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Synthesis and Activity of Largazole Analogues with Linker and Macrocycle Modification

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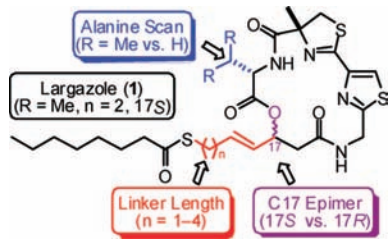
ABSTRACT



To characterize largazole's structural requirements for histone deacetylase (HDAC) inhibitory and antiproliferative activities, a series of analogues with modifications to the side chain or 16-membered macrocycle were prepared and biologically evaluated. Structure−activity relationships suggested that the four-atom linker between the macrocycle and octanoyl group in the side chain and the (*S*)-configuration at the C17 position are critical to repression of HDAC activity. However, the valine residue in the macrocycle can be replaced with alanine without significant loss of activity.

Largazole (**1**, Figure 1) is a cyclic depsipeptide recently isolated from a marine cyanobacterium.[1] It consists of an unusual 16-membered macrocycle incorporating a 4-methylthiazoline linearly fused to a thiazole and an ester of a 3-hydroxy-7-mercaptohept-4-enoic acid unit, part of which constitutes the side chain which has been shown to be essential for the potent histone deacetylase (HDAC) inhibitory and consequently antiproliferative activities.[2] Largazole (**1**), like other inhibitors acting via HDAC inhibition, shows promising selective activity against cancer cells. The biological activity combined with the intriguing yet chemically tractable structure warrants further investigation into the therapeutic potential of **1** or its next-generation analogues. Recently, we reported the first total synthesis of **1** and identified histone deacetylases (HDACs) as molecular targets.[2,3] In addition, we demonstrated that the thiol group generated by hydrolysis of the thioester moiety is the warhead and that **1** exhibits a pronounced selectivity for HDAC class I inhibition, which is desirable for anticancer drug development.[2] Herein, we describe the synthesis and structure−activity relationship (SAR) study of key analogues of **1** to characterize structural requirements for its HDAC inhibitory and antiproliferative activities.

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(1) Taori, K.; Paul, V. J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 1806–1807.

(2) Ying, Y.; Taori, K.; Kim, H.; Hong, J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 8455–8459.

(3) During review of this manuscript, four other syntheses have appeared in the literature confirming our biological data reported in ref 2, see: (a) Nasveschuk, C. G.; Ungermannova, D.; Liu, X.; Phillips, A. J. *Org. Lett.* **2008**, *10*, 3595−3598. (b) Seiser, T.; Kamena, F.; Cramer, N. *Angew. Chem., Int. Ed.* **2008**, in press, DOI: 10.1002/anie.200802043. (c) Bowers, A.; West, N.; Taunton, J.; Schreiber, S. L.; Bradner, J. E.; Williams, R. M. *J. Am. Chem. Soc.* **2008**, *130*, 11219−11222. (d) Ghosh, A. K.; Kulkarni, S. *Org. Lett.* **2008**, *10*, 3907−3910.

