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Phosphonodiamidate prodrugs of *N*-alkoxy analogs of a fosmidomycin surrogate as antimalarial and antitubercular agents

Charlotte Courtens[a], Martijn Risseeuw[a], Guy Caljon[b], Paul Cos[b], Anandi Martin[c], Serge Van Calenbergh[a,*]

[a] Laboratory for Medicinal Chemistry, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium
[b] Laboratory for Microbiology, Parasitology and Hygiene, University of Antwerp, Universiteitsplein 1 (S7), B-2610 Wilrijk, Belgium
[c] Medical Microbiology, Institute of Experimental and Clinical Research, Université catholique de Louvain, Avenue Hippocrate 55, B-1200 Woluwe-Saint-Lambert, Belgium

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ABSTRACT

A series of *N*-alkoxy analogs of a L-leucine ethyl ester phosphonodiamidate prodrug of a fosmidomycin surrogate were synthesized and investigated for their ability to inhibit in vitro growth of *P. falciparum* and *M. tuberculosis*. These compounds originate by merging a previously reported successful phosphonate derivatisation with favorable modifications of the hydroxamate moiety. None of the synthesized compounds showed enhanced activity against either *P. falciparum* or *M. tuberculosis* in comparison with the parent free hydroxamate analog.

Despite international efforts, malaria and tuberculosis (TB) remain among the most problematic infectious diseases worldwide. According to the World Health Organization (WHO), malaria incidence has decreased significantly since 2010. Since 2014, however, the number of malaria cases is steadily increasing, while the number of deaths remains comparable.[1] Resistance to antimalarial drugs is a persisting problem and, alarmingly, elevated resistance to artemisinin combination therapy (ACT) drugs has been observed in recent years.[2,3] Despite a significant drop in TB mortality rates since 2010, the proportion of multidrug-resistant (MDR) TB cases is steadily increasing. Approximately 5% of active TB cases are multidrug-resistant (MDR), of which 6% are extensively drug-resistant (XDR). Treatment success rates of drug-resistant TB cases are relatively low, being 50% for MDR-TB and 30% for XDR-TB.[4,5] In 2009, the first totally drug-resistant (TDR) strains have been detected in India.[6] In order to halt the upsurge of infections with drug-resistant pathogen strains, there is an urgent need for antimalarial and antitubercular agents with a novel mechanism of action (MOA). In this respect, the non-mevalonate pathway (NMP) for isoprenoid biosynthesis represents an interesting potential drug target. Isoprenoids form the largest class of natural compounds and are essential to all living organisms. They are built up of the five-carbon isoprene units isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These building blocks can be synthesized via two evolutionary distinct pathways: the mevalonate (MVA) pathway and the NMP, also known as the methylerythritol phosphate (MEP) pathway. Both malaria-causing *Plasmodium* parasites and *Mycobacterium tuberculosis* (*Mtb*), the causative agent of TB, rely entirely on the NMP, while it is absent in humans. 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (DXR, also known as IspC), catalyzes the second step of the MEP pathway and is the most extensively investigated enzyme of this pathway.[7,8]

L-leucine ethyl ester based prodrug derivatives of *N*-alkoxy analogs of a fosmidomycin surrogate are the focus of this work.

Fosmidomycin (**1**, Fig. 1) and FR900098 (**2**, Fig. 1), the *N*-acetyl analog of fosmidomycin, are natural antibiotics originally isolated from *Streptomyces lavendulae* and *Streptomyces rubellomurinus*, respectively.[9] Fosmidomycin was originally evaluated for the treatment of urinary tract infections. In 1998, however, fosmidomycin and FR900098 were

Abbreviations: AA, amino acid; ACT, artemisinin-based combination therapy; DCM, dichloromethane; DMAPP, dimethylallyl pyrophosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, 1-deoxyxylulose 5-phosphate reductoisomerase; EDC, *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide; Et_3N, triethylamine; GlpT, glycerol-3-phosphate transporter; 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; NADPH, nicotinamide adenine dinucleotide phosphate; NMP, non-mevalonate pathway; PK, pharmacokinetic; POM, pivaloyloxymethyl; SD, standard deviation; SI, selectivity index; TB, tuberculosis; TDR, totally drug-resistant; TFA, trifluoroacetic acid; THF, tetrahydrofuran; WHO, world health organization; XDR, extensively drug-resistant

* Corresponding author.
E-mail address: serge.vancalenbergh@ugent.be (S. Van Calenbergh).

