Bioorganic & Medicinal Chemistry 16 (2008) 7992–8002

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of novel compounds with conjugated structure as anti-tumor agents

Hong Su [a], Angela Nebbioso [b], Vincenzo Carafa [b], Yadong Chen [a], Bo Yang [c], Lucia Altucci [b,*], Qidong You [a,*]

[a] Department of Medicinal Chemistry, China Pharmaceutical University, People's Republic of China
[b] Dipartimento di Patologia generale, Seconda Università degli Studi di Napoli, Italy
[c] Department of Pharmacology, School of Pharmaceutical Science, Zhejiang University, People's Republic of China

ARTICLE INFO

Article history:
Received 12 June 2008
Revised 22 July 2008
Accepted 23 July 2008
Available online 29 July 2008

Keywords:
Histone deacetylase
HDAC inhibitor
Hydroxamic acids
Anti-tumor

ABSTRACT

A series of hydroxamic acids with conjugated structure was designed and synthesized to explore the possible HDAC subtype selectivity by testing these compounds against recombinant human HDAC1 and HDAC4. The most selective compound resulted **5a**, with a SI of 11.9. The enzymatic inhibitory activity of these conjugated compounds was relatively weak; however, some of these compounds showed significant effect in inducing apoptosis. Moreover, the anti-proliferative activity in cancer cells resulted quite promising, especially in the HCT119 cell line.

1. Introduction

The correlation between histones acetylation and gene transcription has been recognized for many years.[1] The acetylation status of histones is determined by histone deacetylases (HDACs) and histone acetyltransferases (HATs). HATs transfer acetyl groups to amino-terminal lysine residues on histones, leading to local expansion of chromatin and increased accessibility of regulatory proteins to DNA,[2] whereas HDACs catalyze the removal of acetyl groups, resulting in chromatin condensation and transcriptional repression.[3] Recently, many non-histone proteins including transcription factors, transcription regulators, signal transduction mediators, DNA repair enzymes have been identified to be subject to acetylation,[4] further suggesting the importance of acetylation in the upstream regulation of gene transcription. HDACs are widely recognized as promising targets for therapeutic intervention in cancer.[3,5,6] More than 80 clinical trials are underway, testing different HDAC inhibitors in malignant diseases from rare leukemias and lymphomas to breast, prostate and ovarian cancers.[7] Half of these inhibitors have the hydroxamic acid moiety, a typical zinc-binding group. One of them, SAHA, is the first HDAC inhibitor that has been approved by the FDA for the second line treatment of cutaneous T-cell lymphoma (CTCL). However, hydroxamic acids usually have metabolic and pharmacokinetic problems such as glucoronidation and sulfation that may result in a short half-life in vivo.[8]

Oxamflatin (**1**, Fig. 1) was originally identified as a compound inducing the morphological reversion of *v*-K-*ras*-transformed NIH3T3 cells from a chemical library. Only later it was shown to be a potent HDAC inhibitor (IC_{50} = 15.7 nM).[9] CG1521 (**2**, Fig. 1), which is orally available, was reported to have anti-proliferative effects in cancer cell lines, displaying also an in vivo activity, causing a 50% reduction in mean tumor volume after intraperitoneal administration. The HDAC inhibitory activity of CG1521 was mod-

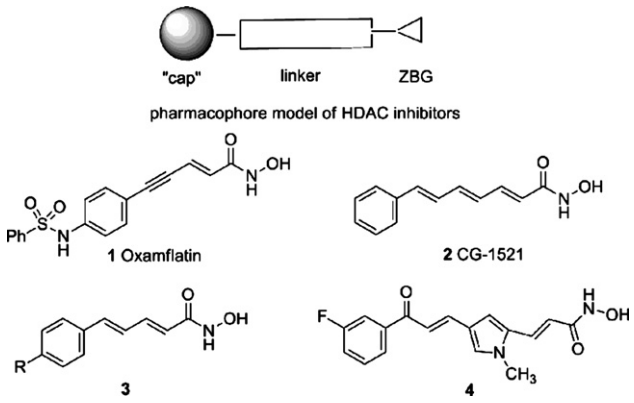


Figure 1. Pharmacophore model of HDAC inhibitors and HDAC inhibitors with stereodefined structures.

* Corresponding authors. Tel.: +39 081 566 7569; fax: +39 081 2144840 (L.A.); tel./fax: +86 025 83141351 (Q.Y.).
E-mail addresses: lucia.altucci@unina2.it (L. Altucci), youqidong@gmail.com (Q. You).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.07.066

erate (IC_{50} = 1.8 μM). The structure of (2*E*,4*E*)-5-arylpenta-2,4-dienoic acid hydroxyamides (**3**, Fig. 1) is similar to **2**, and they act as HDAC inhibitors with moderate inhibitory potency.[10] These findings confirmed that the stereochemical rigidity seems to be advantageous for the enzyme inhibition.

On the other hand, Mai and co-workers have disclosed a series of aroyl-pyrrolyl-hydroxy-amides (APHAs).[11] Some APHAs showed distinct selectivity against HD1-B and HD1-A, two maize deacetylases that are homologous of mammalian class I and class IIa HDACs, respectively. The most selective APHAs is **4** (Fig. 1), and its selective ratio is 176.4. As hydroxamates generally lack selectivity due to their strong zinc-binding ability, and also because of the rarity of class II selective HDAC inhibitors reported so far, we got interested in the selectivity of APHAs. We speculated that the conjugated structure may contribute to the selectivity for its rigid structure directing the 'cap' group of the inhibitor to interact with the rim of the catalytic pocket of the HDAC enzymes. To further exploration the inhibitory activity of hydroxamic acids characterized with conjugated stereodefined structure against mammalian HDACs, as well as their anti-proliferative activity at the cellular level, we designed and synthesized compounds **5a**–**j** and **6a**–**h**.

The structures of compounds **5a**–**j** are quite similar to APHAs, the main difference lying in the aromatic linker group. The furan ring in **5a**–**j** is smaller than *N*-methyl pyrrole ring, and most importantly, the substitutes on the furan ring are at 2,5-position, while the substitutes on the pyrrole ring are at 2,4-position. Thus, the rigid backbones in compound **4** and compounds **5a**–**j** could direct the cap group of these compounds to interact with the rim of the catalytic pocket of the enzyme in a relative different configuration. The structures of **6a**–**h** are similar to that of **3**, with a furan ring connecting the hydroxamic acid group. We presumed that **6a**–**h** with more bulky furan ring could make more favored contacts with the stacked phenyl groups of the two Phe residues than **3**.

We initially carried out plasma stability in vitro studies, validating the positive effect of conjugated structures on the stability of the hydroxamic acid group. The cellular effects of these compounds on apoptosis, granulocytic differentiation, and cell cycle progression in human acute myeloid leukemia (AML) U937 cells have also been assessed. The inhibitory activity of these compounds against human HDAC1 and HDAC4, considered as representative of class I and class IIa HDACs, respectively, was further tested to evaluate their selectivity profile. As direct measure of the anti-proliferative effects in vitro, as well as indirect measure of cell permeability, the anti-proliferative cytotoxic activity was also evaluated by using four different tumor cell lines. Finally, the most effective compounds were tested for their ability to affect acetylation levels and expression of $p21^{WAF1/CIP1}$ taken as histone and non-histone targets.

2. Chemistry

As shown in the Scheme 1, synthesis of compounds **5a**–**j** started from preparation of (*E*)-ethyl 3-(furan-2-yl)acrylate **8** by reacting furfural **7** with triethyl phosphonacetate in absolute ethanol containing anhydrous potassium carbonate. (*E*)-Ethyl 3-(5-formylfuran-2-yl)acrylate **9** was prepared by Vilsmeier-Hacck reaction. Aldol condensation with acetophenone or substituted acetophenone gave the corresponding highly conjugated (*E*)-Ethyl 3-(5-(un)substituted-styrylfuran-2-yl)acrylates, which were hydrolyzed without separation and purification to give **10a**–**j**. Further reaction with oxalyl chloride and hydroxylamine gave the desired hydroxamates **5a**–**j**.

For compounds **6a**–**h**, the starting material was Methyl furan-2-carboxylate **12**, which was chloromethylated. The resulted methyl 5-(chloromethyl) furan-2-carboxylate **13** was converted into the corresponding triphenyl phosphonium salt **14**, which was treated with benzaldehyde or substituted benzaldehyde to give the corresponding (*E*)-methyl 5-(un)substituted-styrylfuran-2-carboxylates **15a**–**h**. The Methyl esters were hydrolyzed; further reaction with oxalyl chloride and hydroxylamine gave the desired hydroxamates **6a**–**h** (Scheme 2).

3. Results and discussion

We chose compounds **5j** and **6a** as representatives to study. Both **5j** and **6a** have neither electro-withdrawing nor electro-donating substitutes, thereby eliminating the possible influence of substitutes on stability.

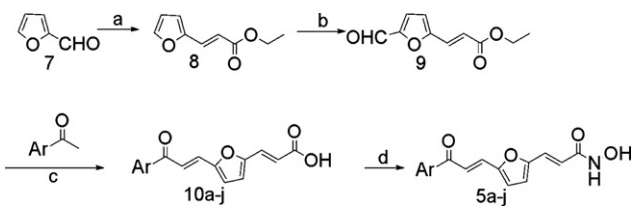
As shown in Figures 2A and B, both **5j** and **6a** show significant increase in plasma stability. **5j** seems more stable than **6a** for its highly conjugated structure.

Then, we analyzed the effect of **5a**–**j** and **6a**–**h** on cell cycle, differentiation, and apoptosis in the human acute myeloid leukemia U937 cell line.

