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Design, synthesis and docking studies on benzamide derivatives as histone deacetylase inhibitors

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ABSTRACT

A series of benzamide derivatives including two scaffolds were designed and synthesized as potential histone deacetylase inhibitors. Most of synthesized compounds showed moderate enzymatic potency at the same order of magnitude, and compound **12b** possessed better potency to the positive control (3.8 μM vs 13.0 μM). It also showed a 50-fold increase in vitro anticancer activity against DU-145 cell-lines. Molecular docking studies were carried out and used to explain the structure–activity relationships observed in vitro. Then we found that the cavity surrounded by ASP104, HIS33, PRO34 and PHE155 may be crucial for the inhibitors' activity. The docking results provide some useful information for future design of more potent inhibitors.

Histone deacetylases (HDACs) and the counteracting histone acetyl transferases (HATs) play an important role in the regulation of gene expression. HDACs catalyze the deacetylation of the acetylated ε-amino groups of specific histone lysine residues,[1] and then the deacetylated histones acquire a net positive charge. Because deacetylated histones interact strongly with the negatively charged DNA, DNA becomes tightly wrapped around the nucleosome core, and then gene expression is impeded. That is to say, increased levels of histone acetylation are associated with increased transcriptional activity, whereas decreased levels of acetylation are associated with repression of gene expression.[2] Association between acetylation and gene expression has stimulated the study of HDACs on the aberrant gene expression, which is often observed in cancer. Many studies show that inhibition of HDAC elicits anticancer activities in several tumors cell lines,[3] which make HDAC an attractive target for the development of new anticancer drugs.

More than 10 HDAC inhibitors are advancing in different clinical trial phases. Currently, several HDAC inhibitors have been approved by FDA, such as SAHA and FK-228 for the treatment of cutaneous T-cell lymphoma. In addition, HDAC inhibitor's pharmacophore is characterized by three portions: a zinc-binding group (ZBG), a hydrophobic group (CAP) for protein surface recognition, and a linker between ZBG and CAP as exemplified by MS-275 (Fig. 1). MS-275 uses N-(2-aminophenyl) formamide as ZBG for the interaction with the catalytic Zn in HDACs' active site. MS-275 is a moderately potent HDAC inhibitor with micromole enzymatic activity, which is in phase II clinical trial. Other companies also pursued new potent HDAC inhibitors usually by modifying linker or CAP, and preserving the same ZBG shown in Figure 1. MGCD-0103 and CS-055 are now both in phase II, and WO2004035525-Ii is the most potent example in a patent.[4] We found that these compounds restricted their linkers' conformation using more rigid fragment compared with MS-275, which is commonly used in drug design to discover more potent and specific compound. Among them, MGCD-0103[5] has a slight improvement on in vitro activity, and CS-055[6] only retains the similar potency in vitro, but WO2004035525-Ii[4] shows reduced potency. Meanwhile, WO2004035525-Ii possesses the most rigid linker, so probably it is beneficial for inhibition that the linker makes a compromise between certain extent rigidity and flexibility.

Based on this, we integrated amide in MS-275 and CS-055, and phencarbonyl in WO2004035525-Ii into a novel linker (Fig. 1), together with other portions, to buildup a new chemical scaffold **1**. After altering amide of scaffold **1** to sulfonamide, we gained another chemical scaffold **2**.

Then the compounds **11a–e**, **12a–c** with new chemical scaffolds mentioned above were prepared as depicted in Scheme 1. The common intermediate 2-(4-(2-nitrophenylcarbamoyl)phenyl)ethanaminium chloride **8** was synthesized in four steps.

Using Phosphorus oxychloride and catalytic pyridine, compound **4** was acylated by **3** to provide **5**, followed by bromination to give **6** as yellow powder which was further aminated by methenamine to obtain white solid **7**, and then hydrolyzed in strongly acidic conditions to give the common intermediate **8**. Compound **8** was dissolved in the mixture of ethyl acetate and potassium carbonate solution, and then acylated by various acylchlorides to form compounds **9a–e** and **10a–c** which were reduced by iron powder

