Brief Articles

Synthesis of Apicidin-Derived Quinolone Derivatives: Parasite-Selective Histone Deacetylase Inhibitors and Antiproliferative Agents

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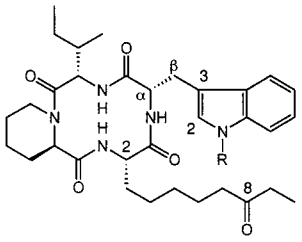
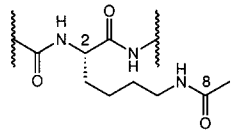
Apicidin's indole was efficiently converted into a series of *N*-substituted quinolone derivatives by indole *N*-alkylation followed by a two-step, one-pot, ozonolysis/aldol condensation protocol. The new quinolones exhibited good parasite selectivity and potency both at the level of their molecular target, histone deacetylase, and in their whole cell antiproliferative activity in vitro.

Apicidin (**1a**) is a novel fungal metabolite recently discovered by Merck scientists.[1] This cyclic tetrapeptide, which was isolated from endophytic fungi on twigs collected in Costa Rica, exhibits potent, broad spectrum cidal activity against the apicomplexan family of protozoan parasites.[2] These protozoa are the causative agents of diverse, economically significant diseases of both humans and animals including *Plasmodium sp.* (malaria), *Cryptosporidium parvum* (cryptosporidosis), *Toxoplasma gondii* (toxoplasmosis), *Sarcocystis neurona* (sarcocystis), and *Eimeria sp.* (coccidiosis). Apicidin exerts its biological activity by inhibiting the zinc metalloamidase, histone deacetylase[3] (HDAC). HDACs are essential eukaryotic enzymes which modulate chromatin structure and function by deacetylating the ϵ-*N*-acetyl lysine residues of histones (**2**), which in turn are formed by the action of histone acetyltransferases.[4,5] Structural similarities between the 2-amino-8-oxodecanoic acid (Aoda) side chain of apicidin and these acetylated histones suggest that **1a** mimics **2**, resulting in HDAC inhibition, histone hyperacetylation, the disruption of transcriptional control, and ultimately cell death. Apicidin, however, is a *nonselective* HDAC inhibitor that displays comparable efficacy against protozoan and mammalian HDAC (see below), for which multiple isoforms exist.[6−8] A functional consequence of this indiscriminate HDAC inhibitory activity is significant toxicity in rats orally dosed with **1a**, which has been termed a hemorrhagic factor.[9] Thus, the development of a novel class of antiprotozoal agents employing HDAC inhibition required the identification of *parasite-selective* and hence potentially nontoxic apicidin analogues.

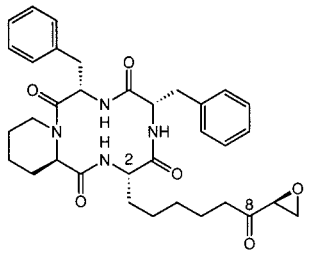
Apicidin is structurally related to a family of α-epoxyketone cyclic tetrapeptides which are known HDAC inhibitors with pronounced anti-neoplastic[10−17] and anti-HIV[18,19] activity. These cyclopeptides, typified by the extensively investigated trapoxin A[14,20] (**3**), also include HC-Toxin,[11,15] chlamydocin,[10,16] Cyl-2,[17] Tan-1746s,[21] and WF-3161.[13] These HDAC inhibitors differ from each other primarily in the hydrophobic substitution patterns displayed on their macrocyclic backbones. Apicidin, however, is unique in that it alone lacks an α-keto-epoxide moiety at the terminus of its Aoda side chain. Epoxide removal from HC-Toxin, for instance, led to a biologically inactive derivative,[22] suggesting that apicidin's tryptophan moiety might be a critical participant in HDAC binding. Consequently, a program was initiated to prepare tryptophan-modified apicidin derivatives in order to directly evaluate its contribution to biological activity and determine if such derivatives might provide an entrée into pathogen-specific HDAC inhibitors. We disclose herein the first syntheses of quinolone-containing apicidin analogues which are potent inhibitors of parasite HDAC exhibiting parasite-selective whole cell antiproliferative activity.



1a: Apicidin (R = OMe)
1b: Apicidin A (R = H)



Acetylated Histone Lysine (2)



Trapoxin (3)

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Scheme 1

Scheme 2

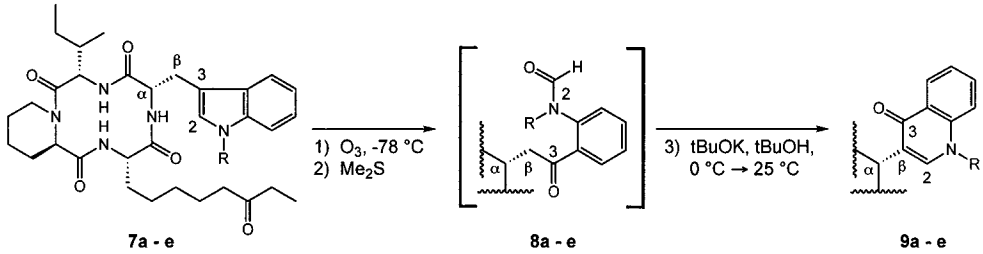

Table 1. Parasite-Selective Apicidin-Derived Quinolones[24]

compd	*E. tenella* binding (IC_{50}, ng/mL)	chick liver binding (IC_{50}, ng/mL)	*E. tenella* HDAC (IC_{50}, nM)	HeLa HDAC (IC_{50}, nM)	malaria (IC_{50}, ng/mL))	HeLa (AP_{50}, ng/mL)
1	8	4	1	1	57	78
5	15	1000			40	>10 000
6	40	1000	3.8	86	40	10 000

