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Synthesis and Biological Evaluation of *N*-Hydroxyphenylacrylamides and *N*-Hydroxypyridin-2-ylacrylamides as Novel Histone Deacetylase Inhibitors

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The histone deacetylases (HDACs) are able to regulate gene expression, and histone deacetylase inhibitors (HDACi) emerged as a new class of agents in the treatment of cancer as well as other human disorders such as neurodegenerative diseases. In the present investigation, we report on the synthesis and biological evaluation of compounds derived from the expansion of a HDAC inhibitor scaffold having *N*-hydroxy-3-phenyl-2-propenamide and *N*-hydroxy-3-(pyridin-2-yl)-2-propenamide as core structures and containing a phenyloxopropenyl moiety, either unsubstituted or substituted by a 4-methylpiperazin-1-yl or 4-methylpiperazin-1-ylmethyl group. The compounds were evaluated for their ability to inhibit nuclear HDACs, as well as for their in vitro antiproliferative activity. Moreover, their metabolic stability in microsomes and aqueous solubility were studied and selected compounds were further characterized by in vivo pharmacokinetic experiments. These compounds showed a remarkable stability in vivo, compared to hydroxamic acid HDAC inhibitors that have already entered clinical trials. The representative compound **30b** showed in vivo antitumor activity in a human colon carcinoma xenograft model.

Introduction

Since the original observation that reversible post-translational modification of histones, such as acetylation or methylation, is linked to transcriptional regulation, the biological function of these modifications has aroused considerable interest.[1] Later it was found that hyperacetylated histone cores are generally allocated in transcriptionally active genes, whereas hypoacetylated histones are in transcriptionally silent regions of the genome.[2] Acetylation occurs at lysine residues on the amino-terminal tails of the histones, which leads to neutralization of the positive charge of the histone tails and thus to a decreased affinity for DNA.[3] The acetylation status of the lysine residues is tightly controlled by two counteracting enzyme families: the histone acetyltransferases (HAT[a]) and the histone deacetylases (HDAC). Up to now, there are 18 known human histone deacetylases, categorized into four classes based on the structure. Classes I (HDACs 1−3 and 8), II (HDACs 4−7, 9, and 10), and IV (HDAC 11) enzymes are Zn^{2+}-dependent enzymes and are the so-called "classical" HDACs, while class III enzymes (also known as sirtuins) are defined by their dependency on NAD^+.[4] The acetylation status of histones is crucial in modulating gene expression and cell fate, and its dysregulation is involved in the development of several cancers. Histone hyperacetylation leads to transcriptional activation of suppressed genes, some of them being associated with cell cycle arrest, differentiation, or apoptosis in tumor cells. Recent studies have shown that acetylation of non-histone proteins is also relevant for tumorigenesis, cancer cell proliferation, and immune functions.[5] Thus, inhibition of HDACs has emerged as a novel therapeutic strategy to reverse aberrant epigenetic changes associated with cancer.[6−10] After the discovery that exposure of cultured cells to sodium *n*-butyrate caused a reversible accumulation of highly acetylated histones,[11] several classes of HDAC inhibitors have been found demonstrating potent anticancer activities in preclinical studies. These include short chain fatty acids, hydroxamic acids, cyclic tetrapeptides/depsipeptides, ketones, and benzamides. In October 2006 SAHA (suberoylanilide hydroxamic acid) **1**[12−14] gained approval by the FDA as the first HDAC inhibitor for the treatment of cutaneous T-cell lymphoma, and various agents are currently at different stages of clinical development. Other examples of inhibitors currently being evaluated in clinical trials are the hydroxamic acids panobinostat or LBH589 **2** (Novartis, phase III),[15] PXD101 or belinostat **3** (Topotarget, phase III),[16] ITF-2357

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[a] Abbreviations: HDAC, histone deacetylases; HAT, histone acetyltransferases; K562, chronic myelogenous leukemia cell line; HCT-116, human colorectal carcinoma cell line; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PK, pharmacokinetics.

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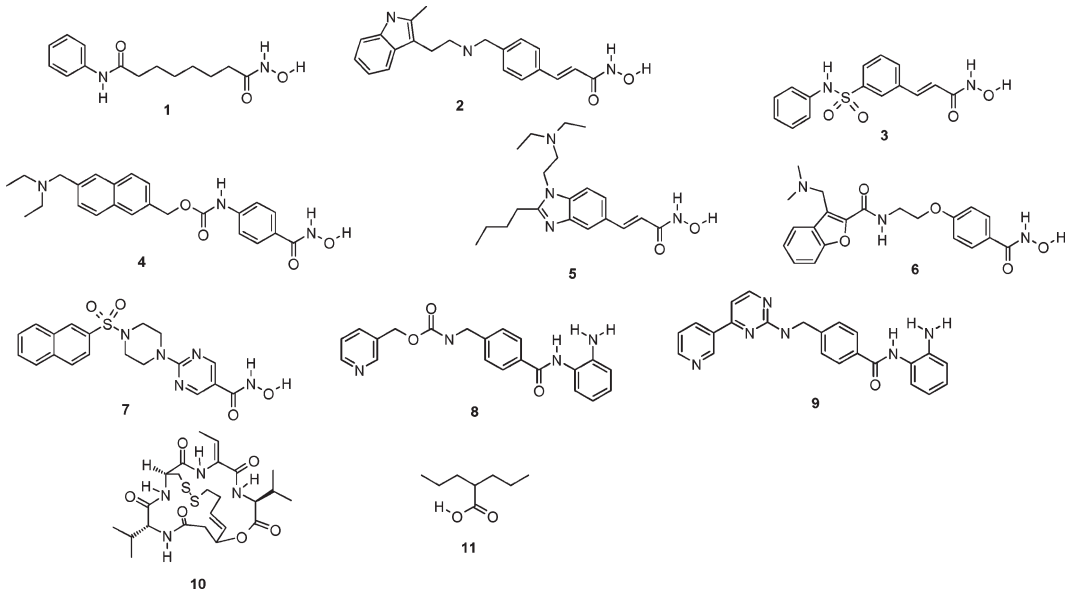


Figure 1. HDAC inhibitors in clinical development.

4 (Italfarmaco, phase II),[17] SB-939 **5** (S*BIO, phase I),[18] PCI-24781 **6** (Pharmacyclics, phase I),[19] and JNJ-16241199 **7** (J&J, phase I),[20] the benzamides SNDX-275 or MS-275 **8** (Syndax, phase II)[21] and MGCD-0103 **9** (Methylgene, phase II),[22] the cyclic depsipeptide romidepsin or FK228 **10** (Gloucester, phase II),[23] and valproic acid **11** (Topotarget, phase II)[24] (see Figure 1).

So far, given their potency, hydroxamic acid derivatives are among the most promising candidates for HDAC inhibition. However, several compounds have been shown to have short half-lives in vitro as well as in vivo. Nevertheless, the compounds showed in vivo activity. As a consequence, a "hit-and-run" mechanism has been suggested whereby the transient and short exposure to relatively high concentrations of the drug might be sufficient for an antitumor effect in vivo.[25,26]

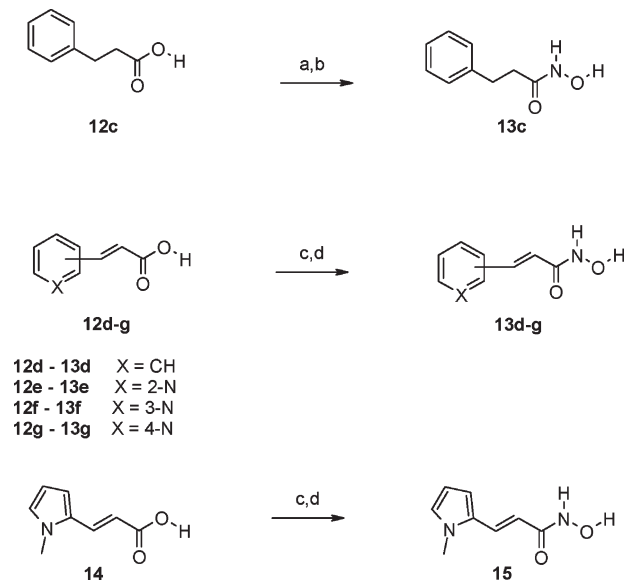
In the present study, we report the synthesis and biological characterization of novel HDAC inhibitors using as starting points *N*-hydroxy-3-phenyl-2-propenamide and *N*-hydroxy-3-(pyridin-2-yl)-2-propenamide, selected among a small series of aryl- and heteroarylhydroxyamide fragments. Their phenyloxopropenyl derivatives, unsubstituted or substituted with different 4-methylpiperazin-1-yl or 4-methylpiperazin-1-ylmethyl moieties, were prepared with the goal of synthesizing novel, highly potent compounds with improved pharmacokinetic properties.

Chemistry

N-Hydroxybenzamide (**13a**) is commercially available, and *N*-hydroxy-2-phenylacetamide (**13b**)[27] was prepared according to the literature. As shown in Scheme 1, *N*-hydroxy-3-phenylpropionamide (**13c**) was prepared by reacting 3-phenylpropionic acid (**12c**) with NH_2OBn (*O*-benzylhydroxylamine) in the presence of EDC (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride) and HOBt (*N*-hydroxybenzotriazole). The benzyl group was then cleaved by hydrogenation with 10% Pd−C and ammonium formate.

Phenylacrylic acid (**12d**) is commercially available; the pyridylacrylic acids **12e**−**g**[28] and the pyrrolylacrylic acid **14**[29] were prepared according to the literature. The acrylic acids **12d**−**g** and **14** reacted with NH_2OTHP (*O*-(tetrahydropyran-2-yl)hydroxylamine) in the presence of EDC and HOBt as coupling agents. Cleavage of the protecting group with hydrogen chloride (HCl) afforded the desired hydroxamates **13d**−**g** and **15**.

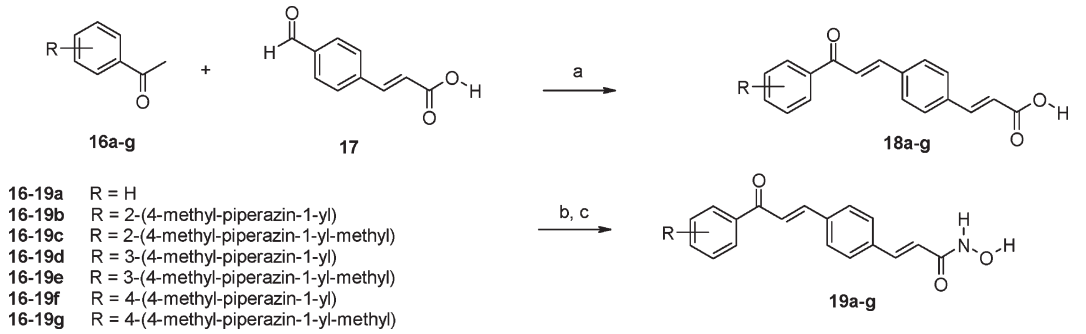
Scheme 1[a]



[a] Reagents and conditions: (a) CH_2Cl_2, EDC, HOBT, NH_2OBn, room temp; (b) MeOH, Pd/C, NH_4HCO_2; (c) CH_2Cl_2, EDC, HOBT, TEA, NH_2OTHP, room temp; (d) CH_2Cl_2, Et_2O, HCl, room temp.

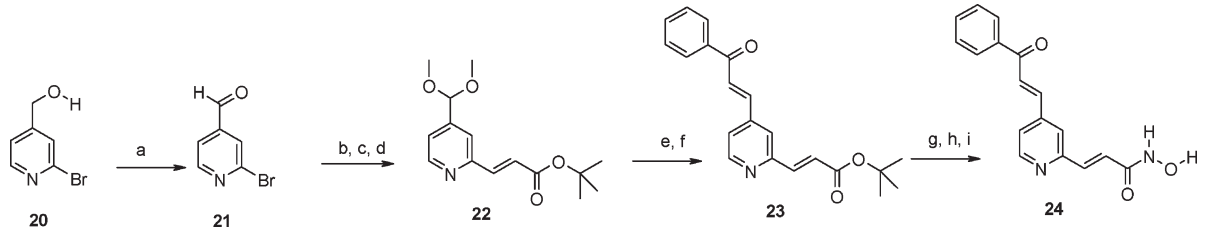
As shown in Scheme 2, condensation of (un)substituted acetophenones **16a**−**g** (see Supporting Information) with commercially available (4-formylphenyl)acrylic acid (**17**) was carried out in ethanol in the presence of aqueous KOH. The acrylic acid **18a** was then treated with ethyl chloroformate and triethylamine, followed by the addition of *O*-(2-methoxy-2-propyl)hydroxylamine.[30] Acidic workup in the presence of the Amberlyst 15 ion-exchange resin furnished the desired hydroxamic acid **19a**. The conversion of the acid intermediates **18b**−**g** into the hydroxamic acids **19b**−**g** was carried out according to the procedures described in Scheme 1 for the preparation of compounds **13d**−**g**.

Scheme 2[a]



[a] Reagents and conditions: (a) EtOH, KOH, room temp; (b) THF, DMF, EDC, HOBT, TEA, NH_2OTHP, room temp, or (for **19a**) $ClCOOC_2H_5$, TEA, THF, 0 °C, and then $NH_2OC(CH_3)_2OCH_3$; (c) CH_2Cl_2, Et_2O, HCl, room temp, or (for **19a**) Amberlyst 15, MeOH, 45 °C.

Scheme 3[a]



[a] Reagents and conditions: (a) CH_2Cl_2, DMSO, TEA, $(COCl)_2$, −60 °C; (b) MeOH, TMOF, PTSA, room temp; (c) THF, *i*-PrMgCl, then DMF, room temp; (d) THF, NaH, $(EtO)_2P(O)CH_2COO^tBu$, room temp; (e) THF, HCl, room temp to 80 °C; (f) **16a**, THF, KOH, room temp; (g) CH_2Cl_2, TFA, room temp; (h) CH_2Cl_2 EDC, HOBT, TEA, NH_2OTHP, room temp; (i) CH_2Cl_2, Et_2O, HCl, room temp.

Commercially available (2-bromopyridin-4-yl)methanol (**20**) was oxidized through Swern oxidation (Scheme 3). After protection of the carbaldehyde **21** with trimethyl orthoformate in the presence of *p*-toluenesulfonic acid, the compound was first treated with isopropylmagnesium chloride (*i*-PrMgCl), then quenched with DMF and finally treated with *tert*-butyl diethyl phosphonacetate according to Horner−Emmons reaction forming the intermediate **22**. After cleavage of the dimethoxy group with HCl in THF the carbaldehyde was treated with acetophenone **16a**. The reaction was carried out in THF instead of ethanol, since Michael addition of acetophenone on the double bond had been observed as side reaction in ethanol (9:1 ratio between the bis-acetophenone adduct and the desired *tert*-butyl ester **23** was found in the reaction mixture). Removal of the *tert*-butyl group with trifluoroacetic acid (TFA) in dichloromethane and introduction of the hydroxamic acid moiety according to the procedure described above in Scheme 1 for compounds **13d**−**g** afforded compound **24**.

Commercially available 6-bromopyridine-3-carbaldehyde (**25**) was protected with trimethyl orthoformate, then treated with *i*-PrMgCl followed by DMF to give 5-dimethoxymethylpyridine-2-carbaldehyde (**26**) (Scheme 4). Horner−Emmons olefination of **26** and cleavage of the dimethoxy group gave the formylpyridinyl ester **27**. Condensation of (un)substituted acetophenones **16a**−**g** with intermediate **27** was carried out in MeOH, EtOH, or THF in the presence of aqueous KOH as outlined in Schemes 2 and 3. Hydrolysis of the *tert*-butyl ester followed by EDC coupling with NH_2OTHP and final cleavage of the tetrahydropyranyl protecting group furnished the hydroxamic acid derivatives **30a**−**g**.

