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Largazole: From Discovery to Broad-Spectrum Therapy

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Abstract

The cyclic depsipeptide largazole from a cyanobacterium of the genus *Symploca* is a marine natural product with novel chemical scaffold and potently inhibits class I histone deacetylases (HDACs). Largazole possesses highly differential growth-inhibitory activity, preferentially targeting transformed over non-transformed cells. The intriguing structure and biological activity of largazole have attracted strong interest from the synthetic chemistry community to establish synthetic routes to largazole and to investigate its potential as a cancer therapeutic. This highlight surveys recent advances in this area with a focus on the discovery, synthesis, target identification, structure–activity relationships, HDAC8–largazole thiol crystal structure, and biological studies, including *in vivo* anticancer and osteogenic activities.

1 Introduction

Pioneering studies particularly by Richard Moore and later William Gerwick have demonstrated the value of cyanobacteria for biomedical research.^{1,2} The antimicrotubule agents cryptophycins³ and dolastatin 10,⁴ various analogues of which have been in cancer clinical trials, may perhaps be considered most significant. In fact, an anti-CD30 monoclonal antibody–monomethyl auristatin E (dolastatin 10 analogue) conjugate was FDA-approved in August 2011 to treat Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (ALCL).⁵

Particularly, cyanobacterial secondary metabolites from the *marine* environment are emerging as a “hot” resource for anticancer agents, many but not all of which disturb cytoskeletal processes (microtubule and actin dynamics) commonly targeted by marine natural products.⁶ The histone deacetylase (HDAC) inhibitor largazole (Fig. 1) is a remarkable example of a novel potential anticancer (and other disease-modifying) agent that has a molecular (enzyme) target relevant to cancer, but with a broad applicability due to its mechanism of action that is similar to that of the drugs vorinostat (SAHA) and romidepsin (FK228), approved for the treatment of cutaneous T-cell lymphoma (CTCL).⁷ Largazole was recently featured in *Newsweek* as the latest victory in bioprospecting the oceans for novel medicines.⁸ We review here the short history of this fairly new, but intensely studied molecule with a promising future and recapitulate some relevant data.

2 The discovery of largazole

Where can new chemical entities that cover biologically relevant (therapeutic) chemical space be discovered? While answers may diverge, the best odds might lie in the investigation of unexplored marine microorganisms.⁹ This was the direction the Luesch and Paul groups took when they started investigating marine cyanobacteria of the genus *Symploca* from coastal Florida. While there are only a few reports on secondary metabolites from *Symploca* spp., the same genus has previously yielded cancer clinical trial agent dolastatin 10.⁴ One *Symploca* extract from Key Largo, Florida, possessed remarkably potent antiproliferative activity. Using bioassay-guided fractionation involving solvent partitioning and silica gel chromatography followed by reversed-phase (C18) HPLC, Luesch and co-workers largely attributed the activity to a new chemical entity, termed largazole (Fig. 1),¹⁰ which also showed synergistic activity with another extract component displaying weak antimitotic activity, symplostatin 4.¹¹ Largazole received its trivial name as a result of combining the collection site of the producing cyanobacterium (Key Largo) with some of the structural features that include two “azole” type units, viz. one thiazole that is linearly fused to a 4-methylthiazoline. The other notable structural characteristic of largazole is the thioester functionality that was the first one ever encountered in a secondary metabolite from a marine cyanobacterium and which also plays a key role in its mechanism of action (see below).