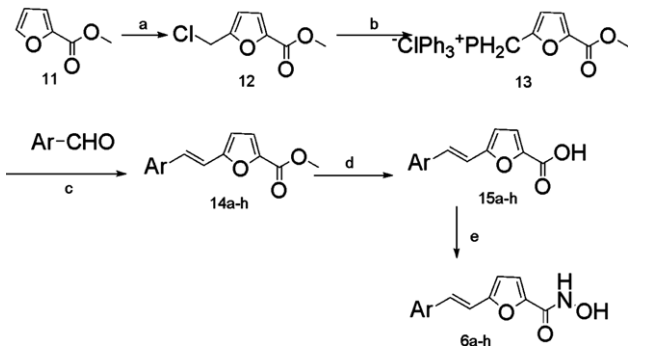
As shown in Figure 3, **5a**, **5e**, **5h**, and **5j** significantly induced accumulation of the cells in the S phase, whereas **5b**–**d**, **5f**, **5g**, and **5i** induced accumulation of the cells in the G_1 phase. **5a**, **5c**, **5e**, **5f**, **5h**, and **5i** were able to induce apoptosis. The effect of **5a**–**j** on differentiation could not be evaluated for the interference of their fluorescence with CD11c FACS analysis.

Compounds **6a**–**c** induce higher percentage of apoptosis (Fig. 4B), whereas they only moderately increase differentiation after 30 h of treatment (Fig. 4A), as assessed by the analysis of the expression of CD11c, a marker for granulocytic maturation; the expression of the CD14, a marker for monocytic differentiation was also measured but did not show signal (data not shown) thus indicating that these compounds did not induce monocytic differentiation in these experimental conditions.

Compound **6f**, **6d**, **6e**, **6g** and **6h** induced high toxicity in the cells at the concentration of 5 μM, thereby the concentration of these compounds was diluted to 0.5 μM and the effects were eval-



Scheme 1. Synthesis of compounds **5a**–**j**[α]. Reagents and conditions: (a) $(C_2H_5)_2P(O)CH_2CO_2C_2H_5$, K_2CO_3, EtOH; (b) $POCl_3$, DMF; (c) KOH, EtOH, H_2O; (d) 1—$(COCl)_2$, DMF, CH_2Cl_2, 0 °C; 2—$NH_2OH{\cdot}HCl$, Et_3N, THF, H_2O.



Scheme 2. Synthesis of compounds **6a**–**h**[α]. Reagents and conditions: (a) paraformaldehyde, $ZnCl_2$, HCl, CH_2Cl_2, rt; (b) PPh_3, CH_2Cl_2, reflux; (c) Et_3N, CH_2Cl_2; (d) 2 N NaOH, EtOH, H_2O; (e) 1—$(COCl)_2$, DMF, CH_2Cl_2, 0 °C; 2—$NH_2OH{\cdot}HCl$, Et_3N, THF, H_2O.

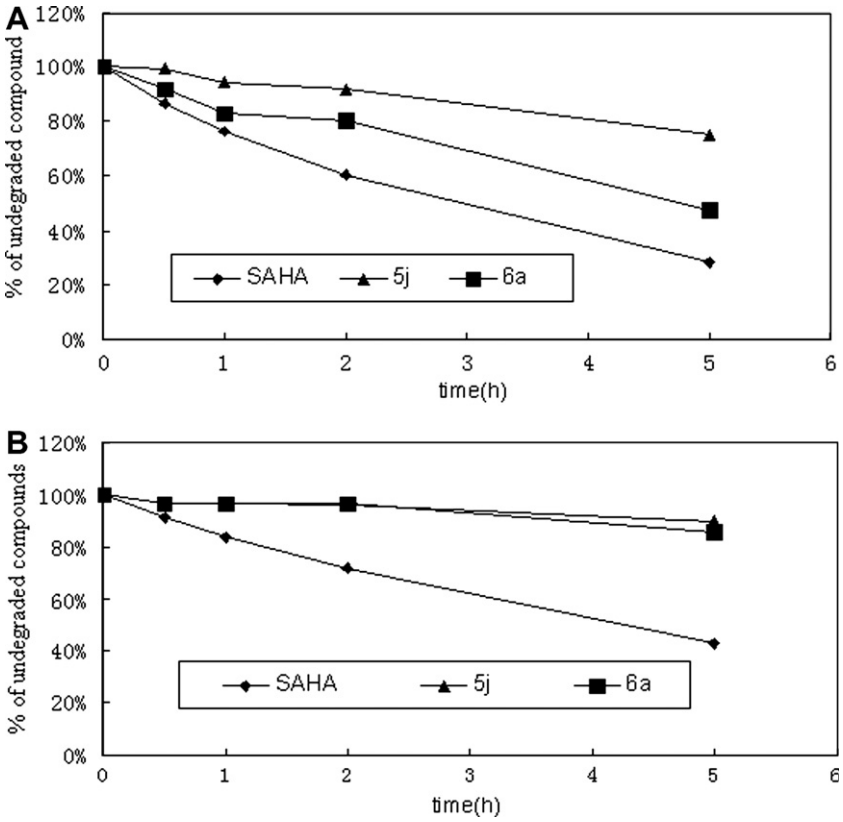


Figure 2. (A) Stability comparison of the indicated compounds in rat plasma at 25 μg/mL. (B) Stability comparison of the indicated compounds in plasma at 100 μg/mL.

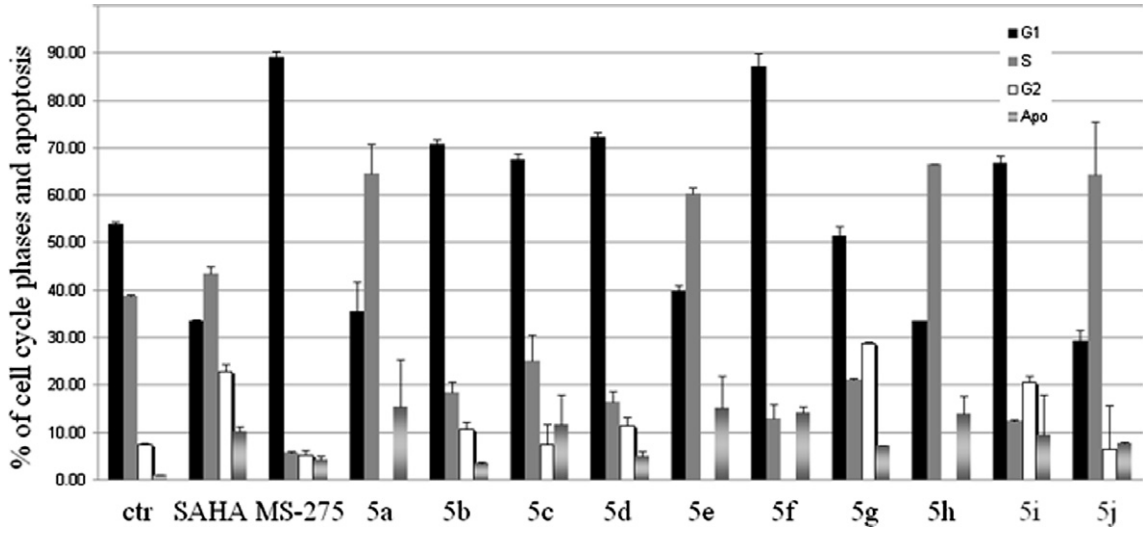


Figure 3. Analysis of cell cycle and apoptosis in U937 cells treated with the indicated compounds at 5 μM for 24 h. The data represent the media of independent duplicates.

uated. Compounds **6d** and **6e** showed no effect on cell cycle, differentiation and apoptosis after dilution. Compounds **6g** and **6h** were able to increase differentiation significantly after 28 h of treatment at the concentration of 0.5 μM (Fig. 5A), and compound **6h** could also induce apoptosis after 28 h of treatment (Fig. 5B). Note that in all differentiation assays PI positive cells have been excluded from the analysis as specified.

We further performed enzymatic assays to evaluate the activity on human recombinant HDAC1 and HDAC4, taken as markers for class I and class II HDACs, respectively. As to the inhibitory potency against HDAC4 (Table 1), compound **5a** is the most potent inhibitor, with IC_{50} of 1.10 μM, whereas compound **5i** is the weakest inhibitor, with IC_{50} of 22.78 μM. The only structural difference between **5a** and **5j** is the substitute in position C_4 of the benzene ring, and the inhibitory potency of compounds **5a**, **5d**, **5i**, and **5j** changed regularly. Their inhibitory potency against HDAC4 increased when the substitutes (H, F, Cl and methyl) in position C_4 of the benzene ring changed bigger, indicating a shallow groove may exist around the rim of the catalytic pocket of HDAC4 and the substitutes in position C_4 of the benzene ring should fit into this groove. The inhibitory potency of compound **5b** with a much bigger substitute (*iso*-propyl) in position C_4 of the benzene ring decreased slightly

