Recent developments in natural product-based drug discovery for tuberculosis

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Natural products (NPs) have been at the origin of several established drugs against tuberculosis (TB). Although the current clinical TB pipeline does not feature any candidates derived from new NP scaffolds, numerous novel NP or NP analogs have been discovered in the recent past with promising activity against *Mycobacterium tuberculosis* (Mtb). This includes newly discovered structures as well as known NP classes that had not been previously recognized to be active against Mtb. These compounds could help to replenish the dry clinical TB pipeline and, thus, contribute to improvements in the treatment of a devastating disease.

Keywords: drug discovery; natural products; tuberculosis.

Teaser: Natural products continue to serve as a prolific source of lead structures for drug discovery against TB.

Introduction

TB is a global threat to public health that is becoming ever more serious because of the widespread emergence of drug-resistant strains of the causative pathogen *Mtb* [1]. At the same time, TB-directed drug discovery has been subcritical for several decades. Although efforts towards the development of new TB drugs have re-intensified in the more recent past, resulting in the first approvals of two new TB drugs in more than 40 years (bedaquiline and delamanid) [2], there continues to be an urgent need for the discovery of novel, potent, and safe TB treatments.

NP have a rich history as lead compounds for drug discovery and development in different disease areas, but particularly so in the area of bacterial infections [3,4]. In fact, most clinically used antibiotics are NP or NP derivatives. Thus, it is no surprise that the first effective TB drug to be developed was the bacterial NP streptomycin, an aminoglycoside antibiotic isolated in 1944 from *Streptomyces griseus* by Waksman and Schatz (Table 1) [5]. Streptomycin and also other aminoglycosides to this day are important components of second-line drug combination regimens that are used in the treatment of multidrug-resistant (MDR)-TB [6]. Since the discovery of streptomycin, a multitude of NPs have been described in the literature to exhibit antimycobacterial activity. Most prominently, this includes rifamycin B, an ansamycin-type macrolactam that was first isolated from *Streptomyces mediterranei* in 1957; rifamycin B was the starting point for the development of the first-line TB drug rifampicin [5], which is a semisynthetic derivative of rifamycin B. In addition to rifampicin and aminoglycosides (streptomycin, amikacin, and kanamycin), further NP-based TB drugs are cycloserine and capreomycin, which are both part of second-line drug combinations [6].

Although the frequent occurrence of NP with the capacity to inhibit the growth of *Mtb* (at least *in vitro*) underscores the lead potential of NP for TB drug discovery, few NP/NP derivatives have been moved into drug development since the introduction of rifampicin in 1968. In fact, the current clinical development pipeline for TB includes only two NP derivatives (high-dose rifampicin and rifapentine-moxifloxacin) (www.newtbdrugs.org/pipeline/clinical), both of which are based on long known scaffolds [5]. In addition to the fact that drug development for TB has generally progressed at a slow pace, several specific issues contribute to the paucity of NP-based drug candidates in the clinical development pipeline for TB. These include: (i) the limited availability of material (because of low isolation yields or issues with source access); (ii) the lack of synthetic follow-up on promising leads, because of (perceived) synthetic difficulties; or (iii) the moderate activity of unmodified NP, whose minimum inhibitory concentration (MIC) are generally <1 Mg/ml. In addition, unmodified NP rarely have the pharmacokinetic properties needed for direct therapeutic use and, therefore, require chemical modification to make them suitable for clinical development. Only *in vitro* data are also available for most antimycobacterial NP and many of the corresponding literature reports do not include any selectivity and/or cytotoxicity data, which makes it impossible to judge the true lead potential of most NP with *in vitro* anti-*Mtb* activity. In fact, the toxicity risk associated with NP might be particularly pronounced, because many of them are produced by the source organisms to eliminate competing species or as a means of defense against predators.

