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Anti-tumor activity of new orally bioavailable 2-amino-5-(thiophen-2-yl) benzamide-series histone deacetylase inhibitors, possessing an aqueous soluble functional group as a surface recognition domain

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ABSTRACT

New orally bioavailable 5-(thiophen-2-yl)-substituted 2-aminobenzamide-series histone deacetylase inhibitors were synthesized. These compounds possess a morpholine or piperadine-derived moiety as an aqueous soluble functional group. Among them, **8b**, having a 4-ethyl-2,3-dioxopiperazine-1-carboxamide group as a surface recognition domain, showed promising inhibitory activities against HCT116 cell growth and HDAC1/2. Notably, unlike MS-275, this compound did not induce apoptosis in the cell cycle tests. We therefore conducted antitumor tests of **8b** and MS-275 against HCT116 cell xenografts in nude mice. Compound **8b** reduced the volume of tumor mass to T/C: 60% and 47% at 45 and 80 mg/kg over 16 days, respectively. These values were comparable to the rate (T/C: 51% at 45 mg/kg) for MS-275. Furthermore, **8b**, at neither 45 nor 80 mg/kg, induced the weight loss which was observed in the mice given MS-275 at 45 mg/kg.

Histone deacetylases (HDACs) have drawn attention as biological molecules, and their overexpression is closely associated with the incidence and growth of carcinomas.[1–5] HDAC inhibitors regulate the growth of cancer cells through the epigenetic activation of gene transcription leading to cell cycle regulation as well as apoptosis. HDACs are grouped into four classes, which are further classified into 18 isoforms on the basis of their size, cellular localization, number of catalytic active sites, protein homology, etc.[6,7] Most of these enzymes contain a zinc ion at the active site, and a number of papers have reported on the docking of these enzymes and HDAC inhibitors, utilizing the X-ray crystal structure of histone deacetylase-like protein (HDLP),[8] HDAC8,[9,10] and HDAC2.[11]

Most HDAC inhibitors comprise a hydroxamic acid or 2-aminobenzamide moiety as a zinc ion-chelating domain. The former compounds, typified by TSA[12,13] and SAHA[8,14] (Zolinza), are broad-ranging inhibitors of HDACs, whereas the latter, typified by MS-275[15–17] and MGCD-0103,[18] are orally available inhibitors that target HDAC 1, 2 and 3 (Fig. 1).

Recently, diverse 2-amino-5-(thiophen-2-yl)benzamide-series HDAC inhibitors were synthesized by the groups of Merck & Co., Takeda Pharmaceuticals and MethylGene. These compounds exert HDAC1/2-selective inhibition by projecting the 2-thienyl ring into the extensive internal cavity adjacent to the zinc-binding site within the HDAC1/2 pocket.[11,19–24] Since both HDAC1 and 2 are deeply involved in canceration,[25–30] the inhibitors selective for these isoforms may provide potential utility as anti-cancer therapeutics.

The Merck & Co. group prepared 5-(thiophen-2-yl)-substituted 2-aminobenzamide derivatives with an aqueous soluble functional group such as a phosphonate/phosphinate or phenylglycine/phenylalanine derivative. Although representative compounds showed inhibitory activity against tumor growth in a HCT116 xenograft mouse model, the efficacy did not seem to reflect the increased selectivity toward HDAC1/2.

In the course of searching for orally bioavailable 2-aminobenzamide-series HDAC inhibitors, we prepared the 5-(thiophen-2-yl)-substituted compound **1a** which had a (benzo[*d*][1,3]dioxol-5-ylmethyl)(2-hydroxyethyl)amino group as a surface recognition domain (Fig. 2). Compound **1a** showed stronger HDAC1/2 inhibitory activities[31] (HDAC1 IC_{50}: 0.06 μM; HDAC2 IC_{50}: 0.74 μM) than the 5-(thiophen-2-yl)-unsubstituted counterpart (**1b**) (HDAC1 IC_{50}: 1.17 μM; HDAC2 IC_{50}: 3.36 μM) as was the case in MS-275 and its 2-thienyl-substituted derivative,[19] though it had a problem with aqueous solubility (<0.1 ml for 10% DMSO) (Table 1). This finding prompted us to synthesize new potential 5-(thiophen-2-yl)-substituted 2-aminobenzamide-series compounds with aqueous soluble functionality. In the present study, we tried