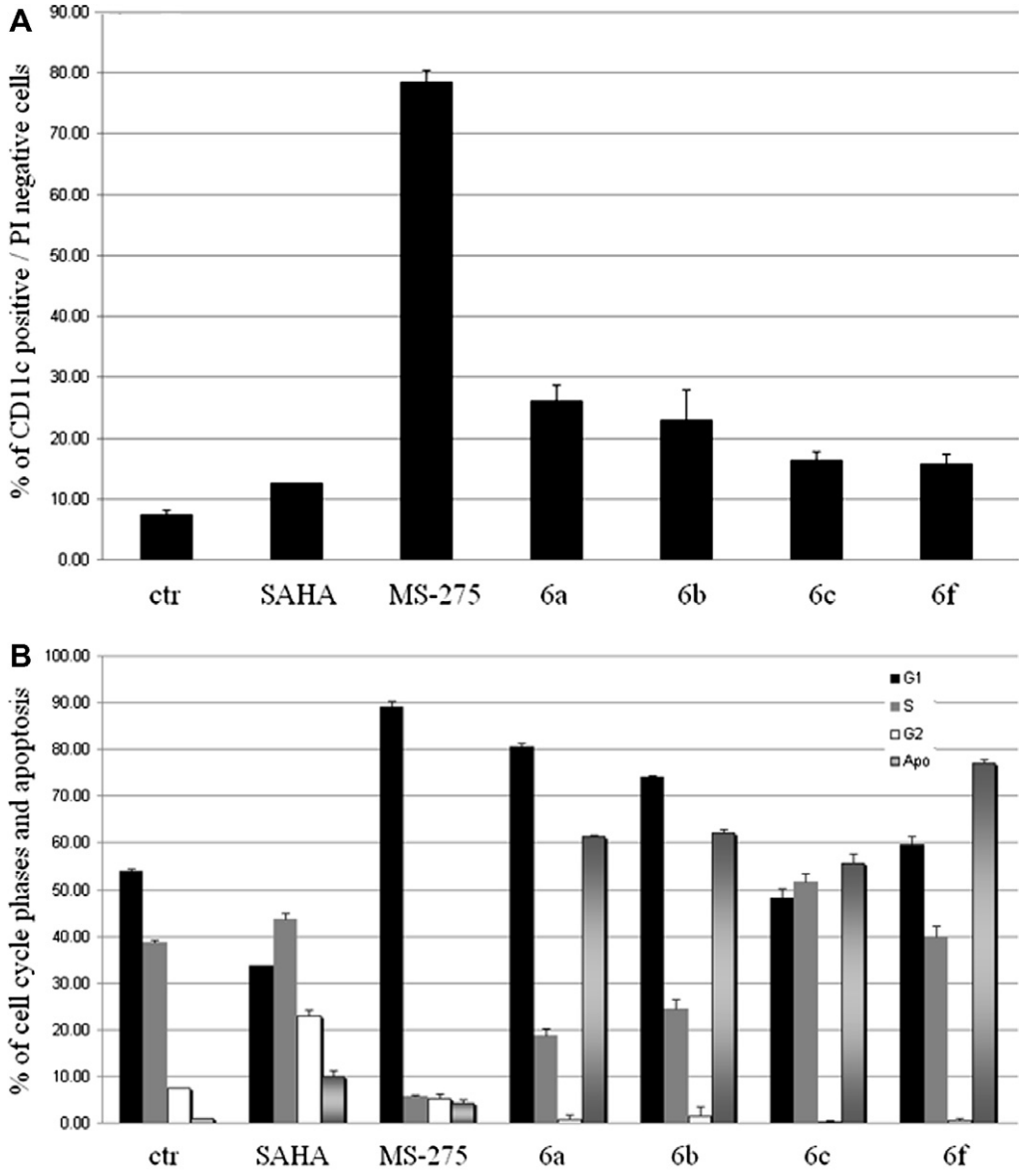


Figure 4. (A) Differentiation analysis in U937 cells after 30 h of treatment with the indicated compounds at 5 μM. The data shown represent the media of independent quadruplicates. (B) Analysis of cell cycle and apoptosis in U937 cells treated with the indicated compounds at 5 μM for 30 h. The data represent the media of independent duplicates.

against HDAC4, compared with compound **5a**, suggesting a strict size restriction of the groove. The inhibitory potency of compounds **5a**, **5d**, **5i**, and **5j** against HDAC1 did not change regularly, verifying the structural difference around the rim of the catalytic pockets between HDAC1 and HDAC4. Compounds **5g** and **5h** bearing two chlorine substitutes in the benzene ring had better inhibitory potency than both **5c** and **5d** bearing only one chlorine substituted in the benzene ring both against HDAC1 and HDAC4. Compound **5e** bearing substituted naphthyl ring was more selective than compound **5f** bearing un-substituted naphthyl ring.

The enzymatic inhibitory potency and selectivity of **6a**–**h** were weak (Table 2); **6h** bearing two bulky substitutes on the benzene ring was the most potent of this compound series, in correspondence with the SAR of **3**.[10]

All compounds were evaluated for their anti-proliferative cytotoxic activity using the PC3 (prostate carcinoma), HCT116 (colorectal carcinoma), A549 (lung carcinoma) and HEPG2 (hepatocellular carcinoma) (Table 3) tumor cell lines. The majority of the compounds displayed excellent anti-proliferative profiles, showing that improved stability of hydroxamic acids may contribute to biological effects of the molecules. In general, these compounds showed higher anti-proliferative activities against PC3 and HCT116 than against A549 and HEPG2 cells.

Tubulin acetylation was taken as potential marker of HDAC6 inhibition. As shown in Figure 6, **5b**–**j** showed significant effect on inducing the tubulin acetylation level, whereas **6a**–**c**, **6f**–**h** had no effect.

Class I- and pan-HDAC inhibitors usually have the capacity to induce over-expression of $p21^{WAF1/CIP1}$. As shown in Figure 5, **5b**–**j** and **6g** were able to induce the expression of $p21^{WAF1/CIP1}$, whereas **5a** and, **6a**–**f** and **6h** did not show any effect (Fig. 7).

4. Conclusion

We have synthesized and evaluated a series of hydroxamic acids with stereodefined-conjugated structures. **5a**–**j**, **6a**–**c**, **6f**, and **6h** showed significant effect on arresting cell cycle progression. Most of these compounds could induce apoptosis. **6a**–**c**, **6f**,

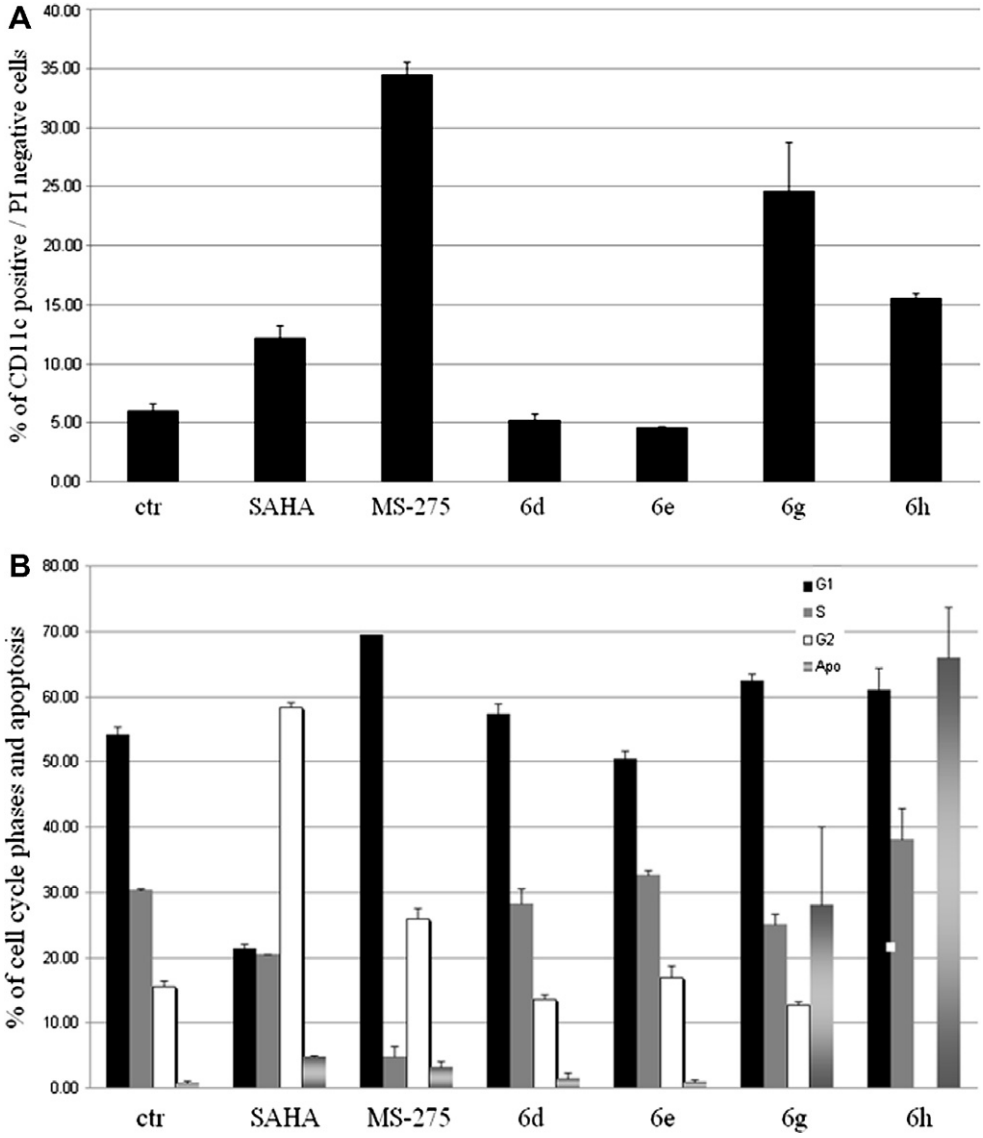


Figure 5. (A) CD11c analysis in U937 cells after 28 h treatment with the indicated compounds. **6d**, **6e**, **6g** and **6h** were analyzed at 0.5 μM while SAHA and MS-275 were at 5μM. The data shown represent the media of independent quadruplicates. (B) Analysis of cell cycle and apoptosis in U937 cells treated with the indicated compounds for 28 h. **6d**, **6e**, **6g** and **6h** were analyzed at 0.5 μM, while SAHA and MS-275 at 5μM. The data represent the media of independent duplicates.

Table 1
HDAC inhibitory activities of compounds **5a**–**j**[a]

Compound	Ar	$IC_{50} \pm SD$ (μM)		SI[b]
		HDAC1	HDAC4	
5a	4-Methyl-Ph	13.20	1.10	11.9
5b	4-*iso*-Propyl-Ph	24.10	4.89	4.9
5c	3-Chloro-Ph	12.60	12.91	1.0
5d	4-Chloro-Ph	15.70	6.03	2.6
5e	2-(6-Methyloxy)-naphthyl	11.04	4.04	2.7
5f	2-Naphthyl	3.77	4.38	0.9
5g	2,5-Dichloro-Ph	4.72	4.31	1.1
5h	2,4-Dichloro-Ph	4.29	8.19	0.5
5i	4-Fluro-Ph	14.98	7.68	0.5
5j	Ph	11.55	22.78	2.0
SAHA	—	0.59	0.55	1.01

[a] Data represent mean values of at least three separate experiments.
[b] SI, selective index, calculated as IC_{50} (HDAC1)/IC_{50} (HDAC4).

