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Double prodrugs of a fosmidomycin surrogate as antimalarial and antitubercular agents

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ABSTRACT

A series of eleven double prodrug derivatives of a fosmidomycin surrogate were synthesized and investigated for their ability to inhibit in vitro growth of *P. falciparum* and *M. tuberculosis*. A pivaloyloxymethyl (POM) phosphonate prodrug modification was combined with various prodrug derivatisations of the hydroxamate moiety. The majority of compounds showed activity comparable with or inferior to fosmidomycin against *P. falciparum*. *N*-benzyl substituted carbamate prodrug **6f** was the most active antimalarial analog with an IC_{50} value of 0.64 μM. Contrary to fosmidomycin and parent POM-prodrug **5**, 2-nitrofuran and 2-nitrothiophene prodrugs **6i** and **6j** displayed promising antitubercular activities.

Introduction

Malaria and tuberculosis (TB) are two of the most lethal infectious diseases worldwide, being together responsible for over 2 million deaths in 2016.[1,2] The malaria targets set by the WHO aim at reducing malaria incidence and mortality by 90% by 2030, eliminating malaria in at least 35 additional countries and preventing resurgence of malaria in malaria-free countries.[1] Challenges for achieving these goals are numerous. The major challenge today is rapid emergence of insecticide and drug resistance. Especially the rise of resistance to artemisinin derivatives in the Greater Mekong region is problematic as no alternative first-line antimalarial treatments are currently available.[3–5] TB mortality rates have dropped significantly in recent years. However, the proportion of multidrug-resistant (MDR) TB-cases is steadily increasing.[2] The WHO End TB Strategy aims at eradication of TB by 2030 with TB mortality reduced by 95% and TB incidence by 90%.[6,7] The current annual decline in TB incidence worldwide is far off from what is necessary to reach the targets set by the WHO.[7] The reasons for the failing TB pushback in recent years are numerous and include failing health care systems in TB endemic countries, the emergence and rise of HIV/AIDS and the expansion of resistant TB strains, at least partially due to the lengthy treatment regimens and their consequent low compliance rates.[8–10] In order to halt the rise of resistant pathogen strains, drugs with a novel mechanism of action (MOA) against both malaria-causing *Plasmodium* parasites and *Mycobacterium tuberculosis* are urgently required.

In this respect, fosmidomycin (**1**, Figure 1) is an interesting lead compound. Fosmidomycin (**1**, Figure 1) and FR900098 (**2**, Figure 1) are natural phosphonate antibiotics, produced by *Streptomyces* bacteria.[12] They are slow, tight-binding inhibitors of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), the second enzyme of the non-mevalonate isoprenoid biosynthesis pathway.[13,14] Isoprenoids or terpenoids are essential molecules for all living organisms. They are built up of five-carbon building blocks, which can be metabolically derived from two evolutionary distinct pathways, the mevalonate (MVA) and the non-mevalonate or 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. Humans exclusively utilize the MVA pathway, whereas both *Plasmodium* parasites and *Mycobacterium tuberculosis* rely solely on the MEP pathway. The absence of homologous enzymes in humans and the essentiality of the pathway in pathogenic organisms renders the

Abbreviations: AIDS, acquired immunodeficiency syndrome; DCM, dichloromethane; DMF, dimethylformamide; DXR, 1-deoxyxylulose 5-phosphate reductoisomerase; EDC, *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide; Et_3N, triethylamine; HIV, human immunodeficiency virus; HOBt, hydroxybenzotriazole; HRMS, high-resolution mass spectrometry; MDR, multidrug-resistant; MEP, methylerythritolphosphate; MIC, minimal inhibitory concentration; MOA, mechanism of action; *Mtb*, *Mycobacterium tuberculosis*; MVA, mevalonate; PK, pharmacokinetic; POM, pivaloyloxymethyl; TB, tuberculosis; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMSBr, trimethylsilyl bromide; WHO, world health organization

