ARTICLE

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Polymer-assisted, multi-step solution phase synthesis and biological screening of histone deacetylase inhibitors

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Received 24th October 2003, Accepted 16th December 2003
First published as an Advance Article on the web 27th January 2004

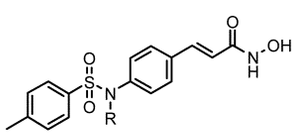
The polymer-assisted solution phase synthesis (PASP) of an array of histone deacetylase (HDAc) inhibitors is described. HDAc inhibitors have considerable potential as new anti-proliferative agents. Selected compounds were shown to inhibit both human endothelial cell proliferation, and the formation of tubules (neovascularisation) in an *in vitro* model of angiogenesis.

Introduction

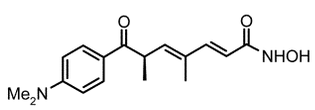
In recent years, the parallel synthesis of focused arrays of analogues of 'lead' structures has evolved into a powerful method to facilitate the rapid determination of structure activity relationships (SAR) and thus drive the lead optimisation process in the pharmaceutical industry.[1] To achieve this objective, attention has increasingly concentrated on the adoption of efficient and versatile solution-phase synthesis approaches, as opposed to solid-phase organic synthesis (SPOS)[2] strategies. In this way, difficulties associated with SPOS, such as unacceptably long reaction development and optimisation times, the need to use large reagent excesses to drive reactions to completion, and the inability to perform the purification of resin-bound intermediates, are largely circumvented. A particularly powerful and widely adopted approach to parallel solution phase synthesis utilises polymer-supported reagents and scavenger resins.[3] Polymer-assisted solution phase (PASP) synthesis reduces reaction work-ups to a series of simple filtrations and facilitates in-line purification by solid-phase extraction and 'catch and release' techniques.[4] It is thus well-suited to enable the rapid, multi-step synthesis of focused compound arrays.

Recently, we have reported a fully automated, multi-step synthesis using polymer supported reagents to prepare a series of histone deactylase (HDAc) inhibitors[5] based upon the reported lead structure **1a**.[6] These are structurally related to the natural product trichostatin A (TSA) **2**[7] and suberoylanilide hydroxamic acid (SAHA) **3**.[8] Here we describe in detail the development of the polymer-assisted synthesis, and the manual preparation and associated biological activity of a 24-member array of HDAc inhibitors **1a**–**x**.

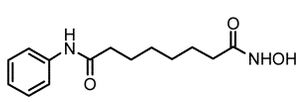
There is increasing evidence that the acetylation and deacetylation of proteins and small molecules is an important regulatory modification in many cellular processes.[9] In particular, the reversible acetylation of the ε-amino functionalities of conserved lysine residues in the *N*-terminal tails of nucleosomal histones results in chromatin remodelling and is a key biological event controlling gene transcription.[10] This post-translational modification of histones is co-regulated by a combination of histone acetyltransferase (HAT) and histone deacetylase (HDAc) enzymes, that cause conformational changes in the nucleosome, altering the accessibility and binding of transcription factors to DNA. In normal cells, this leads to a regulation of cell differentiation and proliferation. However, an imbalance in the level of histone acetylation has been associated with malignant disease.[11] HDAc inhibitors lead to a reversal of transcriptional repression and an associated upregulation of tumour suppressors.[12]



1a: R=H
1b: R=Me



2; Trichostatin A (TSA)



3; SAHA

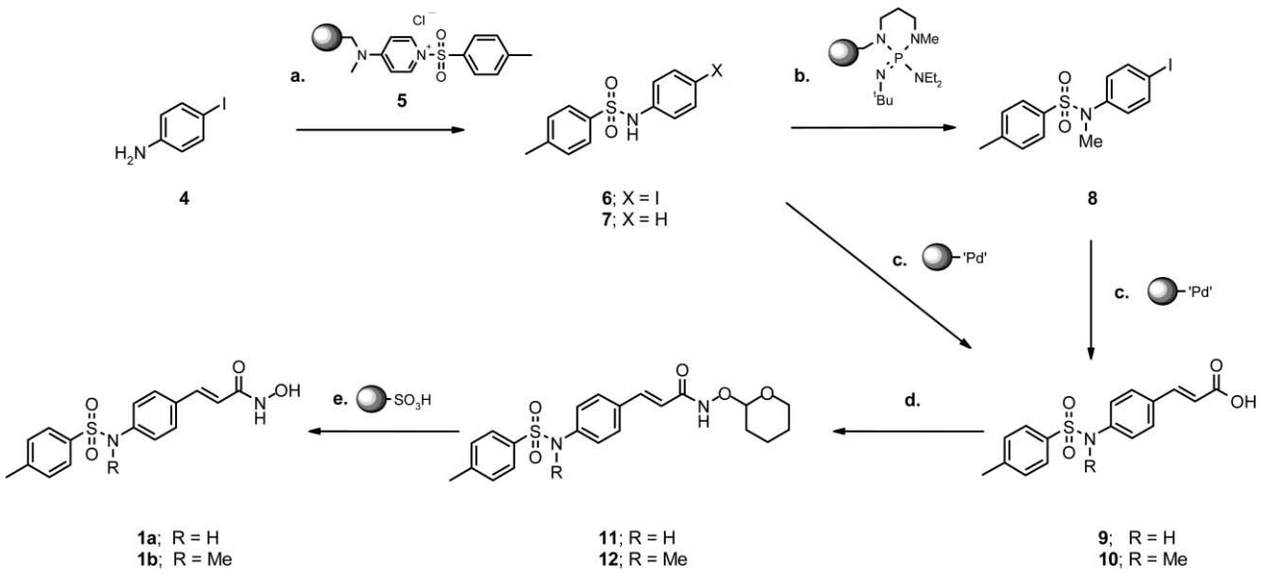
In addition, HDAc inhibitors have been observed to result in an inhibition of angiogenesis[13] and are therefore of considerable interest as potential new anti-cancer agents.[11c] Other studies have suggested that HDAc inhibitors may also offer promise as novel anti-protozoal agents[14] and new anti-virals.[15]

Results and discussion

Chemistry

Previous work has established that the hydroxamic acid functionality in TSA coordinates to a zinc cation that is held by several hydrogen bonds in the active site of HDLP (histone-deacetylase-like-protein), a bacterial homologue of eukaryotic HDACs.[16] The polyene chain in TSA acts as a spacer group that occupies a lipophilic channel leading from the zinc binding domain of the enzyme to a region occupied by the aromatic capping group. Therefore, we elected to prepare a focused array based upon **1a** that retained the core aryl propenoic hydroxamic acid functionality but incorporated three points of diversity in order to investigate the consequences of: (i) sulfonamide *N*-methylation, (ii) regioisomerism about the aniline, and (iii) different substituents on the terminal benzenesulfonamide.

DOI: 10.1039/b313414h



Scheme 1 *Reagents and conditions:* a) (i). DMF, 50 °C, 1 h, ×2; (ii). SCX SPE purification; b) MeI, DMF, rt, 18 h; c) acrylic acid, triethylamine, DMF, 90 °C, 18 h; d) (i). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole, DMF, rt, 20 min; (ii). hydroxylamine-OTHP, 50 °C, 18 h; (iii). NH_2 SPE purification; e) Amberlyst H-15, MeOH, rt, 2 h.

Moreover, we wished to develop a multi-step synthesis that did not require aqueous work-ups and could be realised using only a sequence of immobilised reagents and scavenger resins. In addition, we recognised that the ability to use the same solvent for all transformations would further simplify reaction work-up and establish a route that would subsequently be amenable to full automation.

Initially, we developed a suitable polymer-assisted procedure for each step of the synthesis in turn before combining these protocols to prepare a sample of **1a** in a flow-through manner, without isolation or chromatographic purification of any intermediates (Scheme 1). Finally, the array **1a**–**x** was assembled in a similar way by parallel polymer-assisted multi-step synthesis.

Considering the reported synthesis of **1a**, we wished to avoid the need to first prepare a *bis*-sulfonylated product which then required hydrolysis back to the desired *mono*-sulfonamide. However, although we have reported the sulfonylation of amines using sulfonyl chlorides immobilised on polymer-supported 4-dimethylaminopyridine (PS-DMAP),[17] the sulfonylation of anilines in this way was not precedented. In the event, treatment of the 4-iodoaniline **4** with the supported reagent **5**, prepared from toluenesulfonyl chloride and PS-DMAP at reflux in dichloromethane, gave the desired toluenesulfonamide **6** in 35% isolated yield. No evidence of *bis*-sulfonamide formation was observed, but the presence of the toluenesulfonic acid derived from hydrolysis of the immobilised reagent was evident. A similar result was obtained using acetonitrile or tetrahydrofuran as solvent. However, the use of dimethylformamide proved optimal giving rise to no observable toluenesulfonic acid by-product and a moderate increase in yield to 40%. Ultimately, we determined that repeating this protocol twice using a fresh batch of pre-formed PS-DMAP/tosyl chloride followed by filtration through an Isolute™ SCX cartridge[18] to scavenge unreacted aniline, gave the desired sulfonamide **6** in 64% yield and excellent purity (>95% by HPLC and ^{1}H-NMR).

Next, we turned our attention to the *N*-methylation of the sulfonamide in order to introduce a second point of diversity. This transformation could be reliably achieved with iodomethane in dimethylformamide in the presence of the polymer-supported phosphazene base PS-BEMP[19] to cleanly afford **8** in 90% isolated yield.

