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Structure–activity relationships of aryloxyalkanoic acid hydroxyamides as potent inhibitors of histone deacetylase

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Abstract—Syntheses of aryloxyalkanoic acid hydroxyamides are described, all of which are potent inhibitors of histone deacetylase, some being more potent in vitro than trichostatin A (IC_{50} = 3 nM). Variation of the substituents on the benzene ring as well as fusion of a second ring have marked effects on potency, in vitro IC_{50} values down to 1 nM being obtained.
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Inhibitors of histone deacetylase (HDAC) enzymes have recently gained prominence as an emerging class of anti-cancer agents.[1,2] 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 protonated ϵ-amino lysine residues that interact strongly with DNA, leading to a less accessible, compacted chromatin structure.[3] In this way, HDACs can act as repressors of gene transcription by altering the accessibility of transcription factors to DNA.[4,5] The inappropriate recruitment of HDAC enzymes by oncogenic proteins may alter gene expression in favor of arrested differentiation, and/or excessive proliferation.[6] Conversely, inhibitors of HDACs are able to relieve transcriptional repression of certain genes including the cyclin-dependent kinase inhibitor protein $p21^{WAF1/CIP1}$,[6,7] and such HDAC inhibitors typically inhibit cancer cell proliferation by induction of cell cycle arrest, differentiation and/or apoptosis.[8–10]

Several HDAC inhibitors including suberoylanilide hydroxamic acid (SAHA),[11] (Fig. 1) NVP-LAQ824,[12] MS-275,[13] and the cyclodepsipeptide FK-228[14] have entered clinical trials. Those and the natural product trichostatin A (TSA)[15] induce differentiation in cancer cell lines and suppress cell proliferation. The efficacy of such HDAC inhibitors is generally considered to depend upon the presence of three major features: (1) a terminal group (hydroxamic acid in the present class of novel inhibitors) that can bind to zinc in the active site of HDAC enzymes and which is connected to (2) a linker unit, residing in the channel, which in turn is covalently bonded to (3) an end moiety (or capping group) that principally occupies the external entrance to the channel of the enzyme. Such a model is in agreement with structures of histone deacetylase-like protein (HDLP) co-crystallized with either TSA or SAHA.[16]

As part of our anti-cancer program centered on therapy using small molecules, we have used the framework of trichostatin A to design novel HDAC inhibitors.[17] While both TSA and SAHA contain carbonyl groups (keto and amide, respectively) as part of the linker, we (CMM and SPJ) have also shown that HDAC inhibitory potency can be maintained by a sulfoxide, or merely a thioether of type **1** in the linker unit.[18] Compounds containing those groups showed moderate inhibition in the enzyme assay employed,[18] suggesting that an oxygen

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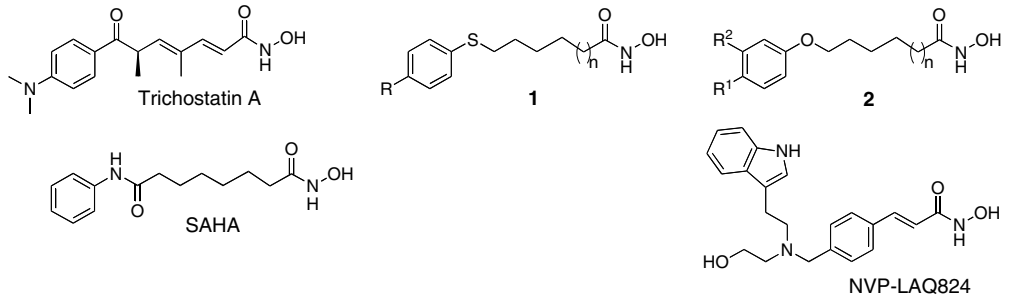
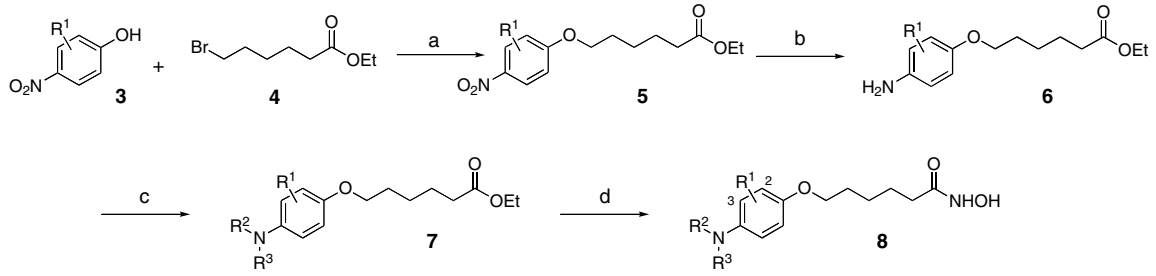


