98 (d, J=15.8 Hz, 1H), 7.61 (d, J=8.4 Hz, 1H), 7.55 (d, J=8.6 Hz, 2H), 7.07 (d, J=8.6 Hz, 2H), 6.81 (d, J=2.2 Hz, 1H), 6.67 (dd, J=8.6, 2.2 Hz, 1H), 6.64 (d, J=15.8 Hz, 1H), 5.27 (s, 2H), 3.93 (s, 3H), 3.92 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=162.5, 159.7, 158.6, 135.6, 129.0, 128.8, 116.8, 114.4, 113.6, 105.8, 99.5, 69.9, 54.5, 54.3 ppm; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{18}H_{20}NO_5$: 330.1341, found: 330.1341.

(*E*)-*N*-Hydroxy-2-[(naphthalen-4-yl)methoxy]-4-methoxycinnamide (13e): Following the procedure as described for **13a**, reaction of **12e** (2.82 g, 8.44 mmol) in THF (40 mL) with ethyl chloroformate (2.24 mL, 12.67 mmol) and Et$_3$N (3.38 mL, 12.67 mmol) gave **13e** (1.38 g, 47%) as a white solid: mp: 152–165 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=10.55 (s, 1H), 8.84 (s, 1H), 8.13 (d, J=8.2 Hz, 1H), 7.98 (m, 2H), 7.94 (d, J=8.2 Hz, 1H), 7.69 (d, J=6.7 Hz, 1H), 7.61 (d, J=16.1 Hz, 1H), 7.55 (m, 2H), 7.45 (d, J=8.5 Hz, 1H), 6.90 (s, 1H), 6.60 (d, J=8.5 Hz, 1H), 6.30 (d, J=16.1 Hz, 1H), 5.65 (s, 2H), 3.80 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=163.9, 162.2, 158.1, 133.8, 133.2, 132.7, 131.6, 129.3, 129.0, 128.7, 127.1, 127.0, 126.5, 125.9, 124.3, 117.0, 106.9, 100.4, 68.7, 55.9 ppm; HRMS-ESI: m/z $[M+H]^+$ calcd for $C_{21}H_{20}NO_4$: 350.1392, found: 350.1390.

(*E*)-*N*-Hydroxy-2-(2-(4-methoxyphenoxyoxy)ethoxy)-4-methoxycinnamide (18a): Following the procedure described for **8a**, reaction of **17a** (900 mg, 2.61 mmol) in THF (10 mL) with ethyl chloroformate (424 mg, 3.92 mmol) and Et$_3$N (0.73 mL, 5.22 mmol) gave **18a** (497 mg, 53%) as a white solid: mp: 110–115 °C; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ=10.12 (s, 1H), 8.63 (s, 1H), 7.85 (d, J=15.8 Hz, 1H), 7.49 (d, J=8.5 Hz, 1H), 6.95 (d, J=8.1 Hz, 2H), 6.86 (d, J=8.1 Hz, 2H), 6.68 (d, J=2.4 Hz, 1H), 6.57 (dd, J=2.4, 8.5 Hz, 1H), 6.54 (d, J=15.8 Hz, 1H), 4.42 (m, 2H), 4.38 (m, 2H), 3.83 (s, 3H), 3.73 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=162.1, 158.4, 158.3, 154.1, 152.8, 133.7, 129.4, 117.2, 116.9, 116.2, 115.1, 113.2, 107.0, 100.0, 67.7, 67.3, 60.4, 55.9, 55.8 ppm; HRMS-EI: m/z $[M]^+$ calcd for $C_{19}H_{21}NO_6$: 359.1369, found: 359.1364.

