

# Ketolide agents HMR 3004 and HMR 3647 (telithromycin) inhibit the growth of *Plasmodium falciparum* in vitro

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## Abstract

**Background:** Malaria is on the increase due to emergence of parasite drug resistance and there is thus an urgent need for the development of new antiparasitic drugs effective at low concentrations. Ketolides antibiotics are used for treatment of various ailments and are relevant candidates to establish antiparasitic activity.

**Objectives:** The present study investigates the activity of ketolide compounds HMR 3004 and HMR 3647 (telithromycin) (0.025-12.5  $\mu$ M) for activity against chloroquine-sensitive and resistant strains of *Plasmodium falciparum* in vitro.

**Methods:** The antiplasmodial activity of the two ketolide agents were determined using microscopic and colorimetric [lactate dehydrogenase assay] procedures.

**Results:** Both HMR 3004 and HMR 3647 caused a dose-dependent inhibition of growth of both parasite strains with IC50 values 3 and 15 nM, respectively. Suppression of parasite growth was evident after 8 hours of exposure to both agents at 12.5  $\mu$ M with total parasite clearance achieved at 40 hours.

**Conclusion:** The results indicate lack of cross-resistance between the ketolide compounds and chloroquine, implying presence of a drug target different from that of chloroquine. The particular drug target has still to be investigated but the stage-specific results indicate that it is expressed in all parasite growth phases. These observations demonstrate the anti-malarial potential of the ketolide antimicrobial agents.

**Key words:** Chloroquine, ketolides, macrolides, malaria, *Plasmodium falciparum*.

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## Introduction

According to the World Health Organization report, 207 million new malaria cases were recorded in 2012<sup>1</sup>. There has since been a observable decline in malaria cases that is attributable to proper intervention strategies like introduction of long-lasting insecticidal nets

(LLINs) and artemisinin-based combination therapy (ACT)<sup>2,3,4,5</sup>. The relevance of use of ACT is currently challenged by recent reports of reduced susceptibility and resistance in South-East Asia<sup>6,7</sup>. Drug development efforts are focused on the design of compounds which have reduced toxicity and increased potency, as well as on the development of agents which potentiate the activities of existing anti-malarial agents<sup>8</sup>. An alternative approach has been to examine the antiplasmodial potential of existing anti-bacterial agents<sup>9,10</sup> and the riminophenazines that have shown promise<sup>11</sup>. Presently, the World Health Organization (WHO) prefers use of artemisin in-based combination therapy (ACT) and hybrid molecules for tailored delayed acquisition of resistance and/or curtailing it in totality<sup>12,13</sup>.

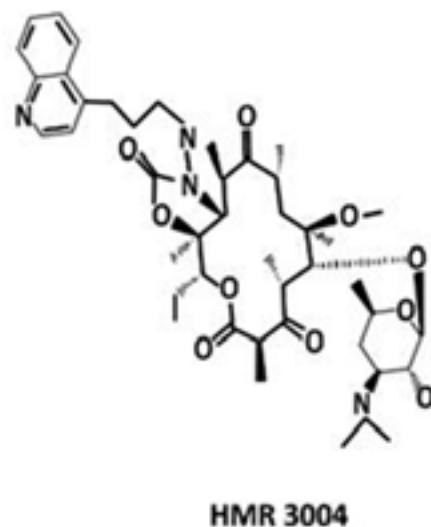
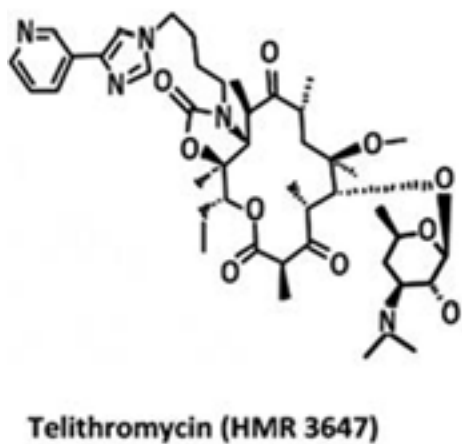
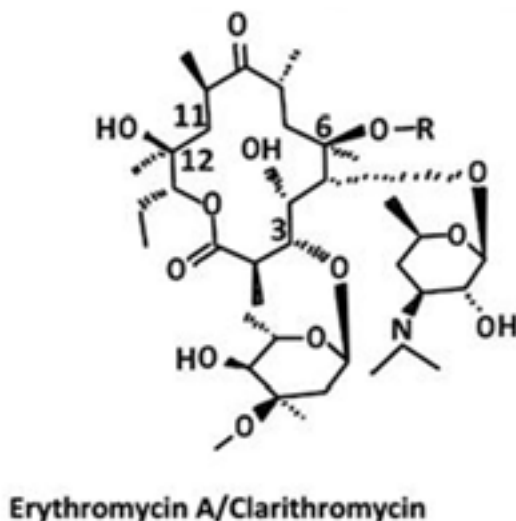
Structural modification of the prototype, 14-membered macrolide, erythromycin A, has led to the development of a novel class of macrolide agents, the ketolides. The major alteration characterising ketolides is

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replacement of the 3-cladinose of the macrolide ring by a keto-group. Ketolides inhibit microbial growth by interfering with the translation of messenger RNA, and exhibit increased potency, broadened antimicrobial spectrum and impressive accumulation in tissues and blood<sup>14,15,16,17</sup>.

