GENERAL INTRODUCTION
Tuberculosis

Tuberculosis (TB) is a common infectious disease, which is caused by bacteria of the *Mycobacterium tuberculosis* complex, among which *M. tuberculosis* is the main causative agent of human TB. *M. tuberculosis* was first identified by the German scientist Robert Koch in 1882. It is an obligate intracellular pathogen that can infect several animal species, with human beings as primary host. *M. tuberculosis* is an aerobic, acid-fast, neither typical Gram positive nor Gram negative bacterium, which due to its lipid-rich cell wall is impermeable to basic dyes. It infects the human host via air and patients who are sick with pulmonary TB expel bacteria, e.g. cough, and sneeze or other transmits respiratory fluids in the air. *M. tuberculosis* can infect the lungs, leading to the active disease, (pulmonary TB), although other parts of the human body can be affected as well. In most cases, inhalation of *M. tuberculosis* does not result in active disease, 90% of the cases will enter into a latent TB infection (LTBI) state. It is estimated that one third of the world’s population is latently infected with *M. tuberculosis*. Humans with LTBI will not show typical TB symptoms and are not infectious, but there are chances the LTBI might develop into active and contagious disease, e.g. when the immune system weakens. About 5-20% of LTBI patients develop active disease (pulmonary TB) during their lifetime, especially when the patients are suffering from e.g. co-infection with HIV. Consequently, the co-infection with HIV has significantly enhanced the mortality of TB infections and treatment options need to be expanded.

Tuberculosis has plagued humankind for ages, with TB infection described during most of human history. In the late of 19th century, TB reached epidemic proportions in Europe, responsible for 1000 deaths per 100,000 individuals annually. At that time, effective methods to eliminate the bacilli and to prevent the infectious disease were not available. Fortunately, between the 1940 and 1960 drugs against TB were discovered, most of which (such as isoniazid and rifampicin) are still in use as front-line antibiotics. However, in the
1980s and 1990s resistance to TB drugs increased rapidly, and the World Health Organization (WHO) declared TB as a global public health emergency in 1990. After that, the WHO decided on global targets within the context of the Millennium Development Goals (MDGs), with the goal to dramatically reduce the global burden of TB by 2015. Although 20 years have passed after the WHO declaration and considerable progress has been made towards the targets, TB remains a global health problem. In 2012, there were 8.6 million newly diagnosed TB cases and nearly 1.3 million deaths (Table 1). As such, TB ranks as the second most deadly infection disease after human immunodeficiency virus (HIV). For a basically curable infectious disease, this situation is unacceptable.

At present, TB is spread worldwide, with the majority of cases occurring in the South-East Asia, Africa and Western Pacific regions. Moreover, multidrug resistant tuberculosis (MDR-TB), which is caused by mycobacteria resistant to at least the first-line anti-TB drugs (isoniazid and rifampicin), increased the severity of the TB problem. The highest levels of MDR-TB are found in Eastern Europe and central Asia. Moreover, extremely drug resistant TB (XDR-TB) was reported by the end of 2012. XDR-TB bacilli are resistant to both first and second line anti-TB drugs, which makes it extremely difficult to treat. On average, an estimated 9.6% of MDR-TB patients have XDR-TB. In addition, *M. tuberculosis* and HIV co-infection makes it even harder to treat TB, because of drug-drug interactions, overlapping drug toxicities, concerns about adherence, and the immune reconstitution inflammatory syndrome.
Prevalence

Incidence
(newly diagnosed TB)

Mortality

<table>
<thead>
<tr>
<th></th>
<th>Prevalence</th>
<th>Incidence (newly diagnosed TB)</th>
<th>Mortality</th>
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<tbody>
<tr>
<td>Tuberculosis (TB)</td>
<td>12</td>
<td>8.6 in which 1.1 HIV positive</td>
<td>1.3 in which 0.32 HIV positive</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>0.45</td>
<td>0.31</td>
<td>0.17</td>
</tr>
<tr>
<td>XDR-TB</td>
<td>0.0432</td>
<td>0.0297</td>
<td>NA</td>
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Attempts to solve the TB problem by vaccination have so far been largely unsuccessful; the only currently licensed TB vaccine is Bacillus Calmette-Guerin (BCG), which has been used for prevention of TB for almost 100 years. Until now, more than 4 billion people have been vaccinated, which makes it the most widely used vaccine in the world. The BCG vaccine is highly effective for protection against meningitis and disseminated TB in children, but the results for adult pulmonary TB are highly variable. BCG is also not suitable for HIV positive infants, because of the risk of disseminated BCG disease. The reasons for the observed variability are not clear but may be connected to loss of some genetic regions during serial passage in vitro in the attenuation process.

