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Broad Spectrum Antiprotozoal Agents that Inhibit Histone Deacetylase: Structure–Activity Relationships of Apicidin. Part 2

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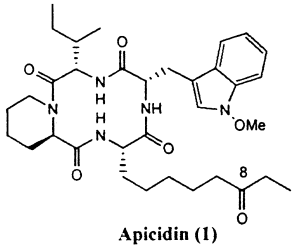
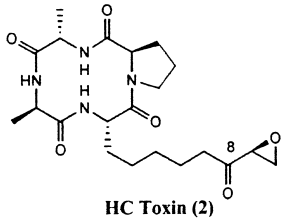
Abstract—Recently isolated at Merck, apicidin inhibits both mammalian and protozoan histone deacetylases (HDACs). The conversion of apicidin, a nonselective nanomolar inhibitor of HDACs, into a series of picomolar indole-modified and parasite-selective tryptophan-replacement analogues is described within this structure–activity study.

Recently isolated at Merck, the natural product apicidin (**1**) is a metabolite generated from endophytic fungi (*Fusarium pallidoroseum*) found on twigs collected in Costa Rica.[1] It exhibits potent broad spectrum cidal activity against the apicomplexan family of protozoa, including *Plasmodium falciparum*, *Cryptosporidium parvum*, *Toxoplasma gondii*, *Sarcocystis neurona*, and *Eimeria tenella*.[2] These protozoan parasites cause infectious diseases such as malaria, cryptosporidiosis, toxoplasmosis, sarcocystis and coccidiosis, respectively. Due to the advent of opportunistic infections related to HIV involving cryptosporidiosis and toxoplasmosis, the increasing multi-drug resistance of malaria and coccidiosis, and the endemic rise of equine sarcocystis, these diseases are in serious need of new therapies.



Apicidin (1)



HC Toxin (2)

In our previous letter we detailed a study of **1** concentrating on the structure–activity relationships (SARs) of the macrocycle and the side chain of the natural product with histone deacetylase (HDAC) inhibition and functional activity.[3] Further studies of epoxide removal from the related natural product HC Toxin afforded the ethyl ketone analogue of **2** (HeLa HDAC, $IC_{50} = 430$ nM),[4] a compound devoid of HDAC affinity relative to the ethyl ketone **1** (HeLa HDAC, $IC_{50} = 1$ nM). This result suggested that apicidin's indole region is a key constituent of enzyme binding and HDAC activity. Consequently, attention was then focused on the synthesis of indole-modified and tryptophan-replacement apicidin derivatives.[4,5] As a result of these studies, the tryptophan of apicidin emerged as a moiety whose structural modification conferred 20- to 100-fold parasite selectivity to the natural product. In certain cases, picomolar HDAC affinity was also realized as a result of these indole permutations.

Chemistry

The syntheses of indole-modified analogues **3**, **6**–**12**, **14**, **15**, **18**, and **19** (Table 1) along with tryptophan-replacement analogues **20**–**23** and **25**–**28** (Table 2) have been disclosed.[4,5] Pyrrolidine **4** (2 equiv pyrrolidine, 2.5 equiv $CH_2O_{(aq)}$, DMF, 23 °C, 20 h, 55%), acetic acid **5** ((i) NaH, THF, $BrCH_2CO_2Me$, DMF, 23 °C, 15 h, 65%; (ii) LiOH, THF–MeOH–H_2O, −10 °C, 2 h, 90%) and aminoethyl **13** ((i) NaH, HMPA–DMF, 23 °C, 15 min, then $BrCH_2CH_2OTBDMS$, TBAI, 100 °C, 2 h, 90%; (ii) HF–pyridine, THF, 0 °C, 1 h, 99%; (iii) $Zn(N_3)_2$-pyr_2, imidazole, DEAD, Ph_3P, CH_2Cl_2, 23 °C, 12 h,