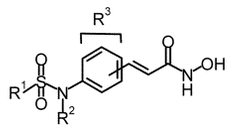
In order to facilitate product clean-up in the subsequent Heck olefination of **6** with acrylic acid, we elected to use an immobilised source of palladium. A polyurea microencapsulated palladium acetate (Pd–EnCat™)[20] and a variety of fibrous polystyrene-supported palladium phosphine complexes (FibreCat™)[21] were profiled in combination with a limiting amount of acrylic acid using a ReactArray™ SK233 automated reaction sampling system.[22] In general, we observed that reducing the amount of Pd catalyst present led, unsurprisingly, to slower conversions, but more significantly, an increase in the amount of the corresponding reduced product **7** derived from the competing reduction pathway was also observed. Under the conditions examined, the use of Pd–EnCat™ resulted in the highest conversion to **9** with least contamination by **7**. In the event, **7** could be readily removed from the crude reaction product by a single recrystallisation. Alternatively, the crude product could be progressed directly to the next stage of the synthesis because **7** does not participate in the subsequent hydroxmate formation and may be removed at a later stage.

Initially, formation of the hydroxamate **11** in high yield proved problematic. A number of coupling reagents including 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and diisopropylcarbodiimide (DIC) were examined under a variety of conditions for their ability to effect this condensation. Ultimately, optimal conditions were determined to be pre-activation in the presence of EDC for 20 min, followed by the introduction of hydroxylamine tetrahydropyranyl ether and subsequent incubation at 50 °C. After non-aqueous work-up by filtration through an amino-SPE cartridge, **11** was obtained in a respectable 70% isolated yield and excellent purity (90% by LC-MS). Finally, the THP *O*-protecting group was removed by treatment with Amberlyst H-15 in the presence of added methanol to give the desired hydroxamic acid **1a** in good purity and 71% isolated yield.

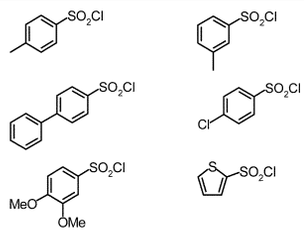
In a similar way, Heck olefination of the *N*-Me analogue **8** with acrylic acid gave the acid **10** (80% yield). This material was converted into the corresponding intermediate tetrahydropyranyl hydroxamate **12** which was deprotected directly to afford the *N*-Me hydroxamic acid **1b** in 76% yield and 95% LC-MS purity.

Having established that the complete synthesis could be performed in DMF without the need for aqueous work-up or the evaporation of solvents at intermediate stages, we next attempted the synthesis of **1a** in a flow-through manner. In a linear synthesis, the need to efficiently wash resins after each step without subsequent solvent evaporation tends to lead to a

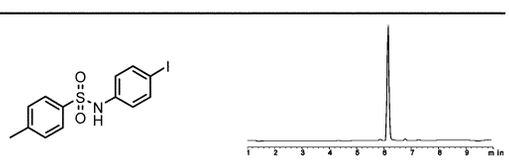
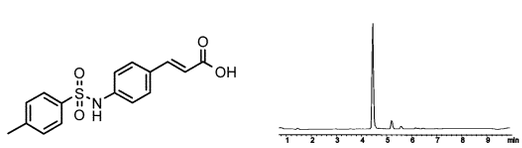
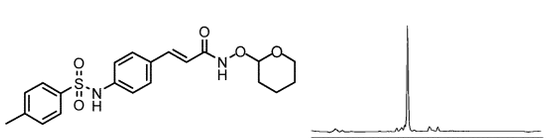
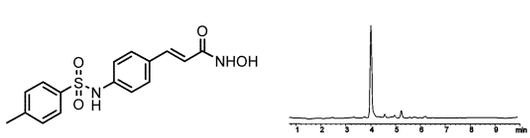
Table 1 Monomer sets chosen for combinatorial array **1a**–**x**



1a-x

R^1-SO_2Cl:		
R^2:	H	Me
I-R^3-NH_2:		

dilution of the reaction solution which, in turn, can have a detrimental effect on reaction rates and yields. Therefore, we initiated the flow-through synthesis at a higher substrate concentration than used previously and carefully minimised resin wash solvent volumes. Using the validated reaction conditions, the complete synthesis was performed in a flow-through manner without any intermediate solvent removal or off-line purification of intermediates to afford the hydroxamic acid **1a** in 47% overall isolated yield and 87% purity according to LC-MS and ^{1}H NMR analysis. The purity profile for this reaction sequence is shown in Fig. 1.

Compound	HPLC trace[a]
6	
9	
11	
1a	

[a] Crude HPLC traces measured at 254 nm plotting absorbance *vs* time.

Fig. 1 Purity profile for the flow-through synthesis of hydroxamic acid **1a**.

With a reliable synthetic route in hand, we next selected a series of monomers to prepare a small focused compound array **1a**–**x** in order to investigate SAR associated with this pharmacophore (Table 1). Initially, a set of six polystyrene-supported sulfonyl pyridinium chlorides were prepared. For a small compound array such as **1a**–**x**, it was feasible to perform reaction quality control by LC-MS throughout the synthesis. In this way, it was noted that in the Heck olefination step, *tert*-sulfonamides tended to lead to lower purity products than secondary sulfonamides. Nevertheless, all the desired products were obtained in reasonable overall yields with acceptable purities (Fig. 2). However, to obtain reliable SAR it is desirable to assay substrates with uniformly high purities. Therefore, prior to screening, a single auto-preparative purification step was performed to ensure that all substrates had purities in excess of 95%.

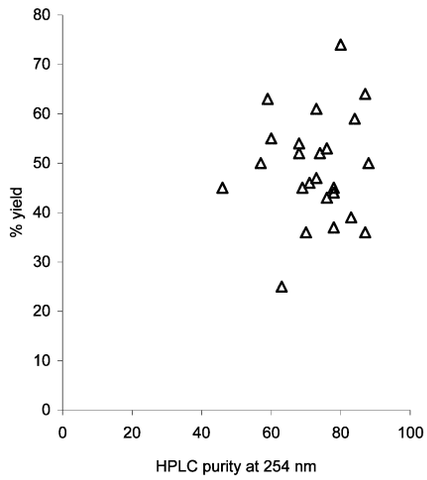


Fig. 2 Isolated yields and purities for crude array compounds **1a**–**x**.

Biology

The *in vitro* biological activity of the compounds **1a**–**x** was measured at 1 μM in a primary HDAc enzyme inhibitory assay using HeLa cell nuclear extract (principally HDAc-1 and 2). The readout was obtained as the amount of deacetylated substrate produced and is inversely proportional to enzyme inhibitory activity. Since HDAc inhibitors are known to induce cell death in cell types that cannot maintain a G_1/G_2 checkpoint, the ability of the compounds to inhibit HUVEC (human umbilical vein endothelial cell) proliferation was also determined.[23] In this assay, the number of viable cells remaining after incubation with the inhibitor for 48 h was measured spectrophotometrically following introduction of the MTS reagent.[24] Endothelial cells play a central role in angiogenesis, which is essential for the growth of solid tumours beyond a few cubic millimeters and hence, the most active analogues were also evaluated for their ability to inhibit tubule formation in a HUVEC-fibroblast co-culture which is an *in vitro* model of angiogenesis. In this model, the endothelial cells initially form small islands within the culture matrix. The HUVECs subsequently begin to proliferate and then enter a migratory phase during which they move through the matrix to form tubules. These tubules eventually join over a period of 12–14 days to form a network of anastomosing tubes that closely resembles a capillary bed (Fig. 3). The significance of angiogenesis in the growth of tumours and the distribution and progression of metastatic disease makes vascular targeting an important strategy for the effective, long term control of cancers.[25]

Results and discussion

In vitro, the most effective enzyme inhibitors (*i.e.* those compounds producing deacetylated substrate in the range 10–20 μM) were found to be the toluenesulfonamide **1a**, the biphenylsulfonamide **1k** and the 3,4-dimethoxysulfonamide **1q** (Table 2). Notably, in this assay, the 3,4-dimethoxy analogue **1q** was found to be equipotent with TSA. Whilst compounds **1a** and **1q** are derived from a *para*-substituted R^3 core motif, compound **1k**, derived from a *meta*-substituted core, was more potent than its *para*-analogue **1i**. The weakest compounds were the thiophenyl analogues **1u**, **1w** and **1x**.

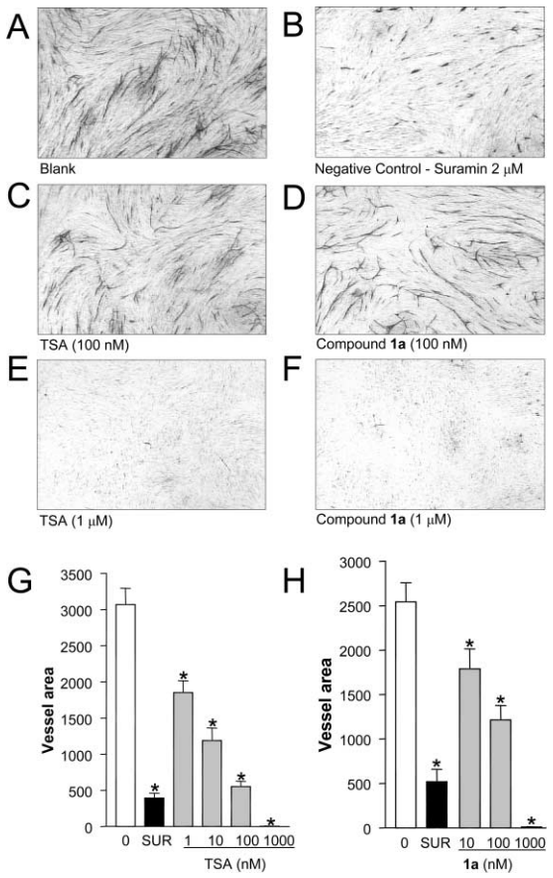


Fig. 3 TSA and **1a** inhibit tube-formation in an *in vitro* angiogenesis assay. The HUVEC-fibroblast co-culture was treated with increasing concentrations of TSA and **1a** and immunocytochemically stained with PECAM-1 antibodies after 11 days. The images were analysed using Angiosys software and the total tubule area was measured in bit map units. **A**. Blank; **B**. Negative control (2 mM suramin); **C**, **E** TSA; **D**, **F** Compound **1a**; **G**, **H** Graph of mean tubule area. Values are ± SEM measured from 8 microscope fields for each conc^{n}.

