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Syntheses of Amamistatin Fragments and Determination of Their HDAC and Antitumor Activity

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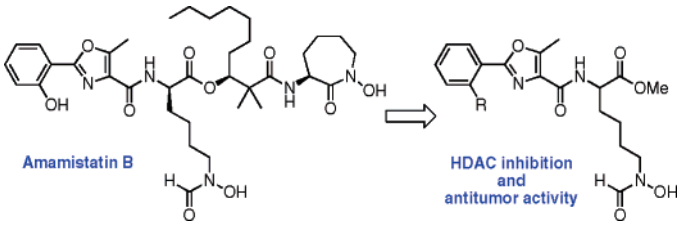
ABSTRACT



Amamistatins A and B are natural products found to have anti-proliferative effects against MCF-7, A549, and MKN45 human tumor cell lines (IC_{50} 0.24–0.56 μM). It was proposed that their activity was due to histone deacetylase (HDAC) inhibition mediated by the *N*-formyl-*N*-hydroxy lysine moiety. Amamistatin B fragment analogs were synthesized and screened for biological activity. These compounds were modest HDAC inhibitors and showed antitumor activity against MCF-7 and PC-3 human tumor cells.

Histone deacetylase (HDAC) enzymes work with histone acetyltransferases (HATs) to modulate levels of histone acetylation, which are directly related to gene expression.[1,2] Aberrant recruitment of histone deacetylases can lead to gene silencing and tumor growth, and small molecule HDAC inhibitors cause differentiation, growth arrest, and apoptosis in tumor cells.[3] Many HDAC inhibitors such as Trichostatin A (TSA)[4] and SAHA[5] (Figure 1) contain a hydroxamic acid moiety, used to chelate the catalytic Zn^{2+} found in the enzyme active site. The *N*-formyl hydroxylamine, or retro-hydroxamate, is an alternative zinc-binding ligand that has also been employed in the synthesis of HDAC inhibitors.[6,7]

Amamistatins A and B are natural products isolated from the actinomycete *Nocardia asteroides*.[8] Amamistatin A was found to have anti-proliferative effects against MCF-7 breast, A549 lung, and MKN45 stomach human tumor cell lines, with IC_{50} values of 0.48, 0.56, and 0.24 μM, respectively. The amamistatin structure, containing a lysine-derived *N*-formyl hydroxylamine, is very similar to that of related natural products formobactin,[9] nocobactin,[10] brasilibactin,[11] and the mycobactins[12] (Figure 2). We proposed that the anti-

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(1) Meinke, P. T.; Liberator, P. *Curr. Med. Chem.* **2001**, *8*, 211−235.
(2) Johnstone, R. W. *Nat. Rev. Drug Discovery* **2002**, *1*, 287−299.
(3) Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097−5116.
(4) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, *265*, 17174−17179.
(5) Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003−3007.
(6) Wu, T. Y. H.; Hassig, C.; Wu, Y.; Ding, S.; Schultz, P. G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 449−453.
(7) Nishino, N.; Yoshikawa, D.; Watanabe, L. A.; Kato, T.; Jose, B.; Komatsu, Y.; Sumida, Y.; Yoshida, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2427−2431.
(8) (a) Suenaga, K.; Kokubo, S.; Shinohara, C.; Tsuji, T.; Uemura, D. *Tetrahedron Lett.* **1999**, *40*, 1945−1948. (b) Kokubo, S.; Suenaga, K.; Shinohara, C.; Tsuji, T.; Uemura, D. *Tetrahedron* **2000**, *56*, 6435−6400.
(9) Murakami, Y.; Kato, S.; Nakajima, M.; Matsuoka, M.; Kawai, H.; Shin-Ya, K.; Seto, H. *J. Antibiot.* **1996**, *49*, 839−845.
(10) Ratledge, C.; Snow, G. A. *Biochem. J.* **1974**, *139*, 407−413.
(11) Tsuda, M.; Yamakawa, M.; Oka, S.; Tanaka, Y.; Hoshino, Y.; Mikami, Y.; Sato, A.; Fujiwara, H.; Ohizumi, Y.; Kobayashi, J. *J. Nat. Prod.* **2005**, *68*, 462−464.
(12) Snow, G. A. *Bacteriol. Rev.* **1970**, *34*, 99−125.