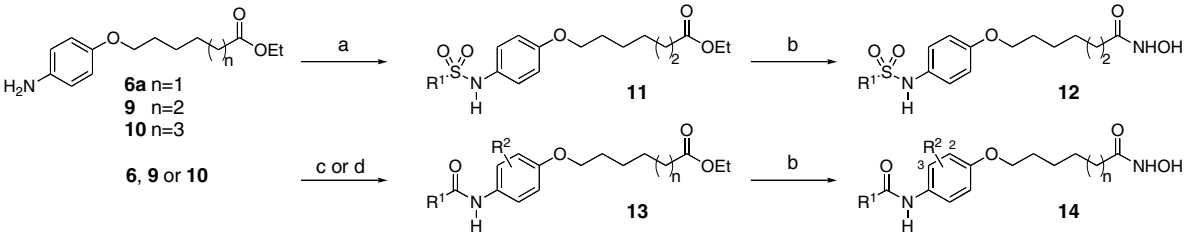
Figure 1.

atom interposed between an aromatic ring and an alkyl chain could also be of interest and perhaps furnish more potent inhibitors of HDAC than the thioether analogues.[19] Here, we report that aromatic ether-linked compounds, principally of type **2**, are indeed compatible with very potent HDAC inhibition, with IC_{50} values extending down to 1 nM.

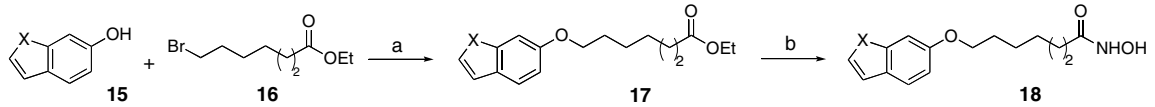
Esters of aryloxyalkanoic acids were conveniently prepared by the Williamson ether synthesis involving alkylation of a suitable phenol with an ω-bromo ester **4** in the presence of Cs_2CO_3 (Scheme 1).[20] Since an N-substituted *p*-aminophenyl moiety was a common feature of many of the inhibitors **2** and their precursors, selective O-alkylation was a requirement of the routes. Moreover, a variety of substituents at nitrogen were to be investigated; both requirements could be met by O-alkylation of a *p*-nitrophenol **3** to give the ethers **5** followed by reduction to the *p*-amino derivatives **6**. Such amines **6** proved satisfactory in a variety of reductive alkylations



Scheme 1. Reagents and conditions: (a) Cs_2CO_3, DMF, 16 h, reflux; (b) H_2, 5% Pd–C, EtOH, 3h; (c) R^2CHO, (R^3CHO), $NaCNBH_3$, (2 equiv), $ZnCl_2$, MeOH; (d) 50% aq NH_2OH (9 equiv), KOH (1.7 equiv) in MeOH–THF. Compound key: a, R^1 = H, R^2 = R^3 = Me; b, R^1 = H, R^2 = Me, R^3 = *p*-$ClC_6H_4CH_2$; c, R^1 = H, R^2 = Me, R^3 = 4-pyridylmethyl; d, R^1 = R^3 = H, R^2 = 5-indolylmethyl; e, R^1 = R^3 = H, R^2 = 4-(3-hydroxy-5-hydroxymethyl-2-methyl)pyridylmethyl; f, R^1 = 3-fluoro, R^2 = H, R^3 = 4-(3-hydroxy-5-hydroxymethyl-2-methyl)pyridylmethyl; g, R^1 = H, R^2 = 3-indolylpropionyl, R^3 = 4-pyridylmethyl.

