Design and synthesis of 4-[(s-triazin-2-ylamino)methyl]-N-(2-aminophenyl)-benzamides and their analogues as a novel class of histone deacetylase inhibitors

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Abstract—Inhibition of histone deacetylases (HDAC) is emerging as a new strategy in human cancer therapy. The synthesis and biological evaluation of a variety of 4-(heteroarylaminomethyl)-N-(2-aminophenyl)-benzamides is presented herein. From the different series bearing a six-membered heteroaromatic ring studied, the s-triazine series showed the best HDAC1 enzyme and in vitro anti-proliferative activities with IC$_{50}$ values below micromolar range. Some of these compounds can also significantly reduce tumor growth in human tumor xenograft models in mice.
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Histone deacetylases (HDACs) are prominent targets for cancer therapy. Evidence has shown that HDAC inhibitors arrest cell growth and lead to differentiation and apoptosis in tumor cells.[1] HDACs are zinc enzymes that catalyze the removal of the acetyl group from the ε-amino groups in histone lysine residues. These are located at the N-terminal tails of histones and their hydrolysis into positively charged amine groups leads to changes in the chromatin structure, blocking the access of transcription factors to DNA during gene expression. Recently, Zolinza™ from Merck (also known as SAHA or Vorinostat)[2] was the first HDAC inhibitor approved by the FDA for the treatment of advanced cutaneous T-cell lymphoma (CTCL). MethylGene's isotype specific HDAC inhibitor MGCD0103 (Fig. 1) is currently undergoing clinical evaluation in solid tumors and hematological malignancies.[3]

Therefore, in our effort to identify new therapeutic agents for the treatment of cancer, the design and synthesis of selective HDAC inhibitors[4] has become one of our major goals. Thus, we have recently disclosed a new series of N-(2-aminophenyl)cinnamide- and benzamide-type derivatives 1a–c which inhibit HDAC enzymes and exhibit in vitro anti-proliferative activities in human tumor cell lines (Fig. 1).[5] As a continuation of these efforts, we report herein the efficient and straightforward synthesis of new compounds of type 2, bearing a six-membered heteroaromatic ring in the amino-benzylic position.

We also report the evaluation of these compounds as HDAC inhibitors and potential anti-tumor agents.
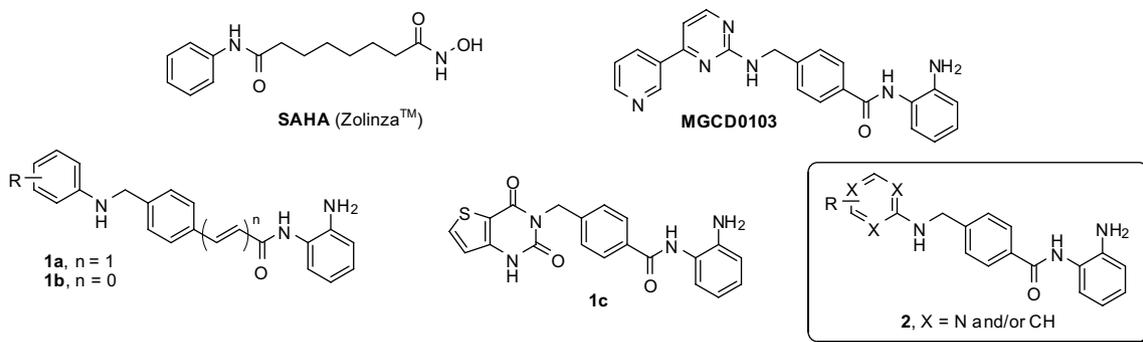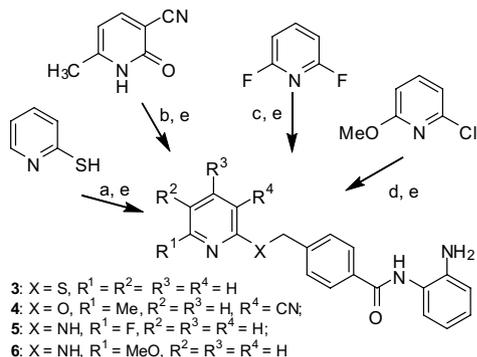
Figure 1.