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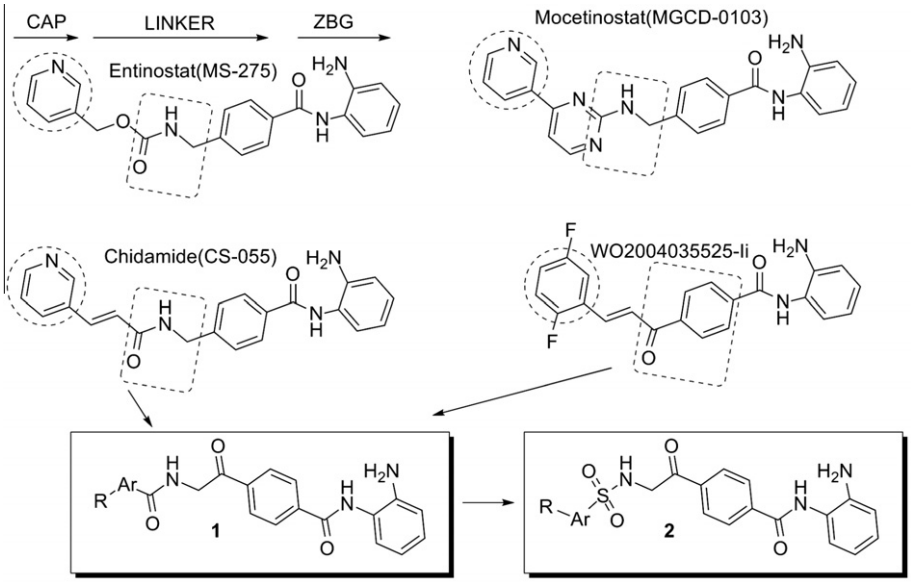
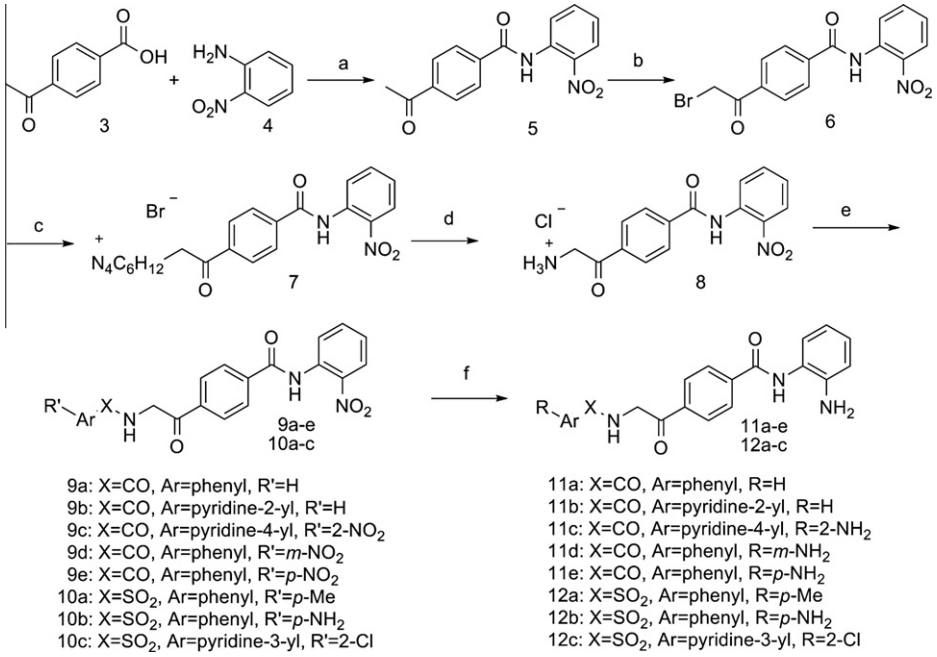


Figure 1. Known HDAC inhibitors classified as benzamides and their novel scaffolds.



Scheme 1. Reagents and conditions: $POCl_3$, Py, DCM, room temp (70%); (b) Br_2, DCM, room temp (74%); (c) $C_6H_{12}N_4$, DCM, 30 °C (93%); (d) HCl, MeOH; (e) R1ArXCl, EA, H_2O, K_2CO_3, rt −40 °C (37–86%); (f) Fe, HAc, EtOH, H_2O, 70 °C (20–75%).

in acid condition to provide target compounds **11a–e** and **12a–c,** respectively.

The synthesized compounds were tested in enzyme assay for HDAC inhibition.[7] The results are summarized in Table 1. As for chemical scaffold **1**, the first notable observation was lack of inhibition activity for compound with naked phenyl CAP (**11a**). After being substituted by amino regardless in *meta*- or *para*-position of phenyl, the analogues showed improved activity. Meanwhile, compounds containing pyridine (**11b**, **11c**) showed certain level of HDAC inhibitory effects, but compound substituted by amino showed no improvement, not behaving as compounds bearing with phenyl CAP (**11d**, **11e**). Obviously, pyridine CAP was better than phenyl CAP for HDAC inhibition, which was consistent with the trend found in MS-275 analogs.[8] As for chemical scaffold **2**, all compounds bearing sulfonamide group showed higher activity than those with scaffold **1**. Though compound **12a** is the least potent inhibitor, it has showed comparative potency to MS-275 (22.4 μM vs 13 μM). using amino group replacing *para*-methyl group in compound **12a**, compound **12b** dramatically improved its potency to 3.8 μM, which showed 6-fold increase to compound **12a,** and 4-fold increase to MS-275. Compound **12c** with halogen substituted on CAP showed only similar potency to MS-275, which resulted in a 3-fold loss of activity compared to compound **12b**. Pyridine based inhibitor Compound **12c** was 2-fold more active than compound **12a**, indicating again that pyridine CAP was better than phenyl CAP for HDAC inhibition. However it was 3-fold less active than compound **12b**, suggesting that *p*-NH_2 substituent is the most favored.