Pergamon

Tetrahedron Letters 41 (2000) 7831–7835

TETRAHEDRON LETTERS

Synthesis of side chain modified apicidin derivatives: potent mechanism-based histone deacetylase inhibitors

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

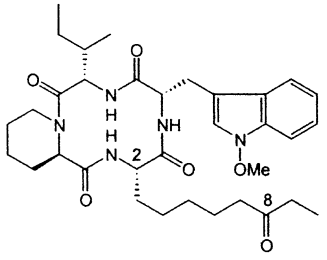
Received 24 March 2000; revised 3 August 2000; accepted 7 August 2000

Abstract

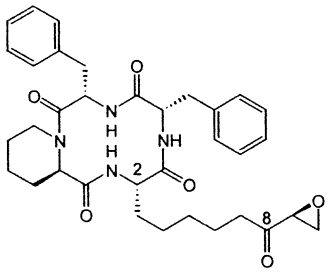
An efficient degradation of apicidin's ketone-containing side chain to two common intermediates (the C7-aldehyde and the C8-methyl ester) is described. From these intermediates, a series of potent mechanism-based histone deacetylase inhibitors was prepared to facilitate biochemical studies.

Keywords: histone deacetylase; apicidin; trapoxin; trichostatin; inhibitor; malaria.

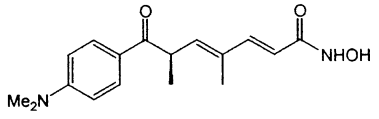
The potent histone deacetylase (HDAC) inhibitor apicidin (**1**) is a novel natural product recently isolated[1] from fungi (*Fusarium* sp.) collected in Costa Rica. This cyclic tetrapeptide exhibits cidal activity against apicomplexan protozoa, preventing the development of intracellular parasites which cause diseases such as malaria, cryptosporidiosis, toxoplasmosis, and coccidiosis.[2] HDAC is an integral component in the functional regulation of gene transcription, contributing to control of the dynamic acetylation/deacetylation that occurs at specific lysine residues in histones.[2] Structural similarities between apicidin's 2-amino-8-oxodecanoic acid (Aoda) moiety and these acetylated lysine residues (**2**) suggests that **1** is a functional mimic of **2**, reversibly inducing histone hyperacetylation, causing altered transcriptional regulation and ultimately cell death.



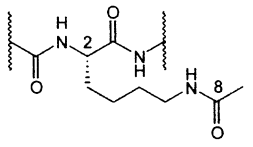
Apicidin (1)



Trapoxin (3)



Trichostatin A (4)



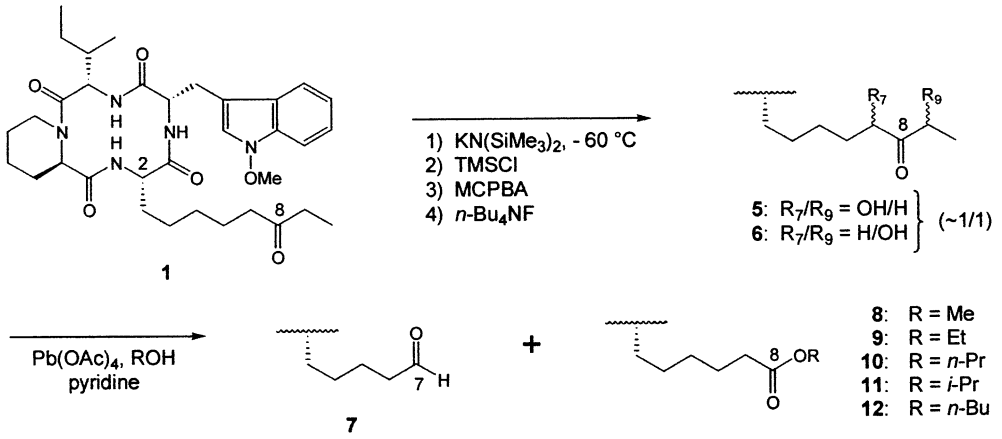
Acylated Histone Lysine (2)

* Corresponding author. Fax: (732) 594-9556; e-mail: peter_meinke@merck.com

0040-4039/00/$ - see front matter
PII: S0040-4039(00)01364-2

Apicidin is structurally related to a family of cyclic tetrapeptides,[3a–f] typified by trapoxin[3f] (**3**), which are known HDAC inhibitors with pronounced anti-neoplastic activity. Additional *potent* inhibitors of this zinc metalloamidase[4] are largely restricted to hydroxamic acid-containing molecules,[5] such as the natural product trichostatin A (**4**)[6] and related synthetic derivatives,[7] or cyclic tetrapeptides containing reactive α-epoxyketone surrogates.[3a,8] Apicidin, however, unlike these other HDAC inhibitors, is unique in that it alone lacks the pharmaceutically undesirable α-epoxyketone or hydroxamate functionality whose presence is a prerequisite for biological efficacy. Consequently, a series of side chain modified apicidin analogs[9] were synthesized in order to delineate the Aoda's contribution to HDAC inhibition, to prepare mechanism-based HDAC inhibitors with increased potency, and to prepare valuable biochemical tools for mechanism of action investigations. Ultimately, these efforts may lead to the design of superior, nonpeptidyl acyclic mimics of apicidin with diverse potential in cancer and antiprotozoal chemotherapy.