Table 2
HDAC inhibitory activities of compounds **6a**–**h**[a]

Compound	Ar	$IC_{50} \pm SD$ (μM)		SI[b]
		HDAC1	HDAC4	
6a	Ph	49.15	47.17	1.04
6b	4-Hydroxyl-Ph	43.1	57.06	0.76
6c	2-Hydroxyl-Ph	47.07	74.24	0.63
6d	[3,4]Dioxol-Ph	29.6	85.6	0.34
6e	2,3-Dichloro-Ph	25.31	49.28	0.51
6f	2-Hydroxyl,3-methyloxy-Ph	50.14	42.81	1.17
6g	4-(*N*,*N*-Dimethyl)-Ph	49.10	50.58	0.97
6h	3-Methyloxy,4-benzyloxy-Ph	13.41	10.25	1.30
SAHA	—	0.59	0.55	1.01

[a] Data represent mean values of at least three separate experiments.
[b] SI, selective index, calculated as IC_{50} (HDAC1)/IC_{50} (HDAC4).

and **6h** also showed promising profile in induction of differentiation. However, the selectivity as well as the inhibitory potency of these compounds against mammalian HDAC1 and HDAC4 resulted limited. We also tested the relatively potent compounds **5f** and **6h** using the commercially available kit AK-500 (BIOMOL), the IC_{50} of **5f** and **6h** are 0.63 μM and 1.89 μM, respectively. In the same assay, the inhibitory IC_{50} of SAHA and MS-275 are 0.042 μM and

Table 3
Anti-proliferative activities of the indicated compounds

Compound	PC3 IC_{50} (μM)	HCT116 IC_{50} (μM)	A549 IC_{50} (μM)	HEPG2 IC_{50} (μM)
5a	16.01	0.54	48.64	16.04
5b	2.27	0.49	56.68	35.90
5c	9.60	1.51	18.92	54.48
5d	1.35	2.01	98.11	6.74
5e	36.27	2.06	—	6.22
5f	15.09	2.22	29.37	9.93
5g	24.70	4.12	27.06	48.27
5h	16.24	6.73	88.11	13.52
5i	40.14	2.82	—	12.00
5j	13.28	2.89	20.15	12.12
6a	40.40	2.49	32.54	12.78
6b	21.78	2.73	86.37	20.92
6c	91.30	5.10	79.15	54.03
6d	45.93	4.98	63.61	33.56
6e	39.25	3.39	23.58	37.20
6f	35.13	—	—	—
6g	3.20	—	46.31	31.69
6h	1.20	—	50.28	30.93
SAHA	8.17	2.69	—	63.48
MS-275	59.06	27.47	—	60.12
Taxol	2.73	6.18	13.05	0.62

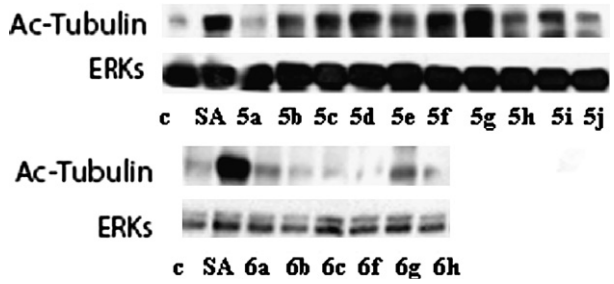


Figure 6. Western blot analyses of α-tubulin acetylation carried out in U937 cells after 24 h of treatment with the indicated compounds. Total ERK levels as account for equal loading control.

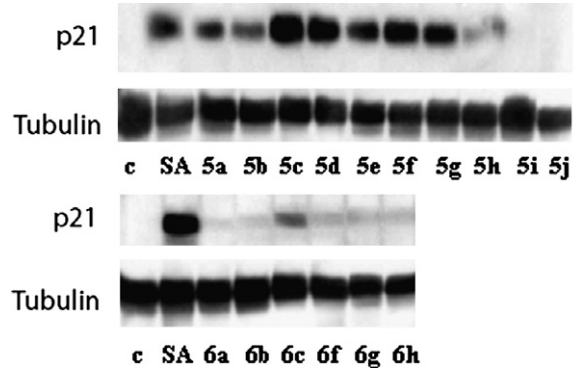


Figure 7. Western blot analysis of $p21^{WAF1/CIP1}$ expression in U937 cells after 24 h treatment with the indicated compounds. Tubulin is used as equal loading control.

2.76 μM, respectively. Thus, the general inhibitory activity of our compounds against HDACs is acceptable. The relative weak inhibitory activities of our compounds were likely due to the unfavorable binding of the stereodefined structure with enzyme catalytic pocket. Comparing the structure of oxamflatin, we could conclude that the shape of the stereodefined structure is very important in HDAC inhibition. Alignment-independent GRIND 3D-QSAR and docking studies on APHAs carried out on homology modeling of maize HD1-A and HD1-B, revealed that in general a bent molecular shape structure is a prerequisite for HD1-A selective inhibitory activity, while straight shape molecular skeleton leads to selective HD1-B compounds.[12] Thus, the shape of the stereodefined structure is also crucial in subtype selectivity. From our enzyme inhibitory data we could deduce that the substitutes on the 'cap' region might also influence the subtype selectivity, high-lightened by the structure and SI relationship of **5a** and **5j**. Although the HDAC inhibitory activity of these compounds is weaker than SAHA, many of these compounds had anti-proliferative activity in several tumor cell lines. This finding might be due to the improved stability of hydroxamic acids, or to off-target effects of these compounds. The existence of 'off-target' effect of **5a**–**h** in tumor cell is compatible with the observation that these compounds had no effects in increasing tubulin acetylation and/or $p21^{WAF1/CIP1}$ levels. Further biological study of these compounds is underway.

5. Experimental

5.1. General

Melting points were determined on a Mel-TEMP II melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^{1}H NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Shimadzu GC–MS 2010 (EI) or a Mariner Mass Spectrum (ESI), or a LC/MSD TOF HR-MS Spectrum. Compound purity was assessed by the reverse phase HPLC (Waters 510, Shimadzu SPD-10A VP, using Hanbang C_{18} 4.6 × 150 mm, 5 μm). All compounds were routinely checked by TLC and ^{1}H NMR. TLCs and preparative thin-layer chromatography were performed on silica gel GF/UV 254, and the chromatograms were performed on silica gel (200–300 mesh) visualized under UV light at 254 and 365 nm. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results are within (0.40% of the theoretical values).

5.1.1. (*E*)-Ethyl 3-(furan-2-yl)acrylate (8)

Furfural (**7**, 0.1 mol, 8.28 mL) in absolute ethanol (10 mL) was added in one portion to a mixture of triethyl 2-phosphonobutyrate (0.12 mol, 23.8 mL) and anhydrous potassium carbonate (0.3 mol, 41.4 g) in absolute ethanol (100 mL). After being stirred at 70 °C for 2 h, the reaction mixture was cooled to room temperature, filtrated and washed with ethanol (2× 20 mL). The filtrate was concentrated and distilled under reduced pressure to give pure yellowish solid (13.6 g) in 82% yield; mp 24–26 °C [lit.[13] 24 °C].

5.1.2. (*E*)-Ethyl 3-(5-formylfuran-2-yl)acrylate (9)

$POCl_3$ (0.13 mol, 11.9 mL) was added to a cooled (0 °C) mixture of (*E*)-ethyl 3-(furan-2-yl)acrylate (0.12 mol, 19.6 g) and DMF (0.13 mol, 10.0 mL) over a period of 20 min. After stirring at 60 °C for 2 h, the mixture was cooled to room temperature and poured onto crushed ice (200 g). The pH of the mixture was adjusted to 7 with 10% NaOH. The resulted precipitate was filtered and purified to give brown solid (19.2 g) in 82.5% yield by crystallization with a mixture of ethanol and water; mp 73.4–74 °C [lit.[14] 74 °C].

5.1.3. Representative procedure for aldol condensation

5.1.3.1. (*E*)-3-(5-((*E*)-3-Oxo-3-*p*-tolylprop-1-enyl)furan-2-yl)acrylic acid (10a). A mixture of **9** (5.0 mmol, 0.97 g), 1-*p*-tolylethanone (5.0 mmol, 0.67 mL), and 2 N KOH (20.0 mmol, 10.0 mL) in ethanol (10 mL)/water (10 mL) was stirred at room temperature for 24 h. Afterward, the solution was poured into water (60 mL) and was made acid with 2 N HCl. The obtained precipitate was fil-

tered and recrystallized to give the pure acid (0.97 g) in 68.5% yield; mp 154–155 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 2.41 (s, 3H), 6.53 (d, 1H, J = 15.5), 6.95 (d, 1H, J = 3.3), 7.19 (d, 1H, J = 3.3), 7.32 (d, 1H, J = 15.5), 7.39 (d, 2H, J = 8.0), 7.52 (d, 1H, J = 15.4), 7.60 (d, 1H, J = 15.4), 7.98 (d, 2H, J = 8.0), 12.4 (s, 1H); MS (ESI) m/z 281 (M−1); Anal. ($C_{17}H_{14}O_4$) C, H.

5.1.3.2. (*E*)-3-(5-((*E*)-3-(4-Isopropylphenyl)-3-oxoprop-1-enyl)-furan-2-yl)acrylic acid (10b). Compound **10b** was synthesized from **9** (1.94 g, 0.01 mol), 1-(4-isopropylphenyl)ethanone (1.62 g, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 19.8% yield (g); mp 160–162 °C; MS (EI) m/z 310 (M^+).

5.1.3.3. (*E*)-3-(5-((*E*)-3-(3-Chlorophenyl)-3-oxoprop-1-enyl)furan-2-yl)acrylic acid (10c). Compound **10c** was synthesized from **9** (1.94 g, 0.01 mol), 1-(3-chlorophenyl)ethanone (1.3 mL, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 66.9% yield; mp 132–134 °C; MS (EI) m/z 302 (M^+).

5.1.3.4. (*E*)-3-(5-((*E*)-3-(4-Chlorophenyl)-3-oxoprop-1-enyl)furan-2-yl)acrylic acid (10d). Compound **10d** was synthesized from **9** (1.94 g, 0.01 mol), 1-(4-chlorophenyl)ethanone (1.3 mL, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 29.8% yield; mp 132–134 °C; MS (EI) m/z 302 (M^+).

