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Substituted *N*-(2-aminophenyl)-benzamides, (*E*)-*N*-(2-aminophenyl)-acrylamides and their analogues: Novel classes of histone deacetylase inhibitors

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Abstract—Inhibition of histone deacetylases (HDACs) is emerging as a new strategy in human cancer therapy. Novel 2-aminophenyl benzamides and acrylamides, that can inhibit human HDAC enzymes and induce hyperacetylation of histones in human cancer cells, have been designed and synthesized. These compounds selectively inhibit proliferation and cause cell cycle arrest in various human cancer cells but not in normal cells. The growth inhibition of 2-aminophenyl benzamides and acrylamides against human cancer cells in vitro is reversible and is dependent on the induction of histone acetylation. Compounds of this class can significantly reduce tumor growth in human tumor xenograft models.

Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are enzymes that regulate transcription in eukaryotic cells by selectively deacetylating or acetylating the ε-amino group of lysine residues located in the NH_2-terminal tails of core histones.[1] Correlations between increased levels of histone acetylation and transcriptionally active genes have long been recognized, and conversely, hypoacetylated nucleosomal histones are enriched in chromatin characterized by a virtual absence of gene expression.[2] The anionic phosphate backbone of DNA strongly interacts with the cationic lysine residues of histone proteins, resulting in a condensed chromatin structure; lysine acetylation attenuates these ionic interactions, relieving the highly condensed structure of

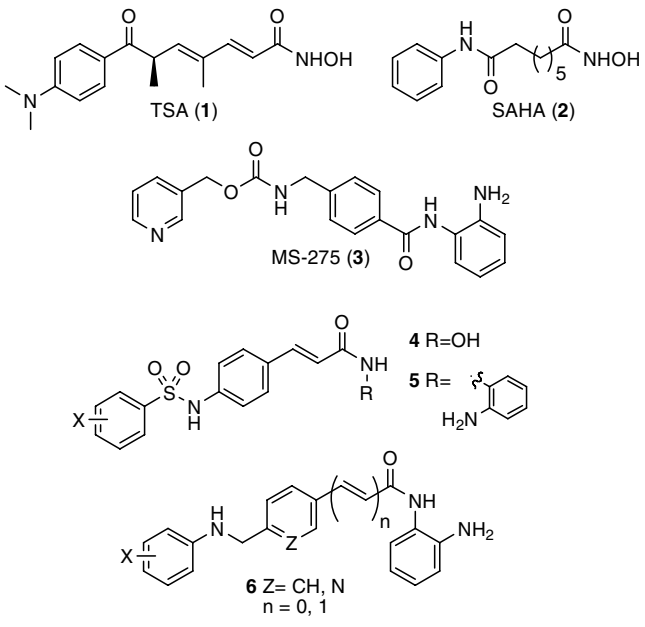

Figure 1.

Keywords: Histone deacetylase inhibitors; 2-Aminophenyl-benzamides; 2-Aminophenyl-acrylamides; HDAC; HDACI.
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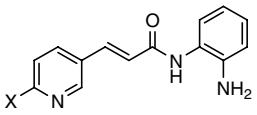
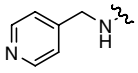
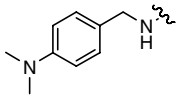
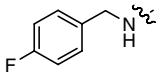
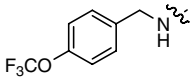
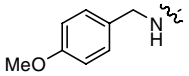
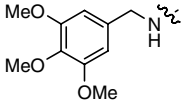
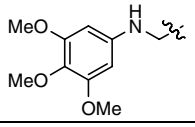
chromatin and facilitating the accessibility of transcription factors to DNA. HDACs and HATs influence many cellular processes such as cell cycle progression and differentiation. The deregulation of HDAC activity has also been linked to carcinogenesis. Consequently, the modulation of HDAC activity could be a potential therapeutic strategy in treating human cancer.[3]