A new pyridine-based series of HDAC inhibitors was synthesized following the procedures described in Scheme 1 (compounds **3–6**).[5a] The thiopyridinyl intermediate was synthesized by alkylation of pyridine-2-thiol with methyl 4-(bromomethyl)-benzoate in the presence of base. The O-alkylation of 6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile with the same alkylating agent gave the methyl ester. Fluorine substitution of 2,6-difluoropyridine with methyl 4-aminomethylbenzoate generated the ester intermediate.

Finally, palladium cross-coupling reaction between 2-chloro-6-methoxypyridine and methyl 4-amino-methyl-benzoate was used to prepare the aminopyridine intermediate. The ester intermediates were then hydrolyzed and coupled with 1,2-phenylenediamine to afford the corresponding final compounds (**3–6**). Compound **7** (Table 1) was obtained similarly to the synthesis of **6**.

A new series of 4-[(pyrimidin-2-ylamino)methyl]-benz-amides was also synthesized (Table 2, compounds **8–12**). Compounds **8–10** were synthesized via a reductive amination[6] as a key step, and an amide coupling with 1,2-phenylenediamine, starting from the substituted 2-aminopyrimidines and 4-formylbenzoic acid. Compound **11** was prepared in three steps (Scheme 2).

Table 1. Pyridine series (**3–7**)

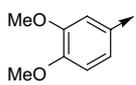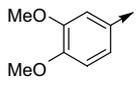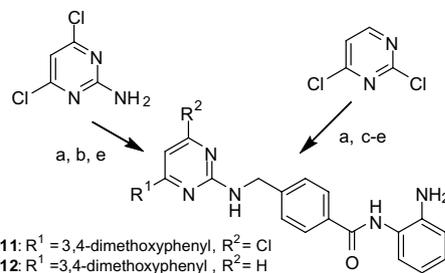| Compound	X	HDAC1[a] IC$_{50}$ (μM)	MTT HCT116[b] IC$_{50}$ (μM)
3		0.3	1
4		0.3	0.5
5		0.1	1
6		0.1	0.8
7		0.1	0.7

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

The first step was the Suzuki coupling of 3,4-dimethoxyphenylboronic acid with 2-amino-4,6-dichloropyrimidine, followed by a reductive amination reaction with the 4-formylbenzoic acid; the final amide coupling with 1,2-phenylenediamine afforded the desired material **11**. In a similar fashion, Suzuki coupling between 2,4-dichloropyrimidine and 3,4-dimethoxyphenylboronic acid, followed by the chlorine displacement with methyl 4-(aminomethyl)-benzoate, then saponification and amide coupling with 1,2-phenylenediamine yielded the desired compound **12**.

Pyrimidine **13** (Table 3) was synthesized using the same procedures as used in the synthesis of **9**. Few purine derivatives were also generated (compounds **14–16**, Table 3). For example, the chlorine substitution of 6-chloro-9H-purin-2-amine or 2,6-dichloro-9H-purine by methyl 4-(aminomethyl)-benzoate followed by a

Scheme 1. Reagents and conditions: (a) methyl 4-(bromomethyl)-benzoate, NaH, DMF, 0 °C–rt; (b) methyl 4-(bromomethyl)-benzoate, K$_2$CO$_3$, DME, reflux; (c) methyl 4-(aminomethyl)-benzoate hydrochloride, K$_2$CO$_3$, MS 4 Å, 18-crown-6, DMF, 110 °C; (d) methyl 4-(aminomethyl)-benzoate, Pd(OAc)$_2$, NaO-t-Bu, 2-(dibutylphosphino)-biphenyl, toluene, 110 °C; (e) i—LiOH·H$_2$O, THF/H$_2$O, rt; ii—1,2-phenylenediamine, BOP, Et$_3$N, DMF.

