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Synthesis and biological evaluation of novel histone deacetylases inhibitors with nitric oxide releasing activity

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ABSTRACT

A novel series of histone deacetylases inhibitors (HDACIs) containing benzofuroxan pharmacophore as nitric oxide (NO) donor were designed based on the combination principle and 'multifunctional drugs' theory. As a novel study on embedding NO donor into the structure of HDACIs, all designed hybrid compounds, especially **19d** and **24d**, displayed remarkable HDACs inhibitory activity and outstanding antiproliferative activity on tumor cells. Besides, they could produce high levels of NO in HCT-116 cells; furthermore, their antiproliferative activity on HCT-116 cells could be diminished by pretreatment with hemoglobin, as the NO scavenger, in a dose-dependent manner. All in all, our designed compounds displayed great inhibitory activities and might offer a prospective avenue to discover novel anti-cancer drugs.

1. Introduction

Epigenetic changes, such as DNA methylation, histone modifications, chromatin remodeling and non-coding RNA regulation, have been recognized being associated with cancers. Histone acetylation, a dynamic and reversible process, is regulated by both histone acetyltransferases (HATs) and histone deacetylases (HDACs).[1] HATs add acetyl groups to lysine residues of histone tails to form a permissive chromatin structure allowing transcription, while HDACs, catalyzing the removal of acetyl groups from *N*-acetyl lysine residues of histone, are found in all modern eukaryotic organisms and play fundamental roles on cellular proliferation, differentiation and homeostasis.[2] HDACs family includes eighteen enzymes and they are grouped into four classes based on their homology to the respective yeast transcriptional control factor sequence. Class I (HDACs 1-3, 8), Class IIa (HDACs 4, 5, 7, 9), Class IIb (HDACs 6, 10) and Class IV (HDAC 11) are Zn^{2+} dependent enzymes,[3] while Class III HDACs (sirtuins) are NAD^{+} dependent, belonging to sirtuins family.[4] Overexpression of HDACs can arrest gene transcription which is associated with a variety of disease states including cancer, cellular metabolism disorders, and inflammation. Therefore, it is no doubt that the design and synthesis of histone deacetylases inhibitors (HDACIs) is an effective way to adjust the dysregulation of HDACs. Currently, more and more HDACIs (Fig. 1) have entered into clinical trials, among which, SAHA (**1**, Fig. 1), romidepsin (Istodax), PXD-101 (**2**, Fig. 1) and LBH589 (**3**, Fig. 1) have been approved by Food and Drug Administration (FDA) for the treatment of cancers. Co-crystals of Zn^{2+} dependent HDACs with inhibitors demonstrated a common molecular model for HDACIs: a Zn^{2+}-binding group (ZBG), such as hydroxamic acid, can bind the Zn^{2+} of the active site; a big hydrophobic cap group, which can occupy the rim of the HDAC active pocket; and a saturated or unsaturated linker can connect the ZBG and cap group[5] (Fig. 1). This special structure is the important design direction to discover new HDACIs as anti-cancer drugs.

Nitric oxide (NO) is a key signaling molecule involved in regulating the numerous physiologic and pathologic processes,[6] such as tumor cells proliferation, angiogenesis, metastasis and accelerate tumor cells apoptosis by regulating functional proteins through S-nitrosylation, nitration.[7] Recently, many synthesized NO-releasing compounds with strong cytotoxicity against human carcinoma cells in vitro and anti-cancer growth and anti-metastasis activity in vivo have been reported.[8,9] High concentration of NO could be generated not only by inducible nitric oxide synthase (iNOS), stimulated by cytokines, but also by NO donor effectively. Recently, the