In this review, we highlight some of the more recent discoveries in relation to antimycobacterial NP or NP derivatives. For the sake of brevity and conciseness, we focus on compounds with MICs >3 Mg/ml; although this particular potency cutoff is rather arbitrary, we firmly believe that NP with lower potency *in vitro* offer little promise as true leads for TB drug discovery (although, obviously, this cannot be excluded with certainty). However, even within this boundary, the list of compounds discussed here (Figure 1) is not exhaustive. The reader is referred to [7–9] for more inclusive reviews on antimycobacterial NP.

Peptides and depsipeptides

Griselimycins

The cyclic peptide griselimycin (**1**) (Figure 1) was first reported in the literature in 1965 (as RP 11072) by Noufflard-Guy-Loe and Berteaux, who described its isolation from two species of *Streptomyces*, *S. griseus* and *S. coelicus*, and showed it to be active against mycobacteria [9]. The structural elucidation of **1** was then reported in a series of papers in 1969 [10] and 1971 [11]. After attempts to improve the unfavorable pharmacokinetic properties of the compound had failed at the time, interest in the compound ceased, especially after rifampicin became available for TB treatment in 1968 (according to [13]). Recently, however, the compound was revisited by Müller and coworkers [13] and they found that introducing alkyl substituents on the pyrrolidine ring of Pro⁸ led to a substantial increase in metabolic stability. In addition, these derivatives, such as cyclohexylgriselimycin (**2**) (Figure 1), showed enhanced antibiotic activity against *Mtb*, with the MIC of **2** against *Mtb* H37Rv being 50-fold lower than for the parent NP **1** (0.06 Mg/ml versus 1 Mg/ml for **1**) [13]. MIC values for **2** against a series of different monoresistant *Mtb* strains were between 0.056 and 0.2 µg/ml. Müller and coworkers were also able to elucidate the protein target of griselimycins as the DNA polymerase sliding clamp (DnaN) by means of resistant mutant sequencing. The genetic findings were further supported by subsequent surface plasmon resonance (SPR) measurements, which showed good selectivity of **2** for bacterial over human DnaN [13]. Ultimately, binding of griselimycin (**1**)/cyclohexylgriselimycin (**2**) to DnaN was firmly established by X-ray crystallography [13]. Cyclohexylgriselimycin (**2**) prevented bacterial growth and the formation of gross lung lesions in an acute mouse model of TB after oral administration. In a chronic model, the compound was shown to be equally effective as rifampicin [13].

Cyclomarins

In 1999, the groups of Clardy and Fenical reported the isolation of a small family of cycloheptapeptides from the marine streptomycete CNB-982, termed 'cyclomarins A–C' (Figure 1) [14]. Although these compounds were noteworthy at the time because of their anti-inflammatory activity, the major metabolite cyclomarin A (**3**) was shown by a group at Novartis in 2011 to be active against *Mtb*, with cidal concentrations of 0.3 µM and 2.5 µM against bacteria in culture broth medium and in human-derived macrophages, respectively [15]. The compound also inhibited the growth of nonreplicating, intracellular bacteria in macrophages (90% kill after exposure to 2.5 mM of **3** for 5 days) and was found to retain activity against different MDR strains of *Mtb*, although no MICs were reported for this activity [15]. No spontaneous resistant *Mtb* mutants could be raised upon exposure of *Mtb* to cyclomarin A (**3**), suggesting a mutation frequency of $>10^{-9}$. Using a proteomic approach with *Mycobacterium bovis* BCG as a model organism, the protein target of cyclomarin A (**3**) was identified to be the ATPase ClpC1 (Rv3596c), the regulatory subunit of the caseinolytic Clp protease. In agreement with these findings, cyclomarin A (**3**) was found to specifically increase proteolysis in *Mycobacterium smegmatis*, as demonstrated by the significantly increased degradation rate of GFP tagged with a Clp recognition sequence, compared with the unmodified protein (40% versus 10% degradation, respectively, after treatment with 0.3 µM **3** for 4 h) [15]. The equilibrium dissociation constant for the complex between cyclomarin (**3**) and ClpC1 was determined as 16 nM for a 1:1 stoichiometry; the binding of **3** to isolated ClpC1 was not competitive with ATP binding. Of three cyclomarin-type structures studied in [15] [including cyclomarin A (**3**)], only those two that showed activity against *Mtb* were able to prevent binding of **3** to ClpC1 in an *Mtb* cell lysate. The Novartis group subsequently also reported significant antimalarial activity of **3**, because of selective, nanomolar inhibition of the diadenosine triphosphate hydrolase (PfAp₃Ase) of *Plasmodium falciparum* (IC₅₀ = 4 nM), whereas the human homolog of PfAp₃Ase (hFHIT; human fragile histidine triad) was not affected [16]. In 2016, Barbie and Kazmaier reported the synthesis of desoxycyclomarin C (**6**), which has the β-hydroxy-L-Trp in cyclomarin C (**5**) replaced with L-Trp. Interestingly, this compound retained antimycobacterial activity, with an MIC of 2 Mg/ml versus 0.5 Mg/ml for cyclomarin C (**5**) (the tested strain is not indicated in the paper) [17]. This latter study was performed in the context of a more comprehensive program by the Kazmaier group on the synthesis of cyclomarins and cyclomarin analogs. This work could open a path to synthetic cyclomarin analogs with improved pharmacokinetic properties over the NPs, which are cited by Kazmaier *et al.* as being extremely susceptible to metabolic degradation and, therefore, cannot serve as drug candidates. (No published original data on the PK properties of cyclomarins are available, however.) As demonstrated by the work on griselimycins, such improvements could be feasible [13].