Table 1. SAR of some pyridin-3-yl-acrylamides



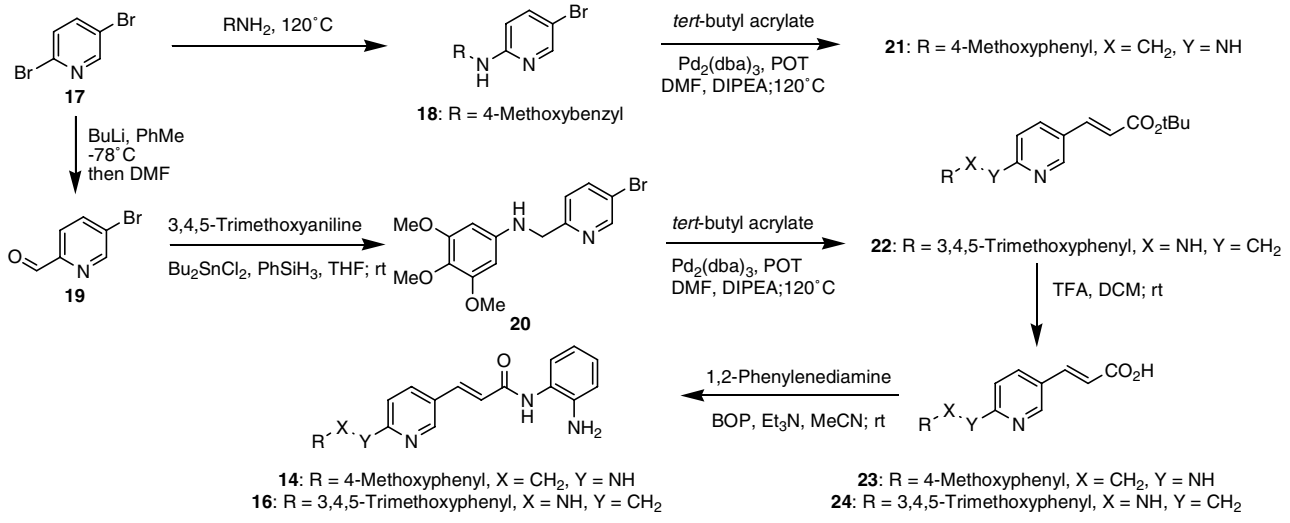
Compound	X	IC_{50} (μM)	
		HDAC1	MTT HCT116
7	$PhCH_2NH$	3	1
8	$Ph(CH_2)_3NH$	3	2
9	$PhNH(CH_2)_2NH$	2	5
10		4	7
11		4	3
12		5	2
13		2	4
14		2	3
15		4	2
16		3	3

Small molecules containing functional groups such as a hydroxamic acid or aminoanilide have been reported to exhibit HDAC inhibitory activity. The natural product trichostatin A (TSA)[4] (**1**) and its analogues,[5] synthetic compounds such as suberoylanilide hydroxamic acid (SAHA)[6] (**2**), and MS-275 (**3**) are examples (Fig. 1).[7] Some of these compounds have been shown to have potent in vivo antitumor effects in tumor-bearing animals and a few of them are currently in clinical trials. In some cases, however, their potential for clinical drug development is limited by lack of selectivity, cytotoxicity, low solubility, and/or low stability.[8]

In the course of searching for novel HDAC inhibitors with potency and good safety profiles, we initially designed hydroxamic acid-based sulfonamides (**4**).[9] Further development of these HDAC inhibitors led to the synthesis of the corresponding aniline-based sulfonamides (**5**) which showed improved pharmaceutical properties.[10] More importantly, our novel arylamine anilides (**6**) resulted in significant improvement in antitumor activities both in vitro and in vivo. The structure–activity relationships (SAR), the antiproliferative activity, and the in vivo efficacy will be discussed.

The first series of compounds, *N*-(2-aminophenyl)-3-(pyridin-3-yl)acrylamides **7**–**16** (Table 1), were synthesized starting from 2,5-dibromopyridine **17** as described in Scheme 1 (exemplified by compounds **14** and **16**).[11]

Compound **18** was obtained by nucleophilic substitution of bromine in **17** with 4-methoxybenzylamine. The formation of 5-bromopicolinaldehyde **19**, using *n*-butyl lithium and DMF followed by a reductive amination with 3,4,5-trimethoxyaniline, gave compound **20**. Heck couplings generated the intermediates **21** and **22** starting from **18** and **20**, respectively. These *tert*-butyl esters were cleaved with TFA and then coupled to 1,2-phenylenedi-



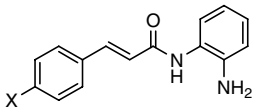
Scheme 1.

amine using BOP as a coupling agent to afford final products **14** and **16**.