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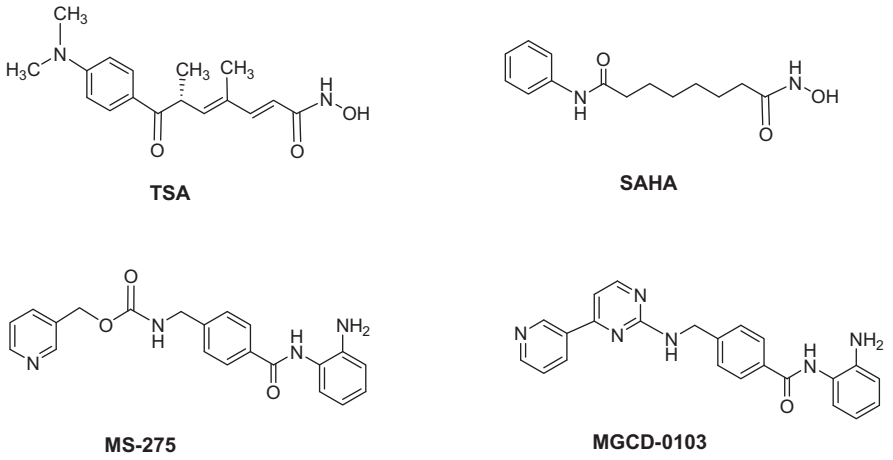


Figure 1. Structure of HDAC inhibitors.

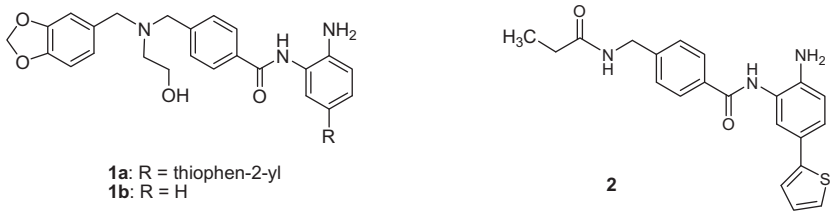


Figure 2. Structure of 5-(thiophen-2-yl)-substituted 2-aminobenzamides.

Table 1
Solubility and effect of HDAC inhibitors against HCT116 cell growth and HDAC1/2 activities