During an unsuccessful attempt to prepare **4** by oxidative cleavage of apicidin's indole 2,3-bond with copper(II) acetate[23] (Scheme 1), trace amounts of two new apicidin analogues were generated. On the basis of ^{1}H NMR, mass spectral analysis, and chemical correlation,[24] these new compounds were identified as quinolones **5** and **6**. These new apicidins were evaluated for biological efficacy and parasite versus host selectivity using the following assays: [^{3}H]-**1b** HDAC competitive binding assays[2] using *E. tenella* and chicken liver extracts; HDAC enzyme inhibition assays[2] using *E. tenella* and mammalian cell nuclear extracts;[25] and mammalian cell (HeLa) and parasite-based antiproliferative assays[2] (Table 1). Competitive binding assays established that both quinolones exhibited high affinity for parasite HDAC which was only slightly reduced relative to apicidin. However, both quinolones possessed significantly lower affinity to avian HDAC relative to apicidin. Thus, an average 67-fold and 25-fold increase in parasite HDAC selectivity was observed for quinolones **5** and **6**, respectively. As expected, these results were confirmed using parasite and host HDAC enzyme activity assays. The enhanced parasite selectivity observed at the enzyme level in vitro correlated well with the parasite selectivity measured at the whole cell level. Quinolones **5** and **6** also exhibited high potency against *Plasmodium falciparum* in vitro, comparable to apicidin. In this assay, the antimalarial agent chloroquine is less efficacious (IC_{50} = 92 ng/mL) than either of these new quinolones. However, both quinolones were virtually inactive in the HeLa cell antiproliferative assay, in marked contrast to the high activity of apicidin in this assay. Thus, as predicted for a parasite-selective HDAC inhibitor, the elimination of host HDAC activity yielded a compound devoid of host cell antiproliferative activity. These results provide further support that HDAC is the principle, if not sole, antiproliferative target of apicidin and that HDAC inhibition may play a role in its reported toxicity.[9]

These encouraging results prompted the development of an improved strategy for a quinolone synthesis which would provide an extended series of apicidin-derived quinolones. The conversion of 3-substituted indoles into quinolones is precedented in the literature, proceeding via ozonolysis of the indole's 2,3-bond followed by an intramolecular aldol cyclization.[26,27] Indeed, a one-pot, solvent-switch protocol as described[24] led to the smooth conversion of apicidin into **6** (66−95% overall yield, depending on scale) and rigorously confirmed the initial quinolone structure assignment (Scheme 2). Similarly, removal of apicidin's *N*-OMe group by hydrogenolysis[22] produced apicidin A (**1b**), which could be converted into **5** in >95% yield.

Treatment of **1b** with a strong base followed by *N*-alkylation (NaH, DMF, RI or RBr, 35 °C → 80 °C) led to the formation of a series of *N*-substituted apicidin analogues **7a**−**e** in reasonable yields (54−65%), along with minor amounts of ring *N*-alkyl products and/or C7/C9 Aoda-alkylated products and unreacted starting **1b**. The indole *N*-alkylation reaction could be accelerated by heat and/or the addition of catalysts such as nBu$_4$NI (3 equiv). Oxidative cleavage of these *N*-substituted apicidins **7a**−**e** (O_3, CH_2Cl_2, −78 °C, 10 min) smoothly formed aldol precursors **8a**−**e**. Intermediates **8a**−**e** generally were not isolated and, when examined by ^{1}H NMR, were a complex mixture of the respective formamides, aldol intermediates, and dehydration products **9a**−**e**. Instead, direct treatment of **8a**−**e**, following a solvent switch to *t*BuOH/THF, with a strong nonnucleophilic base such as *t*BuOK, yielded the desired quinolones **9a**−**e** in high chemical yield for the two-step procedure ranging from 85% for **9a** to 63% for **9e**.