5.1.3.5. (*E*)-3-(5-((*E*)-3-(6-Methoxynaphthalen-2-yl)-3-oxoprop-1-enyl)furan-2-yl)acrylic acid (10e). Compound **10e** was synthesized from **9** (1.94 g, 0.01 mol), 1-(6-methoxynaphthalen-2-yl)ethanone (2.0 g, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 17.8% yield; mp 200–202 °C; MS (EI) m/z 348 (M^+).

5.1.3.6. (*E*)-3-(5-((*E*)-3-(Naphthalen-2-yl)-3-oxoprop-1-enyl)furan-2-yl)acrylic acid (10f). Compound **10f** was synthesized from **9** (1.94 g, 0.01 mol), 1-(naphthalen-2-yl)ethanone (1.70 g, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 50.0% yield; mp 190–191 °C; MS (EI) m/z 318 (M^+).

5.1.3.7. (*E*)-3-(5-((*E*)-3-(2,5-Dichlorophenyl)-3-oxoprop-1-enyl)-furan-2-yl)acrylic acid (10g). Compound **10g** was synthesized from **9** (1.94 g, 0.01 mol), 1-(2,5-dichlorophenyl)ethanone (1.41 mL, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 65.5% yield; mp 188–190 °C; MS (EI) m/z 337 (M^+).

5.1.3.8. (*E*)-3-(5-((*E*)-3-(2,4-Dichlorophenyl)-3-oxoprop-1-enyl)-furan-2-yl)acrylic acid (10h). Compound **10h** was synthesized from **9** (1.94 g, 0.01 mol), 1-(2,4-dichlorophenyl)ethanone (1.43 mL, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 56.9% yield; mp 193–194 °C; MS (EI) m/z 337 (M^+).

5.1.3.9. (*E*)-3-(5-((*E*)-3-(4-Flurophenyl)-3-oxoprop-1-enyl)furan-2-yl)acrylic acid (10i). Compound **10i** was synthesized from **9** (1.94 g, 0.01 mol), 1-(4-fluorophenyl)ethanone (1.23 mL, 0.01 mol), and 2 N KOH according to the procedure used to synthesize **10a** in 55.1% yield; mp 196–198 °C; MS (EI) m/z 286 (M^+).

5.1.3.10. (*E*)-3-(5-((*E*)-3-Oxo-3-phenylprop-1-enyl)furan-2-yl)-acrylic acid (10j). Compound **10j** was synthesized from **9** (1.94 g, 0.01 mol), acetophenone (1.2 mL, 0.01 mol) and 2 N KOH according to the procedure used to synthesize **10a** in 39.2% yield (1.89 g); mp 177–178 °C; MS (EI) m/z 268 (M^+).

5.1.4. Representative procedure for conversion of carboxylic acid to hydroxamic acid

5.1.4.1. (*E*)-*N*Hydroxy-3-(5-((*E*)-3-oxo-3-*p*-tolylprop-1-enyl)furan-2-yl)acrylamide (5a). The compounds were prepared according to the analogous literature.[15] Oxalyl chloride (0.65 mL, 7.54 mmol) was added slowly to a cooled (0 °C) solution of **10a** (0.95 g, 3.36 mmol) and DMF (0.26 mL, 3.36 mmol) in CH_2Cl_2 (50 mL). Gas evolution was noted during this addition. After being stirred for 40 min, this solution was added to a solution of hydroxylamine hydrochloride (0.94 g, 13.5 mmol) and triethylamine (2.8 mL, 20.2 mmol) in THF (20 mL)/water (10 mL). After being stirred for an additional 30 min, the mixture was poured into 2 N HCl and extracted with CH_2Cl_2. The organic phase was concentrated and purified by column chromatography on silica gel by eluting with a mixture of CH_2Cl_2 and methanol (1:10) to give the desired product (0.36 g) as yellowish solid in 36.5% yield; mp 145–146 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 2.41 (s, 3H, CH_3-Ar), 6.53 (d, 1H, J = 15.6, vinyl-H), 6.95 (d, 1H, J = 3.4, furan-H), 7.19 (d, 1H, J = 3.5, furan-H), 7.31 (d, 1H, J = 15.6, vinyl-H), 7.39 (d, 2H, J = 8.0, Ar-H), 7.52 (d, 1H, J = 15.4, vinyl-H), 7.60 (d, 1H, J = 15.4, vinyl-H), 7.98 (d, 2H, J = 8.0, Ar-H), 9.11 (s, 1H, CON*H*OH), 10.84 (s, 1H, CONHO*H*); MS (ESI) m/z 298 (M+H); HRMS calcd for $C_{17}H_{15}NO_4$ $[MH]^+$: 298.1073; found, 298.1079; IR (KBr) ν (cm^{-1}) = 3451, 2911, 1648, 1612.

5.1.4.2. (*E*)-*N*-Hydroxy-3-(5-((*E*)-3-(4-isopropylphenyl)-3-oxoprop-1-enyl)furan-2-yl)acrylamide (5b). Compound **5b** was synthesized from **10b** according to the procedure used to synthesize **5a** in 39.2% yield; mp 118–120 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 1.24 (d, 6H, J = 3.0, CH(CH_3)$_2$), 2.30 (m, 1H, CH(CH_3)$_2$), 6.56 (d, 1H, J = 14.9, vinyl-H), 6.96 (d, 1H, J = 3.4, furan-H), 7.18 (d, 1H, J = 3.4, furan-H), 7.30 (d, 1H, J = 14.9, vinyl-H), 7.45 (d, 2H, J = 7.9, Ar-H), 7.52 (d, 1H, J = 15.4, vinyl-H), 7.58 (d, 1H, J = 15.4, vinyl-H), 7.90 (d, 2H, J = 8.0, Ar-H), 9.1 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 324 (M−H); HRMS calcd for $C_{19}H_{19}NO_4$ $[MH]^+$: 326.1386; found, 326.1382; IR (KBr) ν (cm^{-1}) = 3777, 3453, 1797, 1708, 1644.

5.1.4.3. (*E*)-3-(5-((*E*)-3-(3-Chlorophenyl)-3-oxoprop-1-enyl)furan-2-yl)-*N*-hydroxyacrylamide (5c). Compound **5c** was synthesized from **10c** according to the procedure used to synthesize **5a** in 37.3% yield; mp 155–157 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.53 (d, 1H, J = 15.6, vinyl-H), 6.97 (d, 1H, J = 3.6, furan-H), 7.23 (d, 1H, J = 3.6, furan-H), 7.32 (d, 1H, J = 15.6, vinyl-H), 7.60 (d, 1H, J = 7.9, vinyl-H), 7.62 (d, 1H, J = 7.9, vinyl-H), 7.57–8.1 (m, 4H, Ar-H), 9.1 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 316 (M−H); HRMS calcd for $C_{16}H_{12}NO_4Cl$ $[MH]^+$: 318.0527; found, 318.0528; IR (KBr) ν (cm^{-1}) = 3779, 3459, 1818, 1644.

5.1.4.4. (*E*)-3-(5-((*E*)-3-(4-Chlorophenyl)-3-oxoprop-1-enyl)furan-2-yl)-*N*-hydroxyacrylamide (5d). Compound **5d** was synthesized from **10d** according to the procedure used to synthesize **5a** in 37.7% yield; mp 150–151 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.53 (d, 1H, J = 15.2, vinyl-H), 6.97 (d, 1H, J = 3.4, furan-H), 7.23 (d, 1H, J = 3.4, furan-H), 7.26 (d, 1H, J = 15.2, vinyl-H), 7.62 (d, 1H, J = 7.9, vinyl-H), 7.63 (d, 2H, J = 7.1, Ar-H), 7.74 (d, 2H, J = 7.1, Ar-H), 8.04 (d, 1H, J = 7.9, vinyl-H), 9.1 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 318 (M+H); HRMS calcd for $C_{16}H_{12}NO_4Cl$ $[MH]^+$: 318.0527; found, 318.0527; IR (KBr) ν (cm^{-1}) = 3444, 2069, 1904, 1644.

5.1.4.5. (*E*)-*N*-Hydroxy-3-(5-((*E*)-3-(6-methoxynaphthalen-2-yl)-3-oxoprop-1-enyl)furan-2-yl)acrylamide (5e). Compound **5e** was synthesized from **10e** according to the procedure used to synthesize **5a** in 38.2% yield; mp 199–200 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 3.93 (s, 3H, OCH_3), 6.56 (d, 1H, J = 15.6, vinyl-H), 6.97 (d, 1H, J = 3.4, furan-H), 7.20 (d, 1H, J = 3.4, furan-H), 7.33 (d, 1H, J = 15.4, vinyl-H), 7.28–8.73 (m, 8H, naphthalene-H and vinyl-H), 9.1 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 364 (M+H); HRMS calcd for $C_{21}H_{17}NO_5$ $[MH]^+$:

364.1179; found, 364.1176; IR (KBr) ν (cm^{-1}) = 3587, 3466, 1648, 1623, 1548.

5.1.4.6. (*E*)-*N*-Hydroxy-3-(5-((*E*)-3-(naphthalen-2-yl)-3-oxoprop-1-enyl)furan-2-yl)acrylamide (5f). Compound **5f** was synthesized from **10f** according to the procedure used to synthesize **5a** in 35.2% yield; mp 190–191 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.49 (d, 1H, J = 15.5, vinyl-H), 6.94 (d, 1H, J = 3.5, furan-H), 7.15 (d, 1H, J = 3.5, furan-H), 7.22 (d, 1H, J = 15.6, vinyl-H), 7.28 (d, 1H, J = 15.5, vinyl-H), 7.40 (d, 1H, J = 15.6, vinyl-H), 7.58–8.31 (m, 7H, naphthalene-H), 9.1 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 334 (M+H); HRMS calcd for $C_{20}H_{15}NO_4$ $[MH]^+$: 334.1073; found, 334.1073; IR (KBr) ν (cm^{-1}) = 3623, 3571, 3455, 1637, 1552.

