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Total Synthesis and Biological Evaluation of Largazole and Derivatives with Promising Selectivity for Cancers Cells

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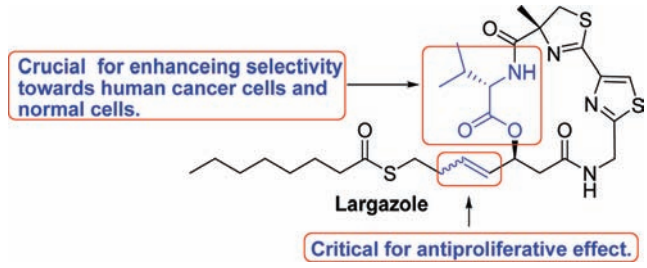
ABSTRACT



The efficient total synthesis of the natural substance largazole is described. Using this strategy, a small library of largazole analogs was developed. Structure−activity relationship studies suggested that the geometry of the alkene in the side chain is critical. While the largazole's analogues with *trans*-alkene are potent for the antiproliferative effect, those with *cis*-alkene are completely inactive. Most importantly, replacement of valine with tyrosine in largazole increased selectivity toward human cancer cells over human normal cells more than 100-fold.

Natural products have traditionally played an important role in drug discovery and are the basis of many important therapeutics that have found broad use in clinics for the treatment of cancer, microbial infections, inflammation, hypercholesterolemia, and tissue rejection in organ transplantation, etc.[1] In particular, more than 60% of currently available anticancer drugs are either natural compounds or their analogues.[2] Very often, the highly potent natural compounds have limited clinical use due to their systemic toxicity caused by lack of selectivity toward cancer cells over normal cells. Largazole **1** is a natural macrocyclic depsipep-

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tide isolated from the marine cyanobacterium *Symploca sp*. by Luesch and co-workers.[3] It has shown potent and selective biological activity for differential growth inhibition in a number of transformed and nontransformed human and murine cell lines *in vitro*. Largazole also exhibits good histone deacetylase 1 (HDAC1) selectivity.[4] These excellent properties of largazole have attracted significant attention, and several research groups have completed its total synthesis and evaluated the biological activities of the natural product and its derivatives against HDACs.[5−9] On the basis of the total synthesis of largazole, several groups conducted a preliminary evaluation of structure−activity relationships (SAR) of the synthetic largazole derivatives.[6−8] Hong and co-workers reported that replacement of valine residue with alanine in the macrocycle decreased the activity by 3-fold.[6] By analyzing molecular modeling of the largazole **1** complex with HDAC1 structure, we revealed that the valine residue has hydrophobic interactions with the side chains of Tyr 196 and Leu 263 of the HDAC1, and these interactions may be crucial for HDAC class/isoform selectivity of largazole (Figure 1).[10] Therefore, we assumed that introducing a more hydrophobic amino acid residue into position 1 of largazole **1** would improve this kind of hydrophobic interactions, and could increase selectivity for HDAC1 over other isoforms. In addition, we envisioned that the geometry of the alkene residue of 3-hydroxy-7-mercaptohept-4-enoic acid unit could also play an important role in biological activities.

