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N-(2-Amino-phenyl)-4-(heteroarylmethyl)-benzamides as new histone deacetylase inhibitors

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Dedicated to the memory of Naomy Bernstein, our colleague and friend.

Abstract—A variety of *N*-(2-amino-phenyl)-4-(heteroarylmethyl)-benzamides were designed and synthesized. These compounds were shown to inhibit recombinant human HDAC1 with IC_{50} values in the sub-micromolar range. In human cancer cells growing in culture these compounds induced hyperacetylation of histones, induced the expression of the tumor suppressor protein $p21^{WAF1/Cip1}$, and inhibited cellular proliferation. Certain compounds of this class also showed in vivo activity in various human tumor xenograft models in mice.

Histone acetylation/deacetylation is essential for chromatin remodeling and regulation of gene transcription in eukaryotic cells. Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are enzymes that catalyze the deacetylation (associated with transcriptional silencing) and acetylation (associated with transcriptional activation), respectively, of lysine residues located in the NH_2 terminal tails of core histones.[1] Perturbations of this balance in the form of histone deacetylation are often observed in human tumors.[2] Thus, inhibition of HDACs has emerged as a novel therapeutic strategy against cancer.[3] Small molecules of different classes such as MGCD0103 (**1**) (MethylGene Inc.),[4,5] CRA-024781 (**2**) (Celera Genomics),[6] PXD-101 (**3**) (Curagen/Topo-Target),[7] LBH-589 (**4**) (Novartis AG),[8] and MS-275 (**5**) (Syndax Pharmaceuticals/Schering AG)[9] are potent HDAC inhibitors, demonstrating in vivo antitumor efficacy and are currently undergoing clinical trials (Fig. 1).

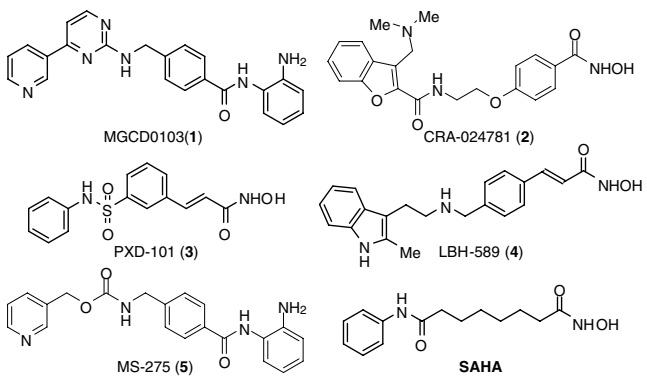

Figure 1.

Keywords: Histone deacetylase inhibitors; HDAC; Benzamides; Cancer cell proliferation.
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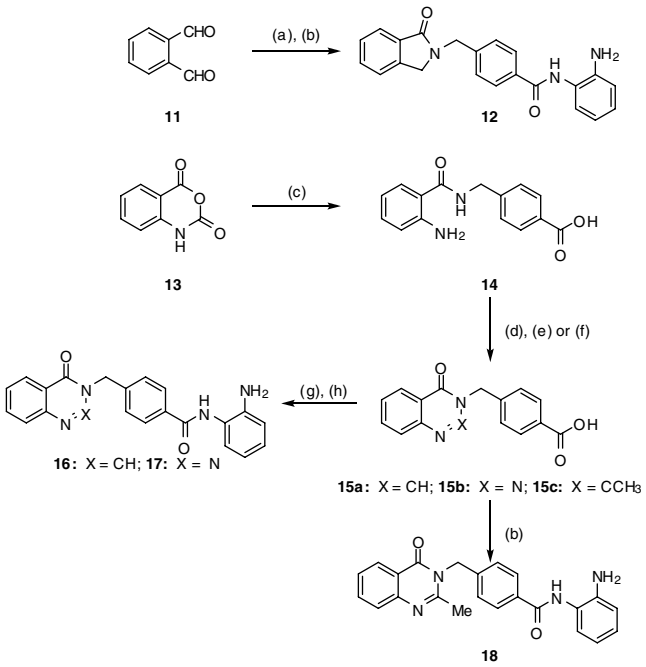
Suberoylanilide hydroxamic acid Zolinza(TM) (vorinostat, SAHA, Merck)[10] has recently been approved for the treatment of advanced cutaneous T-cell lymphoma (CTCL).

