Aromatic Sulfide Inhibitors of Histone Deacetylase Based on Arylsulfinyl-2,4-hexadienoic Acid Hydroxyamides

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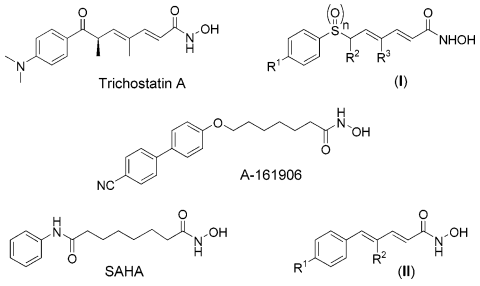
The synthesis of a novel series of potent inhibitors of histone deacetylases is described, based on arylsulfinyl-2,4-hexadienoic acid hydroxyamides and their derivatives. In vitro IC_{50} values down to 40 nM were obtained, and several compounds showed inhibition of CEM (human leukemic) cell viability with IC_{50} of ~1.5 μM, comparable to or better than that of suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase currently in clinical trials.

Introduction

Inhibitors of histone deacetylase (HDAC) enzymes are emerging as a promising class of anticancer agents[1,2] able to regulate gene transcription[3−5] and inhibit cancer cell proliferation by induction of cell cycle arrest, differentiation, and/or apoptosis.[6−8] HDAC enzymes catalyze the deacetylation of ϵ-amino groups of lysine residues in the N-terminal tails of core histones in the nucleosome, resulting in an increased interaction of the protonated ϵ-amino lysine residues with DNA, leading to a compacted and less accessible chromatin structure.[9] Consequently, HDACs can act as repressors of gene transcription by mediating conformational changes in the nucleosome and altering the accessibility of transcription factors to DNA.[10,11] The inappropriate recruitment of HDAC enzymes by oncogenic proteins may alter gene expression in favor of arrested differentiation and/or excessive proliferation.[12] Accordingly, the hyperacetylation of chromatin induced by inhibitors of HDACs usually results in the relief of transcriptional repression of certain genes, e.g., the cyclin-dependent kinase inhibitor protein $p21^{WAF1/CIP1}$.[12,13] Relief of transcriptional repression is known to be central to the therapeutic mode of action of HDAC inhibitors in the treatment of several proliferative diseases, including a variety of leukemias.[14,15]



A number of small-molecule inhibitors of HDAC including trichostatin A (TSA)[16] and suberoylanilide hydroxamic acid (SAHA)[17] induce differentiation in cancer cell lines and suppress cell proliferation. Several HDAC inhibitors including SAHA, NVP-LAQ824,[5] MS-275,[18] and the cyclodepsipeptide FK-228[19] have entered clinical trials. The efficacy of such HDAC inhibitors is generally considered to depend on the presence of (1) a terminal group (hydroxamic acid in the present class of novel inhibitors) that can bind to the metal-containing active site of HDAC enzymes and is connected to (2) a chain of several atoms ("linker") that is linked to (3) an end moiety (or "cap") of sufficient bulk to occupy the cleft outside the pocket that leads to the active site. Such a model is in agreement with crystal structures of histone deacetylase-like protein (HDLP) to which is bound TSA or SAHA.[20]

As part of our anticancer program centered on therapy using small molecules, we have used the framework of trichostatin A to design novel HDAC inhibitors (**II**).[21] We have shown that HDAC inhibitory potency can be maintained (in vitro IC_{50} down to 50 nM)[21] by using a simplified carbon chain although it lacks the keto group and adjacent chiral center present in trichostatin A. As HDAC mimics of trichostatin A, a number of functionalities have been interposed between an aromatic ring and an alkyl or alkylene chain, especially numerous amides,[20,22−25] and recently an oxygen atom in the form of the aromatic ether designated A-161906, which showed potent in vitro HDAC inhibition (IC_{50} = 9 nM) but appeared to have correspondingly less satisfactory in vivo properties.[26] HDAC inhibitors based on aromatic thioethers were not reported and have the advantage of an adjustable oxidation level at sulfur for improving activity, possibly by means of a prodrug mode of action. Such replacement of carbon by sulfur was also structurally appealing since a sulfur isostere, the sulfoxide of **12**, could be prepared and evaluated in which a sulfoxide group replaced the inherently reactive carbonyl group of trichostatin A. Accordingly, we describe here the synthesis and preliminary biological evaluation of a new class of sulfur-containing HDAC inhibitors, principally of type **I**.

