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Design and synthesis of phthalimide-type histone deacetylase inhibitors

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Abstract—Several hydroxamic acid derivatives with a substituted phthalimide group as a linker and/or cap structure, prepared during structural development studies based on thalidomide, were found to have histone deacetylase (HDAC)-inhibitory activity. Structure–activity relationship studies indicated that nature of the substituent introduced at the phthalimide nitrogen atom, introduction of a hydroxamic acid structure, and distance between the *N*-hydroxyl group and the cap structure are important for HDAC-inhibitory activity.

Thalidomide (Fig. 1 **1**) is a sedative and/or hypnotic drug, which was used early in the 1960s, but was subsequently withdrawn from the market when its teratogenicity was discovered.[1–3] However, basic research on thalidomide did come to a halt, and after the serendipitous finding that thalidomide has immunomodulatory activity that can be utilized in the treatment of erythema nodosum associated with leprosy, numerous further studies of thalidomide were reported. These studies clearly indicated the drug's effectiveness against various kinds of ailments, including leprosy, AIDS, and cancers.[2–4] Finally, thalidomide was relaunched in the U.S. in 1998 for the treatment of Hansen's disease, with special precautions for usage. Furthermore, many clinical studies of thalidomide for the treatment of multiple myeloma, breast cancer, prostate cancer, and other conditions are on-going in the U.S. Recently, thalidomide has been included as one of the choices in combination therapy for the treatment of multiple myeloma in Japan.

To explain the pleiotropic effects elicited by thalidomide, we have postulated that thalidomide is a multi-target drug, and we have been engaged in the structural development studies of thalidomide as a scaffold.[2,3,5–17] This systematic search has yielded a wide range of drug leads, such as tumor necrosis factor (TNF)-α production regulators,[2,3,5–7] androgen receptor antagonists,[2,3,8,9] peptidase inhibitors,[3,10–13] glucosidase inhibitors,[15,16] thymidine phosphorylase inhibitors,[16] cyclooxygenase (COX) inhibitors,[17–20,19] and nitric oxide synthase (NOS) inhibitors.[21,22] These fruitful results have indicated that phthalimide structure is a pleiotropic pharmacoprotophore to develop drug lead. We speculated that histone deacetylase (HDAC) might be another anticancer-related molecular target of thalidomide, because some metabolites or degradation products of thalidomide [23] (Fig. 2, **2**, **3**) seem to have structural similarity to previously reported HDAC inhibitors, such as sodium butyrate (Fig. 2, **4**), sodium valproate (Fig. 2, **5**), phenylbutanoic acid (Fig. 2, **6**), and suberoyl anilide hydroxamic acid (SAHA) (Fig. 2, **7**).

Histone acetylation and deacetylation play an important function in modifying the chromatin structure

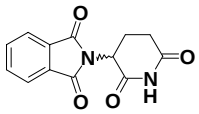

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Figure 1. Structure of thalidomide.

Keywords: Thalidomide; Phthalimide; HDAC inhibitor.
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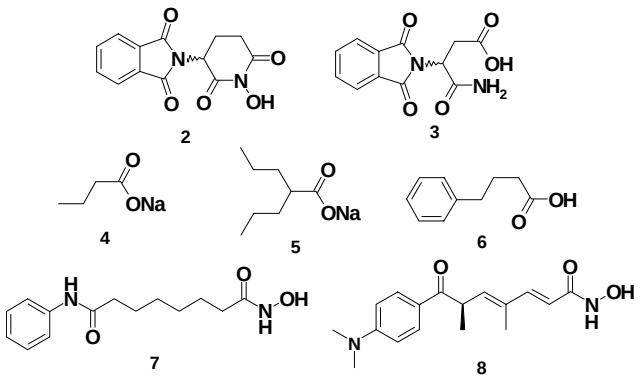


Figure 2. Structures of the representative thalidomide metabolites (**2**, **3**), and HDAC inhibitors (**4**–**8**).