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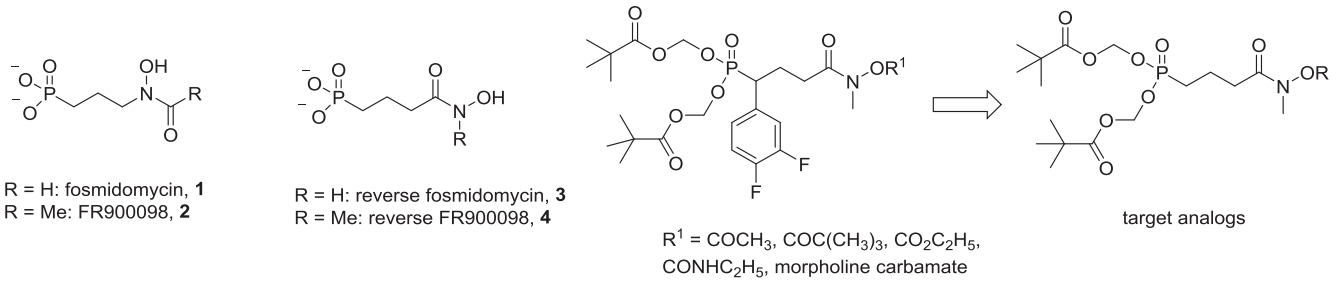
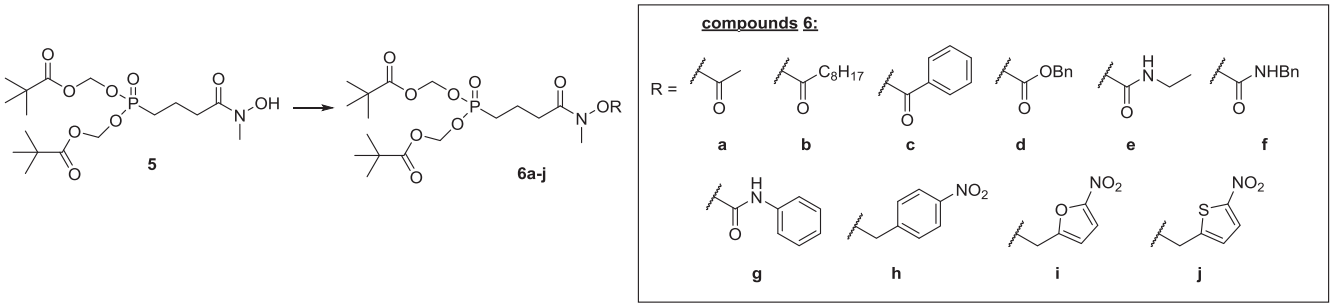


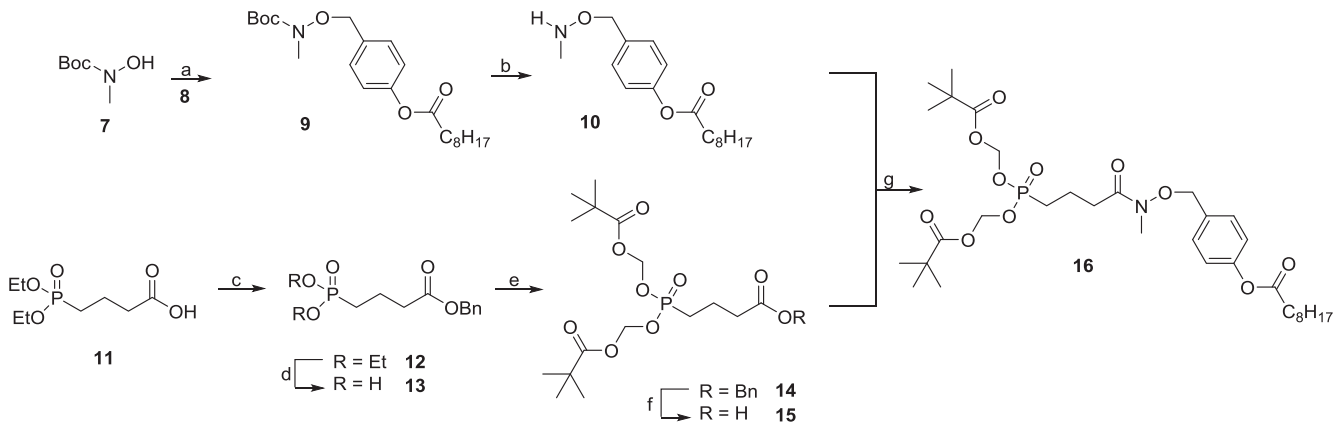
Figure 1. Structural formulae of fosmidomycin, FR900098, their reverse analogs **3** and **4**, and previously reported double prodrug derivatives.[11] Double prodrugs of **4**, with a POM phosphonate prodrug derivatisation and various modifications of the hydroxamate moiety, are the focus of this work.

