Abstract
Tuberculosis (TB) represents a major public health problem. The growing number of (extensively) multi-drug resistance cases indicates that there is an urgent need for discovery of new anti-TB entities, addressed towards new and specific targets, and continuous development of fast and efficient synthetic strategies to access them easily. Microwave-assisted chemistry is well suited for small-scale laboratory synthetic work, allowing full control of reaction conditions, such as temperature, pressure, and time. Microwave-assisted high-speed organic synthesis is especially useful in the lead optimization phase of drug discovery. To illustrate the advantages of modern microwave heating technology, we herein describe applications and approaches that have been useful for the synthesis of new drug-like anti-TB compounds.

Key words: Extensive drug resistance, microwave, multi-drug resistance, Mycobacterium tuberculosis, tuberculosis

Introduction
Tuberculosis (TB) is a common and often lethal infectious disease caused primarily by the bacillus Mycobacterium tuberculosis (Mtb) and occasionally by M. bovis or M. africanum. It typically affects the lungs (pulmonary TB) but can affect other organs or tissues as well (extrapulmonary TB). The disease is transmitted through the air when people with pulmonary TB expel bacteria, for example by coughing. The origin of the disease was unknown until 1882, when Robert Koch showed that TB was caused by Mtb, for which he was later awarded the Nobel Prize (in 1905) (1,2). The only treatment available at that time consisted of rest at sanatoria supplemented with pulmonary collapse procedures, aimed to rest infected parts of lungs and to close cavities. Although this was widely applied, it could not be extended to the entire population affected by TB (3). In 1921, the Bacille Calmette–Guérin (BCG) vaccine was developed, but widespread vaccination campaigns were not initiated until 1943 after the introduction of the freeze-drying technique, which enabled vaccine mass production (4). The vaccine is still used mainly in countries with endemic TB, but its efficacy has been questioned in several studies, most notably in India, where very limited or no protection has been reported (5). Effective drug treatments were first developed in the 1940s–1950s. Streptomycin was the first drug proven to have a clinical effect, but it was not until the introduction of isoniazid, the first highly potent and orally available bactericidal entity, in 1952, that TB could be effectively treated. In 1965, with the advent of combination chemotherapy with isoniazid and rifampicin, the duration of the treatment could be shortened to 6 months. This allowed widespread TB control programmes to be implemented, which rapidly reduced the incidence of TB disease in industrialized countries. Unfortunately, the same success could not be observed in developing countries. The emergence of drug-resistant Mtb strains along with the human acquired immunodeficiency syndrome (AIDS) epidemic in the early 1980s, resulted in a crucial and sharp increase in TB incidence in developed countries. These issues, along with socio-economic problems and ineffective health
care systems in many countries, enabled the disease to re-establish itself (4). The World Health Organization (WHO) in 1990 estimated that one-third of the world’s population was infected with Mtb, with 8 million new infections and millions of death annually (6). Accordingly, in 1993 WHO officially recognized TB as a global emergency. Since then, significant effort has been put into overcoming the disease, and this has resulted in the recent launch of bedaquiline, the first effective drug to reach the market since the introduction of rifampicin almost 50 years ago (in 1965) (7).

**Mycobacterium tuberculosis life cycle**

Infection with Mtb follows a pattern of events that has been established through animal models, as well as observations from human TB (8,9). The infectious bacilli are inhaled as droplet nuclei that have been exhaled into the atmosphere. Initially, these droplets are small enough to remain airborne for several hours. The bacteria are phagocytosed by alveolar (10) macrophages, which then invade the epithelial layer. At this stage, a localized inflammatory response occurs and leads to recruitment of mononuclear cells from neighbouring blood vessels, providing fresh host cells for the expanding bacterial population. These cells are the building blocks for the generation of the granuloma, which initially appears as an amorphous mass of macrophages. Subsequently, it differentiates into several specialized cell types, such as multinucleated giant cells, foamy macrophages, and epithelioid macrophages. With the development of an acquired immune response and the arrival of lymphocytes, the granuloma becomes more organized and adopts a stratified structure, where the macrophage centre is surrounded by a mantle of lymphocytes. The appearance of Mtb lymphocytes, about 2 to 3 weeks post-infection, marks the end of the phase of rapid bacterial replication. At this time the granuloma is extensively vascularized, and cells are actively recruited to the site of the infection. In the late stage, the granuloma becomes hypoxic (11), a condition that can induce a state of non-replicative persistence of Mtb in culture. An active granuloma is highly infective, and ultimately its rupture spills thousands of infectious bacilli into the airways, which results in the development of a productive cough that facilitates aerosol spread of infectious bacilli.