The planar structure of largazole was elucidated by extensive 1D and 2D NMR analysis coupled with high-resolution and tandem mass spectrometry. With only 1.2 mg of largazole in hand, the Luesch lab was able to unambiguously establish the structure including absolute configuration through degradation chemistry (Fig. 2). Largazole contains only one non-modified standard amino acid, valine, and the C2 configuration is easy to establish by conventional methods. The C7 configuration of the methylthiazoline unit could be determined analogously to configurations of other thiazoline 4-carboxylic acids, upon oxidative destruction of the thiazoline ring. The 3-hydroxy-7-mercapto-hept-4-enoic acid, a unit that was encountered for the first time in a marine natural product, had to be converted into a subunit for which enantiomeric standards were readily available, e.g., through oxidation of C18 to a carboxylic acid. The configurations of the three stereogenic centers were assigned after ozonolysis followed by oxidative work-up and acid hydrolysis (Fig. 2). This reaction sequence liberated L-valine, (*R*)-2-methylcysteic acid, and L-malic acid, as verified by comparative enantioselective HPLC analysis with easily accessible enantiomerically pure standards.¹⁰

3 Differential cytotoxicity

The minute amounts of initially isolated natural product were used to assess if largazole had any selectivity towards certain cancer cell types and if there was any selectivity for cancer cells over non-transformed cells, since we reasoned that these would be the first differentiating factors when assessing the compound's therapeutic potential as an anticancer agent. Preliminary results lived up to its promise. The growth of numerous epithelial and fibroblastic cancer cell types, including colorectal carcinoma, breast cancer, neuroblastoma and osteosarcoma cells, was inhibited at nanomolar concentrations, while non-transformed

epithelial cells and fibroblasts were inhibited to a much lesser extent.¹⁰ Even though the genetic background of transformed and non-transformed cells was not identical, the selectivity trend suggested that largazole warranted further study. In comparison, other natural products drugs such as paclitaxel lacked the desired selectivity window in these studies. Cell type specific rather than general cytotoxic effects were later supported by the NCI 60-cell line screen that became possible after we had sufficient synthetic material in hand.¹² Since the initial disclosure of the differential cytotoxicity of largazole, largazole and synthetic analogues have further been tested against colorectal carcinoma,^{13–17} human malignant melanoma,¹⁸ human epithelial carcinoma,¹⁹ breast cancer,^{20,21} lung cancer,¹⁴ prostate cancer,²² and leukemia cell lines.²³ These results indicate that largazole has broad-spectrum yet differential activity against various cancer cell types.

Synthetic material was required to solve the supply problem and provide sufficient amounts for extensive biological testing, including mechanism and target identification studies. The presence of the unusual and presumably labile thioester functionality gave rise to the hypothesis that largazole may be a prodrug that upon hydrolysis is converted into an HDAC inhibitor. The hydrolysis product (reactive species) then bears a Zn²⁺-complexing mercapto group similarly to that released by a disulfide-reduced form of FK228 (redFK228)²⁴ (Fig. 3), FR901375, and spiruchostatins.²⁵ In fact, prodrug strategies are quite commonly employed by Nature.²⁶ The selectivity for cancer cells indeed hinted at a molecular target preferentially expressed or overactive in cancer, as known for HDACs. It was also clear that the thioester linkage in largazole should be unstable, and the isolation of intact largazole was possibly somewhat surprising in that regard, and/or proved that care was taken during the isolation procedure. Yet, it necessitated total synthesis to prove the mechanistic hypothesis.

4 Total synthesis of largazole

Due to its exciting differential cytotoxicity and great potential for a cancer therapeutic, largazole has attracted considerable interest from a number of synthetic groups, culminating in the first total synthesis by Luesch/Hong and co-workers.¹³ To date, eleven total syntheses of largazole have appeared in the literature,^{13,14,18–21,27–31} and they have been extensively reviewed.^{32–34} The synthetic challenges it presents include the preparation of the β -hydroxy carboxylic acid subunit, the formation of the 16-membered cyclic depsipeptide core, and the incorporation of the fairly labile thioester.

Due to the structural simplicity of the natural product, most of the total syntheses reported to date rely on either of the two approaches illustrated in Fig. 4: (1) synthesis of the 16-membered macrocycle followed by installation of the thioester side chain via cross-metathesis; (2) incorporation of the *S*-protected precursors to the side chain followed by macrolactamization and acylation.

