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Short communication

Growth inhibition of *Mycobacterium smegmatis* by prodrugs of deoxyxylulose phosphate reducto-isomerase inhibitors, promising anti-mycobacterial agents

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ABSTRACT

Since *Mycobacterium tuberculosis* sets up several multiple anti-tuberculosis drug resistance mechanisms, development of new drugs with innovative target is urgent. The methylerythritol phosphate pathway (MEP) involved in the biosynthesis of essential metabolites for the survival of mycobacteria, represents such a target. Fosmidomycin **1a** and FR900098 **1b**, two inhibitors of DXR, do not affect the viability of *M. tuberculosis* cells, due to a lack of uptake. To overcome the absence of the mycobacterial cell wall crossing of these compounds, we synthesized and tested the inhibition potency of acyloxymethyl phosphonate esters as prodrugs of fosmidomycin **1a**, FR900098 **1b** and their analogs **2a** and **2b** on *Mycobacterium smegmatis*. Only the prodrugs **4b**–**6b** inhibit the bacterial growth and could be effective anti-mycobacterial agents.

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1. Introduction

Tuberculosis is one of the major infectious diseases: around nine million new cases are annually estimated with approaching two million human deaths [1,2]. *Mycobacterium tuberculosis*, responsible for tuberculosis, owing to its unique cell wall organization, can be considered as a fortress. The development of new drugs to fight this bacterium is a difficult challenge. The first problem encountered by anti-bacterial agents is the crossing of the cell wall. In addition, this *Mycobacterium* sets up several mechanisms of resistance to major anti-tuberculosis drugs [3]. As the continuing increase of tuberculosis risk is in large part due to the development of antibiotic resistant strains, it is urgent to find other targets for the development of new anti-tuberculosis drugs than those presently used. Isoprenoid biosynthesis in *Mycobacterium* species represents such a target in this context. Isoprenoids are found in all living organisms. A number of them including bactoprenyl diphosphate required for the biosynthesis of peptidoglycan, a major cell wall component [4], and the prenyl side-chains of menaquinones involved in electron transport chains is present in mycobacteria and represents essential metabolites for their survival [5]. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the universal precursors of all isoprenoids are synthesized *via* two pathways: the mevalonate pathway, which is present in animals and humans, fungi and the cytosol of plant cells [6], and the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway, which has been found in plant plastids, unicellular green algae, apicomplexan parasites and in most bacteria including many pathogens such as *Mycobacterium* spp [7].

The determination of the nucleotide sequences of the genomic DNA of *Mycobacterium* spp. confirmed the presence of the sole MEP pathway in this genus of bacteria. Accordingly, all enzymes of the MEP pathway can be considered as potential targets for anti-tuberculosis drug development [8]. The knowledge of the DNA sequence also allows obtaining and studying the recombinant enzymes of this pathway.

The 1-deoxy-D-xylulose 5-phosphate reducto-isomerase (DXR), the second enzyme of the MEP pathway [9] (Scheme 1) is inhibited by fosmidomycin (**1a**) and FR900098 (**1b**), two natural antibiotics [10], (Scheme 2) which consequently block the growth of many bacteria possessing the MEP pathway. Owing to the rapid emergence of resistance observed in the case of fosmidomycin and the fast elimination of the antibiotic in the urine [11], several groups attempted to synthesize other DXR inhibitors with improved pharmacokinetic and pharmacodynamic properties. The structures of these new molecules are all inspired from those of the antibiotics **1a** and **1b**, possessing on the one hand a phosphonate or another

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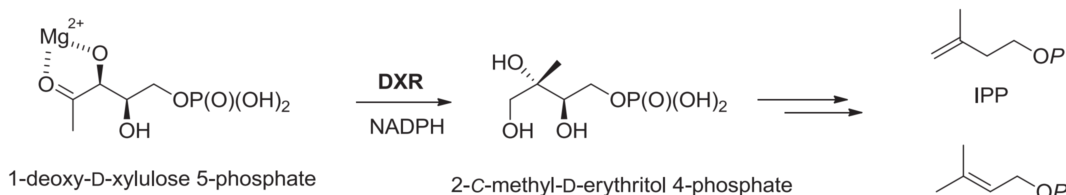


Scheme 1. Biosynthesis of IPP and DMAPP. Second step of MEP pathway: conversion of 1-deoxy-D-xylulose 5-phosphate into 4-methyl-D-erythritol 4-phosphate by the deoxyxylulose phosphate reducto-isomerase.

isosteric moiety fitting into the phosphate-recognition site and on the other side a chelating group tightly binding the metal cation required for enzyme activity [12].