enzymes of the MEP pathway highly interesting drug targets.[15,16] DXR is by far the most extensively investigated enzyme of the non-mevalonate pathway.[14,17] K_i values of fosmidomycin against *P. falciparum* DXR and *Mtb* DXR are 20 nM and 140 nM, respectively.[18,19] Several phase II clinical trials of fosmidomycin in combination therapy with clindamycin as a potential treatment of acute uncomplicated *P. falciparum* malaria have been completed and have repeatedly demonstrated their good synergistic profile. However, the most recently completed study (Mozambique, 2012) found a low cure rate on day 28, resulting in a slowdown of further development of this combination. Possible explanations for these discrepant results are differences in the age of the patients involved and the formulation used.[20,21] Despite its overall promising activity and safety profile, fosmidomycin has several shortcomings. Fosmidomycin is highly polar. This is mainly due to the phosphonate functionality, which is negatively charged at physiological pH. This results in poor passive permeability characteristics and suboptimal pharmacokinetic (PK) properties, including moderate oral bioavailability (20–40%) and a short plasma half-life (1.87 h).[22] Furthermore, *P. falciparum* induced permeability pathways are required for active uptake of fosmidomycin in infected red blood cells.[23] *Mycobacteria* do not have this transporter, and additionally have a highly lipophilic cell wall, resulting in intrinsic resistance of *Mycobacteria* against this phosphonate antibiotic.[24]

In recent years, fosmidomycin research has mainly focused on the development of prodrug derivatives, especially in the interest of improving PK properties and obtaining antibacterial activity. Furthermore, enabling fosmidomycin uptake independent of transporters would be interesting as sole dependence of drug uptake on one transporter increases the chances of resistance development significantly.[25] Prodrugs of fosmidomycin (analogs) have been obtained, mainly by conversion of the phosphonate moiety into acyloxyalkyl and alkoxycarbonyloxyalkyl phosphonate esters and have been shown to possess increased antimalarial activity.[11,26,27] However, sufficiently potent antitubercular analogs have not been obtained. Not only the phosphonate, but also the (retro)hydroxamate functionality contributes to the high polarity of fosmidomycin. Therefore, additionally masking the (retro)hydroxamate might further enhance uptake via passive diffusion. Furthermore, masking the hydroxamate functionality might also result in enhanced plasma stability as hydroxamates are known to be susceptible to plasma hydrolysis.[28,29] Few hydroxamate prodrug modifications have been described in the literature. There have been reports of increased cellular uptake with carbamate[30,31] and *p*-acetoxybenzyl ester[32] prodrug derivatives of hydroxamates. For the *p*-acetoxybenzyl ester prodrugs, improved in vivo oral PK in mice has been demonstrated, with five-fold higher plasma levels of the parent drug after administration as its prodrug.[32] A number of double prodrugs of fosmidomycin analogs has been reported, of which a selection is shown in Figure 1.[11] Inspired by the promising antiplasmodial activities of these compounds, we decided to further expand the double prodrug series. The previously reported double prodrugs are derived from the reverse hydroxamate α-3,4-difluorophenyl analog, which compared to fosmidomycin is more active against *Pf*DXR (IC_{50} = 4 nM), but less active against *Mtb*DXR (IC_{50} = 3.4 μM).[33] As we are interested in not only antiplasmodial, but also antitubercular activities, fosmidomycin surrogate **4** (Figure 1) has been used as a starting point for the development of prodrugs. The hydroxamate counterparts of fosmidomycin and FR900098 (**3–4**, Figure 1) have equal inhibitory activity as fosmidomycin against both *Pf*DXR and *Mtb*DXR. Also IC_{50} values against the *P. falciparum* K1 strain have been shown to be comparable.[34] We combined a POM phosphonate prodrug modification with various prodrug derivatisations of the hydroxamate moiety. Besides simple ester, carbonate and carbamate prodrug derivatisation, 3 prodrug promoieties were selected based on bioreductive properties. The bioreductive prodrug approach in general has mainly been investigated in the interest of the development of anticancer agents which are activated selectively in hypoxic tumor tissue. We hypothesized that this prodrug strategy might also allow targeting to hypoxic TB lesions. The most commonly used prodrug promoieties in this approach are 4-nitrobenzyl, 1-methyl-2-nitroimidazole, 2-nitrothiophene and 2-nitrofuran.[35,36] We have synthesized the synthetically easily accessible 4-nitrobenzyl, 2-nitrothiophene and 2-nitrofuran hydroxamate prodrug derivatives of fosmidomycin surrogate **4**. In view of the results obtained with the *p*-acetoxybenzyl ester prodrug approach by Rais et al., and our own research on acyloxybenzyl prodrug derivatives of a fosmidomycin surrogate as antitubercular agents, we also synthesized the *p*-nonanoyloxybenzyl ester prodrug derivative of **4**.[32,37]