For the synthesis of the β -hydroxy carboxylic acid, Luesch/Hong, Williams, Ye, Doi, and Xie used asymmetric acetate aldol reactions. Phillips, Cramer, and Ghosh utilized an enzymatic resolution of *tert*-butyl 3-hydroxypent-4-enoate. Forsyth uniquely prepared a fully elaborated derivative of 3-hydroxy-7-(octanoylthio)hept-4-enoic acid via *N*-heterocyclic carbene (NHC) mediated acylation of α,β -epoxy aldehyde. Following the

synthesis of the β -hydroxy carboxylic acid and the 4-methylthiazoline–thiazole, each group combined them with the L-valine subunit to prepare precursors to the 16-membered cyclic depsipeptide core. For the synthesis of the 16-membered macrocycle, Luesch/Hong, Doi, and Xie used the macrolactamization reaction of the valine amino group and the 4-methylthiazoline carboxylic acid group (Macrolactamization I, Fig. 4). The other groups formed the amide bond between the β -hydroxy carboxylic acid and the thiazole amino group (Macrolactamization II, Fig. 4). Luesch/Hong and Forsyth attempted the macrolactonization reaction of the valine carboxylic acid and the hydroxy group of the β -hydroxy carboxylic acid, but this approach failed due to ring strain and possible elimination of the β -hydroxy carboxylic acid to a conjugated diene.

To complete the synthesis of largazole, Luesch/Hong, Cramer, Phillips, and Ghosh installed the thioester side chain through the cross-metathesis reaction. Due to the coordination of the thioester with the ruthenium catalyst, the cross-metathesis reaction in the presence of Grubbs' second-generation catalyst required a high loading of the catalyst. Cramer and co-workers found that Grela's *p*-nitro substituted catalyst (15 mol %) gave a better yield (75%). Williams, Ye, Doi, Xie, and Ganesan chose to incorporate the side chain at an early stage by using *S*-protected side chains as substrates for the asymmetric aldol reactions. After the assembly of the 16-membered macrocycle, they removed *S*-protecting groups and used the acylation reaction to complete the synthesis of largazole.

5 Target identification, proof of mechanism of action, and HDAC8–largazole thiol crystal structure

Marine natural products are often notorious for their structural complexity and difficulty of synthesis, which leads to the supply problem. The easy access to largazole accomplished through efficient total syntheses and, consequently, availability of largazole for further testing spurred additional research into the biology of this molecule. Cellular assays using an artificial fluorogenic substrate and assessing endogenous histone (H3) hyperacetylation (Lys9/14) in colon cancer cells demonstrated a nice correlation between largazole's growth-inhibitory and cellular HDAC inhibitory activity.¹³ Our results indicated that the mechanism of action relevant for the antiproliferative activity is indeed mediated by the inhibition of HDAC enzyme(s) that utilize Ac-H3 (Lys9/14) as a substrate. The same finding was independently reported by the Williams group.¹⁸ When we probed how the prodrug activation might occur, we found that largazole, including the thioester linkage, is fairly stable under aqueous conditions at various pHs. However, in the presence of plasma or cellular proteins, largazole is rapidly hydrolyzed to largazole thiol.¹² Largazole activation appears to be induced through a general protein-assisted mechanism, which may explain why largazole itself displayed apparent activity in the *in vitro* enzymatic assay with recombinant HDAC1 enzyme, although with about 10-fold lower potency.^{13,15} This result indicates that even the HDAC enzyme itself is able to catalyze the largazole thiol liberation from largazole and that the nature of the protein is irrelevant. How does largazole thiol interact with the HDAC enzymes? Several groups have used homology modeling to predict how this may happen for HDAC1,^{12,14,35} a class I HDAC isoform relevant to cancer as it is overexpressed and hyperactive in various cancer types.^{36–39} A real visualization of the

interaction with HDAC8 was recently achieved by Christianson and co-workers, who reported the X-ray crystal structure of HDAC8 complexed with largazole thiol at 2.14 Å resolution (Fig. 5).⁴⁰ It was the first structure of an HDAC complex with a macrocyclic depsipeptide inhibitor.