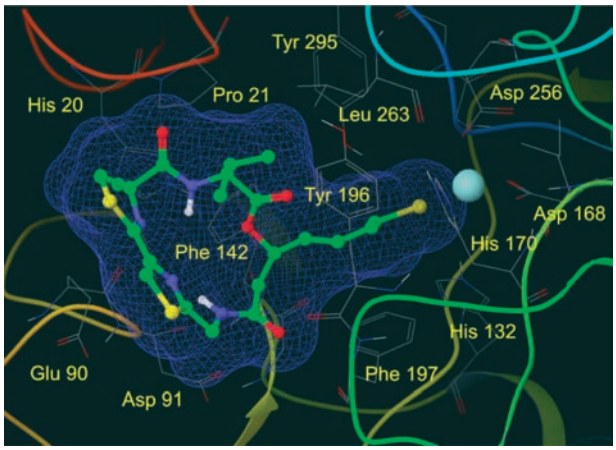
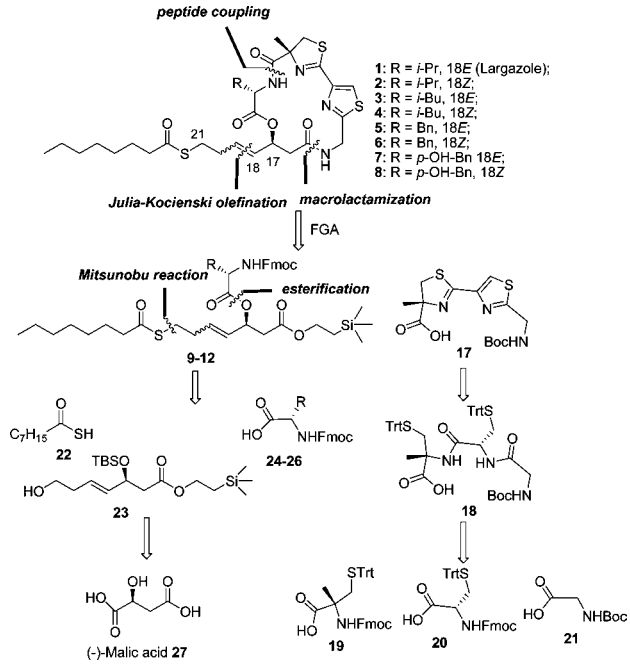


Figure 1. Plausible binding mode of largazole **1** to HDAC1.

To fully explore the potential of largazole derivatives as selective anticancer agents and to further understand SAR of largazole, we chose a synthetic approach that would enable the early stage replacement of Val1 from a common precursor. By using this strategy, a small, focused library was generated efficiently. Here, we report application of this strategy for generation of several compounds that are selectively toxic for cancer cells.

Scheme 1. Retrosynthesis of Largazole **1** and its Analogues **2**−**8**



As shown in Scheme 1, we chose a synthetic approach that allowed for generation of a variety of analogues from a common precursor **23**. By varying the combination of different fragments **24**−**26**, seven analogues of the largazole were obtained by parallel assembly of prefunctionalized units. The structurally significant modification in these compounds was made in the valine region, where a leucine, a phenylalanine, or a tyrosine unit was introduced. In addition, alteration of geometry of the alkene was achieved during the formation of the 3-hydroxy-7-mercaptohept-4-enoic acid unit by Julia-Kocienski olefination.

As shown in Scheme 2, we explored solid-phase synthesis of key fragment **17** intending to develop a more concise and efficient synthetic method to synthesize new largazole analogues. Due to the acid-labile protecting groups present in the intermediates, the synthesis and cleavage from resin had to be performed under either mildly acidic or neutral conditions. For these reasons, the 2-chlorotrityl chloride resin was chosen for

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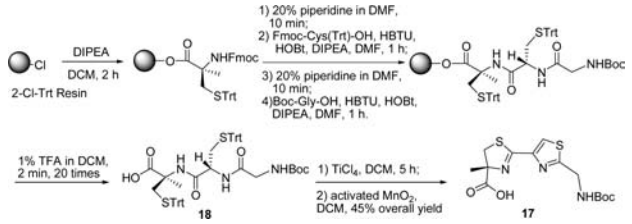
Scheme 2. Synthesis of Key Fragment **17**



the synthesis. In the resin-loading step, a limited incorporation of the Fmoc protected (*R*)-2-methyl cysteine[11] on ClTrt-Cl resin was achieved with *N*,*N*-diisopropylethylamine (DIPEA).[12] Unreacted resin chloride functions were capped with MeOH to avoid the formation of deletion sequences. After deprotection of the amino group (20% piperidine in DMF), Fmoc chemistry-based solid-phase peptide synthesis methodology was used for the synthesis of the linear peptide Boc-Gly-Cys(Trt)-(R-Me)Cys(Trt)-O-2-Cl-Trt-resin. Upon completion of the peptide sequence, the side chain protected peptide was cleaved from the resin with 1% TFA in DCM, affording precursor **18**. The conversion of **18** to thiazoline-thiazole fragment **17** was successfully achieved by using titanium tetrachloride mediated tandem deprotection-cyclodehydration of **18** and subsequent oxidation with activated manganese.

