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Bioorganic & Medicinal Chemistry Letters 17 (2007) 4895–4900
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and evaluation of isoindolinone-hydroxamic acid derivatives as histone deacetylase (HDAC) inhibitors

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Received 21 May 2007; revised 7 June 2007; accepted 11 June 2007
Available online 13 June 2007

Abstract—We designed and synthesized hydroxamic acid derivatives bearing a 4-(3-pyridyl)phenyl group as a cap structure, and found that they exhibit potent histone deacetylase (HDAC) inhibitory activity. A representative compound, **17a**, showed more potent growth-inhibitory activity against pancreatic cancer cells and greater upregulation of $p21^{WAF1/CIP1}$ expression than the clinically used HDAC inhibitor suberoylanilide hydroxamic acid (Zolinza™).

Epigenetic regulation of specific gene expression is mediated by several mechanisms, among which one of the most important is post-translational acetylation of the side-chain amino groups of specific histone lysine residues. The acetylation status of histones is modulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC).[1,2] HAT is generally considered as a transcriptional activator, and HDAC is considered as a transcriptional inhibitor, because histone acetylation is associated with transcriptionally active chromatin, whereas histone deacetylation is associated with transcription repression. Many recent studies have shown that inhibition of HDAC elicits anticancer effects in several lines of tumor cells by inhibiting cell growth and inducing apoptosis.[3–5] Therefore, compounds that inhibit HDAC activity may depress the expression of certain genes, resulting in antiproliferative and antitumor effects.[6] Natural and synthetic HDAC inhibitors have been studied extensively (Fig. 1), and suberoylanilide hydroxamic acid (SAHA; Zolinza™) has been approved by the FDA for once-daily oral treatment of advanced cutaneous T-cell lymphoma (CTCL), and further clinical studies of Zolinza™ for the treatment of various solid tumors are in progress.

Numerous biological studies have indicated that HDACs are heterogeneous, consisting of 18 isozymes, which can be categorized into four classes (class I, class IIa, class IIb, and class III). Class I and class II HDACs are zinc-containing amidehydrolases, and class III HDAC consists of NAD-dependent amidehydrolase. The biological function and distribution of each class of HDACs have been extensively studied from a molecular-pharmacological viewpoint, but much remains to be learnt.

We have been engaged in structural development studies of the multi-drug template thalidomide for the creation of structurally novel drug leads,[7–12] and have already reported the design and synthesis of potent HDAC inhibitors with cyclic amide structure (isoindolinone), such as compounds **8** and **9**.[13,14] In this paper, we report the design, synthesis, and biological activity of novel HDAC inhibitors with a pyridylphenyl group as a cap structure.

Synthetic routes to the present series of cyclic amide derivatives are outlined in Chart 1.

4-Iodobenzoic acid (**10**) was reduced with BH_3·THF to afford 4-iodobenzyl alcohol (**11**). This alcohol was

Keywords: HDAC; HDAC inhibitor; Isoindoklinone; Hydroxamic acid; Pancreatic cancer; p21.
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doi:10.1016/j.bmcl.2007.06.038

