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Discovery of histone deacetylase 8 selective inhibitors

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ABSTRACT

We have developed an efficient method for synthesizing candidate histone deacetylase (HDAC) inhibitors in 96-well plates, which are used directly in high-throughput screening. We selected building blocks having hydrazide, aldehyde and hydroxamic acid functionalities. The hydrazides were coupled with different aldehydes in DMSO. The resulting products have the previously identified 'cap/linker/biasing element' structure known to favor inhibition of HDACs. These compounds were assayed without further purification. HDAC8-selective inhibitors were discovered from this novel collection of compounds.

Eukaryotes package their genome into nucleosomes, where DNA is tightly wrapped around an octamer of core histones (two each of the H2A, H2B, H3 and H4).[1] Modifications and the positioning of histones organize the genome into either open or condensed chromatin and by creating docking sites, and thus regulate the accessibility of DNA for diverse cellular processes, from transcription, replication, to DNA repair.[2] Encoded in the human genome are 11 zinc-dependent human histone deacetylases (HDACs) that catalyze the hydrolysis of ε-acetyl group of acetylated lysine on histone proteins.[3,4] The acetylation status of histone and non-histone proteins plays an important role in the modulation of transcription, microbubule structure and function, the cell cycle, among others.[5] Small-molecule inhibitors of HDACs such as trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), trapoxin B, tubacin and histacin (Fig. 1), played an important role in both the discovery of HDACs and in the elucidation of their functions.[3,6–9] HDAC inhibitors that have undergone or are undergoing clinical investigation have little selectivity towards individual HDACs.[8,10] Selectively inhibiting a single HDAC or a subclass of HDACs is currently a major focus of HDAC inhibitor design. Selective HDAC inhibitors, for example tubacin, not only can elucidate the function of individual HDAC but also provide candidates with less side effects for the treatment of cancer or other diseases.[11] However, only limited progress towards the discovery of these agents has been made to date.[6–8,12,13]

Structural studies of HDACs and related proteins revealed that the active site and the channel that accommodates the aliphatic chain of HDAC inhibitors such as TSA are highly conserved.[14] The structural diversity of the HDACs lies in a region peripheral to the catalytic site.[15] This sequence diversity in the periphery suggests that selective inhibitors may be identified from collections of compounds having varied groups that can interact with the residues in this region. In addition, HDACs exist in numerous protein complexes and the enzymatic activities of HDACs are often dependent on or regulated by these complexes. Small molecules with diverse structures that can interact with the peripheral regions of HDACs may provide selectivity for certain protein complexes.

The coupling of compounds having diverse structural features with compounds that can interact with the conserved active site, such as biasing reagents for HDACs, has provided one strategy for the discovery of isozyme-selective inhibitors.[6,7] Ideally, the coupling chemistry should be compatible with diverse functional groups and efficient—with minimal or ideally no purification required—and suitable for miniaturized high-throughput synthesis and screening.

A two-step process for the conversion of primary alcohols to HDAC inhibitors was envisioned as shown in Figure 2.[16] Primary alcohol **1** was first oxidized to aldehyde **2** using a polymer-supported oxidation reagent.[17,18] Excess reagents can be used to drive the reactions to completion and can be removed by simple filtration. Condensation of the resulting aldehyde **2** with HDAC-biasing compound **3** that possess both hydrazide and hydroxamic groups yields hydrazone **4**. The hydroxamic acid group with a suitable linker element is known to bias the resulting compounds towards the inhibitions of HDACs.[8] For example, trichostatin A has a hydroxamic acid as its metal-chelating group.

