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Design, synthesis, and biological evaluation of largazole derivatives: alteration of the zinc-binding domain

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ABSTRACT

A new series of largazole analogues in which the side chain was replaced with disulfide groups were synthesized, and their biological activities were evaluated. Compound **8** bearing an octyl moiety showed much better selectivity for HDAC1 over HDAC7 than largazole (320-fold). Structure–activity relationships suggested that the length in the disulfide chain of largazole is important for the selectivity toward HDAC1 over HDAC7.

1. Introduction

Histone deacetylases (HDACs) are a family of enzymes that control gene transcription via regulation of lysine acetylation in chromatin.[1] The 18 HDACs are classified into four groups on the basis of their size, cellular localization, number of catalytic active sites, and sequence homology to yeast HDAC proteins: class I, HDAC1, -2, -3, and -8; class IIa, HDAC4, -5, -7, and -9; class IIb, HDAC6 and -10; class III, sirtuin proteins; class IV, HDAC11.[2] The HDAC proteins are associated with basic cellular functions and disease states such as cancer, but the importance of individual isoforms cancer growth and inhibition is not well understood. Class I isoforms are over expressed in solid and hematological tumors, highly correlating with a worse prognosis,[3–7] but not resting endothelial cells and normal organs. Therefore, selective inhibition of class I isoforms is currently a major area of research in cancer chemotherapy.[8]

Recently, two HDAC inhibitors (HDACis) suberoylanilide hydroxamic acid (SAHA, vorinostat) and FK228 (romidepsin) have recently been licensed for the treatment of cutaneous T-cell lymphoma (CTCL). More than 11 new HDACis are at different stages of clinical development for therapy of multiple cancer types.[9] However, most pan-HDAC inhibitors exhibited side effects that might limit their clinical potential.[10] Therefore, looking for new HDAC inhibitors that are specific-inhibition to one kind HDAC family is extremely urgent and necessary.

Largazole is a natural macrocyclic depsipeptide isolated from the cyanobacterium *Symploca* sp. by Luesch and co-workers, which show promising HDAC1 inhibitory activity and selectivity (Fig. 1).[11] These excellent properties of largazole have attracted several research groups to complete its total synthesis and structure–activity relationship (SAR) studies.[12–28] Mechanistic studies showed that largazole is a prodrug that releases a free thiol function group of 3-hydroxy-7-mercapto-4-heptenoic acid side chain through hydrolysis by cellular esterases or lipases.[12,13] The free thiol function group could chelate with the Zn^{2+} cation present at the active site. Up to now, only two groups reported the results through modification of thiol function of largazole.[23,27] On the basis of their results, we envisioned that structural modifications in the zinc-binding domain of largazole may modulate metal-binding affinity and develop isoform-selective inhibitors, which could help in

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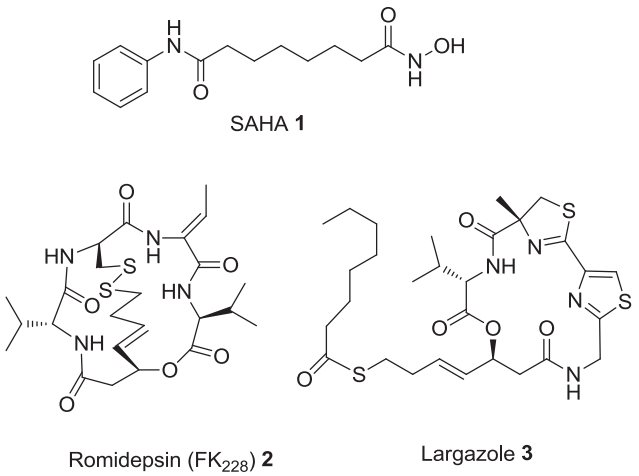

Fig. 1. Structures of SAHA, romidepsin (FK228), and largazole.

understanding the role of different isoforms in disease states. Herein, we report our efforts to modify the metal-binding domain of largazole with the goal of increasing selectivity for HDAC1 over other isoforms (Fig. 2).

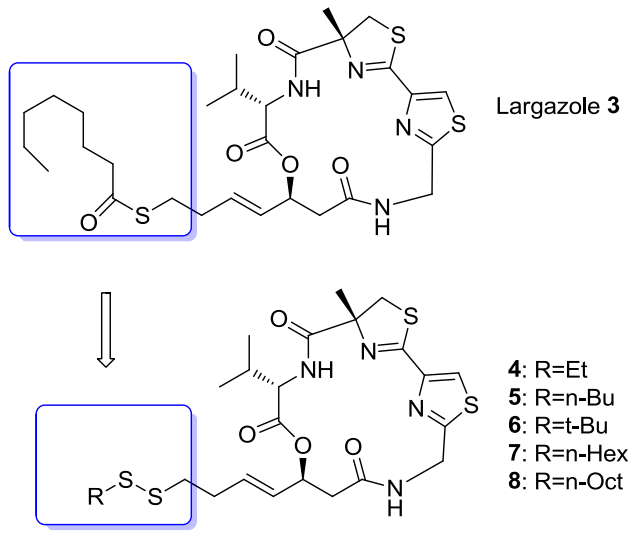

Fig. 2. Design of new analogues of largazole targeted to Zn^{2+} binding motif.

