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4-(Heteroarylaminomethyl)-*N*-(2-aminophenyl)-benzamides and their analogs as a novel class of histone deacetylase inhibitors

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Abstract—The synthesis and biological evaluation of a variety of 4-(heteroarylaminomethyl)-*N*-(2-aminophenyl)-benzamides and their analogs is described. Some of these compounds were shown to inhibit HDAC1 with IC_{50} values below the micromolar range, induce hyperacetylation of histones, upregulate expression of the tumor suppressor $p21^{WAF1/Cip1}$, and inhibit proliferation of human cancer cells. In addition, certain compounds of this class were active in several human tumor xenograft models in vivo.

Histone deacetylases (HDACs) catalyze the hydrolysis of acetyl groups on the NH_2-terminal lysine residues of the core nucleosomal histones.[1] The acetylation status of the core histones correlates with transcriptional activity of certain genes. HDAC activity is generally associated with transcriptional repression. Abnormally increased HDAC activity has been associated with the development of certain human cancers.[2] In recent years, inhibition of HDACs has emerged as a potential strategy to reverse aberrant epigenetic changes associated with cancer.[3] Small molecules with hydroxamic acid functional groups such as natural product trichostatin A (TSA)[4] (**1**) and it's analogues,[5] suberoylanilide hydroxamic acid (SAHA, Zolinza™, Merck & Co., Inc.)[6] (**2**) and synthetic compounds such as the 2-aminoanilide MS-275[7] (**3**) and our isotype specific, oral product candidate **MGCD0103**,[8] are potent HDAC inhibitors (Fig. 1). Some of these compounds demonstrate in vivo anti-tumor activity and are currently under clinical evaluation and SAHA, has recently been approved for the treatment of advanced cutaneous T-cell lymphoma.

In the course of searching for novel HDAC inhibitors with high potency and good safety profiles, we recently designed 4-[(*s*-triazin-2-ylamino)methyl]-*N*-(2-aminophenyl)benzamides (**4**).[9] As a further development of HDAC inhibitors with better pharmaceutical and pharmacokinetic properties, we have synthesized 4-(heteroarylaminomethyl)-*N*-(2-aminophenyl)benzamides (**5**) bearing a 5-membered heteroaromatic ring which showed significant improvement in anti-tumor activities both in vitro and in vivo. The structure–activity relationships (SAR), the anti-proliferative activity and the in vivo efficacy of these novel HDAC inhibitors will be discussed.

The first series of compounds bearing a 5-membered heteroaromatic ring **6**–**12** (Table 1) was synthesized using two different approaches (Scheme 1). To generate

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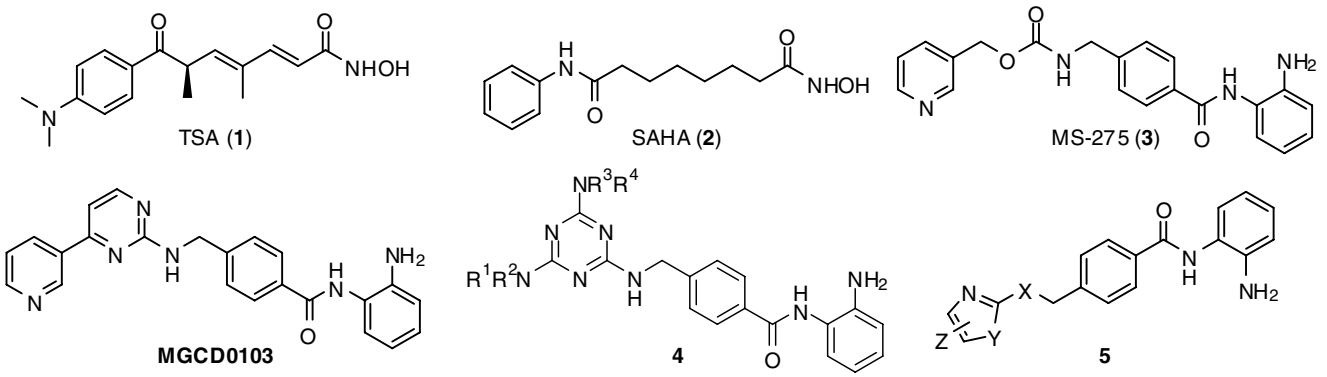
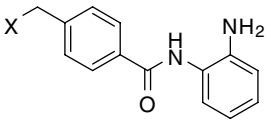
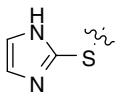
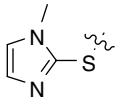
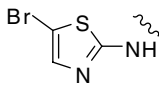
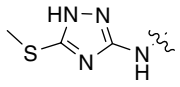
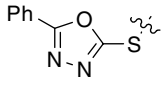
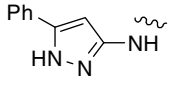
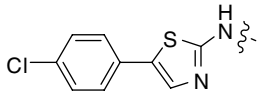


Figure 1. Small molecule HDAC inhibitors.

