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REVIEW ARTICLE

New antituberculous drugs derived from natural products: current perspectives and issues in antituberculous drug development

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Tuberculosis is one of the most common and challenging infectious diseases worldwide. Especially, the lack of effective chemotherapeutic drugs for tuberculosis/human immunodeficiency virus co-infection and prevalence of multidrug-resistant and extensively drug-resistant tuberculosis remain to be serious clinical problems. Development of new drugs is a potential solution to fight tuberculosis. In this decade, the development status of new antituberculous drugs has been greatly advanced by the leading role of international organizations such as the Global Alliance for Tuberculosis Drug Development, Stop Tuberculosis Partnership and Global Health Innovative Technology Fund. In this review, we introduce the development status of new drugs for tuberculosis, focusing on those derived from natural products.
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INTRODUCTION

The majority of patients suffering from the three major infectious diseases (human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis (TB), and malaria) and neglected tropical diseases (a group of 20 diseases)[1] are concentrated in developing countries or among the poverty class. Therefore, these diseases, except for AIDS that has also spread rapidly in developed countries, have received little attention as targets for new drug research and development. Recently, international organizations, national governments and private sectors have established support organizations for research and development of innovative drugs and distribution of drugs to countries burdened by HIV/AIDS, TB and malaria.[2]

It is generally recognized that up to one-third of the world's population may be affected and they are at the risk of subsequent reactivation of TB. According to the World Health Organization, 10.4 million new active cases of TB and 1.8 million deaths occurred in 2015.[3] Additionally, the spread of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) has recently been considered to be another serious problem.[3] Moreover, a case of TB resistant to all currently used drugs, referred to as totally drug-resistant tuberculosis, has already been reported.[4] There is, therefore, an urgent need for the discovery and development of novel antituberculous drugs, especially those that have a different mode of action from currently used drugs.[5]

TB/HIV co-infection further exacerbates the problem. There are over 36.7 million HIV-positive people worldwide; of the 1.1 million who die of HIV annually, 0.4 million (36%) suffered from TB/HIV co-infection.[6] Thus, TB is the largest single cause of death among HIV-infected patients. The principal chemotherapy for TB/HIV co-infection is combination treatment. However, there are some concerns about such combination therapies, including overlapping side-effect profiles of antituberculous and antiretroviral drugs, immune reconstitution inflammatory syndrome and drug–drug interactions. For instance, drug–drug interactions between antibiotic rifamycin and four classes of antiretroviral drugs are particularly important problems for this treatment. Rifamycins induce drug-metabolizing enzymes such as cytochrome P450 enzymes, whereby antiretroviral drugs are known to reduce their serum half-life and serum concentration by being metabolized by that system. The ability to induce cytochrome P450 enzymes differs among rifamycins, and it becomes stronger in the order of rifabutin, rifapentine and rifampicin. Thus, overcoming significant drug–drug interactions is a great issue for the treatment of TB/HIV co-infection.[7–9]

Many pharmaceutical companies have been indifferent to serious development of new antituberculous drugs because they estimate that antituberculous drugs will not generate profits commensurate with the cost and effort for development. However, international organizations such as the Global Alliance for TB Drug Development (TB Alliance; https://www.tballiance.org), Stop TB Partnership (WHO; http://www.stoptb.org) and Global Health Innovative Technology Fund (GHIT; https://www.ghitfund.org/en), have recently taken a leading role in initiating efforts to end the TB epidemic.[2] As a result of this effort, new classes of antitubercular drugs, such as bedaquiline, have been launched for the first time in more than 40 years.[10,11]

Antituberculous drugs currently under development are required to have superior properties to existing drugs, such as achieving a shorter

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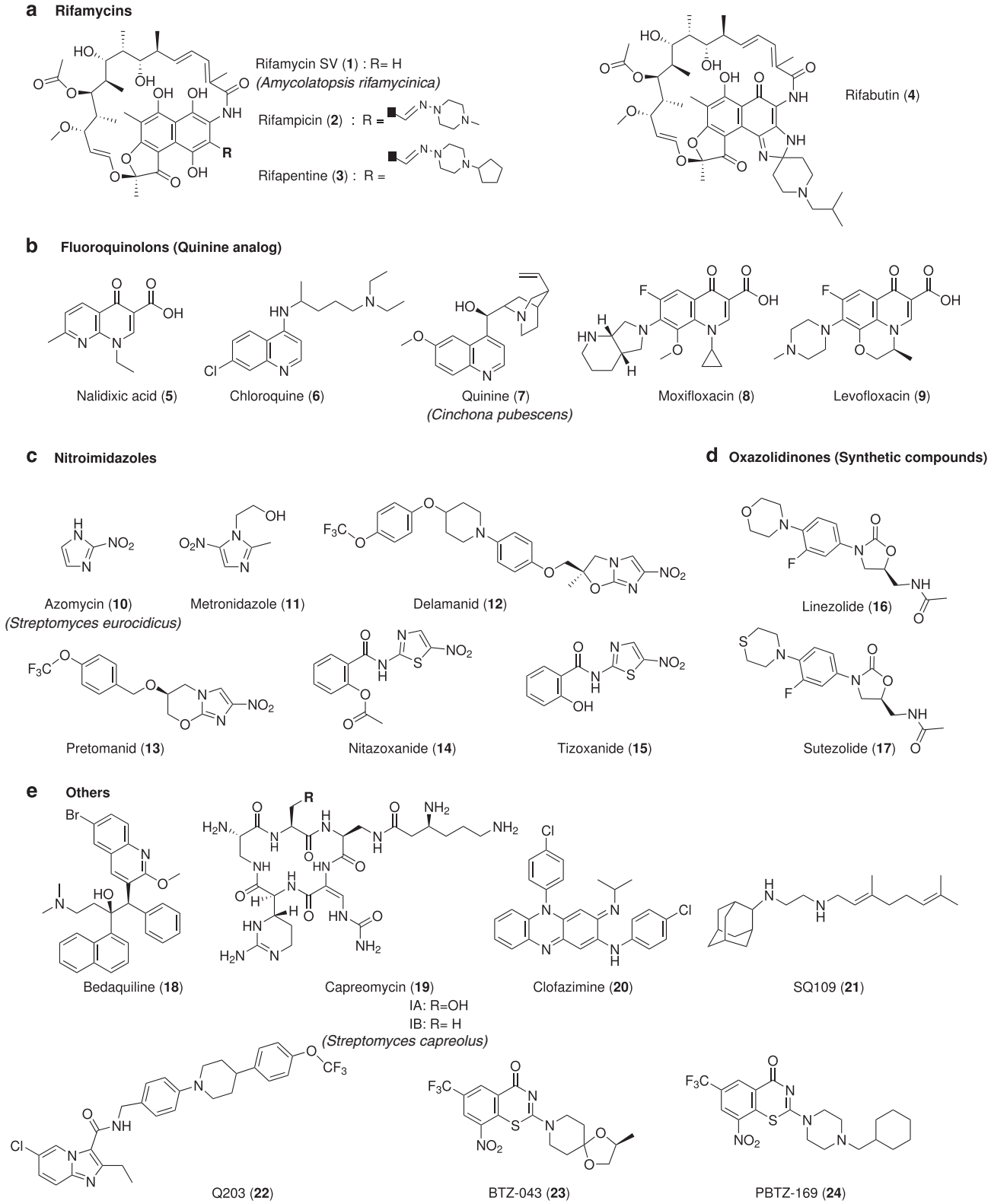