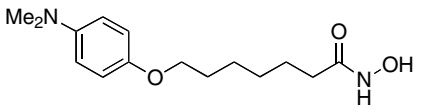
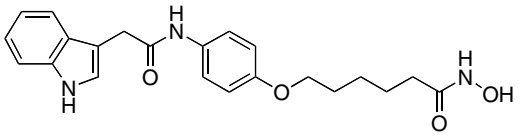
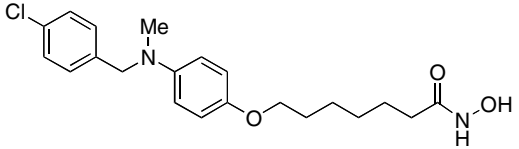
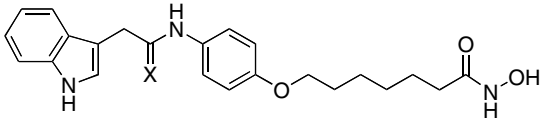
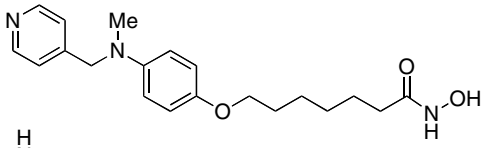
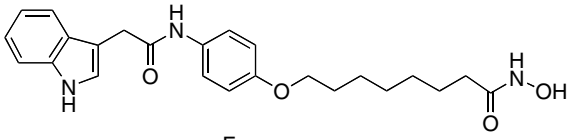
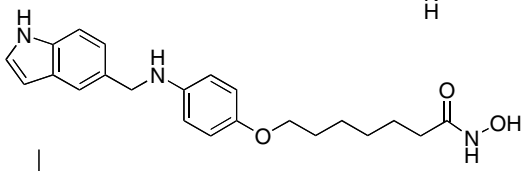
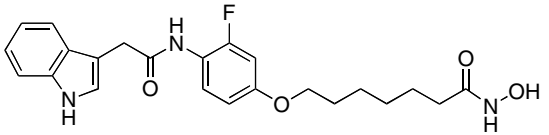
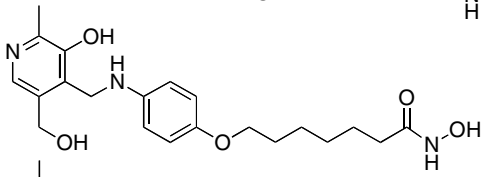
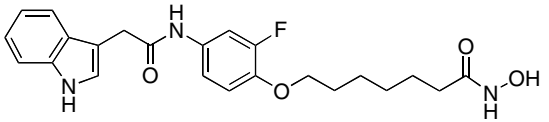
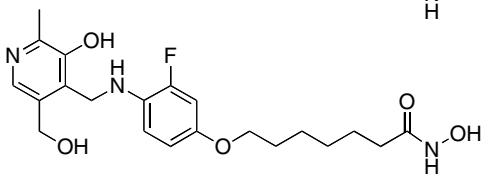
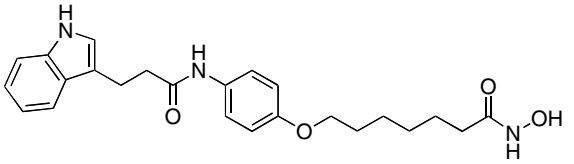
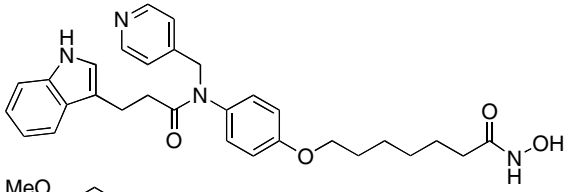
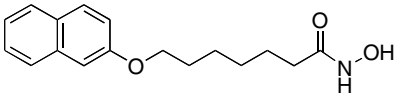
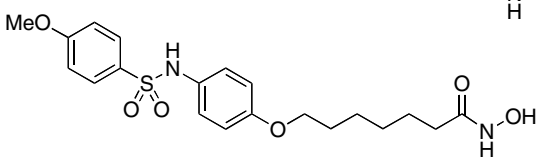
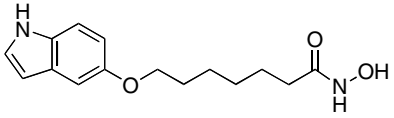


Scheme 2. Reagents and conditions: (a) R^1SO_2Cl, Et_3N, CH_2Cl_2, 2 h; (b) 50% aq NH_2OH (6 equiv), KOH (1 equiv) in MeOH–THF; (c) R^1CO_2H, *iso*-butyl chloroformate, *N*-methylmorpholine, THF; (d) R^1CO_2H, 1-hydroxybenzotriazole, 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide, *N*-methylmorpholine, THF. Compound key: for **11** and **12**: R^1 = *p*-$MeOC_6H_4$; for **13** and **14**: a, R^1 = 3-indolylacetyl, R^2 = H, *n* = 1; b, R^1 = 3-indolylacetyl, R^2 = H, *n* = 2; c, R^1 = 3-indolylacetyl, R^2 = H, *n* = 3; d, R^1 = 3-indolylethyl, R^2 = H, *n* = 2; e, R^1 = 3-indolylacetyl, R^2 = 3-fluoro, *n* = 2; f, R^1 = 3-indolylacetyl, R^2 = 2-fluoro, *n* = 2, R^1 = 3-indolylpropionyl, R^2 = H, *n* = 2.