5.1.4.7. (*E*)-3-(5-((*E*)-3-(2,5-Dichlorophenyl)-3-oxoprop-1-enyl)-furan-2-yl)-*N*-hydroxyacrylamide (5g). Compound **5g** was synthesized from **10g** according to the procedure used to synthesize **5a** in 39.3% yield; mp 158–159 °C; ^{1}H NMR (DMSO-*d* textsubscript6, 300 MHz, δ ppm) 6.49 (d, 1H, J = 15.6, vinyl-H), 6.89 (d, 1H, J = 15.9, vinyl-H), 6.94 (d, 1H, J = 3.5, furan-H), 7.15 (d, 1H, J = 3.5, furan-H), 7.25 (d, 1H, J = 15.9, vinyl-H), 7.30 (d, 1H, J = 15.6, vinyl-H), 7.56–7.78 (m, 3H, Ar-H), 9.11 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 352 (M+H); HRMS calcd for $C_{16}H_{11}NO_4Cl_2$ $[MH]^+$: 352.0137; found, 352.0132; IR (KBr) ν (cm^{-1}) = 3672, 3629, 3459, 1637.

5.1.4.8. (*E*)-3-(5-((*E*)-3-(2,4-Dichlorophenyl)-3-oxoprop-1-enyl)-furan-2-yl)-*N*-hydroxyacrylamide (5h). Compound **5h** was synthesized from **10h** according to the procedure used to synthesize **5a** in 42.3% yield; mp 162–164 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.49 (d, 1H, J = 15.6, vinyl-H), 6.89 (d, 1H, J = 15.9, vinyl-H), 6.94 (d, 1H, J = 3.4, furan-H), 7.16 (d, 1H, J = 3.4, furan-H), 7.25 (d, 1H, J = 15.9, vinyl-H), 7.29 (d, 1H, J = 15.6, vinyl-H), 7.57–7.79 (m, 3H, Ar-H), 9.11 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 352 (M+H); HRMS calcd for $C_{16}H_{11}NO_4Cl_2$ $[MH]^+$: 352.0137; found, 352.0130; IR (KBr) ν (cm^{-1}) = 3637, 3580, 3451, 1651.

5.1.4.9. (*E*)-3-(5-((*E*)-3-(4-Fluorophenyl)-3-oxoprop-1-enyl)furan-2-yl)-*N*-hydroxyacrylamide (5i). Compound **5i** was synthesized from **10i** according to the procedure used to synthesize **5a** in 37.1% yield; mp 160–161 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.53 (d, 1H, J = 15.5, vinyl-H), 6.96 (d, 1H, J = 3.3, furan-H), 7.20 (d, 1H, J = 3.3, furan-H), 7.31 (d, 1H, J = 15.5, vinyl-H), 7.40 (d, 2H, J = 8.6, Ar-H), 7.54 (d, 2H, J = 15.3, vinyl-H), 7.59 (d, 2H, J = 15.3, vinyl-H), 8.15 (d, 2H, J = 8.6, Ar-H), 9.1 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 300 (M−H); HRMS calcd for $C_{16}H_{12}NO_4F$ $[MH]^+$: 302.0823; found, 302.0822; IR (KBr) ν (cm^{-1}) = 3637, 3594, 3459, 2832, 1669.

5.1.4.10. (*E*)-*N*-Hydroxy-3-(5-((*E*)-3-oxo-3-phenylprop-1-enyl)-furan-2-yl)acrylamide (5j). Compound **5j** was synthesized from **10j** according to the procedure used to synthesize **5a** in 40.6% yield; mp 172–174 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.53 (d, 1H, J = 15.5, vinyl-H), 6.95 (d, 1H, J = 3.3, furan-H), 7.19 (d, 1H, J = 3.3, furan-H), 7.32 (d, 1H, J = 15.5, vinyl-H), 7.51–7.70 (m, 6H, benzene-H and vinyl-H), 8.06 (d, 1H, J = 7.2, vinyl-H), 9.10 (s, 1H, CON*H*OH), 10.8 (s, 1H, CONHO*H*); MS (ESI) m/z 282 (M−H); HRMS calcd for $C_{16}H_{13}NO_4$ $[MH]^+$: 284.0917; found, 284.0909; IR (KBr) ν (cm^{-1}) = 3651, 3615, 3580, 3437, 2918, 1626.

5.1.5. Methyl 5-(chloromethyl)furan-2-carboxylate (12)

Dry HCl gas was induced into the stirred mixture of methyl furan-2-carboxylate (25.2 g, 0.2 mol), paraformaldehyde (6.0 g, 0.2 mol), and $ZnCl_2$ (30.0 g, 0.22 mol) in CH_2Cl_2 (100 mL) for 3 h at 30–35 °C. The reaction mixture was poured in 80 mL water. The mixture was extracted with CH_2Cl_2 (3× 30 mL). The organic phase was combined and dried with anhydrous Na_2SO_4. The solution was evaporated to dryness. The remaining residue was distilled and collected the distillate as colorless oil in 32.3% yield (11.3 g); 29–30 °C [lit.[15] 31–33 °C].

5.1.6. ((5-(Methoxycarbonyl)furan-2-yl)methyl)triphenylphosphonium chloride (13)

Methyl 5-(chloromethyl)furan-2-carboxylate (3.48 g, 20 mmol), and triphenyl phosphine (4.76 g, 20 mmol) were dissolved in anhydrous toluene (20 mL), refluxed for 2 h. The mixture was cooled to room temperature. The precipitate was filtrated, washed with anhydrous toluene (2 mL), and dried to give white powder in 87.5% yield (7.64 g); mp 223–225 °C.

5.1.7. Representative procedure for the Wittig reaction

5.1.7.1. (*E*)-Methyl 5-styrylfuran-2-carboxylate (14a). Benzaldehyde (1.02 mL, 10 mmol) diluted in CH_2Cl_2 (2 mL) was slowly added to the solution of **13** (4.37 g, 10 mmol) and Et_3N (1.66 mL, 12 mmol) in CH_2Cl_2 (10 mL). After stirring under reflux for 2 h, the mixture was cooled to room temperature and added in 30 mL petroleum ether. The resulted precipitate was filtered. The filtrate was concentrated and purified by column chromatography on silica gel by eluting with a mixture of ether acetate and petroleum ether (1:100) to give the desired product in 52.6% yield (1.09 g); mp 102–104 °C; MS (EI) m/z 228 (M^+).

5.1.7.2. (*E*)-Methyl 5-(4-hydroxystyryl)furan-2-carboxylate (14b). Compound **14b** was synthesized from **13** (4.37 g, 10 mmol), 4-hydroxybenzaldehyde (1.22 g, 10 mmol) and Et_3N (1.66 mL, 12 mmol) according to the procedure used to synthesize **14a** in 66.3% yield (1.62 g); mp 180 °C; MS (EI) m/z 244 (M^+).

5.1.7.3. (*E*)-Methyl 5-(2-hydroxystyryl)furan-2-carboxylate (14c). Compound **14c** was synthesized from **13** (4.37 g, 10 mmol), 2-hydroxybenzaldehyde (1.1 mL, 10 mmol) and Et_3N (1.66 mL, 12 mmol) according to the procedure used to synthesize **14a** in 86.8% yield (2.12 g); mp 129–130 °C; MS (EI) m/z 244 (M^+).

5.1.7.4. (*E*)-Methyl 5-(2-(benzo[*d*][1,3]dioxol-5-yl)vinyl)furan-2-carboxylate (14d). Compound **14d** was synthesized from **13** (4.37 g, 10 mmol), benzo[*d*][1,3]dioxole -5-carbaldehyde (1.5 g, 10 mmol) and Et_3N (1.66 mL, 12 mmol) according to the procedure used to synthesize **14a** in 60.1% yield (1.63 g); mp 120–121 °C; MS (EI) m/z 272 (M^+).

5.1.7.5. (*E*)-Methyl 5-(2,3-dichlorostyryl)furan-2-carboxylate (14e). Compound **14e** was synthesized from **13** (4.37 g, 10 mmol), 2,3-dichlorobenzaldehyde (1.75 g, 10 mmol) and Et_3N (1.66 mL, 12 mmol) according to the procedure used to synthesize **14a** in 30.6% yield (0.9 g); mp 87–88 °C; MS (EI) m/z 297 (M^+).

5.1.7.6. (*E*)-Methyl 5-(2-hydroxy-3-methoxystyryl)furan-2-carboxylate (14f). Compound **14f** was synthesized from **13** (4.37 g, 10 mmol), 2-hydroxy-3-methoxybenzaldehyde (1.52 g, 10 mmol) and Et_3N (1.66 mL, 12 mmol) according to the procedure used to synthesize **14a** in 53.2% yield (1.46 g); mp 103–105 °C; MS (EI) m/z 274 (M^+).

5.1.7.7. (*E*)-Methyl 5-(4-(dimethylamino)styryl)furan-2-carboxylate (14g). Compound **14g** was synthesized from **13** (4.37 g, 10 mmol), 4-(dimethylamino)benzaldehyde (1.49 g, 10 mmol) and Et_3N (1.66 mL, 12 mmol) according to the procedure used to synthesize **14a** in 65.1% yield (1.76 g); mp 97–99 °C; MS (EI) m/z 271 (M^+).

5.1.7.8. (*E*)-Methyl 5-(4-(benzyloxy)-3-methoxystyryl)furan-2-carboxylate (14h). Compound **14h** was synthesized from **13** (4.37 g, 10 mmol), 4-(benzyloxy)-3-methoxybenzaldehyde (2.42 g, 10 mmol) and Et_3N (1.66 mL, 12 mmol) according to the procedure used to synthesize **14a** in 78.4% yield (2.85 g); mp 93–95 °C; MS (EI) *m*/*z* 364 (M^+).