In our research program directed toward design, synthesis, and biological evaluation of novel HDAC inhibitors with adequate 'drug-like' properties, we identified several distinct classes of such molecules: arylsulfonamide-based hydroxamates exemplified by compound (**6**),[11] arylsulfonamide-based amino-anilides such as (**7**),[12] long-chain SAHA-like ω-substituted hydroxamic acids like (**8**),[13] and 2-amino-phenylamides of ω-substituted alkanoic acids such as (**9**).[14] All these classes of compounds while possessing good in vitro HDAC-inhibitory activity showed, however, marginal in vivo efficacy, which was attributed to their relatively short half-life and poor bioavailability (data not shown). Efforts to overcome these shortcomings led us to the novel class of *N*-(2-amino-phenyl)-4-(heteroarylmethyl)-benzamides (**10**) (Fig. 2), which not only retained high HDAC in vitro potency, but showed significant improvement in antitumor in vivo behavior.

The first series of target compounds representing the class of *N*-(2-amino-phenyl)-4-(heteroarylmethyl)-benzamides (**10**) was the set of compounds bearing a carbonyl group on the left-hand side heterocyclic moiety such as compounds **12**, **16**–**18**. Their synthesis is presented in Scheme 1, while the detailed procedures are described in Delorme et al.[15] It is worth mentioning that all these HDAC inhibitors as well as the ones described below bear the *N*-(2-amino-phenyl)-benzamide fragment which has been shown to be the pharmacophore.[9b] Removal of the amino group is detrimental to the HDAC-inhibitory activity. The amino group, however, can be replaced by an OH-group without the loss of potency and only a few other subtle substitutions in that fragment are allowed.[9b]

The second series of the synthesized target compounds was the series of molecules bearing two carbonyl groups on the left-hand side heterocyclic moiety such as compounds **20**, **22**, and **24**–**29** (Scheme 2). We also explored a replacement of the fused benzene ring in compounds **16**, **24**, and **26** for a thiophene thus preparing the corresponding thienopyrimidines **31**, **34**, and **36** (Scheme 3). To further investigate possible replacements within this class of molecules, we substituted the endocyclic nitrogen atom as an attachment point between heterocycles and the benzamide fragments, for a carbon atom, which resulted in the design and synthesis of the 'carbon analogues' **38**, **41**–**43** (Scheme 4). 4-(4-Oxo-chroman-3-ylidenemethyl)-benzoic acid methyl ester (**40**) turned out to be the key intermediate in the synthesis of target molecules **41**–**43**. Thus, the $RhCl_3$ mediated isomerization of its double bond[16] followed by hydrolysis and a coupling with 1,2-phenylenediamine afforded compound **41**. Reduction of the double bond using phenylsulfonyl hydrazine was the key procedure in the synthesis of the target **42**, while hydrogenation of **40** in the presence of palladium on charcoal ultimately allowed for the formation of the compound **43** devoid of the carbonyl group.



Scheme 1. Reagents and conditions: (a) *p*-aminomethylbenzoic acid, AcOH, 5 min, reflux, 49%; (b) 1,2-phenylenediamine, BOP, Et_3N, DMF, 31–49%; (c) *p*-aminomethylbenzoic acid, Et_3N, H_2O, 3 h, 40 °C 100%; (d) HCOOH, reflux, 6 h (X = CH), 96%; (e) $NaNO_2$, HCl, 0 °C (X = N), 96%; (f) Ac_2O, reflux, 1 h, then AcOH, reflux, 48 h (X = CCH_3), 43%; (g) (2-amino-phenyl)-carbamic acid *tert*-butyl ester, BOP, Et_3N, DMF (58%, X = CH; 62%, X = N); (h) TFA/CH_2Cl_2 (74%, X = CH; 19%, X = N).

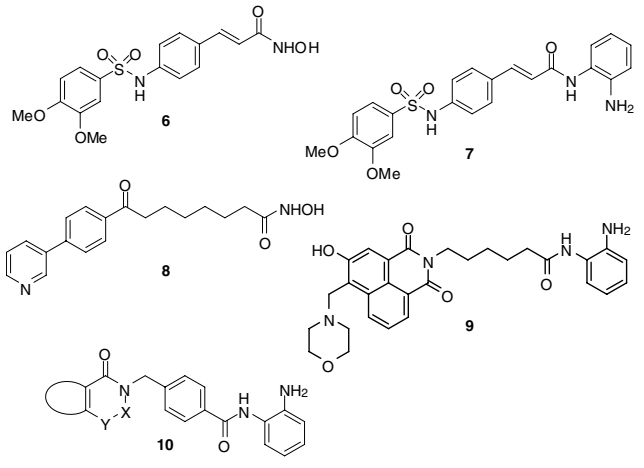


Figure 2.

The data in Table 1 demonstrate the ability of these compounds to inhibit recombinant HDAC1 with an IC_{50} range of 0.1–1.0 μM (measured using BocLys(acetyl)AMC as substrate).[17] Also, these compounds demonstrated good in vitro antiproliferative potency (measured with MTT reagent) in the human colon cancer cell line (HCT116), with desirable selectivity (6- to >50-fold) over normal human mammary epithelial cells