(*E*)-*N*-Hydroxy-2-(2-(4-methoxyphenoxyoxy)propoxy)-4-methoxycinnamide (18b): Following the procedure described for **8a**, reaction of **17b** (300 mg, 0.84 mmol) in THF (10 mL) with ethyl chloroformate (136 mg, 1.26 mmol) and Et$_3$N (0.23 mL, 1.68 mmol) gave **18b** (191 mg, 61%) as a white solid: mp: 115–120 °C; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ=10.60 (s, 1H), 8.86 (s, 1H), 7.57 (d, J=15.9 Hz, 1H), 7.41 (d, J=8.6 Hz, 1H), 6.86 (d, J=9.2 Hz, 2H), 6.82 (d, J=9.2 Hz, 2H), 6.60 (d, J=2.3 Hz, 1H), 6.54 (dd, J=2.3, 8.6 Hz, 1H), 6.38 (d, J=15.9 Hz, 1H), 4.18 (t, J=6.2 Hz, 2H), 4.07 (t, J=6.2 Hz, 2H), 3.75 (s, 3H), 3.66 (s, 3H), 2.19 ppm (q, J=6.2 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=164.2, 162.1, 158.7, 153.9, 152.9, 134.0, 130.1, 117.3, 116.7, 115.9, 115.1, 106.6, 99.6, 65.5, 65.2, 55.9, 55.8, 29.1 ppm; HRMS-ESI: m/z $[M+Na]^+$ calcd for $C_{20}H_{23}NNaO_6$: 396.1408, found: 396.1418.

(*E*)-*N*-Hydroxy-2-(2-(4-methoxyphenoxyoxy)butoxy)-4-methoxycinnamide (18c): Following the procedure described for **8a**, reaction of **17c** (250 mg, 0.67 mmol) in THF (10 mL) was treated with ethyl chloroformate (109 mg, 1.00 mmol) and Et$_3$N (0.19 mL, 1.34 mmol) gave **18c** (166 mg, 64%) as a white solid: mp: 82–88 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=10.62 (s, 1H), 9.46 (s, 1H), 7.57 (d, J=15.9 Hz, 1H), 7.40 (d, J=8.4 Hz, 1H), 6.83 (d, J=9.4 Hz, 2H), 6.79 (d, J=9.4 Hz, 2H), 6.56 (s, 1H), 6.52 (d, J=8.4 Hz, 1H), 6.35 (d, J=15.9 Hz, 1H), 4.06 (t, J=5.7 Hz, 2H), 3.93 (t, J=5.7 Hz, 2H), 3.74 (s, 3H), 3.64 (s, 3H), 1.86 ppm (m, 4H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=164.2, 162.1, 158.8, 153.8, 153.1, 134.0, 129.9, 117.2, 116.7, 115.8, 115.1, 106.5, 99.6, 68.3, 68.0, 55.9, 55.8, 26.1, 25.8 ppm; HRMS-ESI: m/z $[M-H]^-$ calcd for $C_{21}H_{24}NO_6$: 386.1604, found: 386.1609.

(*E*)-*N*-Hydroxy-2-(2-(4-methoxyphenoxyoxy)pentyloxy)-4-methoxycinnamide (18d): Following the procedure described for **8a**, reaction of **17d** (200 mg, 0.52 mmol) in THF (10 mL) with ethyl chloroformate (85 mg, 0.78 mmol) and Et$_3$N (0.15 mL, 1.04 mmol) gave **18d** (118 mg, 57%) as a colorless liquid: ^{1}H NMR (500 MHz, [D$_6$]acetone): δ=7.82 (d, J=15.7 Hz, 1H), 7.47 (d, J=8.4 Hz, 1H), 6.86 (d, J=9.4 Hz, 2H), 6.84 (d, J=15.7 Hz, 1H), 6.82 (d, J=9.4 Hz, 2H), 6.60 (d, J=2.2 Hz, 1H), 6.54 (dd, J=2.2, 8.4 Hz, 1H), 4.12 (t, J=6.4 Hz, 2H), 3.97 (t, J=6.4 Hz, 2H), 3.82 (s, 3H), 3.71 (s, 3H), 1.93 (m, 2H), 1.84 (m, 2H), 1.69 ppm (m, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=162.4, 159.1, 153.9, 153.3, 135.1, 129.8, 116.8, 115.4, 114.5, 105.7, 99.0, 68.2, 68.1, 54.9, 28.9, 28.7, 22.6 ppm; HRMS-ESI: m/z $[M-H]^-$ calcd for $C_{22}H_{26}NO_6$: 400.1760, found: 400.1766.

