Class II (IIa)-Selective Histone Deacetylase Inhibitors. 1. Synthesis and **Biological Evaluation of Novel (Aryloxopropenyl)pyrrolyl Hydroxyamides**

Antonello Mai,*^{,§} Silvio Massa,[#] Riccardo Pezzi,[§] Silvia Simeoni,[§] Dante Rotili,[§] Angela Nebbioso,[⊥] Annamaria Scognamiglio,[⊥] Lucia Altucci,^{*,⊥,‡} Peter Loidl,[∥] and Gerald Brosch^{*,∥}

Istituto Pasteur - Fondazione Cenci Bolognetti, Dipartimento di Studi Farmaceutici, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Roma, Italy, Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, via A. Moro, 53100 Siena, Italy, Dipartimento di Patologia Generale, Seconda Università degli Studi di Napoli, vico L. De Crecchio 7, 80138 Napoli, Italy, Centro di Oncogenomica AIRC, CEINGE Biotecnologia avanzata, Napoli, and Department of Molecular Biology, Innsbruck Medical University, Peter-Mayr-Strasse 4b, 6020 Innsbruck, Austria

Received December 7, 2004

Chemical manipulations performed on aroyl-pyrrolyl-hydroxyamides (APHAs) led to (aryloxopropenyl)pyrrolyl hydroxamates 2a-w, and their inhibition against maize HDACs and their class I or class II HDAC selectivity were determined. In particular, from these studies some benzene meta-substituted compounds emerged as highly class II (IIa)-selective HDAC inhibitors, the most selective being the 3-chloro- and 3-fluoro-substituted compounds 2c (SI = 71.4) and **2f** (SI = 176.4). The replacement of benzene with a 1-naphthyl ring afforded 2s, highly active against the class II homologue HD1-A (IC₅₀ = 10 nM) but less class II-selective than $2c_{,f.}$ When tested against human HDAC1 and HDAC4, 2f showed no inhibitory activity against HDAC1 but was able to inhibit HDAC4. Moreover, in human U937 acute myeloid leukaemia cells **2f** did not produce any effect on apoptosis, granulocytic differentiation, and the cell cycle, whereas 2s (that retain class I HDAC inhibitory activity) was 2-fold less potent than SAHA used as reference.

Introduction

Histone acetylation and deacetylation play an essential role in modifying chromatin structure and regulating gene expression in eukaryotic cells. Hyperacetylated histones are generally found in transcriptionally active genes and hypoacetylated histones in transcriptionally silent regions of the genome. $^{1-4}$ Key enzymes, which modify histone proteins and thereby regulate gene expression, are histone acetyltransferases (HATs) and histone deacetylases (HDACs).⁵⁻⁷ In mammals, both these acetylating/deacetylating enzymes are components of multiprotein complexes containing other proteins known to function in transcriptional activation/ repression.⁸⁻¹⁰ Such multiprotein complexes are recruited to specific regions in the mammalian genome and generate an unique spectrum of expressed and silenced genes. Known repressor multiprotein complexes contain DNA binding proteins (such as Rb and Rb-like proteins, N-CoR, SMRT, MEF, MeCP2, sin3A, etc.) that can recruit HDACs to repress transcription and to block the function of some tumor suppressor genes.¹¹⁻¹⁶ Compounds able to inhibit HDAC activity (HDAC inhibitors, such as trichostatin A (TSA), trapoxin (TPX), suberoylanilide hydroxamic acid (SAHA), sodium butyrate (NaB), sodium valproate (VPA), cyclic hydroxamic

[‡] Centro di Oncogenomica AIRC, CEINGE Biotecnologia avanzata, Napoli.

Innsbruck Medical University.

acid-containing peptides (CHAPs), depsipeptide FK-228, and MS-275) can de-repress these genes, resulting in antiproliferative effects in vitro and antitumor effects in vivo.¹⁷⁻²⁵

To date, eighteen distinct human HDACs have been reported, grouped into three classes (I, II, and III) depending on their primary homology to three Saccharomyces cerevisiae HDACs (RPD3, HDA1, and SIR2, respectively).²⁶ Class I and II HDACs show some degree of homology in their catalytic domain, whereas class III HDACs (SIRT1-7, sirtuins) show no homology to class I/II enzymes. Class I HDACs, comprise HDAC1-3 and 8, are predominantly nuclear enzymes, and are ubiquitously expressed. HDAC11 is most closely related to the class I HDACs, but its overall sequence similarity is too low²⁷ for classifying it into class I, and a class IV of human HDACs (containing the sole HDAC11 until now) has been proposed.²⁸ Class II HDACs comprise HDAC4-7. 9. and 10 and are divided into two subclasses. IIa (HDAC4-7 and 9) and IIb (HDAC6 and 10, containing as a unique feature two deacetylase domains), according to their sequence homology and domain organization.²⁹⁻³¹ Class IIa HDACs shuttle between the nucleus and the cytoplasm, depending on their CaMK-mediated phosphorylation extent and subsequent binding of 14-3-3 proteins. In particular, phosphorylation-dependent binding of 14-3-3 proteins to the N-termini of class IIa HDACs masks the nuclear import signal and prevents nuclear import. Class IIb HDACs are mainly cytoplasmic, but show significant nuclear amounts in several cell lines. Differently from class I HDACs that are ubiquitarian, class II HDACs are expressed in a restricted number of cell types. Most class IIa HDACs (HDAC4, 5, and 9) are abundant in heart, skeletal

^{*} To whom correspondence should be addressed: A.M., Tel.: +396-4991-3392; Fax:+396-491491; e-mail: antonello.mai@uniroma1.it. Biology: L.A., Tel.: +3981-566-7569; Fax: +3981450-169; e-mail: lucia.altucci@unina2.it. Biochemistry: G. B., Tel.: 0512-507-3608; Fax: 0512-507-9880; e-mail: gerald.brosch@uibk.ac.at. [§] Università degli Studi di Roma "La Sapienza".

[#] Università degli Studi di Siena.

Seconda Università degli Studi di Napoli.





muscle, and brain,^{32–36} and HDAC7 is highly expressed in CD4/CD8 double-positive thymocytes.³⁷ Class IIb HDAC6 is predominantly expressed in testis,³² and HDAC10 is expressed in liver, spleen, and kidney.^{38,39}

Class I HDACs are well-known transcriptional corepressors, acting through the block of the expression of some tumor suppressor genes. Class IIa HDACs have been reported to interact with one or more DNA-binding transcription factors, as well as with transcriptional corepressors.²⁹ Among these interactions, the class IIa HDACs-MEF2 interactions are the most studied.⁴⁰ MEF2 plays a critical role in cardiac and skeletal myogenesis, in negative selection of developing thymocytes, and in the transcriptional regulation of Epstein-Barr virus (EBV). Moreover, MEF2 has a role in neuronal resistance to excitotoxicity. By binding to MEF2 several promoters, class IIa HDACs can act as transcriptional repressors in a variety of biological functions, from myogenesis⁴¹ to EBV latency.⁴² Differently, class IIb HDAC6 takes part, together with the class III HDAC SIRT2, in the microtubule network and acts as specific α -tubulin deacetylase in vitro and in vivo.43,44

Most of the HDAC inhibitors described so far inhibit to the same extent class I as well as class II members of the HDAC family. The only exceptions are VPA, 5-fold more potent against HDAC1 than HDAC5 and HDAC6,⁴⁵ and FK-228, a class I-selective prodrug.⁴⁶ Class IIb HDAC6 and HDAC10 have been found to be resistant to the inhibitors TPX-A and -B, CHAPs, and NaB^{47,48} and are selectively inhibited by tubacin, a 1,3-dioxanecontaining hydroxamate discovered through a multidimensional, chemical genetic screen of 7392 molecules as a specific α -tubulin deacetylation inhibitor in mammalian cells (Chart 1).^{49–52} **Chart 2.** From Aroyl-pyrrolyl-hydroxamides (APHAs) to (Aryloxopropenyl)pyrrolyl Hydroxamates **2a**-**w**



Further development of new small molecules with the specificity to selectively perturb only a (sub)class of the HDAC family is a very attractive goal to pursue, because such as compounds could be useful tools to dissect the role of a given HDAC in different protein complexes.⁵³

Since 2001 a new class of HDAC inhibitors, namely aroyl-pyrrolyl-hydroxy-amides (APHAs), have been described by us.⁵⁴⁻⁶⁰ In early studies, APHAs have been tested again HD2,⁶¹ a maize deacetylase different from mammalian HDACs but being a good predictive model for the behavior of class I HDACs with various series of HDAC inhibitors.^{55,56,58,62} Recently, we have undertaken a broad strategy^{59,63,64} by testing the new derivatives against HD1-B^{65,66} and HD1-A enzymes,^{67,68} two maize deacetylases that are homologous of mammalian class I and class II (IIa) HDACs, respectively, to explore the potential selectivity of our compounds. Our maize system offers the advantage of completely separated enzyme forms, while human HDACs act as multiprotein- and, in some cases, multi-HDAC-containing complexes in their active forms.^{69,70} First, anti-HD1-B/ HD1-A data^{59,63} showed that in APHA compounds just one type of chemical manipulation performed on the lead compound 3-(4-benzoyl-1-methyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamide 1 (Chart 2) was efficient to yield highly selective derivatives, that is the insertion of an alkyl/alkenyl spacer between the benzoyl moiety and the carbon atom at the pyrrole-C4 position in the 1 structure.63

Here we report in full details the synthesis and biochemical characterization of novel (aryloxopropenyl)pyrrolyl hydroxyamides $2\mathbf{a}-\mathbf{w}$, structurally related to APHAs and highly selective against the class II (class IIa) histone deacetylase homologue HD1-A. Moreover, on selected compounds the inhibitory activities against human HDAC1 and HDAC4 as well as the cellular effects on apoptosis, granulocytic differentiation, and cell cycle phases in U937 acute myeloid leukemia (AML) cells are described.