Ecumicin

Gao *et al.* recently reported the isolation of ecumicin (**7**) (Figure 1) [18], a head-to-side-chain cyclic tridecapeptide comprising mainly *N*-methylated amino acids, from *Nonomuraea* sp. MJM5123. Given its particular structural features (large fraction of *N*-methyl/D-configured amino acids), ecumicin (**7**) is highly resistant to enzymatic degradation [19]. Similar to cyclomarin A (**3**), ecumicin (**7**) specifically binds to ClpC1 of *Mtb* [19,20] and its *in vitro* anti-*Mtb* activity profile is comparable with that of current first-line TB drugs. Thus, the compound exhibits MIC values against sensitive *Mtb* strains of 0.16–0.58 Mg/ml and it retains activity against monodrug-resistant (MICs <0.12–0.31 Mg/ml) as well as MDR and extensively drug-resistant (XDR) strains of *Mtb* (MICs <0.12–0.31 Mg/ml and 0.31–0.62 Mg/ml, respectively). Ecumicin (**7**) is essentially nontoxic to mammalian cells (IC₅₀ values of <63 µM and <32 µM against Vero and J774 cells, respectively) [19] and it has also been shown to be active in a mouse model of TB after subcutaneous administration [19].

Lassomycin

The 'lasso' hexadecapeptide lassomycin (**8**) was recently isolated from the *Actinomycete* *Lentzea kentuckyensis* spp. IO0009804 by Garvish *et al.* (Figure 1) [21]. Similar to ecumicin (**7**) and cyclomarin A (**3**), **8** has been reported to target and bind to ClpC1, which appears to be emerging as a (clinically) unexplored, high-potential target for new TB drug discovery [19]. Lassomycin (**8**), similar to ecumicin (**7**), leads to a several-fold increase in the ATPase activity ClpC1, but in contrast to the latter, which causes excessive protein degradation, **8** inhibits ClpP1P2-dependent proteolysis [21]. As indicated above, excessive protein degradation is also triggered by cyclomarin A (**3**); no data have been reported for the effect of **3** on the ATPase activity of ClpC1. Lassomycin (**8**) inhibits *Mtb* growth *in vitro* with MICs in the range of 0.8–3 Mg/ml and its activity extends across a variety of *Mtb* strains, including MDR and XDR isolates [21]. However, more recently, the biological evaluation of synthetic lassomycin (**8**) (based on the structure reported in [21]) and its C-terminal amide failed to reveal any activity against *Mtb* for either compound [22]. Although the reasons for this discrepancy have not yet been fully established, temperature-dependent NMR measurements on synthetic lassomycin (**8**) suggest that natural lassomycin (**8**) exists in a threaded conformation that does not unfold upon heating to 40°C, contrary to the original proposal that the peptide would exist in an unthreaded state [21].