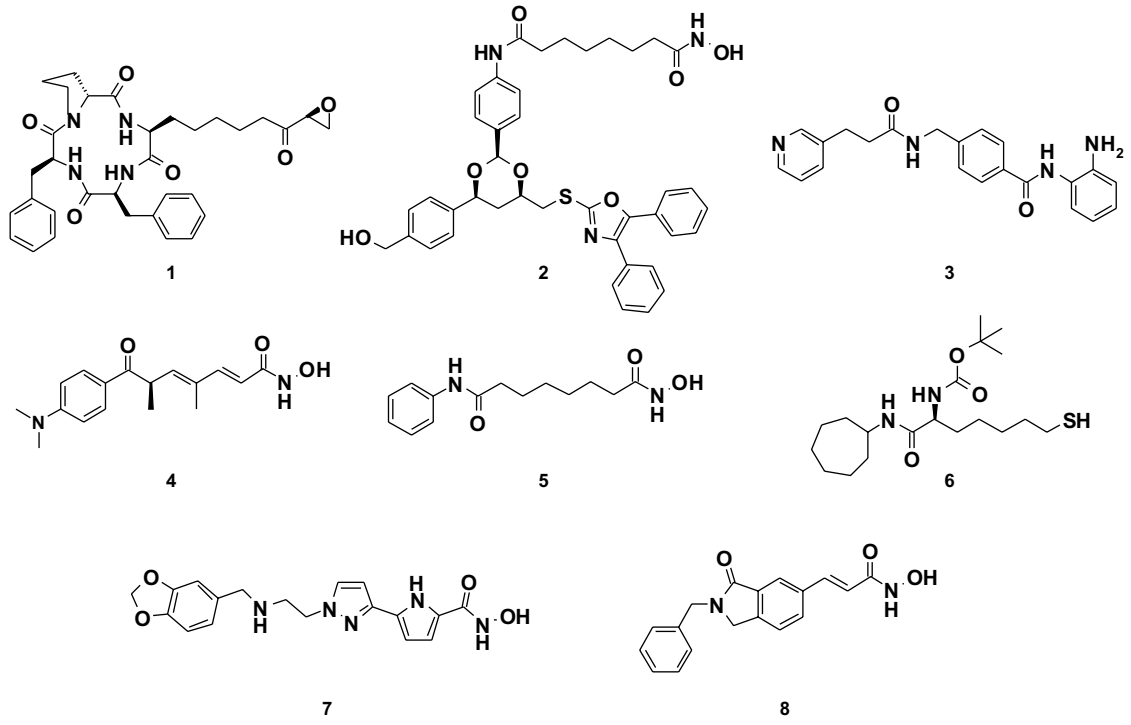


Figure 1. Structures of representative natural HDAC inhibitors (**1** (trapoxin A), **4** (TSA)), and synthetic HDAC inhibitors (**2** (tubacin), **3** (MS-275), **5** (SAHA: Zolinza™), **6** and **7** (ADS102550), and our lead compound **8**).