Chemistry. Vilsmeier–Haack formylation of ethyl 3-(1-methyl-1*H*-pyrrol-2-yl)-2-propenoate⁷¹ with oxalyl chloride/*N*,*N*-dimethylformamide afforded the ethyl 3-(4-formyl-1-methyl-1*H*-pyrrol-2-yl)-2-propenoate **3** as a sole product in high yield. Further condensation of **3** with the properly substituted alkyl aryl ketone in alkaline medium furnished the 3-[4-(3-aryl-3-oxopropen-1-yl)-1-methyl-1*H*-pyrrol-2-yl]-2-propenoic acids **4a**–**w**, key intermediate for the synthesis of the title compounds. By reaction of **4a**–**w** with ethyl chloroformate, followed by addition of *O*-(2-methoxy-2-propyl)hydroxylamine⁷² and acidic treatment in the presence of the Amberlyst 15 ion-exchange resin, the desired 3-[4-(3-aryl-3-oxopropen-1-yl)-1-methyl-1*H*-pyrrol-2-yl]-*N*-hydroxy-2-propenamides **2a**–**w** have been obtained (Scheme 1).

Scheme 1^a



 a (a) (COCl)₂, DMF, dichloroethane, room temp; (b) aryl alkyl ketone, KOH, C₂H₅OH, H₂O, room temp; (c) ClCOOC₂H₅, (C₂H₅)₃N, THF, 0 °C; (d) NH₂OC(CH₃)₂OCH₃; (e) Amberlyst 15, MeOH, 45 °C.

Table 1. Chemical and Physical Data for Compounds 2a-w

compd	R	R_1	mp, °C	recrystn solvent	% yield	formula	anal. ^a
2a	Ph	Н	205 - 207	CH ₃ CN/benzene	54	$C_{17}H_{16}N_2O_3$	C, H, N
2b	2-Cl-Ph	Н	224 - 226	CH ₃ CN/MeOH	58	$C_{17}H_{15}ClN_2O_3$	C, H, N, Cl
2c	3-Cl-Ph	Н	198 - 200	MeOH	72	$C_{17}H_{15}ClN_2O_3$	C, H, N, Cl
2d	4-Cl-Ph	н	>260	CH ₃ CN/MeOH	80	$C_{17}H_{15}ClN_2O_3$	C, H, N, Cl
2e	2-F-Ph	н	220 - 222	CH ₃ CN/MeOH	65	$C_{17}H_{15}FN_2O_3$	C, H, N, F
2f	3-F-Ph	н	212 - 215	CH ₃ CN/MeOH	62	$C_{17}H_{15}FN_2O_3$	C, H, N, F
$2\mathbf{g}$	4-F-Ph	Н	>260	CH ₃ CN/MeOH	53	$C_{17}H_{15}FN_2O_3$	C, H, N, F
2h	2-Br-Ph	Н	168 - 170	CH ₃ CN/MeOH	61	$C_{17}H_{15}BrN_2O_3$	C, H, N, Br
2i	3-Br-Ph	Н	253 - 255	MeOH	56	$C_{17}H_{15}BrN_2O_3$	C, H, N, Br
2j	4-Br-Ph	н	250 - 252	MeOH	61	$\mathrm{C}_{17}\mathrm{H}_{15}\mathrm{BrN}_{2}\mathrm{O}_{3}$	C, H, N, Br
2k	2-Me-Ph	н	222 - 224	CH_3CN	45	$C_{18}H_{18}N_2O_3$	C, H, N
21	3-Me-Ph	Н	215 - 217	CH ₃ CN/MeOH	67	$C_{18}H_{18}N_2O_3$	C, H, N
2m	4-Me-Ph	н	246 - 248	CH ₃ CN/MeOH	61	$C_{18}H_{18}N_2O_3$	C, H, N
2n	2-MeO-Ph	Н	165 - 166	MeOH	69	$\mathrm{C}_{18}\mathrm{H}_{18}\mathrm{N}_{2}\mathrm{O}_{4}$	C, H, N
2o	3-MeO-Ph	Н	194 - 196	MeOH	65	$\mathrm{C}_{18}\mathrm{H}_{18}\mathrm{N}_{2}\mathrm{O}_{4}$	C, H, N
2p	4-MeO-Ph	Н	>260	MeOH/DMF	52	$\mathrm{C}_{18}\mathrm{H}_{18}\mathrm{N}_{2}\mathrm{O}_{4}$	C, H, N
$2\mathbf{q}$	$3-CF_3-Ph$	н	207 - 209	THF/MeOH	46	$C_{18}H_{15}F_3N_2O_3$	C, H, N, F
2r	$3-CF_{3}O-Ph$	н	>260	MeOH/DMF	45	$C_{17}H_{15}FN_2O_3$	C, H, N, F
2s	1-naphthyl	н	184 - 185	CH ₃ CN/MeOH	76	$C_{21}H_{18}N_2O_3$	C, H, N
2t	2-naphthyl	Н	205 - 207	MeOH	68	$C_{21}H_{18}N_2O_3$	C, H, N
2u	4-biphenyl	н	184 - 185	MeOH	69	$C_{23}H_{20}N_2O_3$	C, H, N
2v	\mathbf{Ph}	Me	174 - 175	CH ₃ CN/benzene	61	$C_{18}H_{18}N_2O_3$	C, H, N
$2\mathbf{w}$	Ph	\mathbf{Et}	190 - 192	CH ₃ CN/benzene	63	$C_{19}H_{20}N_2O_3$	C, H, N

 a Analytical results were within $\pm 0.4\%$ of the theoretical values.

Chemical and physical data of compounds $2\mathbf{a}-\mathbf{w}$ are listed in Table 1. Chemical and physical data of the intermediate compounds **3** and $4\mathbf{a}-\mathbf{w}$ are listed in Table 2.

Results and Discussion

The novel (aryloxopropenyl)pyrrolyl hydroxyamides 2a-w have been evaluated for their ability to inhibit maize HD2.^{61,73-76} Two short-chain fatty acids (NaB⁷⁷ and VPA^{37,78}), two hydroxamic acids (TSA⁷⁹ and SA-HA⁸⁰), two cyclic tetrapeptides (TPX⁸¹ and HC-toxin⁸²), and tubacin,⁵⁰⁻⁵² the sole class II (class IIb)-selective HDAC inhibitor reported to date, have been used as reference drugs. The results, expressed as the percent of inhibition at a fixed dose and IC₅₀ (50% inhibitory concentration) values, are reported in Table 3. Compounds 2a-w have been also tested against maize HD1-B^{65,66} and HD1-A,^{67,68} two mammalian class I and class II (IIa) HDAC homologues, respectively, and the resulting fold selectivity values (for class I HDACs: IC_{50-HD1-A}/IC_{50-HD1-B} ratio; for class II HDACs: IC_{50-HD1-B}/

 $IC_{50-HD1-A}$ ratio) have been assessed. TSA, SAHA, and tubacin have been also tested for comparison purpose (Table 4).

HD2 Inhibitory Activity and Structure-Activity Relationships (SARs). (Aryloxopropenyl)pyrrolyl hydroxyamides $2\mathbf{a} - \mathbf{w}$ have been first evaluated against maize HD2, in comparison with NaB, VPA, TSA, SAHA, TPX, HC-toxin, and tubacin (Table 3). From inhibitory data, it is clear that the type and the position of the substituent inserted at the benzene ring of the unsubstituted 3-[4-(3-phenyl-3-oxopropen-1-yl)-1-methyl-1Hpyrrol-2-yl]-N-hydroxy-2-propenamide 2a play a crucial role in determining the inhibitory activity of the tested derivatives. Compound **2a** inhibited HD2 at submicromolar concentrations (IC₅₀ = $0.28 \,\mu$ M). The introduction of a halogen atom (chlorine, fluorine, or bromine) at each of the three positions of the **2a** benzene ring generally decreased or abated the potency of derivatives, the only exception being the 2-bromo analogue **2h** that retained almost the same activity as 2a. The 2-chloro- and 2-fluoro derivatives 2b and 2e were 18- and 25-fold less active than **2a**, respectively; when the halogen was

