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Design and synthesis of histone deacetylase inhibitors: the development of apicidin transition state analogs

Steven L. Colletti,[a,*] Robert W. Myers,[b] Sandra J. Darkin-Rattray,[b] Dennis M. Schmatz,[b] Michael H. Fisher,[a] Matthew J. Wyvratt[a] and Peter T. Meinke[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co., Inc., Rahway, NJ 07065, USA*
[b]*Department of Parasite Biochemistry and Cell Biology, Merck Research Laboratories, Merck & Co., Inc., Rahway, NJ 07065, USA*

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Abstract

A four step degradation of the C8 ethyl ketone of apicidin provided a route to the C6 aldehyde intermediate and several mechanism-based transition state inhibitors of histone deacetylase. The compounds generated herein delineate the significance of apicidin's side chain, highlighted by the high affinity C8 aldehyde and C8-keto-9,10-epoxide analogs of apicidin. © 2000 Elsevier Science Ltd. All rights reserved.

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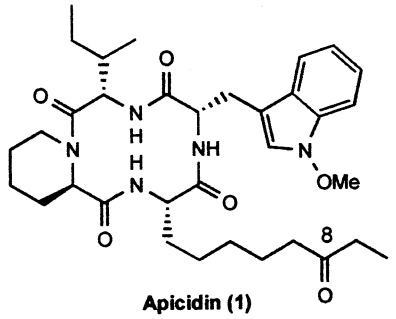
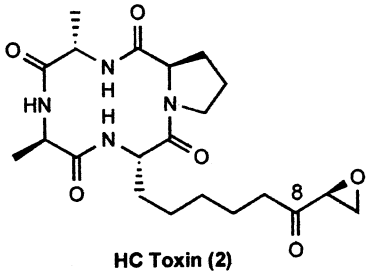
The recently discovered cyclic tetrapeptide apicidin[1] (**1**) is a potent broad spectrum antiprotozoal agent which derives its biological activity from the reversible inhibition of the zinc metallopeptidase, histone deacetylase[2] (HDAC). HDACs are nuclear isozymes that regulate gene transcription via the deacetylation of acetylated lysine residues (**3**→**5**) within the basic N-termini



Apicidin (1)



HC Toxin (2)

* Corresponding author. Fax: 732/594-5966; e-mail: steve_colletti@merck.com

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of their endogenous histone substrates (Fig. 1).[3] This deacetylation event modulates chromatin structure by producing cationic lysine residues (**5**) that facilitate histone binding to DNA through favorable ionic interactions with its anionic phosphodiester backbone. We have recently demonstrated that apicidin exhibits comparable affinities for both mammalian and protozoan HDAC, leading to histone hyperacetylation, subsequent mitotic arrest and ultimately cell death.[4] Consequently, we have been involved in the application of an HDAC inhibitor to the treatment of protozoal infections.[5]

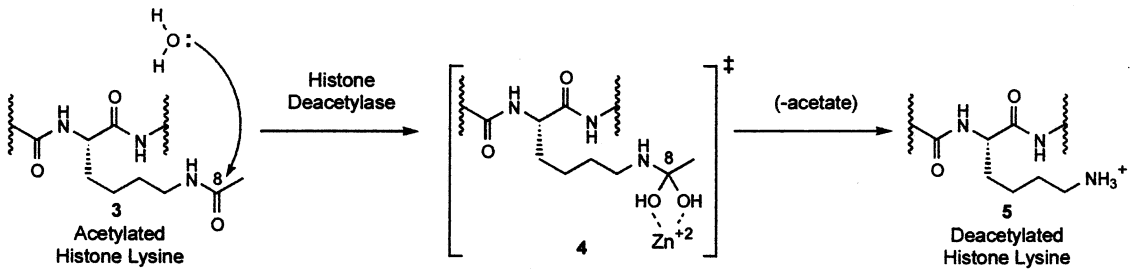
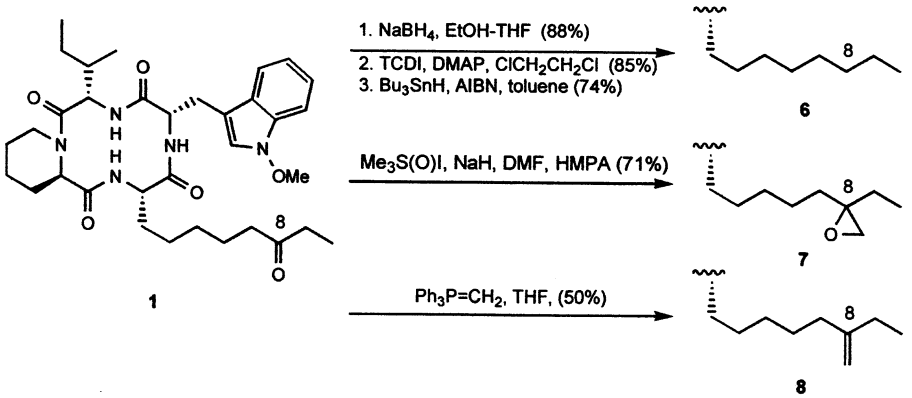