In general, *N*-methylation of the sulfonamide nitrogen led to a reduction in enzyme inhibitory activity. For example, compounds **1o**, **1s** and **1q** were more effective inhibitors than their *N*-Me counterparts **1p**, **1t** and **1r**. An exception to this observation arises when contrasting the analogues **1i** (*N*-H) and **1j** (*N*-Me) where **1j** is more potent than **1i**.

Considering the MTS *in vitro* cell proliferation assay, a number of compounds were found to possess micromolar anti-proliferative activity. However, whilst the thiophenyl analogue **1w**, for example, was one of the weakest enzyme inhibitors in HeLa nuclear extract, it was determined to be a potent anti-proliferative agent in HUVECs. The most potent anti-proliferative agents *in vitro* were the toluenesulfonamides **1a** (*para*-R^3 core) and **1c** (*meta*-R^3 core) both with IC_{50}s of 2 μM. The corresponding 4-biphenyl analogues **1k** (*meta*-core) and **1i** (*para*-core) were also effective with IC_{50}s of 4 and 3 μM respectively. Interestingly, in the case of **1i**, the corresponding *N*-methylated analogue **1j** was also a potent anti-proliferative agent in HUVECs.

The lack of correlation between the *in vitro* enzyme inhibition and anti-proliferative assays suggests that in HUVECs the primary mode of action of **1a**–**x** is likely to be inhibition of HDACs other than HDACs-1 and 2. In addition, in whole cell assay barriers to biological activity may be presented by the need to effectively penetrate cellular and/or nuclear membranes to access the site of action. Therefore, whilst there is no doubt that compounds such as **1a** and **1c** are potent anti-proliferative agents in HUVECs, the precise mode of biological action cannot be ascribed with certainty at this stage and is the subject of ongoing studies. However, it was noted that when compounds **1a** and **1b**, were administered to HUVECs at higher concentrations (>20 μM), a decrease in the number of cells below that present at the start of the assay was observed implying that cell death was also occurring at these concentrations.[26] These observations suggest that the overall anti-proliferative action of HDAc inhibitors such as **1a** and **1c** in HUVECs is a combination of a specific inhibition of cell proliferation and an induction of cell death.

Compounds **1a**, **1c** and TSA were also assayed *in vitro* for their ability to inhibit tubule formation in a HUVEC-fibroblast co-culture (Angiokit).[27] All of these compounds were found to be effective in reducing total vessel area. Compounds **1a** and **1c** were essentially equipotent and effective at sub-micromolar concentrations, whereas TSA was approximately 10 fold more potent and was an effective inhibitor below 10 nM (Fig. 3). Interestingly, both TSA and **1a** are effective at inhibiting angiogenesis at <100 nM, a concentration below that at which a significant anti-proliferative effect was observed in isolated HUVECs.

Conclusions

In summary, we have prepared an array of hydroxamic acids **1a**–**x** which are structurally related to TSA, using a parallel, multi-step polymer-assisted solution phase (PASP) synthesis. Following biological evaluation *in vitro*, a number of these compounds were found to possess micromolar inhibitory activity in a HeLa cell nuclear extract enzyme inhibition assay (HDAc-1 and 2), and anti-proliferative activity in human umbilical vein endothelial cells (HUVECs). In addition, the most active compounds **1a** and **1c** were shown to be potent inhibitors of tube-formation (neovascularisation) in an *in vitro* model of angiogenesis.

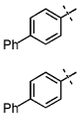
This study serves to further demonstrate the utility of PASP synthesis techniques for the rapid preparation of focused, biologically relevant compound arrays.

Experimental

Synthesis

All solvents and reagents were used as supplied unless otherwise stated. Where appropriate, reactions were carried out under an inert atmosphere of nitrogen. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} pre-coated plates. Analytical high pressure liquid chromatography (HPLC) was performed using a Hewlett Packard Series 1050 instrument. *Column*: Supelcosil™ ABZ$^+$PLUS 3.3 cm × 4.6 mm, 3 μm. *Eluent* A: water, 0.1% TFA, B: acetonitrile 95%, water 5%, TFA 0.05%. *Flow rate*: 1 cm^3 min^{-1}. *Detection*: UV (diode array: 215, 230, 254 nm). *Method*: gradient 10–95% B in A over 7 min. Preparative HPLC was performed using a Gilson auto-preparative instrument. *Column*: Supelcosil™ ABZ$^+$PLUS 10.0 cm × 2.12 cm, 5 μm. *Eluent* A: water, 0.1% TFA, B: acetonitrile 95%, water 5%, TFA 0.05%. *Flow rate*: 6 ml min^{-1}. *Detection*: UV (254 nm). *Method*: gradient 10–95% B in A over 25 min. Infrared spectra were collected on a Perkin-Elmer Spectrum One FT-IR instrument under attenuated total reflectance (ATR) conditions. Liquid chromatography-mass spectra (LC-MS) were recorded using a Hewlett Packard Series 1050 HPLC attached to a Micromass ZQ 2000 mass spectrometer under electrospray positive and negative ionisation conditions. *Column*: Supelcosil™ ABZ$^+$PLUS 3.3 cm × 4.6 mm, 3 μm. *Eluent* A: 10 mM solution of ammonium acetate in water, 0.1% formic acid, B: acetonitrile 95%, water 5%, formic acid 0.05%. *Flow rate*: 3 ml min^{-1}. *Detection*: UV (diode array: 215–330 nm). *Method*: gradient 0–100% B in A over 3.5 min. High resolution mass spectra were obtained using a Micromass Q-TOF 2 under positive or negative ionisation conditions. NMR spectra were recorded on Bruker AM 400 or Bruker

Table 2 Comparison of enzyme inhibitory and anti-proliferative activity of HDAc inhibitors **1a**–**x**

Compound	R^1	R^3	R^2	HeLa extracts (μM)[a]	HUVEC IC_{50} (μM)[b]	Compound	R^1	R^3	R^2	HeLa extracts (μM)[a]	HUVEC IC_{50} (μM)[b]
1a			H	19	2	**1m**			H	29	>20
1b			Me	33	6	**1n**			Me	ND	15
1c			H	32	2	**1o**			H	38	>20
1d			Me	45	10	**1p**			Me	57	13
1e			H	54	>20	**1q**			H	13	8
1f			Me	34	>20	**1r**			Me	43	29
1g			H	50	>20	**1s**			H	34	>20
1h			Me	56	>20	**1t**			Me	54	17
1i			H	58	3	**1u**			H	72	>20
1j			Me	31	4	**1v**			Me	40	ND
1k			H	20	4	**1w**			H	61	3
1l			Me	35	>20	**1x**			Me	75	>20

[a] Amount of deacetylated Color de Lys™ substrate produced after 30 min. [b] Inhibition of HUVEC proliferation determined by spectrophotometric measurement of viable cell count after 48 h following introduction of MTS reagent.

DRX 500 spectrometers at 400 MHz and 500 MHz respectively for ^{1}H NMR, and at 100 MHz and 125 MHz for ^{13}C NMR (proton decoupled) in the indicated solvent. Chemical shifts (δ) are reported in parts per million (ppm). The following abbreviations are used for multiplicities: s = singlet; d = doublet; t = triplet; m = multiplet; dd = doublet of doublets; br = broad; and coupling constant J-values are quoted in Hz. Melting points were measured on a Mettler FP5 automatic melting point apparatus in open capillaries and are uncorrected. Parallel reactions requiring heating were performed with gentle stirring using a Greenhouse™ parallel reaction block.[28] All other reactions were performed in fritted plastic filter tubes on a laboratory shaker.[29] Amino and SCX solid-phase extraction (SPE) cartridges were obtained from Varian.[18]

Polystyrene-supported 4-dimethylaminopyridine-toluene sulfonyl chloride adduct (5). Toluenesulfonyl chloride (190 mg, 1.00 mmol) and dried polymer-supported 4-dimethylaminopyridine (4-DMAP) (0.33 g, 1.00 mmol) were suspended in anhydrous CH_2Cl_2 (5 ml). The suspension was agitated for 1 h, filtered, washed with CH_2Cl_2 (3 × 5 ml) and then dried *in vacuo* to give the polymer-supported reagent **5** as an orange solid (0.47 g).

The combined washings were evaporated and the resin loading was calculated based on the weight of sulfonyl chloride recovered (2.2 mmol g^{-1}).