**Tuberculosis treatment**

TB is not a disease of the past. Rather, TB remains a major public health problem. According to the latest TB global report released by the WHO (12), in 2012 an estimated 8.6 million people developed TB and 1.3 million died from the disease (including 320,000 deaths among HIV-positive people). The African region has the highest TB/HIV burden. The majority of cases worldwide were in the Southeast Asian (29%), African (27%), and Western Pacific (19%) regions. India and China alone accounted for 26% and 12% of total cases, respectively. The currently recommended treatment for new cases of drug-susceptible TB is a 2-month regimen with four first-line drugs (isoniazid, rifampicin, ethambutol, and pyrazinamide), followed by a continuous treatment with rifampicin and isoniazid for an additional 4 months (Figure 1) (12).

![First line anti-TB drugs](image)

Figure 1. First line anti-TB drugs used in combination treatment.
Treatment for multi-drug resistant TB (MDR-TB), defined as resistance to rifampicin and isoniazid (the two most powerful anti-TB drugs) is longer, and requires second-line drugs, which are more expensive and more toxic. In 2013, an estimated 3.6% of new TB cases and 20.2% of previously treated cases have MDR-TB (12). Eastern European countries and especially Asia continue to have the highest numbers of MDR-TB. Globally in 2012, there were an estimated 450,000 new cases of MDR-TB. Extensively drug-resistant *Mtb* (XDR-TB) strains have also been reported in at least 92 countries, and these make up around 10% of all MDR-TB cases (Figure 2). This is a form of multi-drug resistance with additional resistance to at least one fluoroquinolone (DNA gyrase inhibitor) and at least one second-line injectable aminoglycoside antibiotic (ribosome inhibitor). Thirteen of these countries reported more than 10 XDR-TB cases last year. Among these cases, the highest proportion was found in Azerbaijan (12.8%), Belarus (11.9%), Latvia (16.0%), Lithuania (24.8%), and Tajikistan (21%) (12).

Currently available drugs in the market target mainly *Mtb* enzymes, such as DNA gyrase, topoisomerase IV, and ATP-phosphoribosyl transferase (His-G) (13). Isoniazid and ethambutol act as inhibitors for the synthesis of mycolic acids that are essential for building the cell wall of *Mtb*. They can generate electrophilic intermediates during their oxidation, capable of reacting with a nucleophilic group of the enoyl-reductase (InhA), a specific enzyme of *Mtb*, leading to its inactivation (14). Aminoglycosides such as amikacin and streptomycin have several potential antibiotic mechanisms, such as protein synthesis inhibition, inhibition of ribosomal translocation, and disruption of the integrity of the bacterial cell membrane (15). Rifamycins (rifampicin, rifabutin, and rifalazil) act directly on messenger RNA synthesis.

The growing number of MDR-TB and XDR-TB cases highlights the urgent need for development of new anti-TB drugs, addressed towards new and specific targets. This has resulted in the need for the continuous development of new TB entities. Moreover there exists an unmet need for the development of new enabling technologies to accelerate the anti-TB drug discovery and the development process. In this context, this review will focus on the use of controlled microwave irradiation for the synthesis of small molecules designed and exploited as potential anti-TB drugs with novel *Mtb* targets.

**Importance of MW in the synthesis of new anti-TB entities**

Microwave (MW) irradiation has proven to be a highly effective heating source for driving chemical reactions in sealed vessels. Microwaves can accelerate the reaction rate, provide uniform and selective heating,
achieve greater reproducibility of reaction outcome, and help in developing cleaner and greener synthetic routes (16,17). Dedicated microwave equipment is suitable for small-scale laboratory synthetic work, allowing full control of the reaction conditions, such as temperature, pressure (depending on the heating properties of the solvent used and on the selected temperature), and time (generally short) (18). Microwaves heat reaction system directly (in situ heating); therefore, usage of solvents in the chemical reaction can be reduced or eliminated. Microwave-assisted organic synthesis is being widely applied in the pharmaceuticals industry, particularly for developing compounds in the lead optimization phase of drug development (19,20). To illustrate the advantages of using modern microwave heating technology, we herein describe applications and approaches used in the synthesis of anti-TB entities (21).