Scheme 3. Reagents and conditions: (a) Cs_2CO_3, DMF, 16 h, reflux; (b) 50% aq NH_2OH (9 equiv), KOH (1.7 equiv) in MeOH–THF. Compound key: a, X=CH=CH; b, X=NH.

Table 1. In vitro inhibition of histone deacetylase[21,22]

Compound	Structure	HDAC IC_{50} (nM)	Compound	Structure	HDAC IC_{50} (nM)
8a		18.8 ± 6.0	**14a**		50 ± 21
8b		2.0 ± 1.0	**14b** (X=O) **14d** ($X=H_2$)		12.8 ± 5.2 (**14b**) 6.2 ± 3.5 (**14d**)
8c		15.3 ± 6.2	**14c**		22.4 ± 9.0
8d		14.3 ± 5.7	**14e**		23.5 ± 9.0
8e		22.0 ± 12.0	**14f**		13.1 ± 5.0
8f		70 ± 28	**14g**		16.0 ± 5.5
8g		16.4 ± 8.0	**18a**		0.9 ± 0.3
12		22.1 ± 13.3	**18b**		3.6 ± 1.8

In vitro inhibition data for A-161906 (IC_{50} = 9 nM)[19] and trichostatin A (IC_{50} = 3 nM).[21]

(Scheme 1): a one-pot *N*,*N*-dimethylation using aqueous 37% formaldehyde (6 equiv) afforded **7a**. Reductive monoalkylations also proceeded well, affording the secondary amines **7b–f**. Treatment of those esters with aqueous 50% hydroxylamine afforded the corresponding hydroxamic acids **8a–f**. A similar procedure afforded, after acylation with 3-indolylpropionic acid using method c (Scheme 2) the ester **7g** which with aqueous 50% hydroxylamine gave the hydroxamic acid **8g**.

The amino ether **6a** underwent sulfonylation to give **11**. Acylation of the appropriate amine **6a**, **9**, or **10** furnished esters **13a**,**13b**,**13e**, and **13f** using *iso*-butyl chloroformate in the presence of *N*-methylmorpholine, esters **13c**, **13d**, and **13g** using EDCI-HOBt.[20] (The subsequent reduction of the amide with $NaBH_4$ (1 mol equiv) in the presence of acetic acid (1 equiv) at 45 °C for 17 h furnished **13d**). Treatment of those esters with aqueous 50% hydroxylamine afforded the corresponding hydroxamic acids **12** and **14a–g**.

Fused ring phenols **15a** and **15b** were conveniently alkylated with ethyl 7-bromoheptanoate (**16**) to give the corresponding ethers **17a** and **17b**.[20] Treatment of those esters with aqueous 50% hydroxylamine afforded the corresponding hydroxamic acids **18a** and **18b** (Scheme 3).

Table 1 shows in vitro IC_{50} values of various compounds described above as measured against deacetylation of histone H4.[21,22] Preliminary modeling studies indicated that the binding energy of a saturated chain ether **8** is greater than the corresponding α,β,γ,δ-unsaturated system despite the conformational constraints imposed by a diene unit. Accordingly, this work concerns only the more readily obtained saturated compounds **8** which have the additional advantage of not being capable of undergoing conjugate addition. In designing inhibitors, consideration was also given to the need for a branch point such that the aromatic portion can adopt an obtuse angle with respect to the main linker chain in the tunnel of the enzyme (as in Fig. 2). Given the lengths of the linker units in TSA and SAHA, the heptanoic acid hydroxyamides were expected to be of suitable length; at least in the case of series **14a**, **14b**, and **14c**, the heptanoic acid hydroxyamide **14b** was the most potent. Those indolylacetamide derivatives were initially selected partly because of ease of synthesis, but also because in the Novartis LAQ series[12] (Fig. 1), the presence of an indole ring was found to be necessary for good cellular anti-proliferation values.[12a] Introduction of a fluoro substituent (amides **14e** and **14f**) showed no appreciable effect for the 2-fluoro derivative, and somewhat lower potency for the 3-fluoro derivative, although all compounds **14d–f** were potent inhibitors