***N*-(4-Iodo-phenyl)-4-methyl-benzenesulfonamide (6).** To the PS-DMAP:sulfonyl chloride adduct **5** (45 mg, 0.10 mmol) was added a solution of 4-iodoaniline (22 mg, 0.10 mmol) in DMF (1.0 ml). The reaction mixture was heated with gentle stirring at 50 °C for 1 h, then filtered and the resin washed with DMF (0.5 ml × 2). To the filtrate was added an additional quantity of sulfonyl chloride resin **5** (45 mg, 0.10 mmol) and this mixture was heated at 50 °C for 1 h. The reaction was filtered and the resin again washed with DMF (0.5 ml × 2). The combined filtrates were applied to an SCX cartridge (0.5 g) and eluted with methanol to give the sulfonamide **6** as a white solid (24 mg, 64%). Mp: 171–172 °C; R_f 0.35 [EtOAc/hexane (1 : 2)]; Found: C, 41.93; H, 3.07; N, 3.79; S, 8.55%. $C_{13}H_{12}NO_2IS$ requires C, 41.84; H, 3.24; N 3.75; S, 8.59%; HRMS: $C_{13}H_{12}NO_2IS$ requires $(MH)^+$ 373.9712, found 373.9717; IR ν_{max}/cm^{-1}: 3220 (NH), 1320 (SO_2), 1160 (SO_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.3 (br s, 1H), 7.61 (d, 2H, J = 8 Hz), 7.52 (d, 2H, J = 8 Hz), 7.31 (d, 2H, J = 8 Hz), 6.86 (d, 2H, J = 8 Hz), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 142.2, 136.2, 134.3, 133.7, 127.7, 125.2, 121.1, 87.1, 19.5; LC-MS (ESI −ve): m/z 372 $(MH)^-$, t_R = 3.58 min; HPLC: t_R = 4.79 min (97%; 254 nm).

***N*-Methyl-*N*-(4-iodo-phenyl)-4-methyl-benzenesulfonamide (8).** To a solution *N*-(4-iodo-phenyl)-4-methyl-benzenesulfonamide **6** (20.0 mg, 0.05 mmol) in DMF (0.5 ml) was added PS–BEMP (26 mg, 0.05 mmol) and iodomethane (3.7 μl, 0.06 mmol). The suspension was agitated for 18 h, The resin was drained, washed with DMF (0.5 ml × 3), and the washings combined and evaporated *in vacuo* to give the sulfonamide **8** as a white solid (17.5 mg, 90%). Mp: 91–92 °C; R_f 0.43 [EtOAc/hexane (1 : 4)]; Found: C, 43.51; H, 3.56; N, 3.58; S, 8.34%. $C_{14}H_{14}NO_2IS$ requires C, 43.42; H, 3.64; N 3.62; S, 8.28%; HRMS: $C_{14}H_{15}NO_2IS$ requires $(MH)^+$ 387.9868, found 387.9880; IR ν_{max}/cm^{-1}: 1343 (SO_2), 1163 (SO_2); ^{1}H NMR (400 MHz, CD_3OD): δ 7.63 (d, 2H, J = 8 Hz), 7.40 (d, 2H, J = 8 Hz), 7.32 (d, 2H, J = 8 Hz), 6.88 (d, 2H, J = 8 Hz), 3.11 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 146.0, 143.3 139.5, 134.9, 131.1, 129.9, 129.4, 93.2, 38.7, 21.9; LC-MS (ESI +ve): m/z 388 $(MH)^+$, t_R = 3.78 min; HPLC: t_R = 6.23 min (100%; 254 nm).

General conditions for ReactArray™ study. To the sulfonamide **6** (40 mg, 0.10 mmol) and the immobilised palladium catalyst was added a solution of triethylamine in DMF (0.66 M × 0.5 ml) and then a solution of acrylic acid in DMF (0.03 M × 0.5 ml) using a ReactArray SK233 automated workstation. The reaction mixture was then heated at 90 °C with stirring for a total of 13 h. Aliquots (25 µl) were withdrawn automatically over this period, diluted with MeCN (0.5 ml) and subjected to in-line HPLC analysis. Results were plotted as % composition *versus* time.

3-[4-(Toluene-4-sulfonylamino)-phenyl]-acrylic acid (9). To a solution of *N*-(4-iodo-phenyl)-4-methyl-benzenesulfonamide **6** (0.40 g, 1.10 mmol) in DMF (5 ml) was added acrylic acid (75 µl, 1.10 mmol), triethylamine (0.45 ml, 3.21 mmol) and Pd EnCat™ (84 mg, 3 mol% Pd). The reaction mixture was stirred at 90 °C for 18 h, then filtered, and the solvent evaporated *in vacuo* to leave a brown gum. Recrystallisation from hexane/EtOAc afforded the acrylic acid **9** as white crystals (0.29 g, 83%). Mp: 233–234 °C; R_f = 0.21 [MeOH/$CHCl_3$ (1 : 19)]; Found: C, 60.53; H, 4.76; N, 4.55; S, 9.94%; $C_{16}H_{15}NO_4S$ requires C, 60.55; H, 4.76; N, 4.41; S, 10.10%; HRMS: $C_{16}H_{15}NO_4S$ requires $(MH)^+$ 318.0800, found 318.0809; IR ν_{max}/cm^{-1}: 3255 (NH/OH), 1674 (C=O), 1627 (C=C), 1322 (SO_2), 1155 (SO_2); ^1H NMR (400 MHz, CD_3OD): δ 7.66 (d, 2H, J = 8 Hz), 7.53 (d, 1H, J = 16 Hz), 7.43 (d, 2H, J = 8 Hz), 7.27 (d, 2H, J = 8 Hz), 7.11 (d, 2H, J = 8 Hz), 6.33 (d, 2H, J = 16 Hz), 2.33 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.5, 145.4, 145.3, 141.2, 138.1, 131.7, 130.7, 130.2, 128.3, 121.3, 118.4, 21.4; LC-MS: (ESI −ve) m/z 316 $(MH)^-$, t_R = 3.04 min; HPLC: t_R = 4.18 min (93%; 254 nm).

3-{4-[Methyl-(toluene-4-sulfonyl)-amino]-phenyl}-acrylic acid (10). To a solution of *N*-methyl-(4-iodo-phenyl)-4-methyl-benzenesulfonamide **8** (85 mg, 0.22 mmol) in DMF (2 ml) was added acrylic acid (15 µl, 0.21 mmol), triethylamine (91 µl, 0.66 mmol) and Pd EnCat™ (16 mg, 3 mol% Pd). The reaction mixture was stirred at 90 °C for 18 h, then filtered, and the solvent evaporated *in vacuo* to leave a brown solid. Recrystallisation from hexane/EtOAc afforded the acrylic acid **9** as off-white crystals (54 mg, 80%). Mp: 215–216 °C; R_f = 0.21 [MeOH/$CHCl_3$ (1 : 19)]; HRMS: $C_{17}H_{17}NO_4S$ requires $(MH)^+$ 332.0957, found 332.0951; IR ν_{max}/cm^{-1}: 1676 (C=O), 1625 (C=C), 1346 (SO_2), 1172 (SO_2); ^1H NMR (400 MHz, CD_3OD): δ 7.62 (d, 1H, J = 16 Hz), 7.39 (d, 2H, J = 8 Hz), 7.40 (d, 2H, J = 8 Hz), 7.32 (d, 2H, J = 8 Hz), 7.15 (d, 2H, J = 8 Hz), 6.44 (d, 1H, J = 16 Hz), 3.16 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 170.2, 145.6, 145.0, 144.6, 134.6 (2C), 130.6, 129.6, 128.9, 127.6, 120.1, 38.3, 21.5; LC-MS: (ESI +ve) m/z 332 $(MH)^+$, t_R = 3.19 min; HPLC: t_R= 5.04 min (97%; 254 nm).

***N*-(Tetrahydro-pyran-2-yloxy)-3-[4-(toluene-4-sulfonylamino)-phenyl]-acrylamide (11).** To a solution of 3-[4-(toluene-4-sulfonylamino)-phenyl]-acrylic acid **9** (66 mg, 0.20 mmol) in DMF (1 ml), was added a solution of diethylaminopropyl-carbodiimide hydrochloride (EDC) (50 mg, 0.30 mmol) and 1-hydroxybenzotriazole (HOBt) (40.5 mg, 0.30 mmol) in DMF (1.5 ml). The reaction mixture was stirred at room temperature for 20 min when a solution of *o*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine (35 mg, 0.30 mmol) in DMF (500 µl) was added. The resulting mixture was heated at 50 °C for 24 h. Upon cooling to room temperature, the reaction mixture was applied to a NH_2-SPE cartridge (2 g) and eluted with methanol to afford the tetrahydropyranyl ether **11** as an off-white solid (49 mg, 70%). R_f = 0.25 [EtOAc/hexane (1 : 1)]; HRMS: $C_{21}H_{24}N_2O_5S$ requires $(MH)^+$ 417.1484, found 417.1501; ^1H NMR (400 MHz, CD_3OD): δ 7.62 (d, 1H, J = 8 Hz), 7.49 (d, 2H, J = 16 Hz), 7.38 (d, 2H, J = 8 Hz), 7.27 (d, 2H, J = 8 Hz), 7.10 (d, 2H, J = 8 Hz), 6.36 (d, 1H, J = 16 Hz), 4.94 (br s, 1H), 4.01 (t, 1H, J = 8 Hz), 3.61 (m, 1H), 3.34 (s, 3H), 2.35 (s, 3H), 1.8 (m, 3H), 1.5 (m, 3H); LC-MS: (ESI +ve) m/z 417 $(MH)^+$, t_R = 4.77 min; HPLC: t_R = 4.58 min (90%; 254 nm).

