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## Evaluation of class I HDAC isoform selectivity of largazole analogues

Bumki Kim<sup>a</sup>, Heekwang Park<sup>a</sup>, Lilibeth A. Salvador<sup>b,c</sup>, Patrick E. Serrano<sup>a</sup>, Jason C. Kwan<sup>b,†</sup>, Sabrina L. Zeller<sup>a</sup>, Qi-Yin Chen<sup>b,d</sup>, Soyoung Ryu<sup>a</sup>, Yanxia Liu<sup>b,d</sup>, Seongrim Byeon<sup>a</sup>, Hendrik Luesch<sup>b,d,\*</sup>, and Jiyong Hong<sup>a,e,\*</sup>

<sup>a</sup>Department of Chemistry, Duke University, Durham, North Carolina 27708, United States

<sup>b</sup>Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, Florida 32610, United States

<sup>c</sup>Marine Science Institute, College of Science, University of the Philippines, Diliman, Quezon City, 1100, Philippines

<sup>d</sup>Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville, Florida 32610, United States

<sup>e</sup>Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, United States

### Abstract

Largazole is a potent class I selective histone deacetylase (HDAC) inhibitor. The majority of largazole analogues to date have modified the thiazole-thiazoline and the warhead moiety. In order to elucidate class I-specific structure–activity relationships, a series of analogues with modifications in the valine or the linker region were prepared and evaluated for their class I isoform selectivity. The inhibition profile showed that the C2 position of largazole has an optimal steric requirement for efficient HDAC inhibition and that substitution of the *trans*-alkene in the linker with an aromatic group results in complete loss of activity. This data will aid the design of class I isoform selective HDAC inhibitors.

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\*Corresponding authors: Tel.: 1-919-660-1545; fax: 1-919-660-1605; jiyong.hong@duke.edu. Tel.: 1-352-273-7738; fax: 1-352-273-7741; luesch@cop.ufl.edu.

<sup>†</sup>Current address: Pharmaceutical Sciences Division University of Wisconsin-Madison, Madison, WI, USA

Supplemental information

Supplementary data associated with this article can be found...

### Notes

HL is a co-founder of Oceanyx Pharmaceuticals, Inc., which is negotiating licenses for largazole-related patents and patent applications.

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## Keywords

Largazole; HDAC inhibitor; Class I histone deacetylase; Isoform selectivity; Structure–activity relationship

Largazole (**1**, Fig. 1), isolated from a cyanobacterium of the *Symploca* genus, possesses highly differential growth inhibitory activity towards cancer cells.<sup>1</sup> This selectivity is attributed to its very potent class I histone deacetylase (HDAC) inhibitory activity.<sup>2,3</sup> Because of its potency, class I selectivity, and *in vivo* efficacy,<sup>4</sup> largazole (**1**) possesses great potential as an anti-cancer agent.<sup>5</sup> Additionally, largazole (**1**) has shown a variety of other biological activities, such as the *in vitro* and *in vivo* induction of osteoblast differentiation biomarkers,<sup>6</sup> the sensitization of EBV+ tumor cells to the anti-herpes drug ganciclovir (GCV),<sup>7</sup> the inhibition of ubiquitin activating enzyme, E1,<sup>8</sup> and the *in vitro* and *in vivo* induction of apoptosis in hepatic stellate cells (HSC) in liver fibrosis models.<sup>9</sup> These promising biological activities led to 11 total syntheses<sup>2,3,10–18</sup> and a wide variety of analogues of largazole (**1**).<sup>2–4,10,12,13,16,18–27</sup>

To date, the majority of largazole analogues that have been synthesized and studied have altered the warhead or the thiazole-thiazoline moiety. For example, previous attempts to replace the thiol moiety of largazole thiol (**2**, Fig. 1) with other Zn<sup>2+</sup> chelating groups have resulted in significant decreases in inhibitor potency.<sup>2,10,13,21,25</sup> While the active site of HDACs is highly conserved, sequence variety in the cap region is relatively high.<sup>28</sup> It is believed that the interactions between this hydrophobic cap region and the macrocycle of largazole (**1**) influence its class selectivity.<sup>5,29</sup> However, changes in the thiazole-thiazoline unit of largazole (**1**) have not resulted in any significant improvement in its potency or isoform selectivity.<sup>18,21–23,26</sup>

In contrast, relatively little work has been done on the valine subunit at the C2 position or the nature of the linker as only aliphatic linkers have been studied. Furthermore, the limited work on these analogues has mostly focused on anti-proliferative activity rather than HDAC isoform selectivity. Here we report the class I isoform selectivity profile of several C2 and linker analogues of largazole (**1**) to provide valuable insights for future isoform selective analogue design.