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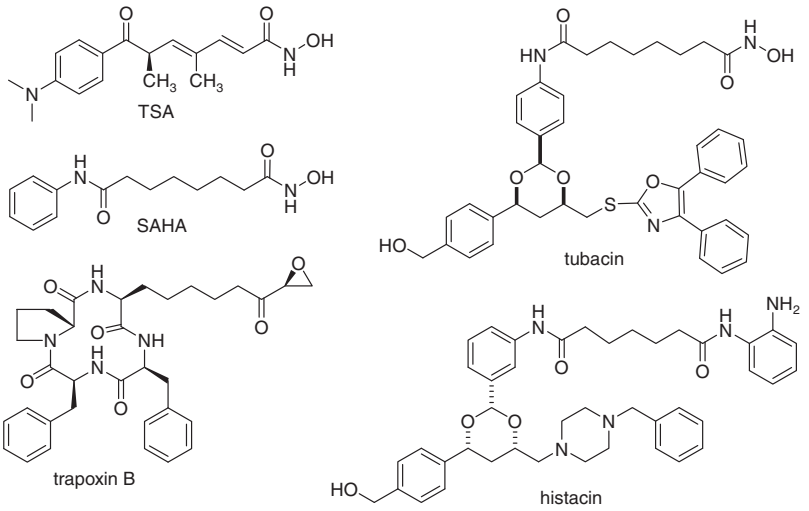

Figure 1. TSA, SAHA, trapoxin B, tubacin, and histacin.

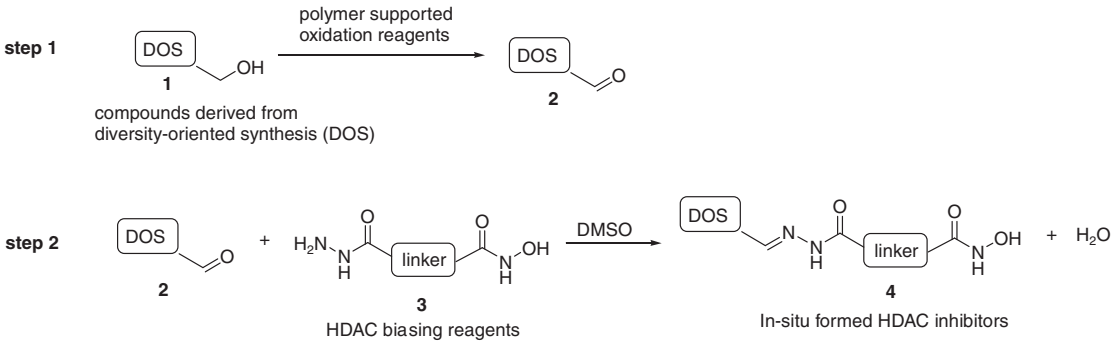

Figure 2. A two-step protocol for the conversion of diverse primary alcohols to HDAC inhibitors is illustrated.

We selected 12-membered macrocyclic lactams functionalized with zinc ion-binding elements as core structural features of compounds comprising an initial pilot library. A number of naturally occurring HDAC inhibitors are 12-membered cyclic tetrapeptides, including trapoxin, chlamydocin, HC toxin, apicidin, Cyl-1, Cyl-2 and FK228.[8] Epoxyketones, ketones, hydroxyketones and thiols function as metal-chelating groups in these macrocyclic HDAC inhibitors. Structurally diverse synthetic analogues of the above natural products have been identified as HDAC inhibitors including a hydroxamic acid-containing cyclic peptide (CHAP).[19] These cyclic tetrapeptides are among the most potent HDAC inhibitors. However, syntheses of theses inhibitors are generally complex, requiring more than 20 steps.[3,20] Macrocyclic lactam **11** shown in Figure 3, on the other hand, can be prepared in a few steps according to previously established methods.[21] The ring closing metathesis (RCM) using Grubbs' first generation catalyst[22] gave *trans* olefin **10** predominantly. Alcohol **11** can then be oxidized efficiently to aldehyde **A6** using solid support reagents.[17] Macrocyclic lactams **A1**–**A11** (Fig. 5) were synthesized according to the procedure shown in Figure 3. The *E*/*Z* selectivity for the RCM reaction varies from 5:1 to over 10:1.[23]

Bifunctional reagents having hydroxamic acid and other chelating groups and linkers with different length and rigidity were synthesized as shown in Figure 4.