The Mtb genome is unique in encoding only two topoisomerases, topoisomerase I and II, also known as DNA gyrase (22) (Escherichia coli genome, for example, encodes four topoisomerases (22)). Unlike other bacteria, Mtb does not possess a topoisomerase type IV. The presence of a single type I topoisomerase and a single type II topoisomerase in Mtb makes the enzyme attractive from a drug discovery perspective, because it can be more vulnerable to inhibition. Very recently, Ghorpade et al. have disclosed the structure-activity relationship (SAR) studies of a library of thiazolopyridine ureas as novel anti-TB compounds active towards Mtb DNA gyrase B (GyrB) (23). Potent compounds with GyrB half maximal inhibitory concentration (IC_{50}) ≤ 1 nM and Mtb minimum inhibitory concentration (MIC) ≤ 0.1 μM were obtained (Figure 3), with a combination of side chains at the C-5 position and introduction of heterocyclic moieties at the C-6 position of the benzothiazole core. Urea formation was performed by microwave heating of benzothiazol-2-amine at 110°C for 20 min in the presence of the appropriate isocyanate. Additionally, heterocyclic groups were introduced at the C-6 position via a microwave-heated Suzuki cross-coupling reaction (24,25). By heating at 110°C the reaction time could be shortened to 30 min compared with a longer time of 4–15 h when cross-coupling was performed under standard heating conditions at 90°C. The best compound in this series, 2a, showed a Mtb MIC of 0.06 μM and was very active against GyrB in M. smegmatis (Msm GyrB IC_{50} < 0.05 nM).

Fluoroquinolones are known to be Mtb DNA GyrB inhibitors (see Introduction). In 2013, De Rosa et al. reported the synthesis of a set of new fluoroquinolones bearing a (hetero)aromatic moiety at the C-7 position and an alkyl group at the N-1 position (Figure 4) that were very active against the Mtb H37Rv strain (26). Compound 3a was found to be the most active (MIC_{50} = 0.25 μM), showing a similar MIC to the reference compound ciprofloxacin. MICs were also determined for Mtb fluoroquinolone-sensitive
and -resistant clinical isolate strains; compound 3a was the most active compound, and it was found to be 4-fold more potent than ciprofloxacin. Diversity at the C-7 position was introduced via an efficient and rapid microwave-heated Suzuki cross-coupling reaction. 3-Quinolone-carboxylic acids were reacted with the appropriate arylboronic acids or esters at 150°C, to afford the desired final inhibitors in moderate to very good yields after only 5 min of irradiation.

In the same year, Kozikowski and co-workers designed and synthesized a series of indole-2-carboxamide structure-based molecules active against resistant and multi-drug resistant Mtb strains (27). Compound 5 showed very good activity against Mtb (MIC = 0.04 μM). Its heterocyclic scaffold was prepared via a microwave-assisted hydrazone formation (4), followed by a Fischer indole cyclization (Figure 5a). Compound 5 was significantly more active than isoniazid against the Mtb H37Rv strain. However, a close analogue to 5, with the two trifluoromethyl groups both replaced by a methyl substituent, showed lower cytotoxicity, good pharmacokinetic profile in mice, and excellent anti-TB activity against a panel of five drug-resistant and multi-drug resistant Mtb strains.

Aminopyrazinamides have been reported as a novel class of Mtb GryB inhibitors, able to kill both

![Figure 4. Preparation of new fluoroquinolones via microwave assisted Suzuki cross-coupling reaction.](image)

![Figure 5. a) Microwave assisted preparation of a Mtb inhibitor via Fischer indolization. b) Synthesis of Mtb inhibitors via a microwave assisted ammonolysis.](image)
replicating and non-replicating mycobacteria (28). The development of compounds active against non-replicating \textit{Mtb} is one of the most promising strategies to achieve new and shorter TB treatment regimes. Inhibitor 6 (Figure 5b) was the most active compound of this class of new molecules (MIC = 0.5 \mu M), and the primary amide moiety was obtained via a microwave-assisted ammonolysis of the precursor methyl ester.

\textit{Mtb} glutamine synthetase (\textit{Mtb}-GS) is an essential enzyme involved in bacterial cell wall biosynthesis (29), the ability of pathogens to inhibit phagosome-lysosome fusion, and in phagosome acidification (30,31). \textit{Mtb}-GS is believed to be a promising TB target since it was shown that L-methionine-(SR)-sulfoximine (MSO, Figure 6a), a well-known inhibitor of this enzyme, was able to inhibit bacterial growth both \textit{in vitro} and \textit{in vivo} (32,33). Recently, Gising et al. reported a new class of imidazole-based \textit{Mtb}-GS inhibitors. Microwave-assisted synthesis played an important role for the preparation of the most potent compound of this series (8, Figure 6b) (34). Intermediate 7 was obtained in excellent yield of 93% via a palladium-catalysed alkoxy carbonylation of the starting-material 2-bromo-6-methoxynaphthalene in ethanol, using Mo(CO)$_6$ as the carbon monoxide source (35), in the presence of palladium(II) acetate as the precatalyst and Xantphos as the ligand. Fluorine was replaced by a primary amine, through a sluggish SN$_2$Ar substitution using diphenylmethaneamine, followed by a deprotection reaction, affording the final inhibitor 8. In both reactions, the superheated microwave conditions were necessary for product formation. When compared with MSO, compound 8 showed 100-times higher activity towards \textit{Mtb}-GS, with an IC$_{50}$ of 0.049 \mu M.