Scheme 1. Synthesis of double prodrugs[a], [a]Reagents and conditions. **esters:** R(C═O)Cl, Et_3N, DCM (63–90%); **carbonates:** benzylchloroformate, Et_3N, DCM (76%); **carbamates:** R-isocyanate, Et_3N, THF (85–96%); **ethers:** RCl or RBr, Na_2CO_3, MeCN, 80 °C (18–24%).



Scheme 2. Synthesis of nonanoyloxybenzyl hydroxamate prodrug[a], [a]Reagents and conditions: (a) (i) NaH, DMF (ii) nonanoyloxybenzyl iodide **8** (17%); (b) TFA, DCM; (c) BnBr, Na_2CO_3, MeCN, 60 °C (q); (d) (i) TMSBr, DCM; (ii) H_2O, THF; (e) POM-Cl, Et_3N, DMF, 80 °C (33%); (f) H_2, Pd/C, MeOH (74%); (g) EDC.HCl, HOBt, Et_3N, DCM (84%).

The synthesis of double prodrugs **6a-j** started from POM prodrug **5** (Scheme 1), which was reported by us previously.[37] Ester prodrugs **6a-c** were synthesized by reaction with the appropriate acid chloride and Et_3N as base. Carbonate prodrug **6d** was synthesized by reaction with benzylchloroformate and Et_3N. Carbamate prodrugs **6e-g** were synthesized by reaction with the appropriate isocyanate and Et_3N. Ether prodrugs **6h-j** were synthesized by heating **5** with the appropriate alkyl halide and Na_2CO_3 as base. For the synthesis of double prodrug **16**, alkylation of **5** with nonanoyloxybenzyl halide was attempted using a range of different reaction conditions, but failed to produce the desired product in acceptable yield. HRMS and ^1H nmr analysis of the obtained products revealed that both the POM-moiety and the nonanoyloxybenzyl moiety degraded during the reaction, resulting in a complex mixture of undesired reaction products. Therefore, an alternative synthesis route was designed.

The synthesis of hydroxylamine ether **10** started from Boc-protected hydroxylamine **7** (Scheme 2). Alkylation with nonanoyloxybenzyl iodide **8** in the presence of NaH as a base, yielded Boc-protected hydroxylamine ether **9** in low, but acceptable yield. Boc deprotection with TFA produced **10**. The synthesis of phosphonate intermediate **15** started from carboxylic acid **11**. Benzyl protection of the carboxylic acid group afforded **12**. TMSBr-mediated phosphonate deprotection and subsequent hydrolysis of the phosphonate esters, provided free phosphonate **13**. Alkylation with POM-Cl gave **14**, which was hydrogenated to the free carboxylic acid **15**. EDC-coupling of **10** and **15** afforded final compound **16**.