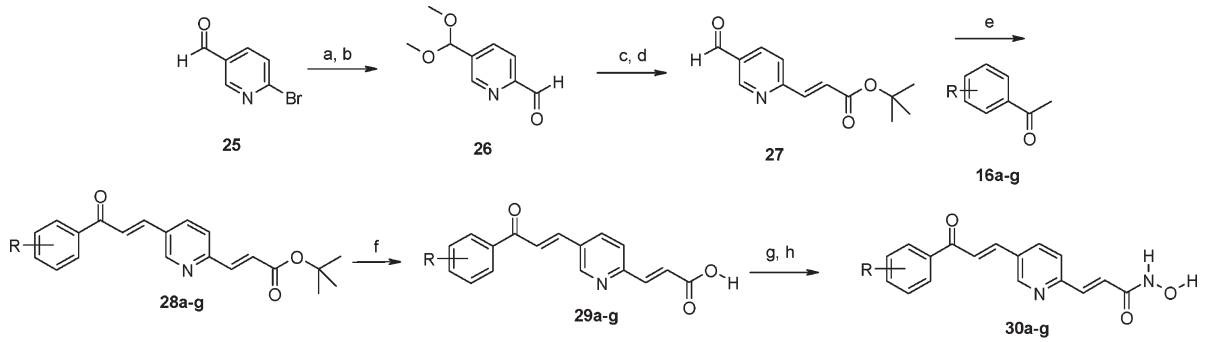
The meta-substituted *tert*-butyl acrylate **32a** was prepared starting from the commercially available 3-bromobenzaldehyde **31a** according to the Heck reaction in the presence of palladium acetate [$Pd(OAc)_2$], PPh_3, triethylamine, and $NaHCO_3$. Different coupling conditions (1,4-diazabicyclo[2.2.2]-octane (DABCO), K_2CO_3, tetrabutylammonium bromide (n-Bu_4NBr), and $Pd(OAc)_2$ under microwave irradiation) were used to prepare the *tert*-butyl acrylate **32b** from 6-bromopyridine-2-carbaldehyde (**31b**) (Scheme 5). Subsequent condensation of these intermediates with acetophenone derivatives (**16a**−**g**), cleavage of the *tert*-butyl ester group, and conversion to the hydroxamic acid afforded **35a**−**n**.

Biological Results and Discussion

The hydroxamic acid derivatives were profiled using a commercially available HDAC assay kit, with HeLa cell nuclear extracts as enzyme source and a fluorogenic acetylated histone peptide fragment (Fluor de Lys) as a substrate. The results are expressed either as the percentage of inhibition at a fixed dose or as IC_{50} (50% inhibitory concentration) values. The antiproliferative activity of the prepared compounds against the chronic myelogenous leukemia cell line K562 was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Furthermore, the ability of selected compounds to inhibit cellular HDACs was evaluated following the modification of the basal level of acetylation in the above-reported cell line.[31] A microsomal stability assay was performed as further characterization. Compounds were exposed to mouse and human microsomes at 37 °C, and the percentage of the unmetabolized compound after 30 min of incubation was established.

Initially, a small series of aryl- and heteroarylhydroxamic acid derivatives were synthesized and tested to identify the best minimal scaffold endowed with HDAC inhibitory activity. As shown in Table 1, **13a** and **13b** were demonstrated to be inactive under the experimental conditions used, whereas **13c** showed a moderate biochemical activity with 36% enzyme

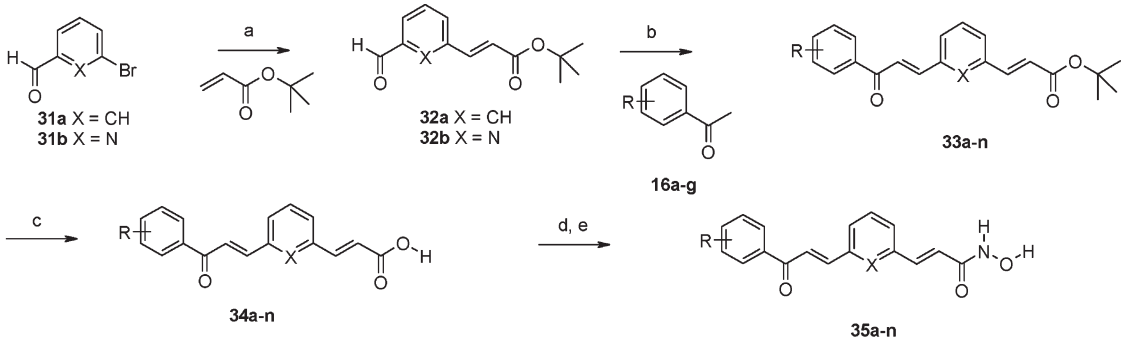
Scheme 4[a]



28-30a R = H
28-30b R = 2-(4-methyl-piperazin-1-yl)
28-30c R = 2-(4-methyl-piperazin-1-yl-methyl)
28-30d R = 3-(4-methyl-piperazin-1-yl)
28-30e R = 3-(4-methyl-piperazin-1-yl-methyl)
28-30f R = 4-(4-methyl-piperazin-1-yl)
28-30g R = 4-(4-methyl-piperazin-1-yl-methyl)

[a] Reagents and conditions: (a) MeOH, TMOF, PTSA, room temp; (b) THF, *i*-PrMgCl, then DMF, room temp; (c) THF, NaH, $(EtO)_2P(O)$-CH_2COO^tBu, room temp; (d) THF, HCl, room temp; (e) EtOH or THF or MeOH, KOH, room temp or 0 °C; (f) CH_2Cl_2, TFA, room temp; (g) CH_2Cl_2, THF, DMF, EDC, HOBT, TEA, NH_2OTHP, room temp; (h) CH_2Cl_2, Et_2O, HCl, room temp.

Scheme 5[a]



33-35a X = CH; R = H
33-35b X = CH; R = 2-(4-methyl-piperazin-1-yl)
33-35c X = CH; R = 2-(4-methyl-piperazin-1-yl-methyl)
33-35d X = CH; R = 3-(4-methyl-piperazin-1-yl)
33-35e X = CH; R = 3-(4-methyl-piperazin-1-yl-methyl)
33-35f X = CH; R = 4-(4-methyl-piperazin-1-yl)
33-35g X = CH; R = 4-(4-methyl-piperazin-1-yl-methyl)

33-35h X = N; R = H
33-35i X = N; R = 2-(4-methyl-piperazin-1-yl)
33-35j X = N; R = 2-(4-methyl-piperazin-1-yl-methyl)
33-35k X = N; R = 3-(4-methyl-piperazin-1-yl)
33-35l X = N; R = 3-(4-methyl-piperazin-1-yl-methyl)
33-35m X = N; R = 4-(4-methyl-piperazin-1-yl)
33-35n X = N; R = 4-(4-methyl-piperazin-1-yl-methyl)

[a] Reagents and conditions: (a) for **32a** DMF, TEA, PPh_3, $NaHCO_3$, $Pd(OAc)_2$, 100 °C, or (for **32b**) DMF, DABCO, K_2CO_3, Bu_4NBr, $Pd(OAc)_2$, 120 °C, microwave; (b) EtOH or THF, KOH, room temp or 0 °C; (c) CH_2Cl_2, TFA, room temp; (d) CH_2Cl_2, THF, DMF, EDC, HOBT, TEA, NH_2OTHP, room temp, or (for **35a**) $ClCOOC_2H_5$, TEA, THF, 0 °C, and then $NH_2OC(CH_3)_2OCH_3$; (e) CH_2Cl_2, Et_2O, HCl, room temp, or (for **35a**) Amberlyst 15, MeOH, 45 °C.

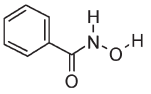
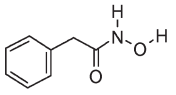
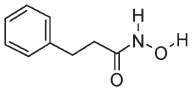
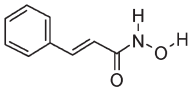
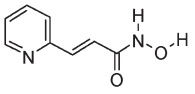
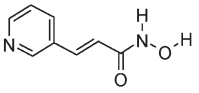
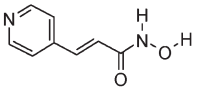
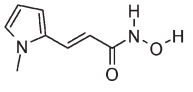
inhibition at 1 μM. Introduction of an acrylic group (**13d**) led to a further increase in activity. Thus, pyrrolyl- and pyridinylacryl hydroxamates were prepared and tested. The pyridine-2-yl derivative **13e** was demonstrated to be the most potent compound among this series, whereas the electron-rich pyrrolyl derivative **15** was less active than the other acryl hydroxamates.

The heteraryl hydroxamate **13e** and the aryl hydroxamate **13d** were selected for further expansion in the first step by preparing scaffolds with a phenyloxopropenyl moiety, which is present in previously reported structures.[32] The HDAC inhibitory activity of the meta phenyl analogue **35a**, with an IC_{50} value of 0.085 μM, was substantially higher than that of the corresponding para derivative **19a** (1.48 μM). This trend did not emerge so clearly in the pyridinyl series, as the 2,5 disubstituted derivative **30a** and the 2,6 disubstituted analogue **35h** showed similar IC_{50} values (~0.02 μM) and are around 15 times more active than the pyridinyl derivative **24**, substituted at the 2 and 4 positions (see Table 2).

Generally, the pyridinyl derivatives showed a better antiproliferative activity than the corresponding phenyl analogues. In detail, the cellular IC_{50} values for **30a**, **35h**, and **24** were between 0.5 and 1 μM, whereas **19a** and **35a** exhibited IC_{50} values of 2.3 and 3.2 μM, respectively.

All five compounds showed a limited stability in mouse microsomes. After 30 min of incubation at 37 °C, the percentage of unaltered compounds was below 20%. Similar data were obtained in human microsomes with the exception of **35h**, which was found to be more stable than the other inhibitors. Furthermore, the compounds showed a limited solubility in the screening buffer (Table 2).

Table 1. HDAC Inhibitory Activity of the Minimal Scaffold[a]

comp		% inhibition at 0.1 μM	% inhibition at 1 μM	% inhibition at 10 μM
13a		0	1	19
13b		0	0	0
13c		0	36	89
13d		18	55	93
13e		34	82	100
13f		13	75	96
13g		0	34	87
15		9	13	53

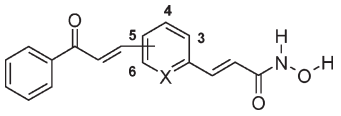
[a] Values are the mean of three experiments. The standard derivations are < 20% of the mean. The numbers represent the percentage of inhibition of HDAC enzymes (HeLa nuclear extract) at 0.1, 1.0, and 10 μM concentration of the inhibitors.

On the basis of the observations reported above, subsequent optimization was directed to obtain compounds with an improved metabolic stability and solubility. As previously mentioned, **24** and the precursor **23** were shown to be reactive and THF had to be used as solvent in place of ethanol in the aldol reaction of **16a** with **22**. Electrophilic reactivity is often associated with in vivo toxicity;[33,34] therefore, no further structures related to compound **24** were synthesized. Prompted by the reactivity of compound **24**, the electrophilic behavior of compounds **19a**, **30a**, **35a**, and **35h** was also examined in more detail. By adaptation of the protocol described by Wienkers et al.,[34] the compounds were incubated at a test concentration of 100 μM with glutathione (100 μM) in a mixture of PBS (pH 7.4)/acetonitrile (65:35, v:v) for 1 h at room temperature. Aliquots were taken at 0 and 1 h and analyzed by LC−UV spectroscopy. **19a**, **30a**, and **35a** were recovered quantitatively after 1 h of incubation, whereas a minor formation of a glutathione adduct of **35h** was detected by mass spectrometry. In this case 84% of **35h** was recovered after 1 h of incubation. Considering the literature benchmark value of less than 20% residual glutathione after reaction with the test compound for electrophilicity,[34] compound **35h** was also considered, together with **19a**, **30a**, and **35a**, for further optimization. The goal toward more soluble compounds was approached by synthesizing derivatives containing a phenyloxopropenyl moiety, which was substituted either by a 4-methylpiperazin-1-yl or by a 4-methylpiperazin-1-ylmethyl group.

Tables 3 and 4 summarize the biological data of the 4-methylpiperazin-1-yl and 4-methylpiperazin-1-ylmethyl derivatives (entries **19b**−**g**, **30b**−**g**, **35b**−**g**, and **35i**−**n**). Overall, the introduction of this moiety led to compounds with good solubility while maintaining or increasing enzymatic inhibitory and antiproliferative activities.

Large differences were found in the enzymatic assay for compounds containing a para-substituted central phenyl or 2,5-disubstituted pyridinyl ring (see Table 3). IC_{50} values ranged from 0.012 μM (**30c**) to 2 μM (**19f**). Side by side comparison of the compounds containing a central para substituted phenyl ring with those with a central 2,5-disubstituted

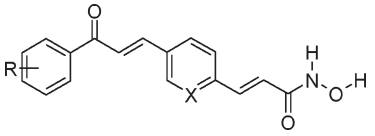
Table 2. HDAC Enzyme, Antiproliferative Activity, Microsomal Stability, and Solubility Data for Unsubstituted Phenyloxopropenyl Derivatives[a]



					microsomal stability		
compd	X	position	enzyme (μM)	antiproliferative activity K562 (μM)	mouse (%)	human (%)	solubility (μM)
19a	CH	5	1.48	2.28	2	5	3
24	N	4	0.323	1.06	14	16	15
30a	N	5	0.022	0.69	6	14	27
35a	CH	6	0.085	3.21	6	9	12
35h	N	6	0.02	0.51	11	38	16

[a] Assays were done in replicates ($n \geq 2$). Mean values are shown, and the standard derivations are $< 30\%$ of the mean.

Table 3. HDAC Enzyme, Antiproliferative Activity, Microsomal Stability, and Solubility Data for Para Substituted Compounds **19b**−**g** and **30b**−**g**[a]



					microsomal stability		
compd	R	X	enzyme (μM)	antiproliferative activity K562 (μM)	mouse (%)	human (%)	solubility (μM)
19b	2-(4-methyl piperazin-1-yl)	CH	0.186	1.60	34	38	210
19c	2-(4-methyl piperazin-1-ylmethyl)	CH	0.350	1.42	4.2	56	453
19d	3-(4-methylpiperazin-1-yl)	CH	0.280	0.85	6.2	44	16
19e	3-(4-methylpiperazin-1-ylmethyl)	CH	0.226	2.06	17	23	287
19f	4-(4-methyl piperazin-1-yl)	CH	1.99	0.90	9.1	33	1
19g	4-(4-methylpiperazin-1-ylmethyl)	CH	0.183	1.28	17	61	244
30b	2-(4-methylpiperazin-1-yl)	N	0.141	1.22	61	65	469
30c	2-(4-methyl piperazin-1-ylmethyl)	N	0.012	2.73	53	93	490
30d	3-(4-methylpiperazin-1-yl)	N	0.030	0.36	20	41	104
30e	3-(4-methyl piperazin-1-ylmethyl)	N	0.053	0.35	46	32	475
30f	4-(4-methylpiperazin-1-yl)	N	0.175	0.41	3.8	43	29
30g	4-(4-methylpiperazin-1-ylmethyl)	N	0.027	0.23	56	47	467

[a] Assays were done in replicates ($n \geq 2$). Mean values are shown, and the standard derivations are $< 30\%$ of the mean.

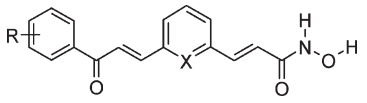
pyridinyl ring showed that inhibitors containing the pyridinyl ring were more potent in the assay. Moreover, a methylene spacer between the piperazine group and the distal phenyl group resulted in an increase in enzyme inhibitory activity in some examples as seen when comparing **19g** and **19f**, **30c** and **30b**, **30g** and **30f**. No clear trends were observed regarding ortho, meta, and para substitution on the distal phenyl ring. For example, the para-substituted 4-methylpiperazin-1-yl derivative **19f**, with an IC_{50} value of 2.00 μM, was substantially less active than the corresponding ortho (**19b**, 0.186 μM) and meta (**19d**, 0.280 μM) analogues. On the contrary, the para-substituted **30f**, with an IC_{50} value of 0.175 μM, and the ortho (**30b**) analogue (0.141 μM) showed comparable potency, whereas the meta (**30d**) derivative had an IC_{50} value of 0.030 μM.

On the other hand (see Table 4), the compounds containing a meta-substituted central phenyl or a 2,6-disubstituted pyridinyl ring displayed consistently good, double digit nanomolar HDAC inhibitory activity with no major differences within this subseries. The compounds **35b**−**n** exhibited IC_{50} values between 15 and 87 nM and were generally more potent than the analogues with the para-substituted aromatic ring. This trend was more evident within the phenyl than in the pyridinyl series.

For a further characterization of their isoform selectivity profile, some representative compounds were tested together with **1** against HDACs 1, 3, 8, 4, and 6 (Amphora Corp., Research Triangle, NC).[35] All compounds showed activities comparable or in some cases superior to **1**, and the structures can be classified as typical pan-HDAC inhibitors (data not shown).

As observed for the unsubstituted phenyloxopropenyl series, the antiproliferative activity of the compounds containing a 2,5-disubstituted pyridinyl ring was generally higher than that of those containing a para-substituted central phenyl (Table 3). Differences in cellular activity were observed between compounds, wherein the distal phenyl ring was substituted on different positions: the ortho-substituted **19b** with a cellular IC_{50} value of 1.6 μM was less active than the meta (**19d**) and the para (**19f**) analogues with IC_{50} values of 0.85 and 0.90 μM, respectively. The same trend was found within the pyridinyl series: **30b** exhibited an IC_{50} value of 1.2 μM, whereas the meta (**30d**) and the para (**30f**) 4-methylpiperazin-1-yl analogues had IC_{50} values of 0.36 and 0.41 μM, respectively.