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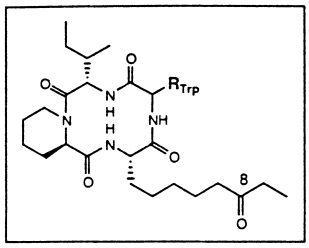
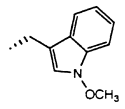
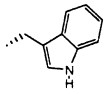
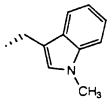
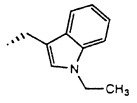
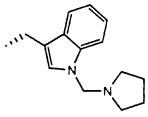
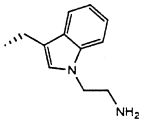
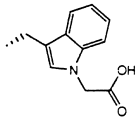
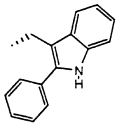
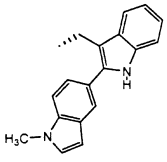
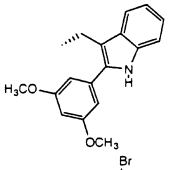
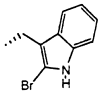
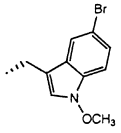
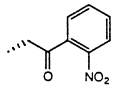
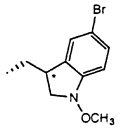
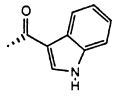
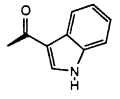
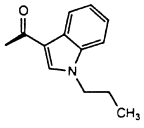
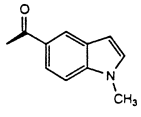
85%; (iv) H_2, Pd–C, CH_2Cl_2, 23 °C, 8 h, 67%) were all prepared from **11**. 5-Bromoindole **16** was prepared from apicidin in two steps via ionic bromination (excess NBS, CCl_4, 80 °C, 45 min, 97%) followed by a reductive cleavage ($Na_2S_2O_4$, $NaHCO_3$, DMF–H_2O, 23 °C, 12 h, 50%) of the undesired *N*-bromide resulting from overbromination of the more kinetically labile tryptophan amide ring nitrogen. The 5-bromoindole **16** could be reduced to the corresponding 5-bromoindoline **17** when this sodium dithionite reaction was run for longer time

Table 1. Indole-modified analogues of apicidin



Compound	R_{Trp}	Binding/HDAC (nM)	*P. falciparum* (ng/mL)	*E. tenella* (ng/mL)	Compound	R_{Trp}	Binding/HDAC (nM)	*P. falciparum* (ng/mL)	*E. tenella* (ng/mL)
1[a]		HeLa[b] 1 *E. tenella* 1	58	94	**11**		HeLa[b] 2 *E. tenella* 1	35	31
3		CL[c] 5 *E. tenella* 5	20	125	**12**		CL[c] 10 *E. tenella* 100	180	50
4		HeLa[b] 0.2 *E. tenella* —	35	31	**13**		HeLa[b] 1.3 *E. tenella* —	100	>1000
5		CL[c] 80 *E. tenella* 100	>1000	>1000	**14**		HeLa[b] 4 *E. tenella* —	700	>1000
6		CL[c] 5 *E. tenella* 25	100	62	**15**		HeLa[b] 0.2 *E. tenella* 0.2	250	125
7		CL[c] 3 *E. tenella* 200	25	15	**16**		CL[c] 60 *E. tenella* 175	550	500
8		HeLa[b] 91 *E. tenella* 33	500	125	**17**		CL[c] 4500 *E. tenella* 2500	>1000	1000
9		CL[c] 12 *E. tenella* 10	16	125	**18**		CL[c] 30 *E. tenella* 23	70	125
10		HeLa[b] 15 *E. tenella* 4	150	125	**19**		CL[c] 20 *E. tenella* 30	15	50

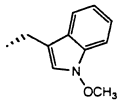
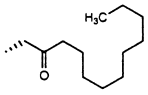
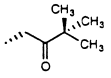
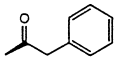
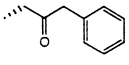
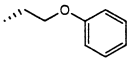
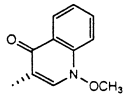
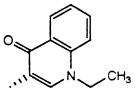
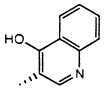
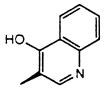
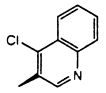
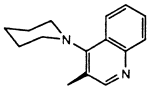
[a]See Table 2 for binding data (CL and *E. tenella*) of apicidin (**1**).
[b]HDAC enzyme inhibition.
[c]Binding assay using 2-tritio-**11**.