Figure 1 Structures of antitubercular compounds in clinical trials and related compounds.

therapy period, a simpler treatment regimen, treatment of MDR/XDR-TB, simultaneous treatment of TB/HIV and higher safety.[5,6,12–14] New antituberculous drugs that display low toxicity and have excellent antituberculous activity and efficacy against dormant bacteria will be beneficial for short-course therapy and against endogenous reactivation. Although long-term combination therapy is currently the basis of TB treatment, the development of short-term TB treatments may be safer for use in children and helps to prevent MDR/XDR-TB by

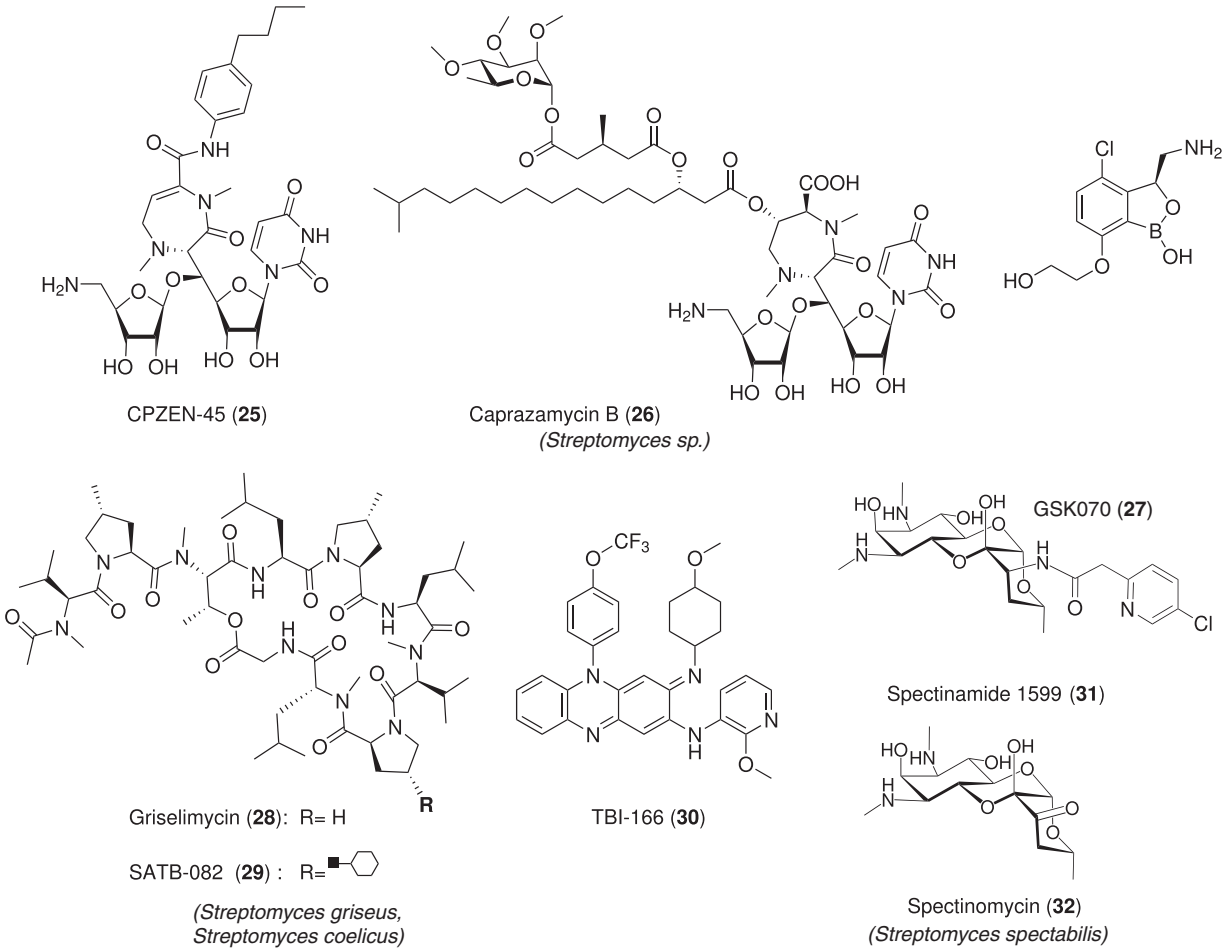

Figure 2 Structures of antitubercular compounds in preclinical trials and related compounds.

increasing patient compliance. Additionally, there is an urgent need for pharmaceutical drugs that are effective against MDR/XDR-TB that possess novel mechanisms of action along with a suitability for TB/HIV or TB/diabetes cotreatment.[5,6,12–15]

Conventionally, oral administration has been demanded in antituberculous drug development; however, inhaled drug delivery systems have recently received remarkable attention as a new method for the treatment of infectious lung diseases. Inhaled drug delivery will enable the achievement of high concentrations of pharmacologic agents in the lungs, resulting in an immediate effect by direct delivery to the lungs with a lower dosage and reduced side effects compared to oral administration.[11,16–20] For instance, natural products and related compounds, for example, tobramycin, colistin and aztreonam, have been developed for cystic fibrosis as inhalation drugs.[21] Additionally, amikacin,[22–25] arbekacin[26] and capreomycin[27] are under development for nontuberculous mycobacterial infection, bacterial pneumonia and tuberculosis, respectively. This method is also important for redevelopment of low bioavailability compounds as antituberculous drugs.[28] However, inhalation therapy is not useful for nonpulmonary infections, and combination therapy of inhalation and oral treatment would be required, which again complicates the therapeutic regimen when considering the patient's adaptability.