Table 4. HDAC Enzyme, Antiproliferative Activity, Microsomal Stability and Solubility Data for Meta Substituted Compounds **35a−n**[a]



					microsomal stability		
compd	R	X	enzyme (μM)	antiproliferative activity K562 (μM)	mouse (%)	human (%)	solubility (μM)
35b	2-(4-methylpiperazin-1-yl)	CH	0.087	1.29	44	62	177
35c	2-(4-methylpiperazin-1-ylmethyl)	CH	0.074	0.98	35	62	473
35d	3-(4-methylpiperazin-1-yl)	CH	0.020	0.99	7.2	55	46
35e	3-(4-methylpiperazin-1-ylmethyl)	CH	0.024	1.09	25	39	405
35f	4-(4-methylpiperazin-1-yl)	CH	0.027	1.17	5.9	40	15
35g	4-(4-methylpiperazin-1-ylmethyl)	CH	0.017	1.10	33	44	134
35i	2-(4-methylpiperazin-1-yl)	N	0.033	0.70	57	58	450
35j	2-(4-methylpiperazin-1-ylmethyl)	N	0.053	0.76	40	67	460
35k	3-(4-methylpiperazin-1-yl)	N	0.032	0.11	29	35	380
35l	3-(4-methylpiperazin-1-ylmethyl)	N	0.056	0.20	40	45	450
35m	4-(4-methylpiperazin-1-yl)	N	0.015	0.14	19	35	7
35n	4-(4-methylpiperazin-1-ylmethyl)	N	0.041	0.20	46	51	447

[a] Assays were done in replicates ($n \geq 2$). Mean values are shown, and the standard derivations are $< 30\%$ of the mean.

Similar trends were found for compounds containing a meta-substituted central phenyl or 2,6-disubstituted pyridinyl ring (Table 4). Pyridinyl derivatives were shown to be more potent than their phenyl analogues. Four derivatives (**35k−n**), wherein the distal phenyl rings were either meta- or para-substituted, were found to be among the most potent compounds of this series with cellular IC_{50} values between 0.1 and 0.2 μM.

By comparison of the antiproliferative activities of the inhibitors, in which the central aromatic ring is meta-substituted, with the corresponding para analogues, the results indicate that the meta analogues generally exhibited a higher potency (Tables 3 and 4).

Tables 3 and 4 also include the microsomal stability data for the 4-methylpiperazin-1-yl and 4-methylpiperazin-1-ylmethyl derivatives. The results are expressed as percentage (%) remaining of the parent compound after 30 min of incubation. The inhibitors of both series, wherein the central aromatic ring is meta- or para-substituted, were in general found to be more stable in human than in mouse microsomes. An increased stability of hydroxamic acid derivatives in human over mice microsomes has been recently described by Venkatesh et al.[36] The authors observed that *N*-hydroxy-3-[2-phenethyl-1-(2-pyrrolidin-1-ylethyl)-1*H*-benzimidazol-5-yl]acrylamide (SB639) showed a limited stability in mice microsomes with a half-life of 3 min, whereas the compound was significantly more stable in human microsomes ($t_{1/2}$ = 59 min). At the same experimental conditions, the observed half-life of compound **1** was 28 min in mouse and 60 min in human microsomes. Comparison of compounds containing a phenyl group as central aromatic ring with those having a pyridinyl ring showed that the latter ones generally demonstrated an increased stability. Compounds **30b**, **30c**, **30g**, and **35i** were the most stable compounds in this test (less than 50% of the product was metabolized in both mouse and human microsomes), and three of them (**30b**, **30c**, and **35i**) are ortho-substituted on the distal phenyl ring. In contrast, compounds with a 4-methylpiperazin-1-yl-moiety in para position are among the most labile compounds in mouse microsomes. As expected, the introduction of the piperazine group led to structures having generally a good solubility. As outlined in Tables 3 and 4, the solubility of most of the inhibitors exceeded 200 μM at a pH value of 7.4. Compounds with solubility lower than 200 μM as well as derivatives that were metabolized more than 80% in mouse and human microsomes were not considered for further tests. Compounds **35j** and **35l** were not further characterized, since both compounds were shown to be potent inhibitors of the cytochromes P-450 CYP2D6 and CYP3A4, inhibiting the enzymes at least 85% at 10 μM.

Table 5. Histone Acetylation Assay of Standard Compound **1** and Nine Representative Derivatives[a]

compd	acetylation increment in presence of 0.5 μM inhibitor
1	3.61
19b	2.87
30b	4.89
30c	2.22
30e	3.78
30g	3.72
35c	2.54
35i	8.35
35k	3.84
35n	3.88

[a] Values refer to a single experiment. The numbers represent the ratio of the acetylation level of treated cells over controls.

Nine compounds (**19b**, **30b**, **30c**, **30e**, **30g**, **35c**, **35i**, **35k**, and **35n**) that fulfilled the requirements discussed above were selected for a more extensive characterization.

First, human cancer leukemia K562 cells were incubated with the compounds in order to assess the ability of the molecules to inhibit HDACs in cells measuring the histone acetylation levels by flow cytometry. Consistent with an ability to inhibit cellular HDACs, all compounds tested induced a 2−8 times increase in the basal level of acetylation in K562 cells at 0.5 μM (Table 5).

The same selected compounds were subsequently submitted to pharmacokinetic studies in mouse. The compounds were administered in single intravenous (iv) and oral doses of 5 and 15 mg/kg, respectively. All the compounds were dissolved in 0.9% NaCl solution for the intravenous dose or in water for the oral dose. The results were compared with SAHA (**1**), which was dissolved in water containing 1% DMSO and 10%

Table 6. Pharmacokinetic Parameters of Standard Compounds **1**−**3** and Nine Representatives Derivatives in Mice

comp	**1**	**1**[37]	**2**[37]	**3**[37]	**19b**	**30b**	**30c**	**30e**	**30g**	**35c**	**35i**	**35k**	**35n**
$AUC_{iv_{0-\infty}}$ (ng h/mL)[a]	657	743	273	431	698	1397	1492	736	1052	849	1898	1506	2524[b]
$t_{1/2}$ (h)	0.5	0.38	1.37	1.21	2	2.3	5.55	6.7	9.7	8.27	8.6	8.5	7.3
Cl ((L/h)/kg)	7.62	6.73	18.3	11.6	7.14	3.58	3.35	6.78	4.75	5.88	2.64	3.32	1.75
V_{ss} (L/kg)	1.2	0.81	15.1	5.03	5.4	2.1	11.7	28.4	31.9	20.1	13.9	18.3	16.07
F (%)	12.1	8.33	4.6	6.66	18.1	6.3	4.5	3.9	5.5	3.1	2.9	2	2.6

[a] Literature AUC data are normalized to the doses used in house. [b] AUC_{iv} from 0 to 24 h (ng h/mL).

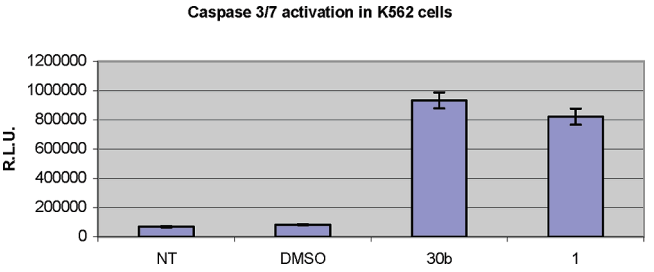


Figure 2. Caspase-3/7 induction in human cancer cells K562, expressed as relative light unit (RLU): untreated or treated with solvent (DMSO), **30b**, or **1** at 3 μM for 24 h.

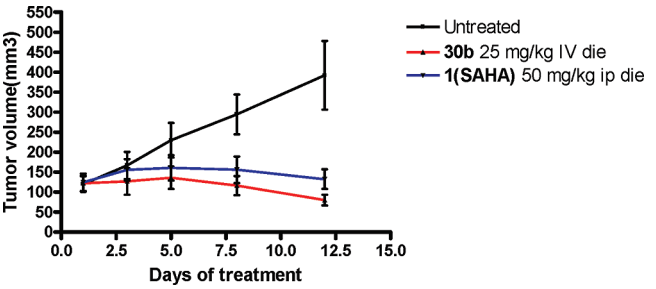


Figure 3. Antitumor activity of **30b** and **1** against HCT-116 human tumor xenografts implanted in mice, expressed as mean tumor volume (expressed as mm^3) ± standard error of the mean (SEM): untreated or treated with **30b** in PBS or **1** in PEG/H_2O/DMSO (45:45:10).

encapsin as iv formulation or in water containing 5% DMSO and 1% PEG400 for the oral dose. The main plasma pharmacokinetic parameters are presented in Table 6, which also include data reported in the literature for **1**, LBH-589 (**2**), and PXD-101 (**3**).[37] The pharmacokinetic profiles for all the compounds tested were quite similar after the iv administration.

As reported in the table, the compounds exhibited in general a medium/high clearance but still lower than the three reference compounds, whose clearance was greater than the hepatic blood flow of 5.4 (L/h)/kg in mice.[38] Furthermore, the estimated elimination half-life of the tested compounds is between 2 h (**19b**) and 9.7 h (**30g**) and is generally higher than that of all three reported reference compounds.[37] **19b** and **30b** exhibited a medium-high estimated steady-state volume of distribution V_{ss} (5.4 and 2.1 L/kg, respectively), whereas the other tested compounds had a high V_{ss} with more than 10 L/kg. In comparison, reference compounds **1** and **3** had a medium-high V_{ss} with 1.2 and 5.0 L/kg, respectively, much lower than **2** (15.1 L/kg). A poor oral bioavailability characterizes the compounds as well as the three reference molecules. **19b** was the only exception with an oral bioavailability higher than 10%.

Despite the poor pharmacokinetic parameters, the three reference compounds had shown antitumor activity in vivo.[25] These results had triggered even more the search for active compounds with a more favorable PK profile, and as described above, compound **30b** emerged as a compound with an acceptable pharmacokinetic profile.

With respect to the apoptotic properties, **30b** was profiled in comparison with **1** in terms of ability to induce caspase activation in human leukemia cancer cells (K562). As shown in Figure 2, **30b**, at the fixed concentration of 3 μM, induced a relevant caspase-3/7 activation after 24 h of treatment, slightly higher than **1**.

Finally, **30b** and reference compound **1** were tested in an in vivo efficacy experiment. In detail, we established a subcutaneous human HCT-116 xenograft model to determine the effect of **30b** or **1** on tumor growth in vivo. Once tumors became palpable (~100 mm^3), mice were treated either with **30b** at a dose of 25 mg/kg iv once a day or with **1** at a dose of 50 mg/kg intraperitoneally once a day, which corresponds, to our best knowledge, to the optimal condition for this derivative. As outlined in Figure 3, compound **30b** was demonstrated to have a better in vivo efficacy than reference compound **1**, characterized by tumor stabilization and by a partial tumor regression in 5 out of 7 mice. No significant body weight difference among the groups of mice and no signs of overt toxicity were observed during the treatment.

Conclusion

A novel series of *N*-hydroxy-3-phenyl-2-propenamide and *N*-hydroxy-3-(pyridin-2-yl)-2-propenamide derivatives containing a phenyloxopropenyl moiety have been described as HDAC inhibitors. Unsubstituted derivatives exhibited low micromolar to double digit nanomolar IC_{50} values in the in vitro HDAC inhibition studies and low micromolar to submicromolar activities in the antiproliferative assay, but their further development was hampered by limited solubility and stability in human and mouse microsomes. The goal toward more soluble compounds was approached by synthesizing derivatives containing a phenyloxopropenyl moiety, which was substituted either by a 4-methylpiperazin-1-yl or by a 4-methylpiperazin-1-ylmethyl group. Overall, the introduction of these moieties led to compounds with good solubility while maintaining or increasing enzymatic inhibitory and antiproliferative activity. Furthermore, an improvement of microsomial stability was observed for several compounds. The compounds (**19b**, **30b**, **30c**, **30e**, **30g**, **35c**, **35i**, **35k**, and **35n**) with the best balance of in vitro inhibitory potency and ADME properties were submitted for a pharmacokinetic profiling in mice. Compared to HDAC inhibitors, which are already in advanced clinical studies (**1**−**3**), the whole series showed in general a lower clearance rate and an increased half-life. A representative example, **30b**, was demonstrated to cause tumor growth inhibition in vivo in a human colon carcinoma xenograft model.

Experimental Section

Chemistry. Reagents and solvents used, unless stated otherwise, were of commercially available reagent grade quality and were used without further purification. Flash chromatography purifications were performed on Merck silica gel 60 (0.04−0.063 mm). Nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker 300 MHz spectrometer at 300 K and are referenced in ppm (δ) relative to TMS. Coupling constants (J) are expressed in hertz (Hz). HPLC−MS experiments were performed on an Acquity UPLC apparatus equipped with a diode array and a Micromass ZQ single quadruple (Waters) using a BEH C18 (1.7 μm, 2.1 mm × 50 mm) column. The flow rate was adjusted to 0.6 mL/min, and the splitting ratio between the amount submitted to the mass spectrometer and to the waste was 1:4. Mobile phase A was composed by a mixture of water and acetonitrile (95:5) containing 0.1% TFA, and mobile phase B was composed by a mixture of water and acetonitrile (5:95) containing 0.1% TFA. Purity refers to UV detection at 220 nm or base peak intensity (BPI), and the compounds used for biological tests were at least 95% pure with the exception of **35a** (92%) (see Supporting Information).

***N*-Hydroxy-3-phenylpropionamide (13c).** A mixture of **12c** (300 mg, 2.0 mmol), *O*-benzylhydroxylamine (NH_2OBn) (0.28 mL, 2.4 mmol), EDC (766 mg, 4.0 mol), and HOBt (540 mg, 4.0 mmol) in dry CH_2Cl_2 (20 mL) was stirred for 3 h at room temperature. The solution was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo to dryness. The resulting benzyl protected hydroxamic acid was purified by flash chromatography (AcOEt/hexane from 3:7 to 4:6). NH_4HCO_2 (813 mg, 12.9 mmol) was added to a suspension of the intermediate and Pd/C (10%, 236 mg) in methanol (30 mL), and the mixture was stirred for 30 min at 40 °C. After filtration of the catalyst, the solvent was evaporated in vacuo to dryness, and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH/CH_3-COOH, 97:3:0.3). The resulting product was triturated with petroleum ether and diethyl ether, affording the requisite hydroxamic acid **13c** (234 mg, 71%). ^{1}H NMR (DMSO-d_6) δ: 10.37 (s, 1H), 8.71 (s, 1H), 7.29−7.25 (m, 2H), 7.20−7.15 (m, 3H), 2.80 (t, J = 7.8 Hz, 2H), 2.25 (t, J = 7.8 Hz, 2H). LC−MS (m/e): no signal.

Typical Procedure for the Synthesis *N*-Hydroxyacrylamides 13d−g and 15. Example: (*E*)-*N*-Hydroxy-3-pyridin-3-ylacrylamide (13f). A mixture of **12f** (546 mg, 3.66 mmol), NH_2OTHP (357 mg, 3.05 mmol), EDC (773 mg, 4.03 mol), and HOBt (540 mg, 4.0 mmol) in dry CH_2Cl_2 (10 mL) and dry DMF (5 mL) was stirred for 24 h at room temperature. The solution was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo to dryness. The resulting crude compound was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5). The resulting intermediate was dissolved in CH_2Cl_2 and treated with 1 M HCl in Et_2O (3.4 mL) for 1 h. The precipitating yellow solid was filtered off to afford **13f** as its hydrochloride salt (422 mg, 69%). ^{1}H NMR (DMSO-d_6) δ: 8.98 (s, 1H), 8.73 (d, J = 5.2 Hz, 1H), 8.42 (d, J = 8 Hz, 1H), 7.80 (dd, J = 5.2 Hz, 8 Hz, 1H), 7.57 (d, J = 16 Hz, 1H), 6.71 (d, J = 16 Hz, 1H). LC−MS (m/e): 165 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-phenylacrylamide (13d). Yield: 273 mg of **13d** (55%). ^{1}H NMR (DMSO-d_6) δ: 10.75 (bs, 1H), 7.56 (m, 2H), 7.45 (d, J = 16 Hz, 1H), 7.43−7.35 (m, 3H), 6.47 (d, J = 16 Hz, 1H). LC−MS (m/e): no signal.