Reactions of hydrazine with dimethyl diester **12** (in excess) yielded mixtures of mono- and dihydrazides. Pure monohydrazides were obtained after silica gel filtration. Treatment of the resulting monohydrazides with hydroxylamine under basic conditions afforded simple bifunctional reagents **B1**–**B3**. The mono hydrazides can also be prepared from corresponding monoacid **13** via activation followed by hydrazinolysis. Bifunctional reagents **B4** and **B5** with a benzene ring within the linker were prepared in two steps. Nonsymmetrical linkers were used in bifunctional reagents **B6** – **B13** via a four-step protocol from corresponding hydroxybenzaldehyde **14** or its substituted counterparts. Bifunctional reagents **B14**–**B16** containing *ortho*-hydroxyanilides and **B17**–**B18** containing carboxylic acids as the biasing reagents were also prepared following similar procedures.

A library of small molecule inhibitors of HDACs was synthesized from 18 bifunctional biasing reagents **B1**–**B18** (Fig. 4) and 15 aldehydes **A1** – **A15** (Fig. 5) in 96-well plates yielding milligram quantities of each final product. LC–MS showed that acylhydrazones are formed as the exclusive products with over 90% purity. The DMSO solution of the reaction products in 96-well plate was directly used for subsequent screening.

Using protocols established previously,[7,10,24,25] the resulting compounds were tested in biochemical assays against HDAC2, HDAC3, and HDAC8 (Table 1). Several HDAC8-selective inhibitors **A8B4**, **A12B4**, and **A14B4** (Fig. 6) were discovered. Reagent **B4** is biased towards HDAC8 as judged by the observation that several products derived from it are selective for HDAC8 (Table 1).[13]

Biasing reagents **B1**–**B18** were also coupled with hundreds of commercially available aldehydes to generate thousands of HDAC inhibitors that showed diverse biological activities.[10,24,26]

In summary, we developed an efficient strategy for rapid assembling and in situ screening of HDAC inhibitors. Biasing

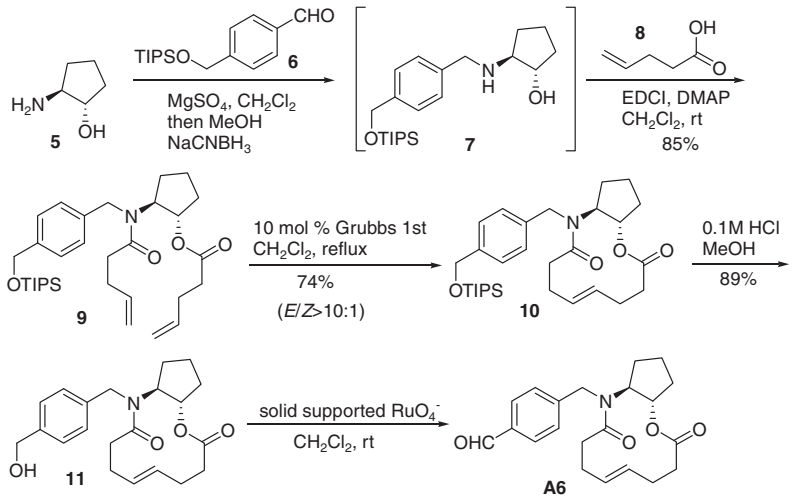

Figure 3. Short syntheses of 12-membered macrocyclic aldehydes are illustrated.