Chemistry

Few arylsulfanylhexa-2,4-dienoic acids are known,[27] and to our knowledge no arylsulfanylhexa-2,4-dienoic acid hydroxyamides have been previously reported. Reaction of methyl sorbate and *N*-bromosuccinimide in chlorobenzene at reflux and under irradiation with a sunlamp afforded methyl (2*E*,4*E*)-6-bromo-2,4-hexadienoate (**2**).[28] Ester **2** or the corresponding ethyl ester proved suitable for alkylation of a variety of aromatic thiols, typically in tetrahydrofuran and in the presence of triethylamine (1.0 equiv) at 20 °C or where the reaction was more sluggish

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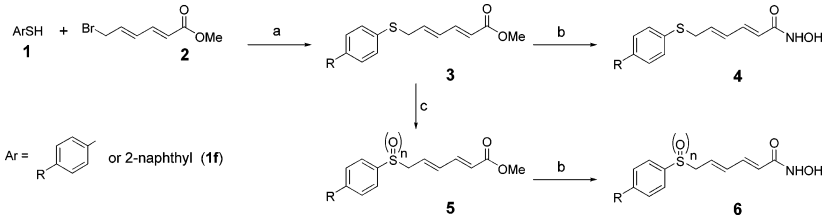
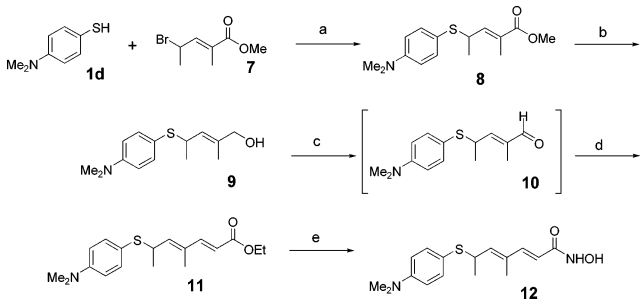
Scheme 1[a]



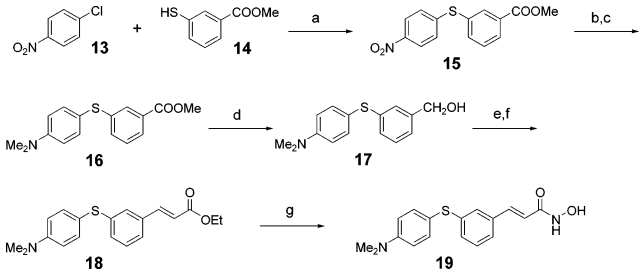
[a] Reagents: (a) Et_3N, Bu_4NI, THF; (b) aqueous NH_2OH; (c) $NaIO_4$. Key: (a) R = H (n = 1); (b) R = Cl (n = 1); (c) R = OMe (n = 1); (d) R = NMe_2 (n = 1); (e) R = H (n = 2); (f) Ar = 2-naphthyl.

Scheme 2[a]



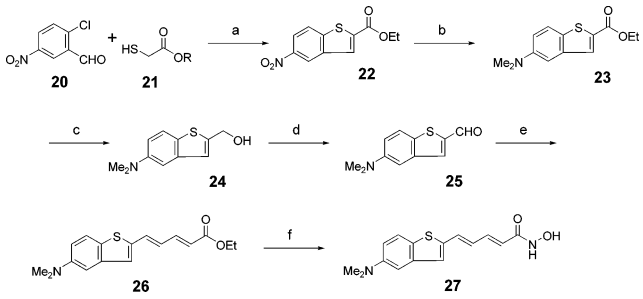
[a] Reagents: (a) Et_3N, Bu_4NI, THF; (b) DIBAL, THF; (c) pyridine−SO_3, Et_3N, DMSO; (d) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2; (e) 50% aqueous NH_2OH, 1 M KOH in MeOH, THF.

Scheme 3[a]



[a] Reagents: (a) NaOMe, MeOH, THF; (b) H_2, Pd/C; (c) 37% aqueous HCHO, $NaBH_3CN$, $ZnCl_2$, MeOH; (d) $LiAlH_4$, THF; (e) pyridine−SO_3, Et_3N, DMSO; (f) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2; (g) 50% aqueous NH_2OH, 1 M KOH in MeOH, THF.

Scheme 4[a]



[a] Reagents: (a) NaH, DMF; (b) H_2, Pd/C, EtOAc, then 37% aqueous HCHO, $NaBH_3CN$, $ZnCl_2$, MeO; (c) $LiAlH_4$, THF; (d) pyridine−SO_3, Et_3N, DMSO; (e) $(EtO)_2P(O)CH_2CH{=}CHCO_2Et$, NaH, DME; (f) 50% aqueous NH_2OH, 1 M KOH in MeOH, THF.