Current drug and regimen to treat and prevent tuberculosis

Without treatment, TB mortality rates are very high. Even within the TB positive/HIV negative cases of pulmonary TB, nearly 70% of the patients died within 10 years; among smear negative but culture positive cases, 20% of the patients died in 10 years. Nowadays, the co-infection of HIV and TB further increases mortality rates. There
are 0.32 million TB /HIV positive death cases among the new 1.3 million TB death cases in 2012 (Table 1). TB is currently treated with six-month combination regimen containing four drugs: ethambutol (EMB or E), isoniazid (INH or H), pyrazinamide (PZA or Z), and rifampicin (RIF or R). Since introduction of this regimen, 56 million TB patients were successfully treated, in 2011 the success rate of treating TB has increased to 87%\(^5\). Nevertheless, newly emerged multidrug resistant and extensive drug resistant tuberculosis (MDR-TB and XDR-TB) make the treatment much more complicated. For MDR-TB patients, the regimen recommended by the WHO takes 20 months and the treatment success rate is much lower. In 2010, only 48% of the MDR-TB patients were effectively treated, resulting in high mortality rates\(^3\). For the treatment of XDR-TB, the outcome is even more severe. Among 623 XDR-TB patients in South Africa treated during an outbreak in 2010, the mortality rate was 49% and only 12% of the patients were successfully cured\(^3\).

6 months treatment duration with the current standard TB chemotherapy cocktail is needed to eliminate all the bacilli, reaching both culture and smear negative state. This long-term therapy raises a number of problems, e.g. decreased compliance, increased development of resistance, and occurrence of relapses. Especially in developing countries, which carry the heaviest burden of TB disease, patients might not complete their treatment, contributing to the emergence of drug resistance. Therefore, improvement of TB chemotherapy is of high importance.

New drugs, which are desperately needed for combating the epidemic TB burden, should meet and address medical demands such as a novel working mechanism that can target MDR- and XDR-TB; the potential to simplify TB chemotherapy and shorten the long-term treatment; as well as co-applicability with HIV drugs. Fortunately, 10 new or repurposed TB drugs are in late phases of clinical development\(^3\), an overview of the current anti-TB drugs pipeline is given in Table 2. Interestingly, several inhibitors of energy metabolism are prominent on this list.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Chemical Class</th>
<th>Mechanism of action</th>
<th>References</th>
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<tbody>
<tr>
<td>First-line antibiotics</td>
<td></td>
<td></td>
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<tr>
<td>Isoniazid</td>
<td>Thioamides</td>
<td>Inhibits mycolic acid synthesis</td>
<td>11-13</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Pyrazines</td>
<td>Depletion of proton motive force, inhibition of translation</td>
<td>14, 15</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Ethylenediamine</td>
<td>Inhibits cell wall synthesis</td>
<td>13, 16</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Rifamycines</td>
<td>Inhibits nucleic acid synthesis</td>
<td>13, 16, 17</td>
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<td>Second-line antibiotics</td>
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<td></td>
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<tr>
<td>Steptomycin, Amikacin,</td>
<td>Aminoglycosides</td>
<td>Inhibits protein synthesis</td>
<td>16, 18, 19</td>
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<tr>
<td>Kanamycin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>capreomycin, viomycin,</td>
<td>Cyclic Polypeptides</td>
<td>Inhibits protein synthesis</td>
<td>16, 19</td>
</tr>
<tr>
<td>enviomycin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ciproflaxcin,levofloxacin</td>
<td>Fluoroquinolones</td>
<td>Inhibits DNA gyrase and DNA topoisomerase; Inhibit DNA replication and transcription</td>
<td>16, 17, 20</td>
</tr>
<tr>
<td>ethionamide, prothionamide</td>
<td>Thioamides</td>
<td>Inhibits mycolic acid biosynthesis</td>
<td>16</td>
</tr>
<tr>
<td>cycloserine</td>
<td>Serine derivatives</td>
<td>Inhibits peptidoglycan synthesis</td>
<td>13, 16</td>
</tr>
<tr>
<td>p-aminosalicylic acid</td>
<td>Organic compound</td>
<td>Inhibits folate acid biosynthesis</td>
<td>16, 21</td>
</tr>
<tr>
<td>Third-line antibiotics</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rifabutin</td>
<td>Derivative of rifamycin</td>
<td>blocking the DNA-dependent RNA-</td>
<td>22</td>
</tr>
<tr>
<td>Drug</td>
<td>Class</td>
<td>Effect</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>---------------</td>
</tr>
<tr>
<td>Thioacetazone</td>
<td>Thioamides</td>
<td>Inhibits mycolic acid synthesis</td>
<td>23</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>Phenothiazine</td>
<td>Inhibits NDH-2 dehydrogenase</td>
<td>24</td>
</tr>
<tr>
<td>Bedaquiline (TMC207)</td>
<td>Diaryquinolines</td>
<td>Inhibits mycobacterial ATP synthase</td>
<td>25</td>
</tr>
<tr>
<td>rifapentine</td>
<td>Derivative of rifamycin</td>
<td>blocking the DNA-dependent RNA-polymerase of the bacteria</td>
<td>26</td>
</tr>
<tr>
<td>PA-824, Delamanid (OPC-67683)</td>
<td>Nitroimidazoles</td>
<td>Inhibits cell wall mycolic acid biosynthesis, inhibits protein synthesis</td>
<td>11, 13, 17, 27</td>
</tr>
<tr>
<td>Linezolid, AZD5847, PNU-100480</td>
<td>Oxazolidinones</td>
<td>Inhibits protein synthesis</td>
<td>11, 13, 16, 17, 28</td>
</tr>
<tr>
<td>SQ109</td>
<td>Ethylenediamide</td>
<td>Inhibits cell wall synthesis, inhibits menaquinone biosynthesis</td>
<td>29</td>
</tr>
<tr>
<td>Gatifloxacin, Moxifloxacin</td>
<td>Fluoroquinolones</td>
<td>Inhibits DNA gyrase and DNA topoisomerase; Inhibit DNA replication and transcription</td>
<td>16, 20, 30</td>
</tr>
</tbody>
</table>