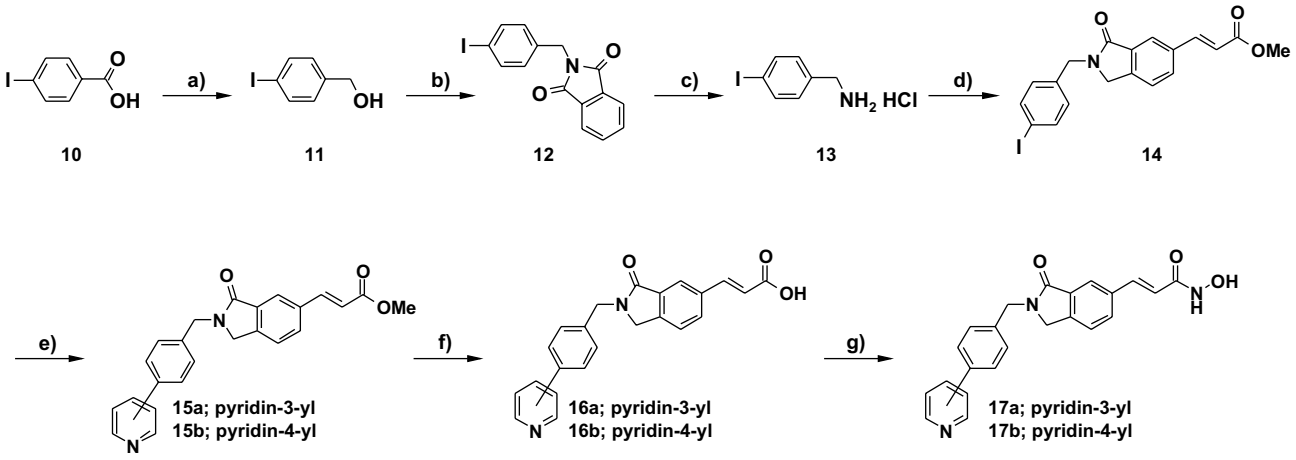


Chart 1. Reagents and conditions: (a) BH_3·THF, THF, 0 °C, 96%; (b) phthalimide, DEAD, $P(Ph)_3$, THF/toluene, rt, 84%; (c) $NH_2NH_2·H_2O$, MeOH, HCl, reflux, quant.; (d) methyl 4-bromomethyl-3-methoxycarbonylcinnamate, TEA, MeOH, reflux, 45%; (e) 3-pyridylboronic acid (or 4-pyridylboronic acid), $Pd(P(Ph)_3)_4$, Na_2CO_3, DME, reflux, 26–74%; (f) NaOH, MeOH, reflux, 18–54%; (g) NH_2OH·HCl, EDCI, HOBt, *i*-Pr_2Net, DMF, rt, 12–39%.

treated with phthalimide under Mitsunobu condition to afford *N*-benzylphthalimide (**12**). Compound **12** was hydrazinolyzed to afford 4-iodobenzylamine·HCl (**13**). Compound **13** was reacted with methyl 4-bromomethyl-3-methoxycarbonylcinnamate in the presence of triethylamine as a base to afford the cyclic amide (**14**). Suzuki coupling of **14** with 3-pyridylboronic acid (or 4-pyridylboronic acid) in the presence of $Pd(P(Ph)_3)_4$ afforded the pyridylphenyl derivatives **15a** and **15b**, respectively. Alkaline hydrolysis of **15a** and **15b** afforded cinnamic acids **16a** and **16b**, respectively. Hydroxylamine·HCl was condensed with **16a** and **16b**, in the presence of EDCI, to give the final hydroxamic acid derivatives, **17a** and **17b**, respectively.[15]

Recent X-ray crystallographic analyses of histone deacetylase-like protein (HDLP),[16] and HDAC 8,[17] complexed with trichostatin A (TSA, **4**)[18] indicate that the HDAC catalytic domain consists of a narrow tube-like pocket, with the zinc ion buried near the bottom of the active site. Therefore, the structural requirements for potent HDAC inhibitors involve three key regions,

that is, (1) a zinc-binding domain, which interacts with the active site zinc, (2) a linking domain, which occupies the channel, and (3) a cap domain, which interacts with the residues on the rim of the active site. Previously we have reported novel HDAC inhibitors based on a new structural scaffold.[13,14] We used a *N*-benzylisoindolinone structure as a novel linker/cap domain that might be suitable to occupy the narrow channel of the HDAC active site. An SAR study indicated that the introduction of a bulky substituent at the 4-position of the distal benzene ring tended to potentiate inhibitory activity toward HDAC, especially HDAC 4, and a phenyl group was the best substituent (compound **9**).[14]

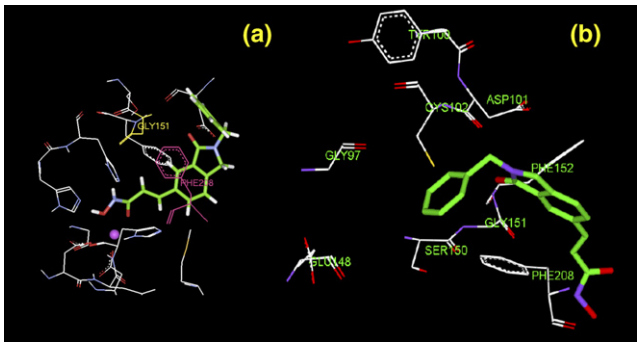


Figure 2. Docking model structures of compound **8** into the HDAC 8 binding pocket.

Our previous molecular modeling study indicated that the backbone-benzene ring of our lead compound (**8**), fused to the cyclic amide skeleton, exhibits a π–π stacking interaction with the side-chain benzyl group of 208Phe of HDAC 8.[14] The environment of the cap structure benzyl group of compound (**8**) contains some important amino acid residues which may be involved in electrostatic interactions, such as Tyr100 and Glu148 (Fig. 2). We expected that the introduction of basic character into the cap structure of **8** might enhance the interaction with the HDAC ligand binding pocket, and further strengthen the HDAC inhibitory activity. Therefore, we planned to synthesize hybrid-type compounds **17a** and **17b** (Fig. 3). The results are summarized in Table 1, together with those for the lead compounds **8**, **9**, and the positive controls Zolinza™ (**5**)