Table 2. Chemical and Physical Data for Compounds 3 and 4a-w

compd	R	R_1	mp, °C	recrystn solvent	% yield	formula	anal. ^a
3			102 - 104	cyclohexane	74	$C_{10}H_{13}NO_2$	C, H, N
4a	Ph	Н	205 - 207	ČH₃CN/MeOH	52	$C_{17}H_{15}NO_3$	C, H, N
4b	2-Cl-Ph	Н	224 - 226	CH ₃ CN/MeOH	57	C ₁₇ H ₁₄ ClNO ₃	C, H, N, Cl
4c	3-Cl-Ph	Н	215 - 217	CH ₃ CN/MeOH	57	C ₁₇ H ₁₄ ClNO ₃	C, H, N, Cl
4d	4-Cl-Ph	Н	>260	CH ₃ CN/MeOH	75	C ₁₇ H ₁₄ ClNO ₃	C, H, N, Cl
4e	2-F-Ph	Н	220 - 222	CH ₃ CN/MeOH	56	$C_{17}H_{14}FNO_3$	C, H, N, F
4f	3-F-Ph	Η	210 - 212	CH ₃ CN/MeOH	51	$C_{17}H_{14}FNO_3$	C, H, N, F
4g	4-F-Ph	Η	>260	CH ₃ CN/MeOH	66	$C_{17}H_{14}FNO_3$	C, H, N, F
4h	2-Br-Ph	Η	255 - 256	CH ₃ CN/MeOH	57	$C_{17}H_{14}BrNO_3$	C, H, N, Br
4i	3-Br-Ph	Η	230 - 232	CH ₃ CN/MeOH	58	$C_{17}H_{14}BrNO_3$	C, H, N, Br
4j	4-Br-Ph	Η	258 - 260	CH ₃ CN/MeOH	65	$C_{17}H_{14}BrNO_3$	C, H, N, Br
4k	2-Me-Ph	Η	258 - 260	$CH_{3}CN$	41	$C_{18}H_{17}NO_3$	С, Н, N
41	3-Me-Ph	Η	>260	CH ₃ CN/MeOH	47	$C_{18}H_{17}NO_3$	С, Н, N
4m	4-Me-Ph	Н	246 - 248	$CH_{3}CN$	45	$C_{18}H_{17}NO_3$	С, Н, N
4n	2-MeO-Ph	Н	205 - 207	CH ₃ CN/MeOH	50	$C_{18}H_{17}NO_4$	С, Н, N
4o	3-MeO-Ph	Н	193 - 195	CH ₃ CN/MeOH	66	$C_{18}H_{17}NO_4$	С, Н, N
4p	4-MeO-Ph	Н	203 - 204	MeOH	74	$C_{18}H_{17}NO_4$	С, Н, N
4 q	$3-CF_3-Ph$	Н	232 - 234	THF/MeOH	86	$C_{18}H_{14}F_3NO_3$	C, H, N, F
4r	3-OCF ₃ -Ph	Н	234 - 236	MeOH	84	$C_{17}H_{13}FNO_3$	C, H, N, F
4s	1-naphthyl	Η	230 - 232	$CH_{3}CN$	71	$C_{21}H_{17}NO_3$	С, Н, N
4t	2-naphthyl	Н	261 - 263	$CH_{3}CN$	69	$C_{21}H_{17}NO_3$	С, Н, N
4u	4-biphenyl	Н	232 - 234	MeOH	70	$C_{23}H_{19}NO_3$	С, Н, N
4v	\mathbf{Ph}	${\rm Me}$	174 - 176	benzene	42	$C_{18}H_{17}NO_3$	С, Н, N
4w	Ph	Et	190 - 192	benzene	37	$C_{19}H_{19}NO_3$	С, Н, N

^{*a*} Analytical results were within $\pm 0.4\%$ of the theoretical values.

Table 5. Maize 11D2 Infibitory Activity of Compounds 2a w
--

			% inhbtn	
compd	R	\mathbf{R}_1	(at a fixed dose, $\mu \mathbf{M})$	$IC_{50}\pm SD~(\mu M)$
2a	Ph	Η	92.3 (26)	0.28 ± 0.01
2b	2-Cl-Ph	Η	79.1 (23.3)	5.2 ± 0.16
2c	3-Cl-Ph	Η	49 (23.3)	24.2 ± 1.21
2d	4-Cl-Ph	Η	45.5(23.3)	25.2 ± 1.26
2e	2-F-Ph	Η	73.9 (24.5)	7.1 ± 0.35
2f	3-F-Ph	Η	56.5 (24.5)	22.0 ± 1.32
$2\mathbf{g}$	4-F-Ph	Η	33.2(24.5)	35.3 ± 1.06
2h	2-Br-Ph	Η	92.4 (20.5)	0.36 ± 0.02
2i	3-Br-Ph	Η	76.8 (20.5)	2.1 ± 0.13
2j	4-Br-Ph	Η	41.7 (20.5)	28.5 ± 1.71
$2\mathbf{k}$	2-Me-Ph	Η	96 (24.8)	0.27 ± 0.008
21	3-Me-Ph	Η	72(24.8)	7.9 ± 0.24
2m	4-Me-Ph	Η	65(24.8)	10.2 ± 0.41
2n	2-MeO-Ph	Η	95.7 (23.6)	0.25 ± 0.01
2o	3-MeO-Ph	Η	78.2(23.6)	0.54 ± 0.02
2p	4-MeO-Ph	Η	74.2(23.6)	0.57 ± 0.03
$2\mathbf{q}$	3-CF ₃ -Ph	Η	46.1 (21.1)	49.0 ± 2.0
$2\mathbf{r}$	3-CF ₃ O-Ph	Η	19.5 (20.2)	176.0 ± 5.3
2s	1-naphthyl	Η	92 (22.2)	0.20 ± 0.006
2t	2-naphthyl	Η	62.9(25.2)	1.8 ± 0.09
2u	4-biphenyl	Η	34 (20.6)	26.5 ± 1.06
2v	Ph	Me	89.9 (24.8)	0.68 ± 0.02
$2\mathbf{w}$	Ph	Et	93 (23.7)	0.42 ± 0.02
NaB			35(5000)	ND^b
VPA				128.0 ± 3.8
TSA				0.007 ± 0.0002
SAHA				0.05 ± 0.001
TPX				0.01 ± 0.0003
HC-toxin				0.11 ± 0.004
tubacin			92.9 (40)	2.0 ± 0.1

 a Data represent mean values of at least three separate experiments. b ND, not determined.

introduced at benzene C_3 as well as C_4 position, only substantially inactive compounds have been obtained, with the exception of the 3-bromo derivative **2i** (IC₅₀ = $2.1 \,\mu$ M), that was 7.5 times less potent than **2a**. Methyl substitution at the benzene C_2 position furnished a compound (**2k**) showing the same activity as **2a**, while the 3- and 4-methyl counterparts (**2l** and **2m**) were from 28 to 36 times less potent than **2a** in inhibiting HD2. In comparison with the unsubstituted **2a**, the three methoxy derivatives **2n**-**p** were equally (**2n**) or slightly less (20,p) active. Differently, 3-trifluoromethyl- and 3-trifluoromethoxy-substituted compounds 2q and 2r as well as 4-biphenyl derivative 2u were substantially inactive. Replacement of the 2a benzene ring with the bulky 1-naphthyl group led to 2s, that was 1.4-fold more potent than 2a, while compound 2t bearing a 2-naphthyl moiety at the 3-propenyl position showed 6.4 times lower activity than 2a. Finally, an alkyl (methyl or ethyl) substituent at the C₂ position of the 3-oxopropenyl chain was tolerated for HD2 inhibition, 2v and 2w being 2.4- and 1.5-fold less active than the unsubstituted 2a, respectively.

HD1-B and HD1-A Inhibitory Assays: Assessment of Class Selectivity. The novel (aryloxopropenyl)pyrrolyl hydroxyamides 2a-w have been tested against maize HD1-B and HD1-A, two mammalian class I and class II (IIa) HDAC homologues, and their class selectivity has been calculated. The unselective inhibitors TSA and SAHA and the class IIb-selective tubacin have been also tested as reference drugs. Data summarized in Table 4 show that the inhibitory trend of **2a**-w against HD1-B as well as against HD1-A is the same as that observed for **2a-w** against HD2, the main difference lying in the diverse degree of susceptibility of HD1-B and HD1-A to (aryloxopropenyl)pyrrolyl hydroxamate inhibitors. The inhibitory activity of the unsubstituted 2a was at submicromolar concentrations $(IC_{50-HD1-B} = 0.26 \,\mu\text{M}; IC_{50-HD1-A} = 0.19 \,\mu\text{M})$, and the potency of substituted compounds against both the two maize enzymes varied according to the order of *ortho* > meta > para-substituted analogues, the last being in general the lowest active (or inactive) derivatives, while the *ortho*-substituted analogues were among the most potent compounds of the series. In anti-HD1-B assay, the chloro- and fluoro-substitution at the benzene C_2 position led to **2b** and **2e**, that were 6- and 13-fold less active than 2a, respectively, while the corresponding C_3 and C₄-substituted 2c,d,f,g were substantially inactive. Bromine and methyl substitutions generated more active compounds, the C₂-substituted analogues being