**Table 2.** Overview of current anti-TB drugs and drug candidates in clinical development.
Energy metabolism in mycobacteria

Aerobic and replicating state

The mycobacterial electron transport chain (ETC) (Figure 1A) can accept reducing equivalents from NADH via NADH dehydrogenase. Mycobacteria do have two types of NADH dehydrogenase, either type I NADH dehydrogenase (NDH-1) or type II NADH dehydrogenase (NDH-2). NDH-1 oxidizes NADH by allotting an electron to menaquinone (MK), coupled to proton pumping from the cytoplasmic side to the periplasmic side, thereby generating the proton motive force (PMF). NDH-2 also reduces menaquinone (MK), however, is not a proton pump\(^{31}\). Although both of the NADH dehydrogenases may be present in the membrane, NDH-2 seems to be the predominately used enzyme\(^{32}\). Sassetti et al have shown that NDH-1 is dispensable for *M. tuberculosis* growth *in vitro*\(^{33}\). Mutagenesis studies for NDH-2 led to a lethal phenotype\(^{34}\), therefore Weinstein et al suggested that NDH-2 might be the sole NADH dehydrogenase enzyme for growth of *M. tuberculosis* in an aerobic environment\(^{35}\).

Upon accepting electrons from NADH dehydrogenase menaquinone converts into menaquinol (MKH\(_2\)). Alternatively, the menaquinol pool can also be filled by succinate dehydrogenase, oxidizing succinate to fumarate. The menaquinol pool can be reoxidized again either by the cytochrome bc\(_1\) complex or by the cytochrome bd oxidase. The cytochrome bc\(_1\) complex, (with subunits QcrA-C) and the cytochrome aa\(_3\) complex (with subunits CatC-F) apparently form a supercomplex\(^{36}\). This branch of the respiratory chain, which is considered as the predominating route for the electron transport under aerobic conditions, translocates protons across the membrane and therefore is energetically more efficient. The cytochrome bd oxidase (with subunits CytA-B), which does not translocate protons, but displays higher oxygen affinity, is mainly used under O\(_2\) limited condition\(^{37}\). During the aerobic growth of the mycobacteria, the terminal oxidase uses the electrons, which flow
through the upstream ETC to reduce the electron acceptor oxygen. In the meantime, the PMF is generated, which is subsequently utilized by the ATP synthase to produce adenosine-5'-triphosphate (ATP). This enzyme consists of two parts: a cytoplasmic F₁ part (subunits α₃β₃γδε) and the transmembrane F₀ part (subunits ab₂c₁₀₋₁₅)³⁸. Proton flow through the membrane-embedded Fo part triggers rotation of subunit c, subunit γ and subunit ε. The rotation of subunit γ within theα₃β₃ hexamer of F₁ in turn drives synthesis of ATP³⁹.
Figure 1: Mycobacterial electron transport chain during aerobic (A) and hypoxia conditions (B). Sdh, succinate dehydrogenase; Cyt, cytochrome oxidase; NarK2, nitrate transporter K2; NarG, nitrate reductase; MK, menaquinone; MKH2, menaquinol; e-, electron.