Figure 1. Histone deacetylase catalyzed amide hydrolysis

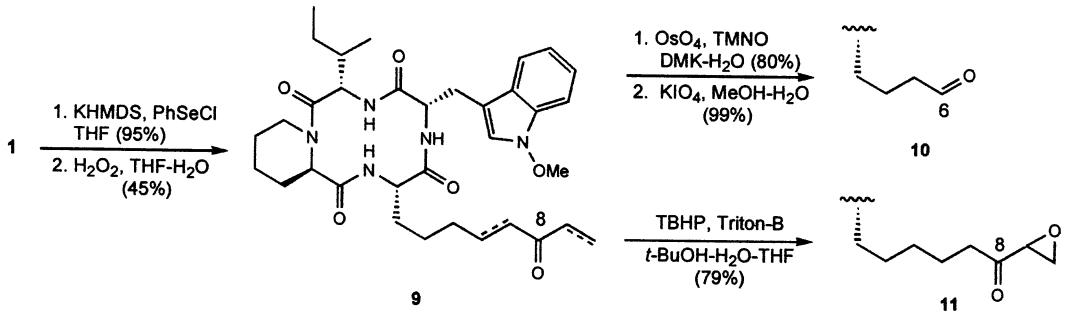
The structural homology between apicidin's 2-amino-8-oxodecanoic acid side chain and **3** suggests that **1** mimics an in-register acetylated histone lysine. Using this model, we sought to develop chemistry of the C6–C9 side chain registers of apicidin, providing access to mechanism-based inhibitors based upon transition state structure **4** (Fig. 1).[6] The synthesis of such analogs assisted the generation of biochemical reagents for HDAC assay development, mode of action studies and structure–activity relationship development.[7]

Initial entry into the study and support of the mode of action described above (Fig. 1) required the synthesis of des-keto apicidin **6**. Optimal conditions involved a Barton–McCombie dehydroxylation[8] of the C8 alcohol via the intermediate thionocarbamate (Scheme 1). Additionally, the C8 disubstituted epoxide **7** was prepared via the sulfur ylide,[9] and the C8 disubstituted olefin **8** was prepared under standard Wittig conditions. All three virtually inactive analogs supported the requirement of the C8 ketone for HDAC binding affinity.



Scheme 1.