Table 4. Maize HD1-B and HD1-A Inhibitory Activities of Compounds 2a-wa

			${ m IC}_{50}\pm 1$	$SD(\mu M)$		SI^b
compd	R	R_1	HD1-B	HD1-A	class I	class II (IIa)
2a	Ph	Н	0.26 ± 0.02	0.19 ± 0.01		
2b	2-Cl-Ph	Η	1.6 ± 0.08	0.05 ± 0.003		32
2c	3-Cl-Ph	Н	31.4 ± 1.26	0.44 ± 0.02		71.4
2d	4-Cl-Ph	Н	29.4 ± 1.18	18.6 ± 1.12		
2e	2-F-Ph	Н	3.4 ± 0.14	0.10 ± 0.004		34
2f	3-F-Ph	Н	38.8 ± 1.16	0.22 ± 0.01		176.4
$2\mathbf{g}$	4-F-Ph	Н	24.9 ± 1.24	15.7 ± 0.94		
2 h	2-Br-Ph	Н	0.18 ± 0.007	0.21 ± 0.008		
2i	3-Br-Ph	Н	1.1 ± 0.04	1.6 ± 0.06		
2j	4-Br-Ph	Н	24.3 ± 0.97	20.3 ± 0.81		
2k	2-Me-Ph	Н	0.39 ± 0.02	0.06 ± 0.002		6.5
21	3-Me-Ph	Н	2.5 ± 0.08	0.17 ± 0.007		14.7
2m	4-Me-Ph	Η	4.8 ± 0.29	0.50 ± 0.02		9.6
2n	2-MeO-Ph	Η	0.17 ± 0.008	0.16 ± 0.01		
2o	3-MeO-Ph	Η	0.60 ± 0.03	0.39 ± 0.02		
2p	4-MeO-Ph	Η	0.80 ± 0.05	0.79 ± 0.02		
2q	3-CF ₃ -Ph	Η	159.0 ± 6.4	9.3 ± 0.46		17.1
$2\mathbf{r}$	3-CF ₃ O-Ph	Η	298.0 ± 14.9	90.0 ± 4.5		3.3
2s	1-naphthyl	Н	0.33 ± 0.02	0.01 ± 0.0007		33
2t	2-naphthyl	Η	1.8 ± 0.07	0.04 ± 0.002		45
2u	4-biphenyl	Η	31.8 ± 1.27	2.2 ± 0.07		14.4
2v	Ph	Me	0.57 ± 0.02	0.78 ± 0.02		
$2\mathbf{w}$	Ph	\mathbf{Et}	4.5 ± 0.22	0.22 ± 0.01		20.4
TSA			0.0004 ± 0.00001	0.0008 ± 0.00003	2	
SAHA			0.03 ± 0.001	0.18 ± 0.009	6	
tubacin			0.98 ± 0.04	0.45 ± 0.02		2.2

^a Data represent mean values of at least three separate experiments. ^bSI, Selectivity Index.

more potent (2h) than or as active (2k) as 2a, and the C₃-substituted **2i** and **2l** were 4- and 10-fold less potent than 2a. 2-Methoxy-substituted 2n showed higher activity than 2a, and the corresponding 3- and 4-isomers 20 and 2p were 2-4 times less active than 2a in inhibiting HD1-B. As it has been observed in the anti-HD2 assay and also against HD1-B, the 3-trifluoromethyl (2q) and 3-trifluoromethoxy (2r) as well as the 4-biphenyl (2u) derivatives failed in inhibiting the enzyme. Compound 2s, in which the 2a benzene ring has been replaced with 1-naphthyl moiety, retained almost the same activity as 2a, while the 2-naphthyl isomer 2t was 7-fold less potent. At last, 2v and 2w carrying methyl and ethyl substituent at the C₂ position of the 3-oxopropenyl chain showed from 2 to 17 times lower activity than 2a in inhibiting HD1-B.

In anti-HD1-A assay the order of potency of substituted compounds (ortho > meta > para) in comparison with the unsubstituted 2a was fully maintained, but the susceptibility of the enzyme to the (aryloxopropenyl)pyrrolyl hydroxamate inhibitors was very high. The 2-chloro- and 2-fluoro-substituted **2b** and **2e** were 4- and 2-fold more active than **2a** in inhibiting HD1-A, and the 3-substituted analogues 2c and 2f showed the same activity (2f) as or 2-fold lower potency (2c) than 2a. Among bromo-substituted derivatives, the benzene-C₂ substituted **2h** retained the same activity than **2a**, the C_3 isomer **2i** being 8 times less potent than the unsubstituted reference. Methyl and methoxy substitutions gave highly active compounds, methyl- being more potent than the corresponding methoxy-containing derivatives. In particular, 2-methyl-, 3-methyl-, and 2-methoxyphenyloxopropenyl-pyrrolyl hydroxamates 2k,l,n were up to 3 times more potent than **2a** in inhibiting HD1-A. Differently, the insertion of trifluoromethyl or trifluoromethoxy substituent at the C_3 position of the benzene ring led to inactive compounds (2q and 2r). Both the naphthyl analogues **2s** and **2t** were more efficient (19- and 5-fold, respectively) than the phenyl counterpart in inhibiting HD1-A, and the 4-biphenyl compound **2u**, inactive against HD2 as well as HD1-B enzymes, showed IC₅₀ = $2.2 \,\mu$ M against HD1-A. Finally, the insertion of a methyl or ethyl substituent at the oxopropenyl chain was well tolerated in anti-HD1-A assay, as phenyloxobutenyl and phenyloxopentenyl derivatives **2v** and **2w** showed slightly lower activity than the phenyloxopropenyl **2a**.

To determine the class selectivity for (aryloxopropenyl)pyrrolyl hydroxamates 2a-w, the IC_{50-HD1-A}/ $IC_{50-HD1-B}$ ratio for class I-selectivity and the $IC_{50-HD1-B}$ / IC_{50-HD1-A} ratio for class II (IIa)-selectivity have been calculated. Only selectivity values ≥ 2 have been reported. Table 4 shows that compounds substituted at C_2 or better at C_3 position of the benzene ring with chloro- (2b,c), fluoro- (2e,f), or methyl (2k,l) groups, as well as the naphthyl derivatives **2s**,t, were highly selective for the class II (IIa) HDAC homologue HD1-A, 3-[4-(3-(3-chlorophenyl)- and 3-[4-(3-(3-fluorophenyl)-3-oxopropen-1-yl)-1-methyl-1H-pyrrol-2-yl]-N-hydroxy-2-propenamides 2c and 2f being the most class II (IIa)selective compounds, with a selectivity ratio of 71.4 and 176.4, respectively. Among the reference drugs, TSA and SAHA showed to be slightly selective (2 and 6 times) toward the class I HDAC homologue HD1-B. Tubacin, a highly "in cell" selective class IIb HDAC inhibitor, was 2.2 times more potent against HD1-A (class II homologue) than HD1-B (class I homologue). This result is as expected, because the tubacin selectivity is truly manifest in "in cell" functional assay, by comparing the induction of acetylated histone (EC₅₀ = $217 \pm 96 \ \mu M$) and acetylated tubulin (EC₅₀ = $2.9 \pm 0.9 \,\mu$ M; selectivity ratio = 75) in mammalian cells,⁵⁰ while in enzyme (HDAC1, HDAC4, and HDAC6) assays tubacin showed

Table 5. Human HDAC1 and HDAC4 Inhibitory Activities of $\mathbf{2f}$ and SAHA^a

	% inhibitory	activity at 5 $\mu { m M}$
assay	2f	SAHA
HDAC1	0	52.9
HDAC4	54.9	37.2

 a Data represent mean values of at least three separate experiments.

about 4-fold selectivity for HDAC6 (J. C. Wong, personal communication).

Human HDAC1 and HDAC4 Inhibitory Assays. The most selective compound in the maize HD1-B/ HD1-A system, i.e. the 3-[4-(3-(3-fluorophenyl)-3-oxopropen-1-yl)-1-methyl-1*H*-pyrrol-2-yl]-*N*-hydroxy-2-propenamide (**2f**) (class II fold selectivity = 176.4), was evaluated against human HDAC1 and HDAC4 in comparison with SAHA as reference drug. Human breast cancer ZR-75.1 cell lysates were immunoprecipitated with antibodies against HDAC1 and HDAC4, and inhibitory assays were performed on such immunoprecipitates (IPs) with **2f** (5 μ M) and SAHA (5 μ M). Data reported in Table 5 clearly show that **2f** lacked any inhibitory activity against human HDAC1 but was effective in inhibiting human HDAC4 enzyme.

Cellular Activities Evaluation: Apoptosis, Granulocytic Differentiation, and Cell Cycle Analysis. To investigate on cellular activities of selected (aryloxopropenyl)pyrrolyl hydroxamides, the effects of **2f** and **2s** on apoptosis, cytodifferentiation, and cell cycle phases in human U937 cell line were determined in comparison with SAHA.