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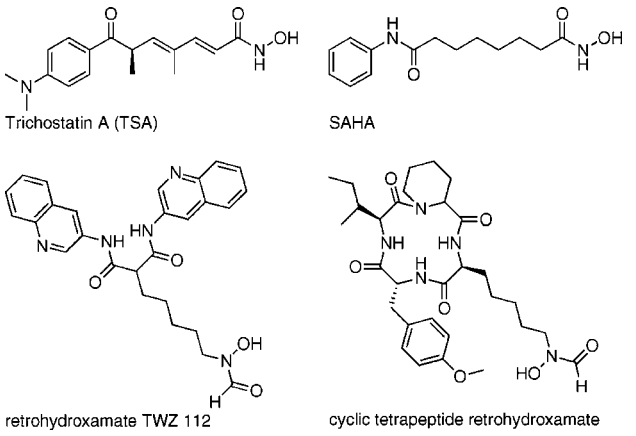


Figure 1. Hydroxamic acid and retrohydroxamate HDAC inhibitors.

cancer activity exhibited by the amamistatins and related compounds was due to HDAC inhibition mediated by the *N*-formyl hydroxylamine ligand.

As an initial test of our hypothesis, we synthesized a set of compounds containing the retrohydroxamate, which corresponds with a portion of the amamistatin structure. With the *N*-formyl hydroxylamine in place for Zn^{2+} coordination, the hydroxyphenyl oxazole moiety should be in a position to mimic the aromatic cap groups found in many HDAC inhibitors.

Synthesis of the hydroxyphenyl oxazole fragment began with salicylic acid and threonine protected as the benzyl ether and benzyl ester, respectively (Scheme 1). EDC-mediated coupling afforded α-hydroxy amide **3**, and one-pot cyclization and oxidation[13] led to oxazole **4** without the need to isolate the intermediate oxazoline. Acid **5** was revealed via hydrogenolytic removal of both benzyl protecting groups.

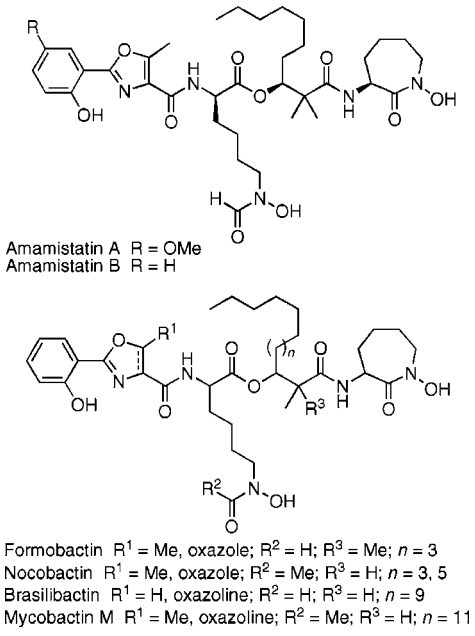
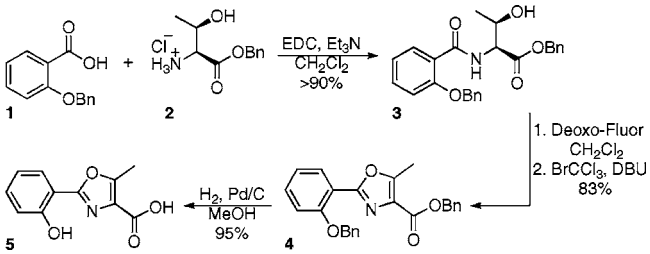


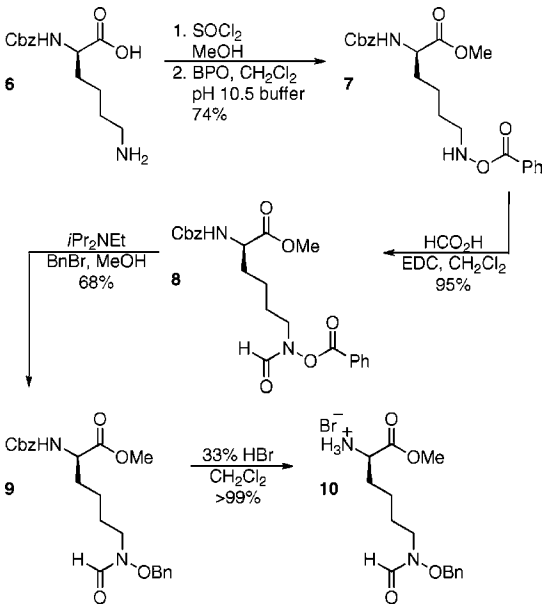
Figure 2. Amamistatins A and B and related natural products.

