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Research paper

Improving potency and metabolic stability by introducing an alkenyl linker to pyridine-based histone deacetylase inhibitors for orally available RUNX3 modulators

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ABSTRACT

RUNX3, a tumor suppressor, is suppressed in various cancers by abnormal epigenetic changes. Histone deacetylases (HDACs) can deacetylate the lysine residues of RUNX3, followed by degradation via a ubiquitin-mediated pathway. Inhibition of HDAC leads to functional restoration of the RUNX3 protein by epigenetic expression and RUNX3 protein stabilization. We previously reported a series of HDAC inhibitors that restored RUNX3 function. In the present study, we introduced an alkenyl linker group to pyridine-based HDAC inhibitors to improve their potencies and chemical properties. This alkenyl linker made the compounds more rigid, facilitating a better fit than alkyl moieties to the active site of HDAC proteins. Most compounds in this series exhibited potent RUNX activities, HDAC inhibitory activities, and inhibitory activities towards the growth of human cancer cell lines. Notably, one of these derivatives, (*E*)-3-(1-cinnamyl-2-oxo-1,2-dihydropyridin-3-yl)-*N*-hydroxyacrylamide (**7k**), showed excellent properties in a microsomal stability study, in a xenograft study, and in an *in vivo* pharmacokinetic evaluation. Modulation of RUNX3 therefore results in highly potent and orally available anticancer chemotherapeutic agents.

1. Introduction

Epigenetics is currently defined as an inheritable change without DNA sequence modifications [1]. Disorders in such changes affect a wide variety of pathologies including cancers [2,3]. There are two major mechanisms of epigenetic regulations involving DNA methylation and covalent histone modification. Histones can undergo multiple posttranslational modifications by diverse enzymes such as histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), and histone demethylases (HDMs) [4]. HATs and HDACs exert their functions within complexes that include multiple HATs, HDACs, transcription coactivators, and corepressors [5]. HDAC activities are modulated in many ways involving protein-protein interactions, post-translational modifications, subcellular localization, and the availability of metabolic cofactors [6]. Although the relationships between aberrant expression of various HDACs and cancer remain largely correlative, the altered expression of HDACs can play an important role in tumor onset and progression, so HDACs are attractive targets for the development of therapeutic agents [7]. Regarding acetylation of multiple HDAC substrates, a single pathway or multiple pathways may be involved in HDAC inhibitor (HDACi)-induced cell death. HDACi can be classified into several structural classes including hydroxamates, cyclic peptides, aliphatic acids, and benzamides [6]. Trichostatin A (TSA, Fig. 1) was the first natural product isolated and shown to inhibit HDACs [8]. SAHA, a structurally close analogue to TSA, was the first HDACi to be approved for clinical use by the Food and Drug Administration (Fig. 1) [9]. A series of hydroxamic acids has been shown to block HDACs and affect cell proliferation at nanomolar concentrations [10]. Despite the outstanding efficacy of HDACs, they have major disadvantages such as nonspecificity and resistance, which are

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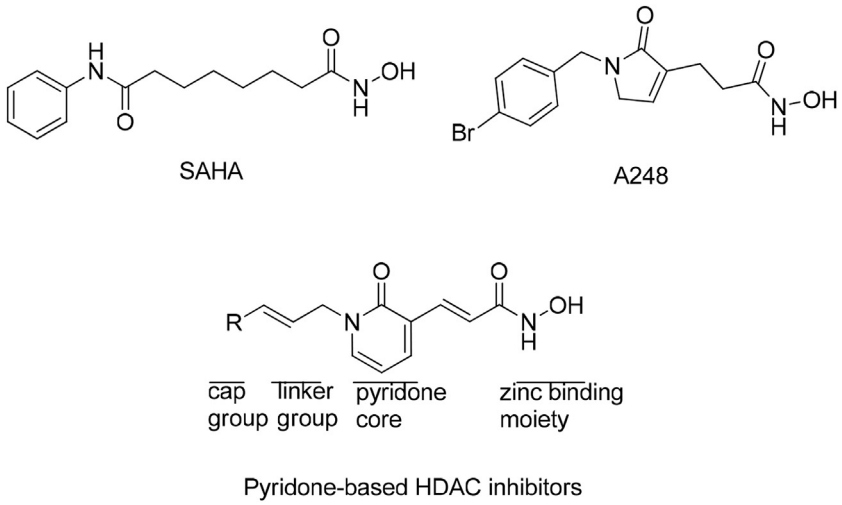

Fig. 1. Chemical structures of hydroxamic acid-based deacetylase inhibitors. A248 was reported in previous study [29].

common disadvantages in epigenetic therapies [11–13]. A new approach is therefore needed to eliminate these disadvantages.

