Parasitol Res (2000) 86: 665–668

ORIGINAL PAPER

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Antimalarial activity of new gossypol derivatives

Received: 27 November 1999 / Accepted: 28 January 2000

Abstract Gossypol, a disesquiterpene extracted from cotton seeds, is known to inhibit strongly the *Plasmodium falciparum* lactate dehydrogenase, but its high toxicity has stopped any antimalarial drug development. A series of Schiff's bases was synthesized from gossypol by modification of the aldehyde groups responsible for its toxicity. A total of 13 compounds showing low cytotoxicity were then selected and were compared with gossypol for activity against 2 chloroquine-resistant strains of *P. falciparum* (PFB, FCB1). These in vitro activities were evaluated using an isotope-based drug-susceptibility semiautomated microdilution test followed by determination of IC_{50} values (50% inhibitory concentration). In all, 12 of the 13 compounds tested were active; 3 of them displayed antimalarial activity comparable with that of gossypol itself.

Introduction

Malaria remains a major tropical public health problem infecting over 200 million people worldwide. This serious problem is worsened by the spread of drug-resistant strains and of vectors resistant to insecticides. The emergence of malaria parasites resistant to conventional drug therapy, especially to that with chloroquine, has stimulated the search for new antimalarial compounds and cellular targets (Peters 1987; WHO 1989; Björkman 1991; Kell 1991).

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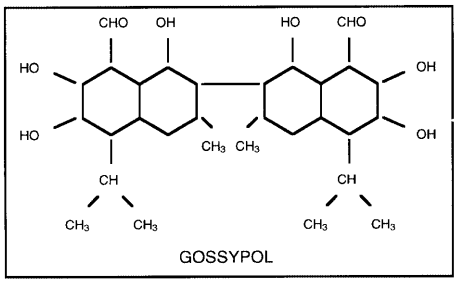
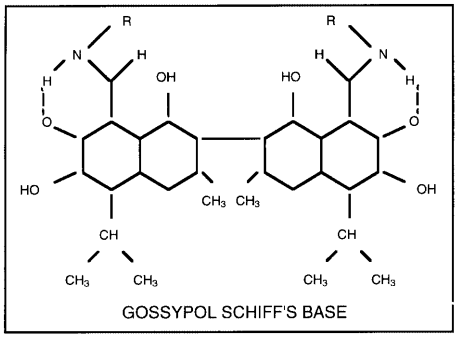
Lactate dehydrogenase (LDH) is one of the most active enzymes expressed by *Plasmodium falciparum* (Vander Jagt et al. 1981, 1990). This enzyme is essential for the anaerobic life cycle of *P. falciparum*, and compounds that inhibit this enzyme also kill the parasite (Royer et al. 1986; Read et al. 1999). This target has become very interesting since gossypol, a toxic disesquiterpene (Fig. 1) isolated from cotton seeds, has been described as an in vitro antimalarial agent against the human pathogen *P. falciparum* (Heidrich et al. 1983; Vander Jagt et al. 1984). Gossypol is a competitive inhibitor of the binding of reduced nicotinamide adenine dinucleotide (NADH) to LDH, and its activity against *P. falciparum* may be attributable to its high affinity to the plasmodial enzyme, which has been reported to show structural and biochemical differences from the host enzyme and could therefore be an attractive target for drug design (Dunn et al. 1996; Vander Jagt et al. 1981).

Gossypol is marginally cytotoxic at high doses. This toxicity may be related to reaction of the two functional aldehyde groups in the molecule, which can easily bind to free amino groups of various proteins of biological importance, leading to the formation of stable Schiff's bases (Clark 1928). Series of gossypol derivatives whose aldehyde groups have been modified have been tested on *P. falciparum* and have been shown to be inhibitors of LDH. The most active gossypol derivatives are gossylic lactone, gossypol deoxy derivatives, and gossypol peryacyl nitriles (Vander Jagt et al. 1984; Royer et al. 1986; Deck et al. 1991, 1998; Gomez et al. 1997). Taking account of these data, in a first attempt we studied molecules with a closely related structure whose functional aldehyde groups had been modified to decrease the toxicity to humans. These molecules were synthesized as Schiff's bases (Fig. 1) by Dr. R. Michelot at the Institute of Natural Subtances, Gif/Yvette, France (Dao Vi et al. 1998). In this article we present data on the antimalarial activity of these new gossypol derivatives.





