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Search for novel histone deacetylase inhibitors. Part II: Design and synthesis of novel isoferulic acid derivatives

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ABSTRACT

Previously, we described the discovery of potent ferulic acid-based histone deacetylase inhibitors (HDACIs) with halogeno-acetanilide as novel surface recognition moiety (SRM). In order to improve the affinity and activity of these HDACIs, twenty seven isoferulic acid derivatives were described herein. The majority of title compounds displayed potent HDAC inhibitory activity. In particular, **IF5** and **IF6** exhibited significant enzymatic inhibitory activities, with IC_{50} values of 0.73 ± 0.08 and 0.57 ± 0.16 μM, respectively. Furthermore, these compounds showed moderate antiproliferative activity against human cancer cells. Especially, **IF6** displayed promising profile as an antitumor candidate with IC_{50} value of 3.91 ± 0.97 μM against HeLa cells. The results indicated that these isoferulic acid derivatives could serve as promising lead compounds for further optimization.

1. Introduction

Histone deacetylase (HDAC) inhibition is a clinically validated therapeutic strategy for cancer treatment. HDAC are involved in remodelling of chromatin and play an essential role in cell proliferation, cell-cycle regulation and apoptosis.[1] An aberrant activity of HDACs has been documented in several human cancers leading to development of histone deacetylase inhibitors (HDACIs) as anticancer agents.[2] Therefore, HDAC is becoming a prominent therapeutic target for the treatment of cancer. Research of HDACIs is becoming an interesting field in anticancer agent design.

Classical HDACs are zinc-dependent enzymes bearing a highly conserved catalytic domain with a zinc ion.[3] So far, a wide range of natural and synthetic derivatives have been identified as potent HDACIs. They are structurally diverse group compounds with attractive antitumor properties. Majority of them consists of a zinc binding group (ZBG) interacting with the zinc ion. Moreover, HDACIs share other common features such as a linker domain, which occupies the narrow channel; a connect unit (CU), which connects SRM and linker; and a surface recognition moiety (SRM), which interacts with residues on the rim of active site (Fig. 1).

Vorinostat (SAHA) is the first HDACI approved by the FDA for the treatment of CTCL in 2006. Belinostat has been granted orphan drug and fast track designation by the FDA. Panobinostat developed by Novartis for the treatment of various cancers is a non-selective HDACI. Entinostat is an oral benzamide HDACI undergoing clinical trials for the treatment of various cancer.[4]

In the course of search for novel HDACIs with novel structure and higher potency, we have developed several ferulic acid derivatives with potent HDAC inhibitory activity and antiproliferative property.[5] It was validated that phenyl-substituted ethylene could serve as rigid linker of HDACIs. Moreover, halogeno-acetanilides were also confirmed to be suitable as SRM of HDACIs. However, in the molecular docking study of ferulic acid derivatives with HDAC, we found that acetanilide did not form any hydrogen binding with SRM as we initially supposed.

On the basis of these observations, we aimed to enhance the structural diversity and affinity with the SRM of HDAC. Rearrangement of acetanilide to *meta*-position was performed to afford corresponding isoferulic acid derivatives. In this study, ferulic acid was replaced by isoferulic acid as rigid linker of HDACIs. The halogeno-acetanilide was incorporated at *meta*-position instead of *para*-position. We supposed that *para*-position might be more suitable for interaction with SRM. These novel HDACIs comprised common hydroxamic acid or 2-aminobenzamide group as ZBG as previously reported (Fig. 2). The conformations of these compounds were more similar to that of SAHA.

As part of our ongoing effort to develop HDACIs with higher affinity and activity, we developed two series of isoferulic acid derivatives bearing halogeno-acetanilide at *meta*-position. The structures of these compounds are quite consistent with common pharmacophore of HDACIs. The binding mode of the most potent

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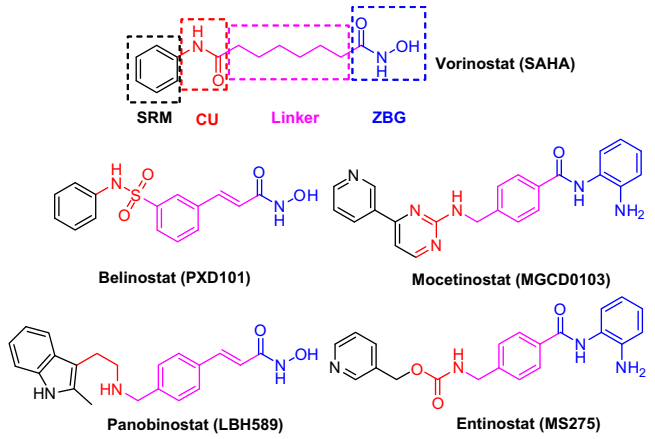


Figure 1. Structures and pharmacophore features of HDACIs.

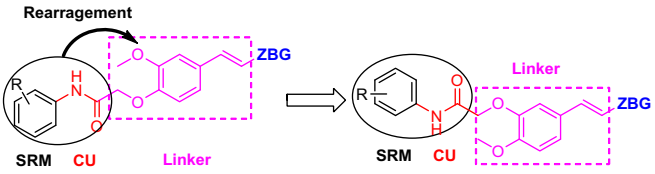


Figure 2. Design strategy and structures of isoferulic acid derivatives.

compound with HDAC was also established in order to analysis the interaction of acetanilide with SRM. Herein, we describe the discovery and evaluation of isoferulic acid-based HDACIs.