2. Results and discussion

The synthesis of key intermediates **20**–**24** was shown in Scheme 1. Hexan-1-ol **9** and octan-1-ol **10** were masked with tosylates **11** and **12**, which further reacted smoothly with TrtSH at room temperature in the presence of potassium *tert*-butoxide in THF, giving compounds **13** and **14** in parallel in 84–85% yield. The ester group of **13** and **14** were oxidized with I_2 to establish the disulfide bridge, which afforded intermediates **15** and **16**. Subsequent reaction of the disulfides **15**–**19** with *m*-CPBA generated sulfinothioates **20**–**24** in quantitative yields.[29–31]

The syntheses of the designed analogues **4**–**8** were illustrated in Scheme 2. Compound **26** was obtained from a commercially available (−) malic acid **25** by following the well-established procedures.[22] Coupling of the alcohol **26** with enantiomerically pure amino acid Fmoc-L-valine in the presence of EDCI and HOAt at room temperature gave compound **27**. Removal of the Fmoc group and HATU-mediated coupling to the thiazoline-thiazole carboxylic acid **29** gave the cyclization precursor **30**. TFA-mediated removal of the Boc and 2-(trimethylsilyl) ethanol groups, and subsequent macrolactamization with the use of HATU/HOAt/DIPEA in anhydrous DCM provided **31** in 50% yield (two steps).[32,33] Deprotection of trityl group furnished the key intermediate **32** in 90% yield in the presence of TFA. Treatment of compound **32** with sulfinothioates **20**–**24** in different combinations in the presence of base at room temperature gave the lagazole's analogues **4**–**8** in high yields.

We evaluated biochemical activities of five largazole analogues **4**–**8** against HDACs 1 and 7 using largazole as the positive control. The results were outlined in Table 1 and exhibited the interesting HDACs isoform selectivity. Largazole showed weak selectivity for the HDAC1 (IC_{50}=0.124 μM) over the HDAC7 (IC_{50}=0.169 μM). For compound **4**, the selectivity between HDAC1 and HDAC7 is 961 fold more potent than that of largazole, in which the octanethioate side chain of largazole may interact with the hydrophobic pocket in the cap of HDAC1, which could increase the selectivity between HDAC1 and HDAC7. Therefore, introducing a more hydrophobic residue in the side chain part of largazole would be able to increase isoform selectivity. The inhibition of compound **5** to HDAC1 increased greatly, the selectivity between HDAC1 and HDAC7 is much better than that of largazole. To evaluate whether the substitute group on the side chain part influences activity or not, compound **6** was designed and synthesized, in which *tert*-butyl group in the side chain. Compared with **5**, the resulting compound **6** showed much less selectivity for HDAC1 over HDAC7. To our delight, compound **8** with octyldisulfide side chain had great binding affinity against HDAC1 (IC_{50}=0.009 μM) and showed much better selectivity for HDAC1 over HDAC7 to that of largazole (320-fold), which may be beneficial for future development of specific therapeutic agents.

The antiproliferative activities of largazole and its analogues **4**–**9** were evaluated against human tumor cell lines, such as Molt-4 (Human acute lymphoblastic leukemia cell line), U937 (human leukemic monocyte lymphoma cell line), MCF-7 (human breast adenocarcinoma cell line), and BGC823 (human stomach cancer cell line), with SAHA as the positive control. The results are summarized in Table 2 and all analogues **4**–**8** show very good GI_{50} values against Molt-4, U937, and BGC823 cancer cell lines, and are more active than SAHA. It is remarkable that compounds **7** and **8**, especially in view of its higher HDAC1 activities, have much better anti-cancer activities than that of other analogues **4**–**6** against Molt-4 and U937 cancer cell lines.

3. Conclusion

In summary, a series of new largazole analogues **4**–**8** have been designed on the basis of alteration of metal-binding domains, and synthesized in high yields. Biological results showed that alteration of octanethioate side chain of largazole had various effects to the selectivity toward HDAC1 over HDAC7. To our delight, compound **8** was identified to show much higher selectivity against HDAC1 over HDAC7 in comparison with largazole. These results clearly indicated that modification of metal-binding domains may be the way forward to the development of isoform-selective HDAC inhibitors.

4. Experimental section

4.1. General

^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance ARX-400 or 500 MHz. Mass spectra were performed on Kompact Axima-CFR MALDI mass spectrometers. Optical rotations were recorded on a Perkin Elmer 341 polarimeter. Anhydrous solvents