***N*-Hydroxy-3-[4-(toluene-4-sulfonylamino)-phenyl]acrylamide (1a).** Amberlyst H-15 resin (80 mg) was added to a solution of the THP ether **11** (48 mg, 115 µM) in methanol (3 ml) and the suspension was agitated at room temperature for 2 h. The resin was then drained, washed with methanol (0.5 ml × 2) and the combined filtrates were evaporated *in vacuo* to afford the hydroxamic acid **1a** as an off-white solid (38 mg, 71%). Mp: 167–168 °C; HRMS: $C_{16}H_{16}N_2O_4S$ $(MH)^+$ requires 333.0909, found 333.0907; IR ν_{max}/cm^{-1}: 1649 (C=O), 1560 (C=C), 1328 (SO_2), 1155 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): δ 10.80 (br s 1H), 10.60 (br s, 1H), 8.93 (br s, 1H), 7.64 (d, 2H, J = 8 Hz), 7.39 (d, 2H, J = 9 Hz), 7.32 (d, 2H, J = 8 Hz), 7.29 (d, 1H, J = 16 Hz), 7.08 (d, 2H, J = 9 Hz), 6.27 (d, 2H, J = 16 Hz), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 145.6, 141.2, 141.1, 138.5, 132.6, 131.1, 130.1, 128.6, 121.9, 117.9, 21.8; LC-MS: (ESI −ve) m/z 331 $(MH)^-$, t_R = 2.69 min; HPLC: t_R = 3.92 min (88%; 254 nm).

***N*-Hydroxy-3-{4-[methyl-(toluene-4-sulfonyl)-amino]-phenyl}-acrylamide (1b).** To a solution of 3-{4-[Methyl-(toluene-4-sulfonyl)-amino]-phenyl}-acrylic acid **10** (70 mg, 0.21 mmol) in DMF (1.5 ml), was added a solution of diethylaminopropyl-carbodiimide hydrochloride (EDC) (52 mg, 0.27 mmol) and 1-hydroxybenzotriazole (HOBt) (42 mg, 0.31 mmol) in DMF (1.5 ml). The reaction mixture was stirred at room temperature for 20 min when a solution of *o*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine (37 mg, 0.31 mmol) in DMF (500 µl) was added. The resulting mixture was heated at 50 °C for 24 h. Upon cooling to room temperature, the reaction mixture was applied to a NH_2-SPE cartridge (2 g) and eluted with methanol. To the resulting methanolic solution of the tetrahydropyranyl ether **12** was added Amberlyst H-15 (300 mg) and this suspension was shaken at room temperature for 3 h. The suspension was filtered and the resin was washed with MeOH (2 ml × 2) to afford the hydroxamic acid **1b** as a colourless gum (55 mg, 76%). IR ν_{max}/cm^{-1}: 1656 (C=O), 1622 (C=O), 1600 (C=C), 1345 (SO_2), 1152 (SO_2); HRMS $C_{17}H_{18}N_2O_4S$ requires $(MH)^+$ 347.1066, found 347.1079; ^1H NMR (500 MHz, CD_3OD): δ 7.58–7.45 (m, 3H), 7.40 (d, 2H, J = 8 Hz), 7.31 (d, 2H, J = 8 Hz), 7.12 (d, 2H, J = 8 Hz), 6.44 (d, 1H, J = 16 Hz), 3.15 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 164.6, 144.1, 142.7, 134.0, 133.6, 133.2, 129.2, 127.7, 127.5, 126.2, 117.7, 36.9, 20.0; LC-MS: (ESI −ve) m/z 345 $(MH)^-$, t_R = 2.83 min; HPLC: t_R = 4.21 min (95%; 254 nm).

'Flow-through' preparation of *N*-hydroxy-3-[4-(toluene-4-sulfonylamino)-phenyl]acrylamide (1a). To the PS-DMAP:sulfonyl chloride adduct **5** (65 mg, 0.13 mmol) was added a solution of 4-iodoaniline (29 mg, 0.13 mmol) in DMF (2 ml) and the reaction mixture gently stirred at 50 °C for 1 h. The mixture was cooled, filtered and the resin was washed with DMF (0.5 ml × 2). The combined filtrates were then added to an additional quantity of resin **5** (65 mg, 0.13 mmol) and stirred at 50 °C for a further 1 h. Upon cooling to rt, the suspension was drained and the resin was washed with DMF (0.5 ml). The combined filtrates were applied to a SCX cartridge (1 g) which was eluted with DMF (1.5 ml). The eluate was collected and triethylamine (35 µl, 0.35 mmol) and a solution of acrylic acid in DMF (25 µl × 4.4 M, 0.09 mmol) were added. Encapsulated Pd catalyst (Pd–EnCat™, 6 mg, 3 mol%) was added and the reaction mixture was stirred at 90 °C for 18 h. The mixture was cooled, filtered and the supported catalyst was washed with DMF (200 µl). To the combined filtrates were added EDC (22 mg, 0.12 mmol) and HOBt (18 mg, 0.14 mmol). The resulting mixture was stirred at room temperature for 20 min when a solution of *o*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (16 mg, 0.14 mmol) in DMF (100 µl) was added and the reaction mixture

heated at 50 °C for 18 h. The resulting mixture was applied to a NH_2-SPE cartridge (1 g) and eluted with methanol. Amberlyst H-15 resin (70 mg) was added to the collected eluate and the suspension was agitated at rt for 2 h. The resin was then drained, washed with methanol (0.5 ml × 2) and the combined filtrates were evaporated *in vacuo* to leave the hydroxamic acid **1a** as an off-white solid (20 mg, 45%); ^{1}H NMR (400 MHz, CD_3OD): δ 7.65 (d, 2H, J = 8 Hz), 7.46 (d, 1H, J = 16 Hz), 7.40 (d, 2H, J = 8 Hz), 7.28 (d, 2H, J = 8 Hz), 7.10 (d, 2H, J = 8 Hz), 2.34 (s, 3H); HPLC: t_R = 3.92 min (87%; 254 nm). Further purification by HPLC gave **1a** as a white solid (10.5 mg, 24%): HPLC: t_R = 3.87 min (>98%; 254 nm). Data as previously.

General procedure for the preparation of polystyrene-supported DMAP:sulfonyl chloride adducts (5). Dried PS-DMAP resin (600 mg, 3.0 mmol g^{-1}, 1.80 mmol) and the sulfonyl chloride (Table 1: 1.80 mmol) were suspended in anhydrous CH_2Cl_2 (10 ml) and shaken for 1 h. The resin was filtered, washed with CH_2Cl_2 (10 ml × 3) and dried *in vacuo* to afford the PS-DMAP:sulfonyl chloride adduct **5**.

Resin loadings were calculated from the mass of the sulfonyl chloride recovered from the combined resin washings (typical loading: 1.9 mmol g^{-1}–2.2 mmol g^{-1}).

General procedure for the preparation of sulfonamides (6). To the PS-DMAP:sulfonyl chloride adducts **5** (0.26 mmol) was added a solution of an iodoaniline **4** (Table 1: 57 mg, 0.26 mmol) in DMF (2 ml). The resulting suspensions were stirred at 50 °C for 1 h, drained, and the resins washed with DMF (0.5 ml). In each case a further quantity of PS-DMAP:sulfonyl chloride resin (0.26 mmol) was added to the collected filtrates and the mixtures were stirred at 40 °C for a further 1 h. Upon cooling to rt, the suspensions were drained and the resins washed with DMF (0.5 ml). The filtrates were applied to SCX cartridges (1 g) and these were eluted with DMF (1 ml) to afford solutions of the sulfonamides **6**.

General procedure for the preparation of methyl sulfonamides (8). Half of each DMF solution of the sulfonamides **6** (1.5 ml) from the previous step were added to suspensions of PS-BEMP (45 mg, 0.10 mmol) in a solution of iodomethane (25 µl × 4.4 M, 0.11 mmol) in DMF. The resulting mixtures were agitated at rt for 18 h, then drained and the resins washed with DMF (0.2 ml) to afford DMF solutions of the methyl sulfonamides **8**. These were used directly in the next step.

General procedure for Heck olefinations to prepare (9) and (10). To the DMF solutions of the sulfonamides **6** or **8** (1.5 ml or 1.7 ml respectively) was added triethylamine (35 µl, 0.35 mmol) followed by a solution of acrylic acid in DMF (25 µl × 3.4 M; 0.09 mmol). Encapsulated Pd catalyst (Pd EnCat™, 6 mg, 3 mol%) was added and the mixtures stirred at 85 °C for 18 h. The reaction mixtures were cooled, filtered and the palladium catalyst washed with DMF (200 µl). These solutions were used directly in the next step.

General procedure for the preparation hydroxamic acids (1a–x). To the DMF solutions of the acrylic acids **9** and **10** in DMF (1.5 ml) was added EDC (22 mg, 0.10 mmol) and HOBt (18 mg, 0.13 mmol). The reaction mixtures were stirred at room temperature for 20 min. A solution of *o*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (10.5 mg, 0.09 mmol) in DMF (100 µl) was added and the resulting mixtures were gently stirred at 50 °C for 18 h. The solutions were applied to NH_2-SPE cartridges (1 g) and the cartridges were eluted with methanol. Amberlyst H–15 resin (70 mg) was added to the column eluates and the resulting suspensions were agitated at rt for 2 h. The resins were drained, washed with methanol (0.5 ml × 2) and the combined filtrates were evaporated *in vacuo*. The products obtained were purified by HPLC to gave the hydroxamic acids **1a**–**x**.

(NB: For the following examples overall yields are quoted for the entire sequence after HPLC purification).

