Short Communication

Double Ester Prodrugs of FR900098 Display Enhanced *In-Vitro* Antimalarial Activity

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Fosmidomycin and FR900098 are inhibitors of the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR; IspC), a key enzyme of the mevalonate-independent isoprenoid biosynthesis pathway. We have determined the *in-vitro* antimalarial activity of two double ester prodrugs **2**, **3** in direct comparison with the unmodified FR900098 **1** against intraerythrocytic forms of *Plasmodium falciparum*. Temporarily masking the polar properties of the phosphonate moiety of the DXR inhibitor FR900098 **1** enhanced not only its oral bioavailability but also the intrinsic activity of this series against the parasites.

Keywords: 1-Deoxy-D-xylulose 5-phosphate reductoisomerase inhibitors / Mevalonate-independent isoprenoid biosynthesis / Prodrug

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Introduction

Malaria is the most important tropical disease and one of the most important infectious diseases altogether. Due to the widespread occurrence of *Plasmodium falciparum* strains resistant against most common antimalarial agents development of new drugs directed against targets hitherto unexploited is mandatory [1].

Fosmidomycin is an inhibitor of the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR; IspC), a key enzyme of the mevalonate-independent isoprenoid synthesis. This biosynthetic pathway is used in most eubacteria as well as by the plastids of algae and higher plants. It is also used exclusively in *P. falciparum*, while being absent in the human host [2 – 4]. In three different clinical studies, fosmidomycin in combination with the antibiotic clindamycin has shown efficacy and good tolerability [5 – 7]. However, due to its high polarity, oral bioavailability is only about 30% [8].

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FR900098 **1** is a structurally closely related derivative of fosmidomycin, and is about twice as active as fosmidomycin against cultured parasites and in animal models [8]. To enhance oral bioavailability we have developed double ester prodrugs [9, 10].

Results and discussion

Synthesis of the prodrugs [10] was achieved starting from the aldehyde **2**, which is readily accessible from the commercially available diethyl acetal (Scheme 1). Treatment of the aldehyde **2** with *O*-benzylhydroxylamine and subsequent reduction with sodium cyanoborohydride gave the *O*-(benzylhydroxylamino)propylphosphonic acid diethyl ester **3**. 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**. Phosphonic acid **5** was then coupled with commercially available chloromethyl pivalate and 2-chloroethyl acetate respectively yielding **6a** and **b**. Removal of the protecting groups with hydrogen and 10% Pd/C in methanol gave the prodrugs **7a** and **b**.

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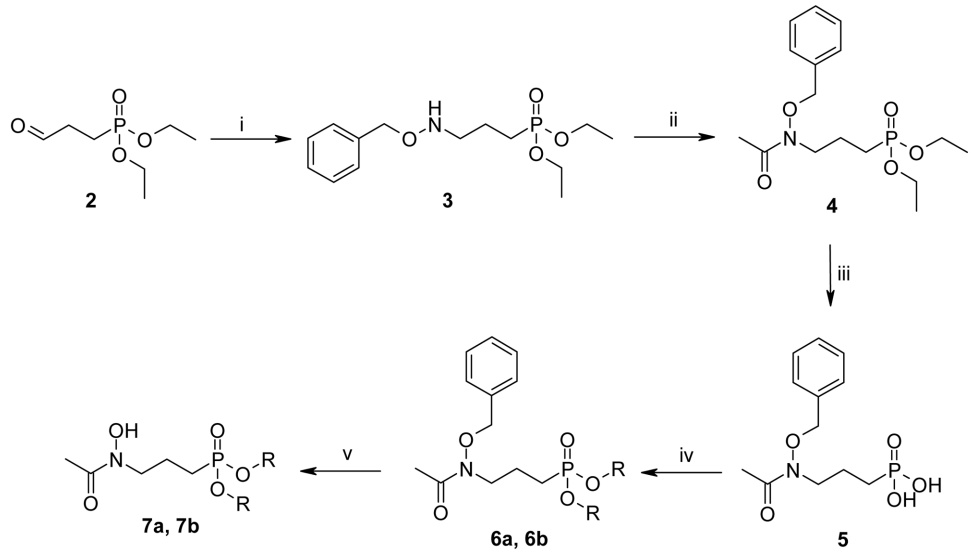

Conditions and yields: (i) *O*-benzylhydroxylamine in methanol, 3 h, 40°C, $NaCNBH_3$ in methanol, HCl, 1 h, rt, 99%; (ii) acetylchloride in CH_2Cl_2, $(H_5C_2)_3N$, overnight, rt, 97%; (iii) trimethylbromosilane in CH_2Cl_2, 30 min, 0°C, overnight, H_2O, rt, 82%; (iv) **6a**: RCl in DMF, $(H_5C_2)_3N$, 6 h, 60°C, **6b**: RCl, DMPU, NaI, $(H_5C_2)_3N$, 6 h, 60°C; (v) H_2, Pd/C, MeOH.