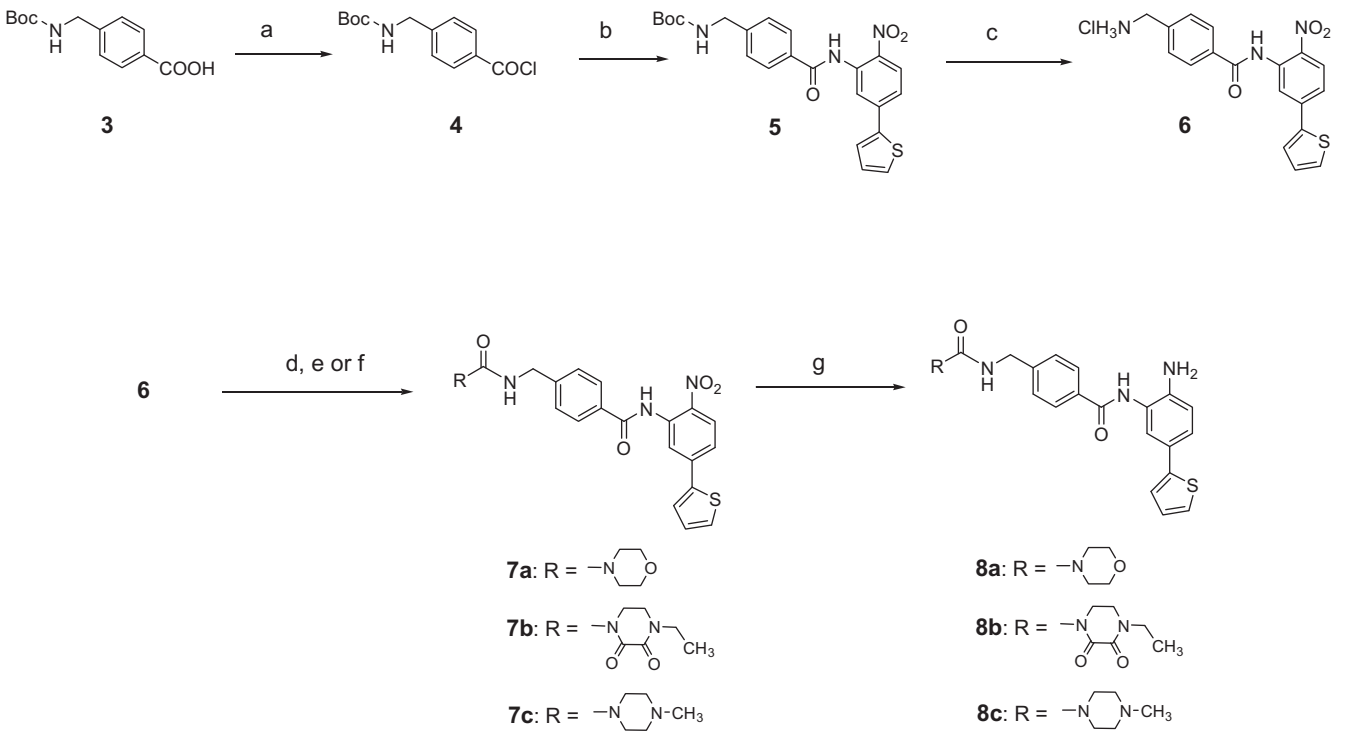
Compound	IC_{50} (μM)[a]	IC_{50} (μM)[b]		10% DMSO/H_2O (mg/mL)
	HCT116	HDAC1	HDAC2	
1a	0.49	0.06	0.74	<0.1
1b	1.20	1.17	3.36	0.2
2	0.56	0.04	0.47	0.5
8a	0.44	0.10	0.88	—[c]
8b	0.51	0.05	0.67	0.6
8c	0.53	0.08	0.80	1.0
MS-275	0.76	0.48	1.51	0.8
TSA		0.006	0.018	

[a] Measured after a 3-day incubation of test compounds with cells. Assays were performed in triplicate.
[b] Assays were performed in duplicate.
[c] Compound **8a** was an oil substance.

to produce the new HDAC inhibitors in which the 4-(((benzo[*d*][1,3]dioxol-5-ylmethyl)(2-hydroxyethyl)amino)methyl) benzamide moiety in **1a** was replaced with a 4-((N-substituted ureido)methyl)benzamide moiety.

New 5-(thiophen-2-yl)-substituted 2-aminobenzamide-series histone deacetylase inhibitors **8a**, **8b** and **8c** were synthesized as illustrated in Scheme 1. Thus, 4-((*tert*-butoxycarbonylamino)methyl)benzoic acid (**3**) was reacted with oxalyl choride to yield the carbonyl chloride **4**. This compound was treated with 2-nitro-5-thiophen-2-yl-phenylamine to yield the condensation product **5**. Deblocking of a Boc group with 15% HCl afforded the amine hydrochloride salt **6**. Compound **6** was reacted with 4-morpholinecarbonyl chloride in the presence of TEA to give the nitrophenyl derivative **7a**, which was reduced to **8a** with $SnCl_2$ and NH_4OAc. Similarly, **8b** was prepared through condensation of **6** and 4-ethyl-2,3-dioxopiperazine-1-carbonyl chloride followed by the reduction of the resulting product **7b**. Compound **8c** was synthesized utilizing **6** and 4-methylpiperazine-1-carbonyl chloride in the conventional way.