A facile and suitably mild degradation of apicidin's Aoda was developed, which yielded two common intermediates and permitted ready access to all oxidation states at positions 7, 8 and 9 (Scheme 1). Although apicidin, not surprisingly, exhibits extremely poor solubility characteristics in many common organic solvents, refluxing **1** in THF (0.02 M) permitted its eventual dissolution, and these solutions remained homogenous at temperatures as low as −60°C. Subsequent treatment of a cooled solution of **1** with $KN(SiMe_3)_2$ (5 equiv.) in the presence of TMSCl (4 equiv.) generated a mixture of C7 and C9 enol ethers.[10,11] These intermediate enol ethers were not purified but, following a rapid workup, were instead subjected to Rubottom oxidation with MCPBA (1.1 equiv.) which produced, following deprotection with n-Bu_4NF, the desired α-hydroxyketones **5** and **6** as a separable mixture of regioisomeric diastereomers (~1:1) in 76% overall yield from **1**. More commonly, however, these alcohols were subjected directly to oxidative cleavage using lead tetraacetate in methanol containing one weight equivalent of pyridine[12] to produce the readily separable C7-aldehyde (**7**, 35%) and the corresponding C8-methyl ester (**8**, 39%). When this reaction was performed utilizing alternative solvents (e.g. EtOH, n-PrOH, i-PrOH, n-BuOH), more lipophilic esters (**9**–**12**, respectively) were obtained in comparable yields.

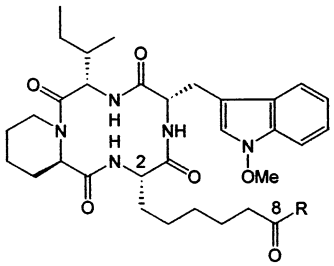


Scheme 1.

Ester **8** served as starting material for the derivatives shown in Table 1. Saponification of **8** with 1N LiOH (1.1 equiv.) in 3:1:1 THF:MeOH:H_2O[13] generated the corresponding carboxylic

acid **13**, which was used directly with minimal purification (extraction only). From this acid in good yields were prepared *N*-methyl amide **14**, hydroxamic acid **15** and Weinreb amide **16** under standard EDCI-coupling conditions. Successful preparation of **15** via its *O*-benzyl protected hydroxamic acid was precluded by the *N*-methoxy-indole's lability to hydrogenolysis, yielding only *N*-desmethoxy-**15** along with the corresponding *N*-desmethoxy-C8-carboxamide (not shown). Exposure of Weinreb amide **16** to a series of Grignard reagents (methyl, *n*-propyl, isopropyl, *n*-butyl, phenyl) in large excess (5–10 equiv.) yielded cleanly the substituted C8 ketones **18**–**21**. The reaction of **16** failed completely when using vinyl Grignard, producing solely the C8-aldehyde **17** in low yield (23%). Also, despite repeated attempts, the synthesis of the C8-trifluoromethyl ketone failed, either by reacting the C8-carboxylic acid **13** with $(CF_3CO)_2O$/DMAP[14] or the C8-aldehyde **17** with Me_3SiCF_3/*n*-Bu_4NF.[15]

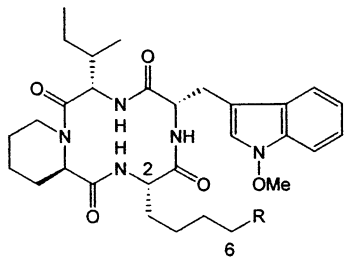
Table 1



8:	R = OMe	Reagents
13:	R = OH	(From **8** using 1N LiOH, 3:1:1 THF:H_2O:MeOH)
14:	R = NHMe	(From **13** using EDCl·HCl, HCl·H_2NMe, DIEA, DMAP, DMF)
15:	R = NHOH	(From **13** using EDCl·HCl, HCl·H_2NOTMS, DIEA, DMF; *n*-Bu_4NF)
16:	R = N(OMe)Me	(From **13** using EDCl·HCl, HCl·HN(OMe)Me, DIEA, DMF)
17:	R = H	(From **16** using 1 M H_2C=CHMgBr)
18:	R = Me	(From **16** using 3 M MeMgBr)
19:	R = *n*-Pr	(From **16** using 2 M *n*-PrMgBr)
20:	R = *i*-Pr	(From **16** using 2 M *i*-PrMgBr)
21:	R = Ph	(From **16** using 1 M PhMgBr)