Scheme 1. Synthesis of compounds **2**–**7**.

Double ester prodrugs displayed 2-3fold higher activities in comparison to the unmodified FR900098 **1** when dosed orally to malaria-infected mice [10].

It has been anticipated that these prodrugs are resorbed from the gastrointestinal tract and cleaved by plasma esterases into the active FR900098 and the corresponding aldehyde and acid. Using the activity of plasma samples against DXR, the concentration of free FR900098 was estimated to be 3 μM 0.5 h after oral administration of 40 mg/kg of prodrug **7b** into mice in comparison to 1.2 μM after administration of FR900098 (40 mg/kg). It was further anticipated that free phosphonate FR900098 **1** then should enter and subsequently kill the parasites.

Independently, it was also shown that double ester prodrugs of several fosmidomycin derivatives display *in-vitro* antimalarial activity [11, 12]. Hitherto, it has been anticipated that the ester prodrugs are readily cleaved under assay conditions to yield the free phosphonates which then enter and subsequently kill the parasites, so that the *in-vitro* activity of the double ester prodrugs is practically the same as the activity of the unmodified parent phosphonic acid. If this is true, there should be no difference in the *in-vitro* activity between the double ester prodrugs of FR900098 and the unmodified FR900098.

To test this hypothesis, we have tested the activity of two double ester prodrugs **7a**, **b** in direct comparison with the unmodified FR900098 **1** against intraerythrocytic forms of the *P. falciparum* strains 3D7 and Dd2 using a semi-automated microdilution assay as described [13–15]. The growth of the parasites was monitored through the incorporation of tritium-labeled hypoxanthine. 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 recombinant DOXP reductoisomerase from *E. coli*. The mixture was incubated with a dilution series of the test compounds on a 96-well 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, Germany).

Unexpectedly, the two double esters **7a**, **b** were about 2-5fold more active against the cultured parasites of the chloroquine sensitive 3D7 and the multi-resistant Dd2 *P. falciparum* strain (Table 1). Both double ester prodrugs **7a** and **b** did not show any activity against *E. coli* DXR at 30 μM, the highest concentration tested (Table 1). Since the activities of a particular compound against DXR form *E. coli* and *P. falciparum* are usually well correlated [16], we conclude that the antiparasitic activity of our esters **7a** and **b** is caused by the action of the free FR900098 molecule on DXR. Would the esters have been cleaved already in the assay medium as previously has been anticipated, their activity should not be higher than that of the unmodified FR900098. But since the esters **7a** and **b** are 2-5fold more active than the unmodified FR900098, we conclude that the double ester prodrugs possess considerable stability under the assay conditions. We propose the following explanation for the higher antiparasitic activity of the ester prodrugs. These uncleaved ester prodrugs are much more lipophilic than the free phosphonate

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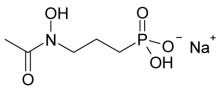
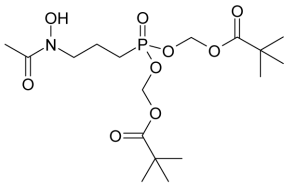
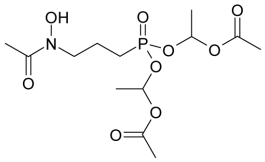
Table 1. *In-vitro* activity of FR900098 and two of its double ester prodrugs.

		IC_{50} (nM)	IC_{50} (nM)	
		E.coli **DXR**	**3D7**	**Dd2**
FR900098 (**1**)		50	868	893
Schl-7150 (**7a**)		>30 000	328	272
Schl-7168 (**7b**)		>30 000	–	174

drugs, and therefore could more readily enter the parasite via diffusion through the parasites' membranes. Possibly, the prodrugs circumvent the glycerol-3-phosphate transport system which, at least in bacteria, is thought to facilitate the uptake of the unmodified phosphonate drug fosmidomycin [17]. Inside the parasites, the double ester prodrugs are then cleaved (since the esters are inactive, see above) by the action of esterases to liberate the free FR900098.

Conclusion

In conclusion, temporarily masking the polar properties of the phosphonate moiety of the DXR inhibitor FR900098 **1** enhances not only the oral bioavailability as shown previously [9, 10], but also the intrinsic activity of these less polar derivatives against the parasites, most probably by achieving a higher intraparasitic drug concentration through enhanced drug uptake. To what degree this enhanced intrinsic antiparasitic activity contributes to the observed superior *in vivo* antimalarial activity remains to be investigated.

The authors have declared no conflict of interest.

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