The synthesis of the key intermediates, **9**−**16**, started with a previously characterized diol **28**, which was obtained from commercially available (−)-malic acid **27** (Scheme 3).[13] The 1,2-diol **28** was sequentially silylated (TBSCl) to afford **29** in 90% yield. The ester group of **29** was saponified with KOH, and then coupled with 2-(trimethylsilyl)ethanol to provide the TSE-protected acid **30**, which afforded alcohol **31** upon selective removal of the TBS protecting group of the primary alcohol. Swern oxidation led to the aldehyde which was subjected to a Julia-Kocienski olefination coupling[14] with sulfone **32**. For the synthesis of olefin **33**, several bases (e.g., LiHMDS, NaHMDS, KHMDS, or LDA) were tested at temperatures ranging from −78 °C to room temperature. We found that utilization of NaHMDS gave the most favorable E/Z (8/1) ratio. After selective removal of the primary TBS protecting group, Mitsunobu reaction was performed with octanethioic acid to afford compound **34**, which was rapidly deprotected to allylic alcohol **35**. Treatment of the allylic alcohol **35** with enantiomerically pure amino acids Fmoc-L-valine, Fmoc-L-leucine, Fmoc-L-phenylalanine, and Fmoc-L-tyrosine in different combinations in the presence of EDCI and HOAt at room temperature gave intermediates **9**−**12**.

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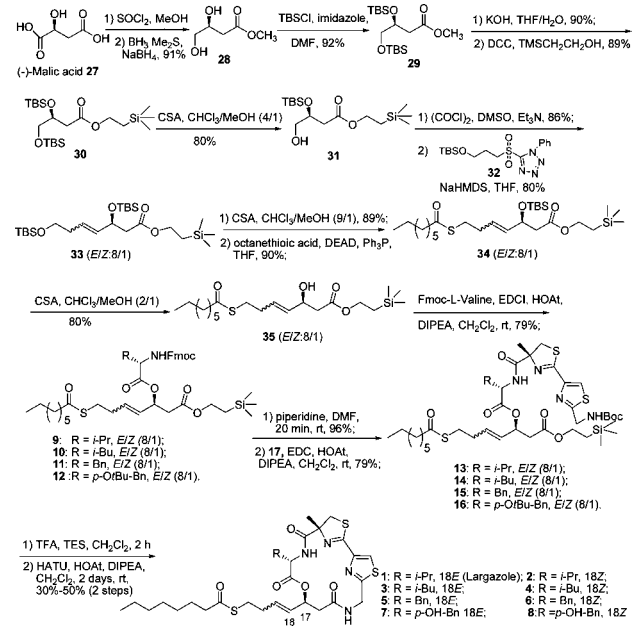
Scheme 3. Synthesis of Lagazole **1** and its Analogues **2**−**8**



Removal of the Fmoc group followed by HATU-mediated coupling to the thiazoline-thiazole carboxylic acid **17** furnished the cyclization precursors **13**−**16**. Formation of the 16-membered cycloamide was achieved in a two-step sequence involving TFA-mediated removal of the Boc and 2-(trimethylsilyl)ethanol groups, and subsequent macrolactamization with HATU/HOAt/DIPEA in anhydrous DCM to provide synthetic (+)-largazole **1** and its analogues **2**−**8** in 30−50% yield (two steps). The spectroscopic data (^{1}H NMR, ^{13}C NMR, and HRMS) and the optical rotation for the synthetic largazole **1** fully matched the data published for the natural product (see Supporting Information).

The effects of largazole and its analogues on human lung cancer cell line (A549), colorectal carcinoma cell line HCT-116, human embryonic kidney cell line (HEK293), and human embryonic lung fibroblast (HLF) cells were tested, and results showed interesting cell line selectivity (Table 1). Synthetic largazole **1** displayed good selectivity for the cancer cell lines (HCT-116: GI_{50} = 80 nM; A549: GI_{50} = 320 nM)

Table 1. Antiproliferative Activity of Largazole and Analogues **2**−**8**

	GI_{50} (μM)			
sample	HCT-116	A549	HEK293	HLF
1	0.08	0.32	1.36	0.98
2	>10	>10	>10	>10
3	0.56	3.28	8.95	6.12
4	>10	>10	>10	>10
5	0.26	0.77	2.57	1.43
6	>10	>10	>10	>10
7	0.39	1.46	>100	>100
8	>10	>10	>10	>10