Previous structure–activity relationships have shown that the valine residue at the C2 position of largazole (**1**) is able to withstand certain variations without significant loss in HDAC inhibitory activity.<sup>4,16,18,19,21</sup> Therefore, compounds **3–6** were prepared by replacing the valine residue with aromatic (Phe, Tyr), basic (His), and acidic (Asp) amino acids to investigate the effect of different chemical functionalities on the class I HDAC isoform selectivity (Fig. 2).<sup>30</sup> Compounds **3–6** have been shown to retain anti-proliferative activity in HCT116 colon cancer cells.<sup>4</sup>

The inhibition profile showed that **3–6** are very weak HDAC8 inhibitors, implying that the largazole scaffold has an intrinsic preference towards HDACs 1, 2, and 3 over HDAC8 (Table 1). Also, while **3–5** were comparable in potency to largazole thiol (**2**), aspartic acid analogue **6** experienced a significant reduction in activity. It is notable that there was an

overall decrease in HDAC2 inhibition and that histidine analogue **5** showed minor selectivity towards HDAC1 over HDACs 2 and 3 (7- and 5.5-fold, respectively).

Based on these observations, a second set of analogues was designed to isolate possible interactions between HDAC1 and the imidazole ring of **5** (Fig. 3). Compounds **7–10** mimicked the position of the two different nitrogens present in histidine. We expected that the addition of an *N*-Boc group in **11** would block the hydrogen bond donating ability of the imidazole. The synthesis of **7–11** very closely followed that of **5** and **6**.<sup>31</sup>

As summarized in Table 2, compounds **7** and **8** showed decreased activity for all HDACs tested. However, the longer alkyl chain analogue **8** showed a higher potency than the shorter alkyl chain analogue **7** for HDACs 1, 2, and 3. The same trend was observed for the corresponding *N*-Boc protected analogues, as **10** was more potent than **9** for all HDACs tested. This suggests that there is an optimal steric requirement at the C2 position for efficient HDAC inhibitory activity. The *N*-Boc protected histidine analogue **11** showed very little change in potency or selectivity, demonstrating that the hydrogen bonding of the histidine might be a non-essential interaction in HDAC inhibition.

There are two phenylalanine residues (Phe150 and Phe205) in the active site of HDAC1.<sup>32</sup> To utilize  $\pi$ - $\pi$  stacking interactions for the improvement of HDAC1 selectivity, we designed linker analogues **12–14** via docking studies<sup>33</sup> on a previously reported homology model of HDAC1 (Fig. 4).<sup>32</sup> Compounds **12–14** were prepared<sup>34</sup> and evaluated for their class I HDAC inhibition. Unfortunately, **12–14** showed no activity against class I HDACs. This complete lack of HDAC inhibition could be due to steric effects of bulky aromatic groups in the linker preventing the adoption of docking poses predicted *in silico*.

In summary, we have prepared and tested a variety of C2 and linker analogues and probed them for their class I HDAC inhibition. Our results provide the following insights into the structure–activity relationship of largazole (**1**). First, the largazole scaffold has an innate preference for HDACs 1, 2, and 3, as every analogue prepared only weakly inhibits HDAC8. Second, the addition of aromatic groups to the linker region is detrimental to HDAC inhibition. Third, the hydrogen bonding of the imidazole ring in the histidine analogue **5** does not play an essential role in HDAC inhibition. Most importantly, the C2 position has an optimal steric requirement that needs to be met for efficient HDAC inhibition. Compounds **7–11** support this insight as the shorter alkyl chain showed weaker inhibition for all class I HDACs. This is further supported by the weak inhibitory activity of the aspartic acid analogue **6**, and the relatively strong inhibition of **2–5** and **11**. The bulkier groups in these analogues did not significantly reduce the inhibitory activity, suggesting that even larger groups could be installed at this position with minimal loss in HDAC inhibition activity. The trends presented here further extend the existing structure–activity relationship of largazole (**1**), and will aid in the design process of class I isoform selective HDAC inhibitors.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