DERIVATIVES	R
Methyl gossypol	- CH_3
Ethyl gossypol	- CH_2 - CH_3
Propyl gossypol	- CH_2 - CH_2 - CH_3
Isopropyl gossypol	- $CH(CH_3)_2$
Butyl gossypol	- CH_2 - CH_2 - CH_2 - CH_3
S-butyl gossypol	- CH_2 - $CH(CH_3)$ - CH_3
T-butyl gossypol	- $C(CH_3)_3$
Pentyl gossypol	- CH_2 - CH_2 - $(CH_2)_2$ - CH_3
Hexyl gossypol	- CH_2 - CH_2 - $(CH_2)_3$ - CH_3
Heptyl gossypol	- CH_2 - CH_2 - $(CH_2)_4$ - CH_3
Dodecyl gossypol	- CH_2 - CH_2 - $(CH_2)_9$ - CH_3
Mephenet [a] gossypol	- $CH_2(CH_3)CH$ - C_6H_5
Phemet [b] gossypol	- $CH(COOCH_3)$ - CH_2 - C_6H_5

[a] mephenet: β–methyl phenylalanine ethyl.
[b] phemet: phenylalanine methyl ester

Fig. 1 Chemical formulae for gossypol and gossypol Schiff's bases

Materials and methods

Strains of *Plasmodium falciparum*

Two chloroquine-resistant strains were used: the Brasilian PFB strain and the Colombian FCB1 strain of *P. falciparum*, both of which had been cloned by the limited-dilution method.

Parasite culture

P. falciparum was maintained in vitro on human type O+ erythrocytes according to the method of Trager and Jensen (1976) in RPMI 1640 medium (Gibco BRL) containing L-glutamine, 25 m*M* $NaHCO_3$, 25 m*M* HEPES, 11 m*M* glucose, 1% penicillin-streptomycin, and 10% heat-treated human O+ serum in a candle-jar atmosphere at 37 °C.

Drugs

Gossypol was extracted from cotton seeds as described by Withers and Carruth (1915) and was purified twice by crystallization as racemic gossypol acetic acid. The following 13 gossypol derivatives were synthesized and stored under argon until used: methyl gossypol, ethyl gossypol, propyl gossypol, isopropyl gossypol, butyl gossypol, *s*-butyl gossypol, *t*-butyl gossypol, pentyl gossypol, hexyl gossypol, heptyl gossypol, dodecyl gossypol, *β*-methylphenylalanine ethyl gossypol, and phenylalanine methyl ester gossypol.

Drug-susceptibility determinations

Assessment of the antimalarial activity of the 14 compounds was performed by the method described by Desjardins et al. (1979) with minor modifications. The incorporation of [^{3}H]-hypoxanthine into cultures of asynchronized parasites was used to assess the antimalarial effect of each drug. The stock solutions were prepared by dissolution of each molecule in dimethylformamide (DMF) followed by serial 2-fold dilution in complete RPMI 1640 medium to final concentrations ranging from 50 to 0.2 μg/ml. The final concentration of DMF in all test sample was $< 0.5\%$. Preliminary tests in the laboratory have shown that this concentration has no effect on parasite growth. Drug testing was performed as follows: the suspension of parasitized blood cells (200 μl/well, 1% parasitemia, 5% hematocrit) was distributed in duplicate in 96-well flat-bottom culture plates (Nalge Nunc International) with gossypol and its derivatives. Plates were incubated for 24 h prior to the addition of 0.5 μCi [^{3}H]-hypoxanthine (Amersham Life Science) to each well. The plates were then incubated for 24 h, the incubation was stopped by a cycle of freeze-thawing, and plates were harvested onto fiberglass filters (FilterMat A 1450-421, Wallac) with a cell harvester (Harvester 96 Mach III M, Tomtec). The filters were washed three times with distilled water and allowed to dry for 2 h at 45 °C, and the radioactivity was then counted in 5 ml of scintillation liquid (OptiScint Hi-safe, Wallac) in a 1450 Microbeta WorkStation counter (Wallac). The final radioactivity count was recorded after subtraction of the nonspecific radioactivity incorporated by control blood cells.

The drug concentration providing a 50% decrease (IC_{50}) in the incorporation of radioactivity with respect to parasites in drug-free medium was determined by linear-regression analysis of the drug concentration/radioactivity incorporated.

Results and discussion

IC_{50} values were obtained from plots of the growth inhibition as shown in Fig. 2. Growth inhibition, measured in counts per minute, was dose-dependent; at low concentrations the drugs had no effect on parasite growth.

The IC_{50} values recorded for gossypol and its derivatives against the two strains of *Plasmodium falciparum*, PFB and FCB1, ranged from 15.3 to 83 μ*M* as shown in Table 1. All of the compounds tested except for pentyl gossypol were active against strain PFB and/or strain FCB1. In all, 3 of the remaining 13 gossypol derivatives (ethyl gossypol, propyl gossypol, and isopropyl gossypol) showed antimalarial activity approximately identical to that of gossypol; these 3 drugs seem more promising than gossypol itself because of their low cytotoxicity to Vero cells (Dao Vi et al. 1998). With regard to the 2 resistant strains of *P. falciparum* studied, no significant difference was observed in their sensitivity to the 13 derivatives.