![Figure 6. a) L-methionine-(SR)-sulfoximine (MSO). b) Synthesis of \textit{Mtb}-GS inhibitors via a multi-step microwave assisted synthetic route. c) Microwave assisted synthesis of \textit{Mtb}-GS inhibitors via multi-component heterocyclization.](image)
Interestingly, compound 8 was found to inhibit bacterial growth and exhibited a MIC of 2 μM against *Mtb*.

Odell et al. and Nordqvist et al. have reported that functionalized 3-amino-imidazo[1,2 a]pyridines, identified via a high-throughput screening (HTS) of AstraZeneca’s corporate library, are potent inhibitors of *Mtb*-GS (36,37). The synthetic approach was based on a microwave-assisted Groebke–Blackburn–Bienaymé heterocyclization, using various substituted 2-aminopyridines, benzaldehydes, and isonitriles (Figure 6c). Compound 9, with an IC₅₀ of 0.38 μM, was the most active compound in this study, being considerably more potent than MSO (51 μM) against *Mtb*-GS.

*Mtb* 1-deoxy-D-xylulose 5-phosphate reductoisomerase (*Mtb*-DXR, also known as IspC) is an important *Mtb* enzyme involved in the non-mevalonate pathway and responsible for the production of isopentyl diphosphate, the precursor of isoprenoid units, essential for the TB bacilli. In eukaryotes, isopentyl diphosphate is instead produced via the mevalonate pathway, making IspC an attractive anti-TB drug target. Fosmidomycin (Figure 7a) is a known inhibitor of this enzyme and is currently in clinical trials for the treatment of malaria, confirming that it is a promising target for drug development (38). In 2013, Dowd et al. used the *Mtb*-DXR-fosmidomycin co-crystal structure complex to design bi-substrate ligands able to bind to both the natural

![Figure 7. a) Structure of fosmidomycin. b) Synthesis of fosmidomycin analogues. c) Synthesis of *Mtb*-IspC inhibitors via a microwave promoted oxidative Heck reaction. d) Synthesis of *Mtb* inhibitors via a heterocyclization.](image-url)
substrate of IspC, 1-deoxy-D-xylulose 5-phosphate (DXP), and nicotinamide adenine dinucleotide phosphate hydrogenated (NADPH), with the aim of improving the whole-cell activity due to increased lipophilicity (Figure 7b) (38). The most potent compound produced in this study (compound 11) exhibited an IC50 of 17.8 μM, and importantly, the diethyl ester of 11 was found to inhibit Mtb growth (H37Rv Mtb MIC = 200 μM). The synthesis of 11 commenced with a microwave-assisted Arbuzov reaction between triethylphosphite and 1,3-dibromopropane to generate the key intermediate 10 (38).

Andaloussi et al. prepared a series of fosmidomycin analogues bearing various aromatic groups in the phosphonic acid α position (Figure 7c) (39). The most potent inhibitor was prepared using a key microwave-assisted oxidative Heck reaction between 3,4-dichloroboronic acid and acrylaldehyde, using palladium(II) acetate and 2,9-dimethyl-1,10-phenanthroline (dmphen) as the catalytic system (40). The corresponding cinnamaldehyde 12 was then easily converted into the final compound 13. This displayed Mtb-IspC inhibition similar to fosmidomycin.

Zhou et al. have reported the synthesis of pyranoannulated 5,6,7,8-tetrahydro-1,6-naphthyridines (Figure 7d), with the aim of discovering novel anti-TB scaffolds (41). The tricyclic core could be easily prepared via a microwave-assisted intramolecular [2 +2+2] cyclization, affording compound 14 in very good yield. Three additional analogues, with different alkyl and aryl groups (R1 and R2) were also prepared, using the same synthetic approach. Removal of the protecting para-methoxybenzyl group (PMB) furnished the corresponding secondary amines, which were subsequently reacted with a diverse set of eight isocyanates, acyl chlorides, and sulfonyl chlorides, yielding the corresponding ureas, amides, and sulphonamides, respectively. Most of the synthesized entities showed potency in the micromolar range, and structure 15 was identified as the most promising anti-TB compound.