***N*-Hydroxy-3-[4-(toluene-4-sulfonylamino)-phenyl]acrylamide (1a).** White solid (11 mg, 26%). Data as previously.

***N*-Hydroxy-3-{4-[methyl-(toluene-4-sulfonyl)-amino]-phenyl}-acrylamide (1b).** White solid (10.5 mg, 23%). Data as previously.

***N*-Hydroxy-3-[3-(toluene-4-sulfonylamino)-phenyl]-acrylamide (1c).** White solid (11 mg, 25%). IR ν_{max}/cm^{-1}: 1658 (C=O), 1603 (C=C), 1330 (SO_2), 1151 (SO_2); HRMS: $C_{16}H_{16}N_2O_4S$ requires $(MH)^+$ 333.0909, found 333.0914; ^{1}H NMR (500 MHz, CD_3OD): δ 7.63 (d, 2H, J = 8 Hz), 7.44 (d, 1H, J = 16 Hz), 7.30–7.20 (m, 5H), 7.06 (m, 1H), 6.38 (d, 1H, J = 16 Hz), 2.01 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 168.3, 147.5, 143.2, 142.1, 140.3, 139.5, 133.0, 132.9, 130.6, 127.2, 125.5, 123.1, 121.5, 23.7; LC-MS: (ESI −ve) m/z 331 $(MH)^-$, t_R = 2.72 min; HPLC: t_R = 3.99 min (100%; 254 nm).

***N*-Hydroxy-3-{3-[methyl-(toluene-4-sulfonyl)-amino]-phenyl}-acrylamide (1d).** Colourless gum (9.0 mg, 20%). IR ν_{max}/cm^{-1}: 1659 (C=O), 1622 (C=O), 1599 (C=C), 1344 (SO_2), 1165 (SO_2); HRMS: $C_{13}H_{12}N_2O_4S$ requires $(MH)^+$ 347.1066, found 347.1057; ^{1}H NMR (500 MHz, CD_3OD): δ 7.51–7.38 (m, 4H), 7.35–7.29 (m, 4H), 7.06 (d, 1H, J = 8 Hz), 6.41 (d, 1H, J = 16 Hz), 3.16 (s, 3H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.9, 145.6, 143.7, 140.6, 137.1, 134.6, 130.6, 130.5, 129.0, 128.6, 127.7, 126.9, 119.5, 38.5, 21.5; LC-MS: (ESI −ve) m/z 345 $(MH)^-$, t_R = 2.98 min; HPLC: t_R = 4.26 min (98%; 254 nm).

***N*-Hydroxy-3-[4-(toluene-3-sulfonylamino)-phenyl]-acrylamide (1e).** White solid (10 mg, 24%). IR ν_{max}/cm^{-1}: 1678 (C=O), 1654 (C=O), 1598 (C=C), 1330 (SO_2), 1146 (SO_2); HRMS: $C_{16}H_{16}N_2O_4S$ requires $(MH)^+$ 333.0909, found 333.0922; ^{1}H NMR (500 MHz, CD_3OD): δ 7.60 (s, 1H), 7.58 (d, 1H, J = 7 Hz), 7.45 (d, 1H, J = 16 Hz), 7.41–7.32 (m, 4H), 7.05 (d, 2H, J = 8 Hz), 6.32 (d, 1H, J = 16 Hz), 2.34 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.0, 140.8, 140.6, 140.5, 134.7, 134.6, 132.2, 130.0, 129.7, 128.4, 125.3, 121.5, 117.5, 21.2; LC-MS: (ESI −ve) m/z 331 $(MH)^-$, t_R = 2.65 min; HPLC: t_R = 3.79 min (96%; 254 nm).

***N*-Hydroxy-3-{4-[methyl-(toluene-3-sulfonyl)-amino]-phenyl}-acrylamide (1f).** Colourless gum (5.7 mg, 13%). IR ν_{max}/cm^{-1}: 1659 (C=O), 1625 (C=O), 1603 (C=C), 1348 (SO_2), 1150 (SO_2); HRMS: $C_{17}H_{18}N_2O_4S$ requires $(MH)^+$ 347.1066, found 347.1080; ^{1}H NMR (500 MHz, CD_3OD): δ 7.57–7.29 (m, 6H), 7.14 (d, 2H), 6.44 (d, 1H, J = 16 Hz), 3.17 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (500 MHz, CD_3OD): δ 166.0, 144.1, 140.6, 140.4, 137.8, 135.1, 135.0, 130.0, 129.2, 129.1, 127.8, 126.0, 119.2, 38.3, 21.2; LC-MS: (ESI −ve) m/z 345 $(MH)^-$, t_R = 2.84 min; HPLC: t_R = 4.26 min (100%; 254 nm).

***N*-Hydroxy-3-[3-(toluene-3-sulfonylamino)-phenyl]-acrylamide (1g).** White solid (10.5 mg, 24%). IR ν_{max}/cm^{-1}: 1661 (C=O), 1603 (C=C), 1332 (SO_2), 1150 (SO_2); HRMS: $C_{16}H_{16}N_2O_4S$ requires $(MH)^+$ 333.0909, found 333.0909; ^{1}H NMR (500 MHz, CD_3OD): δ 7.59 (m, 2H), 7.44 (d, 1H, J = 16 Hz), 7.38–7.21 (m, 5H), 7.06 (m, 1H), 6.39 (d, 1H, J = 16 Hz), 2.34 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.4, 141.4, 141.3, 140.2, 137.6, 135.1, 131.1, 130.3, 128.8, 125.7, 125.4, 123.7, 121.2, 119.6, 21.6; LC-MS: (ESI −ve) m/z 331 $(MH)^-$, t_R = 2.69 min; HPLC: t_R = 3.88 min (100%; 254 nm).

***N*-Hydroxy-3-{3-[methyl-(toluene-3-sulfonyl)-amino]-phenyl}-acrylamide (1h).** Colourless gum (8.8 mg, 20%). IR ν_{max}/cm^{-1}: 1662 (C=O), 1625 (C=O), 1600 (C=C), 1348 (SO_2), 1149 (SO_2); HRMS: $C_{17}H_{18}N_2O_4S$ requires $(MH)^+$ 347.1066, found

347.1075; ^{1}H NMR (500 MHz, CD_3OD): δ 7.51–7.28 (m, 8H), 7.08 (m, 1H), 6.40 (d, 1H, J = 16 Hz), 3.17 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 165.9, 143.7, 140.6, 140.5, 137.4, 137.1, 135.0, 130.5, 129.9, 129.2, 128.7, 127.8, 126.9, 126.1, 119.5, 38.5, 21.2; LC-MS: (ESI −ve) m/z 345 $(MH)^-$, t_R = 2.82 min; HPLC: t_R = 4.19 min (100%; 254 nm).

3-[4-(Biphenyl-4-sulfonylamino)-phenyl]-*N*-hydroxy-acrylamide (1i). White solid (3.2 mg, 6%). IR ν_{max}/cm^{-1}: 1676 (C=O), 1654 (C=O), 1333 (SO_2), 1155 (SO_2); HRMS: $C_{21}H_{18}N_2O_4S$ requires $(MH)^+$ 395.1066, found 395.1080; ^{1}H NMR (500 MHz, CD_3OD): δ 7.85 (d, 2H, J = 8 Hz), 7.73 (d, 2H, J = 8 Hz), 7.61 (d, 2H, J = 8 Hz), 7.48–7.35 (m, 6H), 7.16 (d, 2H, J = 8 Hz, H), 6.33 (d, 1H, J = 16 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 166.7, 146.0, 139.8, 139.3, 138.0, 131.3, 128.8, 128.5, 128.3, 127.5, 127.4, 127.3, 127.0, 120.4; LC-MS: (ESI −ve) m/z 393 $(MH)^-$, t_R = 3.01 min; HPLC: t_R = 4.58 min (97%; 254 nm).

3-{4-[(Biphenyl-4-sulfonyl)-methyl-amino]-phenyl}-*N*-hydroxy-acrylamide (1j). White solid (8.0 mg, 16%). IR ν_{max}/cm^{-1}: 1651 (C=O), 1605 (C=O), 1597 (C=C), 1345 (SO_2), 1153 (SO_2); HRMS: $C_{22}H_{20}N_2O_4S$ requires $(MH)^+$ 409.1222, found 409.1239; ^{1}H NMR (500 MHz, CD_3OD): δ 7.78 (d, 2H, J = 8 Hz), 7.67 (d, 2H, J = 8 Hz), 7.60 (d, 2H, J = 8 Hz), 7.54–7.44 (m, 5H), 7.40 (m, 1H), 7.20 (d, 2H, J = 8 Hz), 6.44 (d, 1H, J = 16 Hz), 3.22 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 147.3, 144.1, 140.4, 140.3, 136.2, 135.2, 130.2, 129.8, 129.6, 129.5, 129.3, 128.5, 128.3, 127.8, 127.0, 119.2; LC-MS: (ESI −ve) m/z 407 $(MH)^-$, t_R = 3.19 min; HPLC: t_R = 5.12 min (100%; 254 nm).

3-[3-(Biphenyl-4-sulfonylamino)-phenyl]-*N*-hydroxy-acrylamide (1k). Colourless gum (9.8 mg, 19%). IR ν_{max}/cm^{-1}: 1660 (C=O), 1596 (C=C), 1331 (SO_2), 1154 (SO_2); HRMS $C_{21}H_{18}N_2O_4S$ requires $(MH)^+$ 395.1066, found 395.1075; ^{1}H NMR (500 MHz, CD_3OD): δ 7.82 (d, 2H, J = 8 Hz), 7.71 (d, 2H, J = 8 Hz), 7.61 (d, 2H, J = 8 Hz), 7.48–7.33 (m, 5H), 7.24 (m, 2H), 7.11 (m, 1H), 6.39 (d, 1H, J = 16 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 164.4, 146.9, 141.4, 141.0, 140.2, 140.1, 137.2, 131.2, 130.5, 129.9, 129.2, 129.0, 128.7, 125.5, 123.7, 121.3, 119.7; LC-MS: (ESI −ve) m/z 393 $(MH)^-$, t_R = 3.07 min; HPLC: t_R = 4.02 min (100%; 254 nm).