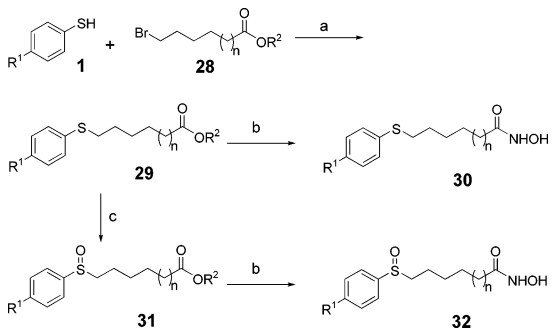
by heating at reflux (Scheme 1). The resulting esters **3** were readily converted into the corresponding sulfoxides **5** (n = 1) by treatment with $NaIO_4$ (1.2 equiv) in aqueous methanol; the sulfone **5e** was prepared by reaction of methyl (2*E*,4*E*)-6-bromo-2,4-hexadienoate (**2**) with sodium benzenesulfinate. The thioethers **3** and the sulfoxides **5** (n = 1) were converted into their corresponding hydroxamic acids **4** and **6** by treatment of a solution of the appropriate ester in THF with 50% aqueous hydroxylamine (9 equiv) at 0 °C to which was subsequently added a solution of potassium hydroxide in methanol (1 M, 1.6 equiv) with stirring over ∼30 min. The corresponding carboxylic acids of the thioethers **3** and the sulfoxides **5** (n = 1) could be prepared by hydrolysis with lithium hydroxide or sodium hydroxide.

Synthesis of the trichostatin thio analogue **12** (Scheme 2) required a sequence compatible with the sensitive nitrogen, sulfur, and dienic functionality. 4-Dimethylaminobenzenethiol (**1d**) was reacted with methyl (*E*)-4-bromo-2-methyl-2-pentenoate (**7**) in the presence of triethylamine to give the key thioether **8** in 95% yield. Ester **7** was prepared by reaction of methyl (*E*)-2-methylpent-2-enoate with *N*-bromosuccinimide in carbon tetrachloride at reflux and under irradiation with a sunlamp.[31] Reduction of ester **8** by diisobutylaluminum hydride (3 equiv) at 0 °C in toluene afforded the corresponding allylic alcohol **9** (88%), which was subjected to Moffatt oxidation (pyridine−sulfur trioxide complex in DMSO followed by addition of triethylamine) to give a solution from which the aldehyde **10** was extracted with diethyl ether; evaporation afforded the crude aldehyde **10**, which was reacted with ethyl (triphenylphosphoranylidene)acetate (1.1 equiv) in THF to give the ethyl ester **11** in 76% yield after purification by column chromatography. Treatment of ester **11** with 50% aqueous hydroxylamine (9 equiv) followed by a solution of potassium hydroxide in methanol gave the trichostatin thio analogue **12** in 52% yield.

Since the cinnamohydroxamic acid moiety was shown to be a favorable linker and terminus for HDAC inhibitors, the diarylthioether **19**, a benzannulated version of the 1,3-dimethyl-substituted hydroxamic acid **12**, was of considerable interest. The likely difficulty in forming an aryl ether linkage by reaction of thiol **1d** with an arene precursor that would be relatively unactivated suggested the reverse combination, i.e., a thiol component such as **14** for reaction with a displaceable halogen atom, as in *p*-chloronitrobenzene (**13**). Such an approach would necessitate conversion of the nitro group into the dimethylamino group present in diarylthioether **19**. The sequence in Scheme 3 proved to be effective; thus, treatment of the thiolate of ester **14** in methanol with *p*-chloronitrobenzene (**13**) first at 0 °C followed by heating at reflux for 2 h gave the thioether **15** (73%), which was reduced quantitatively to the corresponding amino compound, which was reductively aminated with aqueous 37% formaldehyde in the presence of $NaBH_3CN$ and $ZnCl_2$ to give the key *N*,*N*-dimethylamino intermediate **16** in 80% yield. The latter was then reduced quantitatively with $LiAlH_4$ to give the alcohol **17**, which was subjected to a sequence of Moffat oxidation, Wittig olefination, and hydroxamation, similar to that described above for alcohol **9** and resulting in the novel cinnamohydroxamic acid **19**.

In the quest for a more druglike compound (Scheme 4), a fused system, benzo[*b*]thiophene, was considered in which a methyl group in the chain was formally annealed to the aromatic

Scheme 5[a]



[a] Reagents: (a) Et_3N; (b) 50% aqueous NH_2OH, 1 M KOH in MeOH, THF; (c) $NaIO_4$. Key: (a) $R^1 = H$, $R^2 = Me$, $n = 1$; (b) $R^1 = Cl$, $R^2 = Me$, $n = 1$; (c) $R^1 = Cl$, $R^2 = Et$, $n = 2$; (d) $R^1 = NMe_2$, $R^2 = Me$, $n = 1$; (e) $R^1 = p\text{-}ClC_6H_4SO_2NH$, $R^2 = Me$, $n = 1$; (f) $R^1 = NH_2$, $R^2 = Me$, $n = 1$.