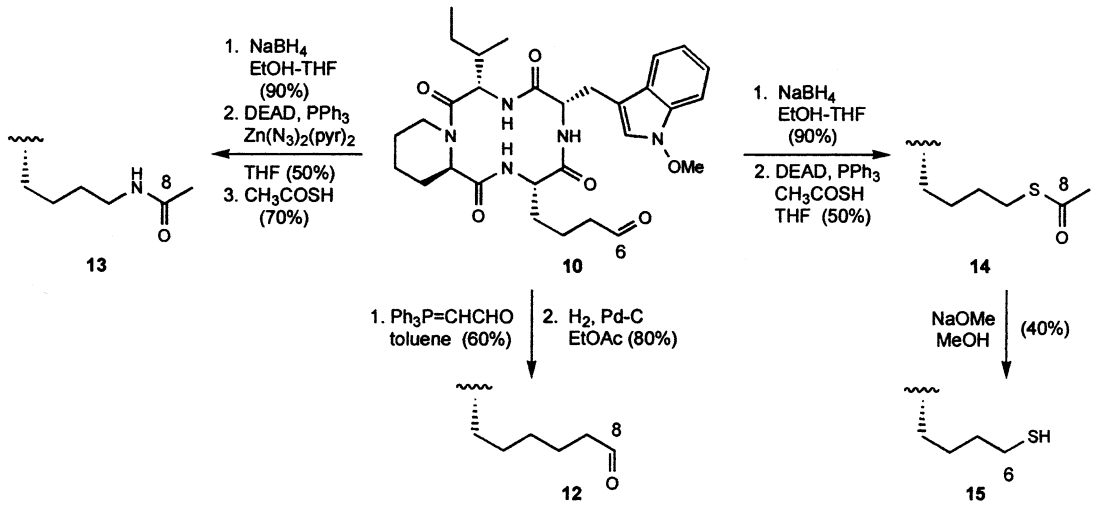
Attention was then focused towards gaining synthetic access to the C6 position of the apicidin side chain, allowing the flexibility to install various functionality at the C8 position. The C6-aldehyde **10** was targeted as an intermediate for this purpose. Accordingly, the successful oxidative degradation of apicidin's side chain required relatively mild reaction conditions in the presence of the acid-labile Pip-Ile juncture, an exceedingly nucleophilic amide ring nitrogen of the *N*-methoxy tryptophan residue[10] and the oxidatively sensitive indole 2,3-bond. A four step approach to **10** was enlisted as shown in Scheme 2.[11] The stoichiometric α-selenation of C8 ketone **1** provided a regiochemical mixture of the 7- and 9-selenides, which were oxidatively eliminated to produce the internal and external enone mixture **9**. Modest regiochemical control of the enone mixture was possible by controlling the enolization site with temperature (−78°C (3:1) 6,7:9,10 enones versus 0°C (1:1) 6,7:9,10 enones). The inseparable enones were then dihydroxylated, and the resulting separable diol mixture was oxidatively cleaved to the desired C6 aldehyde **10**. Alternatively, intermediate **9** was also epoxidized under basic conditions[12] to provide the key external 9,10-α-ketoepoxide **11** (HDAC, IC_{50}<100 pM) along with the separable internal 6,7-α-ketoepoxide (not shown). The increase in observed inhibitory potency of **11** is remarkable, relative to the structurally related α-ketoepoxide cyclic tetrapeptide HC Toxin (**2**)[5,13] (HDAC, IC_{50} = 8 nM) and apicidin (**1**) itself (HDAC, IC_{50} = 1 nM). The basis for the enhanced binding affinity induced by the epoxide moiety in **11** will be addressed in due course.



Scheme 2.

A range of mechanism-based inhibitors was prepared from common intermediate **10** to support a mode of action involving the transition state structure **4** (Fig. 1). Homologation of **10** provided the C8 aldehyde **12** as shown in Scheme 3. In their hydrated form, aldehydes are known zinc chelators and transition state mimics.[14] Accordingly, the C8 aldehyde **12** retained the HDAC binding affinity of apicidin. Thiols are also known zinc chelating ligands.[14] The synthesis of the C6 thiol **15** places sulfur at the 7 position, a compound devoid of HDAC binding. This result is reversed, however, with the C7 thiol[6] (not shown), which places the sulfur atom at the 8 position in register with the C8 ketone of apicidin.

The apicidin acetylated histone lysine surrogate **13** was also prepared from **10** via the Mitsunobu introduction of azide followed by concomitant azide reduction and acetylation with thiolacetic acid.[15] The corresponding thiolacetate **14** was also prepared under analogous Mitsunobu conditions directly with thiolacetic acid.[16] Both the acetamide **13** and thiolacetate **14** displayed diminished HDAC binding affinity, consistent with the expectation that these analogs would be processed by the enzyme. Possessing a cleavable acetate moiety, analogs **13** and **14** were designed as biochemical reagents for the continuous monitoring of time-dependent HDAC



Scheme 3.

enzyme activity through the release of (radioactive) acetate from **13** and/or the detection of the liberated thiol group from **14**. Their utility as surrogate substrates is under investigation.