The X-ray crystal structure showed that the macrocyclic core underwent minimal conformational changes upon binding to HDAC8 and that considerable conformational changes were required by HDAC8 to accommodate the binding of the rigid and bulky inhibitor. The thiol side chain of largazole extended deep into the active site cleft. The overall metal coordination geometry was very close to tetrahedral, with ligand–Zn²⁺–ligand angles ranging between 107.6° and 111.8°. The thiolate moiety exhibited preferred thiolate–metal coordination geometry. It was the first structure of an HDAC complex in which thiolate–Zn²⁺ coordination was observed. The X-ray crystal structure revealed that the ideal geometry of thiolate–Zn²⁺ coordination is crucial to its exceptionally high binding affinity and biological activity. The structure of the HDAC8–largazole thiol complex provided a foundation for understanding structure–activity relationships in a number of largazole analogues prepared by various groups (see below).

Both homology modeling and co-crystal structure will ultimately help in deciphering and improving on isoform selectivity of largazole. Importantly, it is clear that the macrocyclic portion provides the distinguishing interactions with amino acids outside the convergent regions of HDAC isoforms, which contribute to any observed selectivity. Achieving isoform selectivity is indeed one of the most desired goals in HDAC research. The selectivity profile obtained by Luesch/Hong is shown in Table 1. It indicates the class I selectivity of largazole thiol, yet HDACs 1–3 cannot be differentiated by largazole thiol, while HDAC8 was inhibited to a much lesser extent. HDAC11, the only class IV member and related to class I isoforms, is also strongly inhibited by largazole thiol. Among class II isoforms (classified into classes a and b), only HDAC10 (class IIb) activity was severely compromised *in vitro*. Largazole had two orders of magnitude lower potency *in vitro* against the only other class IIb enzyme, HDAC6. Direct comparison with redFK228 also indicates that largazole thiol is slightly more active, proving it to be the most potent natural HDAC inhibitor known.

Selectivity profile studies of largazole have been independently reported by Williams (HDACs 1, 2, 3, and 6),^{18,35,41} Nan (HDACs 1, 2, 3, and 6),¹⁶ de Leca (HDACs 1 and 4),²³ and Tillekeratne (HDACs 1 and 6).¹⁷ Results of these studies are overall consistent with the data shown in Table 1. The class I vs. class IIb selectivity is further manifested and appears even amplified in cellular systems since the α -tubulin acetylation (Lys40) status, which is determined by HDAC6 activity, was not perturbed by largazole up to 10 μ M, while histone H3 (Lys9/14) acetylation, regulated by class I isoforms, was induced at low nanomolar concentration in the same cell line (HCT116).¹²

6 Structure–activity relationship (SAR) studies

Numerous largazole analogues have been prepared to improve potency and isoform selectivity. The main structural changes have focused on the thioester linker region, the L-

valine subunit, and the 4-methylthiazoline–thiazole subunit (Fig. 6). Supplementary Tables S1–7[†] reports additional information on structure–activity relationships of largazole.

First, shortening and lengthening the thiol side chain, changing the olefin geometry from *trans* to *cis*, or changing the configuration of the C17 from (*S*) to (*R*) resulted in a significant loss of activity.^{14,15} Each of these structural modifications would compromise the ideal geometry of thiolate–Zn²⁺ coordination observed in the HDAC8–largazole thiol complex. Several analogues with modified metal-binding motifs were prepared by replacement of the thioester with α -aminobenzamide, α -thioamide, 2-thiomethyl pyridine, 2-thiomethyl thiophene, and 2-thiomethyl phenol groups, but these analogues were significantly less potent than largazole.^{17,41}