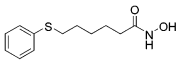
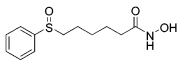
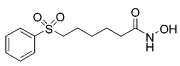
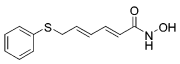
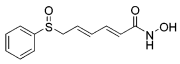
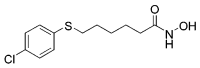
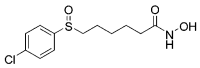
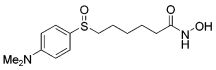
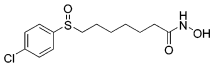
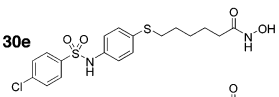
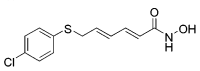
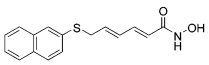
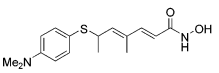
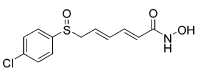
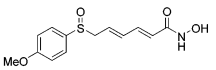
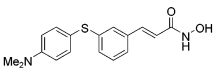
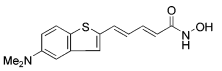
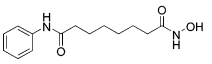
ring present in the previous compound types. The ready accessibility of the suitably functionalized benzo[*b*]thiophene **22**,[32] together with previously successful sequences of chain extension and hydroxamation permitted Scheme 4 to be realized. Thus, ester **22**, prepared from sodium hydride, ethyl 2-mercaptoacetate, and 2-chloro-5-nitrobenzaldehyde, was hydrogenated over 10% palladium on carbon to give the corresponding amine that after filtration was used directly in solution for the reductive amination with aqueous 37% formaldehyde in the presence of $NaBH_3CN$ and $ZnCl_2$ to give the key *N*,*N*-dimethylamino intermediate **23** in 60% yield. The latter was reduced with $LiAlH_4$ to give the alcohol **24**, which was subjected to a sequence of Moffat oxidation, Wittig olefination (differing from the above sequences by the use of triethyl phosphonocrotonate), and hydroxamation, similar to that described above for alcohols **9** and **1** and resulting in the novel cinnamohydroxamic acid **27**.

A variety of compounds containing a saturated chain (Scheme 5) were prepared using methods similar to those for the unsaturated ones shown in Scheme 1. Thus, thiolates **1** were reacted with ω-bromoesters **28** to give thioethers **29** that could be converted into the corresponding sulfoxides **31** using $NaIO_4$. Treatment of **29** and **31** with 50% aqueous hydroxylamine and potassium hydroxide, as described above, afforded the corresponding hydroxamic acids **30** and **32**. Treatment of sulfide **30b** with $NaIO_4$ afforded the sulfoxide **32b** in 68% yield. Upon treatment with *m*-CPBA, sulfoxide **32a** also underwent oxidation, affording the corresponding sulfone **33** in 54% yield. Such oxidations at sulfur in the presence of a hydroxamic acid group provide a useful alternative to establishing the desired oxidation level at sulfur prior to conversion into the hydroxamic acid.

Biology

Table 1 shows that most of the novel sulfur-containing compounds exhibited HDAC IC_{50} of <400 nM in the purified enzyme assay,[33] a promising result since the assay generally gives appreciably higher IC_{50} than assays involving release of tritiated acetic acid from an N-acylated peptide substrate.[4,5] The HPLC-based HDAC assay was modified to permit the determination of HDAC activity in intact cells at a single time-point, reflecting uptake of the compound by cells and inhibition of intracellular HDAC enzymes. Moreover, this measure of cellular HDAC inhibitory activity showed a better correlation with the concentration of inhibitor that induced a 50% reduction in cell viability in CEM cells after a 72 h incubation than with values from the liver HDAC assay (Spearman's rank correlation coefficient for liver IC_{50} vs % viability $IC_{50} = -0.603$, $p = 0.017$; cell IC_{50} vs % viability IC_{50} correlation coefficient $= 0.82$, $p < 0.001$). The cellular HDAC assay may therefore be a more accurate indication of the desired biological endpoint for further screening of HDAC inhibitors that show activity against partially purified enzyme.

Table 1. In Vitro Inhibition of Histone Deacetylase and CEM Cell Viability

Entry	Compound	Structure	HDAC inhibitory activity: Purified liver IC_{50} (μM)	HDAC inhibitory activity: CEM cells IC_{50} (μM)	50% Viability in CEM cells IC_{50} (μM)
1	**30a**		0.12±0.11	9.4±1.9	>25
2	**32a**		0.06±0.02	7.7±1.4	>25
3	**33**		0.04±0.01	15.5±7.4	>25
4	**4a**		0.39±0.12	>25	ND[a]
5	**6a**		0.24±0.03	>25	ND
6	**30b**		0.26±0.07	>25	>25
7	**32b**		0.15±0.01	1.3±0.4	2.6±0.2
8	**32d**		0.18±0.01	2.2±0.1	3.4±1.0
9	**32c**		0.38±0.01	1.6±0.2	12.3±3.0
10	**30e**		0.11±0.01	9.4±4	>25
11	**4b**		0.31±0.02	1.6±0.2	1.6±0.1
12	**4f**		0.70±0.31	9.4±4.0	1.7±0.6
13	**12**		0.34±0.02	5.3±0.9	7.5±0.7
14	**6b**		0.40±0.10	1.2±0.4	ND
15	**6c**		0.41±0.02	5.4±0.5	9.6±0.3
16	**19**		0.16±0.02	8.6±1.8	3.4±0.6
17	**27**		>25	ND	ND
18	**SAHA**		0.44±0.03	0.33±0.05	1.9±0.1

[a] ND = not determined.