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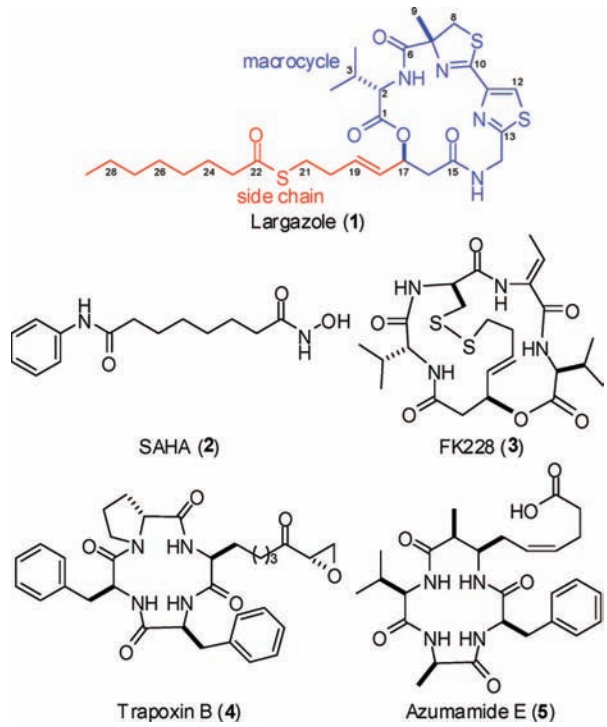
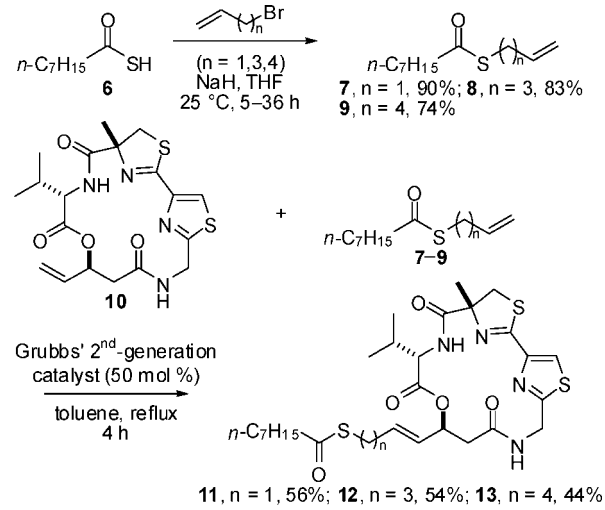


Figure 1. Structure of largazole and representative HDAC inhibitors.

Efficient HDAC inhibitors, including SAHA (**2**),[4] FK228 (**3**),[5] trapoxin B (**4**),[6] and azumamide E (**5**)[7] (Figure 1), should have three features: (1) a hydrophobic region that binds the rim of the active site, (2) a coordinating group (warhead) to chelate Zn^{2+} at the bottom of the channel, and (3) a five- to seven-atom linker from the hydrophobic region to the coordinating group. In efforts to provide insights into the structural requirements for inhibition of HDAC activity of **1**, two areas within the structure of **1** were explored (Figure 1). First, the length of the linker region was altered to determine the effect of chain length on HDAC inhibitory activity. Second, the 16-membered macrocycle was modified to explore the interactions of the macrocycle backbone with the rim of the active site.

The side chain of HDAC inhibitors has a five- to seven-atom linker from the hydrophobic region to the coordinating group which has multiple contacts with hydrophobic residues in the 11 Å deep channel. Since the linker helps with insertion of the warhead into the bottom of the tubular active site, the change in the linker region will likely affect potency and/or binding affinity of **1**. It is noteworthy that **1** as well as FK228 (**3**) have a four-atom linker between the macrocycle and the Zn^{2+}-chelating group. Other cyclic peptide HDAC inhibitors (e.g., trapoxin B, apicidin, azumamide E) have a longer six-atom linker. We envisioned that generation of largazole analogues with different lengths of the linker region (i.e., three-, five-, and six-atom) would establish the impact of linker length on HDAC inhibitory activity.

Scheme 1. Synthesis of Thioester and Side Chain Analogues



As shown in Scheme 1, three-, five-, and six-atom linkers (**7**−**9**) were prepared via coupling the thioacid (**6**)[8] to 3-bromo-1-propene, 5-bromo-1-pentene, and 6-bromo-1-hexene, respectively (NaH, THF, 25 °C, 5−36 h, 74−90%).[2] The olefin cross-metathesis reaction[9] of the 16-membered macrocycle **10**[2] with **7**, **8**, and **9** in the presence of Grubbs' second-generation catalyst (50 mol %, toluene, reflux, 4 h) provided **11**, **12**, and **13**, respectively.

While FK228 (**3**) has a weaker thiol warhead compared to a hydroxamic acid, the additional binding interactions from the macrocyclic backbone result in an overall potency superior to SAHA (**2**) and confer that selectivity as the homology between HDAC isoforms is divergent in the rim region.[10] This underscores the fact that conformation and hydrophobicity of the macrocycle cap of **1** are critical to its potency and isoform specificity. We expected that modification of key residues in **1** which have interactions with the rim region of HDAC would provide insights into the origin of highly potent HDAC inhibitory activity and class I specificity of **1**.