The corresponding (*E*)-*N*-(2-aminophenyl)cinnamides **25**–**35** (Table 2) were also synthesized using the chemistry described in Scheme 1, but starting with 4-bromoaniline or 4-bromobenzaldehyde instead, and introducing the benzylamine bond by reductive amination.

The analogous series of *N*-(2-aminophenyl)-benzamides and *N*-(2-aminophenyl)-nicotinamides **36**–**44** (Table 3) were also synthesized. Nicotinamides **36**–**38** were prepared in three steps from 6-chloronicotinic acid starting

Table 2. SAR of some 2-aminophenyl cinnamides



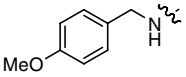
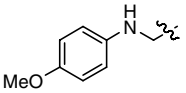
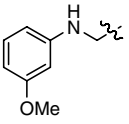
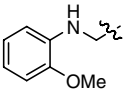
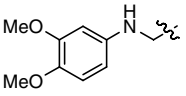
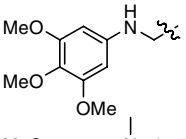
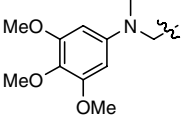
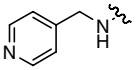
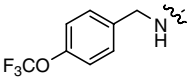
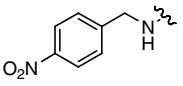
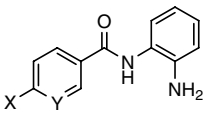
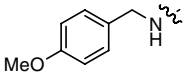
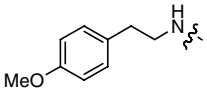
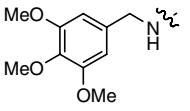
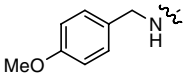
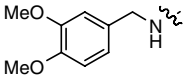
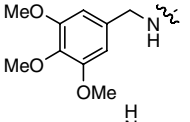
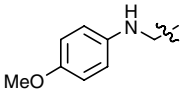
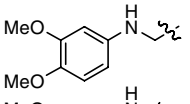
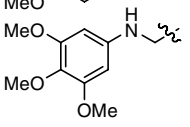
Compound	X	IC_{50} (μM)	
		HDAC1	MTT HCT116
25		4	37
26		4	12
27	$PhNHCH_2$	17	11
28		17	10
29		22	—
30		2	5
31		2	2
32		3	5
33		10	2
34		5	50
35		4	15

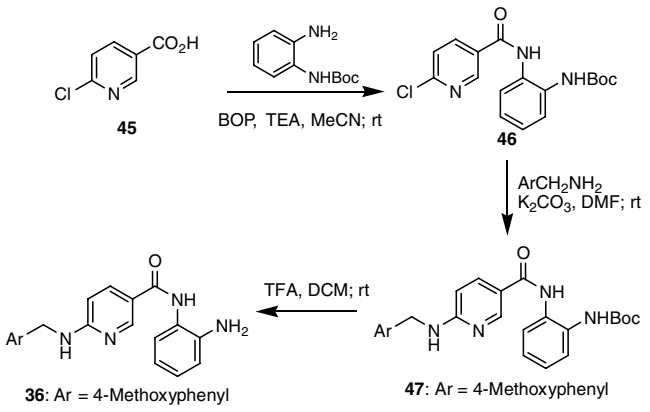
Table 3. SAR of some 2-aminophenyl-benzamides and 2-aminophenyl-nicotinamides



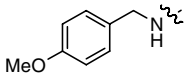
Compound	X	Y	IC_{50} (μM)	
			HDAC1	MTT HCT116
36		N	4	0.5
37		N	3	0.8
38		N	5	3
39		CH	2	1
40		CH	4	0.6
41		CH	3	2
42		CH	0.6	2
43		CH	2	0.4
44		CH	3	2

with amide synthesis followed by nucleophilic substitution of the chloride and then deprotection of the *N*-Boc group, (Scheme 2, exemplified by compound **36**). The *N*-(2-aminophenyl)-benzamides **39**–**44** were synthesized as illustrated in Scheme 3 (exemplified by compounds **41** and **43**). *N*-(2-Aminophenyl)-4-(benzylamino) benzamide **41** and *N*-(2-aminophenyl)-4-((phenylamino)methyl) benzamide **43** were obtained according to Scheme 3; starting, respectively, with 4-aminobenzoic acid **48** and 4-formylbenzoic acid **49**.

