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Potent In Vitro Antimalarial Activity of Metacycloprodigiosin Isolated from *Streptomyces spectabilis* BCC 4785

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Bioassay-guided fractionation of the extract from the fermentation broth of *Streptomyces spectabilis* BCC 4785 led to the isolation of three principle antimalarial agents, metacycloprodigiosin, bafilomycin A_1, and spectinabilin. Metacycloprodigiosin exhibited potent in vitro activity against *Plasmodium falciparum* K1, with a 50% inhibitory concentration of 0.0050 ± 0.0010 μg/ml, while its cytotoxicity was much weaker.

Malaria is a serious endemic disease in many parts of Africa, Asia, Latin America, and Oceania, affecting 5% of the world's population, and mortality is estimated to be over 1 million deaths each year (13, 21). Because of the worsening problems of drug resistance, there has been an urgent need for the discovery of a new chemical class of antimalarial agents (4). As part of an ongoing natural product research program, we have been screening microbial extracts for in vitro antimalarial activity (7–9). Among these, an extract from *Streptomyces spectabilis* BCC 4785 showed significant activity against *Plasmodium falciparum* (K1, a multidrug-resistant strain), with a 50% inhibitory concentration (IC_{50}) of 0.01 μg/ml. Therefore, mass fermentation and activity-guided isolation of antimalarial agents from this strain have been undertaken.

S. spectabilis was isolated from a soil sample collected in Thailand, identified to the species level, and deposited at the BIOTEC Culture Collection as BCC 4785. The strain was incubated in a fermentor containing 75 liters of a liquid medium (10.0 g of soluble starch, 1.0 g of K_2HPO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NaCl, 2.0 g of $(NH_4)_2SO_4$, and 3.0 g of $CaCO_3$ per liter). Chromatographic separation and purification of the methanolic extract from mycelia led to the isolation of three known compounds, metacycloprodigiosin (free-base form, orange powder, 490 mg; further purified as a hydrochloride, magenta powder) (18, 19), bafilomycin A_1 (colorless crystals, 14 mg) (1, 20), and spectinabilin (orange powder, 41 mg) (11). The structures of these compounds (Fig. 1) were identified by spectroscopic analyses and comparison with the literature data.

An assay for activity against *P. falciparum* K1 was performed by using a standard protocol (10) based on the microculture radioisotope technique described by Desjardins et al. (3). The reported IC_{50} represents the concentration that causes a 50% reduction of parasite growth, as indicated by the in vitro uptake of [^{3}H]hypoxanthine by *P. falciparum*. For comparison, the cytotoxicities of the compounds against human epidermoid carcinoma cells (KB cells), human breast cancer cells (BC-1 cells), and African green monkey kidney fibroblasts (Vero cells) were screened by using a colorimetric method (16). The

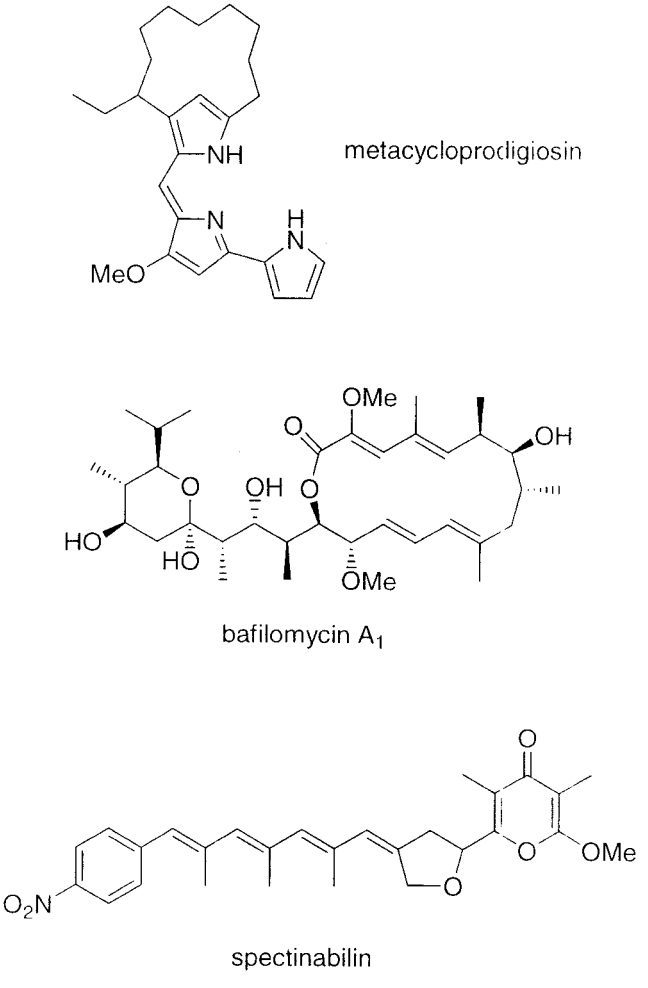

FIG. 1. Structures of the metabolites from *S. spectabilis* BCC 4785.

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TABLE 1. Antimalarial activities and cytotoxicities of compounds isolated from *S. spectabilis* BCC 4785

Compound	Activity (IC_{50}, μg/ml) against *P. falciparum* K1	Cytotoxicity (IC_{50}, μg/ml) for:		
		KB cells	BC-1 cells	Vero cells
Metacycloprodigiosin hydrochloride[a]	0.0050 ± 0.0010	0.36 ± 0.02	0.27 ± 0.01	1.35 ± 0.28
Bafilomycin A_1[a]	0.041 ± 0.010	0.27 ± 0.03	0.20 ± 0.04	1.14 ± 0.04
Spectinabilin	7.8	0.10	0.80	20
Chloroquine diphosphate[b]	0.16	16	>20	>20
Artemisinin[b]	0.0011	>20	>20	>20

[a] Assays were performed in triplicate for these highly active compounds; values are means and standard deviations.
[b] Standard antimalarial compounds.

IC_{50}s of the standard compound, ellipticine, in our system were 0.46 μg/ml for KB cells and 0.60 μg/ml for BC-1 cells.

The bioassay results are summarized in Table 1. Metacycloprodigiosin hydrochloride and bafilomycin A_1 exhibited significant antimalarial activity, while spectinabilin moderately inhibited the proliferation of *P. falciparum* K1. The cytotoxic activity of metacycloprodigiosin hydrochloride was much weaker than its antimalarial activity.

Prodigiosins have been known to exhibit a wide range of biological activities, and recent investigations on their immunosuppressive activities (5, 14, 17) and activities as proton pump inhibitors (12, 15) have sparked renewed interest in these tripyrrole pigments. The in vivo activities of metacycloprodigiosin hydrochloride and several other prodigiosins against *Plasmodium berghei* in mice have been reported (2, 6). According to the literature (6), elongation of the mean survival time of *P. berghei*-infected mice by oral administration of metacycloprodigiosin hydrochloride was observed with a dose of 20 mg/kg. Although members of this class of compounds have shown activity against malaria in an animal model, little has been done concerning their further development. This situation may be due to the lack of availability of compounds in large amounts. To the best of our knowledge, this is the first report on the in vitro activity of a prodigiosin against a human malaria parasite (*P. falciparum*). The high antiplasmodial activity, good selectivity index, and structural novelty of this class of compounds deserve further investigation.

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