The valine residue is expected to interact with the rim region of HDACs, and this interaction could be a determinant

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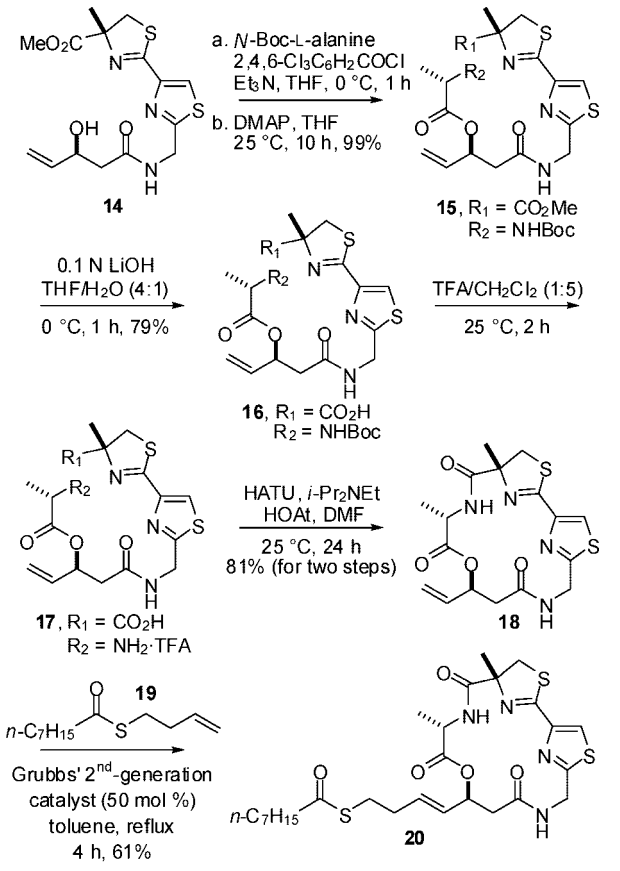
of the HDAC class/isoform specificity of **1**. To evaluate the comparative significance of side-chain functionality in the macrocycle, the valine residue of **1** was replaced with alanine (i.e., alanine scanning)[11]to prepare **20** (Scheme 2).

Scheme 2. Synthesis of the Alanine Analogue



Since alanine is conformationally similar with valine, we anticipated that the synthesis of **20** would smoothly proceed following the previously reported procedures.[2] Briefly, Yamaguchi esterification reaction of **14**[2] and *N*-Boc-L-alanine (2,4,6-trichlorobenzoyl chloride, Et_3N, THF, 0 °C, 1 h; then DMAP, 25 °C, 10 h) afforded the linear depsipeptide **15** in 99%. Hydrolysis of **15** under basic conditions (0.1 N LiOH, THF, H_2O, 0 °C, 1 h) to provide the carboxylic acid **16** and deprotection of the *N*-Boc group in **16** (TFA, CH_2Cl_2, 25 °C, 2 h) provided **17**, a precursor to the 16-membered cyclic depsipeptide core **18**. Without further purification of **17**, macrocyclization reaction of the crude **17** utilizing HATU-HOAt (HATU, HOAt, *i*-Pr_2NEt, DMF, 25 °C, 24 h) proceeded smoothly to give **18** in 81% (for two steps).[12] Olefin cross-metathesis reaction of the macrocycle **18** and the thioester **19**[2] in the presence of Grubbs' second-generation catalyst (50 mol %, toluene, reflux, 4 h) provided **20** in 61% ((*E*)-isomer only).