(*E*)-*N*-Hydroxy-4-methoxy-2-phenylcinnamide (22a): Following the procedure described for **8a**, reaction of **21a** (800 mg, 3.15 mmol) in THF (10 mL) with ethyl chloroformate (512 mg, 4.73 mmol) and Et$_3$N (0.88 mL, 6.30 mmol) gave **22a** (355 mg, 42%) as a white solid: mp: 100–102 °C; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ=8.67 (br s, 1H), 7.66 (d, J=8.2 Hz, 1H), 7.55 (d, J=15.5 Hz, 1H), 7.47 (m, 2H), 7.39 (m, 1H), 7.33 (d, J=8.4 Hz, 2H), 6.98 (dd, J=2.5, 8.7 Hz, 1H), 6.88 (d, J=2.5 Hz, 1H), 6.41 (d, J=15.5 Hz, 1H), 3.89 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]acetone): δ=164.7, 161.3, 145.1, 141.1, 138.6, 130.5, 129.1, 128.8, 128.4, 128.1, 126.4, 117.3, 115.9, 114.9, 114.7, 55.8 ppm; HRMS-ESI: m/z $[M+Na]^+$ calcd for $C_{16}H_{15}NNaO_3$: 292.0950, found: 292.0944.

(*E*)-*N*-Hydroxy-4-methoxy-2-(4-bromophenyl)cinnamide (22b): Following the procedure described for **8a**, reaction of **21b** (500 mg, 1.51 mmol) in THF (10 mL) with ethyl chloroformate (245 mg, 2.27 mmol) and Et$_3$N (0.42 mL, 3.02 mmol) gave **22b** (204 mg, 39%) as a white solid: mp: 141–144 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=10.64 (s, 1H), 8.90 (s, 1H), 7.65 (d, J=8.2 Hz, 2H), 7.63 (d, J=8.7 Hz, 1H), 7.25 (d, J=8.2 Hz, 2H), 7.22 (d, J=15.6 Hz, 1H), 7.02 (dd, J=2.1, 8.7 Hz, 1H), 6.84 (d, J=2.1 Hz, 1H), 6.28 (d, J=15.6 Hz, 1H), 3.80 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=163.4, 160.3, 142.6, 139.4, 136.4, 132.1, 131.8, 128.3, 125.6, 121.7, 118.6, 115.4, 115.1, 55.9 ppm. HRMS-ESI: m/z $[M+Na]^+$ calcd for $C_{16}H_{14}BrNNaO_3$: 370.0055, found: 370.0049.

(*E*)-*N*-Hydroxy-4-methoxy-2-(naphthalen-1-yl)cinnamide (22c): Following the procedure described for **8a**, reaction of **21c** (1.00 g, 3.29 mmol) in THF (15 mL) with ethyl chloroformate (533 mg, 4.93 mmol) and Et$_3$N (0.92 mL, 6.58 mmol) gave **22c** (619 mg, 59%) as a white solid: mp: 125–127 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=8.00 (d, J=8.2 Hz, 2H), 7.74 (d, J=8.8 Hz, 1H), 7.59 (t, J=7.9 Hz, 1H), 7.52 (t, J=7.2 Hz, 1H), 7.42 (t, J=7.9 Hz, 1H), 7.33 (d, J=6.8 Hz, 1H), 7.30 (d, J=8.4 Hz, 1H), 7.11 (dd, J=2.6, 8.8 Hz, 1H), 6.83 (d, J=15.6 Hz, 1H), 6.82 (d, J=2.5 Hz, 1H), 6.25 (d, J=15.6 Hz, 1H), 3.79 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=163.3, 160.3, 142.4, 137.9, 136.2, 133.6, 132.0, 128.8, 128.6, 127.7, 127.5,

127.0, 126.9, 126.6, 125.9, 118.0, 116.3, 115.1, 55.9 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{20}H_{17}NNaO_3$: 342.1106, found: 342.1101.