Hypoxia and non-replicating state

Although *M. tuberculosis* is thought to be an obligate aerobic bacillus, it can certainly adapt to hypoxia by exiting the cell cycle and entering a dormant or non-replicating state

Wayne and Hayes have developed an *in vitro* model (Wayne model), for *M. tuberculosis* in a non-replicating persistence phase (NRP). The bacilli can survive in this system for an extended period without a significant drop in viability. The exact mechanisms facilitating this flexibility and survival in a hypoxic environment and in a NRP state are still unclear. Recent work has begun to shed light on the mechanisms involved in *M. tuberculosis* membrane bioenergetics in hypoxic non-replicating state and the mechanisms underlying ATP production. Georgellis *et al* have shown that the DosRST two-component system and the DosR regulon can mediate the mycobacteria into the hypoxia and NRP state. Further evidence has also shown that DosR regulon proteins are expressed in both active and latent TB disease. Additionally, Koul *et al.* and Rao *et al.* have reported that the intracellular concentration of ATP is five to six times lower in hypoxic non-replicating *M. tuberculosis* cells compared with aerobic replicating bacteria. Moreover, *de novo* ATP synthesis is essential for survival of hypoxic non-growing mycobacteria.

How can the mycobacteria synthesize ATP, since oxygen is absent to act as terminal electron acceptor during hypoxia? The electrons have to be transferred to a different acceptor to maintain the redox balance. In facultative anaerobes, anaerobic respiration is only observed when an external terminal electron acceptor is provided.
But surprisingly, the adding of an exogenous terminal electron acceptor is dispensable for ATP synthesis in hypoxic non-replicating *M. tuberculosis*\textsuperscript{48}. Therefore, it has been proposed that endogenous nitrate and fumarate can act as terminal electron acceptor in hypoxic non-growing *M. tuberculosis*\textsuperscript{50}. The mycobacteria have a membrane bound nitrate reductase, encoded by the *narGHJI* gene locus\textsuperscript{51} (Figure 1B). It was suggested that in an anaerobic environment, the membrane-bound nitrate reductase couples the reduction of nitrate to the generation of PMF across the cytoplasmic membrane, which in turn is used for synthesis of ATP\textsuperscript{51}. Another possible terminal electron acceptor during hypoxia is fumarate. The fumarate reductase (frd), encoded by frdABCD, which converts fumarate to succinate was shown to be up-regulated during low oxygen conditions\textsuperscript{52}. Succinate dehydrogenase, encoded by *sdhCDAB*, could also reduce fumarate by operating in the reverse direction. This enzyme is structurally and enzymatically similar to fumarate reductase and could contribute to the redox balance. Schnorpfeil *et al* have shown that the reverse operating direction of succinate dehydrogenase contributes to the generation of the PMF in *Bacillus subtilis*\textsuperscript{53}, a similar mechanism could operate in mycobacteria. In addition, Boschhoff and Barry also demonstrated that the anaerobic ETC initiated by NDH-2 using fumarate as the terminal electron acceptor\textsuperscript{41}. In conclusion, the ETC and ATP synthase are crucial for the mycobacteria under hypoxic conditions, and the identity of the coupling complex in the mycobacteria anaerobic ETC still needs to be clarified. For an overview of complexes involved in the anaerobic ETC please refer to a schematic view of mycobacterial ETC (Figure 1B).

**Energy metabolism as drug target for antibacterials**

Subpopulation of hypoxic or non-replicating persist (NRP) bacilli are a major factor for the long duration of current TB chemotherapy. These bacilli display only low susceptibility for current front-line
drugs TB drugs such as isoniazid and ethambutol, which makes it difficult to kill and the duration of treatment much longer. The concept that hypoxia and NRP mycobacteria are responsible for the length of the treatment and for the latent TB infection is now well accepted as a working model. The major goal of TB drug discovery and controlling TB prevalence is to find compounds that have a new working mechanism, in particular small molecules that target hypoxic or NRP bacilli and therefore shorten the treatment time. Fortunately, several small-molecule compounds, which interfere with mycobacteria energy metabolism, have been discovered and are in use as TB drugs or clinical development, as outlined below.