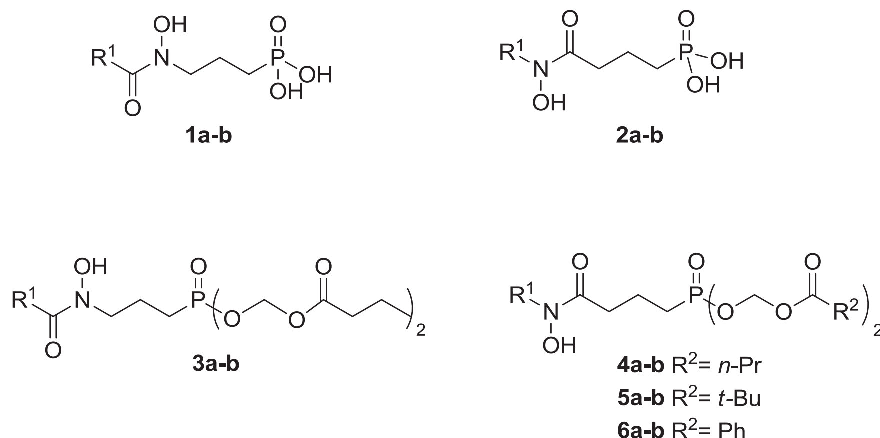
In this context, we synthesized and tested on the DXR of *Escherichia coli* two compounds (Scheme 2, **2a** and **2b**) where the metal cation-binding moiety is a hydroxamic acid group. Compound **2b** inhibits the *E. coli* DXR much like fosmidomycin and has significant anti-bacterial activity against wild type *E. coli* and even against an *E. coli* mutant resistant to fosmidomycin [13]. In the case of recombinant *M. tuberculosis* DXR, fosmidomycin **1a** and FR900098 **1b** were shown to inhibit the enzyme. The two published IC_{50} values differed, probably depending on different experimental conditions: 80 nM and 310 nM for **1a** [11,14] and 160 nM for **1b** [15]. Fosmidomycin does not inhibit the growth or affect the viability of *M. tuberculosis* cells. This lack of effect was interpreted in terms of a lack of uptake of the compound by the bacterium [16]. This fact is not too surprising. The hydrophobic cell wall of mycobacteria is an efficient barrier that prevents passive transport and makes the bacteria naturally resistant to most antibiotics. Mycobacteria cells are surrounded by a hydrophobic, waxy cell wall. Accordingly, hydrophilic compounds cross slowly this cell wall, probably *via* porins that are rare in mycobacteria, whereas lipophilic molecules penetrate by diffusion through the cell wall [17]. For that reason, anti-bacterial agents with lipophilic characteristics are more active against mycobacteria. In this context, less polar FR900098 analogs in which either the phosphonate or the hydroxamate moiety have been replaced by alternative acidic or metal coordinating groups have been prepared, but had no effect on *M. tuberculosis* growth [15,18].

A way to overcome the absence of mycobacterial cell wall crossing of fosmidomycin and its analogs is to mask the polar phosphonate moiety with hydrophobic groups. Owing to their hydrophobic character, such prodrugs may penetrate in the bacterial cells and, after enzymic conversion into the parent drug in the cells, stop the bacterial growth by inhibition of the DXR. The fact that such prodrugs of FR900098 are able to enhance *in vivo* and *in vitro* anti-malarial activity strengthens the relevance of this approach [19]. In this work, we synthesized and tested the inhibition potency of acyloxymethyl phosphonate esters as prodrugs of fosmidomycin, FR900098 and their analogs **2a** and **2b** on the non-pathogenic, fast growing *Mycobacterium smegmatis*. It is expected that the parent compounds are released from such prodrugs intracellularly in two steps: the hydrolysis of the acyl group by an esterase is followed by a rapid and spontaneous hydrolysis of the resulting hemiacetal into the free inhibitor and formaldehyde [20]. Finally, the DXR of *M. smegmatis* was cloned to check whether this enzyme is inhibited by fosmidomycin and its analogs. Such lipophilic fosmidomycin and FR900098 prodrugs have been recently described and tested as anti-bacterial agents [21].

2. Results

2.1. Synthesis of the prodrugs

The propyloxymethyl ester prodrugs **3a** and **3b** of fosmidomycin (**1a**) and FR900098 (**1b**) were synthesized according to a previously described method [19,22]. The synthesis of the prodrugs **4**–**6** was achieved starting from the phosphonate **8**, which is readily accessible by nucleophilic substitution of the commercially available ethyl 4-bromobutyrate with the NaH generated anion of diethyl phosphite (Scheme 3) [12]. The *O*-benzyl hydroxamate derivative **9a** was obtained in one step from **8** using *O*-benzylhydroxylamine hydrochloride in presence of LiHMDS as described by Gissot et al. [23]. The methyl group was introduced by reaction of **9a** with NaH followed by addition of methyl iodide. Deprotection of the phosphonate group using bromotrimethylsilane led to the acid **10** and subsequent alkylation of the crude phosphonic acids with the appropriate chloromethyl esters.



Scheme 2. Chemical structures of the tested compounds. Fosmidomycin **1a**, FR900098 **1b**, phosphono-hydroxamic acids **2a** and **2b** and their acyloxymethyl phosphonate esters prodrugs **3**, **4**, **5** and **6** (**a**: $R^1 = H$; **b**: $R^1 = CH_3$).

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