Using standard synthetic manipulations, the C7-aldehyde **7** was converted into the compounds shown in Table 2. For example, reduction of **7** followed by sulfonylation and displacement yielded thio derivatives **24**–**26**. Reductive amination of **7** with methyl glycinate followed by saponification produced **28**. PDC oxidation[16] of **7** in DMF/MeOH generated the C7-methyl ester **29**, which was hydrolyzed to form **30**. Stabilized Wittig homologation of **7** followed by enoate reduction and ester hydrolysis gave **31**, **32** and **33**, respectively. Lastly, methyl ketone **35** was prepared following treatment of **7** with MeMgBr followed by Dess–Martin oxidation.[17]

Table 2



7:	R = CHO	Reagents
22:	$R = CH_2OH$	(From **7** using $NaBH_4$/MeOH)
23:	$R = CH_2OTs$	(From **22** using (4-Me)$PhSO_2Cl$, pyridine)
24:	$R = CH_2SMe$	(From **23** using NaSMe, MeOH)
25:	$R = CH_2SAc$	(From **23** using KSAc, MeOH)
26:	$R = CH_2SH$	(From **25** using NH_4OH, MeOH)
27:	$R = CH_2NHCH_2CO_2Me$	(From **7** using $H_2NCH_2CO_2Me$, HOAc, $NaBH_3CN$)
28:	$R = CH_2NHCH_2CO_2H$	(From **27** using 1N LiOH)
29:	$R = CO_2Me$	(From **7** using PDC, DMF, MeOH)
30:	$R = CO_2H$	(From **29** using 1N LiOH)
31:	$R = CH{=}CHCO_2Me$	(From **7** using $Ph_3P{=}CHCO_2Me$)
32:	$R = CH_2CH_2CO_2Me$	(From **31** using 5% Pd/C, H_2)
33:	$R = CH_2CH_2CO_2H$	(From **32** using LiOH)
34:	R = C(OH)Me	(From **7** using 3 M MeMgBr)
35:	R = C(O)Me	(From **34** using Dess–Martin reagent)

In summary, the efficient preparation of side chain modified apicidin derivatives proceeding via two common intermediates is described. The availability of these reagents has facilitated biochemical investigations of HDAC and has led to mechanism-based inhibitors having increased potency relative to apicidin [e.g. HeLa HDAC activity[2] $IC_{50} = 240$ pM (**15**); 400 pM (**8**); 3 nM (**25**) versus 1 nM (**1**). Malaria in vitro[2] MIC = 0.24 ng/mL (**15**) versus 125 ng/mL (**1**)].[18] Application of this information may lead to improved HDAC inhibitors with diverse potential applications in cancer, AIDS and antiprotozoal chemotherapy.

Acknowledgements

The authors gratefully thank Drs. Michael Goetz and Sheo Singh for providing apicidin and Ms. Amy Bernick for providing mass spectral data.

References

1. Singh, S. B.; Zink, D. L.; Polishook, J. D.; Dombrowski, A. W.; Darkin-Rattray, S. J.; Schmatz, D. M.; Goetz, M. A. *Tetrahedron Lett*. **1996**, *37*, 8077.