All compounds were initially screened for the ability to inhibit recombinant human HDAC-1. This isozyme was chosen since it is widely implicated in both transcriptional repression and chromatin remodeling.[12] The evaluation of the in vitro antiproliferative activities of the synthesized compounds was performed using 3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay against various human cancer cell lines, in particular, HCT116 human colon cancer cell line. Compound **7**, in the first series (Table 1), showed



Scheme 2.



Scheme 3.

activity against HDAC-1 (3 μM) and good antiproliferative activity (1 μM). Chain elongation (compounds **8** and **9**) or introduction of substituents on the phenyl ring (compounds **10**–**15**) only slightly modified the activity. Interchanging the position of the nitrogen (compounds **15** and **16**) did little to improve the potency.

To continue this SAR, the aminophenyl cinnamide series was explored (Table 2). In this series, interchanging the position of the nitrogen (compounds **25** and **26**) resulted in improvement in antiproliferative activity. The absence or presence of a methoxy substituent and the substitution pattern (compounds **26**–**31**) showed up to a 10-fold difference in HDAC-1 activity, with compound **31** being the most potent. Introduction of a secondary amine (compound **32**) or substituent variety (compounds **33**–**35**) did not prove to be beneficial.

To complete the SAR study, the (pyridin-3-yl)acrylamide (Table 1) and the cinnamide series (Table 2) were compared to the nicotinamide (compounds **36**–**38**) and the benzamide series (compounds **39**–**44**) (Table 3). These new compounds **36**–**44** showed an improved range of antiproliferative activity from 0.5 to 3 μM. Compounds **39**, **42**, and **43** were found to be quite potent; comparable to MS-275 (**3**) which is currently undergoing phase 1 clinical trials.[13]

The cytotoxicity of the most potent compounds toward normal cells was determined by the proliferation of human normal mammary epithelial cells (HMEC). Compounds **31** (HMEC IC_{50} = 39 μM) and **43** (HMEC IC_{50} = 15 μM) proved to have the lowest cytotoxicity.

To confirm the ability of these compounds to inhibit HDAC in whole cells, they were evaluated for their ability to induce histone acetylation in T24 human bladder cancer cells. The anilides **31** and **43** caused dose-dependent histone hyperacetylation in these cells (Fig. 2). Similarly to MS-275, the compounds **31** and **43** also induced expression of p21 protein in T24 human cancer cell lines (Fig. 3), and they caused G2/M cell cycle arrest in the HCT116 human colon cancer cell line (Fig. 4), in accordance with results from the literature.[14,15]

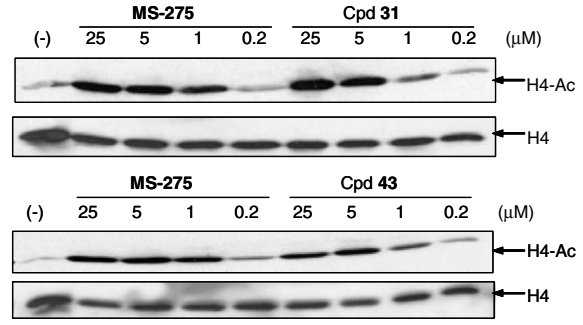


Figure 2. Human bladder carcinoma T24 cells were treated with compounds at 0, 1, 5, and 25 mM for 16 h. Cells were harvested and histones were acid-extracted, analyzed by SDS–PAGE, and immunoblotted with antibodies specific for acetylated H4 histones. Histones were stained by Coomassie blue to reveal their amount loaded on the blots.