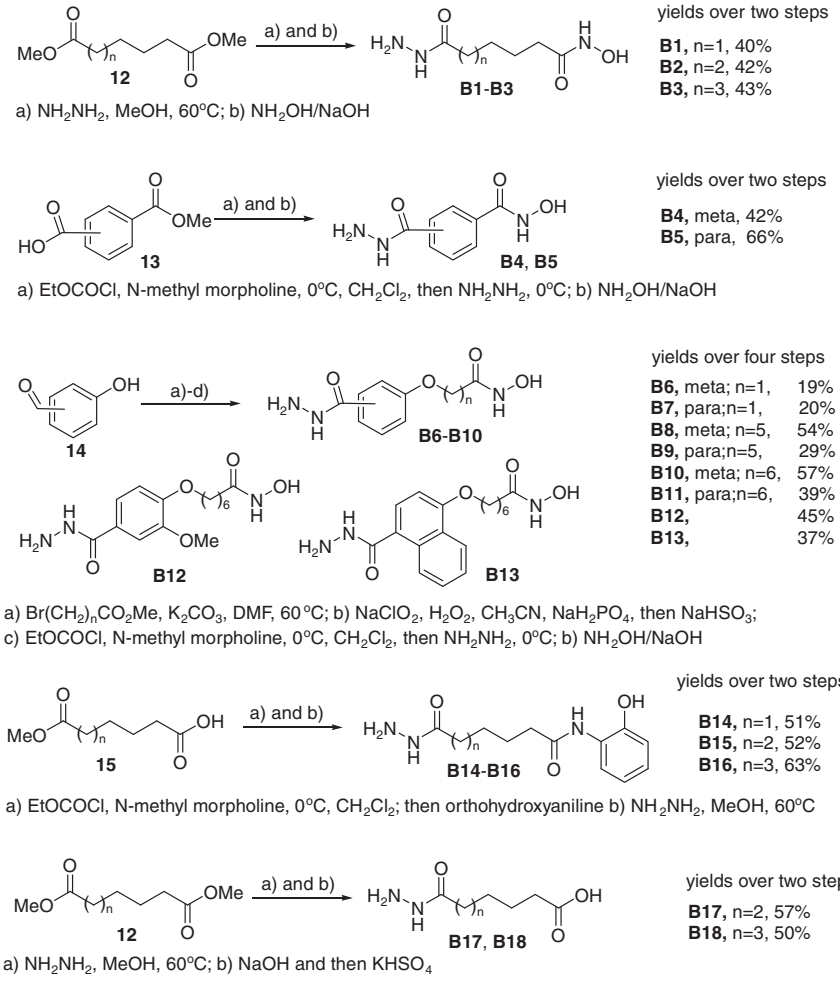

Figure 4. Synthesis of biasing reagents.

reagents **B1**–**B18** were prepared in a few steps in solution and then coupled with macrocyclic aldehydes, which were derived from corresponding primary alcohols using solid supported oxidation reagents. Simple filtration was used to remove the excess oxidation reagents. The coupling step is efficient and does not require purification since its only byproduct is water. The resulting solution from the coupling reaction can be directly used for biological assays since DMSO was used as the solvent. Selective HDAC8 inhibitors, such as **A8B4**, were identified.

Small-molecule probe or tool compounds can be used to illuminate the functions of proteins and to identify new therapeutic targets.[27] The method described here allows efficient coupling of