Table 1. In vitro activities of compounds **6**–**12**



Compound	X	HDAC1[a] IC_{50} (μM)	MTT HCT116 IC_{50}[b] (μM)
6		0.5	5
7		1	5
8		0.4	0.4
9		0.2	0.6
10		0.08	0.4
11		0.07	0.3
12		0.3	0.3

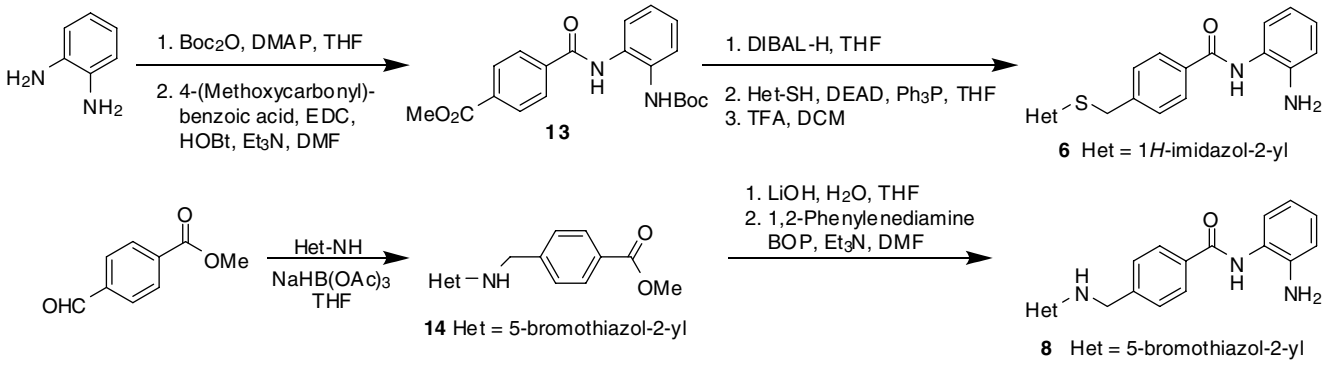
[a] Inhibition of recombinant HDAC1.
[b] Cytotoxicity/proliferation of human cancer HCT116 cells.

the heteroarylthio derivatives, 1,2-phenylenediamine was monoprotected with a Boc-group and coupled with 4-(methoxycarbonyl)benzoic acid to generate amide **13**. The ester functionality was reduced to the alcohol and 1*H*-imidazole-2-thiol was introduced via a Mitsunobu reaction. The Boc-group deprotection with TFA afforded compound **6**. Compounds **7** and **10** were synthesized in a similar fashion. To generate the heteroarylamino derivatives a reductive amination between methyl 4-formylbenzoate and 5-bromothiazol-2-amine was used to generate compound **14**. The ester functionality was hydrolyzed and then coupled with 1,2-phenylenediamine using BOP[§] as a coupling agent to afford final product **8**. Compounds **9**, **11** and **12** were obtained similarly to compound **8**.

The second series was based on benzo-fused heteroaromatic systems (compounds **15**–**25**, Table 2). The reaction between 6-aminobenzothiazole-2-thiol and methyl 4-(bromomethyl)benzoate afforded compound **26** (Scheme 2). The amino group of this material was alkylated with 3-(bromomethyl)pyridine; the ester functionality was hydrolyzed to the corresponding acid which was then coupled with 1,2-phenylenediamine using BOP[§] as a coupling agent, to afford final product **22**. Other benzothiazol-2-ylthio derivatives (compounds **15**, **23** and **24**) and benzimidazol-2-ylthio derivatives (compounds **16** and **21**) were synthesized using the same approach. A reductive amination between 5-bromo-benzothiazol-2-amine and methyl 4-formylbenzoate generated compound **27**. This intermediate was hydrolyzed and the corresponding intermediate acid was coupled with 1,2-phenylenediamine to generate the final compound **19**. Compounds **17**, **18** and **20** were synthesized similarly to compound **19**. Compound **25** was obtained via a reductive amination followed by a Mitsunobu reaction on intermediate **28** and then completed the same way as the other compounds.

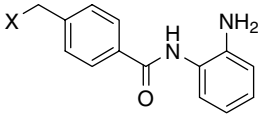
In our HDAC oncology program, we targeted HDAC1 in our design strategy since our work and that of others have clearly linked inhibition of this enzyme with histone hyperacetylation and inhibition of cell proliferation.[10] Thus, the series of compounds presented here are potent HDAC1 inhibitors. As Tables 1 and 2 show, the IC_{50} values range from 30 nM to 1 μM, when tested

[§] Abbreviations: BOP, (benzotriazol-1-yloxy)tris (dimethyl-amino)-phosphoniumhexafluorophosphate; AMC, aminomethylcoumarin; MTT, 3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide.