Teixobactin

In 2015, Ling *et al.* reported the discovery of the head-to-side chain cyclic depsipeptide teixobactin (**9**) (Figure 1) [23], based on a screen for antimicrobial activity against *Staphylococcus aureus* of extracts obtained from uncultured soil bacteria [24]; the species producing teixobactin (**9**) was provisionally named *Eleftheria terrae*. The compound was subsequently found to exhibit excellent activity against Gram-positive pathogens, including drug-resistant strains [23] and also to inhibit *Mtb* H37Rv with an MIC of 0.125 Mg/ml. Teixobactin (**9**) blocks cell wall biosynthesis *in vitro* by binding to a highly conserved motif on the peptidoglycan precursor lipid II and to cell wall teichoic acid precursor lipid III. Given its lack of cytotoxicity against NIH/3T3 and HepG2 cells even at 100 Mg/ml (the highest concentration tested), teixobactin (**9**) is a promising lead for the development of new antibiotics. In fact, the compound has shown *in vivo* activity against *S. aureus* and *Streptococcus pneumoniae* [23]. However, it is unlikely to be orally bioavailable, which clearly limits its potential as a TB drug candidate *per se*.

Initial structure–activity relation (SAR) studies with synthetic teixobactin analogs [25,26] have shown that the *L*-allo-enduracididine residue can be replaced by either Arg or, preferably, Lys. By contrast, modifications of the N-terminal tail lead to a loss of activity.

Pyridomycin

Pyridomycin (**10**) (Figure 1) is a bacterial NP that was first isolated in 1953 by Maeda and coworkers from *Streptomyces albidofuscus* (*S. pyridomyceticus*) [27]. Although the compound was shown to exhibit significant *in vitro* antimycobacterial activity (MIC 5 0.3 Mg/ml, H37Rv) and to display low cytotoxicity [28], no attempts at the further profiling of pyridomycin (**10**) or the elucidation of its SAR were reported in the literature until recently. The *in vitro* antimycobacterial activity of pyridomycin (**10**) against actively growing bacteria was reconfirmed in 2012 by Hartkoorn *et al.* [29], although the compound was not active against nonreplicating *M. tuberculosis* in the streptomycin-starved 18b (ss18b) model. In a subsequent step, pyridomycin-resistant mutants of *Mtb* were selected. Whole-genome sequencing and genetic validation then revealed that pyridomycin (**10**) was targeting the NADH-dependent enoyl-[acyl-carrier-protein] reductase InhA, which is an essential enzyme for cell wall biosynthesis in *Mtb* and is the target of the clinical TB drugs isoniazid and ethionamide [30,31]. As shown by X-ray crystallography, pyridomycin binds to InhA in a unique configuration, because the compound spans both the NADH as well as the substrate-binding sites of the enzyme. This binding mode is distinct from all other known direct InhA inhibitors, which bind to the substrate-binding site of the protein [32,33]. As a consequence of its direct inhibition of InhA, pyridomycin (**10**) retains activity against InhA-resistant strains of *Mtb*, which are mostly characterized by mutations in the isoniazid-activating enzyme KatG rather than in InhA itself.

So far, only one total synthesis of pyridomycin has been reported in the literature, by Kinoshita *et al.* in 1989 [34]. One of the major difficulties in this synthesis was the establishment of the structurally unique enol-ester moiety that is part of the pyridomycin macrocycle. To bypass these difficulties, dihydropyridomycins **11** and **12** have been studied; these have an *iso*-propyl substituent attached to C2 by a single, rather than a double bond [35]. Intriguingly, the *2R* analog **11** retained most of the activity of natural pyridomycin (a fourfold increase in MIC against H37Rv), thus demonstrating that the enol-ester double bond is not a prerequisite for the anti-*Mtb* activity of **10**. The *2S* isomer **12** was 32-fold less potent than the NP. Other pyridomycin analogs that are based on the dihydropyridomycin core structure are currently being investigated.