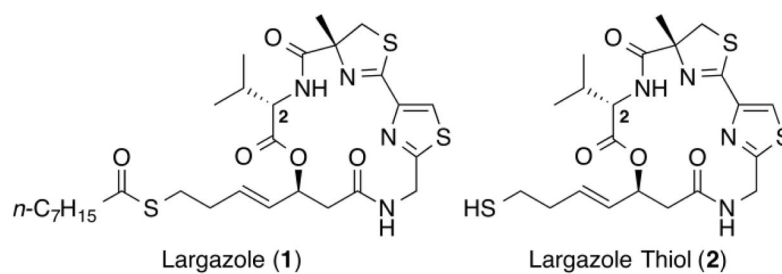
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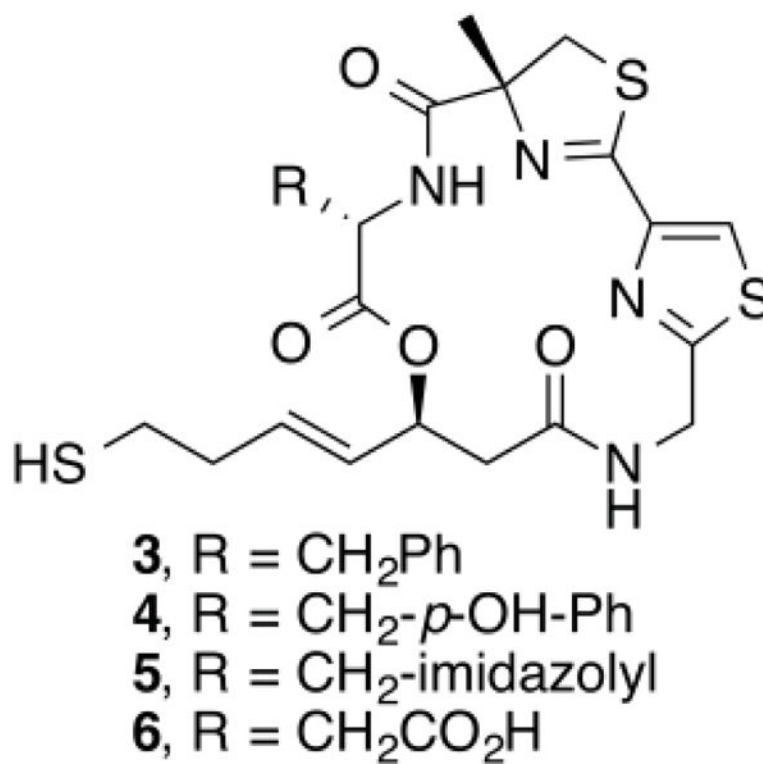
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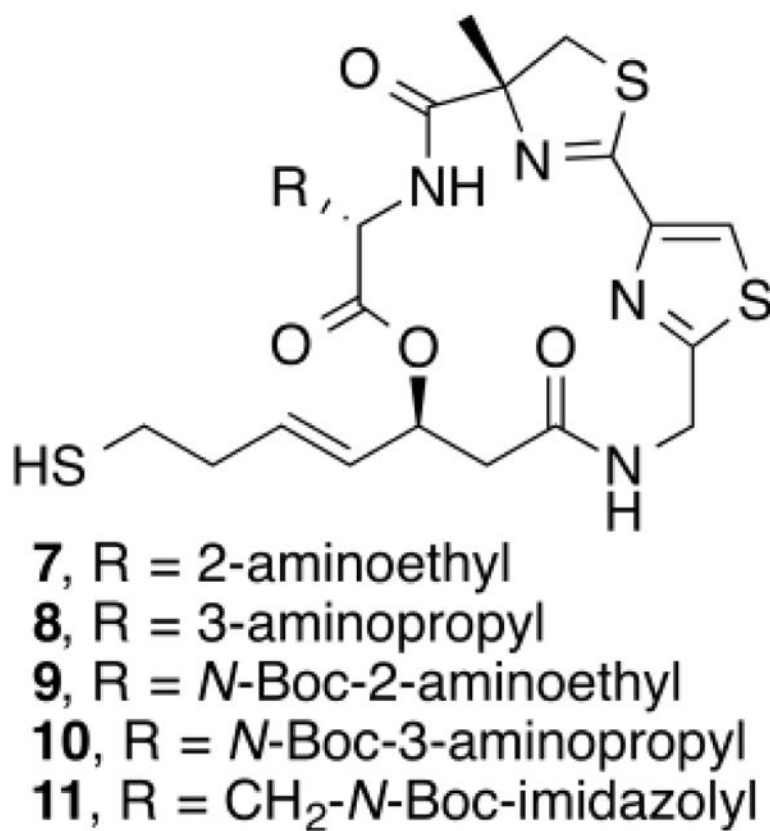
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31. For details on the synthesis of 7–11, see the Supporting Information.
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33. For details on the docking study, see the Supporting Information.
34. For details on the synthesis of 12–14, see the Supporting Information.