(*E*)-*N*-Hydroxy-3-pyridin-2-ylacrylamide (13e). Yield: 404 mg of **13e** (66%). ^{1}H NMR (DMSO-d_6) δ: 8.68−8.67 (m, 1H), 8.02 (t, J = 8.0, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.54−7.50 (m, 2H), 6.98 (d, J = 15.6 Hz, 1H). LC−MS (m/e): 165 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-pyridin-4-ylacrylamide (13g). Yield: 257 mg of **13g** (42%). ^{1}H NMR (DMSO-d_6) δ: 8.85 (d, J = 6.4, 2H), 8.07 (d, J = 6.4, 2H), 7.59 (d, J = 16.0 Hz, 1H), 6.97 (d, J = 16.0 Hz, 1H). LC−MS (m/e): 165 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(1-methyl-1*H*-pyrrol-2-yl)acrylamide (15). Yield: 86 mg of **15** (17%). ^{1}H NMR (DMSO-d_6) δ: 10.5 (bs, 1H), 7.35 (d, J = 15.6 Hz, 1H), 6.90 (bs, 1H), 6.48 (bs, 1H), 6.11 (d, J = 15.6 Hz, 1H), 6.06 (m, 1H), 3.67 (s, 3H). LC−MS (m/e): 189 $[M + Na]^+$.

(*E*)-*N*-Hydroxy-3-[4-((*E*)-3-oxo-3-phenyl-1-propen-1-yl)phenyl]-acrylamide (19a). A mixture of acetophenone (**16a**, 66 μL, 0.56 mmol), 4-formylcinnamic acid (**17**, 99 mg, 0.56 mmol), and 2 M KOH (1 mL) in 10 mL of water/ethanol (1:1) was stirred at room temperature for 24 h. The solution was then acidified with 10% HCl and the formed yellow precipitate was filtered to afford 120 mg (77%) of the acrylic acid **18a**. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.13−8.20 (m, 2H), 8.00 (d, J = 15.73 Hz, 1H), 7.90−7.96 (m, 2H), 7.76−7.81 (m, 2H), 7.75 (d, J = 15.73 Hz, 1H), 7.65−7.72 (m, 1H), 7.62 (d, J = 16.05 Hz, 1H), 7.54−7.63 (m, 2H), 6.64 (d, J = 16.05 Hz, 1H).

Ethyl chloroformate (48 μL, 0.5 mmol) and triethylamine (75 μL, 0.54 mmol) were added to a cooled solution of the carboxylic acid **18a** (117 mg, 0.42 mmol) in dry THF (10 mL), and the mixture was stirred for 10 min. The reaction mixture was filtered, and *O*-(2-methoxy-2-propyl)hydroxylamine (35 μL, 0.47 mmol) was added to the filtrate. The solution was stirred for 15 min at 0 °C, then evaporated under reduced pressure, and the residue was diluted with methanol (10 mL). The solution of the protected hydroxamate was added to an Amberlyst 15 ion-exchange resin (0.3 g), and the resulting mixture was stirred at 45 °C for 1 h. Thereafter, the reaction mixture was filtered, and the filtrate was concentrated in vacuo and then purified by crystallization from acetonitrile affording the desired hydroxamic acid **19a** (76 mg, 62%). ^{1}H NMR (DMSO-d_6) δ: 8.09−8.22 (m, 2 H), 7.96 (d, J = 15.42 Hz, 1 H), 7.87−7.95 (m, 2 H), 7.74 (d, J = 15.73 Hz, 1 H), 7.62−7.71 (m, 3 H), 7.55−7.62 (m, 2 H), 7.49 (d, J = 15.73 Hz, 1 H), 6.58 (d, J = 16.05 Hz, 1 H). LC−MS (m/e): 294 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(4-{(*E*)-3-[2-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (19b). A mixture of 1-[2-(4-methylpiperazin-1-yl)phenyl]ethanone (**16b**, 542 mg, 2.48 mmol), **17** (438 mg, 2.48 mmol), and 1.7 M KOH (2.92 mL) in 10 mL of water/ethanol (1:1) was stirred at room temperature for 24 h. The solution was then acidified with 10% HCl and the formed yellow precipitate was filtered to afford the acrylic acid hydrochloride **18b** (930 mg, 90%). ^{1}H NMR (DMSO-d_6) δ: 12.44 (bs, 1 H), 10.37 (bs, 1 H), 7.81 (m, 4 H), 7.62 (d, J = 15.85 Hz, 1 H), 7.46−7.59 (m, 4 H), 7.28 (d, J = 7.63 Hz, 1 H), 7.11−7.24 (m, 1 H), 6.62 (d, J = 15.85 Hz, 1 H), 3.33−3.57 (m, 2 H), 3.10−3.27 (m, 4 H), 2.77−3.04 (m, 2 H), 2.66 (s, 3 H).

A mixture of **18b** (250 mg, 0.608 mmol), NH_2OTHP (85 mg, 0.73 mmol), EDC (232 mg, 1.22 mol), HOBt (164 mg, 1.22 mmol), and TEA (253 μL, 1.82 mmol) in dry THF (5 mL) and DMF (5 mL) was stirred for 24 h at room temperature. The solution was then diluted with water and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, evaporated in vacuo to dryness, and purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 98:2:0.2). The intermediate was dissolved in CH_2Cl_2 and treated with HCl in Et_2O for 1 h. The resulting yellow solid was filtered to give the desired hydroxamic acid **19b** as its hydrochloride salt (115 mg, 44%). ^{1}H NMR (DMSO-d_6) δ: 10.89 (bs, 1 H), 7.83 (m, 2 H), 7.64 (m, 2 H), 7.43−7.60 (m, 5 H), 7.28 (d, J = 7.92 Hz, 1 H), 7.21 (td, J = 7.63, 0.88 Hz, 1 H), 6.57 (d, J = 15.85 Hz, 1 H), 3.37−3.52 (m, 2 H), 3.09−3.37 (m, 4 H), 2.77−3.00 (m, 2 H), 2.66 (d, J = 4.40 Hz, 3 H). LC−MS (m/e): 392 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(4-{(*E*)-3-[2-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (19c). A mixture of **17** (189 mg, 1.077 mmol), 1-[2-(4-methylpiperazin-1-yl-methyl)phenyl]ethanone (**16c**, 250 mg, 1.077 mmol), and 1.7 M KOH (1.26 mL) in EtOH (5 mL) and H_2O (5 mL) was stirred at room temperature overnight and then acidified with 10% aqueous HCl. The resulting precipitate was filtered to give the carboxylic

acid **18c** (350 mg, 70%, bis-hydrochloride). LC−MS (*m*/*e*): 391 $[M - H]^+$.

A mixture of **18c** (350 mg, 0.756 mmol), HOBt (204 mg, 1.51 mmol), EDC (288 mg, 1.51 mmol), TEA (0.210 mL, 1.51 mmol), and NH_2OTHP (106 mg, 0.907 mmol) in dry DMF (8 mL) was stirred overnight at room temperature and then partitioned between water and AcOEt. The aqueous layer was brought to basic conditions with NH_4OH and extracted with CH_2Cl_2. The collected organic extracts were dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH, 95:5:0.2), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 1 h. The hygroscopic precipitate was filtered and freeze-dried to afford the requisite hydroxamic acid **19c** (229 mg, 63%, bis-hydrochloride salt). ^{1}H NMR (DMSO-d_6, 353 K, +TFA) δ: 7.77 (m, 2 H), 7.61 (m, 2 H), 7.47−7.59 (m, 5 H), 7.43 (d, J = 15.55 Hz, 1 H), 7.33 (d, J = 16.14 Hz, 1 H), 6.63 (d, J = 16.14 Hz, 1 H), 3.91 (s, 2 H), 3.08−3.25 (m, 4 H), 2.79−2.93 (m, 4 H), 2.71 (s, 3 H). LC−MS (*m*/*e*): 406 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(4-{(*E*)-3-[3-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (19d). A mixture of 1-[3-(4-methylpiperazin-1-yl)phenyl]ethanone (**16d**, 406 mg, 1.86 mmol), **17** (328 mg, 1.86 mmol), and 1.7 M KOH (2.19 mL) in 10 mL of ethanol was stirred at room temperature overnight. The solution was then acidified with 10% HCl and the resulting yellow precipitate was filtered to afford the acrylic acid **18d** as its hydrochloride salt (460 mg, 60%). LC−MS (*m*/*e*): 377 $[M - H]^+$.

A mixture of **18d** (224 mg, 0.543 mmol), NH_2OTHP (76 mg, 0.65 mmol), EDC (207 mg, 1.09 mol), HOBt (147 mg, 1.09 mmol), and TEA (151 μL, 1.09 mmol) in dry THF (5 mL) and dry DMF (5 mL) was stirred for 60 h at room temperature. The solution was then diluted with water and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, evaporated in vacuo to dryness, and purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 96:4:0.2). The resulting intermediate was dissolved in CH_2Cl_2 and treated with HCl in Et_2O for 1 h. The precipitating yellow solid was filtered to give the desired hydroxamic acid **19d** (187 mg, 80%, hydrochloride salt). ^{1}H NMR (DMSO-d_6) δ: 11.06 (bs, 1 H), 7.86−8.03 (m, 3 H), 7.74 (d, J = 15.55 Hz, 1 H), 7.61−7.70 (m, 4 H), 7.49 (d, J = 15.55 Hz, 1 H), 7.47 (dd, J = 8.22, 7.63 Hz, 1 H), 7.33 (dd, J = 8.22, 2.05 Hz, 1 H), 6.60 (d, J = 15.85 Hz, 1 H), 3.81−4.09 (m, 2 H), 3.39−3.64 (m, 2 H), 3.03−3.32 (m, 4 H), 2.82 (d, J = 4.69 Hz, 3 H). LC−MS (*m*/*e*): 392 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(4-{(*E*)-3-[3-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (19e). The hydroxamic acid **19e** was prepared according to the procedure described for compound **19c**. The hydroxamic acid **19e** was purified by preparative HPLC (200 mg, 30% from **16d**, bis-trifluoroacetate). ^{1}H NMR (DMSO-d_6, 353 K, +TFA) δ: 7.98−8.16 (m, 2 H), 7.80−7.91 (m, 2 H), 7.82 (d, J = 15.85 Hz, 1 H), 7.77 (d, J = 13.50 Hz, 1 H), 7.61−7.72 (m, 3 H), 7.58 (t, J = 7.48 Hz, 1 H), 7.50 (d, J = 15.85 Hz, 1 H), 6.63 (d, J = 15.55 Hz, 1 H), 3.86 (s, 2 H), 3.25 (t, J = 4.99 Hz, 4 H), 2.81−2.89 (m, 4 H), 2.80 (s, 3 H). LC−MS (*m*/*e*): 406 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(4-{(*E*)-3-[4-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide(19f). A mixture of 1-[4-(4-methylpiperazin-1-yl)phenyl]ethanone (**16f**, 6.59 g, 30.2 mmol), **17** (5.32 g, 30.2 mmol), and 1.7 M KOH (35.5 mL) in ethanol (90 mL) was stirred at room temperature overnight. The solution was then filtered to afford 9.2 g (73%) of the potassium acrylate **18f**. LC−MS (*m*/*e*): 377 $[M - H]^+$.

18f (9.2 g, 22 mmol) was then dissolved in dry THF (60 mL) and dry DMF (60 mL), and TEA (6.2 mL, 44 mmol), NH_2OTHP (3.1 g, 27 mmol), EDC (8.5 g, 44 mmol), and HOBt (6.0 g, 44 mmol) were added to the resulting slurry. The mixture was stirred overnight at room temperature, then diluted with water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, evaporated in vacuo to dryness, and purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 96:4:0.2). The resulting intermediate was dissolved in CH_2Cl_2 and treated with HCl in Et_2O for 6 h. The precipitating yellow solid was filtered to give the requisite hydroxamic acid **19f** as its hydrochloride salt (7.86 g, 83%). ^{1}H NMR (DMSO-d_6) δ: 11.33 (bs, 1 H), 8.11 (m, 2 H), 7.97 (d, J = 15.55 Hz, 1 H), 7.91 (m, 2 H), 7.68 (d, J = 15.55 Hz, 1 H), 7.63 (m, 2 H), 7.49 (d, J = 15.85 Hz, 1 H), 7.11 (m, J = 9.10 Hz, 2 H), 6.60 (d, J = 15.85 Hz, 1 H), 4.01−4.19 (m, 2 H), 3.43−3.56 (m, 2 H), 3.23−3.43 (m, 2 H), 2.97−3.22 (m, 2 H), 2.80 (d, J = 4.69 Hz, 3 H). LC−MS (*m*/*e*): 392 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(4-{(*E*)-3-[4-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (19g). A mixture of 1-[4-(4-methylpiperazin-1-ylmethyl)phenyl]ethanone (**16g**, 392 mg, 1.69 mmol), **17** (300 mg, 1.69 mmol), and 1.7 M KOH (2.0 mL, 3.4 mmol) in H_2O (5 mL) and EtOH (5 mL) was stirred at room temperature for 24 h. The mixture was then acidified with 10% HCl and the resulting yellow precipitate was filtered off to obtain the carboxylic acid **18g** (542 mg, 69%, dihydrochloride). LC−MS (*m*/*e*): 391 $[M - H]^+$.

NH_2OTHP (164 mg, 1.4 mmol), EDC (447 mg, 2.34 mol), HOBt (316 mg, 2.34 mmol), and TEA (488 μL, 3.51 mmol) were added to a solution of **18g** (542 mg, 1.17 mmol) in dry THF (5 mL) and dry DMF (5 mL), and the mixture was stirred for 24 h at room temperature. The mixture was then diluted with water and extracted with AcOEt. The organic layer was rinsed with water, then with brine, dried over Na_2SO_4, and evaporated in vacuo to dryness. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH/NH_3, 98:2:0.2). The resulting intermediate was dissolved in CH_2Cl_2 and treated with HCl in Et_2O for 1 h to obtain a yellow precipitate. The solid was then filtered off to obtain the desired hydroxamic acid **19g** (300 mg, 53%, dihydrochloride). ^{1}H NMR (DMSO-d_6) δ: 11.73 (bs, 1 H), 8.23 (m, 2 H), 8.00 (d, J = 15.55 Hz, 1 H), 7.95 (m, 2 H), 7.86 (m, 2 H), 7.77 (d, J = 15.55 Hz, 1 H), 7.66 (m, 2 H), 7.50 (d, J = 15.85 Hz, 1 H), 6.60 (d, J = 15.85 Hz, 1 H), 4.45 (bs, 2 H), 3.19−3.70 (m, 8 H), 2.82 (s, 3 H). LC−MS (*m*/*e*): 406 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-[4-((*E*)-3-oxo-3-phenyl-1-propen-1-yl)pyridin-2-yl]acrylamide (24). Dry DMSO (1.73 mL, 24.4 mmol) was added dropwise under N_2 atmosphere to a stirred solution of oxalyl chloride (1.08 mL, 12.7 mmol) in CH_2Cl_2 (2 mL) at −78 °C. After the mixture was stirred for 20 min (2-bromopyridin-4-yl)methanol (**20**) (2.0 g, 11 mmol) in CH_2Cl_2 (10 mL) was added, and the mixture was stirred for an additional 10 min at −60 °C. TEA (7.37 mL, 53.0 mmol) was added dropwise, and the resulting solution was allowed to reach room temperature. After 20 min water was added and the product was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, concentrated, and purified by column chromatography (petroleum ether/AcOEt, 8:2) to afford 2-bromopyridine-4-carbaldehyde **21** as a white solid (1.0 g, 62%). ^{1}H NMR ($CDCl_3$) δ: 10.04 (s, 1 H), 8.65 (d, J = 4.99 Hz, 1 H), 7.91 (dd, J = 1.32, 0.73 Hz, 1 H), 7.69 (dd, 1 H).