Table 2. Pyrimidine series (**8**–**12**)

Compound	R^1	R^2	HDAC1[a] IC$_{50}$ (μM)	MTT HCT116[b] IC$_{50}$ (μM)
8	H	H	0.7	2
9	CH$_3$	CH$_3$	0.3	1
10	OCH$_3$	OCH$_3$	0.1	0.6
11	3,4-(MeO)$_2$C$_6$H$_3$	Cl	0.1	0.4
12	3,4-(MeO)$_2$C$_6$H$_3$	H	0.05	0.1

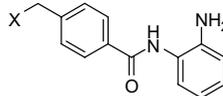[a] Inhibition of recombinant HDAC1.
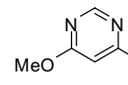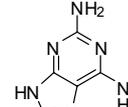[b] Cytotoxicity/proliferation of human cancer HCT116 cells.

Scheme 2. Reagents and conditions: (a) 3,4-dimethoxyphenylboronic acid, Ph$_3$P, Pd(OAc)$_2$, Na$_2$CO$_3$, DME, reflux; (b) 4-formylbenzoic acid, Bu$_2$SnCl$_2$, PhSiH$_3$, THF; (c) methyl 4-(aminomethyl)-benzoate, DIPEA, DMF, 120 °C; (d) LiOH·H$_2$O, THF/H$_2$O; (e) 1,2-phenylene-diamine, BOP, Et$_3$N, DMF.

Mitsunobu reaction with *n*-butanol gave the methyl ester intermediates. Then, the hydrolysis and the final amide coupling generated the desired compounds **15** and **16** (Scheme 3).

To complete the SAR, the HDAC inhibitors of the *s*-triazine series were prepared (compounds **17**–**25**, Table 4). The intermediate **26** was generated via a mono-substitution reaction of the chlorine atom in cyanuric chloride with methyl 4-(aminomethyl)-benzoate (Scheme 4). In pathway A the NH$_2$-group was first introduced using ammonia gas in a sealed flask and the indan-2-yl amino group was introduced at higher temperature to generate the compound **29**. In pathway B, the indan-2-yl amino group was introduced first followed by the cyclopropyl amino group incorporation, to obtain compound **30**.

The ester functionalities of compounds **29** and **30** were hydrolyzed and then coupled with 1,2-phenylenediamine using BOP reagent to give final compounds **17** and **23**. Compounds **18**–**22** and **24**–**25** were synthesized starting

Table 3. Purine series (**14**–**16**)

Compound	X	HDAC1[a] IC$_{50}$ (μM)	MTT HCT116[b] IC$_{50}$ (μM)
13		0.2	1
14		0.2	10
15		0.3	2
16		0.05	0.1

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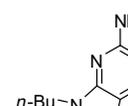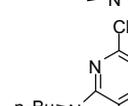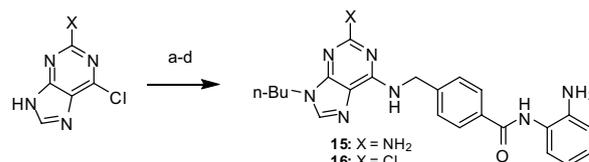[b] Cytotoxicity/proliferation of human cancer HCT116 cells.

Scheme 3. Reagents: (a) methyl 4-(aminomethyl)-benzoate hydrochloride, NaHCO$_3$, H$_2$O; (b) *n*-butanol, Ph$_3$P, DEAD; (c) LiOH·H$_2$O, THF/H$_2$O; (d) 1,2-phenylenediamine, BOP, DIPEA, DMF.

from cyanuric chloride according to the pathways A or B as indicated in Table 4 (also in Scheme 4).