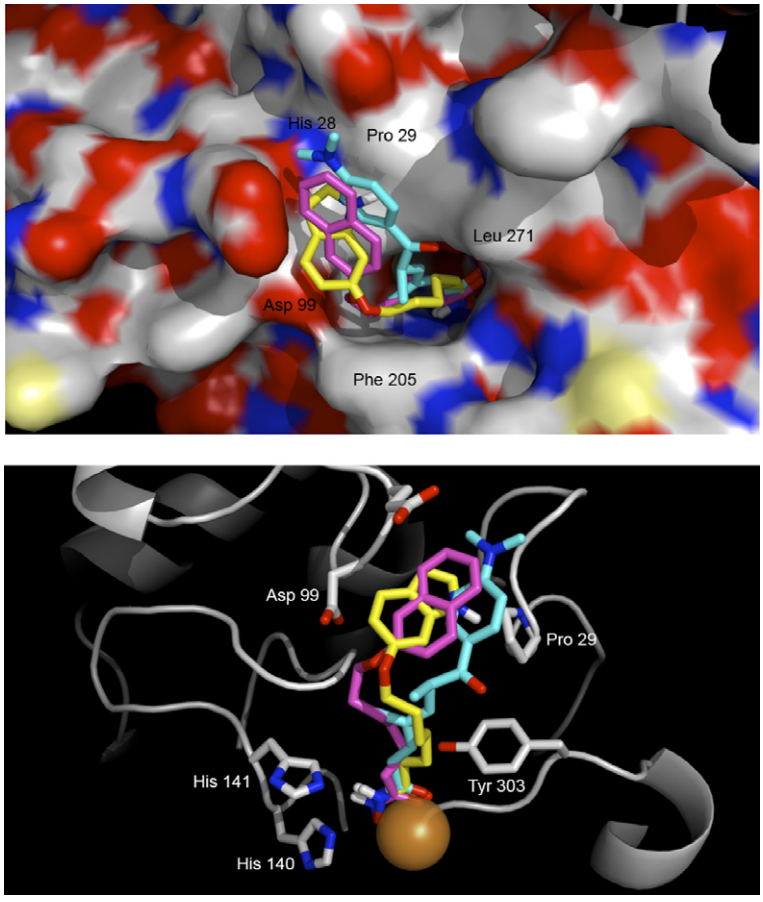


Figure 2. Molecular modeling of the binding of ligands **18a** (magenta), **18b** (yellow), and TSA (turquoise) to HDAC1 using AutoDock3.0[23] and viewed in PyMOL. Upper picture: cap region of the enzyme; lower picture: side-on view of the three ligands and their coordination to zinc (brown sphere) with depiction of residues in and near the catalytic site.[23]

of the HDAC cell extract. The longer indolylpropionamide group of **14g** afforded comparable activity to the indolylacetamide analog **14b**, implying that the additional flexibility arising from an extra methylene group did not confer, overall, more beneficial contacts to the cap region. Perhaps surprisingly, the additional hydrophobic region afforded by the 4-pyridylmethyl group present in **8g** did not confer a greater observed potency than that of **14g**.

Possessing more conformational flexibility than the corresponding amides, the secondary amines **8d** and **14d** were prepared and tested. Both were potent inhibitors of HDAC, the latter somewhat more so, possibly owing to the less rigid chain which could enable more ready presentation of the indole ring to binding regions on the protein (compare also **14b** with **14d**). Among the secondary amines, the polar 4-pyridylmethyl derivatives **8e** and **8f**, bearing polar substituents, were examined; whereas hydroxamic acid **8e** is of comparable potency to both the less polar compounds in the phenoxy series (e.g., **8a**, **8d** and especially **8c**), the 3-fluoro derivative **8f** was much weaker. This marked difference, which is not found in the analogous pair **14e** and **14f**, might be accounted for in terms of diminished capability of the secondary amino nitrogen atom of **8f** to engage in hydrogen bonding.