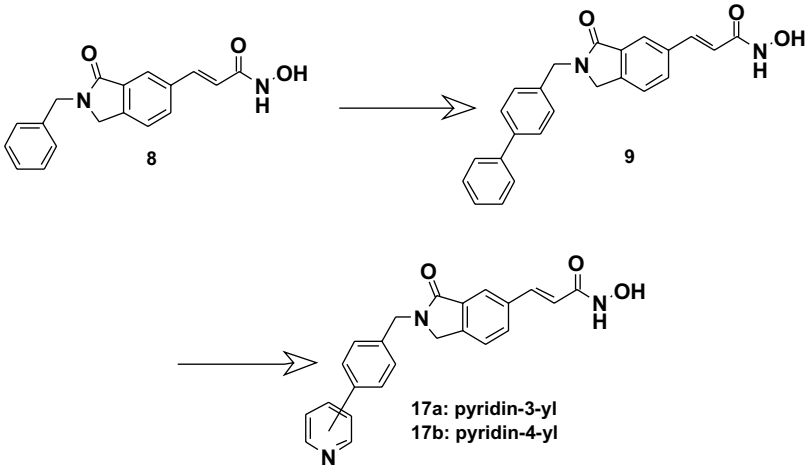
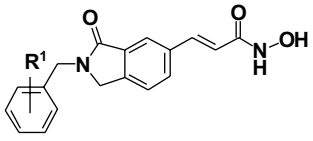


Figure 3. Structural development of our HDAC inhibitors.

Table 1. HDAC inhibitory activities of the prepared compounds



Compound	R^1	HDAC inhibitory activity IC_{50} (nM)		
		HDAC 1	HDAC 4	HDAC 6
8	H	250 ± 20	220 ± 4	230 ± 70
18	4-*t*-Bu	230 ± 20	180 ± 4	990 ± 20
9	4-Ph	220 ± 30	98 ± 6	750 ± 11
17a	4-(Pyridin-3-yl)	92 ± 6	56 ± 7	280 ± 10
17b	4-(Pyridin-4-yl)	3300 ± 200	2100 ± 200	15,000 ± 1800
	Zolinza™(5)[a]	290 ± 50[a]	340 ± 30[a]	200 ± 10[a]
	TSA (4)	16 ± 1	29 ± 5	53 ± 9

[a] Zolinza™ was prepared by Prof. Norikazu Nishino's group (Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology). The HDAC inhibitory activity of Zolinza™ was assayed by Prof. Nishino and two of the present authors, Satoko Maeda and Minoru Yoshida, and we thank Prof. Nishino for disclosure of the results.

and TSA (**4**). TSA is well known as a classical (or representative) potent HDAC inhibitor, which exhibited some toxicity and biological instability. We selected HDAC 1, HDAC 4, and HDAC 6 as representatives of class I, class IIa, and class IIb, respectively, because sufficient amounts of these isozymes were available for our purpose.

Our earlier SAR study indicated that the introduction of a bulky substituent at the 4-position of the distal phenyl group enhanced HDAC 4 inhibitory activity, and decreased HDAC 6 inhibitory activity, while HDAC 1 inhibitory activity was apparently not changed.[14] However, we found here that the appropriate introduction of a basic functionality at the distal benzene ring dramatically affected the HDAC inhibitory activity. The 3-pyridyl derivative (**17a**) exhibited about 2-fold greater HDAC 4 inhibitory activity than the 4-phenyl derivative (**9**), having activity comparable with that of the naturally occurring HDAC inhibitor TSA. As for HDAC 6 inhibitory activity, the introduction of a 3-pyridyl group instead of a phenyl group restored the activity to that of the non-substituted compound (compare **8**, **9**, **17a**). In the case of HDAC 1, the introduction of a 3-pyridyl group also increased the inhibitory activity to some extent. This is interesting, because the introduction of a phenyl group (**9**) did not cause any clear effect. All these data clearly indicated that the introduction of a 3-pyridyl group increases the inhibitory activity toward the HDAC isozymes tested. The reason for this might be that the pyridyl nitrogen atom interacts favorably with acidic amino acid residues located near the cap structure. The 3-pyridyl group of MS-275 (**3**) might also contribute to the positive interaction with acidic amino acid residues located near the cap structure, though the HDAC inhibitory activity of MS-275 is not so high (micromolar order).

