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Acyloxyalkyl Ester Prodrugs of FR900098 with Improved In Vivo Anti-Malarial Activity

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Abstract—FR900098 represents an improved derivative of the new antimalarial drug fosmidomycin and acts through inhibition of the 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, an essential enzyme of the mevalonate independent pathway of isoprenoid biosynthesis. Prodrugs with increased activity after oral administration were obtained by chemical modification of the phosphonate moiety to yield acyloxyalkyl esters. The most successful compound demonstrated 2-fold increased activity in mice infected with the rodent malaria parasite *Plasmodium vinckei*.

Malaria affects over 40% of the world's population, and 1.5–3 million people die of the disease every year, most of them children. The numbers are still rising since available anti-malarials such as chloroquine and sulfadoxin-pyrimethamine increasingly lose efficacy due to the spread of resistant parasite strains. As a result, there is an urgent need for new efficient antimalarial agents.[1]

In malaria parasites, isoprenoids are synthesized by a mevalonate independent pathway, the so-called DOXP pathway, which is absent in humans. In previous studies, we demonstrated that fosmidomycin exerts potent antimalarial activity by inhibition of DOXP reductoisomerase, the second enzyme in the reaction cascade of the DOXP pathway.[2,3] In recent clinical trials conducted in Gabon and Thailand, fosmidomycin proved to be efficient in the treatment of patients suffering from acute, uncomplicated *Plasmodium falciparum* malaria.[4] The acetyl derivative of fosmidomycin, FR900098, is approx. twice as active against *P. falciparum* in vitro and in the *Plasmodium vinckei* mouse model.[2] However, the oral bioavailability of both compounds is only moderate, with a resorption rate of approx 30%, most likely due to very low lipophilicity as a result of high ionization of the phosphono moiety at physiological pH values.[5] In previous work, we synthesized diaryl ester prodrugs of FR900098 in order to increase gastrointestinal resorption.[6] These prodrugs resulted in improved oral efficacy in *P. vinckei* infected mice, but concerns about the toxicity of the phenol derivative generated through processing of the prodrugs prompted us to investigate an alternative strategy. Therefore, a series of acyloxyalkyl esters expected to be hydrolysed by non-specific esterases has been prepared.

Synthesis of the prodrugs was achieved starting from the aldehyde **2**, which is readily accessible from the commercially available corresponding acetale **1** (Scheme 1). Treatment of the aldehyde **2** with *O*-benzylhydroxylamine gave the oxime, which was then treated without prior isolation with sodium cyanoborohydride and hydrochloric acid to yield the *O*-(benzylhydroxylamino)propylphosphonic acid diethyl ester **3**.[7] Acetylation to **4** was carried out with acetylchloride in dichloromethane. Reaction of **4** with trimethylbromosilane and hydrolysis of the resulting silylester with water yielded the *O*-benzyl protected FR900098 **5** (Fig. 1).[8] Phosphonic acid **5** was then coupled with chloroacetic acid methylester or chloromethyl pivalate, respectively, in DMF in the presence of triethylamine giving the phosphonic acid diesters **6a** and **6b**.[9] This method was unsuccessful in the case of 1-chloroethyl pivalate and 1-chloroethyl benzoate. However, when the phosphonic acid **5** and the chloroalkyl esters[10] were