Although the improved anti-bacterial properties of the ketolides are well-recognised and characterised, little or no work appears to have been conducted on the anti-malarial potential of these agents. In the current study, we have investigated the effects of HMR 3004 and HMR 3647 (Figure 1), which have a carbamate group at C11/C12 of the lactone ring with alkyl-aryl extensions on the growth of *Plasmodium falciparum* in vitro.



**Figure 1: Structures of erythromycin A, telithromycin (HMR 3647) and HMR 3004.**

## Materials and methods

### Parasites strains and culturing conditions.

The chloroquine-resistant (FCR-3) and sensitive (FAB-9) strains of *P. falciparum* were maintained in a continuous culture system of human erythrocytes (blood group O+ obtained from the SA Blood Transfusion

Services) in RPMI 1640 culture medium supplemented with 2mM L-glutamine, 25 mM HEPES, 25 mM NaHCO<sub>3</sub> and 10% human AB+ serum. The suspension was maintained at 37°C in an atmosphere of 5% O<sub>2</sub>/5% CO<sub>2</sub> / 90% N<sub>2</sub><sup>10</sup>. Cultures were monitored daily by microscopic analysis of Giemsa-stained blood smears.

Parasites cultures were synchronised to the ring stage by treatment with 5% D-sorbitol<sup>10</sup>.

#### Antimicrobial agents.

HMR 3004 and HMR 3647 were provided by Prof. RA Anderson (Department of Immunology, University of Pretoria, South Africa) and dissolved in 0.1 N HCl to give a stock solution of 5 g/l and further diluted in parasite culture medium.

#### In vitro assays.

For these assays, 20 µl of infected erythrocyte suspension (2% parasitemia and 0.5% final haematocrit) were exposed to 20 µl of the ketolide compounds (0.025-12.5 µM). Parasites (20µl) in culture medium (80µl) and culture medium alone (200µl) served as positive and negative controls respectively. Solvent controls were also run. After 48h incubation at 37°C, parasite growth was determined by microscopic evaluation of Giemsa-stained slides and by use of the LDH assay<sup>18</sup>. For the LDH assay, 20 µl of the parasite culture suspension was added to 100 µl of the Malstat reagent and colour change measured at 620 nm in a microplate spectrophotometer. The FCR-3 (chloroquine-resistant) laboratory strain of *P. falciparum* was used for the stage-specific assays. Synchronised ring-stage parasite culture suspensions (2% parasitemia and 0.5% final haematocrit)

were incubated with antimicrobial agents (12.5 µM) and parasite development monitored at 8 h intervals for 48 hours using microscopy and colorimetry.

#### Statistical analysis

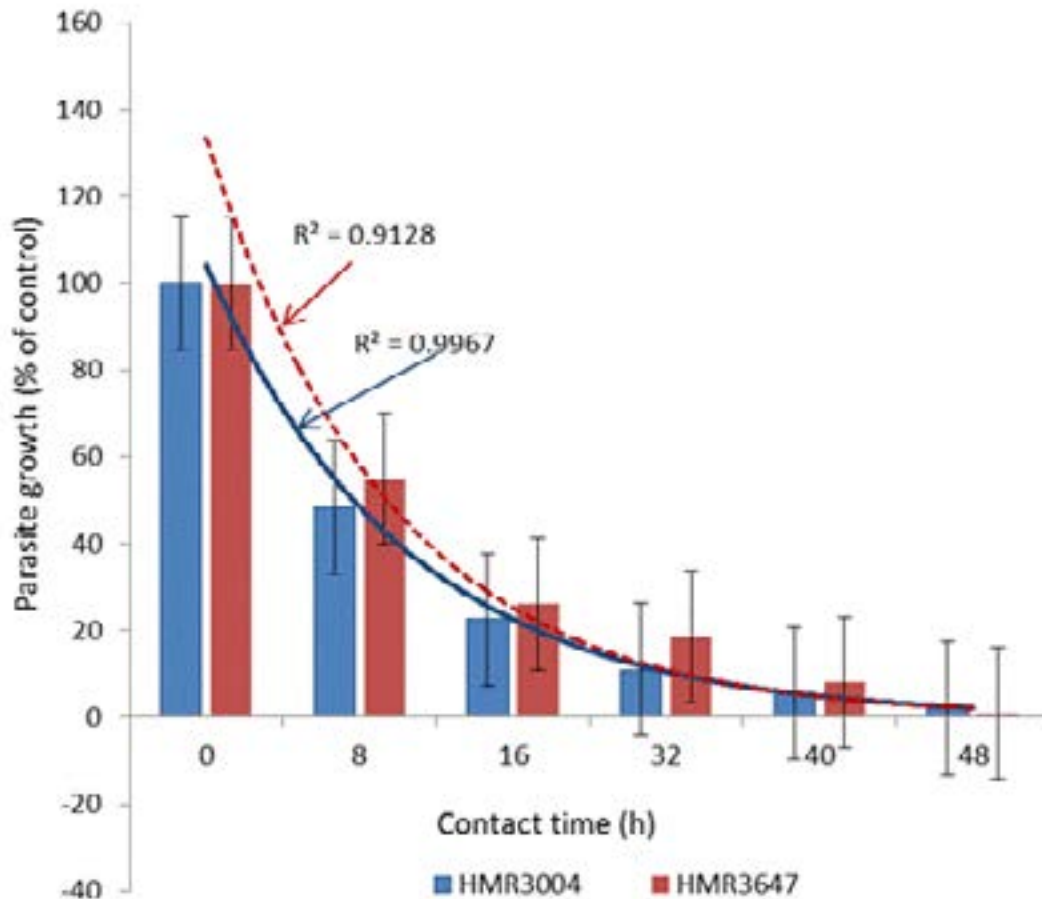
Results are expressed as the mean value ±SEM for four experiments conducted in triplicate. The antiplasmodial activity of the compounds was expressed as percentage parasite growth and the IC<sub>50</sub> values calculated using a Graph Pad Prism software version 5.00. Levels of statistical significance were calculated using Students t test (paired statistics), p≤0.05 was considered significant.