(*E*)-*N*-Hydroxy-4-methoxy-2-(biphenyl-4-yl)cinnamide (22 d): Following the procedure described for **8 a**, reaction of **21 d** (800 mg, 2.42 mmol) in THF (15 mL) with ethyl chloroformate (393 mg, 3.64 mmol) and Et_3N (0.68 mL, 4.84 mmol) gave **22 d** (326 mg, 39%) as a white solid: mp: 76–79 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.76 (d, *J* = 8.1 Hz, 1H), 7.73 (d, *J* = 7.7 Hz, 2H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.39 (d, *J* = 8.1 Hz, 3H), 7.35 (d, *J* = 15.0 Hz, 1H), 7.02 (dd, *J* = 2.6, 8.7 Hz, 1H), 6.90 (d, *J* = 2.6 Hz, 1H), 6.32 (d, *J* = 15.0 Hz, 1H), 3.82 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.5, 160.4, 143.5, 140.0, 139.9, 139.3, 136.9, 130.6, 129.5, 128.3, 128.1, 127.2, 127.1, 125.6, 118.3, 115.4, 114.9, 55.9 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{22}H_{19}NNaO_3$: 368.1263, found: 368.1257.

(*E*)-*N*-Hydroxy-4-methoxy-2-(4-phenoxyphenyl)cinnamide (22 e): Following the procedure described for **8 a**, reaction of **21 e** (800 mg, 2.31 mmol) in THF (15 mL) with ethyl chloroformate (374 mg, 3.47 mmol) and Et_3N (0.65 mL, 4.62 mmol) gave **22 e** (417 mg, 50%) as a white solid: mp: 75–78 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.61 (d, *J* = 8.7 Hz, 1H), 7.42 (t, *J* = 8.1 Hz, 1H), 7.42 (d, *J* = 15.8 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 3H), 7.18 (t, *J* = 7.4 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 6.99 (dd, *J* = 2.6, 8.7 Hz, 1H), 6.85 (d, *J* = 2.6 Hz, 1H), 6.28 (d, *J* = 15.8 Hz, 1H), 3.80 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.5, 160.3, 157.1, 156.5, 143.3, 136.9, 135.0, 131.6, 130.7, 128.3, 125.7, 124.5, 119.8, 118.3, 118.2, 115.5, 114.7, 55.8 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{22}H_{19}NNaO_4$: 384.1212, found: 384.1206.

(*E*)-*N*-Hydroxy-4-methoxy-2-(4-benzoylphenyl)cinnamide (22 f): Following the procedure described for **8 a**, reaction of **21 f** (900 mg, 2.51 mmol) in THF (20 mL) with ethyl chloroformate (406 mg, 3.77 mmol) and Et_3N (0.71 mL, 5.02 mmol) gave **22 f** (487 mg, 52%) as a white solid: mp: 71–73 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.83 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.68 (d, *J* = 7.3 Hz, 1H), 7.67 (d, *J* = 7.6 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 15.6 Hz, 1H), 7.06 (dd, *J* = 2.3, 8.6 Hz, 1H), 6.92 (d, *J* = 2.3 Hz, 1H), 6.31 (d, *J* = 15.6 Hz, 1H), 3.82 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 195.8, 163.4, 160.4, 144.5, 142.8, 137.5, 136.5, 136.4, 133.2, 130.3, 130.2, 130.1, 129.1, 128.5, 125.6, 118.8, 115.4, 55.9 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{23}H_{19}NNaO_4$: 396.1212, found: 396.1206.

(*E*)-*N*-Hydroxy-4-methoxy-2-(dibenzofuran-4-yl)cinnamide (22 g): Following the procedure described for **8 a**, reaction of **21 g** (1.00 g, 2.91 mmol) in THF (20 mL) with ethyl chloroformate (470 mg, 4.36 mmol) and Et_3N (0.82 mL, 5.82 mmol) gave **22 g** (532 mg, 51%) as a white solid: mp: 158–161 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.21 (d, *J* = 7.7 Hz, 1H), 8.19 (d, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.49 (q, *J* = 7.5 Hz, 2H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.11 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.10 (d, *J* = 15.6 Hz, 1H), 7.01 (d, *J* = 2.5 Hz, 1H), 6.30 (d, *J* = 15.6 Hz, 1H), 3.82 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.3, 160.4, 156.0, 153.4, 138.5, 136.2, 129.2, 128.3, 127.9, 126.5, 124.5, 124.3, 124.0, 123.7, 121.8, 121.4, 118.3, 116.3, 115.4, 112.3, 55.9 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{22}H_{17}NNaO_4$: 382.1055, found: 382.1050.