Exploration of natural products is an attractive method for the discovery of new antituberculous drugs. In fact, 63% (12/19) of the currently developing antituberculous drugs listed in this review are natural products, either derived directly from them or inspired derivatives. The utility of natural products is not necessarily as the final drug product; rather, natural products are considered to be a source of new structural leads.[29] Natural products provide organic chemists another direction in the search and discovery of new drugs, which is the provision of novel chemical scaffolds and an inventive hint for target-based mimetic approaches to biosynthetic pathways. This review outlines the development status of current TB drugs, focusing on those derived from natural products.

CANDIDATE DRUGS UNDER DEVELOPMENT

The development of new antituberculous drugs is classified as follows: (1) drugs with new structures and mechanisms of action, (2) reevaluation of existing drugs to optimize the efficacy of first-line drug regimens such as pyrazinamide-containing short-course regimens, (3) next generation of existing antituberculous drugs and (4) drugs used for other infectious diseases. According to the STOP TB Partnership's Working Group on New Drugs (www.newtbdrugs.org/), more than 14 compounds are currently undergoing clinical trials (Figure 1) and more than 5 compounds are in preclinical trials (Figure 2).

Compounds in clinical trials

Rifamycins. Rifamycin (**1**), an ansamycin antibiotic, was originally discovered from the metabolite of *Amycolatopsis rifamycinica* in 1957

by an Italian pharmaceutical company Gruppo Lepetit S.p.A. and shows a strong antibacterial activity against Gram-positive bacteria and *Mycobacterium* spp.[30] Rifampicin (**2**),[31] rifapentine (**3**)[32] and rifabutin (**4**)[33] are semisynthetic derivatives of rifamycin. Rifamycins have been used as essential drugs for current TB treatment in combination therapy, as with isoniazid, for all treatment stages. These drugs inhibit bacterial DNA-dependent RNA polymerase by binding to the site adjacent to the active center in RNA polymerase β subunit.[34,35]

Rifapentine (**3**) was approved for medical use in the US in 1988. In comparison with rifampicin, rifapentine has 2–4-fold stronger antibacterial activities *in vitro* and a longer half-life in blood. Nevertheless, rifapentine penetrates macrophages less well than rifampicin.[36,37] The treatment regimen for rifapentine is anticipated to be once-a-week administration, unlike rifampicin and rifabutin that require more frequent administration. A treatment regimen consisting of high-dose rifapentine (900 mg administered twice a week for 2 months or 1200 mg administered once a week for 4 months) and/or combination therapy with moxifloxacin (**8**) is expected to be effective for short-course therapy and dormant TB.[38,39] Phase III clinical trials are currently underway by Sanofi and the US Centers for Disease Control and Prevention.

Fluoroquinolones. Nalidixic acid (**5**), discovered in 1962 by Lesher *et al.*, is the first identified quinolone and was originally derived as a by-product of chloroquine (**6**), which is an analog of quinine (**7**).[40,41] First-generation quinolones are only active against Gram-negative bacteria, whereas fluoroquinolones, which are fluorinated at the C-6 position, have antibacterial activity against both Gram-negative and Gram-positive bacteria including *M. tuberculosis*.[42] These drugs block bacterial DNA synthesis by inhibiting two type II topoisomerase enzymes, DNA gyrase and topoisomerase IV, which are responsible for controlling DNA topology.[43,44] Fluoroquinolones are particularly important in combination therapy against MDR-TB because they have a different mode of action from other current TB drugs; this class is already used in the clinical setting as key second-line TB drugs.[45,46]

Moxifloxacin (**8**) was launched by Bayer in Germany as an oral antibacterial in 1999. It was approved to treat acute respiratory and uncomplicated skin and soft-tissue infections and has been used as a second-line TB drug.[47–50] Moxifloxacin is currently under development as a new combination regimen with pretomanid (**13**) and pyrazinamide in the clinical trial Shortening Treatment by Advancing Novel Drugs (STAND),[51,52] carried out by TB Alliance for simplification of the cure and treatment of MDR-TB, and in combination with bedaquiline (**18**) and pretomanid in phase III trial New Combination 5 (NC-005).[50] Additionally, the International Union Against Tuberculosis and Lung Disease is testing a new combination therapy for MDR-TB in a phase III trial, and the Evaluation of a Standard Treatment Regimen of Anti-tuberculosis Drugs for Patients with MDR-TB (STREAM) is testing the combination of moxifloxacin, levofloxacin (**9**) and clofazimine (**20**).[52,53] Furthermore, Médecins Sans Frontières (MSF) is developing a new combination therapy using moxifloxacin, bedaquiline, linezolid (**16**) and pretomanid for MDR/XDR-TB in a phase III trial (TB PRACTECAL).[52] Finally, Partners in Health, MSF, Interactive Research & Development and Unitaid created a partnership named 'expand new drug markets for TB (endTB)' and are developing new combination therapies using moxifloxacin, bedaquiline, linezolid and delamanid (**12**) for MDR/XDR-TB in phase III trials.[52]

Levofloxacin (**9**) was launched by Daiichi Pharmaceutical in Japan as an oral antibacterial in 1998. It was approved to treat acute respiratory and uncomplicated skin and soft-tissue infections and has been used as a second-line TB drug.[50,54,55] Levofloxacin has also been under development in combination with bedaquiline and linezolid as a new regimen for MDR-TB in phase III clinical trials by TB Alliance (STAND), University of Cape Town (program name: NExT) and MSF (TB PRACTECAL).[52]