Scheme 1.

Table 2. In vitro activities of compounds **15**–**25**



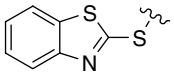
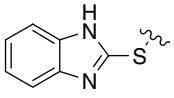
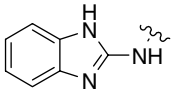
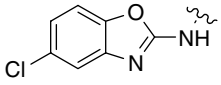
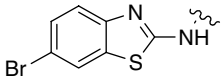
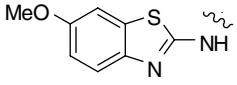
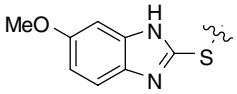
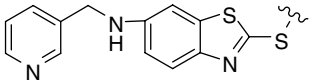
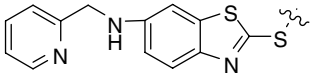
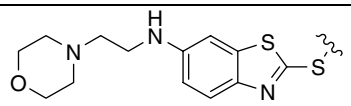
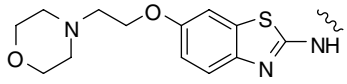
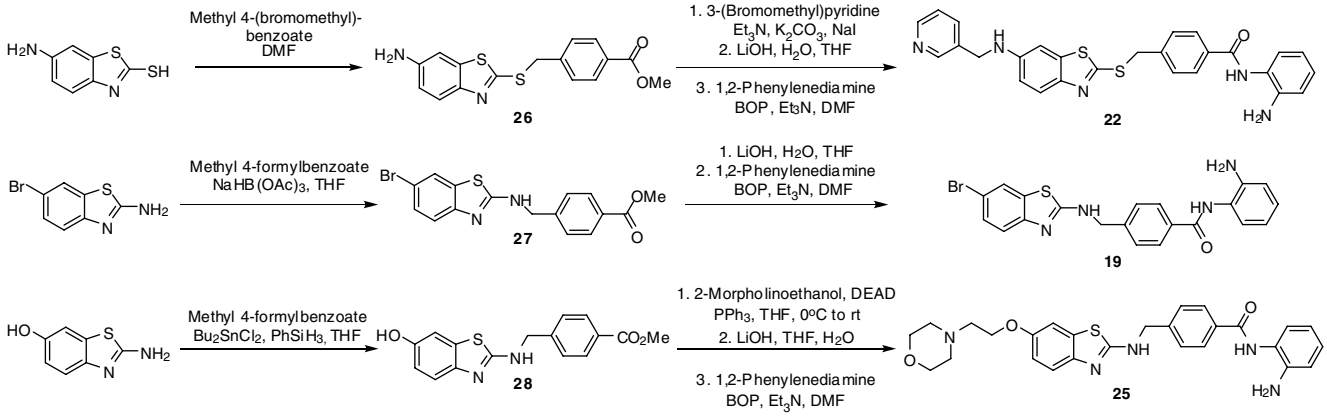
Compound	X	HDAC1[a] IC_{50} (μM)	MTT HCT116[b] IC_{50} (μM)	MTT HMEC[b] IC_{50} (μM)	H4-Ac[c] EC_{50} (μM)
15		0.1	0.8	3	3
16		0.09	0.3	14	<1
17		0.1	0.3	27	1
18		0.1	0.2	17	1
19		0.05	0.4	6	2
20		0.03	0.2	5	1
21		0.07	0.2	10	1
22		0.8	1	>50	2
23		0.07	1	8	3

Table 2 (*continued*)

Compound	X	HDAC1[a] IC_{50} (μM)	MTT HCT116[b] IC_{50} (μM)	MTT HMEC[b] IC_{50} (μM)	H4-Ac[c] EC_{50} (μM)
24		0.07	0.2	15	5
25		0.06	0.2	15	1

[a] Inhibition of recombinant HDAC1.
[b] Cytotoxicity/proliferation in human cancer HCT116 cells and human normal mammary epithelial (HMEC) cells.
[c] Relative effective concentration of compounds in induction of histone H4 acetylation in T24 human cancer cells, relative to MS-275 at 1 μM. Human T24 cells were treated with compounds at 0, 1, 5, 25 μM for 16 h. Cells were harvested and histones were acid-extracted. Histones were analyzed by SDS–PAGE and immunoblotting with antibodies specific for either H4 histones or acetylated H4 histones.



Scheme 2.

using BocLys(acetyl)AMC,[11§] as a substrate. Also shown are data demonstrating the in vitro anti-proliferative potency of these compounds in the human colon cancer cell line, HCT116 (measured using the MTT[§] reagent). A desirable selectivity, ranging from fourfold to greater than 50-fold in potency, is observed in the anti-proliferative activity in cancer cells as compared to normal human mammary epithelial cells (HMEC) (Table 2).