and regulating gene expression. The key enzymes that modify histone proteins and regulate gene expression are histone acetyltransferases (HATs) and histone deacetylases (HDACs).[24,25] Both these acetylating/deacetylating enzymes are included in large protein complexes containing other proteins known to function in transcriptional activation and/or repression.[26,27] Such complexes are recruited to specific regions in the DNA and induce expression and/or silencing of the genes. Well-known targets that are upregulated by HDAC include $p21^{WAF1/CIP129}$. $p21^{WAF1/CIP1}$ is a cyclin-dependent kinase (CDK) inhibitor that associates with a class of CDKs and inhibits their kinase activities, leading to cell cycle arrest and dephosphorylation of Rb.[28] Much evidence suggests that $p21^{WAF1/CIP1}$ plays an important role in determining the fate of cells during growth and differentiation. Therefore, compounds that inhibit HDAC activity may depress these genes, resulting in antiproliferative and antitumor effects.[29] It has not so far been reported whether thalidomide itself, thalidomide metabolites, and/or their degradation products have apparent HDAC inhibitory activity or not. As part of our continuing research directed toward the structural development of thalidomide as a multi-template for drug lead discovery, we report here novel HDAC inhibitors structurally derived from thalidomide and related substituted phthalimide.

Synthetic routes to the present series of phthalimide derivatives are outlined in Charts 1–3.

1,2,4-Benzenetricarboxylic acid anhydride was heated with amines to give the corresponding *N*-substituted phthalimide derivatives (**10a**–**10c**). Compounds (**10a**–**10c**) were condensed with *tert*-butylhydroxylamine via the mixed anhydride to give protected *N*-hydroxybenzamide derivatives (**11a**–**11c**). Deprotection of the *tert*-butyl group of **11a**–**11c** under acidic conditions afforded *N*-hydroxybenzamide derivatives (**12a**–**12c**) (Chart 1). Compound (**10b**) was reduced with BH_3 to give the hydroxymethyl derivative (**13**), and subsequent bromination afforded the bromomethyl derivative (**14**). Dimethyl malonate was alkylated with **14**, and subsequent acid hydrolysis and concomitant decarboxylation afforded the phenylpropanoic acid derivative (**16**). This compound was derivatized to the *N*-hydroxyamide by the mixed anhydride method to give the *N*-hydroxyphenylpropanamide derivative (**17**) (Chart 2). The 4-hydroxyphthalic acid (**18**) was dehydrated (**19**) and subsequent treatment with amines gave the corresponding *N*-substituted-5-hydroxyphthalimide derivatives (**20a**–**20k**).

These compounds were treated with trifluoromethanesulfonic anhydride to give the Heck reaction substrates

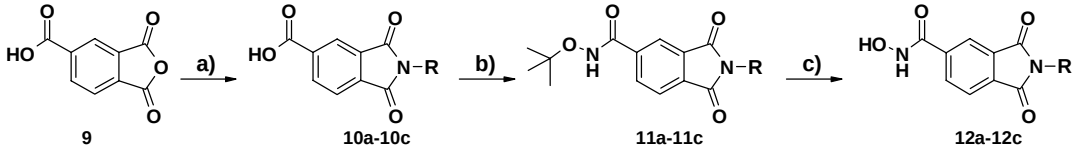


Chart 1. Reagents and conditions: (a) RNH_2, heat, 60–70%; (b) (1) ethyl chloroformate, triethylamine, 0 °C, THF; (2) *t*-butylhydroxylamine, rt, MeOH, 50–60% (two steps); (c) trifluoroacetic acid, rt, CH_2Cl_2, 60–80%.