**Pyrazinamide**

Pyrazinamide (PZA) is a first line anti-TB drug, which was discovered in 1952. Pyrazinamide is a pro-drug, it is hydrolyzed by pyrazinamidase (pncA) after entering the mycobacterial cell to its active form, pyrazinoic acid (POA). Despite having been used for more than 60 years, the working mechanism is the least understood among all the first line anti-TB drugs. Zhang et al proposed that pyrazinoic acid can disrupt the membrane potential and therefore the membrane transport is dysfunctional in an acidic environment. This proposal is in line with the fact that among the front-line anti-TB drugs, PZA has the strongest ability to kill bacilli residing in an acidic environment. It is also postulated that this disruption leads to the interference of the proton motive force, but the impact of POA on respiratory ATP synthesis and on cellular ATP levels has not been investigated. A second, distinctive target for PZA was reported in 2011. Shi et al have shown that POA, the active form of pro-drug PZA binds to RpsA, which inhibits the trans-translation pathways. The trans-translation pathway is of particular importance during stressful and non-replicating conditions. Both targets/mechanisms may explain the effectiveness of PZA against hypoxic or latent cells. The relative importance of the different mechanisms of action of PZA needs to be further investigated. PZA appears to be an unmissable part of the current front-line regimen and shows synergy in many
drug combinations. In particular, drug combinations containing PZA and bedaquiline show synergetic effects in the murine TB model\textsuperscript{57}. The mechanism for this observed synergy is not understood.

**Phenothiazines**

Phenothiazines are a group of compounds with antimycobacterial activity *in vitro* and *in vivo*. Although several of the phenothiazine compounds are in clinical use for decades, the exact target site remained unknown until recently. Weinstein *et al* and Boshoff *et al* reported that phenothiazines inhibit the type II NADH dehydrogenase and thereby interfere with the oxidative phosphorylation system in mycobacteria. Inhibition of the essential NDH-2 dehydrogenase may explain why phenothiazines can exert significant bactericidal activity against mycobacterial strains that are resistant to INH, rifampin, streptomycin, pyrazinamide and ethambutol combined\textsuperscript{58, 59}. It was also reported that trifluoroperazine (TPZ) is active against mycobacteria in a macrophage model of infection, and it shows synergistic effects with both INH and RIF\textsuperscript{60, 61}. Furthermore, it has been shown that thioridazine can increase the \([\text{NADH}]/[\text{NAD}^+]\) ratio, as expected from an NDH-2 inhibitor, and therefore disturbing redox balance in mycobacteria\textsuperscript{48}. The redox state of the menaquinone pool is thought to be an important signal for expression of the Dos regulon, mediating transition into a dormant or non-replicating state\textsuperscript{62}. Phenothazines, which block the electron flow through to menaquinol, may thus prevent this transition. Currently available phenothiazines show severe side effects and therefore are not in use as first line drugs. However, improved phenothiazine derivates or other compounds interfering with NDH-2 may accelerate the treatment of TB by interfering with bacterial persistence and due to synergy in drug combinations.
Clofazimine

Clofazimine (CFZ) was first synthesized by Barry et al as an anti-tuberculosis drug in 1957. Although it shows impressive bactericidal activity against *M. tuberculosis* in vitro, including multidrug-resistant (MDR) strains, the results of CFZ against TB in animal models are highly variable. However, owing to its anti-inflammatory activities it was used in the treatment of leprosy. Due to emergence of MDR- and XDR-TB, clofazimine is re-considered again as potential anti-TB drug. CFZ was first thought to bind to the guanine bases of bacterial DNA, thus inhibiting bacterial proliferation. However, this view was challenged by Yano et al who showed that CFZ interacts with and is reduced by NDH-2, catalyzing the production of reactive oxygen species (ROS). CFZ may play an important role in the treatment MDR-TB by increasing ROS levels and killing of antibiotic tolerant *M. tuberculosis*, as the other companion drugs are likely to be less effective. Recent studies have already shown that CFZ in drug combinations, especially the combination of CFZ, PZA and BDQ, showed significant sterilizing activities in mouse models. In 2010, Van Deun et al also reported that a multidrug regimen with CFZ as prominent component may successful shorten the treatment duration of MDR-TB to 9 months.