The low IC_{50} value for CEM cell viability (1.6 μM) for **4b** encouraged a further study using a modification of the whole-cell assay to allow the determination of intracellular HDAC inhibitory activity after 1 and 24 h of incubation in CEM cells in culture media. The concentration of **4b** required to maintain 50% HDAC inhibition after 24 h was not different from the

concentration at 1 h (3.4 vs 3.4 μM). Additionally, similar studies with **32b** showed comparable stability to **4b** (2.9 μM at 1 h vs 3.4 μM at 24 h). This is in contrast to SAHA, which required a 65% increase in the concentration to achieve 50% HDAC inhibition at 24 h (0.9 vs 2.6 μM), and in marked contrast to TSA for which a >90% increase in concentration was required (0.03 vs 0.37 μM). Those data suggest an improved metabolic stability of **4b** in CEM cells.

All *S*-compounds in Table 1 except **32c** contain five carbon atoms between the sulfur atom and the carbon atom of the hydroxamic acid moiety, the additional methylene group in **32c** (compared with **32b**) conferring lower activity in all three assays and indicating that a linker of six methylene groups may be longer than optimal; that would be consistent with the additional length of ~0.8 Å arising from two carbon−sulfur bonds, compared with the two carbon−oxygen bonds present in A-161906. Both dienic and fully saturated chains were found to possess potent in vivo HDAC inhibitory activities. Potencies of the parent systems (entries 1−3) are high in the enzyme assay but low in both CEM cell assays, a pattern also found for the unsubstituted and unsaturated counterparts (entries 4 and 5). Of the para substituents examined, chloro was usually very effective (entries 7, 11, and 14) in the CEM cell assays. However, additional methyl groups as in **12** did not improve HDAC inhibitory activity, hydroxamic acid **12** showing an IC_{50} of 0.34 μM in the HDAC liver assay and 5.3 μM in CEM cells. Despite the sulfur atom of (±)-**12** being a replacement of the carbonyl group in trichostatin A, the latter (as a single enantiomer) has a much greater potency in the HDAC liver assay (IC_{50} = 0.015 μM) and the CEM cell asays (IC_{50} = 0.019 μM for HDAC inhibition and 0.12 μM for % viablility). In the compounds studied, the presence of a *p*-*N*,*N*-dimethylamino group was generally consistent with moderate but not outstanding potencies (entries 8, 13, and 17).

Although the sulfoxides were generally more potent than the corresponding sulfides in the enzyme assay (entries 1, 2; 4, 5; 6, 7 but contrast 11, 14), sulfide **4b** and sulfoxide **6b** were of comparable potency in the CEM cell HDAC assay. The sulfide **4f**, containing a hydrophobic capping region, is also of notable potency. The effect of sulfoxide **32b** on a further three cell lines was investigated; **32b** was shown to be cytotoxic to HCT116 (percentage viability IC_{50} ± SD: 3.9 ± 0.7 μM), SUD4 (7.5 ± 1.1 μM), and MCF-7 (6.1 ± 0.9 μM) cells. The concentration of **32b** that inhibited proliferation (cell number) by 50% in those cells was 0.9−2.7 μM. The saturated chain sulfides **30b** and **30e** showed potent activities using the purified enzyme assay but performed very poorly in the CEM cell assays; in contrast, the saturated chain sulfoxides **32b**−**d** typically showed good activities in all three assays. The striking difference in CEM cell activity between **30b** and **32b** may indicate the importance of a polar entity adjacent to the aromatic ring (such as the keto group in TSA) or may be associated with a more rapid cellular metabolism of the saturated chain sulfides. The additional hydrophobicity provided by the chlorophenyl terminus in **30e** was found, as expected,[21] to confer improved in vitro enzyme inhibition. While the cinnamohydroxamic acid type **19** provides tolerably good activities, such was not the case for **27** in which a fused ring system is present. Overall, these studies suggest that a sulfide or a sulfoxide linkage as part of an alkyl or possibly an alkylene chain, together with an extended hydrophobic capping region, could provide good potency and good antiproliferative properties with comparable or better metabolic stability than SAHA and much greater metabolic stability than TSA.

Summary

The efficacy of arylpenta-2,4-dienoic acid hydroxyamides indicates for the first time that aromatic thioether units can be present in a novel class of potent inhibitors of histone deacetylase. The sulfides tested, although structurally less similar to trichostatin A than the corresponding sulfones, were generally more potent than the latter, the sulfides probably exhibiting greater cellular uptake than the more polar sulfoxides (or sulfones). Sulfide **4b** was appreciably more metabolically stable than SAHA and far more so than trichostatin A. Since the rat liver assay[22] generally gives significantly higher in vitro IC_{50} than do assays based on deacetylation of tritiated histone-like substrates,[4,5] the present values of under 200 nM for several compounds are considered to be promising. Additionally, **32b** was shown to inhibit proliferation of three cell lines by 50% at 0.9−2.7 μM. One disadvantage of amidic HDAC inhibitors that have been clinically tested is their potential for cleavage by endogenous peptidases, a feature that is absent in most of the novel inhibitors of histone deacetylase herein described and that may confer improved in vivo stability over the more common amidic inhibitors such as SAHA.