It is well-known that class I HDACs induce the expression of some tumor suppressor genes, such as the cyclin-dependent kinase inhibitor $p21^{WAF1/Cip1}$, 17,83 while class II HDACs interact with one or more DNA-binding transcription factors as well as with transcriptional corepressors, such as MEF2.29 A detailed study using small interfering double stranded RNA (siRNA) to selectively knockdown HDACs 1, 3, 4, and 7 in HeLa S3 cells showed that knockdowns of HDACs 1 and 3 produced a significant morphological phenotype similar to that observed with HDAC inhibitor treatment of these cells, giving an antiproliferative effect, whereas knockdowns of HDACs 4 and 7 produced no changes in cell morphology.^{69,84} These studies established the critical role of class I HDACs in tumor cell proliferation/ survival, while class II HDACs may not be involved in the process that controls proliferation and apoptosis in cancer cells, their involvement being important in diverse biological processes such as cardiac and skeletal myogenesis, negative selection of developing thymocytes, and other unknown processes.²⁹

On these bases, the treatment of U937 AML cells with **2s**, that retain class I HDAC activity (IC_{50-HD1-B} = 0.33 μ M; IC_{50-HD1-A} = 0.01 μ M), was expected to induce apoptosis, cell differentiation, and cell cycle arrest, while treatment of U937 AML cells with **2f**, the selective class II (IIa)-inhibitor (IC_{50-HD1-B} = 38.8 μ M; IC_{50-HD1-A} = 0.22 μ M), was expected to have no effect. Indeed, as depicted in Figure 1, **2s** at 5 μ M induced 4% (24 h) and 8% (48 h) apoptosis, 56% CD11c positive cells, and arrest of cell cycle in G1 phase in U937 cells. SAHA, used as reference, at 5 μ M produced 9% (24 h) and 22%

(48h) apoptosis, 73% CD11c positive cells, and cell cycle arrest in G2/M phase. In the same assays, **2f** at 5 μ M showed % values (apoptosis, granulocytic differentiation, cell cycle phases) similar to those of controls.

Conclusion

A novel series of (aryloxopropenyl)pyrrolyl hydroxamates **2a**-**w** have been described as HDAC inhibitors, and their selectivity for maize HD1-A, a mammalian class II (IIa) HDAC homologue, has been determined. In general, the introduction of a substituent (from halogen atoms to methyl or methoxy groups) at the benzene ring of the (phenyloxopropenyl)pyrrolyl skeleton of the molecules did not improve the inhibitory activity, the order of potency of compounds (ortho- > meta > para-substituted) being the same for all the three tested enzymes (HD2, HD1-B, and HD1-A). Nevertheless, in the anti-HD1-A assay some highly active derivatives have been obtained, in particular whit chloro-, fluoro-, methyl- and methoxy-substitutions at the benzene C_2 and, to a lesser extent, C_3 position. Moreover, since such derivatives (mainly the C₃substituted compounds) showed little or no activity against HD1-B, a mammalian class I HDAC homologue, they were highly class II (IIa)-selective, the most selective being the 3-chloro- and 3-fluoro-substituted compounds 2c and 2f, with selectivity ratios of 71.4 (2c) and 176.4 (2f). Also the replacement of the benzene ring with the bulkier 1- and 2-naphthyl moieties furnished highly active derivatives against HD1-A (2s: $IC_{50} = 10 \text{ nM}$; **2t**: $IC_{50} = 40 \text{ nM}$, although less selective than **2c**,**f**. In comparison with TSA and SAHA, the inhibitory concentrations of (aryloxopropenyl)pyrrolyl hydroxyamides against HD1-A are substantially higher than that of TSA and in the range of SAHA. About selectivity, properly substituted (aryloxopropenyl)pyrrolyl hydroxyamides were class II (IIa) HDAC selective inhibitors, while TSA and SAHA showed a little selectivity against class I HDACs.

When tested against immunoprecipitated human HDAC1 and HDAC4, **2f** showed no inhibitory activity against HDAC1, while was able to inhibit HDAC4 (% inhibition at $5 \,\mu$ M = 54.9%). Moreover, in human U937 cancer cells **2f** did not produce any effect on apoptosis, granulocytic differentiation, and the cell cycle (class II HDAC-selective inhibitor behavior), whereas **2s** which retains class I HDAC inhibitory activity was 2-fold less potent than SAHA, used as reference to induce apoptosis and granulocytic differentiation, and blocked the cell cycle in G1 phase.

(Aryloxopropenyl)pyrrolyl hydroxamates $2\mathbf{a}-\mathbf{w}$ are the first reported examples of class II (IIa)-selective compounds and together with the "in cell" class IIbselective tubacin can be useful tools to probe the biology and to elucidate the functions, largely still unknown, of HDACs. Molecular modeling studies are in progress to rationalize the different behaviors of our class II (IIa)selective molecules against HD1-B and HD1-A enzymes.

Experimental Section

Chemistry. Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Perkin-Elmer Spectrum One instrument. ¹H NMR spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer; chemical shifts are reported in



Figure 1. Effects of 2s and 2f on apoptosis, granulocytic differentiation, and cell cycle phases (U937 AML cell line) in comparison with SAHA.

 δ (ppm) units relative to the internal reference tetramethylsilane (Me₄Si). All compounds were routinely checked by TLC and ¹H NMR. TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with spots visualized by UV light. 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 $\pm 0.40\%$ of the theoretical values. A SAHA sample for biological assays was prepared as previously reported by us.85 Tubacin was kindly provided by Jason C. Wong (Harvard University, Cambridge, MA). All chemicals were purchased from Aldrich Chimica, Milan (Italy) or Lancaster Synthesis GmbH, Milan (Italy) and were of the highest purity.

Ethyl 3-(4-Formyl-1-methyl-1H-pyrrol-2-yl)-2-propenoate (3). A 50 mL 1,2-dichloroethane solution of oxalyl chloride (0.06 mol, 5.2 mL) was added to a cooled (0-5 °C) solution of N,N-dimethylformamide (0.06 mol, 4.6 mL) in 1,2-dichloroethane (50 mL) over a period of 5-10 min. After being stirred at room temperature for 15 min, the suspension was cooled $(0-5 \ ^{\circ}C)$ again and treated with a solution of ethyl 3-(1-methyl-1H-pyrrol-2-yl)-2-propenoate⁷¹ (0.06 mol, 10.7 g) in 1,2-dichloroethane (50 mL). The mixture was stirred at room temperature for 1 h and then was poured onto crushed ice (200 g) containing 50% NaOH (50 mL) and stirred for 10 min. The pH of the solution was adjusted to 4 with 37% HCl, the organic layer was separated, and the aqueous one was extracted with chloroform $(2 \times 50 \text{ mL})$. The combined organic solutions were washed with water, dried, and evaporated to dryness. The residual solid was purified by recrystallization. ¹H NMR $(DMSO-d_6) \delta 1.24 (t, 3 H, CH_2CH_3), 3.96 (s, 3 H, NCH_3), 4.20$ (q, 2 H, CH₂CH₃), 6.60 (d, 1 H, CH=CHCO), 6.96 (d, 1 H, pyrrole β -proton), 7.03 (d, 1 H, pyrrole α -proton), 7.60 (d, 1 H, CH=CHCO), 9.58 (s, 1 H, CHO). Anal. (C₁₀H₁₃NO₂) C, H, N.

General Procedure for the Synthesis of 3-[4-(3-Aryl-3-oxopropen-1-yl)-1-methyl-1*H*-pyrrol-2-yl]-2-propenoic Acids 4a-w. Example: 3-[4-(3-(4-Chloro)phenyl-3-oxo-1-propen-1-yl)-1-methyl-1H-pyrrol-2-yl]-2-propenoic Acid 4d. A mixture of ethyl 3-(4-formyl-1-methyl-1H-pyrrol-2-yl)-2-propenoate 3 (6.0 mmol, 1.07 g), 4-chloroacetophenone (6.0 mmol, 0.93 g), and 2 N KOH (24.0 mmol, 12.4 mL) in ethanol (15 mL)/water (15 mL) was stirred at room temperature for 24 h. Afterward, the solution was poured into water (100 mL) and was made acid with 2 N HCl. The obtained precipitate was filtered and recrystallized to give the pure acid 4d. ¹H NMR (DMSO- d_6) δ 3.75 (s, 3 H, NCH₃), 6.34 (d, 1 H, CH= CHCOOH), 6.96 (d, 1 H, pyrrole β -proton), 7.21 (d, 1 H, pyrrole α-proton), 7.54 (d, 1 H, CH=CHCOOH), 7.65 (d, 2 H, benzene H-2,6), 7.73 (d, 1 H, COCH=CH), 7.77 (d, 1 H, COCH=CH), $8.12~(d,\,2~H,\,benzene~H\mathchar`a,5),\,12.22~(s,\,1~H,\,OH).$ Anal. $(C_{17}H_{14}\mathchar`a,5)$ $CINO_3$) C, H, N, Cl.