5.1.8. Representative procedure for conversion of methyl ester to carboxylic acid

5.1.8.1. (*E*)-5-Styrylfuran-2-carboxylic acid (15a). A mixture of **14a** (0.9 g, 3.95 mmol), 2 N KOH (4.0 mL, 7.9 mmol) and EtOH (5 mL) was stirred at 60 °C for 3 h. The solution was poured into water (30 mL) and extracted with ethyl acetate (2× 20 mL). The pH of the aqueous layer was adjusted to 5 by adding 2 N HCl. The precipitate was filtered and recrystallized to give a white solid (0.82 g) in 97.6% yield; mp 211–212 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm) 6.64 (1H, d, *J* = 3.6), 7.12 (1H, d, *J* = 3.6), 7.17 (1H, d, *J* = 8.0), 7.35 (1H, d, *J* = 8.0), 7.34–7.63 (5H, m), 10.2 (1H, s); MS (EI) *m*/*z* 214 (M^+).

5.1.8.2. (*E*)-5-(4-Hydroxystyryl)furan-2-carboxylic acid (15b). Compound **15b** was synthesized from **14b** (1.95 g, 8 mmol) and 2 N KOH (16 mL, 32 mmol) according to the procedure used to synthesize **15a** in 66.3% yield (1.22 g); mp 180 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.61 (1H, d, *J* = 3.6, furan-H), 6.74 (2H, d, *J* = 8.3, Ar-H), 6.76 (1H, d, *J* = 16.9, vinyl-H), 7.16 (1H, d, *J* = 3.6, furan-H), 7.11 (1H, d, *J* = 16.4, vinyl-H), 7.45 (2H, d, *J* = 8.6, Ar-H), 9.72 (1H, s, *H*O-Ar), 12.96 (1H, s, COO*H*); MS (EI) *m*/*z* 230 (M^+).

5.1.8.3. (*E*)-5-(2-Hydroxystyryl)furan-2-carboxylic acid (15c). Compound **15c** was synthesized from **14c** (1.95 g, 8 mmol) and 2 N KOH (16 mL, 32 mmol) according to the procedure used to synthesize **15a** in 89% yield (1.64 g); mp 180–182 °C; MS (EI) *m*/*z* 230 (M^+).

5.1.8.4. (*E*)-5-(2-(Benzo[*d*][1,3]dioxol-5-yl)vinyl)furan-2-carboxylic acid (15d). Compound **15d** was synthesized from **14d** (1.33 g, 4.9 mmol) and 2 N KOH (9.8 mL, 19.6 mmol) according to the procedure used to synthesize **15a** in 99.8% yield (2.85 g); mp 230–231 °C; MS (EI) *m*/*z* 258 (M^+).

5.1.8.5. (*E*)-5-(2,3-Dichlorostyryl)furan-2-carboxylic acid (15e). Compound **15e** was synthesized from **14e** (0.72 g, 2.4 mmol) and 2 N KOH (4.8 mL, 9.6 mmol) according to the procedure used to synthesize **15a** in 66.1% yield (0.45 g); mp 226–228 °C; MS (EI) *m*/*z* 283 (M^+).

5.1.8.6. (*E*)-5-(2-Hydroxy-3-methoxystyryl)furan-2-carboxylic acid (15f). Compound **15f** was synthesized from **14f** (1.46 g, 5.3 mmol) and 2 N KOH (10.6 mL, 21.3 mmol) according to the procedure used to synthesize **15a** in 79.6% yield (1.09 g); mp 217–219 °C; MS (EI) *m*/*z* 260 (M^+).

5.1.8.7. (*E*)-5-(4-(Dimethylamino)styryl)furan-2-carboxylic acid (15g). Compound **15g** was synthesized from **14g** (1.63 g, 6 mmol) and 2 N KOH (12 mL, 24 mmol) according to the procedure used to synthesize **15a** in 77.1% yield (1.19 g); mp 204–205 °C; MS (EI) *m*/*z* 257 (M^+).

5.1.8.8. (*E*)-5-(4-(Benzyloxy)-3-methoxystyryl)furan-2-carboxylic acid (15h). Compound **15h** was synthesized from **14h** (1.82 g, 5 mmol) and 2 N KOH (10 mL, 20 mmol) according to the procedure used to synthesize **15a** in 68.3% yield (1.2 g); mp >250 °C; MS (EI) *m*/*z* 350 (M^+).

5.1.9. Representative procedure for conversion of carboxylic acids to hydroxamates

5.1.9.1. (*E*)-*N*-Hydroxy-5-styrylfuran-2-carboxamide (6a). Compound **6a** was synthesized from **15a** according to the procedure used to synthesize **5a** in 37.4% yield; mp 177–178 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.64 (1H, d, *J* = 3.6, furan-H), 7.12 (1H, d, *J* = 3.6, furan-H), 7.17 (1H, d, *J* = 8.0, vinyl-H), 7.35 (1H, d, *J* = 8.0, vinyl-H), 7.34–7.63 (5H, m, Ar-H), 9.18 (1H, s, CON*H*OH), 11.25 (1H, s, CONHO*H*); MS (EI) *m*/*z* 229 (M^+); HRMS calcd for $C_{13}H_{12}NO_3$ $[MH]^+$: 230.0811; found, 230.0809; IR (KBr) ν (cm^{-1}) = 3452, 3174, 2897, 2384, 2342, 1627, 1584.

5.1.9.2. (*E*)-*N*-Hydroxy-5-(4-hydroxystyryl)furan-2-carboxamide (6b). Compound **6b** was synthesized from **15b** according to the procedure used to synthesize **6a** in 41.4% yield; mp 178–180 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.58 (1H, d, *J* = 3.6, furan-H), 6.84 (2H, d, *J* = 8.4, Ar-H), 6.94 (1H, d, *J* = 16.5, vinyl-H), 7.11 (1H, d, *J* = 3.6, furan-H), 7.26 (1H, d, *J* = 16.5, vinyl-H), 7.46 (2H, d, *J* = 8.4, Ar-H), 9.15 (1H, s, CON*H*OH), 9.74 (1H, s, *H*O-Ar), 11.20 (1H, s, CONHO*H*); MS (ESI) *m*/*z* 244 (M−H); HRMS calcd for $C_{13}H_{12}NO_4$ $[MH]^+$: 246.0760; found, 246.0756; IR (KBr) ν (cm^{-1}) = 3814, 3444, 2840, 1644, 1601.

5.1.9.3. (*E*)-*N*-Hydroxy-5-(2-hydroxystyryl)furan-2-carboxamide (6c). Compound **6c** was synthesized from **15c** according to the procedure used to synthesize **6a** in 42.2% yield; mp 132–134 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.58 (1H, d, *J* = 3.5, furan-H), 6.82 (1H, m, Ar-H), 6.88 (1H, m, Ar-H), 7.06 (1H, d, *J* = 3.5, furan-H), 7.11 (1H, m, Ar-H), 7.13 (1H, d, *J* = 16.5, vinyl-H), 7.44 (1H, d, *J* = 16.5, vinyl-H), 7.49 (1H, m, Ar-H), 9.07 (1H, s, CON*H*OH), 9.91 (1H, s, *H*O-Ar), 11.16 (1H, s, CONHO*H*); MS (ESI) *m*/*z* 244 (M−H); HRMS calcd for $C_{13}H_{12}NO_4$ $[MH]^+$: 246.0760; found, 246.0764; IR (KBr) ν (cm^{-1}) = 3430, 2355, 2840, 1623, 1584.

5.1.9.4. (*E*)-5-(2-(Benzo[*d*][1,3]dioxol-5-yl)vinyl)-*N*-hydroxyfuran-2-carboxamide (6d). Compound **6d** was synthesized from **15d** according to the procedure used to synthesize **6a** in 39.3% yield; mp 170–171 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.04 (2H, s, OCH_2O), 6.54 (1H, d, *J* = 3.5, furan-H), 6.98 (1H, d, *J* = 16.3, vinyl-H), 6.91-7.06 (2H, m, Ar-H), 7.06 (1H, d, *J* = 3.5, furan-H), 7.21 (1H, d, *J* = 16.3, vinyl-H), 7.24 (1H, m, Ar-H), 9.09 (1H, s, CON*H*OH), 11.13 (1H, s, CONHO*H*); MS (EI) *m*/*z* 273 (M^+); HRMS calcd for $C_{14}H_{11}NO_5$ $[MH]^+$: 274.2740; found, 274.2734; IR (KBr) ν (cm^{-1}) = 3437, 3288, 3124, 2790, 1662.

5.1.9.5. (*E*)-5-(2,3-Dichlorostyryl)-*N*-hydroxyfuran-2-carboxamide (6e). Compound **6e** was synthesized from **15e** according to the procedure used to synthesize **6a** in 36.8% yield; mp 180–181 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 6.74 (1H, d, *J* = 3.6, furan-H), 7.11 (1H, d, *J* = 3.6, furan-H), 7.24 (1H, d, *J* = 16.2, vinyl-H), 7.39 (1H, t, Ar-H), 7.55 (1H, d, *J* = 16.2, vinyl-H), 7.57–7.84 (2H, m, Ar-H), 9.17 (1H, s, CON*H*OH), 11.37 (1H, s, CONHO*H*); MS (ESI) *m*/*z* 296 (M−H); HRMS calcd for $C_{13}H_9Cl_2NO_3$ $[MH]^+$: 298.0032; found, 298.0026; IR (KBr) ν (cm^{-1}) = 3460, 2356, 1630, 1503.

5.1.9.6. (*E*)-*N*-Hydroxy-5-(2-hydroxy-3-methoxystyryl)furan-2-carboxamide (6f). Compound **6f** was synthesized from **15f** according to the procedure used to synthesize **6a** in 40.2% yield; mp 122–124 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 3.81 (3H, s, CH_3O), 6.26 (1H, d, *J* = 3.6, furan-H), 6.42 (1H, d, *J* = 12.3, vinyl-H), 6.73 (1H, d, *J* = 12.3, vinyl-H), 6.96 (1H, d, *J* = 3.6, furan-H), 6.67–6.93 (3H, m, Ar-H), 8.80 (1H, s, *H*O-Ar), 9.05 (1H, s, CON*H*OH), 10.87 (1H, s, CONHO*H*); MS (ESI) *m*/*z* 274 (M−H); HRMS calcd for $C_{14}H_{13}NO_5$ $[MH]^+$: 276.0102; found, 276.0106; IR (KBr) ν (cm^{-1}) = 3409, 2932, 2355, 1637.