Experimental Section

Chemistry. Melting points were determined on a microscope hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer PE-983 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC300 instrument at 300 and 75 MHz, respectively; chemical shifts are reported in δ (ppm) relative to the internal reference tetramethylsilane. The homogeneity of the compounds was monitored by ascending thin-layer chromatography, performed on Merck 0.2 mm aluminum-backed silica gel 60 F_{254} plates and visualized using an alkaline $KMnO_4$ spray or by ultraviolet light. Flash column chromatography was performed using Merck 0.040−0.063 mm, 230−400 mesh silica gel. Elemental analysis (C, H, N, Cl, and S) results were obtained on a Perkin-Elmer 2400 CHN elemental analyzer, and all data were consistent with theoretical values (within ±0.4%) unless otherwise indicated. Mass spectra were obtained on a VG7070H mass spectrometer using Finigan Incos II. All solvents were reagent grade and, where necessary, were purified and dried by standard methods. Evaporation refers to the removal of solvent under reduced pressure. Flash column chromatography was performed using Sorbsil C60 40/60A silica gel. The following compounds were prepared according to the literature: (2*E*,4*E*)-6-phenylsulfanyl-2,4-hexadienoic acid,[27a] methyl (2*E*,4*E*)-6-phenylsulfanyl-2,4-hexadienoate,[39] methyl (2*E*,4*E*)-6-bromo-2,4-hexadienoate (**2**),[28] (2*E*,4*E*)-6-benzenesulfinyl-2,4-hexadienoic acid,[30] (2*E*,4*E*)-6-benzenesulfonyl-2,4-hexadienoic acid,[30] (2*E*,4*E*)-6-benzenesulfonyl-2,4-hexadienoic acid hydroxyamide,[30] 4-dimethylaminobenzenethiol (**1d**),[29,30] (*E*)-methyl 2-methyl-2-pentenoate,[35] with methyl 2(*E*)-4-bromo-2-methyl-2-pentenoate (**7**),[31] 5-nitrobenzo[*b*]thiophene-2-carboxylic acid ethyl ester (**22**),[32] methyl (2*E*,4*E*)-2,4-hexadienoate,[34] 3-mercaptobenzoic acid,[36] methyl 3-mercaptobenzoate (**14**),[37] methyl 6-bromohexanoate (**28a**),[38] ethyl 6-phenylsulfanyl hexanoate (**29a**),[39] *N*-(4methyl-7-coumarinyl)-*N*-ω-(*tert*-butyloxycarbonyl)-*N*-ω-acetyllysinamide (MAL),[33] (5-acetylamino-1-carbamoylpentyl)carbamic acid *tert*-butyl ester.[30]

(2*E*,4*E*)-6-(4-Chlorophenylsulfanyl)-2,4-hexadienoic Acid Hydroxyamide (4b). Procedure A. To a solution of methyl (2*E*,4*E*)-6-(4-chlorophenylsulfanyl)-2,4-hexadienoate (0.44 g, 1.64 mmol) in distilled THF (9.0 mL) containing 50% aqueous hydroxylamine (1.0 mL, 15.2 mmol) was added at 0 °C a solution of potassium hydroxide in methanol (1 M, 2.6 mL, 2.6 mmol) over 30 min. After the mixture was stirred at 0 °C for 1 h, distilled water (9.0 mL) was added and the mixture was made neutral by dropwise addition of 2 M HCl at 0 °C. The aqueous solution was extracted with ethyl acetate (3 × 30 mL), and the combined extracts were dried over anhydrous $MgSO_4$ and evaporated. The residue was recrystallized from acetone to give **4b** (0.21 g, 48%) as pale-brown microprisms,

mp 120−122 °C (dec). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.63 (br s, 1H), 8.95 (br s, 1H), 7.34 (s, 4H), 6.98 (dd, J = 15.0, 11.2 Hz, 1H), 6.29 (dd, J = 15.0, 11.2 Hz, 1H), 6.07 (m, 1H), 5.75 (d, J = 15.2 Hz, 1H), 3.75 (d, J = 7.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.7, 137.8, 135.1, 134.4, 131.1, 130.7, 130.4, 128.9, 122.0, 34.5. Anal. ($C_{12}H_{12}NClO_2S$) Calcd: C 53.43%, H 4.48%, N 5.19%. Found: C 52.88%, H 4.59%, N 5.08%.