It is of interest to note that the position of the pyridyl nitrogen is important for potent HDAC inhibitory activity, because the 4-pyridyl derivative (**17b**) exhibited a more than 30-fold decrease in HDAC inhibitory activity, being far less potent than the non-substituted compound (**8**). Although the reason for this is not yet clear, there might be electronic and/or steric interactions between the 4-position substituent and amino acids adjacent to the binding site.

Thus, we obtained the HDAC inhibitor **17a**, which is more potent than Zolinza (**5**), and as potent as TSA (**4**). We therefore thought it worthwhile to examine the potential of **17a** as an anticancer agent.

Pancreatic cancer is the intractable solid tumor, and the fifth leading cause of cancer death in Japan and the United States. The incidence is virtually equal to the mortality, and the 5-year survival rate is only a few percent.[19] The treatment options remain unsatisfactory, although our understanding of the molecular biology of pancreatic cancer has advanced. In general, HDAC activity is increased in cancer cells, including pancreatic cancer cells, and this increase has been shown to induce oncogenic transformation, resulting in altered gene(s) transcription and increased proliferation.[20]

Considering these points, we examined the growth-inhibiting effects of our compounds **17a**, **9**, and the positive control Zolinza™ (**5**) on two human pancreatic cancer cell lines, PANC-1 and PT-45 (Fig. 4). Both cell lines contain a mutation in the *Smad4* gene, and PT-45 is more chemoresistant. As shown in Figure 4, Zolinza™ dose-dependently decreased the proliferation of PANC-1 and PT-45 cells, with IC_{50} values of 450 and 4000 nM, respectively. These results indicate that an HDAC inhibitor can inhibit pancreatic cancer cell growth. Compounds **17a** and **9** also caused dose-dependent decreases in the proliferation of PANC-1 and PT-45 cells. The IC_{50} values of **17a** and **9** for PANC-1 cells were 35 and 150 nM, respectively, and those for PT-45 cells were 200 and 300 nM, respectively. Both **17a** and **9** exhibited more potent antiproliferative activity than Zolinza™, presumably reflecting their more potent HDAC inhibitory activities.

HDAC inhibitors have been reported to upregulate the expression of the tumor repressor, $p21^{WAF1/CIP1}$, and to downregulate cyclin D1 in many types of tumor cells, in parallel with cell cycle arrest in the G1 phase. Activation of the $p21^{WAF1/CIP1}$ gene is associated with the inhibition of proliferation and induction of differentiation and/or apoptosis of tumor cells, both in vitro and in vivo.[5,6] Therefore, we examined the ability of representative compounds to upregulate the expression of $p21^{WAF1/CIP1}$.[21] We also examined the effects of these HDAC inhibitors on the expression of another important gene, $p27^{kip1}$.[22] $p27^{kip1}$ is a cyclin kinase inhibitor, and it plays a central role in the suppression of tumori-

	ID_{50} (nM)	
compd.	**PANC-1**	**PT-45**
17a	**35**	**200**
9	**150**	**300**
Zolinza™	**450**	**4000**

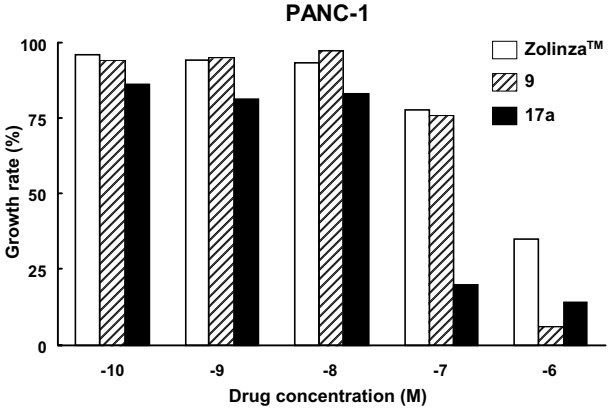


Figure 4. Effect of **9**, **17a**, and Zolinza™ on pancreatic cancer cell growth.

genesis in a variety of human cancers by inhibiting the activity of cyclin E- or A-containing cdk2 (cyclin dependent kinase 2) complexes.