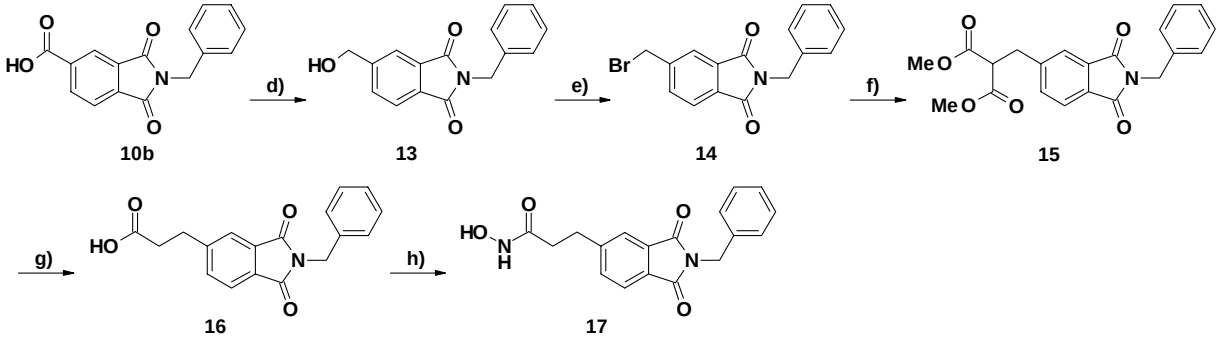


Chart 2. Reagents and conditions: (d) BH_3, 0 °C, THF, 70%; (e) (1) methanesulfonyl chloride, triethylamine, −50 °C, THF; (2) LiBr; 70% (two steps); (f) (1) MeONa, MeOH; (2) dimethyl malonate, reflux, 13%(two steps); (g) 0.5 mol/L HCl, AcOH, reflux; 66%; (h) (1) ethyl chloroformate, triethylamine, 0 °C,THF; (2) hydroxylamine, rt, MeOH, 94% (two steps).

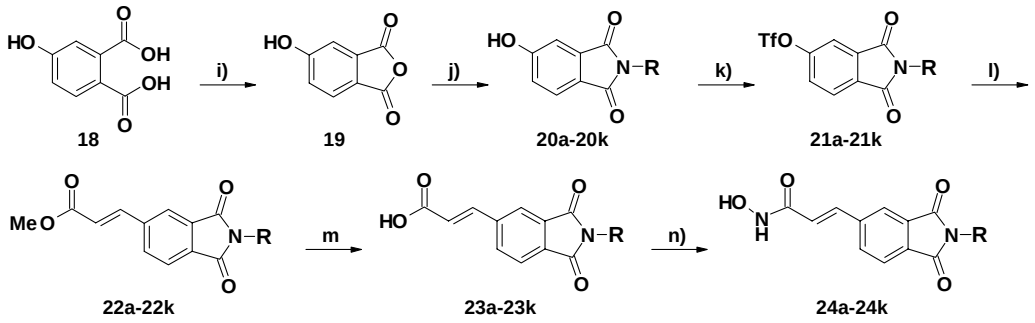


Chart 3. Reagents and conditions: (i) heat, quant.; (j) RNH_2, heat, 85–90%; (k) trifluoromethanesulfonic anhydride, triethylamine, toluene, rt, 80–90%; (l) methyl acrylate, $(P(Ph)_3)_2PdCl_2$, triethylamine, DMF, 50–70%; (m) 0.5 mol/L HCl, AcOH, reflux; 60–70%; (n) (1) ethyl chloroformate, triethylamine, 0 °C, THF; (2) *t*-butylhydroxylamine, rt, MeOH; (3) trifluoroacetic acid, rt, CH_2Cl_2, 33–80% (three steps).

(**21a**–**21k**). Compounds (**21a**–**21k**) were treated with methyl acrylate in the presence of $(P(Ph)_3)_2PdCl_2$ and triethylamine in *N*,*N*-dimethylformamide, and subsequent alkaline hydrolysis afforded cinnamic acid derivatives (**23a**–**23k**). Compounds (**23a**–**23k**) were derivatized to hydroxamic acids (**24a**–**24k**) by means of procedures similar to those employed for the preparation of compound **17** (Chart 3).

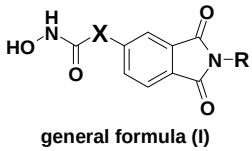


Figure 3. Chemical structure of new HDAC inhibitors.

Kit assays. The assay of HDAC-inhibitory activity was performed using an HDAC fluorescence activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories), according to the supplier's protocol. The assay was performed in triplicate and repeated at least two times. The EC_{50} of SAHA (**7**), using this assay system, was reported to be 280 nM.[30]

Enzyme assays and P21 promoter assay. Assay for inhibitory activities against partially purified HDAC 1, 4, and 6, and P21 promoter assay were done according to the previously reported methods.[31,32]