All compounds were initially screened for their ability to inhibit recombinant human HDAC1. We targeted this isotype following the results of our work and of others which implicated HDAC1 for both transcriptional repression and chromatin remodeling.[7] The compounds were screened as described earlier, using BocLys(acetyl)-AMC as substrate. The in vitro anti-proliferative activities of the synthesized compounds against HCT116 human colon cancer cell line were evaluated using 3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT). The first series studied, compounds **3**–**7** (Table 1), showed good activity against HDAC1 and good anti-proliferative activity, both in the sub-micromolar range. The inhibitory potency (IC$_{50}$) was not sensitive to the small structural changes on the pyridine ring, such as in compounds **5** and **6**, the heteroatom linker in the benzylic position (compounds **3**–**5**), or the pyridine nitrogen position (e.g., **6** vs **7**). The in vitro anti-proliferative activity (MTT assay) followed the same trend.

Table 4. *s*-Triazine series (**17–25**)

| Compound	R^1	R^2	Synthesis pathway	HDAC1[a] IC$_{50}$ (μM)	MTT HCT116[b] IC$_{50}$ (μM)	H4 Ac[c] (T24) EC$_{50}$ (μM)	MTT HMEC[b] IC$_{50}$ (μM)
17	indanyl-2-yl-NH	NH$_2$	A	0.2	0.6	1	8
18	cyclopentyl-NH	NH$_2$	A	0.2	0.7	0.8	11
19	phenethyl-NH	NH$_2$	A	0.1	0.3	1	21
20	indolin-1-yl	NH$_2$	B	0.1	0.8	1	10
21	isoindolin-2-yl	NH$_2$	A	0.04	0.5	1	>50
22	morpholino	NH$_2$	A	0.2	0.8	5	>40
23	indanyl-2-yl-NH	cyclopropyl-NH	B	0.03	1	1	18
24	indanyl-2-yl-NH	morpholino	B	0.08	2	1	>50
25	indanyl-2-yl-NH	morpholinoethyl-NH	B	0.07	0.6	1	16

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.
[a] Inhibition of recombinant HDAC1.
[b] Cytotoxicity/proliferation of 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 pyridin-3-ylmethyl 4-(2-aminophenylcarbamoyl)benzylcarbamate (MS-275),[8] which was used as a positive control in these experiments at 1 μM.
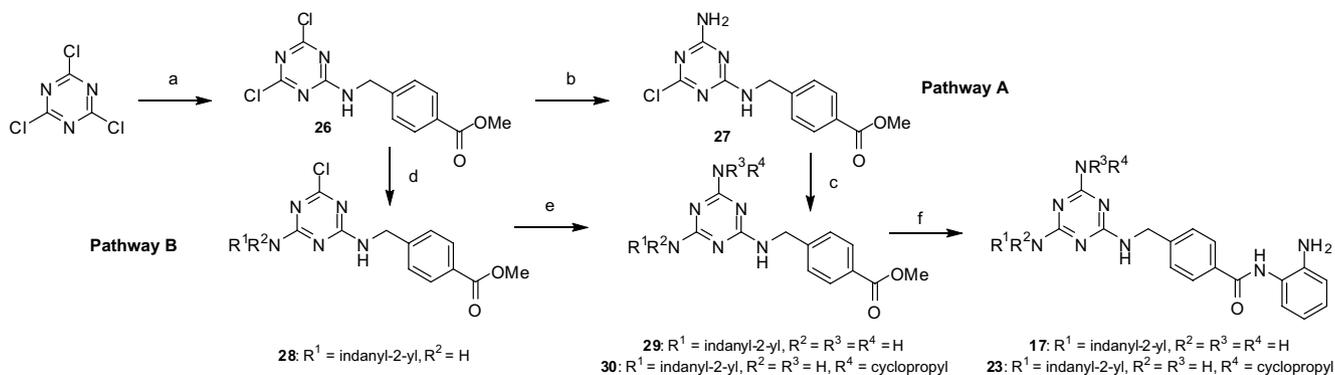
Scheme 4. Reagents and conditions: (a) methyl 4-(aminomethyl)-benzoate hydrochloride, DIPEA, THF, −78 °C; (b) NH$_3$ gas, 1,4-dioxane, sealed flask, 70 °C; (c) R$_1$R$_2$NH, THF or 1,4-dioxane, sealed flask, 120–140 °C; (d) R$_1$R$_2$NH, *i*-Pr$_2$NEt, THF; (e) NH$_3$ or R$_3$R$_4$NH$_2$, THF or 1,4-dioxane, sealed flask, 120–140 °C; (f) i—LiOH·H$_2$O, THF/H$_2$O; ii—1,2-phenylenediamine, BOP, Et$_3$N, DMF.