It has been demonstrated that the L-valine subunit of largazole can be replaced with L-tyrosine, L-alanine, or glycine without drastic loss of activity.^{14,15,21} As seen in the HDAC8–largazole thiol complex, the isopropyl group of L-valine faces the solvent and does not directly influence the enzyme–inhibitor interaction. Therefore, other L-amino acids might be tolerated at this position as long as they do not perturb the overall conformation of the macrocyclic depsipeptide core. 2-*epi*-Largazole was less potent against HDACs 1–3 than largazole by 25-fold,⁴¹ but it was more potent than largazole in inhibiting the viability of prostate cancer cells (LNCaP and PC-3).²²

It has been shown that the methyl group of the 4-methylthiazoline moiety is not essential for the potency of largazole and can be replaced with a hydrogen atom, an ethyl, or a benzyl group without any significant effect on its biological activity.^{23,41} The HDAC8–largazole complex showed that this methyl group is oriented parallel to the protein surface and that it has no interaction with the protein. However, the strained bithiazole analogue was 25–145 times less active than largazole,^{16,41,42} suggesting that the conformation of the macrocycle has a dramatic effect on binding affinity. In addition, Ganesan and co-workers²¹ as well as the Phillips group⁴³ reported the synthesis and biological activity of a slightly simplified analogue by replacing the 4-methylthiazoline moiety with α -aminoisobutyric acid. The analogue modified with α -aminoisobutyric acid showed nanomolar HDAC inhibition, indicating that even further molecular simplification of the largazole scaffold is possible without loss of HDAC inhibitory activity by keeping the overall conformation.

Williams and co-workers reported the synthesis of an analogue in which the thiazole ring was replaced with a pyridine ring, leading to somewhat enhanced potency.⁴¹ The structure of the HDAC8–largazole thiol complex showed that the thiazole ring was positioned away from the protein structure and faced toward the solvent.⁴⁰ They also reported the synthesis of the methyloxazoline–oxazole analogue, in which the two sulfur atoms in the 4-methylthiazoline–thiazole were replaced with oxygen atoms.⁴¹ The methyloxazoline–oxazole analogue was reportedly slightly more active than largazole. Taken together, these results indicate that this position could tolerate additional substitution without significant loss of activity.

[†]Electronic Supplementary Information (ESI) available: Further data on structure–activity relationships of largazole. See DOI: 10.1039/b000000x/

The amide isostere of largazole was prepared and biochemically evaluated by Williams and co-workers.³⁵ The biochemical data revealed that the largazole amide isostere thiol was 4–9 times less potent against HDACs 1–3 than largazole thiol. The loss in binding affinity of the amide isostere was somewhat surprising since the structural perturbation induced by replacement of an oxygen atom with a nitrogen atom was expected to be minimal.

To probe the contribution of the two secondary amide hydrogens to largazole's HDAC inhibitory activity, Luesch/Hong and co-workers synthesized the two corresponding *N*-methylated largazoles.¹² Both compounds were 100- to 1000-fold less active. The crystal structure of the largazole macrocycle suggested a possible hydrogen bonding of the Val-NH with the thiazoline substructure. This hydrogen bonding would be lost upon *N*-methylation, potentially leading to a conformational change and thereby reduced activity.