3-{3-[(Biphenyl-4-sulfonyl)-methyl-amino]-phenyl}-*N*-hydroxy-acrylamide (1l). White solid (4.5 mg, 8%). IR ν_{max}/cm^{-1}: 1656 (C=O), 1604 (C=O), 1596 (C=C), 1346 (SO_2), 1163 (SO_2); HRMS: $C_{22}H_{20}N_2O_4S$ requires $(MH)^+$ 409.1222, found 409.1234; ^{1}H NMR (500 MHz, CD_3OD): δ 7.85 (d, 2H, J = 8 Hz), 7.66 (d, 2H, J = 8 Hz), 7.60 (d, 2H, J = 8 Hz), 7.52–7.41 (m, 4H), 7.38–7.32 (m, 3H), 7.12 (d, 1H, J = 8 Hz), 6.42 (d, 1H, J = 16 Hz), 3.25 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.0, 147.3, 143.7, 140.6, 137.2, 136.2, 130.6, 130.2, 129.7, 129.6, 128.7, 128.5, 128.3, 127.8, 127.0, 119.6, 38.6; LC-MS: (ESI −ve) m/z 407 $(MH)^-$, t_R = 3.19 min; HPLC: t_R = 5.12 min (100%; 254 nm).

3-[4-(4-Chloro-benzenesulfonylamino)-phenyl]-*N*-hydroxy-acrylamide (1m). White solid (4.4 mg, 10%). IR ν_{max}/cm^{-1}: 1654 (C=O), 1599 (C=C), 1337 (SO_2), 1162 (SO_2); HRMS: $C_{15}H_{13}N_2O_4ClS$ requires $(MH)^+$ 353.0363, found 353.0363; ^{1}H NMR (500 MHz, CD_3OD): δ 7.74 (d, 2H, J = 8 Hz), 7.52–7.39 (m, 5H), 7.1 (d, 2H, J = 8 Hz), 6.34 (d, 1H, J = 16 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ 166.4, 140.7, 140.3, 140.2, 139.8, 132.6, 130.4, 129.9, 129.8, 121.9, 117.8; LC-MS: (electrospray −ve) m/z 351 $(MH)^-$, t_R = 2.76 min; HPLC: t_R = 3.98 min (95%; 254 nm).

3-{4-[(4-Chloro-benzenesulfonyl)-methyl-amino]-phenyl}-*N*-hydroxy-acrylamide (1n). Colourless gum (8.7 mg, 18%). IR ν_{max}/cm^{-1}: 1659 (C=O), 1625 (C=O), 1604 (C=C), 1352 (SO_2), 1173 (SO_2); HRMS: $C_{16}H_{15}ClN_2O_4S$ requires $(MH)^+$ 367.0519, found 367.0532; ^{1}H NMR (500 MHz, CD_3OD): δ 7.56–7.49 (m, 7H), 7.16 (d, 2H, J = 9 Hz), 6.46 (d, 1H, J = 16 Hz), 3.19 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.1, 143.8, 140.7, 136.3, 135.4, 130.5, 130.4, 129.4, 127.9, 119.4, 38.5; LC-MS: (ESI −ve) m/z 365 $(MH)^-$, t_R = 2.91 min; HPLC: t_R = 4.48 min (100%; 254 nm).

3-[3-(4-Chloro-benzenesulfonylamino)-phenyl]-*N*-hydroxy-acrylamide (1o). Colourless gum (9.8 mg, 21%). IR ν_{max}/cm^{-1}: 1661 (C=O), 1590 (C=C), 1338 (SO_2), 1153 (SO_2); HRMS: $C_{15}H_{13}N_2O_4ClS$ requires $(MH)^+$ 353.0363, found 353.0360; ^{1}H NMR (500 MHz, CD_3OD): δ 7.72 (d, 2H, J = 8 Hz), 7.54–7.41 (m, 3H), 7.32–7.22 (m, 3H), 7.05 (m, 1H), 6.40 (d, 1H, J = 16 Hz); ^{13}C (125 MHz, CD_3OD): δ 166.0, 140.8, 140.3, 139.7, 139.5, 137.4, 130.9, 130.4, 129.9, 125.3, 123.5, 121.2, 119.4; LC-MS: (ESI −ve) m/z 351 $(MH)^-$, t_R = 2.80 min; HPLC: t_R = 3.98 min (100%; 254 nm).

3-{3-[(4-Chloro-benzenesulfonyl)-methyl-amino]-phenyl}-*N*-hydroxy-acrylamide (1p). White solid (9.7 mg, 20%). IR ν_{max}/cm^{-1}: 1660 (C=O), 1628 (C=O), 1602 (C=C), 1343 (SO_2), 1164 (SO_2); HRMS: $C_{16}H_{15}ClN_2O_4S$ requires $(MH)^+$ 367.0519, found 367.0526; ^{1}H NMR (125 MHz, CD_3OD): δ 7.52–7.46 (m, 6H), 7.35 (m, 2H), 7.08 (dd, 1H, J = 1.5, 9 Hz), 6.43 (d, 1H, J = 16 Hz), 3.20 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.0, 143.4, 140.7, 140.5, 137.3, 136.4, 130.6, 130.4, 128.6, 127.8, 127.1, 119.7, 38.6; LC-MS: (ESI −ve) m/z 365 $(MH)^-$, t_R = 2.89 min; HPLC: t_R = 4.45 min (100%; 254 nm).

3-[4-(3,4-Dimethoxy-benzenesulfonylamino)-phenyl]-*N*-hydroxy-acrylamide (1q). Colourless gum (11 mg, 23%). IR ν_{max}/cm^{-1}: 1665 (C=O), 1603 (C=C), 1508 (Ar–H), 1332 (SO_2), 1262 (C–O), 1136 (SO_2); HRMS: $C_{17}H_{18}N_2O_6S$ requires $(MH)^+$ 379.0964, found 379.0975; ^{1}H NMR (500 MHz, CD_3OD): δ 7.45–7.35 (m, 4H), 7.24 (s, 1H), 7.12 (d, 2H, J = 8 Hz), 6.97 (d, 1H, J = 8 Hz), 6.34 (d, 1H, J = 16 Hz), 3.82 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): 166.7, 154.7, 150.6, 141.2, 132.8, 132.7, 130.1, 122.8, 122.2, 118.0, 112.3, 114, 57.0 (×2); LC-MS: (ESI −ve) m/z 377 $(MH)^-$, t_R = 2.46 min; HPLC: t_R = 3.34 min (95%; 254 nm).

3-{4-[(3,4-Dimethoxy-benzenesulfonyl)-methyl-amino]-phenyl}-*N*-hydroxy-acrylamide (1r). Colourless gum (8.8 mg, 17%). HRMS: $C_{18}H_{20}N_2O_6S$ requires $(MH)^+$ 393.1120, found 393.1137; ^{1}H NMR (CD_3OD): δ 7.56–7.49 (m, 3H), 7.19 (dd, 1H, J = 8, 1 Hz), 7.17 (d, 2H, J = 8 Hz), 7.05 (d, 1H, J = 8 Hz), 6.83 (d, 1H, J = 1 Hz), 6.45 (d, 1H, J = 16 Hz), 3.88 (s, 3H), 3.65 (s, 3H), 3.15 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.1, 154.7, 150.3, 144.4, 140.4, 135.1, 129.1, 128.9, 127.8, 123.0, 119.2, 112.0, 111.7, 56.6, 56.4, 38.2; LC-MS: (ESI −ve) m/z 391 $(MH)^-$, t_R = 2.62 min; HPLC: t_R = 3.83 min (100%; 254 nm).

3-[3-(3,4-Dimethoxy-benzenesulfonylamino)-phenyl]-*N*-hydroxy-acrylamide (1s). White solid (12 mg, 24%). IR ν_{max}/cm^{-1}: 1658 (C=O), 1588 (C=C), 1509 (Ar–H), 1332 (SO_2), 1264 (C–O), 1140 (SO_2); HRMS: $C_{17}H_{18}N_2O_6S$ requires $(MH)^+$ 379.0964, found 379.0977; ^{1}H NMR (500 MHz, CD_3OD): δ 7.46 (d, 1H, J = 16 Hz), 7.38–7.28 (m, 2H), 7.26–7.19 (m, 3H), 7.06 (m, 1H), 6.97 (d, 1H, J = 8 Hz), 6.40 (d, 1H, J = 16 Hz), 3.84 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.5, 154.7, 150.6, 141.3, 140.4, 137.6, 132.6, 131.1, 125.4, 123.9, 122.8, 121.5, 119.6, 112.3, 111.4, 57.0 (×2); LC-MS: (ESI −ve) m/z 377 $(MH)^-$, t_R = 2.50 min; HPLC: t_R = 3.43 min (95%; 254 nm).