Nitroimidazoles. Nitroimidazoles are derivatives of azomycin (**10**)[56,57] that was originally isolated as a metabolite of *Streptomyces eurocidicus* in 1953 by Professor Hamao Umezawa *et al.* One of the most successful derivatives is metronidazole (**11**), which is the first drug for an infectious disease developed by the French pharmaceutical company Rhône-Poulenc to have reduced toxicity.[58] Previously, compounds with nitroimidazole structure were used to treat infectious diseases caused by anaerobic bacteria, *Helicobacter pylori* and protozoa.[59–61] However, in recent years, these compounds have been modified as antituberculous drugs that are activated by an *M. tuberculosis* enzyme and inhibit mycolic acid synthesis.[62,63]

Delamanid (**12**) is a nitroimidazole-type compound developed by Otsuka Pharmaceutical in Japan. Its mechanism of action against *M. tuberculosis* is inhibition of methoxymycolic acid and ketomycolic acid biosynthesis, which constitute the cell wall (Figure 3a).[62] In 2014, this drug was approved for the treatment of MDR-TB in Japan and Europe; it became the new antituberculous drug in Japan in 40 years. The antimicrobial spectrum of delamanid is specific for the *M. tuberculosis* complex, and physicochemical properties resemble the structurally related compound pretomanid (**13**) (described below). Although each drug can result in cross-resistance to the other, delamanid well surpasses pretomanid in terms of antituberculous activity. Since delamanid does not induce the expression of host P450 enzymes,[62,64] it is thought to be a promising candidate for combination treatment with anti-HIV drugs against TB/HIV co-infections.[65,66] Delamanid has also been under development in new combination regimens, such as delamanid/bedaquiline/linezolid/levofloxacin/pyrazinamide, delamanid/linezolid/clofazimine/levofloxacin/pyrazinamide and delamanid/clofazimine/moxifloxacin/pyrazinamide, by endTB. Additionally, the safety and efficacy of delamanid is currently being evaluated in pediatric MDR-TB patients in a phase III trial by Otsuka Pharmaceutical.[52]

Pretomanid (**13**) is a novel nitroimidazole-type compound developed by the pharmaceutical company Pathogenesis in the United States. It possesses two mechanisms of action against *M. tuberculosis*: inhibition of mycolic acid lipid biosynthesis (Figure 3a) and inhibition of bacterial protein synthesis. Its antimicrobial activity is specific to *M. tuberculosis* and does not affect general bacteria.[63] This drug shows bactericidal activity against both active and dormant *M. tuberculosis*. Pretomanid does not induce P450 expression.[7,65,66] Pretomanid has proceeded to a phase III clinical trial (STAND) as a three-drug combination regimen with bedaquiline and pyrazinamide. TB Alliance is also developing a new combination therapy with bedaquiline, pretomanid and linezolid for MDR/XDR-TB in a phase III trial and investigational drugs for XDR-TB (TB Alliance program name: Nix-TB trial).[51,52]

Nitazoxanide (**14**), *N*-(nitrothiazolyl)salicylamide, is related to nitroimidazoles and approved for the treatment of infections caused by protozoans such as *Giardia* and *Cryptosporidium* in the United States and in the European Union.[67] Nitazoxanide is a prodrug that is converted into tizoxanide (**15**) by deacetylation.[68] The active metabolite, tizoxanide, presumed to be activated by pyruvate:ferredoxin oxidoreductase, inhibits the electron transport system and disturbs pH homeostasis in the bacterial membrane.[68,69] Nitazoxanide is