As shown in Figure 5, the $p21^{WAF1/CIP1}$ gene expression of PANC-1 cells was augmented 3-fold as compared to that of the vehicle control after exposure of the cells to 1 μM Zolinza™ for 24 h, and the effect remained for more than 48 h. These results strongly suggest that the enhancement of $p21^{WAF1/CIP1}$ expression is involved in the initial inhibition of PANC-1 cell growth by HDAC inhibitors. Compound **17a** exhibited highly potent $p21^{WAF1/CIP1}$ expression-enhancing activity: 0.1 μM **17a** exhibited almost the same activity as 1 μM Zolinza™, and 1 μM **17a** augmented the $p21^{WAF1/CIP1}$ gene expression 8-fold over the vehicle control in cells exposed for 48 h.

However, none of the HDAC inhibitors tested affected the gene expression of $p27^{kip1}$, although some researchers have reported a positive correlation between HDAC inhibition and $p27^{kip1}$ expression.[23,24] The experimental conditions (cell type, incubation time, etc.) were different, but the reason for the discrepancy remains to be resolved.

In conclusion, we have designed and synthesized hydroxamic acid derivatives bearing a 4-(3-pyridyl)phenyl group as a cap structure, which exhibit more potent HDAC inhibitory activity than the clinically used HDAC inhibitor Zolinza™. Further chemical modification studies based on our present SAR data should provide even more potent and more class-selective HDAC inhibitors. A representative compound, **17a**, exhibited promising antiproliferation activity against human pancreatic cancer cell lines, and is a candidate drug for the treatment of pancreatic cancer, or at least a lead compound for further development.

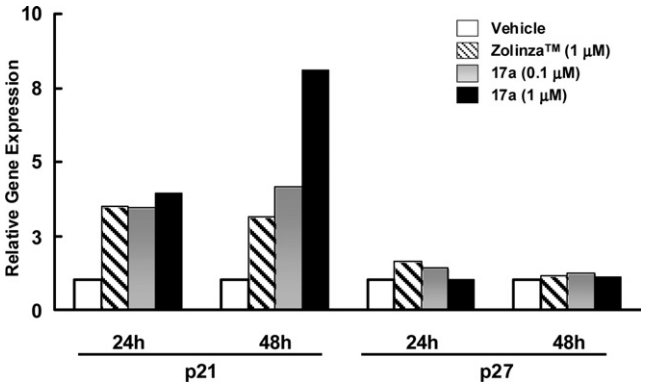


Figure 5. Effects of **17a** and Zolinza™ on mRNA expression of p21 and p27 genes in PANC-1 cells by quantitative real-time RT-PCR. PANC-1 cells were treated with the indicated concentrations of **17a** or Zolinza™ for 24 or 48 h, and mRNA expression was analyzed by quantitative real-time PCR. The ratios of p21 and p27 mRNA levels were normalized to GAPDH, and values shown are relative to vehicle-treated cells.

Acknowledgments

The work described in this paper was partially supported by a Grant-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors thank Prof. Nishino, Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, for data on the HDAC inhibitory activity of Zolinza, and Dr. Keisuke Imai, Lead Discovery Research Labs., Astellas Pharma Inc., for computational docking studies.