We evaluated the antiproliferative activities (IC_{50}s) of the compounds synthesized using a WST-1assay against human colon cancer HCT116 cells. We also measured their inhibitory activities against HDAC1/2 as well as aqueous solubility in 10% DMSO/H_2O (Table 1). In the present experiments, we selected MS-275 and compound **2**[23] (Fig. 2) as reference compounds because the former is a typical 5-(thiophen-2-yl)-unsubstituted 2-aminobenzamide-type HDAC inhibitor, whereas the latter is a 5-(thiophen-2-yl)-substituted 2-aminobenzamide-type HDAC inhibitor exhibiting a highly selective HDAC1/2 inhibition. Compounds **8a**, **8b** and **8c**, as well as **2**, exhibited slightly stronger inhibition than MS-275



Scheme 1. Reagents: (a) oxalyl chloride, DMF, Py; (b) 2-nitro-5-(thiophen-2-yl)-benzeneamine, DMF, Py; (c) 15% HCl, THF, MeOH; (d) 4-morpholinecarbonyl chloride, THF, TEA, DMF; (e) 4-ethyl-2,3-dioxo-1-piperazinecarbonyl chloride, CH_2Cl_2, TEA, DMF; (f) 4-methylpiperazine-1-carbonyl chloride, CH_2Cl_2, TEA, DMF; (g) $SnCl_2$, NH_4OAc, THF, MeOH.

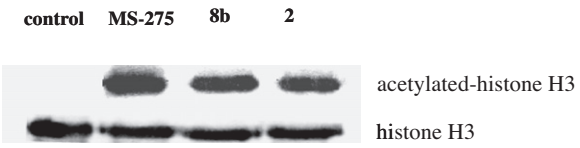


Figure 3. Effects of **8b**, **2** and MS-275 on the histone H3 acetylation. HCT116 cells were treated with **8b**, **2** or MS-275 at 10 μM for 24 h. Cells were lysed to extract crude histone proteins. The Western blot analysis was performed with crude histone samples using antibodies specific to hisotne H3 and total acetylated histone H3. Results are representative of five independent experiments.

against the growth of HCT116 cells. Compound **8b** suppressed the HDAC1/2 activities to nearly the same extent as **2** and promoted the hyperacetylation of core histone H3 as did **2** and MS-275 in the HCT116 cells (Fig. 3). Noticeably, in the cell cycle tests (Table 2), the G_2/M phase cells were more accumulated on exposure of the cells to **8b** or **2** than to MS-275 at 10 μM for 48 h. Additionally, MS-275 induced apoptosis as suggested by the appearance of $subG_1$ population (30.2% in 48 h), whereas neither **8b** nor **2** did (1.4% and 1.4%, respectively). This result suggests that both **8b** and **2** (2-thienyl-substituted derivatives) might have a more cytostatic effect on HCT116 cells than MS-275 (2-thienyl-unsubstituted derivative) though these compounds suppressed the growth of HCT116 cells to the same extent.

Compound **8b** was moderately soluble (0.6 mg/ml) in 10% DMSO and had a pharmacokinetic profile (C_{max}: 8.3 μM; $T_{1/2}$: 2.2 h)[32] similar to HDAC inhibitors[33–37] under development. We thus conducted comparative anti-tumor tests of **8b** and MS-275 using xenografts of HCT116 cells in nude mice.[32] Mice were divided into the following four groups: vehicle (0.1% Tween 80), $n = 6$; MS-275 (45 mg/kg), $n = 7$; **8b** (45 mg/kg), $n = 7$; **8b** (80 mg/kg), $n = 7$. Each compound was administered orally to nude mice with tumor sizes of 90–110 mm^3 on days 0, 2, 4, 6, 8, 10, 12 and 14, and tumor volume and body weight were monitored over 16 days.

Compound **8b** suppressed the growth of tumor xenografts to T/C: 60% at 45 mg/kg and 47% at 80 mg/kg. These values were close to the rate (T/C: 51% at 45 mg/kg) for MS-275 (Fig. 4**A**). Furthermore, a loss of weight was induced by MS-275 at 45 mg/kg, though not by **8b** at either 45 or 80 mg/kg (Fig. 4**B**).