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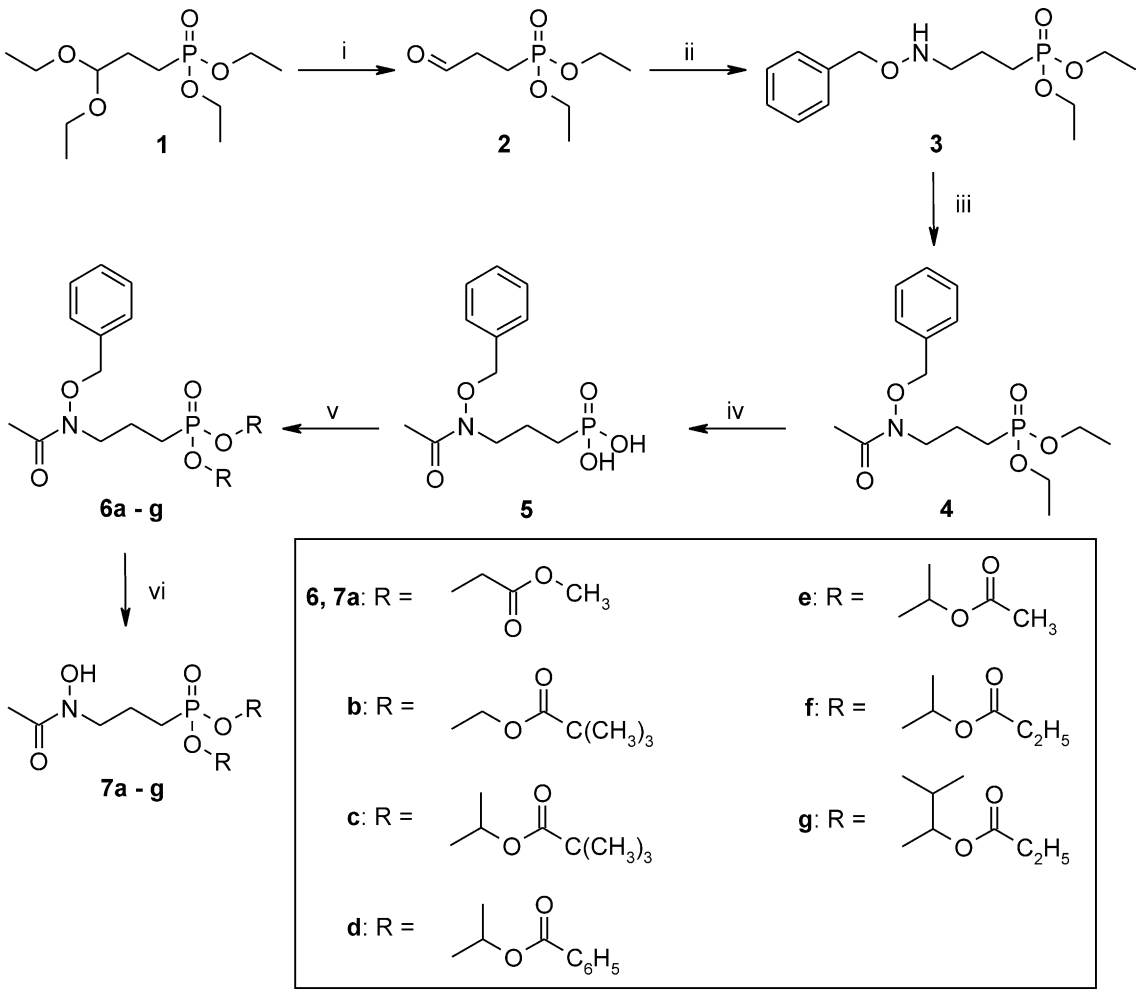

Scheme 1. Conditions and yields: (i) 2 N HCl, overnight, rt, 63%; (ii) *O*-benzylhydroxylamine in methanol, 3 h, 40 °C and then $NaCNBH_3$ in methanol, HCl, 1 h, rt, 99%; (iii) acetylchloride in CH_2Cl_2, $(H_3C)_3N$, overnight, rt, 97%; (iv) trimethylbromosilane in CH_2Cl_2, 30 min, 0 °C and then H_2O, overnight, rt, 82%; (v) (a and b) RCl in DMF, $(H_3C)_3N$, 6 h, 60 °C; (c–g) RCl, DMPU, NaI, $(CH_3)_3N$, 6 h, 60 °C; (vi) H_2, Pd/C, MeOH.

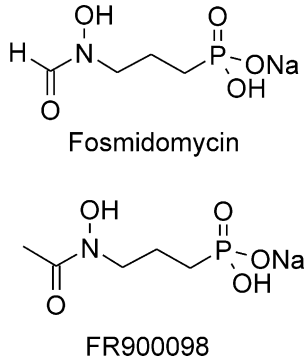

Figure 1. Structures of fosmidomycin and FR900098.

reacted in DMPU in the presence of triethylamine and sodium iodide, the bis(acyloxy)alkyl esters **6c–g** were obtained as diastereomeric mixtures.[9] Removal of the protecting groups with hydrogen and 10% Pd/C in methanol gave the prodrugs **7a–g**.[11]

The anti-malarial activity of the prodrugs was tested in the *P. vinckei* mouse model similar as described before.[6] On day 0, Balb/c mice were inoculated by ip injection of 5×10^7 infected erythrocytes from a donor mouse. On days 1 and 2, the mice were treated by oral administration of 40 mg/kg of the prodrugs.[12] For comparison, another group of mice was treated with an equivalent dosage of FR900098. This dosage was estimated to result in a partial reduction of parasitaemia. On the following days, parasitaemia was monitored to assess the efficacy of the compounds.