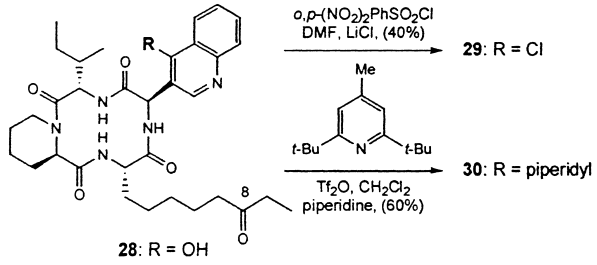
periods. The regiochemistry of indole bromination in **16** was readily determined by a one dimensional ^{1}H NMR NOE experiment with irradiation of the indole *N*-methoxy group. The phenyl ether **24** was prepared from its ethanol precursor via bismuthine chemistry (Ph_3Bi, CH_3CO_3H, $Cu(OAc)_2$, CH_2Cl_2, 60 °C, 3 h, 20%).[6] Lastly, the quinolines **29** (60 °C, 12 h) and **30** (23 °C, 12 h) were prepared from **28** (or **27**) as shown in Scheme 1, providing exclusively the pseudo-equatorial epimers.

Biology[2]

The biological data presented in Tables 1 and 2 was generated primarily from three assays: (a) enzyme inhibition of HDAC derived from partially purified extracts of both human HeLa cells and *E. tenella* protozoa reported as IC_{50} values; (b) an in vitro functional assay using whole red blood cells infected with *P. falciparum*, also reported as IC_{50} values; and (c) an in vitro functional assay using MDBK (Mauden–Downing bovine kidney) host cells infected with *E. tenella* and reported as MIC (minimum inhibitory concentration). In the absence of HDAC inhibition data for certain compounds shown in Tables 1 and 2, IC_{50} values are presented from a competitive enzyme binding assay using radiolabeled 2-tritio-**11** (prepared from 2-bromo-**7**) with crude S100 extracts of both mammalian HDAC from chicken liver (CL) and parasite HDAC from *E. tenella*. To further illustrate the parasite selectivity of the apicidins shown in Table 2, data also is provided from an in vitro mammalian antiproliferative functional assay using either human HeLa cells or foreskin fibroblasts reported as the concentration required for 50% inhibition of cell growth (AP_{50}).

Table 2. Tryptophan-replacement analogues of apicidin

Compound	R_{Trp}	HDAC (nM)	Binding (nM)	*P. falciparum* (ng/mL)	*E. tenella* (ng/mL)	AP_{50} (ng/mL)
1		HeLa 1 *E. tenella* 1	CL 4 *E. tenella* 8	58	94	89
20		HeLa — *E. tenella* —	CL >1000 *E. tenella* >1000	>1000	>1000	>10,000
21		HeLa 444 *E. tenella* 45	CL 2000 *E. tenella* 100	775	>1000	>10,000
22		HeLa 257 *E. tenella* 20	CL 500 *E. tenella* 200	777	500	>10,000
23		HeLa 419 *E. tenella* 50	CL 2000 *E. tenella* 400	850	>1000	>10,000
24		HeLa 34 *E. tenella* 3	CL — *E. tenella* —	—	250	1450
25		HeLa 86 *E. tenella* 4	CL 1500 *E. tenella* 30	40	250	10,000
26		HeLa 330 *E. tenella* 11	CL 2000 *E. tenella* 70	240	>1000	>10,000
27		HeLa — *E. tenella* —	CL 1000 *E. tenella* 10	40	>1000	>10,000
28		HeLa — *E. tenella* —	CL 500 *E. tenella* 250	55	>1000	>10,000
29		HeLa 67 *E. tenella* 2	CL 300 *E. tenella* 50	225	200	10,000
30		HeLa 13 *E. tenella* 0.8	CL 100 *E. tenella* 20	55	125	2500



Scheme 1.