General Procedure for the Synthesis of 3-[4-(3-Aryl-3-oxopropen-1-yl)-1-methyl-1*H*-pyrrol-2-yl]-*N*-hydroxy-2propenamides 2a–w. Example: 3-[4-(3-Phenyl-3-oxo-1propen-1-yl)-1-methyl-1H-pyrrol-2-yl]-N-hydroxy-2propenamide (2a). Ethyl chloroformate (5.0 mmol, 0.5 mL) and triethylamine (5.4 mmol, 0.8 mL) were added to a cooled (0 °C) solution of 3-[4-(3-phenyl-3-oxopropen-1-yl)-1-methyl-1H-pyrrol-2-yl]-2-propenoic acid 4a (4.2 mmol, 1.18 g) in dry THF (10 mL), and the mixture was stirred for 10 min. The solid was filtered off, and O-(2-methoxy-2-propyl)hydroxylamine (12.6 mmol, 1.1 mL)⁷² was added to the filtrate. The solution was stirred for 15 min at 0 °C, then was evaporated under reduced pressure, and the residue was diluted in methanol (30 mL). Amberlyst 15 ion-exchange resin (0.6 g) was added to the solution of the O-protected hydroxamate, and the mixture was stirred at 45 °C for 1 h. Afterward, the reaction was filtered and the filtrate was concentrated in vacuo to give the crude 4a, which was purified by crystallization. ¹H NMR (DMSO-d₆) & 3.73 (s, 3 H, NCH₃), 6.30 (d, 1 H, CH=CH-CONHOH), 6.67 (s, 1 H, pyrrole β -proton), 7.17 (s, 1 H, pyrrole α-proton), 7.32 (d, 1 H, PhCOCH=CH), 7.53 (m, 3 H, benzene H-3,4,5), 7.62 m, 2 H, benzene H-2,6), 8.08 (d, 2 H, CH=CH-CONHOH and PhCOCH=CH). Anal. (C17H16N2O3) C, H, N.

In Vitro Maize HD2, HD1-B, and HD1-A Enzyme Inhibition. Radioactively labeled chicken core histones were used as the enzyme substrate according to established procedures.⁷³⁻⁷⁶ The enzyme liberated tritiated acetic acid from the substrate, which was quantified by scintillation counting. IC_{50} values are results of triple determinations. A 50 μ L sample of maize enzyme (at 30 °C) was incubated (30 min) with 10 μ L of total [³H]acetate-prelabeled chicken reticulocyte histones (2 mg/mL). Reaction was stopped by addition of 50 μ L of 1 M HCl/0.4 M acetate and 800 μ L of ethyl acetate. After centrifugation (10 000g, 5 min), an aliquot of $600 \,\mu\text{L}$ of the upper phase was counted for radioactivity in 3 mL of liquid scintillation cocktail. The compounds were tested at a starting concentration of 40 μ M, and active substances were diluted further. NaB, VPA, TSA, SAHA,85 TPX, HC-toxin, and tubacin were used as the reference compounds, and blank solvents were used as negative controls.

Human HDAC1 and HDAC4 Inhibition. Cell Culture and Ligands. Human breast cancer ZR-75.1 cells were propagated in DMEM medium supplemented with 10% FBS and antibiotics (100 U/mL penicillin, 100 μ g/mL streptomycin, and 250 ng/mL amphotericin-B). Before use, cells were plated at 60% confluence and treated the day after with 5 μ M SAHA⁸⁵ or **2f**. All compounds were resuspended in DMSO (Sigma-Aldrich).

HDAC1 and HDAC4 Assays. A 1000 μ g amount of total protein extracts (RIPA buffer for HDAC1 immunoprecipitation and NP-40 buffer for HDAC4 immunoprecipitation) was immunoprecipitated with 10 μ g of HDAC1 antibody (Alexis) or HDAC4 antibody (Santa Cruz) overnight at 4 °C in slow rotation. As negative control the same amount of protein extracts was immunoprecipitated with purified IgG (Santa Cruz). One day later, the samples were incubated with 40 μ L of protein A/G plus agarose (Santa Cruz) at 4 °C in slow rotation for 2–4 h. The samples were than washed six times with RIPA buffer or NP40-buffer respectively and twice in PBS and resuspended in 20 μ L of sterile PBS. The HDAC assays were carried out according to suppliers instructions (Upstate). Briefly, all samples immunoprecipitated with the HDAC1, HDAC4, or purified IgG were pooled respectively to homogenize all samples. A 10 μ L amount of each HDAC IP was incubated with a previously labeled ³H-Histone H4 peptide linked with streptavidin agarose beads (Upstate). In detail, 100.000 cpm of the H4-3H-acetyl-peptide was used for each tube and incubated in 1X HDAC buffer with 10 μ L of the sample in the presence or absence of the HDAC inhibitor with a final volume of 200 μ L. Those samples were incubated overnight at 37 °C in slow rotation. One day later, 50 μL of a quenching solution was added and 100 μ L of the samples was counted in duplicate after a brief centrifugation in a scintillation counter.

Determination of Apoptosis, Cell Differentiation, and Cell Cycle Effect on U937 AML Cells. Cell Lines and Cultures. U937 cell line was cultured in RPMI with 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, 10 mM HEPES, and 2 mM glutamine.

Ligands. SAHA⁸⁵ was dissolved in DMSO and used at 5 *µ*M. Compounds **2f** and **2s** were dissolved in DMSO and used at 5 μM.

Granulocytic Differentiation. U937 cells were harvested and resuspended in 10 μ L of phycoerythrine-conjugated CD11c (CD11c-PE). Control samples were incubated with 10 μ L of PE conjugated mouse IgG1, incubated for 30 min at 4 °C in the dark, washed in PBS, and resuspended in 500 μ L of PBScontaining propidium iodide (0.25 μ g/mL). Samples were analyzed by FACS with Cell Quest technology (Becton Dickinson) as previously reported.⁸⁶ Propidium iodide positive samples have been excluded from the analysis.

Cell Cycle Analysis. 2.5×10^5 Cells were collected and resuspended in 500 μ L of an hypotonic buffer (0.1% Triton X-100, 0.1% sodium citrate, 50 μ g/mL propidium iodide, 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.⁸⁶ All the experiments were performed 3 times.

FACS Analysis of Apoptosis. Apoptosis was measured with Annexin V/Propidium iodide double staining detection (Roche) as recommended by the supplier; samples were analyzed by FACS with Cell Quest technology (Becton Dickinson) as previously reported.87

Acknowledgment. The authors thank Dr. Jason C. Wong (Harvard University, Cambridge, MA) for the kind gift of tubacin. This work was partially supported by grants from Progetto Finalizzato Ministero della Salute 2002 (A.M.), AIRC 2003 (A.M.), PRIN 2004 (A.M.), European Union (QLG1-CT2000-01935 and QLK3-CT2002-02029), Ministero della Salute R. F. 02/ 184 (L.A.), PRIN 2004 (L.A.), and the Austrian Science Foundation Grant P13620 (P.L.).

Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

- (1) Hassig, C. A.; Schreiber, S. L. Nuclear histone acetylases and deacetylases and transcriptional regulation: HATs off to HDACs. Curr. Opin. Chem. Biol. 1997, 1, 300–308.
- Strahl, B. D.; Allis, C. D. The language of covalent histone modifications. Nature 2000, 403, 41-45.
- (3)Wu, J.; Grunstein, M. 25 Years after the nucleosome model: chromatin modifications. Trends Biochem. Sci. 2000, 25, 619-623
- (4) Wolffe, A. P.; Guschin, D. Chromatin structural features and targets that regulate transcription. J. Struct. Biol. 2000, 129, $10\bar{2}-122.$
- Cheung, W. L.; Briggs, S. D.; Allis, C. D. Acetylation and (5)chromosomal functions. Curr. Opin. Cell Biol. 2000, 12, 326-333