5.1.9.7. (*E*)-5-(4-(Dimethylamino)styryl)-*N*-hydroxyfuran-2-carboxamide (6g). Compound **6g** was synthesized from **15g** according to the procedure used to synthesize **6a** in 41.3% yield; mp 116–117 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 2.94 (6H, s, (CH_3)$_2$N), 6.46 (1H, d, J = 3.3, furan-H), 6.72 (2H, d, J = 8.7, Ar-H), 6.81 (1H, d, J = 16.3, vinyl-H), 7.01 (1H, d, J = 3.3, furan-H), 7.17 (1H, d, J = 16.3, vinyl-H), 7.39 (2H, d, J = 8.7, Ar-H), 9.07 (1H, s, CON*H*OH), 11.1 (1H, s, CONHO*H*); MS (ESI) m/z 273 (M+H); HRMS calcd for $C_{15}H_{16}N_2O_3$ [MH]$^+$: 273.1233; found, 273.1233; IR (KBr) ν (cm^{-1}) = 3451, 2903, 2355, 1633.

5.1.9.8. (*E*)-5-(4-(Benzyloxy)-3-methoxystyryl)-*N*-hydroxyfuran-2-carboxamide (6h). Compound **6h** was synthesized from **15h** according to the procedure used to synthesize **6a** in 43.6% yield; mp 180–181 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm) 3.83 (3H, s, CH_3O), 5.11 (2H, s, CH_2O), 6.54 (1H, d, J = 3.5, furan-H), 7.03 (1H, d, J = 16.5, vinyl-H), 7.06 (1H, d, J = 3.5, furan-H), 7.42 (1H, d, J = 16.5, vinyl-H), 7.20–7.46 (8H, m, Ar-H), 9.08 (1H, s, CON*H*OH), 11.13 (1H, s, CONHO*H*); MS (ESI) m/z 364 (M−H); HRMS calcd for $C_{21}H_{19}NO_5$ [MH]$^+$: 366.1335; found, 366.1333; IR (KBr) ν (cm^{-1}) = 3430, 2882, 2362, 1634.

5.2. Bioactivity test

5.2.1. General

5.2.1.1. Cell lines and cultures. The U937 cell line was cultured in RPMI with 10% fetal calf serum, 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 250 ng/mL of amphotericin-B, 10 mM Hepes and 2 mM glutamine. U937 cells were kept at the constant concentration of 200,000 cells per milliliter of culture medium.

5.2.1.2. Ligands and materials. SAHA (kind gift of Merck) was dissolved in DMSO and used at 5 μM. MS-275 (kind gift from Schering AG) was dissolved in ethanol and used at 5 μM. All other compounds were dissolved in DMSO and used at the indicated concentrations.

5.2.2. Stability studies in rat plasma

The stock standard solutions of SAHA, **5j** and **6a** were prepared by dissolving the respective compound in fresh blank rat plasma to yield a concentration of 1 mg/mL. The working standard solutions were made by a serial dilution of the stock solution with blank plasma. Aliquots of 1 mL plasma samples were incubated at 37 °C and retrieved at different times. Aliquots of 100 μL plasma samples were then treated with two volumes of acetonitrile to precipitate plasma proteins. The resulting samples were centrifuged at 16,000 rpm for 5 min. Aliquots of 20 μL supernatant solutions were taken for HPLC analysis.[16]

5.2.3. Fluorimetric HDAC1 and 4 assays

The HDAC Fluorescent Activity Assay is based on the Fluor de Lys Substrate and Developer combination (BioMol) and has been carried out according to supplier's instructions. First, the *Fluor de Lys* Substrate, which comprises an acetylated lysine side chain, has been incubated with purified recombinant HDAC1, 4 enzymes in presence or absence of the inhibitors. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the Developer produces a fluorophore. Fluorescence has been quantified with a TECAN inphinite M200 station.

5.2.4. Cell cycle analysis in U937 cells

Cells (2.5 × 105) were collected and resuspended in 500 μL of hypotonic buffer (0.1%Triton X-100, 0.1% sodium citrate, 50 μg/mL of propidium iodide (PI), and RNAse A). Cells were incubated in the dark for 30 min. Samples were acquired on a FACS-Calibur flow cytometer using the Cell Quest software (Becton–Dickinson) and analyzed with standard procedures using the Cell Quest software (Becton–Dickinson) and the ModFit LT version 3 Software (Verity) as previously reported.[17] All of the experiments were performed at least two times.

5.2.5. FACS analysis of apoptosis in U937 cells

Apoptosis was measured with the active caspase 3 detection (B-Bridge) and samples were analyzed by FACS with Cell Quest technology (Becton–Dickinson) as previously reported.[18]

5.2.6. Granulocytic differentiation

Granulocytic differentiation was carried out according to Altucci et al.[19] Briefly, U937 cells were harvested and resuspended in 10 μL of phycoerythrine-conjugated CD11c (CD11c-PE). Control samples were incubated with 10 μL of PE-conjugated mouse IgG1, incubated for 30 min at 4 °C in the dark, washed in PBS, and resuspended in 500 μL of PBS containing propidium iodide (0.25 μg/mL). Samples were analyzed by FACS with Cell Quest technology (Becton–Dickinson). Propidium iodide positive cells have been excluded from the analysis.

5.2.7. Determination of α-tubulin acetylation

For α-tubulin, an amount of 50 μg of total protein extracts was separated on 10% polyacrylamide gels and blotted. Western blots were shown for acetylated α-tubulin (Sigma, dilution of 1:500), and total ERKs (Santa Cruz) were used to normalize for equal loading.

5.2.8. Determination of p21$^{WAF1/CIP1}$ induction in U937 cells

Total protein extracts (50 μg) were separated on a 15% polyacrylamide gel and blotted as previously described.[17] Western blots were shown for p21 (Transduction Laboratories, dilution 1:500), and tubulin (Sigma) were used to normalize for equal loading.

5.2.9. Cellular proliferation assay

All compounds were dissolved in DMSO with the stock concentration of 10 mg/mL, and diluted with medium freshly before drug administration. Cell lines were seeded into 96-well flat bottom plates at density of 4000 cells/well. Twenty-four hours after seeding, each compound dilution was added in duplicate and incubation continued at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 h, add 20 μL MTT at 5 mg/mL in PBS (filter sterilized, light protected, and stored at 4 °C) per well, and after 4 h of incubation at 37 °C, the fluorescence was measured at 570 nm using Thermo Multiskan Spectrum.

Acknowledgements

The authors thank Dr. Yulan Rao and Teng Wang (Department of Pharmaceutical Analysis, China Pharmaceutical University) for their efforts in stability studies in rat plasma. This work was supported by the Natural Science Foundation of Jiangsu Province (No. BK2007171), and by grants from Ministero dell'Istruzione Università e Ricerca, PRIN 2006 (L.A.), European Union (APO-SYS Project No. 200767) (L.A.), Regione Campania, L.5 annualità 2005 (L.A.).

References and notes

1. Allfrey, V. G.; Faulkner, R.; Mirsky, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **1964**, *51*, 786.
2. Roth, S. Y.; Denu, J. M.; Allis, C. D. *Annu. Rev. Biochem.* **2001**, *70*, 81.
3. Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Disc.* **2006**, *5*, 769.
4. Xu, W. S.; Parmigiani, R. B.; Marks, P. A. *Oncogene* **2007**, *26*, 5541.
5. Altucci, L.; Clarke, N.; Nebbioso, A.; Scognamiglio, A.; Gronemeyer, H. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 1752.

6. Minucci, S.; Pelicci, P. G. *Nat. Rev. Cancer* **2006**, *6*, 38.
7. Garber, K. *Nat. Biotechnol.* **2007**, *25*, 17.
8. Mulder, G. J.; Meerman, J. H. *Environ. Health Perspect* **1983**, *49*, 27.
9. Wiech, N. L.-H. H.; Lea, M.; Jung, M. *AACR 93rd Annual Meeting Abstract*, 2002.
10. Marson, C. M.; Serradji, N.; Rioja, A. S.; Gastaud, S. P.; Alao, J. P.; Coombes, R. C.; Vigushin, D. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2477.
11. Mai, A.; Massa, S.; Pezzi, R.; Simeoni, S.; Rotili, D.; Nebbioso, A.; Scognamiglio, A.; Altucci, L.; Loidl, P.; Brosch, G. *J. Med. Chem.* **2005**, *48*, 3344.
12. Ragno, R.; Simeoni, S.; Rotili, D.; Caroli, A.; Botta, G.; Brosch, G.; Massa, S.; Mai, A. *Eur. J. Med. Chem.* **2008**, *48*, 621.
13. Haru, O.; Haruo, S. *Synthesis* **1972**, 138.
14. Brochet, C.; Syssa, J. L.; Mouloungui, Z.; Delmas, M.; Gaset, A. *Synth. Commun.* **1991**, *21*, 1735.
15. Finan, P. A. *J. Chem. Soc.* **1963**, 3917.
16. Du, L.; Musson, D. G.; Wang, A. Q. *J. Pharm. Biomed. Anal.* **2006**, *42*, 556.
17. Nebbioso, A.; Clarke, N.; Voltz, E.; Germain, E.; Ambrosino, C.; Bontempo, P.; Alvarez, R.; Schiavone, E. M.; Ferrara, F.; Bresciani, F.; Weisz, A.; de Lera, A. R.; Gronemeyer, H.; Altucci, L. *Nat. Med.* **2005**, *11*, 77.
18. Mai, A.; Rotili, D.; Tarantino, D.; Ornaghi, P.; Tosi, F.; Vicidomini, C.; Sbardella, G.; Nebbioso, A.; Miceli, M.; Altucci, L.; Filetici, P. *J. Med. Chem.* **2006**, *49*, 6897.
19. Altucci, L.; Rossin, A.; Raffelsberger, W.; Reitmair, A.; Chomienne, C.; Gronemeyer, H. *Nat. Med.* **2001**, *7*, 680.