Similarly, in the pyrimidine series (Table 2), the size and the electronic effects of substituents have a minimal effect on HDAC1 inhibitory activity (0.05–0.3 μM), except for compound **8** which shows slightly weakened cytotoxicity (2 μM). Replacing the pyrimidine ring with a purine system (compounds **14–16**, Table 3) generates compounds that are at least as potent as the compound **13** against the HDAC1 enzyme. With respect to the activity in the cell proliferation assay it looks like the polar fragments are detrimental and the hydrophobic substituents are favorable (e.g., **14** vs **15** and **15** vs **16**).

Finally, the *s*-triazine series (compounds **17–25**, Table 4) can accommodate a plethora of substituents. The structural diversification brought about increased potency against HDAC1 with IC_{50} less than 0.2 μM and with concomitant increase in anti-proliferative activity. Furthermore, as the data in Table 4 show, the anti-proliferative activity of these compounds is selective to the cancer cell line tested (HCT116) with a toxicity index that in some cases exceeds a 100-fold when compared to a normal cell line (HMEC). The optimal R^1 substituent producing the best HDAC1 inhibitory activity was found to be either indanyl-2-amino- (**23**) or iso-indolinyl- (**21**). Likewise, the optimal R^2 substituent seems to be an amino- or alkylamino- group. In general, compounds of the triazine series show better profiles than their pyridine, pyrimidine, and purine analogues.

A representative set of compounds (**17**, **18**, **22**, and **25**) were profiled against the different HDAC isoforms. In class I, the compounds showed potent activity against HDAC1, 2, and 3 in the sub-micromolar range, and were very weak or inactive against HDAC 8. The same compounds exhibited no activity against the class II HDACs (HDAC4–7). Consistent with the ability of the *s*-triazine-based compounds to inhibit cellular HDAC, they were able to induce histone acetylation in T24 human bladder cancer cells (Table 4) and induce p21[WAF1/Cip1] (data not shown). A few HDAC small molecule inhibitors from the *s*-triazine series were evaluated in vivo in HCT116 (human colon) tumor xenograft model in mice. Compounds were administered daily by intraperitoneal (ip) injection. Compound **25** displayed anti-tumor activity with 49% tumor growth inhibition relative to vehicle control when dosed at 20 mg/kg per day for two weeks. Compounds **18** and **22** showed both around 50% inhibition when dosed at 40 mg/kg. The three compounds displayed little toxicity as revealed by body and spleen weight measurements and white blood cell counts.

A novel class of selective HDAC class I inhibitors of general structure **2** was designed and the synthesis of these new compounds was developed. *s*-Triazine-based analogues turned out to be the most active compounds having HDAC1 inhibitory potency and anti-proliferative activity in the sub-micromolar range. These inhibitors selectively induced hyperacetylation of histones in whole cells. Compounds **18**, **22**, and **25** showed significant anti-tumor activity in vivo in HCT116 human colon cancer xenograft models in mice. These results represent an important step toward the development of selective HDAC small molecule inhibitors with favorable drug-like characteristics.

References and notes

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