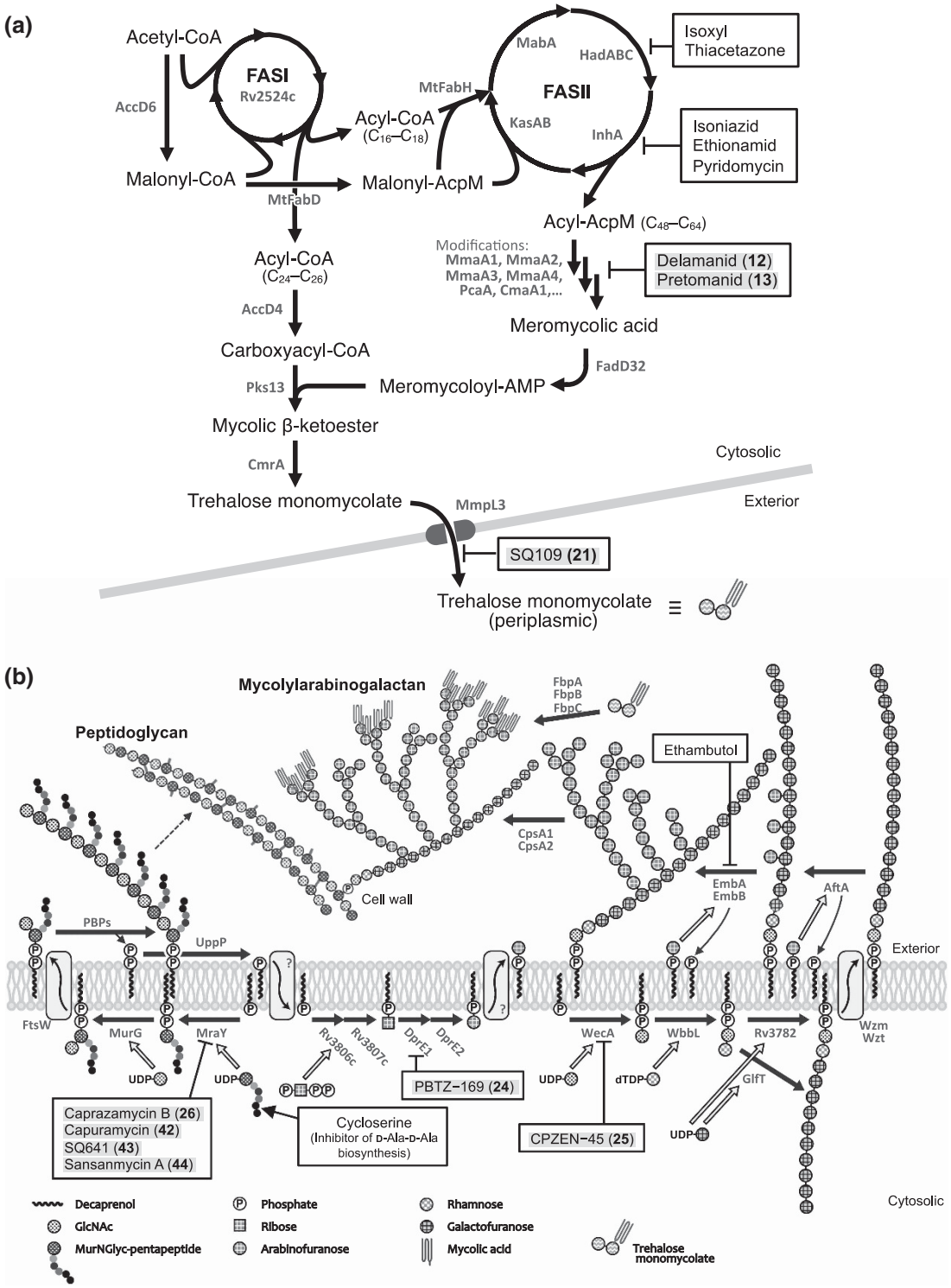

Figure 3 The biosynthetic pathways for mycolic acid. (**a**) and for mycolylarabinogalactan–peptidoglycan complex (**b**). The inhibitors described in the text are highlighted. For details of the genes shown in these figures, see refs 149,150. A full colour version of this figure is available at the *Journal of Antibiotics* journal online.

currently in a phase II trial for drug-susceptible TB by Weill Cornell Medical College in the United States.

Oxazolidinones. Oxazolidinones are an unprecedented class of antibacterials developed in the 1980s. Linezolid (**16**), the first member of the oxazolidinones class, was launched by Pfizer in the United States as an oral antibacterial agent in 2000, which was approved to treat vancomycin-resistant *Enterococcus faecium* infection, nosocomial pneumonia and complicated skin and skin-structure infections.[70–72] Linezolid shows antibacterial activity by binding domain V of 23S rRNA of the 50S ribosomal subunit and inhibiting the subsequent formation of the 70S translation initiation complex.[73,74] Linezolid is

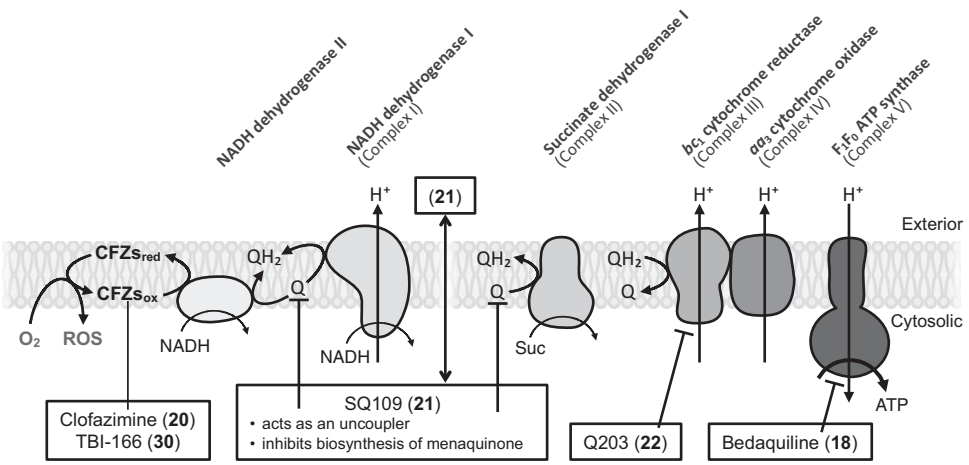

Figure 4 The mycobacterial electron transport chain and antimycobacterial agents that target this pathway. Q, QH_2, Suc, ROX, $CFZs_{red}$ and $CFZs_{ox}$ stand for menaquinone, menaquinol, succinate, reactive oxygen species, reduced and oxidized clofazimine compounds, respectively. For details of these enzymes shown here, see ref [148] . A full colour version of this figure is available at the *Journal of Antibiotics* journal online.

now in phase III trials for XDR-TB (Nix-TB and endTB) and has been under development for combination therapy against MDR-TB (STREAM).

Sutezolid (**17**) is a structural analog of linezolid, in which morpholine is replaced by a thiomorpholine at the C-4′ position.[75] Sutezolid is in a phase II trial by Sequella in the United States for the treatment of both drug-resistant and drug-sensitive TB.[76,77]

The primary challenge with the use of this drug class in TB is bone marrow toxicity associated with long-term use.[78] Thus, a new regimen or compounds that have less or no such toxicity must be developed.

Others. Bedaquiline (**18**), a novel chemically synthesized diarylquinoline compound, was developed by the pharmaceutical company Johnson & Johnson and approved by the US Food and Drug Administration as a treatment for MDR-TB in 2012.[79] Bedaquiline was discovered in a screening campaign for new antituberculous compounds with novel mechanisms of action.[79,80] It shows a strong activity against *M. tuberculosis* including MDR/XDR-TB and non-tuberculous mycobacteria including *Mycobacterium avium* complex and *M. leprae in vitro* and *in vivo*.[81,82] Bedaquiline inhibits bacterial ATP synthase and subsequent energy supply (Figure 4).[80,83] Bedaquiline is oxidized by host cytochrome P450; thus, reduced bedaquiline blood-level concentrations are observed when coadministered with rifampicin, which strongly induces P450.[84] Additionally, bedaquiline has a remarkable synergistic effect in combination therapy with pyrazinamide.[85] Bedaquiline has proceeded to phase II (NC-005) and III trials (Nix-TB, NExT, TB PRACTECAL, STREAM and endTB).[51–53] Additionally, a phase II clinical trial is currently underway at Johns Hopkins University in the United States as a combination therapy with delamanid.