In 2009, Lin et al. described the synthesis of oxathiazol-2-ones derived from a HTS targeting the Mtb proteasome (42). Proteasomes represent an important class of complex enzymes involved in protein degradation and have been investigated as potential targets for the treatment of several human diseases. Interestingly, the active compounds were found to react covalently but reversibly with the enzyme. Structure 16 (Figure 8) was one of the most active compounds in this class and exhibited a 13-fold selectivity for the rate of inactivation (kobs)
over the human proteasome. The heterocyclic core was prepared by reacting a (hetero)aryl-amide with chlorocarbonylsulfenyl chloride, at room temperature for 16 h or by microwave irradiation at 100°C for only 15 min, both reactions affording similar yields.

Öhrngren et al. have described a non-resonant microwave reactor designed specifically for continuous-flow chemistry applications (43). One of the reported applications was the synthesis of compound 16 (Figure 8). The reaction was performed by pumping two stock solutions of benzamide and chlorocarbonylsulfenyl chloride through a mixer before reaching the microwave applicator. Under optimized conditions (353 μL/min of flow, residence time of 1 min and a temperature of 200°C) product 16 was isolated in 62% yield, with a throughput of 3.3 mol/h.

In 2008, the group of Biava reported on the activity of 1,5-diarylpyrrole derivatives towards Mtb (44). Under microwave conditions, a Stetter reaction was carried out reacting cuminaldehyde and methyl vinyl ketone (Figure 9). The reaction was driven by the addition of 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide which gave the 1,4-diketone 17 in good yield. Intermediate 17 was then cyclized according to Paal–Knorr conditions with 4-chloroaniline, yielding the tri-substituted pyrrole 18. The reaction temperature could be safely raised to 160°C (double the boiling-point of the solvent EtOH) by using an appropriate microwave reactor and sealed vials, and full conversion of starting-material was observed within 30 min. One of the most active compounds, 19, was twice as effective as isoniazid on wild-type Mtb. It was also active towards isoniazid-

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and rifampicin-resistant \textit{Mtb} (MIC = 8.0 and 0.125 \textmu M, respectively). The same research group later on presented further exploration of the 1,5-diarylpirerole derivatives (45).

In a report from 2010, Manna et al. described the synthesis of anti-TB 1,3,5-trisubstituted-4,5-dihydro-1\textit{H}-pyrazoles anti-TB molecules (46). The heterocyclization reaction was carried out using a single-mode microwave reactor, affording high yield and decreased reaction time, when compared with a previous protocol using standard conditions (12–22 min instead of reflux for 6–10 h). The final products were prepared by reacting a set of 14 \alpha,\beta-unsaturated ketones with two hydrazides (Figure 10a). Out of 28 substituted pyrazoles synthesized, inhibitor 20 was the most active compound and was twice as active as isoniazid against \textit{Mtb}. Furthermore, compound 20 was studied in a mouse model and was shown to lower the \textit{Mtb} counts in lung and spleen two log units further than isoniazid.

In an effort to develop new anti-TB entities, Alvey et al. investigated a Wittig-type cyclization to prepare benzofurans (47). Using microwave irradiation, the reaction times could be shortened from 88 h to 1 h, compared with traditional reflux conditions. The inhibitors were assembled in a one-pot two-step synthesis, involving an initial coupling between acids or acyl chlorides and the hydroxyl group of the phosphonium species. The esters were subsequently cyclized via Wittig reaction, yielding the desired benzofurans in moderate yields (Figure 10b). Compound 21 was found to be active, showing moderate activity in the whole bacterial assay.

In 2009, Kim and co-workers exploited a structure–activity relationship study of nitroimidazoles active towards \textit{Mtb} (48). One of the synthetic pathways, an intramolecular \textit{SNAr} reaction, was performed under single-mode microwave irradiation (Figure 10c). Compound 22 was obtained in modest yield, by heating the chloroimidazole starting-material at 150°C for 30 min in the presence of DIPEA. Importantly, inhibitor 22 exhibited a low MIC of 0.8 \textmu M.

**Conclusions**

Herein, we have depicted a number of examples of microwave-promoted synthetic approaches used in discovery projects for the development of new anti-TB molecules. In many of these applications, the use of microwave technology enabled the fast, smooth, and reliable preparation of a diverse range of biologically active molecules. In a number of cases, microwave superheating allowed a dramatic reduction in reaction times and the preparation of compounds that are difficult to synthesize using traditional synthetic methods. Microwave-assisted synthesis is not restricted to anti-TB research but is today widely used in many medicinal chemistry projects to accelerate lead optimization. It is believed that microwave chemistry will continue to play an important role in accelerating the development process of all kinds of new drug-like molecules, including novel antibiotics.

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