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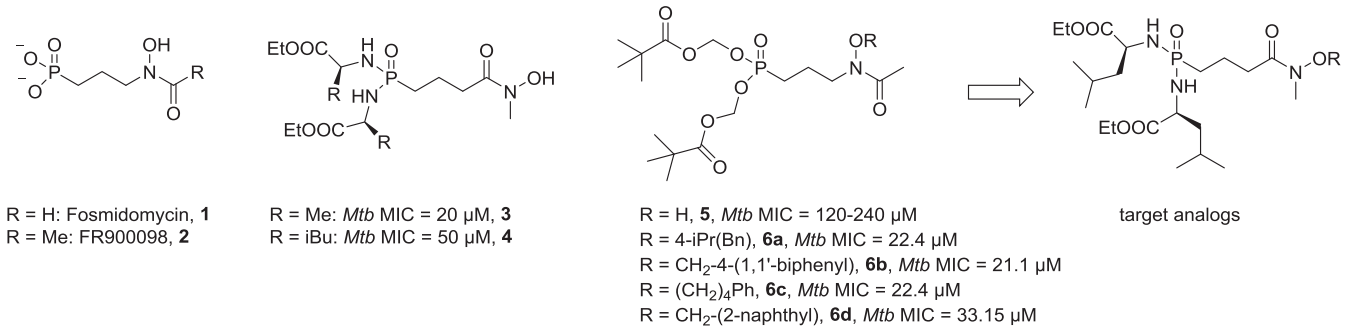
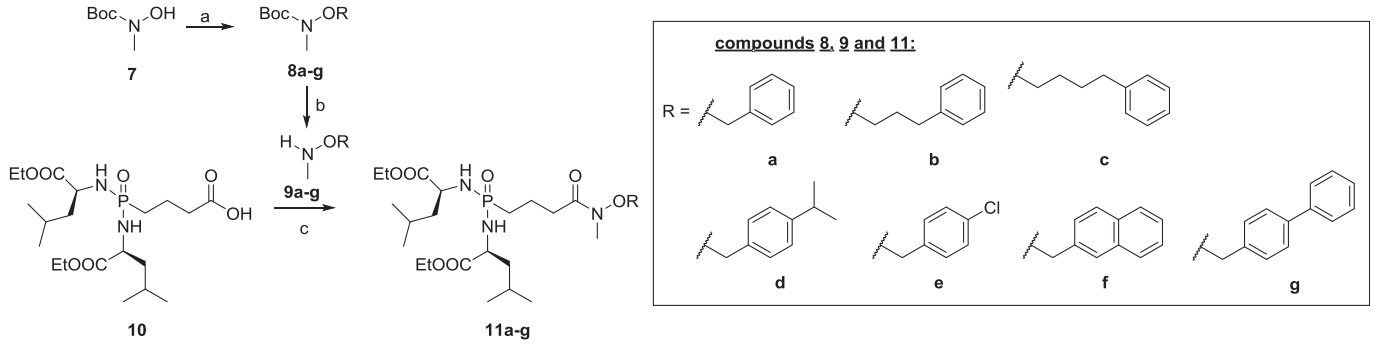


Fig. 1. Structural formulae of fosmidomycin, FR900098, previously reported prodrug derivatives **3–5**, and *N*-alkoxy derivatives **6a-d**.



Scheme 1. Synthesis of L-leucine ethyl ester based prodrug derivatives of *N*-alkoxy analogs of a fosmidomycin surrogate. [a]Reagents and conditions. (a) (i) NaH, dry THF; (ii) RX, 70 °C (82–95%); (b) TFA, DCM; (c) EDC.HCl, HOBt, Et_3N, DCM (59–98% over 2 steps).

shown to be potent DXR inhibitors, resulting in regained interest in these phosphonates, now as antimalarial agents.[10] Fosmidomycin has been shown to be safe and well-tolerated as an antimalarial agent in combination therapy with clindamycin. However, fosmidomycin is a highly polar compound, mainly due to its phosphonate functionality, which exists mainly as its dianion at physiological pH. Primarily as a result of its high polarity, fosmidomycin displays suboptimal pharmacokinetic (PK) properties including moderate oral bioavailability (20–40%) and a short plasma half-life (1.87 h).[11] Therefore, it needs to be administered multiple times a day at relatively high doses.[12–14] Furthermore, fosmidomycin displays poor permeation via passive diffusion. This has important consequences not only for oral bioavailability, but also for the inhibitor to reach its intracellular target. Fosmidomycin uptake in *E. coli* has been shown to be dependent on the presence of a glycerol 3-phosphate transporter (GlpT).[15] Also *P. falciparum* infected erythrocytes have been shown to use parasite-induced permeability pathways to facilitate uptake of fosmidomycin in infected red blood cells.[16] *Mycobacteria* lack an active fosmidomycin uptake system and additionally have a highly lipophilic cell wall. As a result, fosmidomycin is unable to penetrate *Mycobacteria* to reach its target.[17] Lipophilic phosphonate prodrug derivatives of fosmidomycin (analogs) have previously been reported to display significantly enhanced antiplasmodial and antitubercular activities.[18,19] We previously reported amino acid (AA) phosphonodiamidate derivatives of a fosmidomycin surrogate (**3** and **4**, Fig. 1) with promising whole cell antitubercular activities.[20] The Dowd research group has recently reported *N*-acyl and *N*-alkoxy analogs of FR900098 as bisubstrate DXR inhibitors. These compounds were found to be competitive with both the natural substrate (1-deoxy-D-xylulose 5-phosphate, DXP) and cofactor (NADPH). Especially interesting are the antitubercular activities of the pivaloyloxymethyl (POM)-prodrugs of *N*-alkoxy analogs of FR900098 (**6a-d**, Fig. 1) with MIC values up to 5.3–21 μM.[21–23] These results demonstrate that a free hydroxamate OH functionality is not essential for DXR inhibition. This is of interest as the heightened lipophilicity of the *N*-alkoxy analogs is expected to be beneficial for penetration of the highly lipophilic mycobacterial cell wall.