#### Results

The effects of the test agents on the growth of the chloroquine-sensitive and resistant strains of *P. falciparum* as assessed by the colorimetric procedure are shown as IC<sub>50</sub> values in Table 1. HMR 3004 and HMR 3647 exhibited a dose-related antiplasmodial activity (p≤0.05) comparable to that of chloroquine. HMR 3004 and HMR 3647 were also evaluated at 12.5 µM for in vitro stage-specific activity and the results are shown in Figure 2. After an 8 h incubation period, parasite growth was suppressed by more than 50% with extended treatment leading to complete inhibition of parasite growth at around 35 hours with both antimicrobial agents, indicating a time dependent effect. These observations were confirmed by microscopy (data not shown).

**Table 1: Effects of the compounds (0.025-12.5µM) HMR 3004 and HMR 3647 on the growth of the FAB-9 (chloroquine-sensitive) and FCR-3 (chloroquine-resistant) laboratory strains of *P. falciparum*.** Data are expressed as mean IC<sub>50</sub> values ± SEMs of four repeat experiments conducted in triplicates. Results on the activity of chloroquine not shown.

Strain	IC <sub>50</sub> (nM) ± SEM		
	CQ	HMR 3004	HMR 3647
FAB-9	31	3 ± 0.9	7 ± 0.12
FCR-3	125	9 ± 2.1	15 ± 1.34



**Figure 2: Stage-specific antimalarial activity of ketolide compounds HMR 3004 and HMR 3647 (12.5& PubMed micro;M) on the FCR-3 laboratory strain of *P. falciparum*.** Data from four repeat experiments with triplicate determinations in each are expressed as the mean percentage parasitemia of the control system  $\pm$  SEMs.  $R^2$ = coefficient of determination.

## Discussion

The results of the present study indicates that the ketolide compounds HMR 3004 and HMR 3647 inhibit the growth of *P. falciparum* in vitro at concentrations probably comparable to those of chloroquine. Importantly, chloroquine-sensitive and resistant strains of the parasite were equally affected by the ketolides, demonstrating that the mechanisms of antiplasmodial action of these agents is likely to be distinct from that of chloroquine.

Antibiotics have been screened previously for in vitro antiplasmodial activity<sup>19,20,21,22</sup>. Of interest in one of the publications is the laboratory evaluation of macrolide erythromycin and azithromycin for activity against malaria parasites in combination with classical antimalarials. This study did show synergistic and additive activities of drug interactions<sup>20</sup>. Our study unfortunately did not explore drug combination activities in vitro. Telithromycin was also assayed in both laboratory and

a mice models of malaria infection and did show activities that are not comparable to that in our present study<sup>23,24</sup>. The differences in the results might be due to employment of different experimental platforms and parasite strains. For example, the kinetics model extends to over 48 hours thus including the re-infection stage, which is dissimilar to the same assay in the present study. The efforts to develop macrolides as antimalarials have proved fruitless.

HMR 3004 and HMR 3647 also inhibited the growth of the parasite increasingly with time, it does suggest that these chemicals inhibited parasite growth in a time-dependent manner. The observation may indicate that the molecular targets of these agents are expressed by the parasite and are affected as the parasite is exposed over time.

Ketolides inhibit bacterial growth by interfering with its ribosomal translation processes<sup>14</sup>. *Plasmodium* spp. have also been shown to express two forms of riboso-

mal RNA, A- and S-type RNA found in the asexual and sexual forms of the parasite respectively<sup>17</sup>. Although these are potential targets of the ketolides in the parasite, existence of alternative or additional targets cannot be excluded.

### Conclusion

The ketolide antimicrobials HMR 3004 and HMR 3647 possess antiparasite activity in vitro, and should further be evaluated with respect to molecular/biochemical mechanisms of action and efficacy in animal models of experimental chemotherapy.

### Acknowledgements

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### Transparency declarations

None to declare.

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