References and notes

1. Grunstein, M. *Nature* **1997**, *389*, 349.
2. Cheung, W. L.; Briggs, S. D.; Allis, C. D. *Curr. Opin. Cell Biol.* **2000**, *12*, 326.
3. Roth, S. Y.; Denu, J. M.; Allis, C. D. *Annu. Rev. Biochem.* **2001**, *70*, 81.
4. Cress, W. D.; Seto, E. *J. Cell. Physiol.* **2000**, *184*, 1.
5. Rosato, R. R.; Wang, Z.; Gopalkrishnan, R. V.; Fisher, P. B.; Grant, S. *Int. J. Oncol.* **2001**, *19*, 181.
6. Weidle, U. H.; Grossmann, A. *Anticancer Res.* **2000**, *20*, 1471.
7. Hashimoto, Y. *Curr. Med. Chem.* **1998**, *5*, 163.
8. Hashimoto, Y. *Bioorg. Med. Chem.* **2002**, *10*, 461.
9. Miyachi, H.; Azuma, A.; Ogasawara, A.; Uchimura, E.; Watanabe, N.; Kobayashi, Y.; Kato, F.; Kato, M.; Hashimoto, Y. *J. Med. Chem.* **1997**, *40*, 2858.
10. Miyachi, H.; Ogasawara, A.; Azuma, A.; Hashimoto, Y. *Bioorg. Med. Chem.* **1997**, *5*, 2095.
11. Miyachi, H.; Azuma, A.; Kitamoto, T.; Hayashi, K.; Kato, S.; Koga, M.; Sato, B.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1483.
12. Miyachi, H.; Kato, M.; Kato, F.; Hashimoto, Y. *J. Med. Chem.* **1998**, *41*, 263.
13. Shinji, C.; Nakamura, T.; Maeda, S.; Yoshida, M.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4427.
14. Shinji, C.; Maeda, S.; Imai, K.; Yoshida, M.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2006**, *14*, 7625.
15. *(E)-3-{2-[4-(Pyridin-3-yl)benzyl]-1-oxoisoindolin-6-yl}-N-hydroxyacrylamide (***17a***).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.75 (br, 1H), 9.09 (br, 1H), 8.86 (d, J = 1.7 Hz, 1H), 8.55 (dd, J = 4.0, 1.7 Hz, 1H), 8.05 (td, J = 8.1, 1.7 Hz, 1H), 7.88 (s, 1H), 7.78 (dd, J = 8.1, 1.3 Hz, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 16 Hz, 1H), 7.48–7.46 (m, 1H), 7.41 (d, J = 8.1 Hz, 2H), 6.57 (d, J = 16 Hz, 1H), 4.79 (s, 2H), 4.43 (s, 2H); FAB MS *m/z* 386 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 68.47; H, 5.25; N, 10.42. Found: C, 67.98; H, 5.16; N, 10.23. Mp 178–179 °C.
*(E)-3-{2-[4-(Pyridin-4-yl)benzyl]-1-oxoisoindolin-6-yl}-N-hydroxyacrylamide (***17b***).* ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (d, J = 6.0 Hz, 2H), 7.80–7.41 (m, 9H), 7.08–6.92 (m, 1H), 6.64 (d, J = 16 Hz, 1H),4.79 (s, 2H), 4.41(s, 2H); HR FAB MS: $(M+H)^+$ Calcd for $C_{23}H_{19}N_3O_3$: 385.1426. Found: 385.1464.
16. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188.
17. Vannini, A.; Volpari, C.; Filocamo, G.; Casavola, E. C.; Brunetti, M.; Renzoni, D.; Chakravarty, P.; Paolini, C.; Francesco, R.; Gallinari, P.; Steinkuhler, C. D.;

Marco, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15064.
18. Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, *265*, 17174.
19. Warshaw, A. L.; Castillo, F. C. *N. Engl. J. Med.* **1992**, *326*, 455.
20. Glozak, M. A.; Sengupta, N.; Zhang, X.; Seto, E. *Gene* **2005**, *363*, 15.
21. Furumai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; Horinouchi, S. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 87.
22. Slingerland, J.; Pagano, M. *J. Cell. Physiol.* **2000**, *183*, 10.
23. Park, W. H.; Jung, C. W.; Park, J. O.; Kim, K.; Kim, W. S.; Im, Y. H.; Lee, M. H.; Kang, W. K.; Park, K. *Int. J. Oncol.* **2003**, *22*, 1129.
24. Chen, J. S.; Faller, D. V. *J. Cell. Physiol.* **2005**, *202*, 87.