2. Darkin-Rattray, S. J.; Gurnett, A. M.; Myers, R. W.; Dulski, P. M.; Crumley, T. M.; Allocco, J. J.; Cannova, C.; Meinke, P. T.; Colletti, S. L.; Bednarik, M. A.; Singh, S. B.; Goetz, M. A.; Dombrowski, A. W.; Polishook, J. D.; Schmatz, D. M. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13143.
3. (a) Bernardi, E.; Fauchere, J.-L.; Atassi, G.; Viallefont, P.; Lazaro, R. *Peptides* **1993**, *14*, 1091–1093. (b) Gross, M. L.; McCrery, D.; Crow, F.; Tomer, K. B.; Pope, M. R.; Ciuffetti, L. M.; Knoche, H. W.; Daly, J. M.; Dunkle, L. D. *Tetrahedron Lett*. **1982**, *23*, 5381. (c) Stahelin, H.; Trippmacher, A. *Eur. J. Cancer* **1974**, *10*, 801. (d) Umehara, K.; Nakahara, K.; Kiyoto, S.; Iwami, M.; Okamoto, M.; Tanaka, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot*. **1983**, *36*, 478. (e) Hirota, A.; Suzuki, A.; Aizawa, K.; Tamura, S. *Agric. Biol. Chem*. **1973**, *37*, 955. (f) Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S.; Beppu, T. *J. Biol. Chem*. **1993**, *268*, 22429.
4. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavietich, N. P. *Nature* **1999**, *401*, 188.
5. Two recent reports described a series of *ortho*-aminobenzamide HDAC inhibitors exhibiting micromolar potencies in vitro and interesting antitumor activity in vivo. (a) Suzuki, T.; Ando, T.; Tsuchiya, K.; Fukazawa, N.; Saito, A.; Mariko, Y.; Yamashita, T.; Nakanishi, O. *J. Med. Chem*. **1999**, *42*, 3001. (b) Saito, A.; Yamashita, T.; Mariko, Y.; Nosaka, Y.; Tsuchiya, K.; Ando, T.; Suzuki, T.; Tsuruo, T.; Nakanishi, O. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4592.
6. Yoshida, M.; Horinouchi, S.; Beppu, T. *Bioessays* **1995**, *17*, 423.
7. (a) Kim, Y. B.; Lee, K.-H.; Sugita, K.; Yoshida, M.; Horinouchi, S. *Oncogene* **1999**, *18*, 2461–2470. (b) Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3003. (c) Jung, M.; Brosch, G.; Kolle, D.; Scherf, H.; Gerhauser, C.; Loidl, P. *J. Med. Chem*. **1999**, *42*, 4669.
8. (a) Shute, R. E.; Dunlap, B.; Rich, D. H. *J. Med. Chem*. **1987**, *30*, 71–78. (b) Bernardi, E.; Fauchere, J.-L.; Atassi, G.; Viallefont, P.; Lazaro, R. *Amino Acids* **1994**, *6*, 315.
9. Colletti, S. L.; Myers, R. W.; Darkin-Rattray, S. J.; Schmatz, D. M.; Fisher, M. H.; Wyvratt, M. J.; Meinke, P. T. *Tetrahedron Lett*. **2000**, *41*, 7837.
10. Shih, T. L.; Mrozik, H.; Holmes, M. A.; Fisher, M. H. *Tetrahedron Lett*. **1990**, *31*, 3525.
11. Synthesis of **5** and **6**: Apicidin (600 mg, 963 μmol) in THF (45 mL) was refluxed until homogenous. The clear solution was then cooled to −60°C and TMSCl (486 μL, 3.85 mmol) was added followed immediately by $KN(SiMe_3)_2$ (9.63 mL, 0.5 M in toluene, 4.82 mmol). The solution was aged for 20 min, then quenched by addition of cold saturated $NaHCO_3$(aq.), quickly extracted with CH_2Cl_2 and dried (Na_2SO_4). TLC: R_f=0.45 (1:2 acetone:hexanes). The volatiles were removed under reduced pressure and the residue dissolved in CH_2Cl_2 (25 mL) at rt. To this solution was added solid $NaHCO_3$ (400 mg) followed by MCPBA (216 mg, 1.06 mmol, 80–90% pure). The solution was aged for 15 min and then the volatiles were removed under reduced pressure. TLC: R_f=0.64 for intermediate α-siloxyketones (1:1 acetone:hexanes). To the residue at rt was added THF (20 mL) and *n*-Bu_4NF (1.8 mL, 1.8 mmol, 1 M in THF). The solution was aged for 20 min, then poured into saturated $NaHCO_3$ (aq.):brine (1:1), extracted with EtOAc and dried (Na_2SO_4). Pure product (477 mg white powder, 76%), was obtained following flash chromatography on silica gel using a gradient elution (1:2→1:1 acetone:hexanes). TLC: R_f=0.42 for **5**; R_f=0.35 for **6** (1:1 acetone:hexanes). All compounds were characterized by ^1H NMR and MS. This supporting data, including HPLC, is available (103 pages).
12. Corey, E. J.; Xiang, Y. B. *Tetrahedron Lett*. **1988**, *29*, 995.
13. Boger, D. L.; Colletti, S. L.; Honda, T.; Menezes, R. F. *J. Am. Chem. Soc*. **1994**, *116*, 5607.
14. Boivin, J.; Kaim, L. E.; Zard, *Tetrahedron* **1995**, *51*, 2573.
15. Krishnamurti, F.; Bellew, D. R.; Prakash, G. K. S. *J. Org. Chem*. **1991**, *56*, 984.
16. Corey, E. J.; Schmidt, G. *Tetrahedron Lett*. **1979**, *5*, 399.
17. Dess, D. B.; Martin, J. C. *J. Org. Chem*. **1983**, *48*, 4155.
18. Complete biological results for these compounds will be published in a full report elsewhere.