Capreomycin (**19**) is a polypeptide antibiotic that is obtained from *Streptomyces capreolus* and was developed as an antituberculous drug in 1962.[86] Capreomycin binds to the binding domain (A-site) of aminoacyl-tRNA in 16S rRNA of the 30S ribosomal subunit and inhibits protein synthesis.[87,88] Its mechanism of action is quite similar to that of aminoglycoside antibiotics. Capreomycin has been under development by the Lilly TB Drug Discovery Initiative as an inhalant, which increases the drug concentration at the site of infection (that is, lungs), and is expected to have high efficacy and reduced side effects.[16,27]

Clofazimine (**20**) is an iminophenazine dye originally developed as an antituberculous drug by the pharmaceutical company Geigy in Switzerland, but it is now used for leprosy.[89,90] Clofazimine shows bacteriostatic action against *M. leprae*, weak bactericidal action and anti-inflammatory action.[89,90] Clofazimine is a prodrug that is reduced by NADH dehydrogenase II to release reactive oxygen species upon reoxidation by O_2 in *M. tuberculosis* (Figure 4).[91,92] It has been under development as a new TB therapy, called New Combination 3 (NC003), by Novartis.

SQ109 (**21**) was found among synthetic ethambutol analogs and is considered to be a next-generation drug that replaces ethambutol.[93] Its mechanism of action is thought to be the inhibition of cell wall synthesis, and it shows a bactericidal effect against ethambutol-susceptible and ethambutol-resistant *M. tuberculosis* including XDR-TB *in vitro*.[94] Unlike ethambutol, the target of SQ109 was originally identified as MmpL3, a transmembrane transporter for trehalose monomycolate, the carrier of mycolic acid that is a major component of the outer mycomembrane of the cell envelope (Figure 3a).[95] Additionally, it is reported that SQ109 blocks ATP synthesis by acting as an uncoupler and inhibits MenA and MenG, which are required for menaquinone biosynthesis (Figure 4).[96] SQ109 shows a synergistic antibacterial activity with isoniazid and rifampicin and is expected to shorten the treatment period by replacing ethambutol in the standard regimen. A synergistic effect was also observed in combination with bedaquiline *in vitro*.[97] SQ109 has been under development in phase II clinical trials by Sequella.[98]

Q203 (**22**) is an imidazopyridine derivative synthesized by QURIENT in South Korea.[99] It blocks the cytochrome b subunit of the cytochrome bc_1 complex, which is an essential component of the respiratory electron transport chain in *M. tuberculosis* (Figure 4).[99,100] Q203 has been under development in phase I clinical trials in the United States.

PBTZ-169 (**24**) is a piperazine-containing benzothiazinone derived from BZT-043 (**23**).[101,102] PBTZ-169 inhibits decaprenyl-phosphoribose-2′-epimerase (DprE1) involved in biosynthetic pathways for arabinogalactan and lipoarabinomannan, which are essential components found in the cell wall of *M. tuberculosis* (Figure 3b).[102] It is in a phase I clinical trial by the Innovative Medicines for Tuberculosis Foundation in Switzerland.[98]

OPC-167832 is a 3,4-carbostyril derivative synthesized by Otsuka Pharmaceutical and its structure is not disclosed. OPC-167832 is effective against both susceptible and resistant *M. tuberculosis* and has a different mechanism of action from the existing drugs. OPC-167832 has been under development in a phase I clinical trial and is scheduled to be used in combination with delamanid.[98]

Compounds in preclinical development

CPZEN-45 (**25**)[103] is a semisynthetic derivative of liponucleoside antibiotic caprazamycins,[104] the products of *Streptomyces* sp. MK730-62F2. Caprazamycin B (**26**) shows antibacterial activity against Gram-positive bacteria including mycobacteria by blocking translocase I involved in peptidoglycan biosynthesis (Figure 3b, see also below 'Translocase I (MraY) inhibitors'). Caprazamycin B, which has the strongest activity among caprazamycins against mycobacteria, has MICs of 1.56–6.25 μg/ml. CPZEN-45, the most promising derivative of caprazamycins, which has superior antituberculous activity and specifically shows antibacterial activity against slowly growing mycobacteria including *M. tuberculosis*, has MICs of 0.2–3.13 μg/ml.[103] The specificity results from an interesting mode of action: CPZEN-45 inhibits a novel target, phospho-*N*-acetylglucosamine transferase, WecA, involved in arabinogalactan biosynthesis (IC_{50} of 4 ng/ml) (Figure 3b).[105] Because of this novel target, CPZEN-45 does not show cross-resistance to other antituberculous drugs and exhibits antituberculous activity against MDR/XDR-TB. Additionally, CPZEN-45 showed excellent therapeutic efficacy in the treatment of mice infected with an XDR-TB strain resistant to 10 drugs and showed notable synergetic effects with isoniazid and rifampicin in mice infected with a drug-susceptible TB strain.[106] CPZEN-45 is under a preclinical trial carried out with collaboration between the Institute of Microbial Chemistry (BIKAKEN) in Japan and the Lilly TB Drug Discovery Initiative for XDR-TB treatment.

GSK 070 (**27**)[107] is a benzoxaborole derivative that shows a strong antibacterial activity against *M. tuberculosis* including XDR-TB. GSK 070 inhibits leucyl-tRNA synthetase of *M. tuberculosis* by forming a complex with AMP.

Griselimycin (**28**) is a depsipeptide produced by actinomycetes, *Streptomyces griseus*, and *Streptomyces coelicus*.[108–110] This compound was discovered as a particularly effective antimycobacterial antibiotic in 1971 by the pharmaceutical company Rhône-Poulenc in France and is shown to bind to DNA polymerase sliding a clamp.[111] Redevelopment of this compound was recently resumed by Sanofi. In this development, SATB-082 (cyclohexylgriselimycin, **29**) was synthesized as a promising derivative with improved drug kinetics by synthetically replacing proline of griselimycin with 4-cyclohexylproline based on information regarding structural activity relationships.[111]

TBI-166 (**30**) is an analog of clofazimine and has a stronger antibacterial activity than clofazimine against mycobacteria such as drug-susceptible and drug-resistant *M. tuberculosis* including MDR-TB.[112]

Spectinamide 1599 (**31**) is a derivative of spectinomycin (**32**) that was discovered by the US pharmaceutical company Upjohn from the culture broth of a *Streptomyces spectabilis* strain in 1961.[113–116] Spectinomycin is an aminoglycoside antibiotic with an activity against Gram-positive and Gram-negative bacteria; however, this antibacterial activity is weak and thus it is used only for the treatment of urinary gonococcal infections.[117] Spectinomycin has a different mechanism of action from kanamycin and streptomycin; it blocks the attachment of elongation factor G and prevents the translocation of peptidyl-tRNA from the ribosomal A-site, subsequently interfering with bacterial protein synthesis.[118] Spectinamide 1599 shows a strong activity against *M. tuberculosis* with an MIC of 1.6 μg/ml, whereas spectinomycin has an MIC of 50 μg/ml. It was demonstrated that synthetic modifications to the classical antibiotic will overcome the challenges of efflux pump-mediated tolerance.[119]