Described herein is the chemical synthesis of mechanism-based transition state inhibitors derived from apicidin and the discovery of novel compounds displaying high affinity to HDAC. A portion of apicidin's pharmacophore has been identified, leading to the development of potential antiprotozoal agents.[17]

Acknowledgements

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10. The amide ring nitrogen of the *N*-methoxy tryptophan residue was readily hydroxylated (MCPBA), methoxylated ($Pb(OAc)_4$–MeOH), brominated (NBS) and formylated (*N*-formylbenzotriazole), all at room temperature.
11. Synthesis of **10**: Amorphous apicidin (**1**) (2 g, 3.2 mmol) (prepared by dissolution into hot CH_2Cl_2, cooled, conc. in vacuo and vacuum pumped) was dissolved in THF (32 mL) and cooled to ca. −78°C under nitrogen. A solution of KHMDS in toluene (14 mL, 0.5 M) was slowly added, and the reaction mixture maintained for 15 min. Neat PhSeCl (2.5 g, 12.8 mmol) was then added at −78°C, and the mixture aged for 1 h; TLC control (quenched aliquots, partitioned and dried as below): SiO_2, 1:3:96, NH_4OH:MeOH:$CHCl_3$, $R_f=0.34$ (**1**); $R_f=0.53$ (selenides). The reaction mixture was then quenched at −78°C with saturated $NaHCO_3$ (aq.), warmed to 23°C, partitioned with CH_2Cl_2, dried over Na_2SO_4 and conc. in vacuo. The light brown solid was purified by flash column chromatography (SiO_2, 8×14 cm, hexane→50% EtOAc–hexane→50% acetone–hexane gradient) to provide 2.36 g (95%) of the selenide product as a white powder. The pure selenide mixture (90 mg, 0.11 mmol) was dissolved in THF (2.3 mL), cooled to 0°C, and 30% H_2O_2 (aq.) (0.26 mL, 2.3 mmol) was added. The mixture was quickly warmed to 50°C for 10 min, immediately cooled to 0°C and quenched by the slow addition of saturated $Na_2S_2O_3$ (aq.). The mixture was partitioned between CH_2Cl_2 and brine, and the organics dried over Na_2SO_4 and conc. in vacuo; TLC (SiO_2, 1:3:96, NH_4OH:MeOH:$CHCl_3$, $R_f=0.34$). The product was purified by preparative reverse phase HPLC (C-8) using a gradient elution of 40% acetonitrile–H_2O→acetonitrile over 60 min at 20 mL/min to provide 30 mg of **9** (45%). Enone mixture **9** (13 mg, 0.021 mmol) was dissolved in (8:1) acetone:H_2O (1 mL), $TMNO{\cdot}2H_2O$ (5 mg, 0.047 mmol) was added, the mixture cooled to 0°C, aqueous OsO_4 (0.088 mL, 0.024 M) was added and the mixture warmed to 23°C. After 2 h, the mixture was quenched with 10% $NaHSO_3$ (aq.), aged 10 min and partitioned between brine and 30% isopropanol–$CHCl_3$. The organics were dried over Na_2SO_4, conc. in vacuo and purified by PTLC to provide 11 mg (80%) of the diol mixture [SiO_2, 250 micron, 20×20 cm, 50% acetone–hexane (1 development)→1:3:96, NH_4OH:MeOH:$CHCl_3$ (1 development), $R_f=0.27$ (6,7-diol); $R_f=0.18$ (9,10-diol)]. The internal 6,7-diol (8 mg, 0.011 mmol) was dissolved in (5:1) MeOH:H_2O (1 mL) and KIO_4 (5 mg, 0.023 mmol) was added, the mixture aged at 23°C for 8 h and then conc. in vacuo. The residue was partitioned between EtOAc and H_2O, the organics dried over Na_2SO_4, conc. in vacuo and purified by PTLC to provide 7 mg (99%) of **10** [SiO_2, 250 micron, 20×10 cm, 50% acetone–hexane, $R_f=0.50$ (**10**); $R_f=0.70$ (**1**)]. All compounds were characterized by MS and ^1H NMR. This supporting data, including HPLC, is available (34 pages).
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