The imidazole derivatives (compounds **6** and **7**, Table 1) showed reasonable potency against HDAC1 but low anti-proliferative activity (5 μM). The introduction of a non-polar substituent on the 5-membered ring heterocycle (compounds **8**–**12**) improved anti-proliferative activity (0.3–0.6 μM) while maintaining high enzymatic inhibitory potency (0.07–0.40 μM).

To further explore the SAR of heteroarylaminomethyl-based HDAC inhibitors we have designed and synthesized analogues bearing bicyclic annealated heteroaromatic systems **15**–**25** (Table 2). These compounds (regardless of the nature of the heteroaromatic ring or substitution pattern) exhibited sub-micromolar HDAC1 inhibitory activities and they also discriminated between cancer cells (exemplified by the HCT116 colon cancer cell line) and normal cells (Table 2). Compounds **22** and **23** bearing bulky aromatic substituents were weaker in inhibiting HCT116 cell proliferation (1 μM) compared to compounds **15**–**21** which showed sub-micromolar activities in the same assay. Interestingly, compounds **24** and **25** with a large non-aromatic substituent were as potent as compounds **15**–**21** in both assays. Consistent with inhibiting cellular HDACs, these inhibitors induced histone hyperacetylation in T24 human cancer cells (EC_{50} 1–5 μM, Table 2).

Also consistent with their mechanism of action, such inhibitors induced the expression of the cyclin-dependent kinase inhibitor $p21^{WAF1/Cip1}$ ($EC_{50} \sim 2$ μM) in the T24 human cancer cell line. For example, compounds **20**, **21**, and **25** gave EC_{50}s of 0.8, 0.6, and 1.0 μM, respectively. Furthermore, all three compounds caused G2/M cell cycle arrest in the HCT116 human colon cancer cell line (Fig. 2, data shown only for **25**).[10]

Compounds **20**, **21**, and **25** were evaluated in vivo in several different human tumor xenograft models in mice representing the major forms of human cancers: PANC-1 (pancreatic), SW48 (colon), COLO205 (colon) and A549 (lung). Compounds were administered daily for 14 days by either intraperitoneal (ip) injection or orally (po). Compound **25** showed the best anti-tumor activity quantified as % of tumor growth relative to vehi-

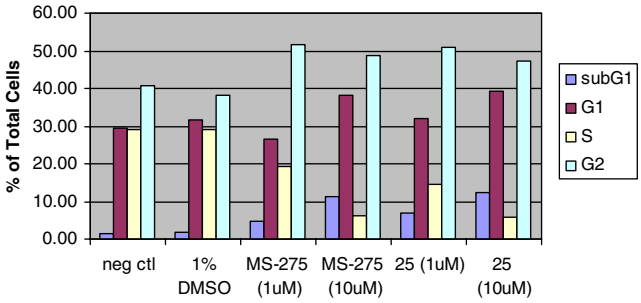


Figure 2. HDAC inhibitors caused G2/M arrest of human cancer cells. Human HCT116 cancer cells were treated with compound **25** or MS-275 in 1% DMSO for 16 h before harvesting by trypsinization. Propidium iodide stained DNA contents in fixed cells were analyzed by flow cytometry.

Table 3. In vivo anti-tumor activity of compound **25**

Dose (mg/kg)	% Tumor growth inhibition
20 (ip)	74% (SW48), 65% (A549), 70% (PANC-1)
90 (po)	72% (SW48), 65% (A549), 62% (COLO205)

cle treated controls, (Table 3, also see supplementary material for the anti-tumor effect of compound **25** compared to MS-275 in PANC-1 Human Pancreatic Carcinoma Model).

This in vivo anti-tumor activity was obtained without any associated body weight loss as a measure of gross toxicity. We designed and synthesized a novel class of HDAC inhibitors, the 4-(heteroarylaminomethyl)-*N*-(2-aminophenyl)-benzamides and their analogues. These compounds exhibited in vitro anti-proliferative activities in numerous human cancer cells with little or no activity on normal cells, induced $p21^{WAF1/Cip1}$ expression and induced hyperacetylation of core histones. They also caused cell cycle arrest of human cancer cells in a dose-dependent manner. The most promising HDAC inhibitors of the 4-(heteroarylamino/thiomethyl)-*N*-(2-aminophenyl)-benzamide series were found to be compounds **20**, **21** and **25**. These compounds have attractive in vitro profiles and show significant anti-tumor activity in vivo in several human cancer xenograft model models. These results represent a significant step towards the development of small molecule HDAC inhibitors with favorable pharmaceutical properties.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.057.

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