7 Downstream activities in cultured cancer cells and in solid tumors *in vivo*

It is well documented that tumor suppressors and negative regulators of the cell cycle (cell cycle inhibitors) are silenced or heavily downregulated in cancer cells.^{44–47} HDAC research has shown that the corresponding genes can be reactivated through treatment with HDAC inhibitors. Largazole induced p21 expression at around 3 nM in HCT116 colon cancer cells, the same concentration that induced G1 cell cycle arrest.¹² Expression of p21 in NB4 leukemia cells was also induced by largazole.²³ Other inhibitors of cyclin-dependent kinases (CDKs) involved in cell cycle progression were induced upon largazole treatment (p19, p15, p57) as well, while some of the corresponding CDKs and cyclins, including CDK6 and cyclin D1, were strongly downregulated.¹² These opposing effects presumably potentiate or contribute to the strong antiproliferative effect of largazole. Interestingly, at higher concentrations, largazole caused G2/M arrest, demonstrating that there is a balance and concentration-dependency of genes that are regulated by largazole.¹² In fact, this phenomenon is not unique to largazole but rather a characteristic feature of HDAC inhibitors.^{48,49} Higher concentrations (>30 nM) additionally induced apoptosis, measured by activation of caspases 3/7. On the transcript and protein levels, the same concentration of largazole strongly upregulated pro-apoptotic members of the BCL2 family of proteins intimately tied to this event. Since HDACs are major regulators of gene transcription, it is not surprising that largazole induced and repressed hundreds of genes directly or indirectly through secondary effects as determined by transcriptomic profiling, which revealed a large overlap of genes regulated by largazole and the two approved HDAC inhibitor drugs, SAHA and FK228.¹² The downregulation of multiple receptor tyrosine kinases (RTKs) that are commonly overexpressed in cancers and drive proliferation is likely also related to their antiproliferative effect. Immunoblot validation was carried out for a variety of highly relevant RTKs, including HER-2, EGFR and MET. Particularly striking was the intense downregulation of insulin growth factor (IGF) receptor substrate 1 (IRS-1) on both transcript and protein level, suggesting that IGF signaling, known to have antiapoptotic activity via AKT activation, is compromised. This pathway was severely affected *in vivo* as well, when HCT116 tumor-bearing mice were treated with largazole, suggesting mechanistic correlations in cultured cells and in solid tumor xenografts.¹² Importantly, this also meant sufficient bioavailability of largazole *in vivo* when administered i.p., another critical issue in HDAC research where most inhibitors show undesirable pharmacokinetic

characteristics due to lack of stability.⁵⁰ While largazole is prone to protein-assisted thioester hydrolysis, it is irrelevant for activity: the resulting HDAC inhibitor largazole thiol appears fairly stable in its free form as well as a reversible adduct which was detected in plasma, microsomes and cellular extract, suggesting that largazole thiol may hijack proteins for delivery to the target site. However, *denatured* proteins were unable to form adducts with largazole thiol, as determined by incubation with inactivated microsomes. Largazole thiol's stability ultimately translated into *in vivo* solid tumor activity.¹² Furthermore, our study showed that largazole lacked acute toxicity, suggesting that largazole treatment may be a safe new anticancer HDAC inhibitor template compared to toxic agents with rather small therapeutic windows. Of note, largazole showed activity in both colon cancer cells with microsatellite and chromosomal instability, indicating a broader applicability for colon cancer treatment.¹² Certain colon cancer cells (HCT15) were moderately resistant, but HDAC inhibition still correlated with the antiproliferative effect at higher concentrations.¹² The NCI 60-cell line screen data further indicates that largazole's activity is not restricted to colon cancer cells.¹²

8 Osteogenic activity of largazole

Due to their capability of modifying chromatin structure and thereby regulating gene transcription, HDACs have been reported to play important roles in osteogenesis and are considered promising potential therapeutic targets for bone diseases, including osteoporosis.⁵¹

Kim, Hong, Luesch, and co-workers showed that largazole exhibits *in vitro* and *in vivo* osteogenic activity (Fig. 7).⁵² Up to 50 nM, largazole significantly induced the expression of markers of osteoblast differentiation such as alkaline phosphatase (ALP) and osteopontin (OPN) in a dose-dependent manner. The osteogenic activity of largazole was mediated through the increased expression of Runx2 and bone morphogenetic proteins (BMPs). In addition, largazole inhibited the formation of multinucleated osteoclasts, suggesting that largazole might elicit the dual action to stimulate bone formation and suppress bone resorption.

