Summary

Malaria and tuberculosis (TB) are two of the most lethal infectious diseases worldwide, being together responsible for over 2 million deaths in 2016. The WHO aims at eradication of both diseases by 2030. Challenges for achieving this goal are numerous. The major challenge today is rapid emergence of drug resistance. Malaria causing *Plasmodium* parasites are increasingly resistant to all currently available antimalarials. 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. Also the proportion of multidrug-resistant (MDR) TB cases is steadily increasing. The expansion of resistant TB strains is at least partially due to the lengthy treatment regimens and their consequent low compliance rates. In order to halt the rise of resistant pathogen strains, drugs with a novel mechanism of action (MOA) against both *Plasmodium* parasites and *Mycobacterium tuberculosis* (*Mtb*) are urgently required.

In this respect, the non-mevalonate pathway for isoprenoid biosynthesis, which is vital for both *Plasmodium* parasites and *Mtb*, and absent in humans, constitutes a highly interesting and validated drug target. Fosmidomycin and its acetyl congener FR900098 are natural phosphonate antibiotics, which have been validated to be slow, tight-binding inhibitors of 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR), the second enzyme of this pathway. Furthermore, fosmidomycin has been demonstrated to be safe and efficacious as an antimalarial in combination therapy with clindamycin in Phase II clinical trials. However, fosmidomycin has several drawbacks. Mainly as a result of its high polarity, fosmidomycin displays suboptimal pharmacokinetic (PK) properties, including a short plasma half-life and moderate oral bioavailability. Due to its highly polar character, mainly due to the phosphonate functionality, which is charged at physiological pH, fosmidomycin permeates cells only poorly via passive diffusion. This has important consequences not only for oral bioavailability, but also for biological activity. *Plasmodium* parasites have been shown to actively transport fosmidomycin inside infected red blood cells (RBCs). However, *Mtb* does not have an active fosmidomycin uptake system, and as a result is naturally resistant to this phosphonate antibiotic. A lot of research has been dedicated to improve the potency of fosmidomycin. However, the problem of low bioavailability and cellular penetration remains. Therefore, we decided to investigate if the application of a broad range of prodrug strategies could lead to derivatives with enhanced antimalarial and antitubercular activities.
In Chapter I, a general overview of malaria and TB is given, with special focus on currently available therapeutic options and the challenges in the control of these infectious diseases. We also introduce the general concept of prodrugs and zoom in on phosph(on)ate prodrug strategies that have previously been shown to lead to increased cellular uptake. The introduction is concluded with a discussion of the non-mevalonate pathway and DXR, and a synopsis of previously reported structural modifications and prodrug derivatisations of fosmidomycin (analogs).

In the form of a compilation of the scientific publications, Chapter III describes the research carried out over the course of this PhD.

The first publication, ready for submission, describes an expansion of the acyloxymethyl and alkoxycarbonyloxymethyl prodrug series. A number of prodrug derivatives of fosmidomycin (analogs) applying this phosphonate prodrug strategy, have been previously reported. We expanded this prodrug series with several alkyl and acyl groups with diverse electronic and lipophilic properties. Additionally, we designed 4 prodrug promoieties based on natural siderophores. Our work resulted in the identification of highly active antiplasmodial prodrug derivatives. Furthermore, some of the most lipophilic derivatives displayed significant antibacterial activity against *Acinetobacter baumannii*.

In a second manuscript, published in ACS Med Chem Lett (DOI: 10.1021/acsmedchemlett.8b00223), we describe an efficient synthesis involving a cross metathesis reaction, for the preparation of both acyloxybenzyl as well as long-chain alkoxypropyl derivatives of a fosmidomycin surrogate. The acyloxybenzyl prodrug strategy yielded highly potent, yet also cytotoxic, antitubercular analogs.

Not only the phosphonate, but also the (retro)hydroxamate functionality contributes to the high polarity of fosmidomycin (analogs). Therefore, in the third paper, published in Bioorg Med Chem Lett (DOI: 10.1016/j.bmcl.2019.03.009), we investigated double prodrug derivatives, in which the phosphonate functionality is masked as pivaloyloxymethyl (POM)-prodrug, in combination with various modifications of the hydroxamate moiety. In particular nitroaromatic hydroxamic acid derivatives demonstrated promising antitubercular activities.
The fourth manuscript, published in Bioorg Med Chem (DOI: 10.1016/j.bmc.2019.01.016), describes the application of two amino acid based prodrug approaches to a fosmidomycin surrogate: the phosphonodiamidate prodrug approach and the tyrosine ester prodrug approach. Both interesting antiplasmodial and antitubercular activities have been obtained. Furthermore, 3 analogs were evaluated in a malaria mouse model.

In the fifth and final manuscript, published in Bioorg Med Chem Lett (DOI: 10.1016/j.bmcl.2019.03.008), we report about the development of a series of L-leucine ethyl ester phosphonodiamide prodrug derivatives of N-alkoxy analogs of a fosmidomycin surrogate. These compounds originate by merging a successful phosphonate derivatisation with favorable modifications of the hydroxamate moiety. However, none of the synthesized compounds showed enhanced activity against either \textit{P. falciparum} (Pf) or \textit{Mtb} in comparison with the parent free hydroxamate analog.

A general conclusion is given in Chapter IV.

In Chapter V, the broader international context and the relevance of the research conducted as well as the results obtained, are discussed and the work is critically evaluated. To conclude, future perspectives are outlined.