(*E*)-*N*-Hydroxy-4-propoxy-2-phenylcinnamide (27 a): NaOH (120 mg, 3.01 mmol) in 50% $NH_2OH_{(aq)}$ (2 mL) in an ice bath was added to a solution of **26 a** (170 mg, 0.60 mmol) in MeOH/THF (1 mL:1 mL). The resulting solution was then warmed to room temperature and stirred for an additional 3 h. The reaction was diluted with distilled water (50 mL), acidified with 1 N $HCl_{(aq)}$ to pH 6–7, and extracted with EtOAc (25 mL × 3). The organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo. The residue was purified by silica gel chromatography (MeOH/CH_2Cl_2, 3:97) to give **27 a** (90 mg, 51%) as a white solid: mp: 147–150 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.22 (s, 1H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.54 (d, *J* = 15.4 Hz, 1H), 7.42 (m, 3H), 7.33 (m, 2H), 6.97 (dd, *J* = 2.5, 8.5 Hz, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 15.4 Hz, 1H), 4.03 (t, *J* = 6.6 Hz, 2H), 1.78 (m, 2H), 1.01 ppm (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.5, 159.8, 140.0, 140.2, 136.9, 130.0, 128.8, 128.2, 128.1, 125.4, 118.0, 116.0, 115.1, 69.7, 22.5, 10.8 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{18}H_{19}NNaO_3$: 320.1263, found: 320.1257.

(*E*)-*N*-Hydroxy-4-butoxy-2-phenylcinnamide (27 b): NaOH (100 mg, 2.50 mmol) in 50% $NH_2OH_{(aq)}$ (2 mL) in an ice bath was added to a solution of **26 b** (162 mg, 0.50 mmol) in MeOH/THF (1 mL:1 mL). Following the procedure as described for **27 a** gave **27 b** (88 mg, 57%) as a white solid: mp: 140–144 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.61 (s, 1H), 8.87 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 15.7 Hz, 1H), 7.42 (m, 2H), 7.28 (m, 3H), 6.99 (dd, *J* = 2.3, 8.7 Hz, 1H), 6.82 (d, *J* = 2.3 Hz, 1H), 6.28 (d, *J* = 15.7 Hz, 1H), 4.02 (t, *J* = 7.4 Hz, 2H), 1.69 (m, 2H), 1.41 (m, 2H), 0.91 ppm (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.5, 159.8, 144.0, 140.2, 136.9, 130.0, 128.8, 128.1, 125.4, 118.0, 115.9, 115.2, 67.9, 31.2, 19.2, 14.2 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{19}H_{21}NNaO_3$: 334.1419, found: 334.1414.

(*E*)-*N*-Hydroxy-4-pentoxy-2-phenylcinnamide (27 c): NaOH (100 mg, 2.50 mmol) in 50% $NH_2OH_{(aq)}$ (2 mL) in an ice bath was added to a solution of **26 c** (169 mg, 0.50 mmol) in MeOH/THF (1 mL:1 mL). Following the procedure as described for **27 a** gave **27 c** (86 mg, 53%) as a white solid: mp: 121–124 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.61 (s, 1H), 9.47 (s, 1H), 7.62 (d, *J* = 8.7 Hz, 1H), 7.47 (d, *J* = 15.6 Hz, 1H), 7.46 (m, 1H), 7.42 (m, 1H), 7.29 (m, 3H), 7.00 (dd, *J* = 2.6, 8.7 Hz, 1H), 6.83 (d, *J* = 2.6 Hz, 1H), 6.29 (d, *J* = 15.6 Hz, 1H), 4.02 (t, *J* = 6.5 Hz, 2H), 1.71 (m, 2H), 1.36 (m, 4H), 0.88 ppm (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.5, 159.8, 158.4, 144.0, 140.2, 136.9, 130.0, 128.8, 128.1, 128.0, 125.4, 118.0, 115.9, 115.2, 68.2, 28.8, 28.1, 22.3, 14.4 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{20}H_{23}NNaO_3$: 348.1576, found: 348.1570.