Final compounds **6a-j** and **16** were screened for growth inhibition of asexual blood stage parasites of *P. falciparum* (Pf-K1) and an avirulent *M. tuberculosis* strain (H37Ra) and/or a virulent *M. tuberculosis* strain (H37Rv). Additionally, toxicity on MRC-5 fibroblasts was assessed (Table 1). Ester prodrugs **6a-c** displayed antiplasmodial activities comparable to both fosmidomycin and parent prodrug **5**, while carbonate prodrug **6d** was inactive. Only *N*-benzyl substituted carbamate prodrug **6f** displayed good antiplasmodial activity with an IC_{50} value of 0.64 μM. Remarkably, all ester prodrugs **6a-c**, carbonate **6d** and carbamates **6e-g** were inactive or only very weakly active against *M. tuberculosis*. Also nonanoyloxybenzyl ester prodrug **16** displayed poor antiplasmodial activity and a poor antitubercular MIC value of 50 μM. It is unclear whether this is due to inadequate stability of the prodrug, inefficient release of the parent drug or poor permeation by passive diffusion. Compared to parent prodrug **5**, prodrugs **6i** and **6j** displayed significantly improved antitubercular activity. Unfortunately, these analogs also decreased cell viability of MRC-5 fibroblasts. Despite the observed toxicity, it would be interesting to expand this prodrug series in order to see if superior activity and enhanced selectivity can be obtained. Additionally, it would be valuable to evaluate the activity of the hypothetical bioreductive prodrugs under non-replicating conditions and to validate their mechanism of bioactivation. At this point, it is unclear whether derivatives **6h-j** are being activated by reduction and/or simple hydrolysis, or whether they are binding as such in the active site. It is also possible that reactive metabolites formed during the reduction of the nitro heterocycles at least contribute to the observed activity. We did not further investigate the lack of activity of 4-nitrobenzyl prodrug **6h**. However, it is known that 2-nitrofuran and 2-nitrothiophene prodrug moieties are more easily reducible than 4-nitrobenzyl moieties.[35] We hypothesize that as a result, the 4-nitrobenzyl prodrug moiety is not activated as efficiently, resulting in insufficient parent drug release.

Table 1
Biological evaluation of double prodrugs of a fosmidomycin surrogate.

Compound	Pf-K1 IC_{50} (± SD) [μM]	H37Rv MIC [μM]	MRC-5 CC_{50} (± SD) [μM]
1	1.73[38]	> 64	> 64
5	0.73	> 50	> 64
6a	1.34 (± 0.52)	[> 64][a]	> 64
6b	1.41	50	> 64
6c	1.08 (± 0.80)	50	> 64
6d	> 36	> 50	> 42
6e	2.52 (± 2.76)	> 50	25.9 (± 1.7)
6f	0.64 (± 0.02)	> 50	27.9 (± 1.6)
6g	3.34 (± 3.86)	[> 64]	33.4 (± 6.9)
6h	8.79 (± 4.88)	> 50	26.5 (± 1.7)
6i	4.42	12.5	26.3
6j	4.69	12.5	20.5
16	19.0	50	> 64

[a] values in square brackets are IC_{50} (μM) values, determined against avirulent *M. tuberculosis* strain H37Ra

In conclusion, we report on the synthesis, antiplasmodial and antimycobacterial activity of a series of 11 double prodrug derivatives of fosmidomycin surrogate **4** (Figure 1). *N*-benzyl substituted carbamate derivative **6f** displayed good antiplasmodial activity. Hypothetical bioreductive 2-nitrofuran and 2-nitrothiophene derivatives **6i** and **6j** displayed promising antitubercular activities. These findings suggest that a bioreductive prodrug approach may enable uptake and release of fosmidomcyin surrogate **4** in mycobacterial cells. Further research is necessary to confirm these findings.

Author contributions

All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.03.009.

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