HDAC small molecule inhibitors **31** and **43** were evaluated in vivo in several different tumor xenograft models

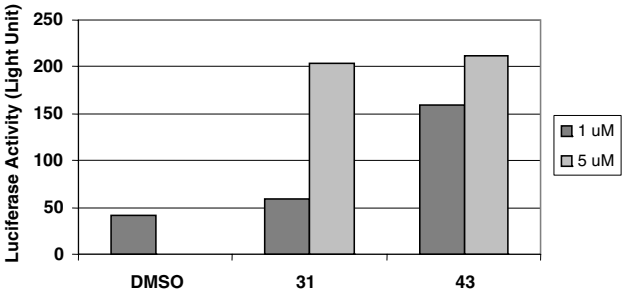


Figure 3. Induction of $p21^{WAF1/Cip1}$ transcription by HDAC inhibitors in Human HCT116 Cancer Cells. HCT116 cells were stably transfected with a luciferase reporter plasmid driven by the p21 promoter. Cells were treated with HDAC inhibitors for 16 h before harvest. Cells were lysed and luciferase activity was analyzed using substrate from Promega.

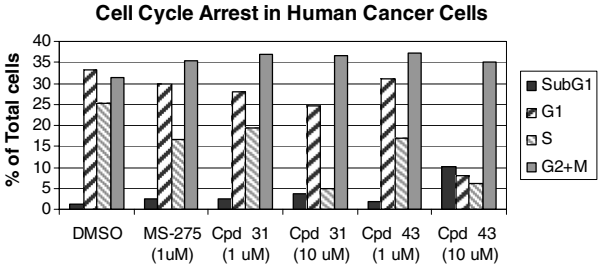


Figure 4. HDAC Inhibitors Caused G2/M arrest of Human Cancer Cells. Human HCT116 cancer cells were treated with in-house HDAC inhibitors or MS-275 in 1% DMSO for 16 h before harvesting by trypsinization. Propidium iodide-stained DNA contents in fixed cells were analyzed by flow cytometry. The (G2+M)/S ratio was calculated using the percentage of cells among total gated cells in G2/M phase divided by the percentage of cells in S phase. Percentages of cells in G1 phase were not affected by HDAC inhibitors.

in mice. These models represent the major forms of human cancers: A549 (non-small cell lung), PANC-1 (pancreatic), and SW48 and HCT116 (colon). Compounds were administered daily by either intraperitoneal injection (ip) or orally (po). Both compounds showed good antitumor activity (% of tumor growth inhibitions relative to vehicle controls, %TGI). Compound **31** showed up to 73% in SW48 and up to 80% in HCT116 when dosed at 25 and 20 mg/kg per day (ip route) for 3 weeks. Compound **43** showed up to 73% in A549 (ip, 40 mg/kg), up to 84% in PANC1 (po, 50 mg/kg), up to 66% and 86% in HCT116 (ip, 50 and 75 mg/kg), and up to 66% and 100% in SW48 (ip, 50 and 75 mg/kg). This activity was obtained without any associated body weight loss as a measure of gross toxicity. Moreover, the activity was comparable or better than that of MS-275 (**3**) (%TGI: 56, 68, and 46 for HCT116, SW48, and A549-bearing mice, respectively, when dosed at 20 mg/kg ip) that was used as a positive control in these experiments.

A novel class of HDAC inhibitors, which induce core histone acetylation in human cancer cells, arylamine anilides (general structure **6**), was designed. Active arylamine anilides exhibit in vitro anti-proliferative activities in various human cancer cells, but not in normal cells. Selective induction of cell cycle arrest and apoptosis of human cancer cells in a dose-dependent manner was observed. At the protein expression level, compounds **31** and **43** induce $p21^{WAF1/Cip1}$ expression and downregulate cyclin A and B1 expression in cancer cells. Induction of $p21^{WAF1/Cip1}$ by compounds **31** and **43** starts as early as 8 h, and can last up to 72 h, post-treatment. It was found that compounds **31** and **43** have significant antitumor activity in vivo in several mice tumor xenograft models. Compounds **31** and **43** showed acceptable pharmacokinetic profiles in rats (iv half-life 1.8 and 0.7 h; bioavailability 65% and 20%, respectively). Little toxicity of compounds **31** and **43**, compared to MS-275 (**3**), was revealed by body weight, white blood cell counts, and spleen weight measurement. These results represent a significant step toward the development of HDAC small molecule inhibitors with favorable pharmaceutical characteristics.