Results and Discussion

Table 1 presents the SAR of indole-modified apicidins. When the *N*-methoxy group of **1** was replaced with the isosteric ethyl moiety in **12**, a decrease in enzyme binding affinity and functional activity against *P. falciparum* was observed, whereas methyl substituted **3** retained the enzyme binding affinity and functional activity against *E. tenella* relative to **1** with an additional increase in *P. falciparum* functional activity. Removal of the *N*-methoxy group in apicidin also resulted in a slight increase of biological activity, illustrated by the free indole **11**. The phenomenon that was illustrated in the previous letter[3] concerning the reduced activity of negatively charged analogues relative to **1** is also exemplified here with *N*-carboxymethylene **5**. Conversely, the inclusion of a basic moiety into apicidin, such as **4** (HDAC inhibition = 200 pM), provided an enzyme inhibitor that more closely resembles the highly basic nature of histones, resulting in an overall improvement of biological activity relative to **1**. This effect is greatly reduced with the less basic primary amine **13**, relative to tertiary amine **4**. Sterics appear to be better tolerated at the 2-indole position versus the 5-indole position of **1** as exemplified by bromides **7** and **16**, respectively. The cell-based functional activity of **7** surpasses both **11** and **1**. Remarkably, when 2-bromo **7** was converted into the bulky 2-aryl analogues **6**, **14**, and **15** the HDAC inhibition and binding affinity of **1** was retained. In fact, the HDAC inhibition observed with **15** (200 pM) was greatly enhanced relative to **11** and **1**. Whether the indole 2,3-bond was reduced (**17**) or oxidized and cleaved (**8**), resulted in very different biological profiles. While indoline **17** lost virtually all activity relative to indole **16**, nitrophenone **8** displayed 3-fold parasite selectivity in the HDAC inhibition assay. Other ketones were studied, such as the β-oxo-tryptophan analogue **9** which displayed a 16 ng/mL IC_{50} against *P. falciparum*. The epimer of this compound, **18**, was less active, but alkylation of the vinylogous amide to provide ketone **10** resulted once again in 4-fold parasite selectivity. A regioisomeric hybrid of **10**, the *N*-methyl *epi*-5-indole ketone **19**, did not retain parasite selectivity, but displayed improved potency across both cell-based assays. In general, the indole modifications that are presented in Table 1 provided improved enzyme affinity and/or in vitro potency relative to apicidin, but resulted in only modest advances toward parasite selectivity, a property that would require more drastic changes to the indole region of **1**.

Thus, Table 2 illustrates the SAR of tryptophan-replacement analogues of apicidin. Stemming from the modestly selective ketones, **8** and **10**, a series of ketone homologues was studied further to elucidate parasite selectivity. Undecanone analogue **20** demonstrates the intolerance of excessively large moieties in this region of the natural product, although the *t*-butyl ketone **21** displayed 10- and 20-fold parasite selectivity in the HDAC inhibition and binding assays, respectively. Likewise, benzyl ketones **22** and **23** along with phenyl ether **24** displayed 10-fold parasite selectivity in the HDAC inhibition assay. Most importantly, when apicidin's indole was ring-expanded to a quinolone (e.g., **25** and **26**), 22- to 50-fold parasite selectivity was realized in both the HDAC inhibition and binding assays. Furthermore, *N*-methoxy quinolone **25** displayed significant activity (40 ng/mL) against *P. falciparum*. Removal of the *N*-methoxy moiety in **25** provided the 4-hydroxyquinoline **27**, a 100-fold selective compound in the binding assay which retained the activity of quinolone **25** against *P. falciparum*. 4-Hydroxyquinoline **27** represents the most parasite-selective apicidin analogue in the series, and combined with the increased potency against *P. falciparum* relative to **1**, marks a vast improvement over the natural product as an antiprotozoal agent. Although the epimer **28** revealed a decrease in selectivity relative to **27**, substitution of the hydroxyl in **28** with a chlorine (**29**) or piperidine (**30**) provided *epi*-quinolines which displayed 34- and 16-fold parasite selectivity in the HDAC inhibition assay, respectively. Once again, the trend that histone-like basicity imparts increased enzyme affinity and functional activity to HDAC inhibitors is illustrated with the highly basic analogue **30** (*E. tenella* HDAC, IC_{50} = 800 pM). Lastly, the parasite selectivity of the compounds presented in Table 2 is further represented by their relative inactivity in the AP_{50} assay, with the exception of **24**.

In summary, Part 2 of this SAR study describes a pharmacophore of apicidin consisting of the indole region of the natural product. The biological data reported herein has delineated the tryptophan moiety of **1** as a key site amenable to the incorporation of up to 100-fold selectivity into the natural product. Another discovery includes the potency-enhancing effect of basic inhibitors related to the highly basic histone endogenous substrates.

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