- (6) Grunstein, M. Histone acetylation in chromatin structure and transcription. Nature 1997, 389, 349–352.
- (7) Kouzarides, T. Histone acetylases and deacetylases in cell
- proliferation. Curr. Opin. Genet. Dev. **1999**, 9, 40–48. Roth, S.; Denu, J. M.; Allis, C. D. Histone acetyltransferases. Annu. Rev. Biochem. **2001**, 70, 81–120. (8)
- (9) Grant, P. A.; Berger, S. L. Histone acetyltransferase complexes. Semin. Cell Dev. Biol. 1999, 10, 169–177.
- (10) Cress, W. D.; Seto, E. Histone deacetylases, transcriptional control, and cancer. J. Cell Physiol. 2000, 184, 1-16
- Ng, H. H.; Bird, A. Histone deacetylases: silencers for hire. (11)Trends Biochem. Sci. 2000, 25, 121-126.
- Ferreira, R.; Magnaghi-Jaulin, L.; Robin, P.; Harel-Bellan, A.; (12)Trouche, D. The three members of the pocket proteins family share the ability to repress E2F activity through recruitment of a histone deacetylase. Proc. Natl. Acad. Sci. U.S.A. 1998, 95, 10493-10498.
- (13) Stiegler, P.; De Luca, A.; Bagella, L.; Giordano, A. The COOHterminal region of pRb2/p130 binds to histone deacetylase 1 (HDAC1), enhancing transcriptional repression of the E2Fdependent cyclin A promoter. *Cancer Res.* **1998**, *58*, 5049–5052. Nagy, L.; Kao, H.-Y.; Chakravarti, D.; Lin, R. J.; Hassig, C. A.;
- (14)Ayer, D. E.; Schreiber, S. L.; Evans, R. M. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 1997, 89, 373-380.
- (15) Alland, L.; Muhle, R.; Hou, H.; Potes, J.; Chin, L.; Schreiber-Agus, N.; DePinho, R. A. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature 1997, 387, 49-55.
- (16) Heinzel, T.; Lavinsky, R. M.; Mullen, T.-M.; Soderstrom, M.; Laherty, C. D.; Torchia, J.; Yang, W.-M.; Brard, G.; Ngo, S. D.; Davie, J. R.; Seto, E.; Eisenman, R. N.; Rose, D. W.; Glass, C K.; Rosenfeld, M. G. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature **1997**, 387, 43–48. Weidle, U. H.; Grossmann, A. Inhibition of Histone Deacety-
- (17)lases: a New Strategy to Target Epigenetic Modifications for Anticancer Treatment. Anticancer Res. 2000, 20, 1471-1486.
- (18) Kramer, O. H.; Göttlicher, M. G.; Heinzel, T. Histone deacetylase as a therapeutic target. Trends Endocrinol. Metab. 2001, 12, 294 - 300.
- (19) Marks, P. A.; Richon, V. M.; Breslow, R.; Rifkind, R. A. Histone deacetylase inhibitors as new cancer drugs. Curr. Opin. Oncol. **2001**, 13, 477-483.
- Marks, P. A.; Rifkind, R. A.; Richon, V. M.; Breslow, R.; Miller, (20)T.; Kelly, W. K. Histone deacetylases and cancer: causes and therapies. Nat. Rev. Cancer 2001, 1, 194-202.
- (21) Jung, M. Inhibitors of Histone Deacetylase as New Anticancer Agents. Curr. Med. Chem. 2001, 8, 1505-1511.
- (22)Vigushin, D. M.; Coombes, R. C. Histone deacetylase inhibitors in cancer treatment. Anti-Cancer Drugs 2002, 13, 1-13.
- Johnstone, R. W. Histone deacetylase inhibitors: novel drugs (23)for the treatment of cancer. Nat. Rev. Drug Discovery 2002, 1, 287 - 299
- (24) Kelly, W. K.; O'Connor, O. A.; Marks, P. A. Histone deacetylase inhibitors: from target to clinical trials. Expert Opin. Investig. Drugs 2002, 11, 1695–1713.
 Miller, T. A.; Witter, D. J.; Belvedere, S. Histone deacetylase
- inhibitors. J. Med. Chem. **2003**, 46, 5097–5116. (26) Grozinger, C. M.; Schreiber, S. L. Deacetylase Enzymes: Biologi-
- cal Functions and the Use of Small-Molecule Inhibitors. Chem. Biol. 2002. 9. 3-16.
- Gao, L.; Cueto, M. A.; Asselbergs, F.; Atadja, P. Cloning and functional characterization of HDAC11, a novel member of the (27)human histone deacetylase family. J. Biol. Chem. 2002, 277, 25748 - 25755
- (28)Gregoretti, I. V.; Lee, Y.-M.; Goodson, H. V. Molecular Evolution of the Histone Deacetylase Family: Functional Implications of Phylogenetic Analysis. J. Mol. Biol. 2004, 338, 17-31.
- Verdin, E.; Dequiedt, F.; Kasler, H. G. Class II histone deacety-(29)lases: versatile regulators. Trends Genet. 2003, 19, 286-293. Bertos, N. R.; Wang, A. H.; Yang, X.-J. Class II histone
- (30)deacetylases: Structure, function, and regulation. Biochem. Cell Biol. 2001, 79, 243-252
- (31) Fischle, W.; Kiermer, V.; Dequiedt, F.; Verdin, E. The emergency role of class II histone deacetylases. Biochem. Cell Biol. 2001, 79.337-348.
- (32) Verdel, A.; Khochbin, S. Identification and characterization of a new family of higher eukaryotic histone deacetylases. Coordinate expression of differentiation-dependent chromatin modifiers. J. Biol. Chem. 1999, 274, 2440-2445.
- (33) Fischle, W.; Emiliani, S.; Hendzel, M. J.; Nagase, T.; Nomura, N.; Voelter, W.; Verdin, E. A new family of human histone deacetylases related to Saccharomyces cerevisiae HDA1p. J. Biol. Chem. 1999, 274, 11713-11720.
- Grozinger, C. M.; Hassig, C. A.; Schreiber, S. L. Three proteins (34)define a class of human histone deacetylases related to yeast Hda1p. Proc. Natl. Acad. Sci. U.S.A. 1999, 96, 4868-4873.

- (35) De Ruijter, A. J. M.; van Gennip, A. H.; Caron, H. N.; Kemp, S.; van Kuilenburg A. B. P. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem. J. 2003, 370, 737 - 749
- (36) Zhang, C. L.; McKinsey, T. A.; Chang, S.; Antos, C. L.; Hill, J. A.; Olson, E. N. Class II histone deacetylases act as signalresponsive repressors of cardiac hypertrophy. Cell 2002, 110, 479 - 488.
- (37) Dequiedt, F.; Kasler, H. G.; Fischle, W.; Kiermer, V.; Weinstein, M.; Herndier, B. G.; Verdin, E. HDAC7, a thymus-specific Class II histone deacetylase, regulates Nur77 transcription and TCRmediated apoptosis. Immunity 2003, 18, 687-698.
- (38) Fischer, D. D.; Cai, R.; Bhatia, U.; Asselbergs, F. A.; Song, C.; Terry, R.; Trogani, N.; Widmer, R.; Atadja, P.; Cohen, D. Isolation and characterization of a novel class II histone deacetylase, HDAC10. J. Biol. Chem. 2002, 277, 6656-6666.
- (39) Kao, H. Y.; Lee, C. H.; Komarov, A.; Han, C. C.; Evans, R. M. Isolation and characterization of mammalian HDAC10, a novel histone deacetylase. J. Biol. Chem. **2002**, 277, 187–193. (40) McKinsey, T. A.; Zhang, C. L.; Olson, E. N. MEF2: a calcium-
- dependent regulator of cell division, differentiation and death.
- (41) McKinsey, T. A.; Zhang, C. L.; Olson, E. N. Control of muscle development by dueling HATs and HDACs. *Curr. Opin. Genet.* Dev. 2001, 11, 497–504. (42) Gruffat, H.; Manet, E.; Sergeant, A. MEF2-mediated recruitment
- of class II HDAC at the EBV immediate early gene BZLF1 links latency and chromatin remodeling. EMBO Rep. 2002, 3, 141-146.
- (43) Hubbert, C.; Guardiola, A.; Shao, R.; Kawaguchi, Y.; Ito, A.; Nixon, A.; Yoshida, M.; Wang, X. F.; Yao, T. P. HDAC6 is a microtubule-associated deacetylase. Nature 2002, 417, 455-458.
- (44) Matsuyama, A.; Shimazu, T.; Sumida, Y.; Saito, A.; Yoshimatsu, Y.; Seigneurin-Berny, D.; Osada, H.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Horinouchi, S.; Yoshida, M. In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. EMBO J. 2002, 21, 6820-6831.
- (45) Göttlicher, M.; Minucci, S.; Zhu, P.; Krämer, O. H.; Schimpf, A.; Giavara, S.; Sleeman, J. P.; Lo Coco, F.; Nervi, C.; Pelicci, P. G.; Heinzel, T. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J. 2001, 20, 6969 - 6978
- (46) Fumurai, R.; Matsuyama, A.; Kobashi, N.; Lee, K.-H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. FK228 (Depsipeptide) as a Natural Prodrug That Inhibits Class I Histone Deacetylases. Cancer Res. **2002**, *6*2, 4916–4921.
- (47) Fumurai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; Horinouchi, S. Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. Proc. Natl. Acad. Sci. U.S.A. 2001, 98, 87-92.
- (48) Guardiola, A. R.; Yao, T.-P. Molecular Cloning and Characterization of a Novel Histone Deacetylase HDAC10. J. Biol. Chem. 2002, 277, 3350-3356.
- (49) Sternson, S. M.; Wong, J. C.; Grozinger, C. M.; Schreiber, S. L. Synthesis of 7200 Small Molecules Based on a Substructural Analysis of the Histone Deacetylase Inhibitors Trichostatin and Trapoxin. Org. Lett. 2001, 3, 4239-4242.
- Wong, J. C.; Hong, R.; Schreiber, S. L. Structural Biasing Elements for In-Cell Histone Deacetylase Paralog Selectivity. (50)J. Am. Chem. Soc. 2003, 125, 5586-5587. (51) Haggarty, S. J.; Koeller, K. M.; Wong, J. C.; Butcher, R. A.;
- Schreiber, S. L. Multidimensional Chemical Genetic Analysis of Diversity-Oriented Synthesis-Derived Deacetylase Inhibitors Using Cell-Based Assays. *Chem. Biol.* **2003**, *10*, 383–396. (52) Haggarty, S. J.; Koeller, K. M.; Wong, J. C.; Grozinger, C. M.;
- Schreiber, S. L. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc. Natl. Acad. Sci. U.S.A. 2003, 100, 4389-4394.
- (53) Tong, J. K. Dissecting Histone Deacetylase Function. Chem. Biol. 2002, 9, 668-670.
- Massa, S.; Mai, A.; Sbardella, G.; Esposito, M.; Ragno, R.; Loidl, (54)P.; Brosch, G. 3-(4-Aroyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides, a New Class of Synthetic Histone Deacetylase Inhibitors. J. Med. Chem. 2001, 44, 2069-2072.
- (55) Mai, A.; Massa, S.; Ragno, R.; Esposito, M.; Sbardella, G.; Nocca, G.; Scatena, R.; Jesacher, F.; Loidl, P.; Brosch, G. Binding Mode Analysis of 3-(4-Benzoyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2propenamide: A New Synthetic Histone Deacetylase Inhibitor Inducing Histone Hyperacetylation, Growth Inhibition, and Terminal Cell Differentiation. J. Med. Chem. 2002, 45, 1778 1784.
- (56) Mai, A.; Massa, S.; Ragno, R.; Cerbara, I.; Jesacher, F.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2alkylamides as a New Class of Synthetic Histone Deacetylase Inhibitors. 1. Design, Synthesis, Biological Evaluation, and Binding Mode Studies Performed Through Three Different Docking Procedures. J. Med. Chem. 2003, 46, 512-524.