The new *N*-substituted apicidin-derived quinolones **9a**−**e**, along with the corresponding precursor indole derivatives **7a**−**e**, were evaluated as described above for HDAC affinity and selectivity using both competition

Table 2. *N*-Substituted Apicidin-Derived Indoles and Quinolones[24]

compd	R group	*E. tenella* binding (IC_{50}, ng/mL)	chick liver binding (IC_{50}, ng/mL)	*E. tenella* HDAC (IC_{50}, nM)	HeLa HDAC (IC_{50}, nM)	malaria (IC_{50}, ng/mL)	HeLa (AP_{50}, ng/mL)
7a	CH_3	12	5			20	225
7b	CH_2CH_3	100	10			180	
7c	$CH_2CH_2CH_3$	30	10			250	625
7d	$CH_2CO_2CH_3$	7	5			250	150
7e	CH_2CO_2H	65	65			1000	
9a	CH_3	60	1000	9	241	117	>10 000
9b	CH_2CH_3	70	2000	11	330	240	>10 000
9c	$CH_2CH_2CH_3$	125	700	28	230	440	>10 000
9d	$CH_2CO_2CH_3$	300	2000			790	>10 000
9e	CH_2CO_2H	2000	5000			550	>10 000

binding and enzyme inhibition assays (Table 2). In each case, the quinolone derivatives were parasite-selective both at the level of the HDAC target and whole cell antiproliferative activity. On the basis of binding data, parasite HDAC selectivity ranged from 28-fold for **9a** to 17-fold for **9b** to ~7-fold for **9d** and **9e**. These results were again entirely consistent with data generated using the enzyme inhibition assays for **9a**, **9b**, and **9c** (30-, 30-, and 8-fold parasite selectivity, respectively). The observed parasite selectivity of the *N*-substituted quinolones **9a**−**e** did not derive from the *N*-alkyl groups since the corresponding *N*-alkyl indole derivatives **7a**−**e** were either nonselective or modestly host-selective. In addition, it is clear that acidic functionality is poorly tolerated in this position (see **9e**). Unfortunately, modest increases in steric bulk on the quinolone nitrogen (e.g. **9c** or **9d**) led to a significant decrease in both parasite HDAC affinity and parasite selectivity at the whole cell level, limiting the utility of such modifications.

In summary, the efficient and high yielding synthesis of a series of potent and selective protozoal histone deactylase inhibitors derived from apicidin has been described. These parasite-selective HDAC inhibitors are also effective and selective antiprotozoal agents in vitro. These results firmly establish that the synthesis of inhibitors which specifically target protozoan HDAC relative to mammalian HDAC is viable. These observations may presage the discovery of superior, parasite-selective HDAC inhibitors with significant potential for antiparasitic chemotherapy.

Experimental Section

Synthesis of Quinolone 6. Apicidin (**1a**, 600 mg, 0.963 mmol) was dissolved in CH_2Cl_2 (60 mL) and cooled to −78 °C. A gentle stream of ozone was bubbled through the solution until a faint blue color was noted. After 10 min, the solution was purged with nitrogen for 5 min, dimethyl sulfide (1 mL) was added, and the solution was warmed to 25 °C. The volatiles were removed under reduced pressure, and additional CH_2Cl_2 (2 × 20 mL) was added and removed under reduced pressure. The resultant residue was dissolved in 1/1 THF/*t*BuOH, the mixture was cooled to 0 °C, and *t*BuOK (216 mg, 1.93 mmol) was added. After aging for 1 h, the solution was poured into saturated $NaHCO_3$(aq) and extracted with CH_2Cl_2, and the organic layers were washed with saturated brine and dried (Na_2SO_4). Pure **6** (470 mg, 76%) was obtained following flash column chromatography on silica gel using 1/1 hexanes/acetone as eluant. TLC: 1/1 hexanes/acetone, R_f = 0.32. RP-HPLC: t_R = 3.17 min (YMC 50 × 4.6 mm column, ODS (120 Å), 2.5 mL/min, gradient elution, 10 → 95% B in 4.5 min then 95% B for 0.5 min; Solvent A: 0.06% TFA in water; Solvent B: 0.05% TFA in MeCN). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.48 (d, J = 8.3 Hz, 1H), 8.14 (br s, 2H), 8.06 (d, J = 10.0 Hz, 1H), 7.82 (t, J = 7.1 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 6.45 (d, J = 10.3 Hz, 1H), 5.31 (d, J = 5.5 Hz, 1H), 5.22 (d, J = 8.7 Hz, 1H), 4.68 (t, J = 10.1 Hz, 1H), 4.60 (q, J = 9.6 Hz, 1H), 4.20 (s, 3H), 4.04 (d, J = 13.5 Hz, 1H), 3.07 (t, J = 12.9 Hz, 1H), 2.37 (t, J = 8.0 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 2.45 (m, 2H), 2.04 (m, 1H), 1.80 (br s, 1H), 1.50−1.70 (m, 6H), 1.24−1.48 (m, 6H), 1.03 (m, 1H), 1.02 (t, J = 7.3 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.77 (t, J = 7.3 Hz, 3H). Low resolution MS: m/z 638.3 ($M^+ + 1$). High resolution MS: calculated for $C_{34}H_{47}N_5O_7$, 638.3548; found, 638.3523 $[M + H]^+$.

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Supporting Information Available: The 500 MHz proton NMR spectra for compounds **5, 6**, **7a**−**e,** and **9a**−**e** are available along with appropriate analytical data (TLC, RP-HPLC, low and high resolution mass spectral data). This material is available free of charge via the Internet at http://pubs.acs.org.

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