**Figure 1.**  
Structure of largazole (1) and largazole thiol (2).

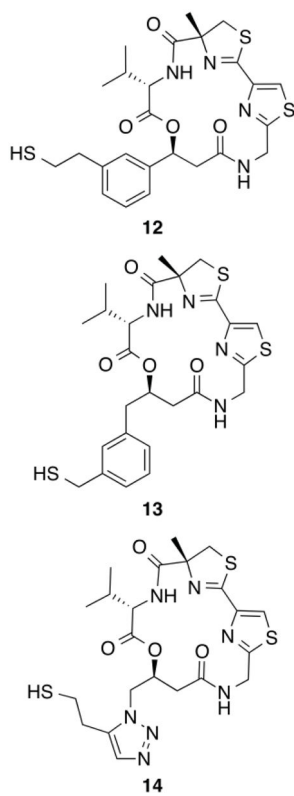


**Figure 2.**  
Structure of analogues 3–6.



**Figure 3.**  
Structure of analogues 7–11.

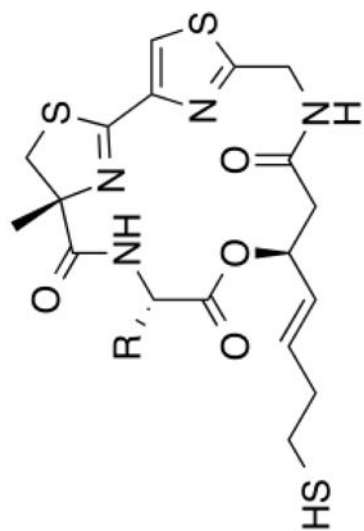




**Figure 4.**  
Structure of analogues **12–14**.

Table 1

Class I HDAC isoform selectivity of 3–6

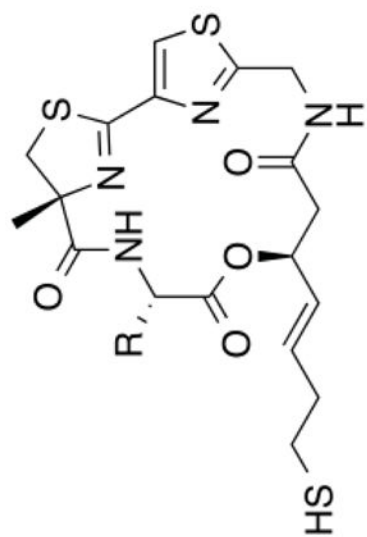


compound	R	IC <sub>50</sub> (nM)					
		HDAC1	HDAC2	HDAC3	HDAC8	IC <sub>50</sub> (HDAC2) IC <sub>50</sub> (HDAC1)	IC <sub>50</sub> (HDAC3) IC <sub>50</sub> (HDAC1)
2 <sup>a</sup>		0.40	0.90	0.70	102	2.3	1.8
3		0.29	1.70	0.68	NI <sup>b</sup>	5.9	2.3
4		0.21	1.10	0.38	NI <sup>b</sup>	5.2	1.8
5		0.20	1.40	1.10	NI <sup>b</sup>	7.0	5.5
6		39	150	100	NI <sup>b</sup>	3.9	2.6

<sup>a</sup>Data from ref. 5.

<sup>b</sup>No inhibition up to 1  $\mu$ M.

Table 2

Class I HDAC isoform selectivity of **7–11**

compound	R	IC <sub>50</sub> (nM)							
		HDAC1	HDAC2	HDAC3	HDAC8	HDAC10	HDAC11	HDAC12	HDAC13
<b>2<sup>a</sup></b>		0.40	0.90	0.70	102	2.3	1.8		
<b>7</b>		5.50	21.00	7.80	NI <sup>b</sup>	3.8	1.4		
<b>8</b>		3.30	6.30	6.00	NI <sup>b</sup>	1.9	1.8		
<b>9</b>		2.30	3.10	4.20	NI <sup>b</sup>	1.4	1.8		
<b>10</b>		0.96	1.40	1.80	790	1.5	1.9		
<b>11</b>		0.62	1.10	1.10	540	1.8	1.8		

<sup>a</sup>Data from ref. 5.

No inhibition up to 1  $\mu$ M.

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