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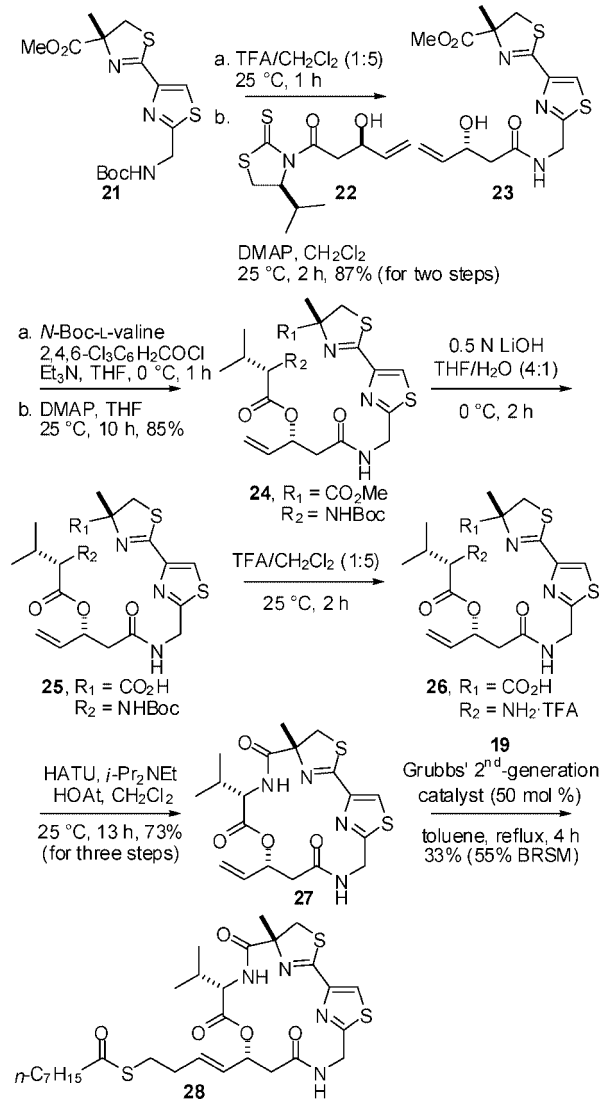
Scheme 3. Synthesis of the C17-Epimer Analogue



In addition to the alanine analogue **20**, we prepared the C17-epimer analogue **28** to study the effect of the configuration at the C17 position on HDAC inhibitory activity. As described in our previous report,[2] Nagao's aldol reaction provided a 3:1 diastereomeric mixture of (3*S*)- and (3*R*)-hydroxy-carboxylic acid.[13] We incorporated (3*R*)-hydroxy-carboxylic acid (**22**, the minor aldol adduct) in the macrolactone backbone to prepare the C17-epimer analogue **28** (Scheme 3).

After completing the synthesis of **11**−**13**, **20**, and **28**, we assessed their antiproliferative activity against HCT-116

(12) For examples of macrocyclization reactions in the synthesis of biologically active cyclic peptides and depsipeptides, see:(a) Hamada, Y.; Shioiri, T. *Chem. Rev.* **2005**, *105*, 4441–4482. (b) Li, W. R.; Ewing, W. R.; Harris, B. D.; Joullie, M. M. *J. Am. Chem. Soc.* **1990**, *112*, 7659–7672. (c) Jiang, W.; Wanner, J.; Lee, R. J.; Bounaud, P.-Y.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 5288–5290. (d) Chen, J.; Forsyth, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 8734–8735.

(13) (a) Nagao, Y.; Hagiwara, Y.; Kumagai, T.; Ochiai, M.; Inoue, T.; Hashimoto, K.; Fujita, E. *J. Org. Chem.* **1986**, *51*, 2391–2393. (b) Hodge, M. B.; Olivo, H. F. *Tetrahedron* **2004**, *60*, 9397–9403.

colon cancer cells and HDAC inhibitory activity using HeLa nuclear extract as the enzyme source side-by-side with largazole (**1**). Compound **20** showed only approximately 2- to 3-fold decreased activity in both assays compared with **1**, suggesting that the valine residue is largely replaceable (Table 1). Homologation to five-atom and six-atom linkers in **12** and **13**, respectively, reduced the cell growth and HDAC inhibitory activity by several orders of magnitude (Table 1), while chain shortening of the linker by one methylene group (compound **11**) completely abolished activity up to 20 μM. This result suggests that a four-atom chain length between the acyloxy methine (C17) and the side-chain sulfur atom, as found in **1**, is optimal for HDAC inhibitory activity. Largazole C17-epimer **28** lacked significant HDAC activity at 20 μM and only weakly inhibited HCT-116 cell growth with >500-fold reduced activity compared with **1** (Table 1). We then subjected protein extracts derived from HCT-116 cells, which had been treated for 8 h with (at least marginally active) largazole analogues, to immunoblot analysis of acetylated histone H3, an endogenous HDAC substrate (Figure 2). Dose−response analysis indicated that the inhibition of histone H3 deacetylation correlated with the GI_{50} values in the cell viability assay (Table 1).