NATURAL PRODUCTS AS PROBES FOR POTENTIAL NEW TARGETS

Caseinolytic proteases dysregulators/inhibitors

ATP-dependent caseinolytic proteases (Clp) are a protein complex that is found widely among prokaryotes and participate in protein homeostasis by hydrolyzing mistranslated or denatured proteins. A typical Clp consists of two subunits: a tetradecamer of the protease subunit ClpP and a hexamer of one of the ATP-binding subunits (for example, ClpA, ClpC and ClpX), which unfold substrate proteins and translocate them into the ClpP subunit. Mycobacteria have two ClpP paralogs, ClpP1 and ClpP2, and their heptamers are substituted for the ClpP tetradecamer in typical Clp proteases. Although Clp is not essential for most bacterial species, Bayer discovered that an acyldepsipeptide antibiotic, A54556 A (**33**), produced by a *Streptomyces hawaiiensis* strain showed antibacterial activity against *Bacillus subtilis* by binding to the ClpP subunit and subsequently dysregulating protein degradation.[120,121] Another interesting observation is that Clp is essential for *M. tuberculosis in vitro*[122] and *in vivo*,[123] unlike other common bacteria. This indicates that a Clp inhibitor may be a highly specific antituberculous drug. A representative Clp dysregulator/inhibitor is shown in Figure 5a.

Cyclomarin A (**34**), which is a cyclic heptapeptide containing four unusual amino acids, is a product of marine *Streptomyces* sp. CNB-982.[124] This compound shows antibacterial activity against *M. tuberculosis* by causing dysregulation of Clp protease activity (MIC of 0.1 μM).[125] A structural study revealed that cyclomarin A binds to the N-terminal domain of ClpC1, which is highly conserved among mycobacteria.[126] A structurally related compound ilamycin (**35**),[127] isolated from the culture of a *Streptomyces islandicus* strain, also shows specific antimycobacterial activity against *M. tuberculosis* (MIC of 0.4 μg/ml).[128]

Ecumicin (**36**),[129] the product of a strain of rare actinomycete *Nonomuraea* sp. MJM5123, is a highly methylated cyclic depsipeptide composed of 13 amino acids including eight valine residues. It shows antimycobacterial activity (MICs < 0.6 μM) against not only drug-susceptible strains but also against MDR/XDR-TB. Additionally, ecumicin exhibits a bactericidal effect (MBC of 1.5 μM) against nonreplicating cultures. The target of ecumicin was identified as ClpC1 by genome sequencing of an ecumicin-resistant TB strain and was confirmed by enzyme assays in which ecumicin enhanced the ATPase activity of ClpC1 but inhibited the caseinolytic activity of Clp protease. To date, ecumicin is the only Clp inhibitor to have demonstrated an activity in an experimental mouse model.[129]

Lassomycin (**37**)[130] is a 16-membered ribosomal cyclic peptide produced by a strain of a rare actinomycete *Lentzea kentuckyensis*. It shows a specific antimycobacterial activity (MICs of 0.78–3.1 μg/ml) against *M. tuberculosis* including MDR/XDR-TB and bactericidal effects (MBCs of 1–4 μg/ml) against *M. tuberculosis* and *M. avium* subsp. *paratuberculosis*. Lassomycin enhances the ATPase activity of ClpC1 without stimulating the caseinolytic activity of Clp protease.[130]

Translocase I (MraY) inhibitors

Translocase I (phospho-*N*-acetylmuramoyl-pentapeptide transferase, generally called MraY) is an enzyme involved in the biosynthesis of bacterial peptidoglycan encoded by *mraY* (also called *murX* in *M. tuberculosis*). This enzyme catalyzes the translocation of phospho-