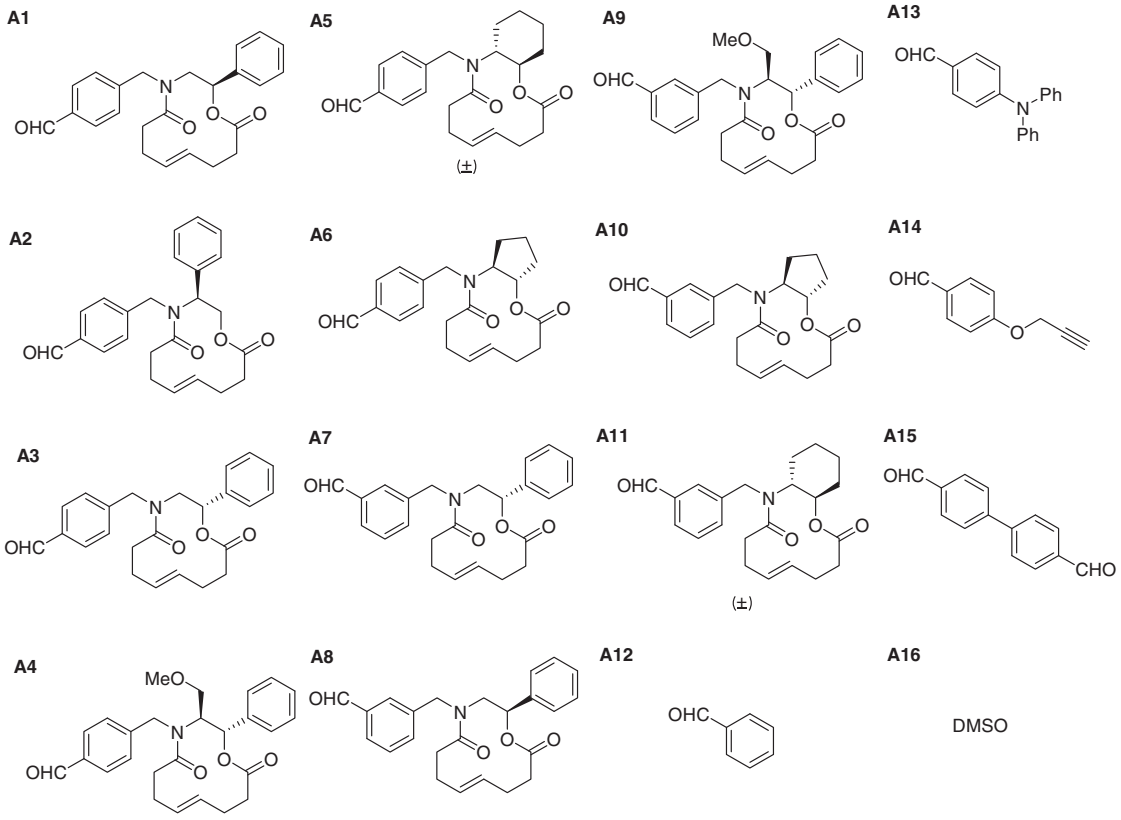

Figure 5. Aldehyde building blocks used in the pilot library.

Table 1
Discovery of HDAC8-selective inhibitors using a biochemical assay

Compound	HDAC2 (IC_{50}, μM)	HDAC3/NCoR2 (IC_{50}, μM)	HDAC8 (IC_{50}, μM)
A12B4	20	18	0.052
A14B3	0.0021	0.0031	0.29
A14B4	6.3	6.2	0.029
A8B4	3.6	15	0.023
A7B4	5	15	0.11
SAHA	0.066	0.034	1.1

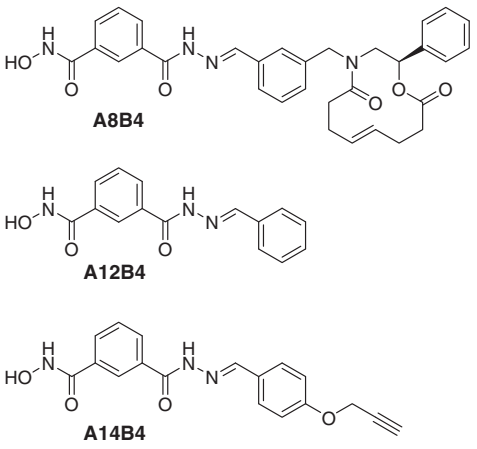

Figure 6. Structures of selected HDAC8-selective inhibitors.

structurally diverse compounds and reagents having structural features that facilitate the inhibition of HDACs. This two-step protocol is also applicable to primary alcohols derived from many other diversity-oriented syntheses since many functional groups can be tolerated under these mild conditions.

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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.2011.01.134.

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