More importantly, largazole showed *in vivo* bone-forming efficacy in two independent models: the mouse calvarial bone formation assay and the rabbit calvarial bone fracture healing model. In the mouse calvarial bone formation assay, when collagen sponges soaked with largazole were implanted in calvarial bones, largazole induced woven bone formation over the periosteum of the calvarial bones. In the rabbit calvarial bone fracture healing model, while an incomplete bone formation was observed with macroporous biphasic calcium phosphates (MBCPs) alone, newly formed bones in direct contact with MBCPs mixed with largazole were observed. With these data together, largazole shows great clinical potential as a novel treatment for bone-related diseases in addition to its already proven potential as an antitumor agent.

9 Outlook

Since the initial disclosure by the Luesch group in 2008, largazole has been one of the most popular targets for synthesis and biological studies. As described above, eleven total

syntheses of largazole have been reported to date, and a broad range of biological studies has appeared in the literature. Largazole is one of the most isoform-selective HDAC inhibitors developed so far; however, there is still a great need for further improvement on its isoform selectivity. With access to the X-ray structure of largazole complexed with HDAC8, homology modeling, and information on details of their molecular interactions, we expect that the design of more isoform-selective largazole analogues will be one of the most active areas of research. Due to their sensitizing effects, HDAC inhibitors, including largazole, will likely be most effective in combination with other cytotoxic agents. Even the producing cyanobacteria appear to employ largazole in combination with other agents; largazole and the co-produced antimitotic agent symplostatin 4 exerted synergistic activities against colon cancer cell growth.¹¹ HDACs have been implicated in pathological processes other than cancer. Beneficial effects of largazole are actively being evaluated for other disease indications where cellular reprogramming may be desirable. Another exciting avenue in largazole research emerging now is the study of the biosynthetic pathway by the Luesch and Paul groups, since genetic material from the producing cyanobacterium is available (unpublished). Clearly, largazole has inspired multidisciplinary research and continues to do so. Undoubtedly, new discoveries with largazole are to be expected.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 2. Chemical degradation to determine the absolute configuration of stereogenic centers. a) O_3 , CH_2Cl_2 , 25 °C, 30 min; b) H_2O_2 – HCO_2H (1:2), 70 °C, 20 min; c) 6 N HCl, 110 °C, 24 h.

Fig. 3. Putative mechanism of largazole action. A) Modes of action of largazole and FK228 to liberate potent HDAC inhibitors. B) Molecular docking of largazole thiol into an HDAC1 homology model. PG = Protecting group.

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Fig. 5. HDAC8–largazole thiol complex. The catalytic Zn^{2+} ion (red sphere) is coordinated by D178, H180, and D267 (blue sticks). Largazole thiol is shown as a stick figure (C = magenta, N = blue, O = red, and S = yellow). Structural K^+ ions appear as green spheres. Reprinted with permission from K. E. Cole, D. P. Dowling, M. A. Boone, A. J. Phillips and D. W. Christianson, *J. Am. Chem. Soc.*, 2011, **133**, 12474–12477. Copyright 2011 American Chemical Society.

Fig. 6.
Structure–activity relationships of largazole.

Fig. 7.

In vitro and *in vivo* osteogenic activities of largazole. Arrows indicate the region of newly forming woven bone. Reprinted with permission from S.-U. Lee, H. B. Kwak, S.-H. Pi, H.-K. You, S. R. Byeon, Y. Ying, H. Luesch, J. Hong and S. H. Kim, *ACS Med. Chem. Lett.*, 2011, **2**, 248–251. Copyright 2011 American Chemical Society.

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Zn²⁺-dependent (class I, II, IV) HDAC isoform selectivity profile of largazole thiol in direct comparison with redFK228 (IC₅₀ in nM).

Table 1

	Class I			Class II			Class I			Class II			Class IV		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HDAC	1	2	3	4	5	6	7	8	9	10	11				
Largazole thiol	0.4	0.9	0.7	>1,000	>1,000	42	>1,000	102	>1,000	0.5	3				
redFK228*	0.8	1	1.3	647	>1,000	226	>1,000	>1,000	>1,000	0.9	0.3				

* Liberated in situ from FK228 through reduction with DTT.