COMMUNICATIONS TO THE EDITOR

In Vitro and *In Vivo* Antimalarial Activities of a Carbohydrate Antibiotic, Prumycin, against Drug-resistant Strains of *Plasmodia*

Sir:

In the course of our program to discover antimalarial antibiotics active against drug-resistant parasites, by screening soil microorganisms and antibiotic library of the Kitasato Institute for Life Sciences, we previously reported on various microbial metabolites exhibiting potent antimalarial properties[1~4]. Now, we find that prumycin[5~7], a compound in the antibiotic library of our institute, has potent and moderately selective antimalarial activity *in vitro* and *in vivo*. We report here the antimalarial profiles of prumycin and its derivatives (Fig. 1) in comparison with those of clinically used antimalarial drugs, and also present some conclusions on structure-activity relationships.

Prumycin was purified from the cultured broth of *Streptomyces* sp. strain No. F-1028[5,6]. The derivatives (**2**~**4**) were synthesized according to our previous report[7]. Benzyl 4-amino-2-(benzyloxycarbonyl) amino-2,4-dideoxy-α-L-arabinopyraside (**5**) was a generous gift of Nippon Kayaku Co. LTD., (Japan). The compounds (**6**~**8**) were prepared from **5** with the protected amino acids (DCC, CH_2Cl_2, r.t.), followed by deprotections.

In vitro activities against *Plasmodium falciparum* strains K1 (drug-resistant) and FCR3 (drug-sensitive), and cytotoxicity against human diploid embryonic cell line MRC-5, were measured as described previously[1]. Rodent malaria-derived strains for *in vivo* 4-days suppressive testing, *P. berghei* N (drug-sensitive) and *P. yoelii* ssp. NS (chloroquine-resistant) were used to assess *in vivo* efficacy as reported previously[1,2]. Prumycin was dissolved in water, while other test compounds were solubilized in 10% DMSO-Tween 80 aqueous solution. The formulated samples were administered subcutaneously (s.c.) to mice two hours after infection with parasites (Day 0), and then once a day for 3 days (Days 1~3). On the day after the last treatment (Day 4), thin blood films were made from the tail blood of the mice, and the parasitaemia was determined as described previously[2].

Table 1 shows the *in vitro* antimalarial activities of prumycin, its derivatives and some standard antimalarial drugs. Prumycin and α-prumycin had similar activity to choroquine against the drug-resistant K1 strain of *P. falciparum*, but were less potent than the clinically used antimalarials artemether, artemisinin and artesunate. Furthermore, prumycin and α-prumycin showed similar activities against the drug-sensitive FCR3 strain of *P. falciparum*. However, the IC_{50} values of prumycin derivatives (**3**~**8**) against the K1 strain were $>12.5\ \mu$g/ml.

Fig. 1. Structures of prumycin and its derivatives.

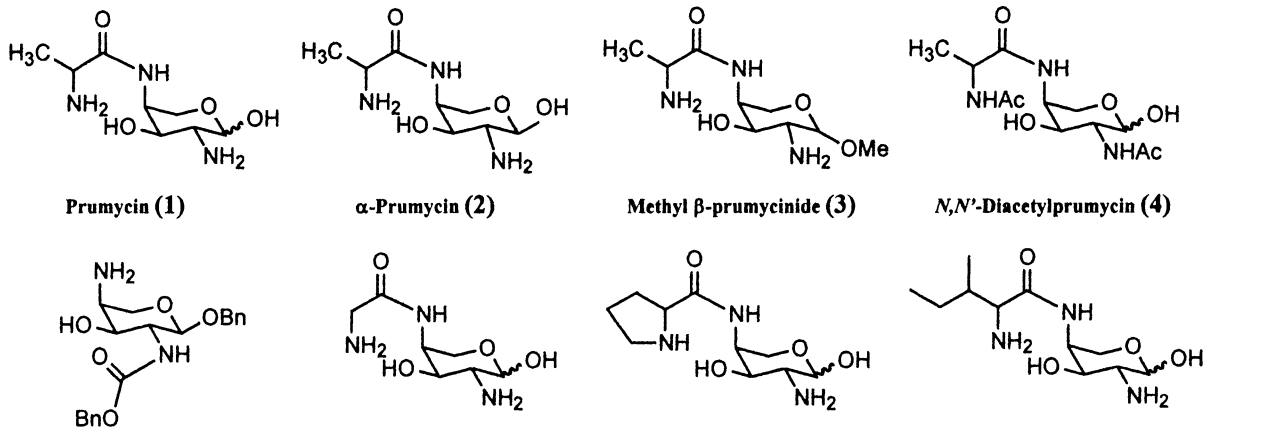

Table 1. Antimalarial activities of prumycin, its derivatives and the antimalarial drugs against K1 and FCR3 strains of *Plasmodium falciparum*.