2. Chemistry

The synthetic routes of the title compounds were outlined in Schemes 1 and 2. An efficient synthesis of hydroxamic acids was developed in 5-step reaction sequence (Scheme 1). Isoferulic acid **2** was prepared from isovanillin **1** utilizing classical Knoevenagel condensation reaction conditions.[6] Isoferulic acid was esterified in the presence of concentrated H_2SO_4 to afford isoferulic acid methylester **3**. The hydroxyl group at *meta*-position of (**2**) was etherified with various substituted acetanilides in anhydrous acetone in the presence of K_2CO_3 to afford corresponding intermediates **4a–4i**.[7] These resulting esters **4a–4i** were treated with methanolic NH_2OK at room temperature to yield corresponding hydroxamic acid derivatives **IF1–IF9**.[8]

Here again, we have developed a new two-step procedure for preparation of isoferulic acid derivatives bearing 2-aminobenzamide as ZBG (Scheme 2). Isoferulic acid was converted into corresponding imidazolide derivative by reaction with *N*,*N*′-carbonyldiimidazole (CDI) in THF at room temperature. This was further reacted with benzene-1,2-diamine or 4-methylbenzene-1,2-diamine in the presence of trifluoroacetic acid to afford key intermediates **5a–5b**.[9] The hydroxyl group in **5a–5b** was etherified with haloacylanilines in anhydrous acetone in the presence of K_2CO_3 to afford corresponding 2-aminobenzamide-containing derivatives **IF10–IF27**.[10]

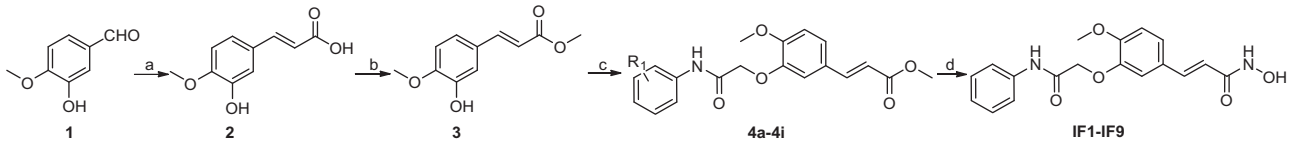
3. Results and discussion

All the synthesized compounds were tested for their HDAC inhibitory activity with SAHA as positive control. It was indicated from Table 1 that majority of them displayed moderate to high inhibitory activity against HDAC. Some of the title compounds were shown to inhibit HDAC with IC_{50} values below the micromolar range. In general, hydroxamic acids were found to be more potent than benzamides. **IF6** was the most potent HDAC inhibitor with an IC_{50} value of 0.57 ± 0.16 μM, while **IF4** and **IF5** also displayed potent HDAC inhibitory activities with IC_{50} values of 0.94 ± 0.26 μM and 0.73 ± 0.08 μM, respectively. The variety and position of halogen-substitution on aniline played important role in biological activity. Benzamides **IF15** and **IF11** were active at higher doses with IC_{50} values of 15.60 ± 3.28 μM and 22.16 ± 2.37 μM, respectively. In contrast, six benzamide derivatives were inactive at 200 μM. According to the results, both substitution on aniline and structure of ZBG played important role in potency. It was indicated that compounds bearing substituents like fluorine, bromine and trifluoromethyl on aniline possessed potent anticancer activity. For ZBG, hydroxamic acid was more suitable for these HDACIs than benzamide.

To further investigate their antiproliferative activity, six compounds (five of hydroxamic acids and one of benzamide) were selected to test for their antiproliferative potential by MTT method. The test compounds were evaluated for their anticancer potency against HeLa and MDA-MB-231 cell lines. The results were described in Table 2. It was found that they displayed potent antiproliferative activities with IC_{50} values ranging from 1.90 ± 0.04 μM to 20.51 ± 1.36 μM. Some of them exhibited promising antiproliferative activity against MDA-MB-231. **IF3** displayed the most potent antiproliferative potency against MDA-MB-231 comparable with SAHA. Moreover, **IF6** exhibited the most potent growth inhibition against HeLa cell line. The antiproliferative results were consistent with the HDAC inhibitory assays. It was indicated that inhibition of HDAC might be one of the basis for anticancer activity.

The most potent inhibitor **IF6** was selected for computational studies. Docking studies were carried out to understand interaction between inhibitors and HDAC. **IF6** was docked into active site of HDAC (PDB ID: 3F07) by SYBYL-X 2.0. Molecular insights based on molecular docking indicated favorable binding mode of **IF6** with HDAC (Fig. 3). The results suggested that hydroxamic group was bonded to zinc ion as ZBG. Hydroxamate OH made two hydrogen bond interactions with His142 and His143 with distance of 1.96 Å and 2.41 Å, respectively. N–H could also form a hydrogen bond to His143 with distance of 1.79 Å. Carbonyl group accepted a hydrogen bond from Tyr306 with distance of 1.75 Å. The distance between zinc ion and two oxygen atoms were 1.89 Å and 2.17 Å, respectively.[11] Based on these results, the binding mode of isoferulic acid-based HDACI was the same as ferulic acid-based HDACI.

However, the direction of binding with surface of active site was totally difference (Fig. 4). Moreover, there was an additional hydrogen bond between oxygen atom of acetyl and Gly151 on the surface. This interaction might contribute to affinity and activity of isoferulic



Scheme 1. Preparation of compounds **IF1–IF9**. Reagents and conditions: (a) malonic acid, DBU, pyridine; (b) CH_3OH, H_2SO_4; (c) K_2CO_3, acetone; (d) NH_2OK, NH_2OH, DMF.