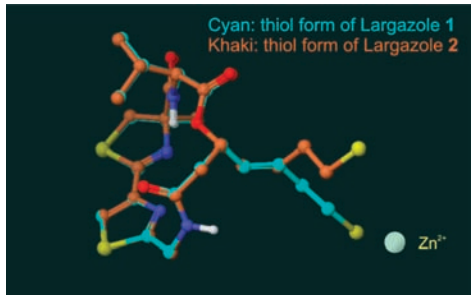


Figure 2. Comparison of global minimal conformations of Largazole **1** (trans-form) and **2** (cis-form).

over the normal cell lines (HEK293: $GI_{50} = 1.36\ \mu M$; HLF: $GI_{50} = 0.98\ \mu M$). Practically no activity was found with all the analogues **2**, **4**, **6**, **8** with cis geometry of the alkene against either human tumor cell lines or normal cell lines even at a concentration of 10 μM. Molecular modeling studies corroborate that the orientations (*Z*)-alkenes could not form chelation bond with the zinc ion (Figure 2). When Val 1 was replaced with leucine and phenylalanine, the inhibitory activity of the resulting analogues **3** and **5** against cancer cell lines slightly decreased. Compounds **3** and **5** retained selectivity for cancer cells over normal cells similar to largazole **1**. To our delight, the replacement of Val 1 with tyrosine in compound **7** resulted in slightly lower potency but much improved selectivity for cancer cell lines (HCT-116: $GI_{50} = 0.39\ \mu M$; A549: $GI_{50} = 1.46\ \mu M$) over the normal cell lines (HEK293: $GI_{50} > 100\ \mu M$; HLF: $GI_{50} > 100\ \mu M$). The improved therapeutic index indicates that the tyrosine residue of compound **7** could be a determining factor for the selectivity toward cancer cells over normal cells. The proposed binding modes of largazole **1** and **7** are shown in Figure 3**A** and 3**B**, in which a methyl group in Val1 of largazole 1 is in the hydrophobic pocket wrapped by Leu 263 and Tyr 196, whereas, the *p*−OH-benzyl group in the corresponding position of **7** forms additional intramolecular $\pi-\pi$ stacking interaction with the two five-membered rings, which increased selectivity.

In summary, a new class of largazole's analogues (**2**−**8**) was designed based on the molecular modeling of the complex structure of HDAC1 with largazole. Largazole and its analogues were synthesized enantioselectively (9% overall yield). This methodology may potentially be applicable to the synthesis of other analogues of this family, as well as

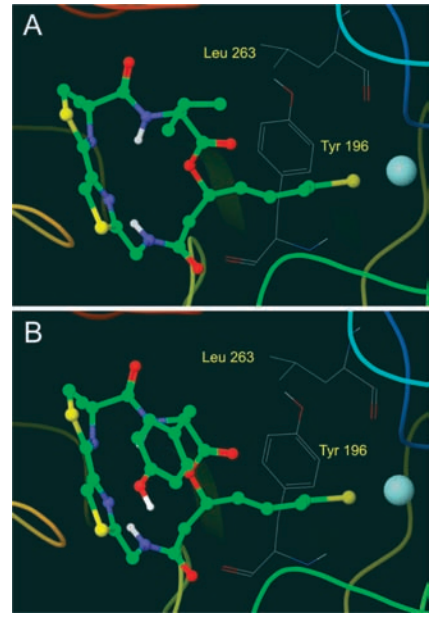


Figure 3. Proposed differences in binding to HDAC1 between Largazole **1** and **7**.

other focused libraries. The biological evaluation of the analogues suggests that the geometry of the alkene is critical for the antiproliferative effect of largazole. Most notably, we have demonstrated that replacement of Val 1 with tyrosine can increase selectivity toward human cancer cells over normal cells more than 100-fold. The synthetic strategy presented herein may accelerate further discovery of more potent and selective antitumor agents. Various biological studies, including inhibitory studies on metastatic tumors in animal models and the activities on a molecular level are currently in progress in our laboratory.

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Supporting Information Available: Moleular modeling and full experimental data described in the paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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