- (57) Mai, A.; Massa, S.; Cerbara, I.; Valente, S.; Ragno, R.; Bottoni, P.; Scatena, R.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1-methyl-1H-2pyrrolyl)-N-hydroxy-2-propenamides as a New Class of Synthetic Histone Deacetylase Inhibitors. 2. Effect of Pyrrole C2 and/or C4 Substitutions on Biological Activity. J. Med. Chem. 2004, 47, 1098 - 1109
- (58) Ragno, R.; Mai, A.; Massa, S.; Cerbara, I.; Valente, S.; Bottoni, P.; Scatena, R.; Jesacher, F.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1methyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides as a New Class of Synthetic Histone Deacetylase Inhibitors. 3. Discovery of Novel Lead Compounds through Structure-Based Drug Design and Docking Studies. J. Med. Chem. 2004, 47, 1351-1359.
- Mai, A.; Massa, S.; Rotili, D.; Cerbara, I.; Valente, S.; Pezzi, R.; (59)Simeoni, S.; Ragno, R. Histone Deacetylation in Epigenetics: an Attractive Target for Anticancer Therapy. Med. Res. Rev. 2004, DOI 10.1002/med.20024.
- (60)Mai, A.; Cerbara, I.; Valente, S.; Massa, S.; Walker, L. A.; Tekwani, B. L. Antimalarial and Antileishmanial Activities of Aroyl-Pyrrolyl-Hydroxyamides, a New Class of Histone Deacetylase Inhibitors. Antimicrob. Agents Chemother. 2004, 48, 1435-1436.
- (61) Lusser, A.; Brosch, G.; Loidl, A.; Haas, H.; Loidl, P. Identification of maize histone deacetylase HD2 as an acidic nucleolar phosphoprotein. *Science* **1997**, *277*, 88–91. Wittich, S.; Scherf, H.; Xie, C.; Brosch, G.; Loidl, P.; Gerhäuser,
- (62)C.; Jung, M. Structure-Activity Relationships on Phenylalanine-Containing Inhibitors of Histone Deacetylase: In Vitro Enzyme Inhibition, Induction of Differentiation, and Inhibition of Proliferation in Friend Leukemic Cells. J. Med. Chem. 2002, 45, 3296-3309.
- (63)Mai, A.; Massa, S.; Pezzi, R.; Rotili, D.; Loidl, P.; Brosch, G. Discovery of (Aryloxopropenyl)pyrrolyl Hydroxamides as Selective Inhibitors of Class IIa Histone Deacetylase Homologue HD1-A. J. Med. Chem. 2003, 46, 4826-4829.
- (64) Mai, A.; Massa, S.; Pezzi, R.; Valente, S.; Loidl, P.; Brosch, G. Synthesis and Biological Evaluation of 2-, 3-, and 4-Acylaminocinnamyl-N-hydroxyamides as Novel Synthetic HDAC Inhibitors. Med. Chem. 2005, 25, 261-309.
- (65) Kölle, D.; Brosch, G.; Lechner, T.; Pipal, A.; Helliger, W.; Taplick, J.; Loidl, P. Different types of maize histone deacetylases are distinguished by a highly complex substrate and site specificity. Biochemistry 1999, 38, 6769–6773.
 (66) Lechner, T.; Lusser, A.; Pipal, A.; Brosch, G.; Loidl, A.; Goralik-
- Schramel, M.; Sendra, R.; Wegener, S.; Walton, J. D.; Loidl, P. $\label{eq:RPD3-Type} RPD3-Type\ histone\ deacetylases\ in\ maize\ embryos.\ Biochemistry$ **2000**, *39*, 1683–1692. (67) Brosch, G.; Goralik-Schramel, M.; Loidl, P. Purification of histone
- deacetylase HD1-A of germinating maize embryos. FEBS Lett. **1996**, 393, 287-291.
- (68) Brosch, G.; Georgieva, E.; Lopez-Rodas, G.; Lindner, H.; Loidl, P. Specificity of zea mays histone deacetylase is regulated by phosphorylation. J. Biol. Chem. 1992, 267, 20561-20564.
- (69)Curtin, M.; Glaser, K. Histone Deacetylase Inhibitors: The Abbott Experience. Curr. Med. Chem. 2003, 10, 2373-2392.
- Li, J.; Staver, M. J.; Curtin, M. L.; Holms, J. H.; Frey, R. R.; (70)Edalji, R.; Smith, R.; Michaelides, M. R.; Davidsen, S. K.; Glaser, K. B. Expression and functional characterization of recombinant human HDAC1 and HDAC3. Life Sci. 2004, 74, 2693-2705.
- (71) Sinisterra, J. V.; Mouloungui, Z.; Delmas, M.; Gaset, A. Barium hydroxide as catalyst in organic reactions. V. Application in the Horner reaction under solid-liquid phase-transfer conditions. Synthesis 1985, 1097-1100.
- (72) Mori, K.; Koseki, K. Synthesis of trichostatin A, a potent differentiation inducer of Friend leukemic cells, and its antipode. Tetrahedron 1988, 44, 6013-6020.
- (73)HD2-activity was extensively purified by anion exchange chromatography (Q-Sepharose), affinity chromatography (Heparin-Sepharose, Histone-Agarose), and size exclusion chromatography (Superdex S200) as described elsewhere.74-76
- Lechner, T.; Lusser, A.; Brosch, G.; Eberharter, A.; Goralik-Schramel, M.; Loidl, P. A comparative study of histone deacety-(74)lases of plant, fungal and vertebrate cells. Biochim. Biophys. Acta 1996, 1296, 181–188.
- (75) Brosch, G.; Lusser, A.; Goralik-Schramel, M.; Loidl, P. Purification and characterization of a high molecular weight histone deacetylase complex (HD2) of maize embryos. Biochemistry 1996, 35, 15907 - 15914.
- (76) Kölle, D.; Brosch, G.; Lechner, T.; Lusser, A.; Loidl, P. Biochemical methods for analysis of histone deacetylases. Methods 1998, 15.323-331.
- (77) Kruh, J. Effects of Sodium Butyrate, a New Pharmacological Agent, on Cells in Culture. Mol. Cell. Biochem. 1982, 42, 65-82
- (78)Phiel, C. J.; Zhang, F.; Huang, E. Y.; Guenther, M. G.; Lazar, M. A.; Klein, P. S. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J. Biol. Chem. 2001, 76, 36734-36741.

- (79) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. Potent and Specific Inhibition of Mammalian Histone Deacetylase both In Vivo and In Vitro by Trichostatin A. J. Biol. Chem. **1990**, 265, 17174– 17179.
- (80) Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003–3007.
- (81) Kijima, M.; Yoshida, M.; Suguta, K.; Horinouchi, S.; Beppu, T. Trapoxin, an Antitumor Cyclic Terapeptide, Is an Irreversible Inhibitor of Mammalian Histone Deacetylase. J. Biol. Chem. 1993, 268, 22429–22435.
- (82) Brosch, G.; Ransom, R.; Lechner, T.; Walton, J.; Loidl, P. Inhibition of maize histone deacetylases by HC toxin, the hostselective toxin of *Cochliobolus carbonum*. *Plant Cell* **1995**, *33*, 1941–1950.
- (83) Sambucetti, L. C.; Fischer, D. D.; Zabludoff, S.; Kwon, P. O.; Chamberlin, H.; Trogani, N.; Xu, H.; Cohen, D. Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. J. Biol. Chem. 1999, 274, 34940–34947.

- (84) Glaser, K. B.; Li, J.; Staver, M. J.; Wei, R. Q.; Albert, D. H.; Davidsen, S. K. Role of class I and class II histone deacetylases in carcinoma cells using siRNA. *Biochem. Biophys. Res. Commun.* 2003, 310, 529–536.
- (85) Mai, A.; Esposito, M.; Sbardella, G.; Massa, S. A new facile and expeditious synthesis of N-hydroxy-N'-phenyloctanediamide, a potent inducer of terminal cytodifferentiation. Org. Prep. Proced. Int. 2001, 33, 391–394.
 (86) Nebbioso, A.; Clarke, N.; Voltz, E.; Germain, E.; Ambrosino, C.;
- (86) 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. Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells. *Nat. Med.* **2005**, *11*, 77-84.
- (87) Altucci, L.; Rossin, A.; Raffelsberger, W.; Reitmair, A.; Chomienne, C.; Gronemeyer, H. Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL. *Nat. Med.* **2001**, *7*, 680–686.

JM049002A