Epigenetic alteration can lead to a variety of transcriptional effects involving the imbalance between HDACs and HATs. Tumor suppressors, such as RUNX3, are suppressed in various cancers by abnormal epigenetic changes [14,15]. All RUNX family members share the central Runt domain, which is a well-conserved domain that recognizes specific DNA sequences. RUNX1 and RUNX2 are involved in hematopoiesis and osteogenesis, respectively, and are genetically altered in leukemia and bone disease [16]. In contrast, RUNX3 is required for the control of cell proliferation of the gastric epithelium, neurogenesis of the dorsal root ganglia, and differentiation of T-cells [17]. RUNX3 interacts with p300 HAT and is acetylated at lysine residues, which is necessary for its transcriptional activity. This acetylation is reversed by HDACs that are in competition with Smurf1-mediated ubiquitination [18]. HDACs cause the deacetylation of RUNX3, followed by degradation by the ubiquitin-mediated pathway. HDAC inhibitors therefore increase the acetylation of RUNX3, and improve the stability and transcriptional activity of RUNX3 [19]. RUNX3 inactivation is usually caused by epigenetic changes, rather than rare mutations [15]. RUNX3 may therefore be an excellent target for anti-cancer therapy because it functions as a tumor suppressor and can be recovered by RUNX3-targeted agents [20].

In a previous study, we reported that the restoration and stabilization of RUNX3 levels by HDAC inhibition could be a new approach in cancer treatment [21]. Furthermore, a series of HDAC inhibitors introduced a pyridone core and an olefin moiety to improve chemical and metabolic stabilities [22]. It is probable that the olefin moiety of HDAC inhibitors is important for activity. In the present study, we designed a new series of pyridone-based HDAC inhibitors that contained an alkenyl group as a linker group to improve the potencies and efficacies as an anticancer agent. A total of 22 compounds were synthesized and tested using various biological assays. We conducted *in vitro* biological evaluations including evaluations of RUNX transcriptional activation levels and HDAC inhibitory activities, plus cancer cell growth inhibitory activities. The synthesized compounds were tested for their effects on the posttranslational acetylation of RUNX3 involving protein stabilization through epigenetic regulation, and were tested for microsomal stability to monitor their physicochemical properties. Furthermore, *in vivo* xenograft studies and pharmacokinetic profiles were performed using the screened compounds to identify orally available candidates. The high potency, stability, and *in vivo* efficacy of synthesized compounds suggested that the RUNX3 modulators can be used for cancer therapy.

2. Results, methods, and discussion

2.1. Strategies and chemistry

In a previous study, the pyridone core HDAC inhibitors with a hydroxamate conjugated system were reported to improve the chemical and metabolic stabilities of hydroxamic acid [22]. A three-dimensional quantitative structure-activity relationship (3D QSAR) and docking simulation study indicated that hydroxamic acid chelated to zinc ion in the HDAC active site passes through a narrow hydrophobic tunnel, and that the bulky aromatic cap groups are important for inhibitory activity [23]. Based on these studies, we synthesized potent and orally available RUNX3 modulators based on HDAC inhibitors (Fig. 1). We designed and synthesized a new scaffold composed of an alkenyl group on the three carbon linker group between the cap group and the pyridone core. This olefin moiety made the compounds more rigid and more stable than the alkyl moiety. Moreover, this rigidity facilitated a better fit to the HDAC active site, and the increased chemical stability protected the compounds from enzymatic hydrolysis to improve their activities and physicochemical properties as RUNX3 modulators. These compounds also contained diverse functional phenyl, bromophenyl, chlorophenyl, methoxyphenyl, naphthyl, trifluoromethylphenyl, and trifluoromethoxyphenyl groups as the cap groups that were evaluated in structure-activity relationship studies (SARs).

The general procedures for the synthesis of pyridone-based RUNX3 modulators are outlined in Scheme 1. Based on the previous SAR study, more alkyl linker and new alkenyl linker derivatives were designed and synthesized [22]. Scheme 1 shows the procedure for the olefin linker (between the cap group and the pyridone core) in the pyridone-based derivatives that were used to produce a new series of RUNX3 modulators. Commercially available carbaldehyde and Wittig reagent were refluxed in dichloromethane to produce the Wittig products, **1**. Compounds **3** and **6** were produced by an N-alkylation reaction of compound **1** [(*E*)-methyl 3-(2-oxo-1,2-dihydropyridin-3-yl) acrylate] with synthetic or commercial aromatic alkyl halides **2** and **5,** respectively. The hydroxamic acid analogues, **4** and **7,** were obtained by reacting the esters of the pyridones with $KONH_2$ (1.7 M in MeOH) in MeOH at low temperature.