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Synthesis and biological evaluation of largazole analogues with modified surface recognition cap groups

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ABSTRACT

Several largazole analogues with modified surface recognition cap groups were synthesized and their HDAC inhibitory activities were determined. The C7-epimer **12** caused negligible inhibition of HDAC activity, failed to induce global histone 3 (H3) acetylation in the HCT116 colorectal cancer cell line and demonstrated minimal effect on growth. Although previous studies have shown some degree of tolerance of structural changes at C7 position of largazole, these data show the negative effect of conformational change accompanying change of configuration at this position. Similarly, analogue **16a** with D-1-naphthylmethyl side chain at C2 too had negligible inhibition of HDAC activity, failed to induce global histone 3 (H3) acetylation in the HCT116 colorectal cancer cell line and demonstrated minimal effect on growth. In contrast, the L-allyl analogue **16b** and the L-1-naphthylmethyl analogue **16c** were potent HDAC inhibitors, showing robust induction of global H3 acetylation and significant effect on cell growth. The data suggest that even bulky substituents are tolerated at this position, provided the stereochemistry at C2 is retained. With bulky substituents, inversion of configuration at C2 results in loss of inhibitory activity. The activity profiles of **16b** and **16c** on Class I HDAC1 vs Class II HDAC6 are similar to those of largazole and, taken together with x-ray crystallography information of HDAC8-largazole complex, may suggest that the C2 position of largazole is not a suitable target for structural optimization to achieve isoform selectivity. The results of these studies may guide the synthesis of more potent and selective HDAC inhibitors.

1. Introduction

Aberrant epigenetic silencing of tumor suppressor genes can be reversed; thus targeting the enzymes responsible for these modifications has become an attractive therapeutic approach in cancer [1]. Mechanisms of epigenetic silencing include DNA methylation and modification of key residues of histone tails. Lysine residues of histone tails can be acetylated/deacetylated, which can alter gene expression [2]. Histone acetylation status is regulated by two enzymes, histone acetyl transferases (HAT), which acetylates lysine tails of histone proteins and histone deacetylases (HDAC), which deacetylate them [3]. FDA approval of two HDAC inhibitors (HDACi) suberoylanilide hydroxamic acid (**1**, SAHA, vorinostat) [4] and FK228 (**2**, romidepsin) [4] (Fig. 1) for the treatment of cutaneous T-cell lymphoma (CTCL) has stimulated extensive research to find more potent and isoform selective HDACis which, in addition to their therapeutic potential, will also be useful as probes to determine the physiological/pathogenic role of individual HDAC isoforms [5]. Three major structural motifs characteristic of a histone deacetylase inhibitor are: i) a metal binding domain which chelates with the active site Zn^{2+} cation ii) a linker, which occupies the hydrophobic channel iii) a surface recognition cap group, which interacts with hydrophobic residues on the rim of the active site [6]. All these three sectors may be targeted for structural alteration to achieve HDAC class and/isoform selectivity. The 14 Å internal cavity

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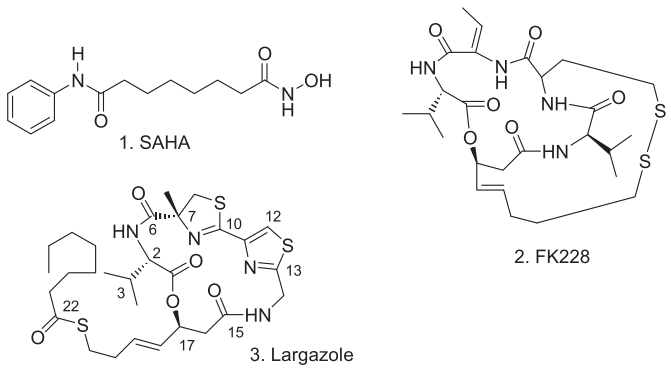

Fig. 1. HDAC inhibitors.

at the bottom of the active site [7–14] and the hydrophobic rim at the entrance to the active site [15] are being specially targeted for developing isoform selective HDAC inhibitors by exploiting variations in the amino acid residues in these two regions.

Largazole **3** (Fig. 1), a potent and selective HDACi recently isolated from a marine cyanobacterium, has attracted the attention of medicinal chemists as an important lead molecule for chemical modification to develop anticancer agents [16]. As HDACs proteins are associated with many basic cellular processes and aberrant HDAC activity is also linked to human disorders other than cancer, the effect of largazole on other disease states such as inflammation and rheumatoid arthritis too are being explored [17–19]. A number of largazole analogues have been synthesized and their HDAC inhibitory activities determined revealing some of the structure–activity relationship (SAR) requirements of the molecule [20–39]. Changes in all three sectors of the molecule have been effected. We recently reported the synthesis and biological activity of several largazole analogues incorporating multiple heteroatoms in the metal binding domain [39]. In continuation of our efforts in the search for selective and potent anticancer agents [39–43], we report here the synthesis and biological activity of several largazole analogues with modifications in the depsipeptide ring, which serves as the cap group that interacts with the hydrophobic rim of the active site. These modifications include inversion of stereochemistry at C7 of largazole to generate the C7-epimer **12** as well as structural and stereochemical changes in the valine side chain at C2 to generate analogues **16a**–**c**.