A new orally bioavailable 2-amino-5-(thiophen-2-yl)benzamide-series HDAC inhibitor, **8b**, comprising a 4-ethyl-2,3-dioxopiperazine-1-carboxamide group, had a promising pharmacological profile, including the tumor-shrinking activity comparable to that of MS-275 as well as no weight loss in nude mice. This in vivo efficacy could be attributed to the cytostatic inhibitory effect of **8b** on

Table 2
Effect of HDAC inhibitors on the cell cycle in HCT116 cells

	% of $subG_1$		% of G_0/G_1		% of S		% of G_2/M	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control (0.1%) DMSO	5.7	2.3	74.2	81.9	10.6	10.4	9.5	5.4
8b	2.3	1.4	71.5	75.3	3.8	2.8	22.4	20.5
2	4.6	1.4	79.5	75.0	5.2	2.8	10.7	20.8
MS-275	8.8	30.2	72.7	62.1	4.9	2.2	13.6	5.5

HCT116 cells (1.0×10^6), after incubating for 24 h, were treated with **8b**, **2** or MS-275 (each 10 M) for 24 or 48 h. The cells were then treated with a BD Cycletest Plus DNA reagent Kit. Data are representative of three independent experiments.

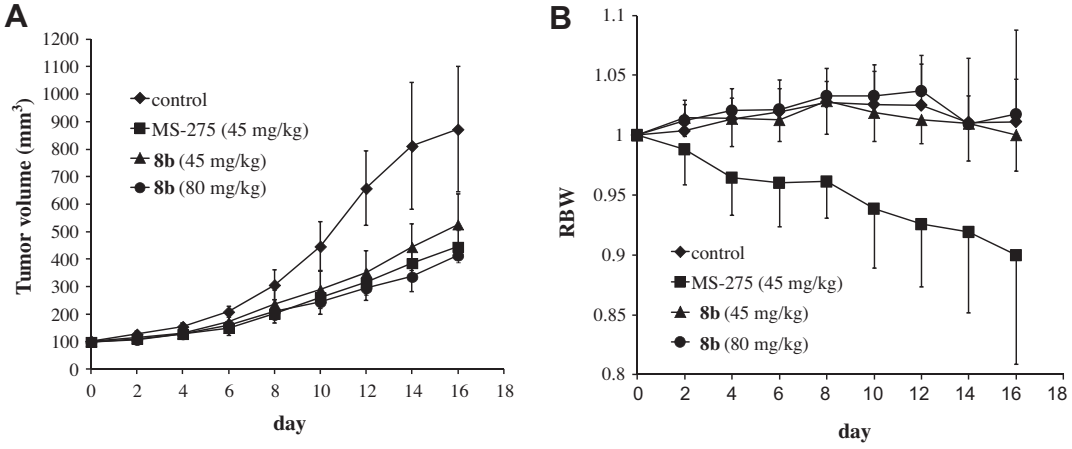


Figure 4. Effects of **8b** and MS-275 on HCT116 cell-inoculated xenografts in vivo. The effects of **8b** and MS-275 on tumor volume (**4A**) as well as relative body weight (RBW) changes (**4B**) were examined. Compounds were orally administered to mice at the indicated time points. Vertical bars indicate standard errors. Significant differences between tumor volumes: $p < 0.05$ (by two-tailed Student *t*-test) for **8b** or MS-275 versus control at all the time points. Significant differences between body weights: $p < 0.05$ for MS-275 versus control, **8b** (45 mg/kg) or **8b** (80 mg/kg) at all the time points except for day16 as well as for MS-275 versus **8b** (80 mg/kg) at day16.

the HCT116 cells, as suggested by the low distribution (1.4–2.3%) of the subG$_1$ cells. Thus, **8b** warrants further tests with other human cancer xenografts to identify its effectiveness as an antitumor agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.053.

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