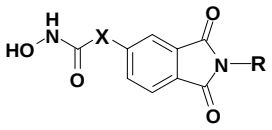
Recent X-ray crystallographic analysis of histone deacetylase-like protein (HDLP),[33] and HDAC8[34], complexed with SAHA and/or TSA (**8**), has revealed that the HDAC catalytic domain consists of a narrow tube-like pocket spanning a length equivalent to a straight chain of four to six carbons, and the zinc ion is buried near the bottom of the active site. Therefore, the structural requirements for an inhibitor to exhibit potent HDAC inhibitory activity involve three pharmacophores of importance, i.e., (1) a zinc binding motif, which interacts with the active site zinc, (2) a linking domain, which occupies the channel, and (3) a surface recognition domain, which interacts with residues on the rim of the active site. Based on these concepts, many HDAC inhibitors have been reported.[35]

To create novel HDAC inhibitors based on a new structural scaffold, we designed the general formula (I), depicted in Figure 3. Hydroxamic acid structure is a well-known zinc binding motif frequently used in already disclosed HDAC inhibitors. Phthalimide structure is a novel linker domain that might be suitable to occupy the narrow channel of the HDAC active site. Also, introduction of aromatic ring at this position might be expected to interact favorably with the side chain of the aromatic amino acids located at the active site. An *N*-substituent can interact with the hydrophobic surface recognition domain of HDAC. Previous structure-activity relationship studies clearly indicated the importance of the distance between the zinc binding motif and the surface recognition domain. We anticipated that the distance between these two important pharmacophores could be regulated by the introduction of various kinds of X groups.

First, we evaluated the effects of *N*-substituents at the phthaloyl nitrogen on HDAC-inhibitory activity (**12a**–**12c**). As can be seen from Table 1, the *N*-phenyl derivative (**12a**) exhibited a very weak HDAC-inhibitory activity (EC_{50} 125 μM). The introduction of a benzyl group (**12b**) at the phthaloyl nitrogen greatly increased the inhibitory activity, and **12b** exhibited about 30-fold more potent activity than **12a**. This result suggests the importance of the shape of the substituents for interaction with the hydrophobic surface recognition domain of HDAC. Further elongation by one methylene chain to afford the *N*-phenethyl derivative (**12c**) decreased the activity to some extent.

We next turned our attention to the linking X group, and prepared the propionyl derivative (**17**) and the acryloyl derivative (**24b**). The natural product TSA contains an α,β-unsaturated carbonyl group, while a saturated carbonyl group is contained in many previously disclosed synthetic HDAC inhibitors, such as SAHA. The propionyl derivative (**17**) exhibited a more potent HDAC-inhibitory activity than the benzoic acid derivative (**12b**). Moreover, the acryloyl derivative (**24b**) exhibited more potent HDAC-inhibitory activity than the benzoic acid derivative (**12b**) and the propionyl derivative (**17**), respectively. This is consistent with the importance of the distance between the zinc binding motif and distal hydrophobic tail part of the molecule. Consider-

Table 1. HDAC-inhibitory activity of the prepared compounds



Compound	X	R	EC_{50} (nM)
12a	—	Ph	125000
12b	—	CH_2Ph	4160
12c	—	CH_2CH_2Ph	6630
17	CH_2–CH_2	CH_2Ph	407
24b	CH_2=CH_2	CH_2Ph	148

ing the fact that the narrow channel of the HDAC active site is composed mainly of aromatic amino acids (Y91, F141, and F198 of HDLP), π-electron conjugation might be favorable for potent inhibitory activity.

Based on these results, we adopted **24b** as the next lead compound and performed further modification focusing on the nitrogen substituent at the phthaloyl group. The results are given in Table 2. Although the number of compounds examined is too small to allow a detailed discussion of the SAR, some tendencies are apparent. Roughly speaking, the *N*-phenyl derivative (**24a**) exhibited moderate activity (micromolar order), while methylene elongation to afford the *N*-benzyl derivative (**24b**) resulted in about 10-fold more potent inhibitory activity than **24a**. Further elongation of the methylene chain afforded the *N*-phenethyl derivative (**24c**), which showed comparable inhibitory activity to that of **24b**. These effects are similar to those seen in *N*-substituted phthaloyl acid derivatives (**12a**–**12c**), and might indicate that the nitrogen substituents of the phthaloyl group interact with the same site of the hydrophobic surface recognition domain of HDAC. Introduction of substituents at the 4-position of the benzene ring of the benzyl group (**24d**, **24e**) had little effect on the inhibitory activity. The hydrophobic surface recognition domain of HDAC is thought to be wide, so we prepared **24f** and **24g** as compounds with a bulkier capped structure. But, to our surprise, these compounds exhibited a much weaker inhibitory activity than **24b**. We cannot yet interpret this finding, but it might imply that there is some distinct structural requirement for potent HDAC-inhibitory activity. Although, in the case of a 1-phenylethyl group (**24h**, **24i**), enantio difference is not clear, but in case of a (2-(benzyloxy)-1-phenylethyl) group (**24j**, **24k**), the (*S*)-enantiomer exhibited a more potent HDAC-inhibitory activity than the antipode. Compound **24b** was found to be the most potent HDAC inhibitor in the present series of phthalimide-based compounds, at least in vitro.