It seems that derivatives with ethyl, propyl, or isopropyl side chains are better recognized by the cellular target of the parasite than are molecules with very short

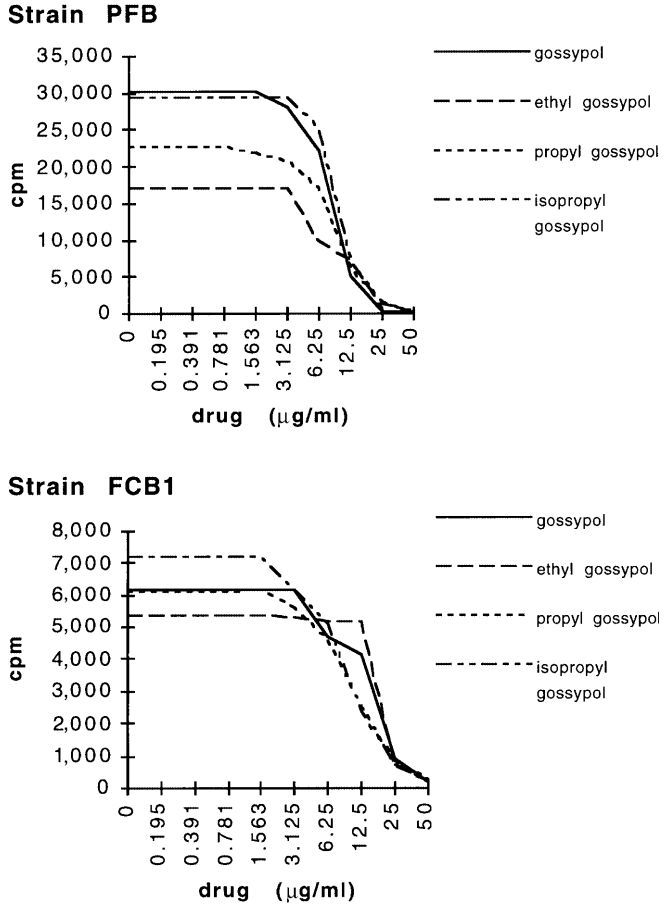


Fig. 2 Inhibition of growth of *Plasmodium falciparum* strains PFB and FCB1 by gossypol and its derivatives as determined by uptake of [^{3}H]-hypoxanthine. The *curves* represent the three drugs displaying antimalarial activity identical to that of gossypol

Table 1 Antimalarial activity of gossypol and its derivatives against *Plasmodium falciparum* in vitro (*MW* Molecular weight, *Mephenet* β-methyl phenylalanine ethyl, *Phemet* phenylalanine methyl ester, *ND* not determined)

Drug	MW	IC_{50} (μ*M*)[a]	
		Strain PFB[b]	Strain FCB1[b]
Gossypol	578.6	15.3	28.8
Methyl gossypol	544.6	ND	66.2
Ethyl gossypol	572.7	22	22.5
Propyl gossypol	600.7	16	20.8
Isopropyl gossypol	600.7	16.6	17.6
Butyl gossypol	628.8	42.4	ND
s-Butyl gossypol	628.8	37.8	54
t-Butyl gossypol	628.8	39.5	40
Pentyl gossypol	656.8	ND	ND
Hexyl gossypol	684.9	67.2	ND
Heptyl gossypol	712.9	ND	33.2
Dodecyl gossypol	853.2	43.2	37
Mephenet gossypol	752.9	47	56
Phemet gossypol	784.9	83	70.3

[a] IC_{50} represents the drug concentration producing 50% inhibition of the growth of *P. falciparum* in drug-free control wells. IC_{50} values were obtained from plots of the growth-inhibition data as shown in Fig. 1. All experimental points were obtained in duplicate
[b] Chloroquine-resistant strains of *P. falciparum*

(methyl) or much longer (butyl, pentyl, hexyl) side chains, which confirms the observation that minor structural modifications produce marked changes in selectivity of inhibition.

Our results were compared with those previously obtained using optimally active gossypol derivatives such as gossylic nitrile 1–1′-divalerate, which shows an IC_{50} value of 16 μ*M* (Royer et al. 1986). These new gossypol Schiff's bases display IC_{50} values very close to those of gossypol itself and of gossylic nitrile 1–1′-divalerate, and their very low toxicity could open a new field in malaria therapy if in vivo tests on *P. berghei*-parasitized mice confirm these results.

Acknowledgements The authors are grateful to Prof. Schrevel for providing access to laboratory facilities and to Dr. Malka Robert Gero and Prof. Joseph Blum for helpful comments on the manuscript.

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