The derivative **8a** showed that a simple *N*,*N*-dimethylamino substituent (corresponding to the cap region of TSA) is sufficient to give good potency. However, extension of the hydrophobic area of the cap group can greatly increase potency; this can be achieved by means of a flexible (aminomethyl) unit, as in **8b**, or by ring fusion, as in **18a** and **18b**. In all three cases, significantly greater potency than that of A-161906 was achieved, showing the potential for further improvements in such aryloxyalkanoic acid hydroxyamides. Modeling of TSA in HDAC1 using AutoDock3.0[23] gave a binding of TSA (Fig. 2) consistent with previous modeling[24] and with its location in HDLP as determined by crystallography.[16] The planar rings of **18a** and **18b** contribute to the potency, probably by preferentially occupying the relatively planar and hydrophobic portion of the cap region of the enzyme as shown in Figure 2.[23]

In conclusion, the aryloxyalkanoic acid hydroxyamides herein described are some of the most potent HDAC inhibitors known in relation to their structural simplicity. The potencies of aryloxyalkanoic acid hydroxyamides indicate that neither a *carbonyl group (as present in trichostatin A and SAHA) nor a rigid (alkylene) chain is essential for low nanomolar HDAC enzyme inhibition to be achieved*. General features of structure–activity relationships can be discerned for this series of inhibitors, and a mode of docking similar to that accepted for TSA[16,24] is proposed for some of the compounds herein described. These aryloxy inhibitors of histone deacetylase may prove to be of superior in vivo stability compared with the well-studied amidic HDAC inhibitors that can be cleaved by peptidases. Additionally, hydroxamic acids **14d** and **18b** inhibited proliferation of HeLa ($IC_{50} = 7\ \mu M$ for both compounds) and MCF-7 cell lines ($IC_{50} = 7\ \mu M$ for **14d** and $5\ \mu M$ for **18b**).

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19. A 4′-cyanobiphenylyl ether designated A-161906 from an Abbott compound library has been reported to be an HDAC inhibitor, but details were scant: (a) Glaser, K. B.; Li, J.; Pease, L. J.; Staver, M. J.; Marcotte, P. A.; Guo, J.; Frey, R. R.; Garland, R. B.; Heyman, H. R.; Wada, C. K.; Vasudevan, A.; Michaelides, M. R.; Davidsen, S. K.; Curtin, M. L. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 683; (b) Frey, R. R.; Wada, C. K.; Garland, R. B.; Curtin, M. L.; Li, J.; Pease, L. J.; Glaser, K. B.; Marcotte, P. A.; Bouska, J. J.; Murphy, S. S.; Davidsen, S. K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3443.
20. Yields for compounds in Scheme 1: **5a** (93%), **5b** (85%); **6a** (98%), **6b** (83%); **5e** (74%), **5f** (79%); **6e** (95%), **6f** (89%); **7a**

(69%), **7b** (68%), **7c** (72%), **7d** (67%), **7e** (77%), **7f** (70%); **8a** (38%), **8b** (31%), **8c** (32%), **8d** (31%), **8e** (59%), **8f** (40%); **7g** (85%); **8g** (81%). Yields for compounds in Scheme 2: **11** (63%); **13a** (85%), **13b** (78%), **13e** (83%), **13f** (94%); **13c** (82%), **13d** (78%), **13g** (79%); **13d** (53%); **12** (64%); **14a** (59%), **14b** (36%), **14c** (42%), **14d** (69%), **14e** (77%), **14f** (28%); **14g** (39%). Yields for compounds in Scheme 3: **17a** (79%), **17b** (60%); **18a** (24%), **18b** (66%).

21. Vigushin, D. M.; Aboagye, E.; Buluwela, L.; Coombes, C. *Clin. Cancer Res.* **2001**, *7*, 971.
22. Histone deacetylase activity was measured by incubation of HeLa cell nuclear extract (a source of histone deacetylase enzymes) prepared according to Dignam, J. D.; Lebovitz, R. M.; Roeder, R. G. *Nucleic Acids Res.* **1983**, *11*, 1475, with a [^{3}H]acetate-radiolabeled peptide substrate followed by extraction with a [^{3}H]acetate-radiolabeled peptide substrate and subsequent extraction of the released product ([^{3}H]acetic acid) with ethyl acetate and quantification by liquid scintillation counting. The substrate was a synthetic peptide corresponding to the N-terminal of the released product ([^{3}H]acetic acid) with ethyl acetate and quantification by liquid scintillation counting. Each test compound was assayed in duplicate whilst positive control (trichostatin A) and negative control samples were assayed in triplicate. Further details are given in note 16 of Ref. 17.
23. (a) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639 http://www.scripps.edu/pub/olson-web/doc/autodock; (b) AutoDockTools can be downloaded free of charge from this website: http://www.scripps.edu/pub/olson-web/doc/autodock/tools.html. For use of PyMOL and viewing of protein-ligand structures see: http://www.pymol.org/funding.html.
24. Wang, D.-F.; Helquist, P.; Wiech, N. L.; Wiest, O. *J. Med. Chem.* **2005**, *48*, 6936.