Table 1. Cancer Cell Growth and HDAC Inhibition (GI_{50} and IC_{50} in nM)

compound	HCT-116 growth inhibition (GI_{50})	HeLa nuclear extract HDACs (IC_{50})[14]
1	6.8 ± 0.6	32 ± 13
11	>10000	>20000
12	620 ± 50	7600 ± 900
13	2500 ± 600	4100 ± 430
20	21 ± 2	72 ± 21
28	3900 ± 450	>20000

To determine if the structural modifications also affected the selectivity for HDAC1 versus HDAC6,[2] we profiled **12**, **13**, and **20** against the recombinant human HDAC1 and HDAC6 (Table 2). All compounds showed a similar decrease in HDAC1 inhibitory activity as in antiproliferative activity compared with **1**. Although the Val→Ala change reduced the discriminatory power of the macrocycle by 3-fold, **20** exhibited strong selectivity for HDAC1 over HDAC6.

In conclusion, a series of largazole analogues (**11**−**13**, **20**, and **28**) were prepared and tested for HDAC inhibitory activity which correlated with their ability to inhibit cancer cell growth. The biological evaluation of the analogues suggested that the four-atom linker between the macrocycle and the octanoyl group in the side chain and the (*S*)-configuration at the C17-position are critical to potent HDAC inhibitory activity of **1**. In contrast, the valine residue in the macrocycle can be replaced with alanine without compromising activity to a large extent. These SAR results would provide insights into structural requirements for HDAC inhibitory activity including the observed HDAC selectivity of largazole (**1**) and help in the design of isoform-specific HDAC inhibitors based on **1**.

(14) It should be noted that the HDAC inhibitory activities of **1**, **12**, **13**, and **20** are presumably derived from the corresponding thiols by thioester hydrolysis,[2] and hydrolysis rates of the compounds are assumed close to equivalent.

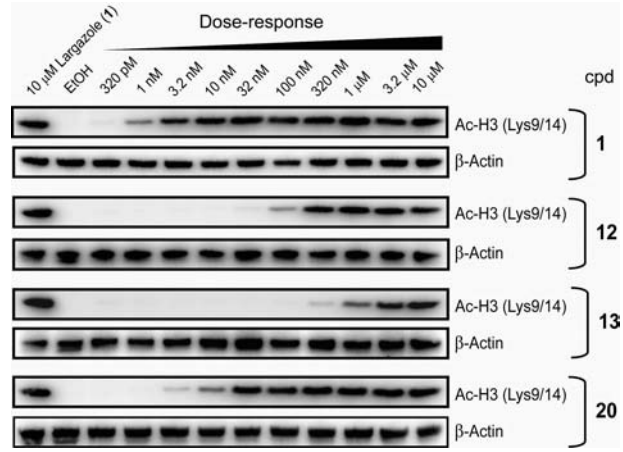


Figure 2. Immunoblot analysis showing various degrees of inhibition of histone H3 deacetylation by largazole analogues in HCT-116 cells upon 8 h of treatment (β-Actin = control).

Table 2. IC_{50} Values for HDAC1 and HDAC6 Inhibition (nM)[14]

compound	HDAC1 (class I)	HDAC6 (class II)
1[a]	7.6	1800
12	690	>10000
13	1900	>10000
20	44	3300
trichostatin A[b]	5.2	1.8

[a] Under the same assay conditions, the pure thiol analogue of largazole[2] exhibited IC_{50} values of 0.77 and 510 nM for HDAC1 and HDAC6, respectively. [b] Standard and nonselective HDAC inhibitor.

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Supporting Information Available: General experimental procedures including spectroscopic and analytical data for **7**−**9**, **11**−**13**, **15**−**18**, **20**, and **23**−**28** along with copies of ^1H and ^{13}C NMR spectra and detailed assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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