In the first experiment, the efficacy of **7a** and **7b** was tested (Fig. 2). The treatment with the acetyl ester derivative **7a** resulted in a marked reduction of parasitaemia compared with untreated control mice, but was less effective than FR900098. In contrast, the pivaloyloxymethyl ester **7b** was significantly more active in suppressing the development of parasitaemia than the parent compound. Based on this result, additional acyloxyalkyl derivatives of FR900098 were investigated. In order to avoid the release of formaldehyde, the dioxymethylen group was replaced by a 1,1-dioxyethylen group generating the less toxic acetaldehyde. The pivaloyloxyethyl ester **7c** and the benzoyloxyethyl ester **7d** displayed activities comparable with but not superior to FR900098. Therefore, two derivatives which carry less bulky acyl residues were tested. Both the acetyloxyethyl

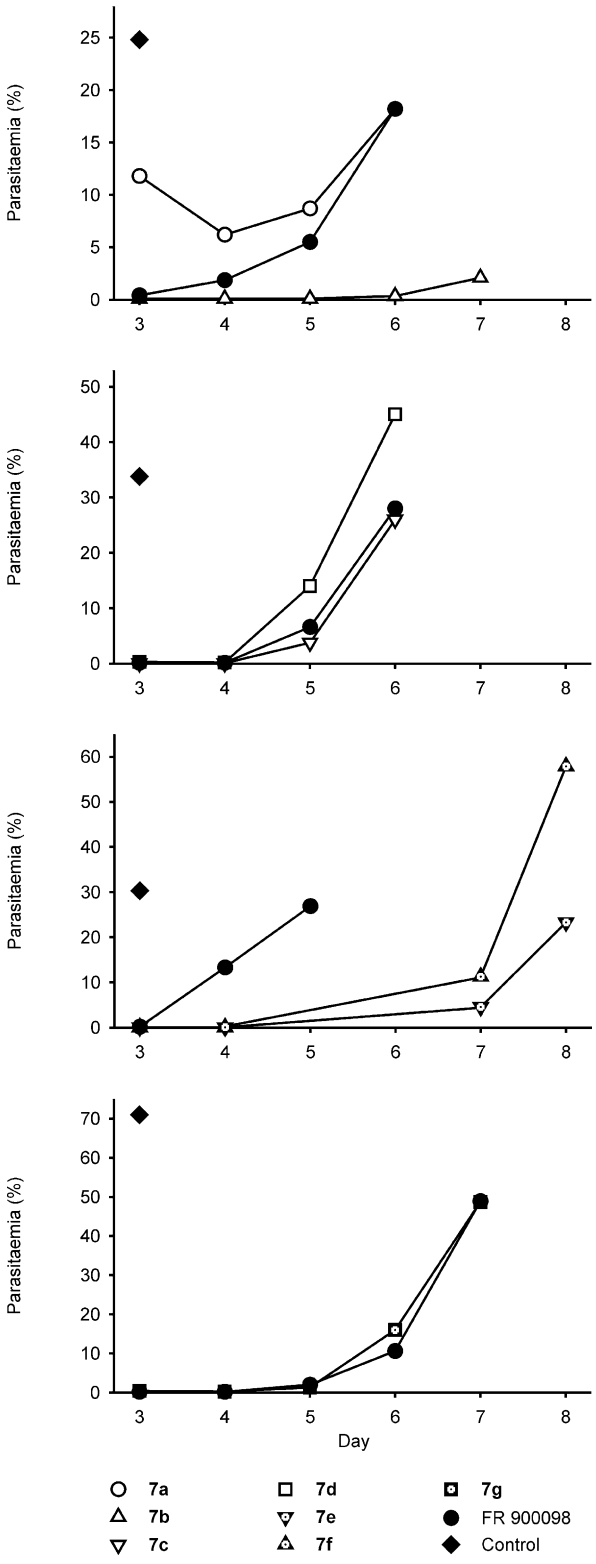

Figure 2. Antimalarial efficacy of the prodrugs **7a–g** in comparison to FR900098. Mice were infected on day 0 and treated with 40 mg/kg of the respective compound on day 1 and 2. Parasitaemia was monitored on days 3–8.

ester **7e** and propionyloxyethyl ester **7f** proved to be more effective than FR900098, with **7e** being slightly more active than **7f**. In contrast, the propionyloxyisobutyl derivative **7g** did not lead to an improved activity.