Nonpeptidic structures

Tryptanthrins

Tryptanthrin (**13**) (Figure 1) is a natural indolo[2,1-*b*]quinazoline-6,12-dione that was first isolated in 1979 by Honda and coworkers [36] from the indigo plant *Strobilanthes cusia* O. Kuntze (Acanthaceae). In 1998, Mitscher and Baker [37] described the antiTB activity of tryptanthrin and several analogs, with PA-505 (**14**) and PA-510 (**15**) as the most promising compounds at the time, but little efficacy was observed in a mouse model of acute TB (without this being commented on).

Tryptanthrin (**13**) is poorly water soluble (5.4 mM) and, because of its planar structure, it might also act as a DNA intercalator, which would lead to cytotoxicity. In light of these shortcomings, Hwang *et al.* [38] recently embarked on the design and synthesis of a series of new tryptanthrin analogs with less planar structures and incorporating solubilizing groups. The most promising of these compounds, **16** (Figure 1), exhibited MICs of 0.5 mg/ml, in the microplate Alamar Blue assay (MABA) against H37Rv, and 11.5 mg/ml, in a low-oxygen recovery assay (LORA); the oral bioavailability of **16** in rats was 30%. However, the compound failed to show any efficacy *in vivo* in a murine model of acute TB. The discrepancy between the *in vitro* and *in vivo* data could not be explained, but could result from insufficient plasma and/or target tissue concentrations. We have included this example, because the study highlights the fact that potent *in vitro* anti-*Mtb* activity is an insufficient measure for the true lead potential of a compound for drug discovery. Although this is in fact a trivial conclusion, there is a vast amount of literature on compounds (including some of those discussed in here), which have been claimed to be promising leads for TB-directed drug discovery solely based on MIC values. The results of Hwang *et al.* on their tryptanthrin analogs put these claims in perspective.

Tiacumicin B

The antimicrobial glycosylated macrolide tiacumicin B (**17**) (Figure 1) was isolated in 1987 from the soil bacterium *Dactylosporangium aurantiacum* subsp. *hamdenensis* subsp. nov. AB718C-41 [39] and was later shown [40] to be identical with the previously reported lipiarmycin A3 [41]. Tiacumicin B (**17**) is a bacterial RNA polymerase inhibitor [42] and has been approved (as fidaxomicin; Dificlir®, Dificid®) for the treatment of infections with *Clostridium difficile* [43]. The compound exhibits activity against a broad range of Gram-positive organisms [44]; more importantly, in the context of this review, it was also found in 2008 to be a potent inhibitor of *Mtb*, including several MDR-*Mtb* strains (MICs of 0.01–1 Mg/ml) [45]. The major obstacle for the development of tiacumicin B (**17**) for TB therapy is its low bioavailability and acid sensitivity [46]. Recently, two independent syntheses of the tiacumicin B aglycone [47,48] and one total synthesis of the complete NP [49] were described, which provide a basis for SAR studies on synthetic tiacumicin analogs that might eventually lead to compounds with a property profile suitable for oral therapy. As illustrated by the recent advances on the pharmacokinetic profile of griselimycins, such improvements are not impossible to achieve.

Caprazamycins

Caprazamycins (CPZs) (Figure 1) are liponucleoside antibiotics that were first isolated in 2003 by Igarashi *et al.* from *Streptomyces* sp. MK730-62F2 [50]. They are generally active against acid-fast bacteria, including *Mtb*, with CPZ B (**18**) being the most potent antimycobacterial agent (MIC against *Mtb* H37Rv = 3.13 Mg/ml) [51]. The antibiotic activity of CPZs results from inhibition of the biosynthetic enzyme MraY (translocase I), which is essential for bacterial cell growth. Although CPZ B (**18**) showed no significant toxicity in mice, its difficult isolation process (the compound has to be separated from a complex mixture by HPLC) and its poor water solubility made it unsuitable for drug development.