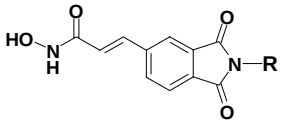
HDACs have been divided into three distinct classes (I–III), and HDAC classes I/II are considered to be therapeutic targets for the treatment of cancer and other diseases. Therefore, we analyzed the inhibitory activity of **24b** toward representative HDACs, i.e., HDAC 1 (class I), HDAC4 (class IIa), and HDAC6 (class IIb), in comparison with that of TSA. As shown in Table 3, **24b** is a nanomolar-order pan-HDAC inhibitor, such as TSA, and the inhibitory activity of **24b** is about 1/10 to 1/30 of that of TSA.

In a majority of tumor cells, HDAC inhibitors were reported to upregulate the expression of $p21^{WAF1/CIP1}$ and to downregulate cyclin D1, in parallel with cell cycle arrest in the G1 phase, and activation of the $p21^{waf1/cip1}$ gene is associated with inhibition of proliferation, induction of differentiation, and/or apoptosis of tumor cells, in vitro and in vivo.[28,29] Therefore, we then assessed the activity of **24b** to upregulate the expression of $p21^{WAF1/CIP1}$ by means of reporter gene assay,[30] and found that the EC_{1000} value of **24b** is 3.29 μM (the EC_{1000} value of **TSA** is 0.0087 μM). This result indicates that **24b** exhibits significant HDAC-inhibitory activity not only in vitro, but also at the cellular level. In conclusion, we have designed and synthesized phthalimide-based pan-HDAC inhibitors derived from thalidomide as a multi-drug template. Compound **24b** provides a structurally novel type of HDAC inhibitor, and further structural development of **24b** with the aim of finding class-selective and/or isozyme-selective HDAC inhibitors is on-going.

Table 3. Inhibitory activity of compound **24b** on HDAC1, HDAC4, and HDAC6

	Inhibitory activity IC_{50} (nM)		
	HDAC1	HDAC4	HDAC6
24b	572	358	709
TSA	20	39	91

Table 2. HDAC-inhibitory activity of the prepared compounds



Compound	R	EC_{50} (nM)	Compound	R	EC_{50} (nM)
24a	Ph	2080	24g	CH_2-$CH(Ph)_2$	7890
24c	$(CH_2)_2Ph$	177	24h	(*S*)-CH(Me)Ph	250
24d	CH_2Ph(4Me)	474	24i	(*R*)-CH(Me)Ph	291
24e	CH_2Ph(4MeO)	186	24j	(*S*)-$CH(CH_2OBn)Ph$	763
24f	$CH(Ph)_2$	2090	24k	(*R*)-$CH(CH_2OBn)Ph$	3790