Compounds **3** and **4** (Fig. 1) display significantly more potent antitubercular activity than POM-prodrug **5** (Fig. 1).[24] Mtb DXR inhibitory activity of the parent compound from prodrug **3** and **4** has been reported to be 1.15 μM.[25] Mtb DXR inhibitory activity of FR900098, the parent compound from prodrug **5**, has been reported to be 2.39 μM.[26] These inhibitory values are comparable, while the difference in antitubercular activities of compounds **3** and **4** in comparison with compound **5** are up to 12-fold better. Therefore, we hypothesized that the improvement in antitubercular activity could be mainly attributed to the prodrug promoiety used. We envisaged that combining the L-leucine ethyl ester phosphonodiamidate moiety with the hydroxamate substituents of **6a-d** (Fig. 1) might further improve antitubercular activity. To this end, we report the synthesis of a series of *N*-alkoxy analogs of the L-leucine ethyl ester phosphonodiamidate derivatives of a fosmidomycin surrogate with an inversed hydroxamate group (see Scheme 1).

We gained access to all target compounds from the common intermediate **10**, which was synthesized as described previously (see Supporting Information).[20] Alkylation of Boc-protected *N*-methyl hydroxylamine **7** with the appropriate alkyl halides in the presence of NaH provided alkoxy carbamates **8a-g**, which were deprotected to give the required hydroxylamine ethers **9a-g**. Coupling of these diverse *O*-substituted *N*-methylhydroxylamines with intermediate **10** using EDC.HCl and HOBt yielded the desired compounds **11a-g** in good yields.

Final compounds **11a-g** were screened for growth inhibition of asexual blood stage parasites of *P. falciparum* (Pf-K1) and an avirulent *M. tuberculosis* strain (H37Ra). Additionally, toxicity on MRC-5 fibroblasts was assessed (Table 1). While most compounds retained moderate activity against *P. falciparum*, none surpassed the *M. tuberculosis* activity of the free hydroxamate analog **4**. The poor antitubercular activities of the compounds were additionally confirmed against the reference H37Rv *M. tuberculosis* strain, against which none of the *N*-alkoxy analogs proved active up to the highest concentration tested (50 μM). Furthermore, contrary to parent compound **4**, all analogs (except benzyl ether **11a**) decreased cell viability of MRC-5 fibroblasts,

Table 1
Biological evaluation of L-leucine ethyl ester based prodrug derivatives of *N*-alkoxy analogs of a fosmidomycin surrogate.[a]

Compound	R	Pf-K1 IC_{50} (± SD) [μM]	H37Ra IC_{50} (± SD) [μM]	MRC-5 CC_{50} (± SD) [μM]	SI[b] (Pf-K1)	SI[b] (H37Ra)
4	H	4.83 (± 2.29)	29.4 (± 18.3)	> 64	> 20	> 2.2
11a	Bn	4.22	> 64	> 64	> 15	
11b	$(CH_2)_3Ph$	8.93 (± 0.13)	> 64	23.1 (± 0.0)	2.6	
11c	$(CH_2)_4Ph$	6.72 (± 1.39)	50.3 (± 19.4)	7.23 (± 1.01)	1.1	0.14
11d	4-iPr Bn	5.28 (± 1.52)	49.1 (± 21.0)	7.22 (± 0.09)	1.4	0.15
11e	4-Cl Bn	7.12 (± 0.22)	49.0 (± 21.2)	18.4 (± 0.9)	2.6	0.37
11f	CH_2-(2-naphthyl)	6.22 (± 0.10)	48.2 (± 19.9)	5.47 (± 0.06)	0.88	0.11
11 g	CH_2-4-(1,1′-biphenyl)	5.25 (± 0.16)	35.2 (± 4.1)	5.94 (± 0.24)	1.1	0.17

[a] Values shown are the calculated mean values of at least two measurement results (except for **11a**, which was tested only once).
[b] SI = selectivity index.

indicating human cell toxicity. As a result, the selectivity index of all target compounds was insufficient both with respect to antiplasmodial and antitubercular activity, as shown in Table 1. Possibly, due to the reverse orientation of the hydroxamate in comparison with FR900098, the *O*-alkyl groups are no longer able to bind the NADPH binding pocket, resulting in decreased DXR inhibitory activity and decreased in vitro growth inhibition. In conclusion, in our hands, the *O*-alkyl substituents do not provide added value for antiplasmodial and/or antitubercular activities in comparison with the corresponding free hydroxamate analog.

Author contributions

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The authors declare no competing financial interest.

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Appendix A. Supplementary data

Experimental details and characterization data for the reported compounds, NMR spectra, biological data (PDF). Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.03.008.

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