Treatment of the mixture of CPZs A–G with aqueous acid leads to cleavage of the ester-linked side chain and the release of the (inactive) common core structure, which is referred to as ‘caprazene’ (**19**) (CPZEN) [52]. The investigation of various non-natural ester derivatives of CPZEN led to the discovery of CPZEN-45 (**20**) as the most active CPZEN analog; the compound exhibits an MIC against *Mtb* H37Rv of 1.56 Mg/ml, is active against MDR and XDR *Mtb* strains, and shows excellent water solubility (>200 mg/ml). [MIC values against monodrug-resistant *Mtb* strains have only been published in various patent applications (e.g., [53]) and are reported to be around 6 µg/ml.] Intriguingly, CPZEN-45 (**20**) was also active against CPZ-resistant *Bacillus subtilis* strains, but was not effective against a strain overexpressing undecaprenyl-phosphate–GlcNAc-1-phosphate transferase (TagO), an enzyme involved in the synthesis of teichoic acid. At the same time, a mutation was found in the *tagO* gene of a spontaneous CPZEN-45-resistant strain, suggesting that the mechanism underlying the antibiotic activity of CPZEN-45 (**20**) in *Bacillus subtilis* was the inhibition of TagO. This was subsequently confirmed by Ishizaki *et al.*, who showed that CPZEN-45 inhibited glycerol incorporation in *B. subtilis* using radiolabeled precursors and evaluated its activity using the sonicated cell lysate of *B. subtilis* strains overexpressing MraY or TagO [54]. Furthermore, and more importantly in the context of this review, more recent work reported by Ishizaki *et al.* also suggests that CPZEN-45 (**20**) inhibits WecA, which is the ortholog of TagO in *Mtb*, with an IC₅₀ of 4.4 ng/ml (in membrane fractions of *Mtb* mc²6230).

Neither MraY nor WecA are targeted by current TB drugs and, thus, the successful clinical development of inhibitors of these enzymes would lead to TB drugs with an entirely new mode of action. Such compounds would be devoid of any (mechanism-related) cross-resistance with existing drugs. CPZEN-45 (**20**) is currently in preclinical development for TB.

Diazaquinomycins

Diazaquinomycins (DAQs) are biologically active secondary metabolites with a diaza-anthracene structure (Figure 1). Several representatives of this class have been investigated as antibiotics and also as anticancer agents [55,56]. However, it was only recently that Mallowney *et al.* discovered the potent antimycobacterial activity of some DAQs, in the context of the isolation of the new DAQs E–G from *Streptomyces* sp. [57] and H and J from *Micromonospora* sp. [58]. DAQH (**21**) and DAQJ (**22**) exhibited MICs of 0.04 and 0.07 Mg/ml, respectively, against *Mtb* H37Rv; potent anti-*Mtb* activity was also found for DAQA (**23**) and DAQE (**24**) (MICs of 0.1 and 0.04 Mg/ml, respectively).

DAQA (**23**) was also found to be active against nonreplicating bacteria (MIC of 0.72 µg/ml in the LORA assay) and it retained high potency against a panel of various monodrug-resistant strains of *Mtb* (MIC values <1 µg/ml), suggesting absence of cross-resistance with current TB agents [58]. Mullooney *et al.* [58] also re-evaluated the cytotoxicity of DAQA (**23**) and found it to be well tolerated compared with earlier studies [56]. DAQs have been reported to inhibit thymidylate synthase from Ehrlich ascites and it has been implied that this enzyme is the target of DAQs in both cancer cells as well as Gram-positive bacteria [56]. However, more recent studies have demonstrated that thymidylate synthase is not the target of DAQs in *Mtb* [58].

Spectinamides

Instead of screening for agents that inhibit *Mtb* and trying to optimize the hits produced, an alternative strategy to develop new TB drugs is the repurposing of existing antibacterials that are known to be safe and effective against other infections, but that have not been used extensively (or not at all) to treat TB. A prime example for how such a strategy can lead to highly interesting, new anti-*Mtb* agents is the work recently reported by Lee *et al.* [59] on new spectinomycin derivatives.