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References and notes

1. Hales, B. F. *Nat. Med.* **1999**, *5*, 489.
2. Hashimoto, Y. *Curr. Med. Chem.* **1998**, *5*, 163.
3. Hashimoto, Y. *Bioorg. Med. Chem.* **2002**, *10*, 461.
4. Calabrese, L.; Fleisher, A. B. *Am. J. Med.* **2000**, *108*, 487.
5. Miyachi, H.; Azuma, A.; Ogasawara, A.; Uchimura, E.; Watanabe, N.; Kobayashi, Y.; Kato, F.; Kato, M.; Hashimoto, Y. *J. Med. Chem.* **1997**, *40*, 2858.
6. Miyachi, H.; Ogasawara, A.; Azuma, A.; Hashimoto, Y. *Bioorg. Med. Chem.* **1997**, *5*, 2095.
7. Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S. *Chem. Pharm. Bull* **1996**, *44*, 156.
8. Miyachi, H.; Azuma, A.; Kitamoto, T.; Hayashi, K.; Kato, S.; Koga, M.; Sato, B.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1483.
9. Ishioka, T.; Kubo, A.; Koiso, Y.; Nagasawa, K.; Itai, A.; Hashimoto, Y. *Bioorg. Med. Chem.* **2002**, *10*, 1555.
10. Miyachi, H.; Kato, M.; Kato, F.; Hashimoto, Y. *J. Med. Chem.* **1998**, *41*, 263.
11. Komoda, M.; Kakuta, H.; Takahashi, H.; Fujimoto, Y.; Kadoya, S.; Kato, F.; Hashimoto, Y. *Bioorg. Med. Chem.* **2001**, *9*, 21.
12. Shimazawa, R.; Takayama, H.; Kato, F.; Kato, M.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 559.
13. Shimazawa, R.; Takayama, H.; Fujimoto, Y.; Komoda, M.; Dodo, K.; Yamasaki, R.; Shirai, R.; Koiso, Y.; Miyata, K.; Kato, F.; Kato, M.; Miyachi, H.; Hashimoto, Y. *J. Enzyme Inhib.* **1999**, *14*, 259.
14. Sou, S.; Mayumi, S.; Takahashi, H.; Yamasaki, R.; Kadoya, S.; Sodeoka, M.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1081.
15. Takahashi, H.; Sou, S.; Yamasaki, R.; Sodeoka, M.; Hashimoto, Y. *Chem. Pharm. Bull. (Tokyo)* **2000**, *48*, 1494.
16. Kita, T.; Takahashi, H.; Hashimoto, Y. *Biol. Pharm. Bull.* **2001**, *24*, 860.
17. Shimazawa, R.; Miyachi, H.; Takayama, H.; Kuroda, K.; Kato, F.; Kato, M.; Hashimoto, Y. *Biol. Pharm. Bull.* **1999**, *22*, 224.
18. Noguchi, T.; Shimazawa, R.; Nagasawa, K.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1043.
19. Sano, H.; Noguchi, T.; Tanatani, A.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2005**, *13*, 3079.
20. Sano, H.; Noguchi, T.; Tanatani, A.; Miyachi, H.; Hashimoto, Y. *Chem. Pharm. Bull. (Tokyo)* **2004**, *52*, 1021.
21. Noguchi, T.; Sano, H.; Shimazawa, R.; Tanatani, A.; Miyachi, H.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4141.
22. Shimazawa, R.; Sano, H.; Tanatani, A.; Miyachi, H.; Hashimoto, Y. *Chem. Pharm. Bull. (Tokyo)* **2004**, *52*, 498.
23. Lu, J.; Palmer, B. D.; Kestell, P.; Browett, P.; Baguley, B. C.; Muller, G.; Ching, L. M. *Clin. Cancer Res.* **2003**, *9*, 1680.
24. Grunstein, M. *Nature* **1997**, *389*, 349.
25. Cheung, W. L.; Briggs, S. D.; Allis, C. D. *Curr. Opin. Cell Biol.* **2000**, *12*, 326.
26. Roth, S.; Denu, J. M.; Allis, C. D. *Annu. Rev. Biochem.* **2001**, *70*, 81.
27. Cress, W. D.; Seto, E. J. *Cell Physiol.* **2000**, *184*, 1.
28. Rosato, R. R.; Wang, Z.; Gopalkrishnan, R. V.; Fisher, P. B.; Grant, S. *Int. J. Oncol.* **2001**, *19*, 181.
29. Weidle, U. H.; Grossmann, A. *Anticancer Res.* **2000**, *20*, 1471.
30. Suzuki, T.; Matsuura, A.; Kouketsu, A.; Nakagawa, H.; Miyata, N. *Bioorg. Med. Chem. Lett.* **2005**, *157*, 331.
31. Furumai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; Horinouchi, S. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 87.
32. Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K.-H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. *Cancer Res.* **2002**, *62*, 4916.
33. 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.
34. 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. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15064.
35. Miller, T. A; Witter, D. J; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097, and references cited therein.