(*E*)-*N*-Hydroxy-2,4-diphenylcinnamide (27 d): NaOH (191 mg, 4.78 mmol) in 50% $NH_2OH_{(aq)}$ (4 mL) in an ice bath was added to a solution of **32** (300 mg, 0.96 mmol) in MeOH/THF (2 mL:2 mL) was added. Following the procedure as described for **27 a** gave **27 d** (133 mg, 44%) as a white solid: mp: 135–137 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.64 (d, *J* = 15.4 Hz, 1H), 7.74 (d, *J* = 7.4 Hz, 2H), 7.71 (dd, *J* = 1.5, 8.2 Hz, 1H), 7.63 (d, *J* = 1.5 Hz, 1H), 7.49 (m, 3H), 7.45 (m, 1H), 7.42 (m, 3H), 7.32 (m, 1H), 6.58 ppm (d, *J* = 15.4 Hz, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 163.1, 142.9, 141.4, 140.2, 139.6, 136.8, 132.2, 130.2, 129.5, 129.4, 128.9, 128.4, 128.1, 127.4, 127.3, 126.7, 120.5 ppm; HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{21}H_{17}NNaO_2$: 338.1157, found: 338.1151.

Biology

Preparation of HDAC4 and HDAC8: Genes encoding HDAC4 (residues 648–1057) and HDAC8 (residues 1–377) flanked with NdeI and EcoRI sites at the 5′- and 3′-ends, respectively, were synthesized by GenScript Corporation (NJ, USA) and subcloned into expression vectors pET-28a(+) and pET-24b(+), respectively. Proteins were expressed in BL21(DE3) cells by induction with IPTG (1 mM) at 20–25 °C for overnight and purified from cleared cell lysates by sequential chromatography on Ni-Sepharose 6 fast flow, Mono Q

5/50 GL, and Superdex 75 10/300 GL columns (GE Healthcare). Protein concentrations were quantified with Bradford Reagent (Bio-Rad).

HDAC activity assay: The HDAC activity assay was carried out as described previously.[31] Enzyme, inhibitors, and substrate were diluted with HDAC buffer (15 mM Tris·HCl pH 8.1, 0.25 mM EDTA, 250 mM NaCl, 10% *v/v* glycerol). Briefly, 10 μL of diluted HDAC such as HeLa nuclear extract, HDAC6, HDAC4, and HDAC8, as well as 50 μL test compound solution at different concentrations were added to each well of a 96-well microtiter plate and pre-incubated at 30 °C for 5 min. The enzymatic reaction was started by addition of 40 μL substrate such as Boc-Lys(Ac)-AMC (Bachem) for HeLa nuclear extract and HDAC6, Boc-Lys(TFA)-AMC (Bachem) for HDAC4 or 8, and KI177 (Enzo) for HDAC1, 2, 3/NCoR2, 10, or 11 in HDAC buffer. After incubation at 30 °C for 30 min, the reaction was terminated by addition of 100 μL trypsin solution (10 mg mL^{-1} trypsin in 50 mM Tris·HCl pH 8, 100 mM NaCl, 2 μM SAHA). After incubation at 30 °C for a further 20 min, fluorescence was measured (extinction $\lambda = 355$ nm, emission $\lambda = 460$ nm). For calculation of IC_{50} values, the fluorescence in wells without test compound (0.1% DMSO, negative control) was set as 100% enzymatic activity and the fluorescence in wells with 2 μM SAHA (positive control) was set at 0% enzymatic activity. All experiments were carried out in triplicate.

MTT cytotoxicity assays: Cells were seeded at 5×104 cells per well in 12-well plates and treated with various concentrations of *N*-hydroxycinnamide for 48 h, followed by 0.5 mg mL^{-1} 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma–Aldrich, St. Louis, MO, USA) for 0.5 h at 37 °C in a 5% CO_2 humidified incubator to determine their cytotoxic effects. Cell cytotoxicity was expressed as the percentage loss in cell viability compared with control (DMSO), and the 50% inhibition concentration (IC_{50}) of cell lines was calculated.

Western blot analysis: The cells were lysed on ice. Lysates were centrifuged at 13000 rpm for 15 min at 4 °C. SDS gel loading buffer was added to cell lysates, and samples containing equal amounts of protein (50 mg) were separated on a 12% SDS-PAGE then electroblotted onto a Immobilon-P membrane (Millipore, Bedford, MA) in transfer buffer. Immunoblotting was performed for various proteins, using the antibodies with conditions described in Table S1.

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