Spectinomycin (**25**) (Figure 1) itself is a bacterial NP that has a long history of safe use for the treatment of gonorrhea in patients who are allergic (or resistant) to penicillins. Based on the careful literature analysis of available SAR data and the construction of a homology model of the purported spectinomycin binding site in the *Mtb* 16S ribosomal subunit (derived from the structure of the *Escherichia coli* 30S ribosomal subunit/spectinomycin complex), Lee *et al.* concluded that the replacement of the 3'-keto group in spectinomycin by an acylated amino group should be well tolerated for an *R* configuration of the newly created stereocenter at C3. Using spectinomycin as the starting material, a series of such analogs, termed 'spectinamides' (**26**) (Figure 1) was produced in a convergent four-step (semi)synthesis. The optimization process, which heavily relied on the use of the above homology model for the selection of improved acyl residues, finally led to three candidate molecules with MIC values against *Mtb* H37Rv of 0.8–1.6 Mg/ml; these compounds were also active against MDR and XDR clinical isolates (with MICs against different monodrug-resistant *Mtb* strains between 0.8 and 6.3 µg/mL) [59]. For one variant of **26** [R 5 2-(5-chloropyridin-2-yl)acetyl; Figure 1], activity was also assessed against hypoxic bacilli and the compound was determined to be 'highly active' (<2% viable bacteria at 10 µg/mL of **26**). The greatly improved anti-*Mtb* activity of these compounds over the parent NP was found to result from the reduced susceptibility of the former to drug efflux. The importance of drug efflux for the activity of spectinamides (**26**) was initially suggested by the lack of a correlation between their anti-TB activity and the inhibition of mycobacterial ribosomal translation, and was subsequently confirmed by experiments with knockout mutants for the multidrug transporter Rv1258c, which is known to induce intrinsic spectinomycin resistance [60]. Given their different ribosomal binding sites, spectinamides **26** lack cross-resistance with other protein synthesis inhibitors, such as streptomycin, amikacin, kanamycin, capreomycin, or linezolid. The optimized spectinamides **26** exhibit favorable pharmacokinetic profiles and they are not cytotoxic. They have also shown excellent *in vivo* activity in both acute and chronic TB models in mice.

Concluding remarks

NPs have contributed in a major way to the currently available portfolio of drugs for the treatment of TB, with one first-line drug and several second-line agents being either NP themselves or being derived from a NP lead. At the same time, no drug derived from a new NP scaffold has been approved for clinical use since the first introduction of rifamycin-based drugs during the 1960s. However, this situation is nothing more than a reflection of several decades of subcritical efforts on TB drug development in general, which has led to a serious shortage of new drugs and drug candidates in clinical development against this devastating disease. In fact, several NPs have been found over the past few years to exhibit promising activity against the causative pathogen *Mtb*. In light of continuous advances in NP research at various levels (isolation techniques, methods for structural characterization, combinatorial biosynthesis, mutasynthesis, activation of silent gene clusters, synthetic methodology, etc.), we will likely continue to see the emergence of new structures with interesting anti-*Mtb* activity and acting through new modes of action. Obviously, there are many challenges that need to be overcome on the way from a bioactive NP to a viable therapeutic; one of the most critical issues often being the achievement of sufficient metabolic stability and oral bioavailability. However, as illustrated by the example of cyclohexylgriselimycin, improvements in these parameters is possible, even by limited structural modification of the original NP lead. Also, the poor economic status of those countries and regions that are most affected by the disease imposes severe restrictions on the affordable cost of goods for drugs against TB. Despite these challenges, we believe that NP can continue to serve as source of new and innovative drugs against TB, if sufficient funding is made available for research and development.

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Figure 1. Molecular structures of natural products or natural product derivatives that have recently been shown to exhibit anti-*Mycobacterium tuberculosis* (*Mtb*) activity.

Figure 1

Griselimycin (**1**):

Cyclohexylgriselimycir