(2*E*,4*E*)-6-(Naphthalen-2-ylsulfanyl)-2,4-hexadienoic Acid Hydroxyamide (4f). To a solution of methyl (2*E*,4*E*)-6-(naphthalen-2-ylsulfanyl)-2,4-hexadienoate (0.505 g, 1.78 mmol) in distilled THF (10 mL) containing 50% aqueous hydroxylamine (1.08 mL, 16.4 mmol) was added at 0 °C a solution of potassium hydroxide in methanol (1 M, 2.5 mL, 2.5 mmol) over 30 min. Continuation as in procedure A gave a residue that was recrystallized from ethyl acetate to give **4f** (0.315 g, 62%) as white microprisms, mp 141−142 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82−7.43 (m, 7H), 7.37 (m, 1H), 6.28 (m, 2H), 5.97 (d, J = 15.4 Hz, 1H), 3.74 (d, J = 5.9 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.5, 145.3, 142.8, 143.9, 139.3, 135.8, 133.8, 133.4, 132.6, 131.1, 129.5, 121.6, 51.9, 37.4. Anal. ($C_{16}H_{15}NO_2S$) C, H, N.

(2*E*,4*E*)-6-(4-Chlorobenzenesulfinyl)-2,4-hexadienoic Acid Hydroxyamide (6b). To a solution of methyl (2*E*,4*E*)-6-(4-chlorobenzenesulfinyl)-2,4-hexadienoate (0.80 g, 2.79 mmol) in distilled THF (15 mL) containing an aqueous solution of hydroxylamine (50%, 1.7 mL, 25.8 mmol) was added at 0 °C a solution of potassium hydroxide in methanol (1 M, 4.5 mL, 4.5 mmol) over 30 min. After the mixture was stirred at 0 °C for 1 h, distilled water (15 mL) was added and the mixture was made neutral by dropwise addition of 2 M HCl at 0 °C. Workup as for procedure A gave a residue that was recrystallized from acetone to give **6b** (0.45 g, 56%) as pale-brown microprisms, mp 159 °C (dec). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.68 (br s, 1H), 9.21 (br s, 1H), 7.62 (s, 4H), 6.97 (dd, J = 15.0, 11.2 Hz, 1H), 6.23 (dd, J = 15.0, 11.2 Hz, 1H), 5.81 (d, J = 15.0 Hz, 1H), 5.79 (m, 1H), 3.88 (dd, J = 13.0, 7.7 Hz, 6H), 3.65 (dd, J = 13.0, 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.5, 142.1, 137.4, 136.0, 135.6, 129.1, 127.1, 126.2, 123.0, 58.6. Anal. ($C_{12}H_{12}NClO_3S$) C, H, N, S.

6-Phenylsulfanylhexanoic Acid Hydroxyamide (30a). To a stirred solution of ethyl 6-phenylsulfanylhexanoate (0.40 g, 1.59 mmol) at 0 °C in anhydrous THF was added a solution of hydroxylamine (1 M, 16 mL) in methanol, followed immediately by dropwise addition of a solution of potassium hydroxide (1 M, 2.07 mL) in methanol. The mixture was warmed to 25 °C and stirred for 16 h. Ice was then added and the pH adjusted to 6−7 by addition of 2 M HCl. Workup as in procedure A gave a residue that was recrystallized from acetone to give **30a** (0.26 g, 68%) as white microprisms, mp 132−135 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.03 (br s, 1H), 8.78 (br s, 1H), 7.41−7.30 (m, 5H), 2.90 (t, J = 7.1 Hz, 2H), 1.88 (t, J = 7.1 Hz, 2H), 1.50−1.32 (m, 4H), 1.27 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.8, 136.1, 130.0, 129.3, 129.0, 32.3, 32.0, 28.7, 27.7, 24.7. Anal. ($C_{12}H_{17}NO_2S$) C, H, N.

6-(Benzenesulfinyl)hexanoic Acid Hydroxyamide (32a). To a stirred solution of ethyl 6-(benzenesulfinyl)hexanoate (0.50 g, 1.87 mmol) at 0 °C was added a solution of hydroxylamine (1 M, 18.7 mL) in methanol, followed immediately by dropwise addition of a solution of potassium hydroxide (1 M, 2.43 mL) in methanol. The mixture was warmed to 25 °C and stirred for 16 h. Ice was then added and the pH adjusted to 6−7 by addition of 2 M HCl. Workup as in procedure A gave a residue that was recrystallized from acetone to give **32a** (0.31 g, 65%) as white microprisms, mp 164−165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.09 (br s, 1H), 8.71 (br s, 1H), 7.60 (m, 5H), 3.02 (t, J = 7.2 Hz, 2H), 2.20 (t, J = 7.2 Hz, 2H), 1.47−1.35 (m, 4H), 1.28 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.9, 136.4, 130.1, 129.8, 128.5, 54.1, 32.2, 29.4, 27.7, 25.0. Anal. ($C_{12}H_{17}NO_3S$) C, H, N.