2. Chemistry

The synthetic strategy we previously employed in the assembly of largazole [39] was conveniently adopted in the synthesis of these analogues. Using (*S*)-α-methylcysteine HCl (**6**), instead of (*R*)-α-methylcysteine HCl used in largazole synthesis, gave the C7-epimer **12** (Scheme 1). (*S*)-α-Methylcysteine HCl **6** formed as a product during the synthesis of (*R*)-α-methylcysteine HCl reported earlier was used in this synthesis [39]. The nitrile **5** [39] was condensed with (*S*)-α-methylcysteine **6** to obtain the thiazole-thiazoline carboxylic acid **7** [22]. After conversion to methyl ester and removal of Boc protecting group, the acyl group was transferred [44] from **8** to obtain the alcohol **9**. Compound **8** was synthesized by stereoselective aldol reaction as previously reported [39]. The conversion of the nitrile **5** to the carboxylic acid **7** and the one-pot conversion of the latter to alcohol **9** gave 67% yield for 3 steps. Yamaguchi esterification [20,45] of **9** with Fmoc-valine afforded the acyclic precursor **10** in 93% yield. After removal of protecting groups, macrocyclization with HOAt, HATU, and Hunig's base yielded the cyclized product **11** in 31% yield over the 3 steps [20]. The cyclized core **11**, upon removal of trityl group and thioesterification with octanoyl chloride, produced analogue **12** in 72% overall yield for two steps [21].

Synthesis of analogues with C2 side chain modification is shown in Scheme 2. Yamaguchi coupling of alcohol **13**, the synthesis of which was reported earlier [39], with Fmoc-protected amino acids D-1-naphthylmethylglycine, L-allylglycine and L-1-naphthylmethylglycine produced the acyclic precursors **14a**–**c**, respectively. Macrocyclization, after removal of protecting groups, gave the cyclic cores **15a**–**c** which were converted to analogues **16a**–**c** as described earlier [39].

3. Biological evaluation

The effects of these compounds on cytoproliferation were measured in the colorectal cancer cell line HCT116 using a standard 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) reduction assay (Promega) as previously reported [46]. Cells were treated with compounds **12**, **16a**, **16b**, **16c** and largazole as a positive control for 96 h and cytoproliferation was quantified. Varying effects on cell growth were observed indicating these structural modifications have gross differences on activities from the parent compound (Fig. 2). Compounds **16b** and **16c** showed the greatest effect with $GI_{50's}$ of 10 nM and 56 nM, respectively. No significant effect on cytoproliferation was observed for compounds **16b** and **16c** at lower concentrations (0.01, 0.1 and 1.0 nM) (data not shown). Additionally, analogues **12** and **16a** demonstrated little effect on cell growth even at the highest concentration tested 5 μM.

Analogues were also evaluated for their in vitro effect on HDAC activity measured by a fluorimetric assay using either recombinant HDAC1 or HDAC6 with a substrate specific for class I and class II HDAC activity respectively (Fig. 3). Largazole showed the greatest inhibition of the recombinant enzymes, but both analogues **16b** and **16c** showed significant inhibition of HDAC1. These results were similar to what was observed with the cytoproliferation assay, with both these analogues showing significant activity, but slightly less than the parent compound. Largazole, as well as **16b** and **16c** showed more than 90% inhibition of HDAC1 activity at 1 μM. Interestingly, there was a dramatic reduction in their inhibitory activity on HDAC1 at 0.1 μM, with both largazole and **16c** inhibiting HDAC1 activity by only about 15% while **16b** had hardly any effect. Analogues **16a** and **12** inhibited HDAC1 activity only by about 15% or less at 1 μM. Importantly, both **16b (~55%)** and **16c** (~50%) inhibited HDAC6 to a lesser extent than largazole (~75%) at 5 μM, suggesting somewhat greater selectivity of the newer analogues for HDAC1 as compared to largazole. However, at more moderate concentration of 1 uM, HDAC6 inhibitory activity became comparable for all three compounds (~50%). Analogue **16a** showed the least amount of HDAC6 specific inhibition, but was inactive in the HCT116 cell growth inhibition assay as well. These data suggest that given the lack of activity against HDAC6, these structural modifications have no effect on the ability of largazole to inhibit class II HDACs like HDAC6.

To determine how these compounds affected cellular HDAC activity in vitro, the colorectal cancer cell line HCT116 was treated with increasing concentrations of each of the compounds for 24 h. Global histone H3 lysine 9 (H3K9) acetylation was assayed as an indicator of the efficacy of these compounds in global HDAC inhibition. Consistent with what was observed with the in vitro enzyme assays, analogues **12** and **16a** produced very little induction of global H3K9 acetylation. Analogue **12** showed a slight increase of global H3K9 acetylation at high concentration, which corresponds to the results of the cell growth assay. Of the analogues synthesized,