A solution of **21** (850 mg, 4.57 mmol), trimethyl orthoformate (0.678 mL, 5.88 mmol), and *p*-toluenesulfonic acid (87 mg, 0.13 mmol) in MeOH (15 mL) was stirred overnight at room temperature, then partitioned between 5% $NaHCO_3$ and Et_2O. The organic layer was dried over Na_2SO_4 and evaporated in vacuo to give 880 mg (83%) of 2-bromo-4-dimethoxymethylpyridine. The intermediate was dissolved in dry THF (20 mL) and added to a stirred solution of *i*-PrMgCl (2 M in Et_2O, 2.5 mL) under N_2 atmosphere. The resulting mixture was stirred at room temperature under N_2, and further *i*-PrMgCl (2.5 mL) was added over 3 h. DMF (1.0 mL, 13 mmol) was added, and the mixture was stirred at room temperature for 2 h and then partitioned between water and Et_2O. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude solid was purified by column chromatography (CH_2Cl_2/AcOEt, 9:1) to give 420 mg (60%) of 4-dimethoxymethylpyridine-2-carbaldehyde, which was then dissolved in dry THF (10 mL) and added dropwise to a stirred solution of *tert*-butyl diethyl phosphonacetate (0.710 mL,

3.02 mmol) and NaH (60% oil dispersion, 140 mg, 3.48 mmol) in dry THF (10 mL). The solution was stirred for 1 h at room temperature under N_2 and was then partitioned between water and Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The crude mixture was purified by column chromatography (petroleum ether/AcOEt, 9:1) to give the (*E*)-3-(4-dimethoxymethylpyridin-2-yl)acrylic acid *tert*-butyl ester **22** (550 mg, 86%). ^{1}H NMR ($CDCl_3$) δ: 8.65 (d, J = 4.99 Hz, 1 H), 7.62 (d, J = 15.85 Hz, 1 H), 7.46−7.56 (m, 1 H), 7.34 (dd, J = 4.99, 1.47 Hz, 1 H), 6.86 (d, J = 15.85 Hz, 1 H), 5.41 (s, 1 H), 3.35 (s, 6 H), 1.55 (s, 9 H).

The *tert*-butyl ester **22** (550 mg, 1.99 mmol) was dissolved in THF (10 mL) and 1 M HCl (10 mL), stirred overnight at room temperature, and then heated to 80 °C for 4 h. The mixture was partitioned between 5% $NaHCO_3$ and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo to afford 186 mg (40%) of (*E*)-3-(4-formylpyridin-2-yl)acrylic acid *tert*-butyl ester. A mixture of the resulting intermediate, acetophenone (**16a**) (96 mg, 0.80 mmol), and 1.7 M KOH (0.52 mL) was dissolved in THF (8 mL) and stirred at room temperature overnight. The solution was then partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude solid was purified by column chromatography (petroleum ether/AcOEt, 9:1) to afford 168 mg of *tert*-butyl (*E*)-3-[4-((*E*)-3-oxo-3-phenyl-1-propen-1-yl)pyridin-2-yl]acrylate **23** (62%). ^{1}H NMR (DMSO-d_6) δ (ppm): 8.72 (d, J = 4.99 Hz, 1H), 8.28−8.33 (m, 1H), 8.24 (d, J = 15.85 Hz, 1H), 8.13−8.22 (m, 2H), 7.81 (dd, J = 4.99, 1.47 Hz, 1H), 7.68−7.76 (m, 1H), 7.70 (d, J = 15.55 Hz, 1H), 7.57−7.66 (m, 2H), 7.59 (d, J = 15.85 Hz, 1H), 6.91 (d, J = 15.85 Hz, 1H), 1.51 (s, 9H).

A mixture of **23** (168 mg, 0.5 mmol) and TFA (3 mL) in CH_2Cl_2 (9 mL) was stirred for 2 h at room temperature, and then the solvent was removed in vacuo. The acrylic acid trifluoroacetate salt was then dissolved in CH_2Cl_2 (5 mL), and TEA (0.49 mL, 3.5 mmol), EDC (191 mg, 1.0 mmol), HOBt (135 mg, 1.0 mmol), and NH_2OTHP (70 mg, 0.6 mmol) were added. The solution was stirred overnight at room temperature. The mixture was then partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 97:3:0.1). The resulting intermediate was then dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h at room temperature. The precipitate was filtered and crystallized from EtOH to give **24** as its hydrochloride salt (32 mg, 20%). ^{1}H NMR (DMSO-d_6) δ: 11.00 (bs, 1 H), 8.72 (d, J = 4.99 Hz, 1 H), 8.23 (d, J = 15.55 Hz, 1 H), 8.14−8.23 (m, 3 H), 7.85 (dd, J = 4.99, 1.17 Hz, 1 H), 7.71 (d, J = 15.85 Hz, 1 H), 7.66−7.78 (m, 1 H), 7.58−7.66 (m, 2 H), 7.55 (d, J = 15.26 Hz, 1 H), 7.02 (d, J = 15.26 Hz, 1 H). LC−MS (*m*/*e*): 295 $[M - H]^+$.

(*E*)-3-(5-Formylpyridin-2-yl)acrylic Acid *tert*-Butyl Ester (27). A solution of 6-bromopyridine-3-carbaldehyde (**25**) (8.01 g, 43.1 mmol), *p*-toluenesulfonic acid (819 mg, 4.31 mmol), and trimethyl orthoformate (TMOF, 9.42 mL, 86.1 mmol) in MeOH (80 mL) was stirred at room temperature for 3 h. The mixture was brought to basic pH with 5% aqueous K_2CO_3 and extracted with diethyl ether. The organic layer was dried over Na_2SO_4 and then evaporated to dryness. The crude solid was purified by silica gel chromatography (petroleum ether/AcOEt, 9:1) to give 7.18 g (72%) of 2-bromo-5-dimethoxymethylpyridine as a pale-yellow oil. The intermediate (7.16 g, 30.9 mmol) was dissolved in THF (20 mL) and added dropwise to a stirred solution of *i*-PrMgCl (2 M in diethyl ether, 20.1 mL) in THF (20 mL) under N_2, keeping the temperature below 25 °C. The resulting mixture was stirred for 4 h, DMF (3.09 mL, 40.1 mmol) was added dropwise, and the solution was stirred for additional 2 h. The mixture was then partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (petroleum ether/AcOEt, 9:1) to afford 5-dimethoxymethylpyridine-2-carbaldehyde **26** as a yellow oil (3.27 g, 60%). ^{1}H NMR ($CDCl_3$) δ: 10.00 (d, J = 0.59 Hz, 1 H), 8.80 (ddd, J = 1.76, 0.59 Hz, 1 H), 8.01 (dddd, J = 7.92, 2.05, 0.59 Hz, 1 H), 7.95 (dd, J = 7.92, 0.88 Hz, 1 H), 5.59 (s, 1 H), 3.31 (s, 6 H).

tert-Butyl diethylphosphonoacetate (5.00 g, 19.8 mmol) was dissolved in dry THF (20 mL) and added dropwise to a stirred suspension of NaH (60% oil dispersion, 936 mg, 23.4 mmol) in dry THF (20 mL). After 10 min at room temperature, a solution of **26** (3.26 g, 18.0 mmol) in dry THF (30 mL) was added dropwise under N_2, keeping the temperature below 25 °C. The resulting mixture was stirred for 1 h and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash chromatography (petroleum ether/AcOEt, 95:5) to obtain 3.86 g (77%) of (*E*)-3-(5-dimethoxymethylpyridin-2-yl)acrylic acid *tert*-butyl ester as a pale-yellow oil. The compound was then stirred for 4 h at room temperature in THF (27 mL) and 1 M HCl (27 mL), brought to basic pH with 10% aqueous K_2CO_3, and extracted twice with AcOEt. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo to give **27** as a white solid (2.97 g, 92%). ^{1}H NMR ($CDCl_3$) δ: 10.13 (s, 1 H), 9.08 (d, J = 1.47 Hz, 1 H), 8.18 (dd, J = 8.07, 2.20 Hz, 1 H), 7.63 (d, J = 15.85 Hz, 1 H), 7.57 (d, J = 7.92 Hz, 1 H), 6.99 (d, J = 15.55 Hz, 1 H), 1.56 (s, 9 H).

(*E*)-*N*-Hydroxy-3-[5-((*E*)-3-oxo-3-phenyl-1-propen-1-yl)pyridin-2-yl]acrylamide (30a). A mixture of **27** (364 mg, 1.56 mmol), **16a** (188 mg, 1.56 mmol), and 1.7 M KOH (1.8 mL) in MeOH (10 mL) was stirred at 0 °C for 3 h. The precipitating product was filtered off to afford the *tert*-butyl ester **28a** as a yellow powder (130 mg, 25%). ^{1}H NMR ($CDCl_3$) δ: 8.88 (d, 1H), 8.02−8.09 (m, 2H), 7.97 (dd, J = 8.22, 2.35 Hz, 1H), 7.81 (d, J = 15.85 Hz, 1H), 7.63 (d, J = 15.55 Hz, 1H), 7.62 (d, J = 15.85 Hz, 1H), 7.59−7.64 (m, 1H), 7.51−7.58 (m, 2H), 7.49 (d, J = 8.22 Hz, 1H), 6.91 (d, J = 15.55 Hz, 1H), 1.56 (s, 9H).

A solution of **28a** (130 mg, 0.38 mmol) in CH_2Cl_2 (4 mL) and TFA (1 mL) was stirred for 4 h at room temperature. Then the solvent was removed in vacuo and the resulting oil was crystallized from Et_2O to give the acrylic acid **29a** (165 mg, quantitative, trifluoroacetate salt). ^{1}H NMR (DMSO-d_6) δ: 9.09 (d, J = 2.05 Hz, 1H), 8.45 (dd, J = 8.22, 2.05 Hz, 1H), 8.14−8.23 (m, 2H), 8.13 (d, J = 15.85 Hz, 1H), 7.86 (d, J = 8.22 Hz, 1H), 7.79 (d, J = 15.55 Hz, 1H), 7.66−7.74 (m, 1H), 7.64 (d, J = 15.55 Hz, 1H), 7.54−7.64 (m, 2H), 6.92 (d, J = 15.85 Hz, 1H).

HOBt (113 mg, 0.83 mmol), EDC (160 mg, 0.83 mmol), TEA (126 mg, 1.26 mmol), and NH_2OTHP (59 mg, 0.50 mmol) were added to a solution of **29a** (165 mg, 0.41 mmol) in THF/DMF (1:1, 10 mL). The mixture was stirred for 6 h at room temperature and then partitioned between water and Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, evaporated in vacuo, and purified by silica gel chromatography (petroleum ether/AcOEt, 4:6). The resulting oil was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 1.5 h. The precipitate was filtered to give the desired hydroxamic acid **30a** (47 mg, 33%, hydrochloride salt). ^{1}H NMR (DMSO-d_6) δ: 9.06 (d, J = 1.89 Hz, 1 H), 8.45 (dd, J = 8.34, 2.04 Hz, 1 H), 8.15−8.23 (m, 2 H), 8.11 (d, J = 15.73 Hz, 1 H), 7.79 (d, J = 15.73 Hz, 1 H), 7.73 (d, J = 7.87 Hz, 1 H), 7.66−7.72 (m, 1 H), 7.57−7.64 (m, 2 H), 7.53 (d, J = 15.73 Hz, 1 H), 7.04 (d, J = 15.42 Hz, 1 H). LC−MS (*m*/*e*): 295 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(5-{(*E*)-3-[2-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (30b). A mixture of **27** (7.0 g, 30 mmol), 1.7 M KOH (14.7 mL), and **16b** (5.5 g, 25 mmol) in THF (200 mL) was stirred at room temperature overnight. Further **27** (0.35 g, 1.5 mmol) was added, and stirring continued overnight. The mixture was partitioned between water and AcOEt, and the aqueous layer was washed three times with AcOEt. The combined collected organic layers were dried over Na_2SO_4 and evaporated in vacuo. The crude solid was purified by column chromatography (petroleum ether/AcOEt, 1:1, and then CH_2Cl_2/MeOH/NH_4OH, 485:15:1) to afford the *tert*-butyl ester **28b** as a yellow powder (9.25 g,

85%). ^{1}H NMR ($CDCl_3$) δ: 8.88 (d, J = 2.05 Hz, 1 H), 7.92 (dd, J = 8.07, 2.20 Hz, 1 H), 7.71 (d, J = 16.14 Hz, 1 H), 7.64 (d, J = 15.85 Hz, 1 H), 7.60−7.64 (m, 1 H), 7.62 (d, J = 15.85 Hz, 1 H), 7.46−7.54 (m, 2 H), 7.08−7.18 (m, 2 H), 6.90 (d, J = 15.85 Hz, 1 H), 2.98−3.21 (m, 4 H), 2.38−2.54 (m, 4 H), 2.22 (s, 3 H), 1.56 (s, 9 H).

A mixture of **28b** (9.25 g, 20.1 mmol) and TFA (2 mL) in CH_2Cl_2 (8 mL) was stirred for 6 h at room temperature. The solvent was removed in vacuo and the residue triturated in Et_2O to give the acrylic acid **29b** (13.6 g, quantitative, trifluoroacetate salt). ^{1}H NMR ($CDCl_3$) δ: 8.96 (d, J = 2.35 Hz, 1 H), 7.97 (dd, J = 8.22, 2.05 Hz, 1 H), 7.67 (d, J = 15.85 Hz, 1 H), 7.47−7.62 (m, 4 H), 7.42 (d, J = 16.14 Hz, 1 H), 7.08−7.27 (m, 2 H), 6.91 (d, J = 15.85 Hz, 1 H), 3.50−3.71 (m, 2 H), 3.21−3.48 (m, 4 H), 2.89−3.13 (m, 2 H), 2.80 (s, 3 H).

29b (13.6 g, 20.1 mmol) was suspended in DMF (100 mL) and CH_2Cl_2 (50 mL). TEA (8.91 mL, 64.1 mmol), HOBt (5.77 g, 42.7 mmol), EDC (8.16 g, 42.7 mmol), and NH_2OTHP (2.25 g, 19.2 mmol) were added, and the resulting mixture was stirred at room temperature for 4 h. The solution was then partitioned between water and AcOEt, and the organic layer was washed with water, dried over Na_2SO_4, and evaporated in vacuo. The crude solid was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH from 98:2:0.2 to 96:4:0.4). The resulting intermediate was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 8 h. The solvent was evaporated, the residue was dissolved in 2 M HCl and washed with AcOEt. The aqueous layer was freeze-dried to give the tris-hydrochloride salt of the hydroxamic acid **30b** (5.5 g, 55%). ^{1}H NMR (DMSO-d_6) δ: 11.34 (bs, 1 H), 9.01 (d, J = 1.76 Hz, 1 H), 8.39 (dd, J = 8.22, 2.05 Hz, 1 H), 7.76 (d, J = 8.22 Hz, 1 H), 7.68 (d, J = 16.14 Hz, 1 H), 7.61 (d, J = 16.14 Hz, 1 H), 7.47−7.61 (m, 3 H), 7.29 (d, J = 7.92 Hz, 1 H), 7.21 (t, J = 7.34 Hz, 1 H), 7.06 (d, J = 15.55 Hz, 1 H), 3.35−3.50 (m, 2 H), 3.22−3.34 (m, 4 H), 2.77−3.01 (m, 2 H), 2.66 (d, J = 4.40 Hz, 3 H). LC−MS (m/e): 393 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(5-{(*E*)-3-[2-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (30c). A mixture of **16c** (829 mg, 3.57 mmol), the *tert*-butyl ester **27** (1.00 g, 4.29 mmol), and 1.7 M KOH (2.09 mL) in THF (40 mL) was stirred overnight at room temperature. The resulting solution was diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude reaction mixture was purified by column chromatography (eluent, from petroleum ether/AcOEt, 1:1, to CH_2Cl_2/MeOH/NH_4OH, 93:7:0.2) to give the *tert*-butyl ester **28c** (1.31 g, 82%). ^{1}H NMR ($CDCl_3$) δ: 8.74 (d, J = 2.05 Hz, 1 H), 7.85 (dd, J = 8.22, 2.35 Hz, 1 H), 7.59 (d, J = 15.85 Hz, 1 H), 7.45 (d, J = 8.22 Hz, 1 H), 7.31−7.42 (m, 4 H), 7.22 (d, J = 16.43 Hz, 1 H), 7.11 (d, J = 16.43 Hz, 1 H), 6.88 (d, J = 15.55 Hz, 1 H), 3.60 (s, 2 H), 2.21−2.53 (m, 8 H), 2.18 (s, 3 H), 1.54 (s, 9 H).

