ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2004, p. 1435–1436 Vol. 48, No. 4
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.4.1435–1436.2004

Antimalarial and Antileishmanial Activities of Aroyl-Pyrrolyl-Hydroxyamides, a New Class of Histone Deacetylase Inhibitors

Members of the genus *Leishmania* are parasitic protozoans that infect about two million people per annum (5), and they are emerging as serious opportunistic infective agents in human immunodeficiency virus-infected patients (4). Malaria parasites are responsible for 1.5 to 2.7 million deaths annually, primarily in Africa (10). The effort to find new antimalarial agents is still a high priority given the increasing malaria emergency largely due to multidrug-resistant *Plasmodium falciparum* strains. The histones of *P. falciparum* have recently been proposed as targets for drug treatment of blood stage parasites (6). They also play an important role in chromatin remodeling in trypanosomatids, which include *Leishmania* species and trypanosomes (3).

Apicidin, a cyclic tetrapeptide isolated from *Fusarium* spp., was reported to block the in vitro development of apicomplexan parasites by inhibiting parasite (including *Plasmodium* species) histone deacetylase (HDAC) (6). Another HDAC inhibitor, suberoyl bishydroxamic acid, showed an in vivo cytostatic effect against the acute murine malaria *Plasmodium berghei*, and one round of treatment with the compound failed to select for resistant mutations (1).

Recently, Mai et al. reported a novel series of hydroxamate compounds, namely, 3-(4-aroyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamides, acting as HDAC inhibitors in the range of low micromolar-submicromolar concentrations (7, 8). The aim of the present study was to investigate the in vitro antimalarial and antileishmanial activities of lead compound 1 and some analogues (compounds 2 to 10) to identify potential chemical tools with selective toxicity for protozoa.

The antimalarial activity of compounds 1 to 10 (Table 1) was determined in vitro for chloroquine-sensitive (CQS) (D6, Sierra Leone) and chloroquine-resistant (CQR) (W2, Indochina) strains of *P. falciparum*. Growth of cultures of *P. falciparum* was determined by a parasite lactate dehydrogenase assay using Malstat reagent (9). Chloroquine was used as the positive control, while dimethyl sulfoxide was tested as the negative control. Suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA), two well-known HDAC inhibitors, were also tested. Antileishmanial activity of compounds 1 to 10 (Table 1) was tested on a transgenic cell line of *Leishmania donovani* promastigotes expressing firefly luciferase (assay with Steady Glo reagent; Promega, Madison, Wis.) obtained from Dr. Rafael Balana-Fouce, University of Leon, Leon, Spain. Pentamidine was tested as a reference drug together with SAHA and TSA. All the compounds were simultaneously tested for cytotoxicity on Vero (monkey kidney fibroblast) cells by a Neutral Red assay (2).

Among compounds 1 to 10, only compound 7 showed antimalarial activity against *P. falciparum* strains; however, its 50% inhibitor concentration (IC_{50}) values were 22- to 100-fold higher than those of chloroquine and 4.8- to 8.5-fold and 33- to 93-fold higher than those of SAHA and TSA, respectively. Compounds 1 to 4 showed little *Plasmodium* inhibition activity (Table 1). This biological behavior of compounds 1 to 10 resembles their corresponding anti-HDAC effect against maize HD2 (compound 7, IC_{50} = 0.1 μM; compounds 1 to 4, IC_{50} = 2 to 4 μM; compounds 5, 6, and 8 to 10, low-level activity or totally inactivity) (7, 8), thus confirming an inhibiting action of compound 7 and, to a lesser extent, of compounds 1 to 4 on parasite HDAC enzymes.

Surprisingly, the majority of compounds 1 to 10 were found endowed with interesting anti-*Leishmania* activity (in this case, activity not directly related to their anti-HD2 action) (Table 1). Compounds 2 and 3, the most potent of the series, were as active as pentamidine, slightly less potent than TSA, and >10-fold more potent than SAHA. Interestingly, compounds 2 and

TABLE 1. Antimalarial and antileishmanial activities of compounds 1 to 10

Compound	Compound[a]	IC_{50} (μg/ml) for *P. falciparum*[b]:		IC (μg/ml) for *L. donovani*		Cytotoxicity (μg/ml)
		D6 (CQS)	W2 (CQR)	IC_{50}	IC_{90}	
1	1	>4.8 (46)	>4.8 (45)	2.4	11.3	NC[c]
2	2	>4.7 (19)	>4.7 (34)	1.7	5.4	NC
3	5	>4.7 (35)	>4.7 (49)	1.6	5.1	NC
4	7	3.8	3.5	2.4	14.3	NC
5	27	NA[d]	NA	NA	NA	NC
6	29	NA	NA	NA	NA	NC
7	8	1.2	4	16	>50	NC
8	25	NA	NA	NA	NA	NC
9	26	NA	NA	8.3	32	NC
10	28	NA	NA	6.8	>50	NC
SAHA		0.25	0.47	22	50	1.2
TSA		0.036	0.043	0.89	25	0.095
Pentamidine		NT[e]	NT	1.25	4.1	NC
Chloroquine		0.014	0.18	NT	NT	NC

[a] From reference 7.
[b] Numbers in parentheses represent percentages of inhibition at the tested dose.
[c] NC, not cytotoxic at concentrations of up to 23.8 μg/ml.
[d] NA, not active at the maximum dose tested (4.8 μg/ml in the case of the antimalarial assays and 50 μg/ml in the case of the antileishmanial assays).
[e] NT, not tested.

3 were less cytotoxic than the reference drugs. Further studies to elucidate the mechanism of anti-*Leishmania* activity of such derivatives are in progress.

The work was supported by "Progetto Finalizzato Ministero della Salute 2002" and AIRC 2003 grants (A.M.), by Centers for Disease Control Cooperative Agreement grants U50-CCU418839 (EIDs) and UR3-CCU418652 (malaria), and by U.S. Department of Agriculture Cooperative Agreement grant 58-6408-2-0009 to the National Center for Natural Products Research (L.A.W. and B.L.T.).

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