Bedaquiline

Bedaquiline (BDQ), earlier named TMC-207 and R207910, is the lead compound of the new drug class of diarylquinoline, first reported by Johnson & Johnson in 2005. BDQ has a minimal inhibitory concentration (MIC) in the lower nanomolar range against *M. tuberculosis* and other mycobacterial strains. Genetic data indicated that ATP synthase is involved in the mechanism of action of this drug and binding studies and biochemical assays validated subunit c of ATP synthase as targets. BDQ shows highly selectivity for mycobacterial ATP synthase compared with human ATP synthase or
other mainline cells\textsuperscript{25, 75}. The molecular basis for this electivity is not well understood. Another surprisingly fact is that bedaquiline is active on both replicating and non-replicating \textit{M. tuberculosis} strains\textsuperscript{25, 47}. In line with this finding, a strong sterilizing effect for BDQ was found in murine TB models,\textsuperscript{25, 76, 77} Several studies have shown that TB combination regimen including BDQ show a synergistic effect, thus shortening of treatment duration might become possible\textsuperscript{71, 77, 78}. In a murine MDR-TB model, a combination of BDQ with second-line drugs resulted in negative cultures after 2 months of treatment, while nine months of treatment were necessary without BDQ\textsuperscript{79}. Addition of BDQ to the standard MDR-TB regimen in humans may decrease treatment time from 20 months to 6 months\textsuperscript{78}. All of these characteristics makes this compound potentially the most promising drug candidate in recent TB drug discover history. Recently, the U.S FDA and the European EMA approved BDQ for treatment of MDR-TB, which makes it the first approved anti-TB drug in the last 40 years\textsuperscript{80}.

As inhibition of ATP synthase by BDQ depletes cellular ATP levels, this drug may also interfere with the function of efflux pumps\textsuperscript{47}. ATP-driven efflux pun can be expressed by drug-resistant mycobacterial strains to expel antibacterials. BDQ might thus indirectly increase the effectiveness of other TB drugs applied in combination therapy. In summary, BDQ with a new novel working mechanism is an attractive drug for combating TB, and regimen based on BDQ could be shorter and better tolerated compared with the current standard one.

Scope of the thesis

The aim of the work described in this thesis was to characterize key components of oxidative phosphorylation in mycobacteria and to explore their suitability as target of (new) drugs. We also investigated the mechanism of drugs acting on energy metabolism and evaluated their usage in drug combinations, which may be suitable for combating tuberculosis.
The ATP synthase is known as key enzyme in oxidative phosphorylation of mycobacteria and as target of diarylquinolines. In chapter 2, an overview of current knowledge on mycobacterial ATP synthase is given, idiosyncratic features are described and the potential implications for utilization as drug target are discussed.

In **chapter 3**, the working mechanism of pyrazinamide is deliberated. Pyrazinamide (PZA), an important first-line drug employed in tuberculosis chemotherapy, played a key role in shortening the duration of tuberculosis treatment from 9 months to 6 months. Despite the importance of PZA, its mechanism of action is probably the least understood among all first- and second-line anti-tuberculosis drugs. We here tested and extended the hypothesis that PZA via its active entity pyrazinoic acid acts as an uncoupler and interferes with mycobacterial bioenergetics. A better understanding of PZA action may help in development of new drugs to further shorten tuberculosis treatment.

**Chapter 4** builds up on results from **chapter 3**, investigating the combination of PZA and BDQ in an *in vitro* model. This drug combination appears highly promising for further shortening TB treatment. In this chapter we used static *M. bovis BCG* culture to understand the combination use of these two drugs *in vitro*.

Multidrug resistant TB (MDR-TB) is become a major obstacle in controlling tuberculosis. MDR mycobacterial strains can express efflux pumps to extrude the anti-tubercular drugs. BDQ, an inhibitor of ATP synthesis, which depletes cellular ATP reserves, may concomitantly indirectly inactivate ATP-dependent efflux pumps. The study described in **chapter 5** tests this hypothesis.

**Chapter 6** deals with the cytochrome bd, one of the two terminal oxidases in the mycobacterial respiratory chain. Using mutants in which one of the two branches of the mycobacterial respiratory chain was inactivated, we show that cytochrome bd plays an important role in protection against peroxide stress and
antibacterials. Cytochrome bd might be a future target in TB drug development.

Finally, the obtained results in this thesis are summarized and discussed in chapter 7.
Reference List


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