The resulting *tert*-butyl ester **28c** (1.31 g, 2.93 mmol) was dissolved in CH_2Cl_2 (15 mL) and TFA (4 mL) and stirred at room temperature for 6 h. The solvent was evaporated to give the carboxylic acid **29c** (2.12 g, 99%, tris-trifluoroacetate salt). A mixture of the carboxylic acid **29c** (2.12 g, 2.90 mmol), HOBt (783 mg, 5.8 mmol), EDC (1.1 g, 5.8 mmol), TEA (1.2 mL, 8.7 mmol), and NH_2OTHP (306 mg, 2.61 mmol) in DMF (15 mL) was then stirred overnight at room temperature. The solution was diluted with water, brought to basic conditions with NH_4OH, and extracted with AcOEt and CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH, 96:4:0.2), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h. The hygroscopic precipitate was filtered, crystallized from isopropanol, and freeze-dried to give the requisite hydroxamic acid **30c** (73.3 mg, 5%, tris-hydrochloride). ^{1}H NMR (DMSO-d_6, 353 K, +Na_2CO_3) δ: 8.79 (d, J = 2.05 Hz, 1 H), 8.09 (dd, J = 8.22, 2.35 Hz, 1 H), 7.52 (d, J = 8.22 Hz, 1 H), 7.31−7.48 (m, 4 H), 7.25−7.30 (m, 2 H), 7.23 (d, J = 15.55 Hz, 1 H), 6.92 (d, J = 15.55 Hz, 1 H), 3.58 (s, 2 H), 2.27−2.33 (m, 4 H), 2.11−2.21 (m, 4 H), 2.05 (s, 3 H). LC−MS (m/e): 407 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(5-{(*E*)-3-[3-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (30d). A mixture of **27** (610 mg, 2.62 mmol), 1.7 M KOH (1.54 mL), and **16d** (521 mg, 2.38 mmol) in THF (3 mL) was stirred at 0 °C for 2 h. The resulting solution was partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was used in the next step without any further purification (1.00 g, 97%). ^{1}H NMR (DMSO-d_6) δ: 9.07 (d, J = 2.05 Hz, 1 H), 8.42 (dd, J = 8.22, 2.35 Hz, 1 H), 8.08 (d, J = 15.85 Hz, 1 H), 7.85 (d, J = 8.22 Hz, 1 H), 7.76 (d, J = 15.85 Hz, 1 H), 7.53−7.65 (m, 2 H), 7.59 (d, J = 15.55 Hz, 1 H), 7.41 (t, J = 8.07 Hz, 1 H), 7.15−7.31 (m, 1 H), 6.87 (d, J = 15.85 Hz, 1 H), 3.09−3.27 (m, 4 H), 2.42−2.50 (m, 4 H), 2.24 (s, 3 H), 1.51 (s, 9 H).

The resulting *tert*-butyl ester **28d** (1.00 g, 2.31 mmol) was dissolved in CH_2Cl_2 (5 mL) and TFA (2.5 mL). The solution was stirred at room temperature for 18 h. The solvent was then removed and the residue was triturated in MeOH to afford **29d** (0.90 g, 64%, bis-trifluoroacetate salt). **29d** (900 mg, 1.49 mmol) was dissolved in DMF (9 mL), and HOBt (372 mg, 2.75 mmol), EDC (527 mg, 2.75 mmol), TEA (0.254 mL, 1.83 mmol), and NH_2OTHP (322 mg, 2.75 mmol) were added. The mixture was stirred at room temperature for 7 h and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo, and the crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 98:2:0.2). The resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 1 h. The precipitate was filtered off to provide the desired hydroxamic acid **30d** (290 mg, 42%, bis-hydrochloride salt). ^{1}H NMR (DMSO-d_6) δ: 11.09 (bs, 1 H), 9.08 (d, J = 1.76 Hz, 1 H), 8.45 (dd, J = 8.22, 2.05 Hz, 1 H), 8.09 (d, J = 15.55 Hz, 1 H), 7.78 (d, J = 15.85 Hz, 1 H), 7.61−7.78 (m, 3 H), 7.54 (d, J = 15.26 Hz, 1 H), 7.48 (dd, 1 H), 7.34 (dd, J = 8.22, 1.76 Hz, 1 H), 7.04 (d, J = 15.55 Hz, 1 H), 3.80−4.16 (m, 2 H), 3.42−3.64 (m, 2 H), 3.02−3.32 (m, 4 H), 2.82 (d, J = 4.70 Hz, 3 H). LC−MS (m/e): 393 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(5-{(*E*)-3-[3-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (30e). KOH (1.7 M, 0.634 mL) was added dropwise to a stirred mixture of **27** (250 mg, 1.08 mmol) and **16e** (250 mg, 1.08 mmol) in EtOH (15 mL). The resulting solution was stirred at 0 °C for 7 h and then diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by chromatographic column (CH_2Cl_2/MeOH/NH_4OH from 97:3:0.1 to 95:5:0.2), and the desired *tert*-butyl ester was dissolved in CH_2Cl_2 (4 mL) and TFA (1 mL). The mixture was stirred at room temperature for 6 h and then the solvent was removed in vacuo to give the desired carboxylic acid **29e** (200 mg, tris-trifluoroacetate salt). LC−MS (m/e): 392 $[M - H]^+$.

A mixture of the unpurified **29e** (194 mg), HOBt (83 mg, 0.616 mmol), EDC (118 mg, 0.616 mmol), TEA (0.127 mL, 0.920 mmol), and NH_2OTHP (44 mg, 0.38 mmol) in DMF (10 mL) was stirred at room temperature for 5 h. The resulting solution was diluted with water and extracted with AcOEt and CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH from 97:3:0.1 to 95:5:0.2), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 3 h. The precipitate was filtered and purified by preparative HPLC to give the desired hydroxamic acid **30e** (9 mg, 1% from **16e**, tris-trifluoroacetate salt). ^{1}H NMR (DMSO-d_6+TFA) δ: 11.20 (bs, 1 H), 9.09 (d, J = 2.05 Hz, 1 H), 8.50−8.60 (m, 1 H), 8.47 (dd, J = 8.07, 2.20 Hz, 1 H), 8.15−8.22 (m, 1 H), 8.21 (d, J = 15.85 Hz, 1 H), 7.86−7.93 (m, 1 H), 7.83 (d, J = 15.85 Hz, 1 H), 7.70 (d, J = 7.92 Hz, 1 H), 7.67 (t, J = 7.63 Hz, 1 H), 7.54 (d, J = 15.26 Hz, 1 H), 7.03 (d, J = 15.55 Hz, 1 H), 4.44 (bs, 2 H), 3.09−3.91 (m, 8 H), 2.84 (s, 3 H). LC−MS (m/e): 407 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(5-{(*E*)-3-[4-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (30f). A mixture of **27** (261 mg, 1.12 mmol), **16f** (246 mg, 1.12 mmol), and 1.7 M KOH (125 mg, 2.24 mmol) in EtOH (10 mL) was stirred at room temperature for 16 h. The formation of a precipitate could be observed, and the solid was then filtered off to obtain the *tert*-butyl ester **28f** (222 mg, 45%). **28f** (222 mg, 0.513 mmol) was then treated with TFA (2 mL) in CH_2Cl_2 (5 mL) at room temperature for 5 h. The solvent was evaporated in vacuo to dryness to give the desired carboxylic acid **29f** (310 mg, quantitative, bis-trifluoroacetate). A mixture of **29f** (310 mg, 0.51 mmol), NH_2OTHP (78 mg, 0.67 mmol), EDC (155 mg, 0.81 mmol), HOBt (109 mg, 0.80 mmol), and TEA (280 μL, 2 mmol) in THF (5 mL) and DMF (5 mL) was stirred for 72 h at room temperature. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with water, then with brine, dried over Na_2SO_4, and evaporated in vacuo to dryness. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH/NH_3, 96:4:0.2). The obtained intermediate was dissolved in CH_2Cl_2 and treated with HCl in Et_2O for 3 h to obtain a dark-brown solid. The solid was then filtered off and washed with CH_2Cl_2 to obtain the requisite hydroxamic acid **30f** (156 mg, 66%, dihydrochloride salt). ^{1}H NMR (DMSO-d_6) δ: 11.37 (bs, 1 H), 9.09 (d, J = 1.76 Hz, 1 H), 8.53 (dd, J = 8.22, 2.05 Hz, 1 H), 8.15 (d, J = 15.85 Hz, 1 H), 8.13 (m, 2 H), 7.81 (d, J = 8.22 Hz, 1 H), 7.73 (d, J = 15.55 Hz, 1 H), 7.56 (d, J = 15.55 Hz, 1 H), 7.12 (m, 2 H), 7.08 (d, J = 15.55 Hz, 1 H), 4.03−4.19 (m, 2 H), 3.44−3.55 (m, 2 H), 3.26−3.42 (m, 2 H), 3.01−3.22 (m, 2 H), 2.80 (d, J = 4.69 Hz, 3 H). LC−MS (m/e): 393 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(5-{(*E*)-3-[4-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (30g). KOH (1.7 M, 1.54 mL) was added dropwise to a stirred mixture of **27** (610 mg, 2.62 mmol) and **16g** (608 mg, 2.62 mmol) in THF (20 mL). The resulting solution was stirred at room temperature overnight and then diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by chromatographic column (petroleum ether/AcOEt, then CH_2Cl_2/MeOH/NH_4OH, 485:15:1) to give the *tert*-butyl ester **28g** (620 mg, 53%). LC−MS (m/e): 448 $[M - H]^+$.

28g (620 mg, 1.39 mmol) was dissolved in CH_2Cl_2 (4.5 mL) and TFA (1.5 mL), and the mixture was stirred at room temperature for 6 h. Then the solvent was removed in vacuo to give the desired carboxylic acid **29g** as its tris-trifluoroacetate salt (1.02 g). A mixture of **29g** (1.02 g), HOBt (375 mg, 2.78 mmol), EDC (531 mg, 2.78 mmol), TEA (0.576 mL, 4.17 mmol), and NH_2OTHP (146 mg, 1.28 mmol) in DMF (18 mL) was then stirred at room temperature for 5 h. The resulting solution was diluted with water and extracted with AcOEt and CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH from 485:15:1 to 480:20:1), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 3 h. The precipitate was filtered, washed with CH_2Cl_2, and freeze-dried to give the desired hydroxamic acid **30g** (85.3 mg, 12% from **28g**, tris-hydrochloride salt). ^{1}H NMR (DMSO-d_6 + TFA) δ: 11.20 (bs, 1 H), 9.06 (d, J = 2.18 Hz, 1 H), 8.42 (dd, J = 8.21, 2.25 Hz, 1 H), 8.23 (m, 2 H), 8.12 (d, J = 15.70 Hz, 1 H), 7.80 (d, J = 15.85 Hz, 1 H), 7.78 (m, 2 H), 7.71 (d, J = 8.16 Hz, 1 H), 7.53 (d, J = 15.36 Hz, 1 H), 7.02 (d, J = 15.42 Hz, 1 H), 4.31 (bs, 2 H), 2.95−3.76 (m, 8 H), 2.82 (s, 3 H). LC−MS (m/e): 407 $[M - H]^+$.

***tert*-Butyl (*E*)-3-(3-Formylphenyl)acrylate (32a).** 3-Bromobenzaldehyde (**31a**, 5.0 g, 27 mmol) was dissolved in DMF (70 mL) and TEA (8.5 mL, 61 mmol). PPh_3 (354 mg, 1.35 mmol), $Pd(OAc)_2$ (121 mg, 0.54 mmol), $NaHCO_3$ (4.5 g, 54 mmol), and *tert*-butyl acrylate (4.0 mL, 27 mmol) were added under N_2 atmosphere, and the solution was heated to 100 °C for 3 h. Additional $Pd(OAc)_2$ (60 mg, 0.26 mmol) was added, and after 1 h at 100 °C the slurry was partitioned between water and Et_2O. The organic layer was dried over Na_2SO_4, evaporated in vacuo, and purified by column chromatography (petroleum ether/AcOEt, 95:5) to give the desired intermediate **32a** as a pale-yellow oil (3.5 g, 56%). ^{1}H NMR (DMSO-d_6) δ: 10.04 (s, 1 H), 8.24 (t, J = 1.76 Hz, 1 H), 8.04 (dt, J = 7.70, 1.28 Hz, 1 H), 7.92 (dt, J = 7.63, 1.32 Hz, 1 H), 7.65 (d, J = 16.14 Hz, 1 H), 7.64 (t, J = 7.63 Hz, 1 H), 6.65 (d, J = 16.14 Hz, 1 H), 1.50 (s, 9 H).

(*E*)-*N*-Hydroxy-3-[3-((*E*)-3-oxo-3-phenyl-1-propen-1-yl)phenyl]-acrylamide (35a). The hydroxamic acid **35a** was prepared according to the procedure described for compound **19a**. Yield: 105 mg (64%). ^{1}H NMR (DMSO-d_6) δ: 10.77 (s, 1H), 9.03 (bs, 1H), 8.15−8.22 (m, 2H), 8.11−8.18 (m, 1H), 8.01 (d, J = 15.55 Hz, 1H), 7.87 (d, J = 7.63 Hz, 1H), 7.77 (d, J = 15.85 Hz, 1H), 7.66−7.73 (m, 1H), 7.44−7.67 (m, 5H), 6.58 (d, J = 15.85 Hz, 1H). LC−MS (m/e): 294 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(3-{(*E*)-3-[2-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (35b). A mixture of **32a** (220 mg, 0.95 mmol), **16b** (200 mg, 0.92 mmol), and 1.7 M KOH (102 mg, 1.82 mmol) in EtOH (5 mL) and H_2O (1 mL) was stirred at room temperature for 12 h. The mixture was then partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was dissolved in CH_2Cl_2 (2 mL) and TFA (1 mL), and the resulting solution was stirred for 6 h at room temperature. The solvent was then removed in vacuo to give the carboxylic acid **34b** (0.220 g, 49% from **32a**, trifluoroacetate salt). **34b** (220 mg, 0.45 mmol), HOBt (95 mg, 0.702 mmol), EDC (134 mg, 0.70 mmol), TEA (0.245 mL, 1.75 mmol), and NH_2OTHP (68 mg, 0.58 mmol) were dissolved in THF (5 mL) and DMF (1 mL), stirred for 12 h at room temperature, and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo, and the crude product was purified by column chromatography (AcOEt to AcOEt/MeOH, 9:1). The resulting compound was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h. The precipitate was filtered and purified by preparative HPLC to obtain the hydroxamic acid **35b** as its trifluoroacetate salt (53 mg, 23%). ^{1}H NMR (DMSO-d_6) δ: 10.75 (bs, 1 H), 9.69 (bs, 1 H), 9.05 (s, 1 H), 7.98 (s, 1 H), 7.79 (d, J = 7.34 Hz, 1 H), 7.65 (d, J = 7.63 Hz, 1 H), 7.43−7.60 (m, 6 H), 7.29 (d, J = 7.63 Hz, 1 H), 7.21 (td, J = 7.50, 0.88 Hz, 1 H), 6.55 (d, J = 15.85 Hz, 1 H), 3.23−3.53 (m, 4 H), 3.02−3.22 (m, 2 H), 2.84−3.00 (m, 2 H), 2.71 (s, 3 H). LC−MS (m/e): 392 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(3-{(*E*)-3-[2-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (35c). KOH (1.7 M, 0.634 mL) was added dropwise to a stirred mixture of **16c** (250 mg, 1.08 mmol) and **32a** (250 mg, 1.08 mmol) in EtOH (15 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 4 h and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (petroleum ether/AcOEt, 7:3, and then CH_2Cl_2/MeOH/NH_4OH, 95:5:0.1) to give the *tert*-butyl acrylate **33c** (384 mg, 80%). LC−MS (m/e): 447 $[M - H]^+$.

The *tert*-butyl acrylate **33c** (384 mg, 0.861 mmol) was dissolved in a mixture of TFA (1.5 mL) and CH_2Cl_2 (6 mL) and stirred for 6 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in DMF (15 mL). HOBt (232 mg, 1.72 mmol), EDC (328 mg, 1.71 mmol), TEA (0.239 mL, 1.72 mmol), and NH_2OTHP (121 mg, 1.03 mmol) were added, and the resulting mixture was stirred at room temperature for 5 h. Water was added, and the product was extracted with AcOEt followed by CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 97:3:0.1), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h. The precipitate was filtered, crystallized from *i*-PrOH/MeOH/diisopropyl ether and then purified by preparative HPLC to give the desired hydroxamic acid **35c** (124 mg, 23% from **33c**, bis-trifluoroacetate salt). ^{1}H NMR (DMSO-d_6, 353 K) δ: 7.91 (s, 1 H), 7.72 (d, J = 7.63 Hz, 1 H), 7.59−7.68 (m, 3 H), 7.56 (td, J = 7.34, 1.47 Hz, 1 H), 7.41−7.53 (m, 4 H), 7.37 (d, J = 16.14 Hz, 1 H), 6.65 (d, J = 15.55 Hz,

1 H), 3.97 (s, 2 H), 3.08−3.36 (m, 4 H), 2.93 (bs, 4 H), 2.69 (s, 3 H). LC−MS (*m*/*e*): 406 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(3-{(*E*)-3-[3-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (35d). A solution of **16d** (350 mg, 1.6 mmol) in EtOH (5 mL) was added within 2 h to a stirred mixture of **32a** (372 mg, 1.6 mmol) and 1.7 M KOH (89 mg, 1.6 mmol) in EtOH (10 mL) at −20 °C. The resulting mixture was stirred at room temperature overnight and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was dissolved in CH_2Cl_2 (10 mL) and TFA (2 mL), and the resulting solution was stirred at room temperature for 6 h. The solvent was then removed in vacuo and the residue was triturated with Et_2O to give 308 mg of the carboxylic acid **34d** as its trifluoroacetate salt. The crude **34d** (308 mg) was then dissolved in DMF (7 mL), and HOBt (170 mg, 1.25 mmol), EDC (240 mg, 1.25 mmol), TEA (262 μL, 1.88 mmol), and NH_2OTHP (88 mg, 0.75 mmol) were added. The mixture was stirred overnight at room temperature and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo, and the crude reaction mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 98:2:0.2). The resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 1 h. The precipitate was filtered and purified by preparative HPLC, providing the requisite hydroxamic acid **35d** (24.7 mg, 3% from **32a**, trifluoroacetate salt). ^{1}H NMR (DMSO-d_6 + TFA) δ: 9.74 (bs, 1 H), 8.10 (s, 1 H), 7.96 (d, J = 15.55 Hz, 1 H), 7.87 (d, J = 7.34 Hz, 1 H), 7.75 (d, J = 15.85 Hz, 1 H), 7.59−7.71 (m, 3 H), 7.40−7.58 (m, 3 H), 7.34 (dd, J = 7.92, 2.35 Hz, 1 H), 6.57 (d, J = 15.85 Hz, 1 H), 3.87−4.08 (m, 2 H), 3.40−3.70 (m, 2 H), 2.97−3.30 (m, 4 H), 2.89 (s, 3 H). LC−MS (*m*/*e*): 392 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(3-{(*E*)-3-[3-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (35e). KOH (1.7 M, 0.634 mL) was added dropwise to a stirred mixture of **16e** (250 mg, 1.08 mmol) and **32a** (250 mg, 1.08 mmol) in EtOH (15 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 4 h and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH from 485:15:1 to 465:35:1) to give the *tert*-butyl acrylate **33e** (330 mg, 69%). LC−MS (*m*/*e*): 447 $[M - H]^+$.