Compound	IC50 (μg/ml)	
	K1 strain*	FCR3 strain**
Prumycin (1)	0.16	0.17
α-Prumycin (2)	0.34	0.14
3	>12.5	ND***
4	>12.5	ND
5	>12.5	ND
6	>12.5	ND
7	>12.5	ND
8	>12.5	ND
Artemisinin	0.01	0.006
Artemether	0.0023	0.0007
Artesunate	0.0042	0.001
Chloroquine	0.3	0.02

* drug-resistant strain ** drug-sensitive strain *** not determined

Table 2. *In vivo* subcutaneous antimalarial activities of prumycin, artemether, artesunate and chloroquine against *P. berghei* N and *P. yoelii* ssp. NS.

Parasite	Compound	ED50 (mg/kg)	ED90 (mg/kg)
P. berghei N*	Prumycin	6.2	17.0
	Artemether	0.95	3.8
	Artesunate	1.7	10.0
	Chloroquine	1.5	2.5
P. yoelii ssp. NS**	Prumycin	10.2	27.8
	Artemether	1.1	5.1
	Artesunate	0.4	26.0
	Chloroquine	4.5	>100.0

* drug-sensitive strain ** chloroquine-resistant strain

We then investigated the cytotoxicities of prumycin and α-prumycin against MRC-5 cells and found them to be, respectively, 3.6 and 1.2 μg/ml. Prumycin and α-prumycin showed moderate to low selectivity indexes, with ratios in the ranges of 22.5~3.5 and 21.3~8.6 for the MRC-5 cells/K1 strain and MRC-5 cells/FCR3 strain, respectively.

The lack of antimalarial activity of prumycin derivatives (**3**~**8**) in comparison to prumycin provides interesting information on structure-activity relationships. Thus introduction of a methyl group on the hydroxyl (to give **3**) and acetylating the two primary amino groups (resulting in **4**) destroys activity. Furthermore, replacement of the L-alanine group on the amino moiety at C-4 with, respectively, L-glycine (**6**), L-proline (**7**), and L-isoleucine (**8**) also destroys activity. These results suggest that a free anomer at C-1, amino groups at C-2, and the methyl group at L-alanine all play an important role in the antimalarial activity of prumycin.

Further studies are necessary to extend the structure-activity relationships of prumycin-related antimalarial compounds.

Table 2 shows a preliminary comparison of the *in vivo* subcutaneous antimalarial activities of prumycin and the standard antimalarial drugs. Prumycin had moderate activity against both rodent malaria-derived *P. berghei* N and *P. yoelii* ssp. NS, but was less effective than the clinically used antimalarials artemether and artesunate. The ED_{90} value of prumycin (27.8 mg/kg) against the chloroquine-resistant strain (*P. yoelii* ssp. NS) was much lower than that of chloroquine (>100 mg/kg), and was similar to that of artesunate.

It is known that prumycin has inhibitory activities against phytopathogenic fungi such as *Sclerotinia sclerotiorum* and *Botorytis cineria*[5,6], and against tumor cells[8~11], and that its mode of anti-fungal action in growing cells of *B. cineria* involves selective inhibition of protein synthesis[12]. Furthermore, we previously reported that it inhibits both protein synthesis and DNA synthesis in cultured HeLa S3 cells[8]. However, the finding of the antimalarial activities of prumycin and its derivative is novel and the above data are the first report of such properties.

We previously reported that the LD_{50} values (i.v. and i.p., in mice) of prumycin were 144 and 155 mg/kg, respectively. However, we did observe toxicity (loss of weight, mortality) when the compound was delivered by the s.c. route at >30 mg/kg×4. These effects are being investigated further.

The above results reveal that prumycin is a promising lead compound for a new type of antimalarial drug. Further investigation of the antimalarial potential of prumycin is in progress.

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