Scheme 1. Synthesis of Hydroxyphenyl Oxazole



The first step in the synthesis of the *N*-formyl hydroxylamine fragment was methyl esterification of *N*-α-Cbz-D-lysine **6** (Scheme 2). Oxidation of the terminal amino group

Scheme 2. Synthesis of Protected Lysine Retrohydroxamate



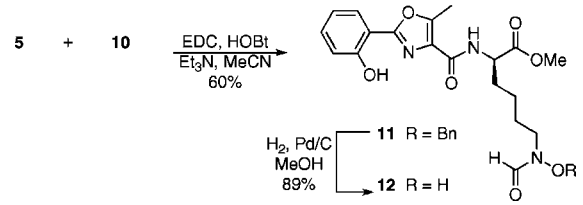
with dibenzoyl peroxide[14] was followed by formylation. Removal of the benzoyl group and reprotection as the benzyl hydroxamate led to the more stable compound **9**. Cbz deprotection with HBr gave the hydrobromide salt **10** in quantitative yield.

Oxazole **5** and retrohydroxamate **10** were joined via an EDC-mediated coupling to give amide **11**. Deprotection by hydrogenolysis revealed the *N*-formyl hydroxylamine (**12**, Scheme 3). A small set of related retrohydroxamates was prepared using the same method starting from benzoic acid and *N*-α-Cbz-L-lysine.

The amamistatin-based retrohydroxamates **12**−**16** were screened in an enzymatic HDAC assay, as well as in cellular

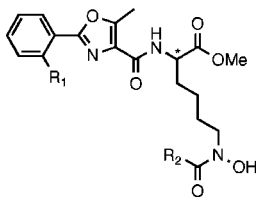
(13) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165−1168.
(14) Wang, Q. X.; King, J.; Phanstiel, O., IV. *J. Org. Chem.* **1997**, *62*, 8104−8108.

Scheme 3. Synthesis of Amamistatin-Based HDAC Inhibitors



assays against MCF-7 (breast cancer) and PC-3 (prostate cancer) cells. The assay results are summarized in Table 1.

Table 1. HDAC Inhibition and Antitumor Activity of Amamistatin-Based Retrohydroxamates



				inhibition (%)		
	R_1	R_2	*	HDAC (10 μM)	MCF-7 (20 μM)	PC-3 (20 μM)
12	OH	H	*R*	45	19	63
13	H	H	*R*	22	18	22
14	OH	H	*S*	73	65	43
15	H	H	*S*	53	65	23
16	OH	Me	*S*	0	50	18

The compounds containing *N*-formyl hydroxylamine (**12**−**15**) showed modest HDAC inhibition, whereas the *N*-acetyl hydroxylamine **16** was inactive. This mirrored what has been reported in the literature for retrohydroxamate HDAC inhibitors.[6,7] Interestingly, the level of HDAC inhibition for these compounds did not correlate with growth inhibition in either the MCF-7 or PC-3 cellular assays. This indicates that although the amamistatin-based *N*-formyl hydroxylamines may have an effect on tumor cell growth via HDAC inhibition, alternate mechanisms of action must also be involved.

To follow up on this work, the syntheses of Amamistatin B and analogs were completed. These compounds, also containing the *N*-formyl hydroxylamine ligand, showed no HDAC inhibition, even at a concentration of 100 μM. This same lack of HDAC inhibition was also observed with synthetic brasilibactin A, as reported by Shaw.[15] Amamistatin B and analogs were, however, extremely potent growth inhibitors of MCF-7 cells, with IC_{50} values of 120−200 nM. Full details regarding the synthesis and biological activity of these compounds will be reported shortly.

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Supporting Information Available: Experimental procedures and NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Mitchell, J. M.; Shaw, J. T. *Org. Lett.* **2007**, *9*, 1679−1682.