33e (330 mg, 0.740 mmol) was dissolved in a mixture of TFA (1 mL) and CH_2Cl_2 (4 mL) and stirred for 6 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in DMF (15 mL). HOBt (200 mg, 1.48 mmol), EDC (283 mg, 1.48 mmol), TEA (0.206 mL, 1.48 mmol) and NH_2OTHP (104 mg, 0.888 mmol) were added, and the resulting mixture was stirred at room temperature overnight. Then water was added, and the product was extracted with AcOEt followed by CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH from 485:15:1 to 475:25.1), then dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h. The precipitate was filtered, crystallized from *i*-PrOH/MeOH/diisopropyl ether and purified by preparative HPLC to give the desired hydroxamic acid **35e** (83.7 mg, 18% from **33e**, bis-trifluoroacetate salt). ^{1}H NMR (DMSO-d_6) δ: 8.12−8.18 (m, 1 H), 8.09 (dt, J = 7.34, 1.76 Hz, 1 H), 7.98−8.05 (m, 1 H), 7.87 (d, J = 15.55 Hz, 1 H), 7.82 (dt, J = 8.22, 1.47 Hz, 1 H), 7.74 (d, J = 15.55 Hz, 1 H), 7.71 (dt, J = 7.34, 1.47 Hz, 1 H), 7.64 (dt, J = 7.70, 1.28 Hz, 1 H), 7.59 (t, J = 7.78 Hz, 1 H), 7.53 (d, J = 15.55 Hz, 1 H), 7.50 (t, J = 7.63 Hz, 1 H), 6.67 (d, J = 15.55 Hz, 1 H), 3.96 (s, 2 H), 3.22−3.41 (m, 4 H), 2.88−3.05 (m, 4 H), 2.79 (s, 3 H). LC−MS (*m*/*e*): 406 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(3-{(*E*)-3-[4-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (35f). A mixture of **32a** (425 mg, 1.83 mmol), **16f** (400 mg, 1.83 mmol), and 1.7 M KOH (202 mg, 3.60 mmol) in EtOH (5 mL) and H_2O (1 mL) was stirred at room temperature for 6 h. The mixture was diluted with water, and the resulting precipitate was filtered off and dried. The solid was dissolved in CH_2Cl_2 (4 mL) and TFA (2 mL), and the solution was stirred at room temperature overnight. The solvent was then removed in vacuo and the product was triturated with diisopropyl ether to give the carboxylic acid **34f** (0.360 g, trifluoroacetate salt). **34f** (150 mg) was then dissolved in THF (5 mL) and DMF (1 mL). HOBt (65 mg, 0.47 mmol), EDC (90 mg, 0.47 mmol), TEA (0.166 mL, 1.19 mmol), and NH_2OTHP (46 mg, 0.40 mmol) were added, and the mixture was stirred at room temperature overnight. The mixture was partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo, and the crude product was purified by column chromatography (AcOEt to AcOEt/MeOH, 9:1). The resulting intermediate (70 mg, yellow powder) was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h. The precipitate was filtered and triturated with MeOH/H_2O to give the hydroxamic acid **35f** (35 mg, 11% from **32a**, hydrochloride salt). ^{1}H NMR (DMSO-d_6 + TFA) δ: 10.36 (bs, 1 H), 8.07−8.17 (m, 3 H), 8.00 (d, J = 15.55 Hz, 1 H), 7.85 (d, J = 7.63 Hz, 1 H), 7.69 (d, J = 15.55 Hz, 1 H), 7.63 (d, J = 7.63 Hz, 1 H), 7.52 (d, J = 15.85 Hz, 1 H), 7.49 (t, J = 7.63 Hz, 1 H), 7.12 (m, 2 H), 6.58 (d, J = 15.85 Hz, 1 H), 4.01−4.22 (m, 2 H), 3.38−3.69 (m, 2 H), 3.04−3.32 (m, 4 H), 2.85 (s, 3 H). LC−MS (*m*/*e*): 392 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(3-{(*E*)-3-[4-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}phenyl)acrylamide (35g). A mixture of **32a** (209 mg, 0.90 mmol), **16g** (194 mg, 0.83 mmol), and 1.7 M KOH (0.512 mL) in EtOH (10 mL) was stirred at 0−4 °C overnight and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by chromatographic column (CH_2Cl_2/MeOH/NH_4OH, 97:3:0.1) to give the *tert*-butyl acrylic ester **33g** (148 mg, 40%). ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1 H), 8.07 (m, 2 H), 7.89 (d, J = 15.85 Hz, 1 H), 7.78−7.84 (m, 1 H), 7.72 (d, J = 15.85 Hz, 1 H), 7.66−7.73 (m, 1 H), 7.60 (d, J = 15.85 Hz, 1 H), 7.41−7.53 (m, 3 H), 6.61 (d, J = 15.85 Hz, 1 H), 3.59 (s, 2 H), 2.42−2.48 (m, 4 H), 2.33−2.41 (m, 4 H), 2.20 (s, 3 H), 1.53 (s, 9 H).

33g (148 mg, 0.33 mmol) was dissolved in a mixture of TFA (0.50 mL) and CH_2Cl_2 (1 mL) and stirred at room temperature for 4 h. The solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (3 mL) and DMF (1 mL). HOBt (81 mg, 0.6 mmol), EDC (115 mg, 0.59 mmol), TEA (0.167 mL, 1.2 mmol), and NH_2OTHP (70 mg, 0.6 mmol) were added, and the resulting mixture was stirred at room temperature overnight. CH_2Cl_2 was removed and the residue partitioned between water and AcOEt. The organic layer was washed with water, dried over Na_2SO_4, and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 95:5:0.1), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 3 h. The precipitate was filtered, crystallized from *i*-PrOH, and then triturated with MeOH to give hydroxamic acid **35g** (54 mg, 34% from **33g**, bis-hydrochloride). ^{1}H NMR (DMSO-d_6, 353 K) δ: 8.13 (m, 2 H), 8.03 (t, J = 1.17 Hz, 1 H), 7.87 (d, J = 15.85 Hz, 1 H), 7.81 (dt, J = 7.63, 1.17 Hz, 1 H), 7.74 (d, J = 15.85 Hz, 1 H), 7.69 (m, 2 H), 7.60−7.66 (m, 1 H), 7.53 (d, J = 15.85 Hz, 1 H), 7.50 (t, J = 7.63 Hz, 1 H), 6.67 (d, J = 16.14 Hz, 1 H), 4.05 (s, 2 H), 3.22−3.49 (m, 4 H), 2.96−3.21 (m, 4 H), 2.78 (s, 3 H). LC−MS (*m*/*e*): 406 $[M - H]^+$.

***tert*-Butyl (*E*)-3-(6-Formylpyridin-2-yl)acrylate (32b).** Three batches of 6-bromopyridine-2-carbaldehyde (**31b**, 1.0 g, 5.4 mmol), *tert*-butyl acrylate (2.1 g, 16 mmol), 1,4-diazabicyclo[2.2.2]octane (DABCO, 25 mg, 0.22 mmol), K_2CO_3 (734 mg, 5.31 mmol), Bu_4NBr (1.73 g, 5.38 mmol), and $Pd(OAc)_2$ (25 mg, 0.11 mmol) in DMF (10 mL) were heated by microwave irradiation at 120 °C for 5 h. The mixtures were combined and partitioned between water and Et_2O. The organic layer was washed with water, dried over Na_2SO_4, evaporated to dryness, and purified by column chromatography (petroleum ether/AcOEt, 95:5) to afford **32b**

(2.8 g, 57%). ^{1}H NMR ($CDCl_3$) δ: 10.08 (d, J = 0.61 Hz, 1 H), 7.80−7.97 (m, 2 H), 7.65 (d, J = 15.55 Hz, 1 H), 7.62 (dd, 1 H), 6.98 (d, J = 15.69 Hz, 1 H), 1.56 (s, 9 H).

(*E*)-*N*-Hydroxy-3-[6-((*E*)-3-oxo-3-phenyl-1-propen-1-yl)pyridin-2-yl]acrylamide (35h). 16a (116 μL, 1.0 mmol) was added dropwise to a solution of **32b** (240 mg, 1.0 mmol) and 1.7 M KOH (0.90 mL) in EtOH (5 mL), cooled to −15 °C. The mixture was kept at −15 °C overnight and then at 0 °C for a further 3 h. The solution was then diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated to dryness, and the crude product was purified by column chromatography (petroleum ether/AcOEt, 8:2). LC−MS (m/e): 336 $[M - H]^+$.

The *tert*-butyl ester **33h** was dissolved in CH_2Cl_2 (4 mL) and TFA (1 mL) and stirred for 6 h at room temperature. Then the solvent was removed in vacuo and the resulting white powder was triturated with diisopropyl ether to give the acrylic acid **34h** (96 mg, trifluoroacetate salt). HOBt (37 mg, 0.27 mmol), EDC (56 mg, 0.29 mmol), TEA (103 μL, 0.74 mmol), and NH_2OTHP (29 mg, 0.25 mmol) were added to 93 mg of **34h** dissolved in THF/DMF (1:1, 10 mL). The mixture was stirred for 12 h at room temperature and then partitioned between water and AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, evaporated in vacuo, and purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH, 98:2:0.2). The resulting oil was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 4 h. The precipitate was filtered to give the desired hydroxamic acid **35h** (40 mg, 12% from **32b**, hydrochloric salt). ^{1}H NMR (DMSO-d_6) δ: 8.17 (d, J = 15.55 Hz, 1 H), 8.07−8.15 (m, 2 H), 7.96 (t, J = 7.34 Hz, 1 H), 7.90 (d, J = 7.04 Hz, 1 H), 7.55−7.79 (m, 5 H), 7.54 (d, J = 15.55 Hz, 1 H), 7.09 (d, J = 15.26 Hz, 1 H). LC−MS (m/e): 295 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(6-{(*E*)-3-[2-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (35i). A solution of **32b** (200 mg, 0.86 mmol), **16b** (187 mg, 0.86 mmol), and 1.7 M KOH (0.504 mL) in THF (10 mL) was stirred at room temperature overnight and then partitioned between water and AcOEt. The aqueous layer was extracted with AcOEt, and the combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 97:3:0.2 to 96:4:0.2) to give the *tert*-butyl ester **33i** (240 mg, 64%). ^{1}H NMR (DMSO-d_6) δ: 8.01 (d, J = 15.85 Hz, 1 H), 7.91 (t, J = 7.92 Hz, 1 H), 7.66−7.73 (m, 2 H), 7.59 (d, J = 15.55 Hz, 1 H), 7.58 (d, J = 15.55 Hz, 1 H), 7.48−7.55 (m, 2 H), 7.22 (d, J = 9.10 Hz, 1 H), 7.09−7.15 (m, 1 H), 6.91 (d, J = 15.55 Hz, 1 H), 3.00−3.05 (m, 2 H), 2.89−2.98 (m, 2 H), 2.35−2.46 (m, 4 H), 2.08 (s, 3 H), 1.53 (s, 9 H).

33i (240 mg, 0.55 mmol) was dissolved in a mixture of TFA (1 mL) and CH_2Cl_2 (4 mL) and stirred at room temperature for 8 h. The solvents were removed in vacuo, and the residue was dissolved in DMF (15 mL). HOBt (150 mg, 1.11 mmol), EDC (212 mg, 1.11 mmol), and TEA (0.231 mL, 1.66 mmol) were added, and the resulting solution was stirred for 10 min. NH_2OTHP (77.8 mg, 0.665 mmol) was added, and the mixture was stirred at room temperature for 7 h. Further NH_2OTHP (7.78 mg, 0.067 mmol) was added, and the solution was stirred at room temperature overnight and then partitioned between water and CH_2Cl_2. The aqueous phase was washed twice with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 97:3:0.2 to 95:5:0.2). The resulting product was dissolved in CH_2Cl_2 and treated with Et_2O/HCl for 4 h. The precipitate was filtered and freeze-dried to give the desired hydroxamic acid **35i** (40 mg, 16% from **33i**, bishydrochloride). ^{1}H NMR (DMSO-d_6) δ: 10.92 (bs, 1 H), 10.58 (bs, 1 H), 7.96 (d, J = 15.55 Hz, 1 H), 7.95 (t, J = 7.63 Hz, 1 H), 7.74−7.81 (m, 1 H), 7.47−7.70 (m, 5 H), 7.29−7.37 (m, 1 H), 7.24 (t, J = 7.48 Hz, 1 H), 7.04 (d, J = 15.26 Hz, 1 H), 3.30−3.55 (m, 4 H), 3.12−3.30 (m, 2 H), 2.82−3.01 (m, 2 H), 2.58 (d, J = 4.40 Hz, 3 H). LC−MS (m/e): 393 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(6-{(*E*)-3-[2-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (35j). A solution of **16c** (110 mg, 0.47 mmol) in THF (4 mL) was added dropwise within 30 min to a stirred solution of **32b** (110 mg, 0.47 mmol) and 1.7 M KOH (0.276 mL) in THF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The solution was partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 98:2:0.1) to give the *tert*-butyl ester **33j** (135 mg, 64%). LC−MS (m/e): 448 $[M - H]^+$.

33j (135 mg, 0.30 mmol) was dissolved in a mixture of TFA (0.456 mL) and CH_2Cl_2 (1 mL) and stirred at room temperature for 4 h. The solvents were evaporated to dryness and the residue was dissolved in CH_2Cl_2 (4 mL) and TEA (0.581 mL, 4.18 mmol). EDC (145 mg, 0.756 mmol), HOBt (103 mg, 0.76 mmol), and NH_2OTHP (66.7 mg, 0.570 mmol) were added, and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 93:7:0.1) and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 1.5 h. The precipitate was filtered off, triturated with *i*-PrOH, and purified by preparative HPLC to afford the requisite hydroxamic acid **35j** (36 mg, 16% from **33j**, tristrifluoroacetate). ^{1}H NMR (DMSO-d_6 + TFA) δ: 10.92 (bs, 1 H), 9.47 (bs, 1 H), 7.92 (t, J = 7.63 Hz, 1 H), 7.79 (d, J = 7.34 Hz, 1 H), 7.41−7.68 (m, 7 H), 7.30 (d, J = 15.85 Hz, 1 H), 7.00 (d, J = 15.55 Hz, 1 H), 3.75 (bs, 2 H), 3.24−3.40 (m, 2 H), 2.73−3.00 (m, 4 H), 2.71 (s, 3 H), 2.30−2.45 (m, 2 H). LC−MS (m/e): 407 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(6-{(*E*)-3-[3-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (35k). A solution of **16d** (137 mg, 0.63 mmol) in THF (10 mL) was added dropwise within 30 min to a stirred solution of **32b** (146 mg, 0.63 mmol) and 1.7 M KOH (370 μL) in THF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The solution was partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 98:2:0.1) to give the *tert*-butyl ester **33k** as an orange solid (188 mg, 69%). LC−MS (m/e): 434 $[M - H]^+$.