6-(Benzenesulfonyl)hexanoic Acid Hydroxyamide (33). To a stirred solution of 6-(benzenesulfinyl)hexanoic acid hydroxyamide (0.31 g, 1.22 mmol) in anhydrous dichloromethane (6.0 mL) at 0 °C was added *m*-CPBA (0.21 g, 1.22 mmol) in small portions. The mixture was maintained at 0 °C for 1 h with stirring, after which the pH was adjusted to 6−7 by dropwise addition of aqueous 1 M NaOH. The organic layer was separated and washed with saturated aqueous ammonium chloride (10 mL) and then with brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The oily residue was recrystallized from acetone to give **34** (0.18 g, 54%) as white microprisms, mp 184−186 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.19 (br s, 1H, OH), 8.68 (br s, 1H, NH), 7.70−7.55 (m, 5H), 2.95 (m, 1H), 2.87 (m, 1H), 1.90 (t, J = 7.1 Hz, 2H), 1.70−1.20 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.8, 143.2, 135.3, 129.2, 125.9, 55.1, 31.9, 27.4, 24.6, 21.0. Anal. ($C_{12}H_{17}NO_4S$) C, H, N.

Biology. HDAC Enzyme Assay. The procedure described in Hoffmann[33] was modified for use as described below and was further modified for use in the whole-cell HDAC activity assay.

Isolated Liver HDAC Activity Assay. The supernatant resulting from the high-speed centrifugation of rat liver homogenates was partially purified on a Q-Sepharose column using a sodium chloride gradient (10−500 mM), with a Tris (15 mM), EDTA (0.25 mM), glycerol (10%) buffer at pH 7.9. Fractions were collected and analyzed for HDAC activity using the method described herein. Those fractions containing HDAC activity were pooled and aliquots stored at −40 °C prior to use. The assay mixture comprised 100 μL of pooled HDAC enzyme, 100 μL of buffer (Tris-HCl (10 mM), NaCl (10 mM), $MgCl_2$ (15 mM), EGTA (0.1 mM), glycerol (10% v/v), and mercaptoethanol (0.007%), 100 μL of HDAC inhibitor (dissolved in DMSO and diluted with HEPES buffer (50 mM, pH 7.4) to working concentrations), and 100 μL of substrate (MAL, diluted to 20 μg/mL with 50 mM HEPES buffer, pH 7.4, 0.5 mL aliquots stored at −40 °C, diluted to 5 μg/mL for use in the assay). Samples were vortexed for 15 s and incubated at 37 °C for 60 min with gentle agitation every 15 min. Acetonitrile (100 μL) was then added to terminate the reaction, after which samples were centrifuged at 10 000 rpm for 10 min and then placed on ice prior to analysis by HPLC of substrate (MAL) and deacetylated product (ML) concentration in the supernatant.

Whole-Cell HDAC Activity Assay. The 1×10^6 CEM cells in RPMI medium (1 mL; Sigma Chemicals, Poole, Dorset U.K.) were exposed to HDAC inhibitors at six concentrations for 60 min at 37 °C, after which MAL (20 μg/mL final concentration) was added and the mixture incubated for a further 30 min. Cells were then rapidly washed with phosphate buffered saline at 4 °C and lysed by sonication. The reaction was stopped with acetonitrile (100 μL), and intracellular MAL and ML concentrations in the supernatant were determined by HPLC analysis.

Quantitation of MAL and ML by HPLC Analysis. Chromatographic separation of MAL and ML was carried out using a 15 cm Apex ODS 5 μm column with an acetonitrile/distilled water (40:60), 2% TFA (v/v) mobile phase at a flow rate of 1.2 mL/min. MAL and ML were quantified by fluorescence detection at excitation/emission wavelengths of 330/395 nm. The activity of each inhibitor was assessed at a minimum of five nonzero concentrations, determined from initial concentration studies. The peak heights of MAL and ML were used to derive the % MAL in the mixture as the ratio MAL/(MAL+ML). The % MAL in the absence of inhibitor (typically 22−25%) was taken as 100% HDAC activity, and the % HDAC activity at each inhibitor concentration was derived from $(100 - \%\,MAL_{DRUG})/(100 - \%\,MAL_{NODRUG}) \times 100$. These data (minimum of $n = 4$ at each concentration for each inhibitor) were fitted to a sigmoidal E_{MAX} model (Graphpad Prism, version 2.01) to derive the IC_{50} concentration for each compound.

Cytotoxicity Assay of HDAC Inhibitors. CEM (human leukemic) cells were exposed to HDAC inhibitors at a minimum of five nonzero concentrations for 3 days under standard cell culture conditions (RPMI medium, 37 °C, 5% CO_2 in a humidified atmosphere). Cell number and percentage viability were assessed by trypan blue staining. The % viability IC_{50} was determined using Graphpad Prism. For **32b** these studies were extended to include HCT116 (colorectal cancer), MCF-7 (breast cancer), and SUD4 (lymphoma) cells.

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Supporting Information Available: Experimental procedures, ^{1}H and ^{13}C NMR spectra for all new compounds, and microanalytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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