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References and notes

1. Rundlett, S. E.; Carmen, A. A.; Kobayashi, R.; Bavykin, S.; Turner, B. M.; Grunstein, M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14503.
2. (a) Hassig, C. A.; Schreiber, S. L. *Curr. Opin. Chem. Biol.* **1997**, *1*, 300; (b) Kouzarides, T. *Curr. Opin. Genet. Dev.* **1999**, *9*, 40; Review (c) Strahl, B. D.; Allis, C. D. *Nature* **2000**, *403*, 41.
3. Lin, R. J.; Nagy, L.; Inoue, S.; Shao, W.; Miller, W. H.; Evans, R. M. *Nature* **1998**, *391*, 811.
4. (a) Beppu, T.; Iwamoto, Y.; Yoshida, M. U.S. Patent 4,690,918, 1987; *Chem. Abstr.* **1987**, *106*, 9389; (b) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, *265*, 17174; (c) Fleming, I.; Iqbal, J.; Krebs, E. P. *Tetrahedron* **1983**, *39*, 841; (d) Mori, K.; Koseki, K. *Tetrahedron* **1988**, *44*, 6013.
5. (a) Jung, M.; Brosch, G.; Kölle, D.; Scherf, H.; Gerhäuser, C.; Loidl, P. *J. Med. Chem.* **1999**, *42*, 4669; (b) Su, G. H.; Sohn, T. A.; Ryu, B.; Kern, S. E. *Cancer Res.* **2000**, *60*, 3137; (c) Massa, S.; Mai, A.; Sbardella, G.; Esposito, M.; Ragno, R.; Loidl, P.; Brosch, G. *J. Med. Chem.* **2001**, *44*, 2069.
6. (a) Kim, Y. B.; Lee, K. H.; Sugita, K.; Yoshida, M.; Horinouchi, S. *Oncogene* **1999**, *18*, 2461; (b) Meinke, P. T.; Liberator, P. *Curr. Med. Chem.* **2000**, *8*, 211.
7. Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4592.
8. Piekarz, R.; Bates, S. *Curr. Pharm. Des.* **2004**, *10*, 2289.
9. Lavoie, R.; Bouchain, G.; Frechette, S.; Woo, S. H.; Abou-Khalil, E.; Leit, S.; Fournel, M.; Yan, P. T.; Trachy-Bourget, M.-C.; Beaulieu, C.; Li, Z.; Besterman, J.; Delorme, D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2847.
10. Bouchain, G.; Leit, S.; Frechette, S.; Abou-Khalil, E.; Lavoie, R.; Moradei, O.; Woo, S. H.; Fournel, M.; Yan, P. T.; Kalita, A.; Trachy-Bourget, M.-C.; Beaulieu, C.; Li, Z.; Robert, M.; MacLeod, R.; Besterman, J.; Delorme, D. *J. Med. Chem.* **2003**, *46*, 820.
11. Full experimental protocols for Schemes 1–3 are described in Delorme, D.; Woo, S. H.; Vaisburg A.; Moradei, O. et al. WO 03/024448.
12. Carmen, A. A.; Rundlett, S. E.; Grunstein, M. *J. Biol. Chem.* **1996**, *271*, 15837.
13. (a) Jaboin, J.; Wild, J.; Hamidi, H.; Khanna, C.; Jai Kim, C.; Robey, R.; Bates, S. E.; Thiele, C. J. *Cancer Res.* **2002**, *32*, 6108; (b) Acharya, M.; Sparreboom, A.; Sausville, E.; Conley, B.; Doroshow, J.; Venitz, J.; Figg, W. *Cancer Chemother. Pharmacol.* **2006**, *57*, 275.
14. Maeda, T.; Nagaoka, Y.; Kawai, Y.; Takagani, N.; Yasuda, C.; Yogosaga, S.; Sowa, Y.; Sakai, T.; Uesato, S. *Biol. Pharm. Bull.* **2005**, *28*, 849.
15. Hirose, T.; Sowa, Y.; Takahashi, S.; Saito, S.; Yasuda, C.; Shindo, N.; Furuichi, K.; Sakai, T. *Oncogene* **2003**, *22*, 7762.