33k (188 mg, 0.434 mmol) was then dissolved in a mixture of TFA (1.5 mL) and CH_2Cl_2 (3 mL) and stirred at room temperature for 2 h. The solvents were evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (4 mL) and TEA (0.800 mL, 5.80 mmol). EDC (198 mg, 1.03 mmol), HOBt (140 mg, 1.04 mmol), and NH_2OTHP (100 mg, 0.854 mmol) were added, and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 97:3:0.1), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h. The precipitate was filtered, triturated with *i*-PrOH, and purified by preparative HPLC to give the desired hydroxamic acid **35k** (27 mg, 10% from **33k**, bistrifluoroacetate salt). ^{1}H NMR (DMSO-d_6) δ: 10.94 (bs, 1 H), 9.80 (bs, 1 H), 8.12 (d, J = 15.55 Hz, 1 H), 7.96 (t, J = 7.34 Hz, 1 H), 7.91 (dd, J = 7.63, 1.17 Hz, 1 H), 7.71 (d, J = 15.55 Hz, 1 H), 7.55-7.67 (m, 3 H), 7.51 (d, J = 2.93 Hz, 1 H), 7.51 (d, J = 15.85 Hz, 1 H), 7.37 (dd, J = 8.36, 1.91 Hz, 1 H), 7.06 (d, J = 15.26 Hz, 1 H), 3.90−4.12 (m, 2 H), 3.43-3.64 (m, 2 H), 3.14-3.33 (m, 2 H), 2.98−3.12 (m, 2 H), 2.89 (s, 3 H). LC−MS (m/e): 393 $[M - H]^+$.

(*E*)-*N*-Hydroxy-3-(6-{(*E*)-3-[3-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (35l). A mixture of **16e** (200 mg, 0.85 mmol), **32b** (200 mg, 0.85 mmol), and 1.7 M

KOH (0.504 mL) in THF (10 mL) was stirred at room temperature overnight and then partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 97:3:0.2) to give the *tert*-butyl ester **33l** (300 mg, 78%). ^{1}H NMR (DMSO-d_6) δ: 8.06 (d, J = 15.55 Hz, 1 H), 7.89−8.00 (m, 3 H), 7.81−7.88 (m, 1 H), 7.71−7.77 (m, 1 H), 7.67 (d, J = 15.55 Hz, 1 H), 7.60 (d, J = 15.85 Hz, 1 H), 7.49−7.62 (m, 2 H), 6.89 (d, J = 15.85 Hz, 1 H), 3.61 (s, 2 H), 2.35−2.47 (m, 8 H), 2.22 (s, 3 H), 1.54 (s, 9 H).

33l (300 mg, 0.671 mmol) was then dissolved in a mixture of TFA (1 mL) and CH_2Cl_2 (4 mL) and stirred at room temperature for 5 h. Additional TFA (1 mL) was added, and the mixture was stirred for further 4 h. The solvents were removed in vacuo, and the residue was dissolved in DMF (15 mL). EDC (256 mg, 1.33 mmol), HOBt (181 mg, 1.34 mmol), and TEA (0.280 mL, 2.01 mmol) were added. After 10 min NH_2OTHP (94.1 mg, 0.804 mmol) was added, and the resulting mixture was stirred overnight at room temperature. Further NH_2OTHP (63 mg, 0.54 mmol) was added in two portions over 24 h, and then the resulting solution was partitioned between water and CH_2Cl_2. The aqueous layer was extracted three times with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH from 97:3:0.2 to 96:4:0.2), and the resulting product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 4 h. The precipitate was filtered, rinsed with CH_2Cl_2, and purified by preparative HPLC to give the hydroxamic acid **35l** (30 mg, 6% from **33l**, tris-trifluoroacetate salt). ^{1}H NMR (DMSO-d_6+TFA) δ: 8.15 (d, J = 15.55 Hz, 1 H), 8.06−8.14 (m, 2 H), 7.96 (t, J = 7.78 Hz, 1 H), 7.89 (d, J = 7.63 Hz, 1 H), 7.74 (d, J = 15.55 Hz, 1 H), 7.60−7.74 (m, 3 H), 7.55 (d, J = 15.85 Hz, 1 H), 7.05 (d, J = 15.26 Hz, 1 H), 3.95 (s, 2 H), 3.05−3.99 (m, 8 H), 2.81 (s, 3 H). LC−MS (m/e): 407 [M − H]$^+$.

(*E*)-*N*-Hydroxy-3-(6-{(*E*)-3-[4-(4-methylpiperazin-1-yl)phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (35m). KOH (1.7 M, 0.580 mL) was added dropwise to a stirred mixture of **16f** (215 mg, 0.987 mmol) and **32b** (229 mg, 0.987 mmol) in EtOH (15 mL) at 0 °C. The resulting solution was stirred at 0 °C for 8 h, diluted with water, and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 10:4:0.2) to give a mixture (0.34 g) of *tert*-butyl (*E*)-3-(6-{1-hydroxy-3-[4-(4-methylpiperazin-1-yl)phenyl]-3-oxopropyl}pyridin-2-yl)acrylate and **33m**. The mixture was dissolved in CH_2Cl_2 (10 mL) and TFA (2 mL), and the resulting solution was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was dissolved in THF (10 mL) and 1.7 M KOH (2.64 mL). The solution was stirred at room temperature for 1 h and then acidified with HCl/Et_2O and evaporated in vacuo. The resulting solid was washed with water and filtered to give the carboxylic acid **34m** (0.14 g, bis-hydrochloride).

EDC (118 mg, 0.62 mmol), HOBt (100 mg, 0.62 mmol), TEA (0.258 mL, 1.86 mmol), and NH_2OTHP (44 mg, 0.37 mmol) were added to a solution of **34m** (140 mg) in DMF (5 mL). The mixture was stirred at room temperature for 6 h, then diluted with water and extracted with AcOEt and CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The resulting crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 96:4:0.2). The product was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 2 h. The precipitate was filtered and rinsed with CH_2Cl_2 and Et_2O to give the desired hydroxamic acid **35m** (68 mg, 15% from **32b**, bis-hydrochloride). ^{1}H NMR (DMSO-d_6, 353 K, +TFA) δ: 8.08 (d, J = 15.85 Hz, 1 H), 8.00−8.05 (m, 2 H), 7.90 (t, J = 7.78 Hz, 1 H), 7.78 (d, J = 7.63 Hz, 1 H), 7.63 (d, J = 15.55 Hz, 1 H), 7.58 (dd, J = 7.78, 0.73 Hz, 1 H), 7.52 (d, J = 15.55 Hz, 1 H), 7.02−7.18 (m, 3 H), 3.75 (bs, 4 H), 3.34 (bs, 4 H), 2.84 (s, 3 H). LC−MS (m/e): 393 [M − H]$^+$.

(*E*)-*N*-Hydroxy-3-(6-{(*E*)-3-[4-(4-methylpiperazin-1-ylmethyl)-phenyl]-3-oxo-1-propen-1-yl}pyridin-2-yl)acrylamide (35n). A solution of **16g** (189 mg, 0.815 mmol) in THF (8 mL) was added dropwise within 30 min to a stirred solution of **32b** (190 mg, 0.815 mmol) and 1.7 M KOH (0.48 mL) in THF (20 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The solution was partitioned between water and AcOEt, and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 96:4:0.1) to give the *tert*-butyl ester **33n** (245 mg, 67%). ^{1}H NMR ($CDCl_3$) δ: 8.16 (d, J = 15.26 Hz, 1 H), 8.06 (m, 2 H), 7.77 (t, J = 7.63 Hz, 1 H), 7.76 (d, J = 15.26 Hz, 1 H), 7.64 (d, J = 15.55 Hz, 1 H), 7.51 (m, 2 H), 7.39−7.47 (m, 2 H), 6.97 (d, J = 15.55 Hz, 1 H), 3.62 (s, 2 H), 2.55 (bs, 8 H), 2.35 (s, 3 H), 1.59 (s, 9 H).

33n (245 mg, 0.548 mmol) was dissolved in a mixture of TFA (3 mL) and CH_2Cl_2 (10 mL) and stirred at room temperature for 2 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (6 mL) and TEA (1.1 mL, 8.14 mmol). EDC (283 mg, 1.47 mmol), HOBt (200 mg, 1.48 mmol), and NH_2OTHP (86.6 mg, 0.74 mmol) were added, and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was partitioned between water and AcOEt. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 96:4:0.1), and the resulting intermediate was dissolved in CH_2Cl_2 and treated with HCl/Et_2O for 3 h. The precipitate was filtered, rinsed with CH_2Cl_2, and purified by preparative HPLC to give the hydroxamic acid **35n** (14 mg, 4% from **33n**, tris-trifluoroacetate salt). ^{1}H NMR (DMSO-d_6 + TFA) δ: 8.16 (d, J = 15.26 Hz, 1 H), 8.13 (m, 2 H), 7.96 (t, J = 7.63 Hz, 1 H), 7.89 (d, J = 7.04 Hz, 1 H), 7.72 (d, J = 15.26 Hz, 1 H), 7.65 (d, J = 7.63 Hz, 1 H), 7.58 (m, 2 H), 7.54 (d, J = 15.55 Hz, 1 H), 7.06 (d, J = 15.26 Hz, 1 H), 3.86 (s, 2 H), 2.88−3.55 (m, 8 H), 2.81 (s, 3 H). LC−MS (m/e): 407 [M − H]$^+$.

HDAC Enzyme Assay. The in vitro activity of HDAC inhibitors was assayed using the HDAC fluorescent histone deacetylase activity assay kit (Biomol Research Laboratories, Plymouth Meeting, PA). Briefly, 15 μL of a nuclear extract from HeLa cells was diluted to 50 μL with the assay buffer containing the HDAC inhibitor and the substrate (peptide containing acetylated lysine) at 200 μM. The samples were incubated for 15 min at room temperature and then exposed to a developer for a further 10 min. Substrate fluorescence was measured using an excitation at 355 nm and emission at 460 nm, and the % inhibition was calculated relative to untreated samples. IC_{50} values were determined using a regression analysis of the concentration/inhibition data.

Cell Growth Assay. The effect of the HDAC inhibitors on cell proliferation was measured using an adaptation of published procedures.[39] K562 cells (derived from human lymphoma) were incubated for 72 h with different concentrations of the inhibitors. Then 5 mg/mL of MTT in PBS was added at different time points and the solution was incubated for 3−4 h. All incubations were carried out at 37 °C. The supernatant was then removed, the formazan crystals were dissolved in a mixture of DMSO and absolute EtOH (1:1, v:v), and the absorption of the solution was measured at a wavelength between 550 and 570 nm. IC_{50} values are determined using a regression analysis of the concentration/inhibition data.

Metabolic Stability in Hepatic Microsomes. By adaptation of the protocols described by Di et al.,[40] the compounds at 1 μM were preincubated for 10 min at 37 °C in potassium phosphate buffer (pH 7.4) together with 0.5 mg/mL mouse or human hepatic microsomes (Xenotech, Kansas City). The cofactor mixture comprising NADP, G6P, and G6P-DH was added, and aliquots were taken after 0 and 30 min. Samples were analyzed on an Acquity UPLC, coupled with a sample organizer, and interfaced with a triple quadrupole Premiere XE (Waters, Milford, MA). Mobile phases consisted of a phase A

[0.1% formic acid in a mixture of water and acetonitrile (95:5, v:v)] and phase B [0.1% formic acid in a mixture of water and acetonitrile (5:95, v:v)]. Separations were achieved at 40 °C on Acquity BEH C18 columns (50 mm × 2.1 mm × 1.7 μm with a flow rate of 0.45 mL/min or 50 mm × 1 mm × 1.7 μm with a flow rate of 0.2 mL/min). The column was conditioned with 2% of phase B for 0.2 min, then brought to 100% of phase B within 0.01 min and maintained at these conditions for 1.3 min. The operating parameters of the mass spectrometer were set as follows: capillary voltage 3.4 kV, source termperature 115 °C, desolvation temperature 450 °C, desolvation gas flow 900 L/h, cell pressure 3.3×10^{-3} mbar. Cone voltage and collision energy were optimized for each compound. LC−MS/MS analyses were carried out using a positive electrospray ionization (ESI(+)) interface in MRM (multiple reaction monitoring) mode with verapamil as internal standard. The percentage of the compound remaining after a 30 min incubation period was calculated according the following equation: [(area at time 30 min)/(area at time 0 min)] × 100%.

Histone Acetylation Assay. K562 cells were incubated with the compounds at a final concentration of 0.5 μM for 3 h, then fixed with 1% formaldehyde in PBS and permeabilized with a solution containing 0.1% Triton X-100 in PBS. After being washed, the cells were preincubated with 10% goat serum in PBS for 30 min at 4 °C, exposed to a monoclonal antibody against acetylated histones for 1 h at room temperature, and then incubated for 1 h with a secondary antibody conjugated with FITC. Histone acetylation levels were measured by cytofluorometry (FACS) and expressed as ratio of the acetylation level of treated versus untreated cells.[31]

In Vivo Drug Pharmacokinetic Studies. Pharmacokinetic experiments were performed using 4 week old male nude CD-1 mice (Charles River Laboratories, Calco, Italy). Animals were quarantined for approximately 1 week prior to the study. They were housed under standard conditions and had free access to water and standard laboratory rodent diet.

Compound **1** was dissolved in a mixture of 1% DMSO and 10% Encapsin in water at a concentration of 1 mg/mL for the iv (rapid bolus) administration or 5% DMSO and 1% PEG400 in water for the oral (gavage) dose at a concentration of 3 mg/mL. Compounds **19b**, **30b**, **30c**, **30e**, **30g**, **35**c, **35i**, **35k**, and **35n** were dissolved in normal saline solution at a concentration of 1 mg/mL for the iv formulation and in water for the oral administration at 3 mg/mL of the base. Each experimental group contained 27−30 animals. The compounds were administered to mice either by iv or oral route, and blood samples were collected after different time points after dosing. Plasma was separated immediately after blood sampling by centrifugation, plasma proteins were precipitated using Sirocco filtration plates or Oasis HLB elution plates according to the distributor instructions, and the plasma samples were kept frozen (−80 °C) until submission to LC/MS/MS analysis. Sample analysis was performed on an Acquity UPLC using either a Acquity BEH C18 column (50 mm × 2.1 mm × 1.7 μm) or a Acquity HSS T3 column (50 mm × 2.1 mm × 1.8 μm), coupled with a sample organizer and interfaced to a triple quadrupole Premiere XE (Waters, Milford, MA). The mass spectrometer was operated using electrospray interface (ESI) with a capillary voltage of 3−4 kV, cone voltage of 25−52 V, source temperature of 115−120 °C, desolvation gas flow of 800 L/h, and desolvation temperature of 450−480 °C. Collision energy was optimized for each compound. LC−MS/MS analyses were carried out using a positive electrospray ionization (ESI(+)) interface in MRM (multiple reaction monitoring) mode.

Pharmacokinetic parameters were calculated by a noncompartmental method using WinNolin 5.1 software (Pharsight, Mountain View, CA).

Induction of apoptosis. Induction of apoptosis was examined by measuring the activities of caspase-3 and caspase-7 in K562 cells, using the caspase-GLO3/7 assay (Promega, Madison, WI) according to the manufacturer's instructions.

K562 cells, plated at 10^6/well in 96-multiwell plates, were incubated for 24 h with the compounds at a final concentration of 3 μM, or solvent (DMSO) prior to direct lysis by caspase-Glo 3/7 reagent. The luminescence produced, determined by the cleavage of the substrate by the activated caspases, is expressed as relative light unit (RLU), and results are expressed as the mean and the standard deviation of triplicate data.

In Vivo Efficacy Experiments. For in vivo antitumor efficacy studies, 5 million human cancer cells (HCT-116) were injected subcutaneously in the flank of female CD-1 nude mice (aged 8−10 weeks, Charles River Laboratories, Wilmington, MA). Tumors were allowed to grow until a volume of around 100 mm^3 was reached. Mice bearing a tumor xenograft were randomized into treated and control groups of 7 animals per group. HDAC inhibitors were dissolved in vehicles (compound **1** in a mixture of 45% PEG400, 45% H_2O, and 10% DMSO and compound **30b** in PBS) and administered daily for 5 days per week for 2 weeks.

Tumor volumes were measured regularly by vernier caliper during the experiments, and body weight was monitored three times a week. Dimensions of the tumors were calculated using the equation $V = 0.5ab^2$, where a and b are the longest and shortest diameter, respectively,[41] and are expressed as mm^3. The reported tumor volumes are expressed as the mean ± SEM.

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Supporting Information Available: Synthesis and characterization of compounds **16b**, **16c**, **16e**, and **16g**; purity of key compounds as determined by HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

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