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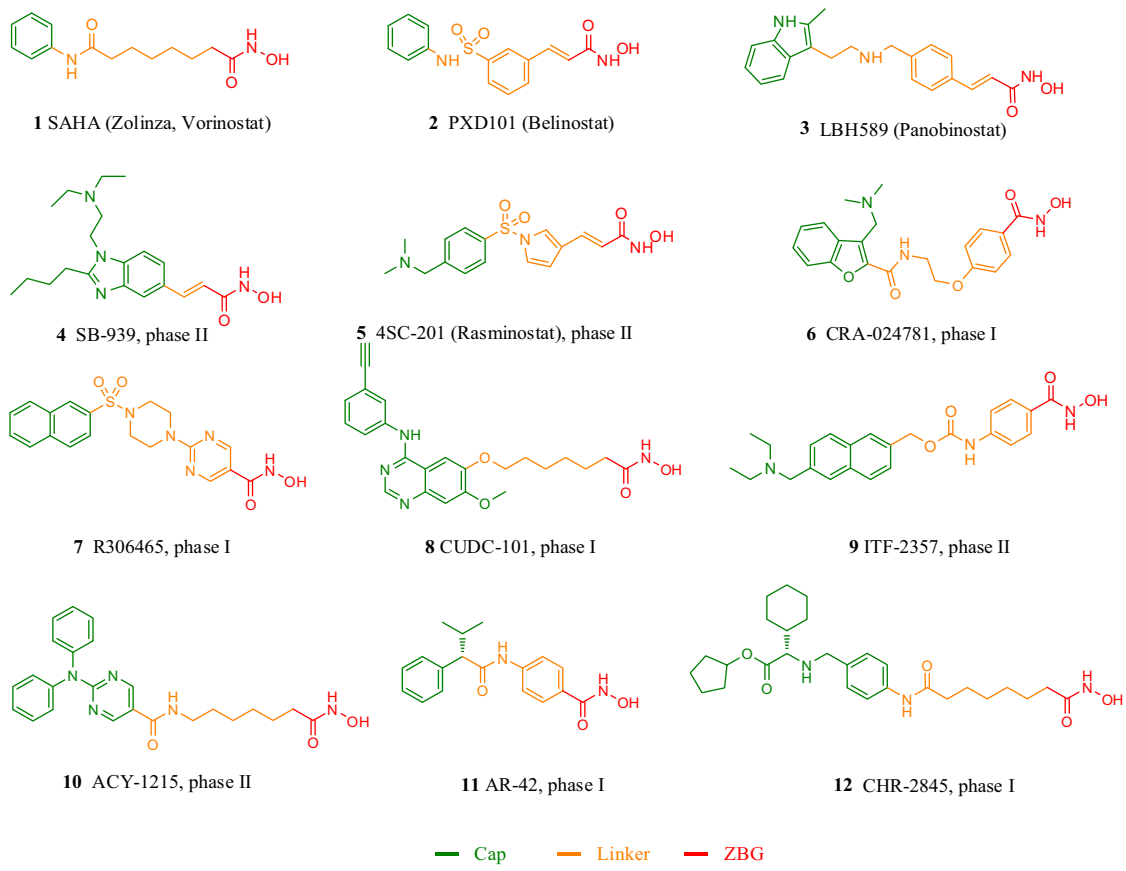

Figure 1. Pharmacophore model and structures of representative HDACIs.

oxadiazole moiety (**13**, Fig. 2) has increasingly attracted medicinal chemists' attention and been applied in many studies because it is stable against acid and base, its broad and interesting bioactivities and it can produce high levels of NO in vitro and inhibit the growth of tumors in vivo.[10] Benzofuroxan (benzofurzan oxide; benzo[1,2-c]1,2,5-oxadiazole *N*-oxide, **14**, Fig. 2) is an important motif, which was exploited previously by numerous researchers for its important biological activities, showed a wide spectrum of relevant pharmacological properties, such as antiprotozoal, antifungal and anti-platelet aggregation activities. Additionally, it has the potential for the treatment of cardiovascular diabetic complications due to its prominent NO-releasing activity.[11]

It is worth noting that several reports have displayed that NO-dependent regulation of HDACs functions.[12] NO, being a key regulator of HDAC function in mammalian neurons,[13] could regulate chromatin remodel in neuronal development[14] and skeletal muscle homeostasis[15] through S-nitrosylation of HDAC2. Furthermore, NO is also an upstream signal that controls the balance between HATs and HDACs.[16] Besides, some documents have shown that HDACIs and NO are synergistic in many diseases such as cardiac hypertrophy and wound healing.[17]

Enlightened by these findings, together with the theory of 'multifunctional drugs' and our previous studies on HDACIs equipped with phenylsulfonylfuroxan module as NO donor,[18] we thereof designed a novel series of HDACIs containing NO donor to improve their antiproliferative activities on tumor cells. In the structures of novel NO-HDACIs, benzofuroxan, as the NO donor, was designed to be the 'cap group' of HDACIs. What's more, the saturated aliphatic chains without any branched chains were designed as the 'linker' in the structures of HDACIs (Fig. 3). In this paper, we described the synthesis and relevant biological evaluation of all compounds and we hope this novel study uniting HDACIs and NO donor could provide and develop a prospective direction on the discovery of anticancer drugs.

2. Results and discussion

2.1. Chemistry

The synthetic routes of compounds **19a–19e** and **24a–24e** are outlined in Schemes 1 and 2. To synthesize the target compounds,

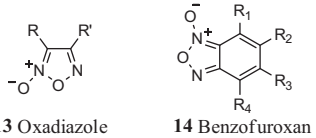

Figure 2. The chemical structure of oxadiazole and benzofuroxan.

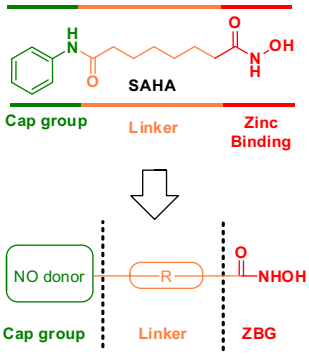

Figure 3. Design strategy of the target compounds.