3-{3-[(3,4-Dimethoxy-benzenesulfonyl)-methyl-amino]-phenyl}-*N*-hydroxy-acrylamide (1t). White solid (9.8 mg, 19%). IR ν_{max}/cm^{-1}: 1662 (C=O), 1625 (C=O), 1588 (C=C), 1508 (Ar–H), 1347 (SO_2), 1261 (C–O), 1137 (SO_2); HRMS: $C_{18}H_{20}N_2O_6S$ requires $(MH)^+$ 393.1120, found 393.1129; ^{1}H NMR (500 MHz, CD_3OD): δ 7.53–7.46 (m, 2H), 7.37–7.33 (m, 2H),

7.21 (dd, 1H, J = 1, 8 Hz), 7.11 (d, 1H, J = 8 Hz), 7.06 (d, 1H, J = 8 Hz), 6.83 (d, 1H, J = 1 Hz), 6.43 (d, 1H, J = 16 Hz), 3.88 (s, 3H), 3.65 (s, 3H), 3.16 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 165.9, 154.7, 150.3, 143.9, 140.6, 137.1, 130.5, 128.8, 128.7, 127.7, 126.9, 123.1, 119.5, 112.0, 111.8, 56.7, 56.5, 38.4; LC-MS: (ESI −ve) m/z 391 $(MH)^-$, t_R = 2.63 min; HPLC: t_R = 3.78 min (100%; 254 nm).

***N*-Hydroxy-3-[4-(thiophene-2-sulfonylamino)-phenyl]-acrylamide (1u).** Colourless gum (7.8 mg, 18%). IR ν_{max}/cm^{-1}: 1655 (C=O), 1604 (C=C), 1336 (SO_2), 1151 (SO_2); HRMS: $C_{13}H_{12}N_2O_4S$ requires $(MH)^+$ 325.0317, found 325.0332; ^{1}H NMR (500 MHz, CD_3OD): δ 7.70 (dd, 1H, J = 5, 1.5 Hz), 7.52 (dd, 1H, J = 4, 1.5 Hz), 7.50–7.42 (m, 3H), 7.17 (d, 2H, J = 8 Hz), 7.06 (dd, 1H, J = 5, 4 Hz), 6.38 (d, 1H, J = 16 Hz); LC-MS: (ESI −ve) m/z 323 $(MH)^-$, t_R = 2.48 min; HPLC: t_R = 3.64 min (100%; 254 nm).

***N*-Hydroxy-3-{4-[methyl-(thiophene-2-sulfonyl)-amino]-phenyl}-acrylamide (1v).** White foam (8.4 mg, 19%). IR ν_{max}/cm^{-1}: 1655 (C=O), 1602 (C=C), 1349 (SO_2), 1148 (SO_2); HRMS: $C_{14}H_{14}N_2O_4S_2$ requires $(MH)^+$ 339.0473, found 339.0473; ^{1}H NMR (CD_3OD): δ 7.80 (dd, 1H, J = 5, 1.5 Hz), 7.56–7.49 (m, 3H), 7.38 (dd, 1H, J = 4, 1.5 Hz), 7.18 (d, 2H, J = 8 Hz), 7.15 (m, 1H), 6.39 (d, 1H, J = 16 Hz), 3.24 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 166.1, 143.8, 140.4, 137.4, 135.3, 134.3, 134.2, 129.2, 128.7, 127.9, 119.2, 38.5; LC-MS: (ESI −ve) m/z 337 $(MH)^-$, t_R = 2.67 min; HPLC: t_R = 3.88 min (100%; 254 nm).

***N*-Hydroxy-3-[3-(thiophene-2-sulfonylamino)-phenyl]-acrylamide (1w).** Colourless gum (8.9 mg, 21%). IR ν_{max}/cm^{-1}: 1654 (C=O), 1615 (C=O), 1604 (C=C), 1326 (SO_2), 1147 (SO_2); HRMS: $C_{13}H_{12}N_2O_4S$ requires $(MH)^+$ 325.0317, found 325.0323; ^{1}H NMR (500 MHz, CD_3OD): δ 7.70 (dd, 1H, J = 5, 1.5 Hz), 7.50 (dd, 1H, J = 4, 1.5 Hz), 7.46 (d, 1H, J = 16 Hz), 7.34 (br s, 1H), 7.30–7.28 (m, 2H), 7.12 (m, 1H), 7.04 (dd, 1H, J = 5, 4 Hz) 6.40 (d, 1H, J = 16 Hz); LC-MS: (ESI −ve) m/z 323 $(MH)^-$, t_R = 2.51 min; HPLC: t_R = 3.50 min (100%; 254 nm).

***N*-Hydroxy-3-{3-[methyl-(thiophene-2-sulfonyl)-amino]-phenyl}-acrylamide (1x).** Colourless gum (7.3 mg, 17%). IR ν_{max}/cm^{-1}: 1662 (C=O), 1625 (C=O), 1351 (SO_2), 1165 (SO_2); HRMS: $C_{14}H_{14}N_2O_4S_2$ requires $(MH)^+$ 339.0473, found 339.0467; ^{1}H NMR (500 MHz, CD_3OD): δ 7.82 (dd, 1H, J = 5, 1.5 Hz), 7.55–7.46 (m, 3H), 7.22–7.08 (m, 4H), 6.42 (d, 1H, J = 16 Hz), 3.25 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 165.9, 143.4, 140.5, 134.3, 134.2, 130.6, 128.8, 128.7, 128.6, 128.0, 126.9, 119.6, 38.6; LC-MS: (ESI −ve) m/z 337 $(MH)^-$, t_R = 2.65 min; HPLC: t_R = 3.84 min (100%; 254 nm).

Biology

***In vitro* HDAC enzyme assay.** The enzyme inhibitory activity of TSA analogues was measured using a HDAC colorimetric assay/Drug discovery kit.[30] Test compounds were dissolved in DMSO and diluted with assay buffer to a final concentration of 1 μM. HeLa cell nuclear extract (enzyme) was diluted to a final concentration of 7 mg ml^{-1}. 10 μl HDAC buffer, 10 μl diluted compound, 5 μl HeLa extract and 25 μl Color de Lys™ substrate were added in the above order to each well of a microtitre plate and the plate was incubated at room temperature for 30 min. The reaction was stopped by the addition of 50 μl of developer and incubating at 37 °C for 20 min. Deacetylated substrate was measured by reading optical density at 405 nm and the amount present in μM was calculated from a standard curve to give a measure of enzyme inhibition.

HUVEC proliferation assay. HUVECs (human umbilical vein endothelial cells) were cultured in Medium 199, containing L-glutamine, Earle's salts and $NaHCO_3$ (Sigma), and supplemented with 10% foetal bovine serum (FBS) (Invitrogen), 1% penicillin/streptomycin, 30 μg ml^{-1} endothelial cell growth supplement (ECGS) from bovine neural tissue and 90 μg ml^{-1} bovine intestinal mucosal heparin (Sigma). The cells were seeded in tissue culture-ware coated with 1.5% gelatin. HUVECs were used between passage 2 and 7. For the proliferation assay, four thousand cells were plated on gelatin-coated 96-well plates in 1% FBS-containing medium and allowed to adhere overnight. Serial dilutions of test compounds were added and cells were incubated at 37 °C, 5% CO_2 in a humidified incubator. Cell number was determined using the CellTiter 96® AQ_{ueous} One Solution Reagent (Promega) at the end of 48 h by reading optical density/absorbance at 492 nm.

AngioKit tube-formation assay. The AngioKit was purchased from TCS Cellworks.[27] Test compounds were dissolved in DMSO and diluted in the optimised medium provided with the kit such that the final DMSO concentration was always below 0.1%. The medium was replaced on day 1, 4, 7, and 9 with fresh medium containing appropriate compounds. Suramin (2 μM) containing medium provided a negative control, and medium without any compound was used as blank. Tubules were visualized at the end of 11 days by immunostaining with CD31/PECAM-1 antibodies (TCS). Primary antibody was diluted 1 : 4000 and secondary, goat anti-mouse IgG alkaline phosphatase conjugated antibody was diluted 1 : 500. Colour was developed using BCIP/NBT (Sigma) and images captured with a Nikon Diaphot microscope and analysed using AngioSys™ image analysis software.

Statistics. All assays were performed in triplicate. For the HDAC enzyme inhibition assay, an average of three OD readings at 405 nm were converted to the amount of deacetylated substrate remaining, reading off from a pre-determined calibration curve. The HUVEC proliferation assay was performed in triplicate and statistical significance was calculated using ANOVA followed by Tukey's test. For the AngioKit assay 8 image fields were captured and the mean tubule area was calculated. Values were considered statistically significant at $P \leq 0.05$.

Acknowledgements

We thank GlaxoSmithKline for generous financial support of this work, The Cambridge Commonwealth Trust and the Department of Pharmacology for funding to AB. Gabrielle Bradfield and Colin Chan are thanked for assistance with the biological screening.

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21 In this case, the most successful of a series of trial fibrous Pd catalysts kindly provided for evaluation by Johnson Matthey was FibreCat™ 1001 (2.71 mol% Pd) which gave **11** and **6** in 64% and 12% yield respectively (www.chemicals.matthey.com).

22 This study was performed using a ReactArray™ SK233 workstation (www.reactarray.com) connected to an Agilent HP1100 HPLC (www.chem.agilent.com).

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27 AngioKit was obtained from TCS Cellworks (www.tcscellworks.co.uk).

28 Greenhouse™ parallel reaction block commercially available from Radleys Discovery Technology catalogue no: RR99600 (www.radleys.co.uk).

29 Laboratory shaker used was an IKA Orbital shaker KS 125 basic with a VX2 test tube attachment. Commercially available from IKA, part numbers 2476000 and 0568900 respectively.

30 HDAc colorimetric activity assay kit AK-501 was obtained from BIOMOL® Research Laboratories Inc, USA (www.biomol.com).