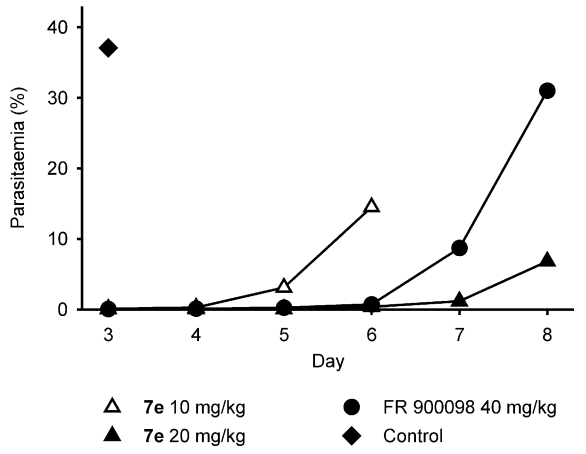

Figure 3. Antimalarial efficacy of 10 and 20 mg/kg **7e** in comparison to 40 mg/kg FR900098. Mice were infected on day 0 and treated on days 1 and 2. Parasitaemia was monitored on days 3–8.

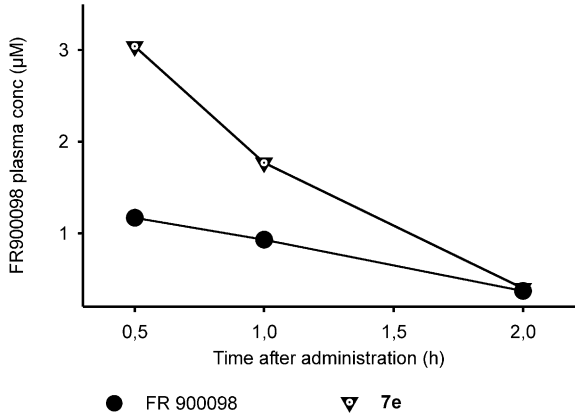

Figure 4. Plasma concentration of FR900098 in mice after oral administration of 40 mg/kg FR900098 and **7e**.

In a subsequent experiment, **7e** as the most successful compound of this series was characterized further. In order to obtain a quantitative assessment of the increase in efficacy, mice were treated with 10 mg/kg and 20 mg/kg of **7e** (Fig. 3), respectively, and compared with another group of mice receiving 40 mg/kg FR900098. The treatment with 10 mg/kg **7e** resulted in a reduced efficacy compared with 40 mg/kg FR900098. The treatment with 20 mg/kg **7e**, however, was slightly more effective than 40 mg/kg FR900098.

In order to confirm whether the improved efficacy of **7e** correlates with an increased bioavailability, the plasma levels of FR900098 were monitored at different time points after oral administration of identical doses of FR900098 and **7e**. Since currently no chromatographic methods for the determination of low amounts of FR900098 are available, the inhibitory activity of the plasma samples against recombinant DOXP reductoisomerase was measured in order to provide an estimation of the FR900098 plasma concentration.[13] As expected, the FR900098 plasma concentration was significantly higher after administration of the prodrug (Fig. 4). At 0.5 h, after administration the plasma level was 3.0 µM after administration of **7e** in comparison to

1.2 μM after administration of FR900098. After 2 h, the plasma concentration fell to the limit of quantification at approx. 0.4 μM in both groups.

In summary, prodrugs of FR900098 with increased oral anti-malarial efficacy were obtained by masking the polar phosphonate moiety as acyloxyalkyl esters. The acetyloxyethyl ester **7e**, which is expected to release only acetic acid and acetaldehyde upon hydrolysis in addition to the active compound, was at least twice as active than FR900098.[14]

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11. Target compounds **7a–g** were characterized by IR, ^{1}H NMR, MS and microanalysis.
12. Prodrugs were dissolved in DMSO at 150 mg/mL and further diluted in SSV (standard suspension vehicle; 0.9% NaCl, 0.5% Na-carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80). FR900098 was dissolved in SSV. Parasitaemia was monitored by Giemsa-stained blood smears. Mice were sacrificed when parasitaemia was exceeding approx 40%. Four mice were used for each treatment group, and three mice for the control group. Results were expressed as geometric mean values for each group.
13. At each time point, one mouse of each group was sacrificed, and the blood collected. The enzyme inhibition assay was performed in a reaction mixture containing 100 mM Tris–HCl (pH 7.5), 0.2% BSA, 1 mM $MnCl_2$, 1 mM NADPH, 0.3 mM DOXP, and 1 μg/mL DOXP reductoisomerase from *Escherichia coli*. The mixture was incubated with a dilution series of the plasma samples on a 96-well microtiter plate, and the reaction started by addition of DOXP. The decrease of absorption was monitored at 340 nm using a SpectraMax 340PC microplate reader (Molecular Devices, Ismaning). The plasma concentrations were calculated from the enzyme inhibition values obtained with FR900098 standard solutions analyzed in parallel.
14. When calculating the dosages in molar units instead of mass units (40 mg/kg FR900098 = 0.18 mmol/kg; 20 mg/kg **7e** = 0.054 mmol/kg), **7e** has 3-fold increased activity compared with FR900098.