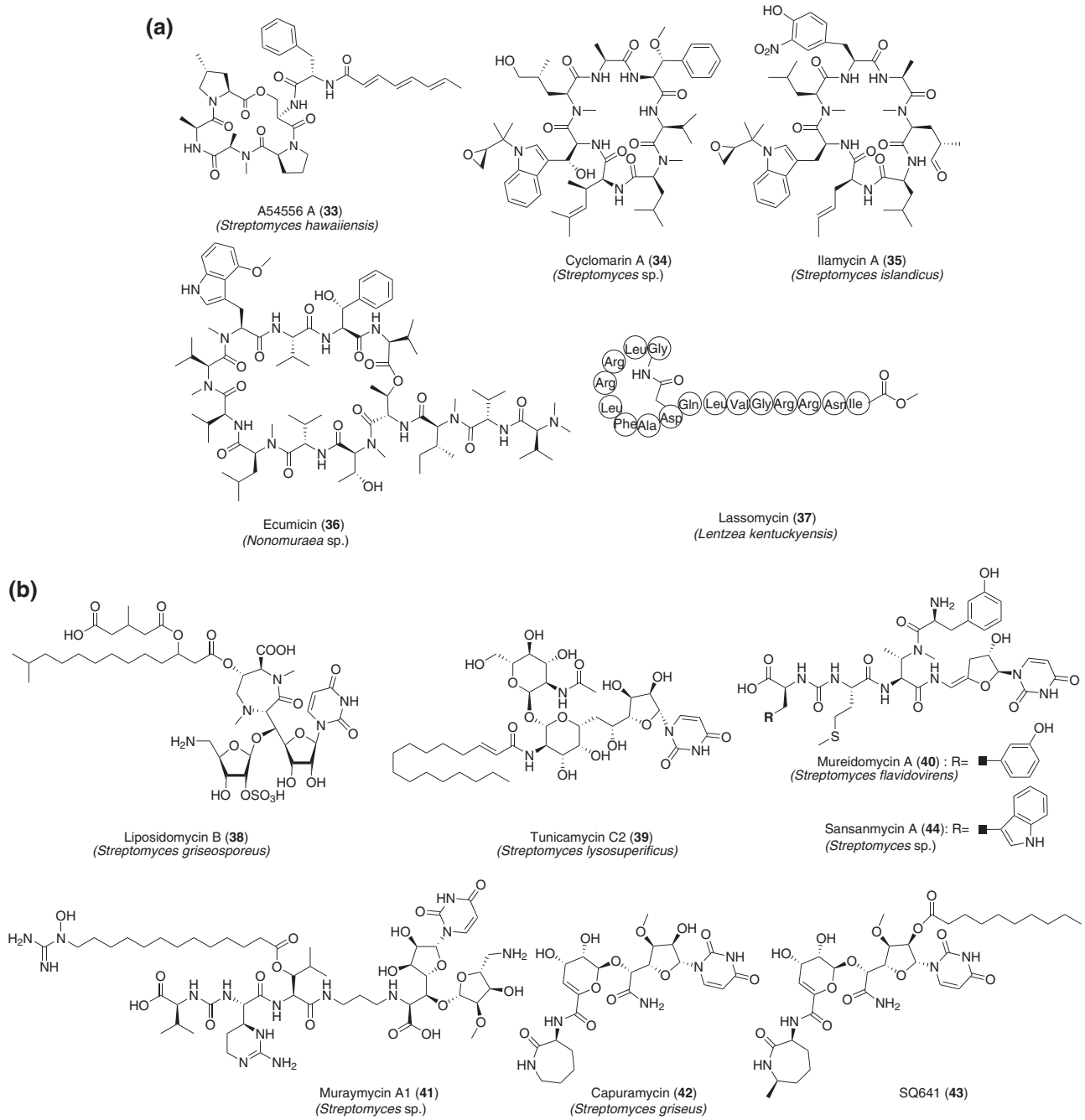

Figure 5 Structures of caseinolytic protease dysregulators/inhibitors. (**a**) and translocase I inhibitors (**b**).

MurNAc from UDP-MurNAc pentapeptide to polyprenyl phosphate. Since the basic structure of peptidoglycan is highly conserved and MraY is commonly found among bacteria, this enzyme is a promising target of antibacterial agents including antituberculous drugs. A representative MraY inhibitor is shown in Figure 5b.

More than a few MraY inhibitors have been isolated from natural sources. Most of them contain uridine (or its derivative) in their structure, such as caprazamycin B described above and the structurally related liponucleoside antibiotic class liposidomycins (for example, liposidomycin B, **38**)[131] isolated from *Streptomyces griseosporeus*. MraY inhibitors also include tunicamycins (for example, tunicamycin C2, **39**),[132] originally isolated from *Streptomyces lysosuperificus*, which contain a unique 11-carbon dialdose sugar; peptidylnucleoside antibiotic mureidomycins (for example, mureidomycin A, **40**),[133] isolated from *Streptomyces flavidovirens* and nucleoside–lipopeptide muraimycins (for example, muraimycin A1, **41**),[134] isolated from *Streptomyces* sp. LL-AA896.[134,135] Among them, selected compounds, as well as caprazamycins, have been focused on as potential targets for antituberculous drugs.

Capuramycin (**42**) was isolated from the culture of *S. griseus* 446-S3 as an antibiotic specifically effective against *Streptococcus pneumoniae* and *Mycobacterium smegmatis*.[136] Capuramycin is also effective against *M. tuberculosis* (MIC of 12.5 μg/ml) and shows an inhibitory effect against MraY of *M. tuberculosis* (IC_{50} of 0.13 μM). One of the

derivatives of capuramycin, SQ641 (**43**),[137] shows a much stronger activity against *M. tuberculosis* (MIC of 0.5 μg/ml). It has a bactericidal activity and kills *M. tuberculosis* cells faster than currently used drugs including rifampicin and isoniazid. Additionally, SQ641 shows a notable synergetic effect with ethambutol *in vitro*.[138]

Sansanmycin A (**44**), isolated from *Streptomyces* sp. SS, contains an unusual 4′,5′-enamide linkage in its structure and is structurally related to mureidomycin A.[139] A derivatization study revealed that one of the derivatives has excellent activity against *M. tuberculosis* with an MIC of 0.037 μM, whereas sansanmycin A, which has the strongest antituberculous activity among parent natural products, has an MIC of 18.5 μM.[140]

New tuberculosis diagnostics

Tuberculin reaction has been used as a diagnostic method for TB infection; however, it has the drawback of being affected by bacillus Calmette–Guérin inoculation and nontuberculous mycobacteriosis. In particular, there is a high possibility of missing an appropriate diagnosis of TB in patients complicated with extrapulmonary TB such as miliary TB.[141]

In recent years, diagnostics for TB have advanced. The interferon-γ release assay is superior to conventional diagnostic methods in terms of rapidity, sensitivity and accuracy.[142] In this assay, interferon-γ released from effector T cells or the number of interferon-γ-releasing cells in response to *M. tuberculosis* antigen stimulation is quantified.[142] This method is particularly effective for diagnosis of latent TB infection (LTBI)[143] and extrapulmonary TB, which are difficult to diagnose conventionally. Because LTBI is a dormant phase of the disease, which is a different metabolic state than the normal state, patients with LTBI do not respond to currently used antituberculous drugs.[143] As described above, a useful diagnostic method for LTBI and extrapulmonary TB has been developed; thus, the development of a new drug effective against these diseases is required.

CONCLUSIONS

Looking back on history, the appearance of key drugs, such as the discovery of streptomycin, the development of rifampicin and reevaluation of pyrazinamide, has been directly linked to improvement in therapeutic outcome.[144] New antituberculous drugs will not only shorten and simplify the treatment duration but will also reduce the interruption/dropout rate of treatment and improve the cure rate. With the advent of multiple new drugs with new mechanisms of action, it will be possible to establish a more effective remedy for MDR/XDR-TB and TB/HIV co-infection. Additionally, these drugs may be more effective against nontuberculous mycobacteriosis such as *M. avium* complex infectious disease, *Mycobacterium abscessus* complex infectious disease and Buruli ulcer disease, for which there is currently no effective drug.[145–148] Future objectives should also include the construction of a legal and/or financial assistant system, while protecting the intellectual property rights/interests of scientists who are endlessly toiling towards the ideal goal, to manufacture these new antituberculous drugs and devices inexpensively and to provide them to developing countries, which are TB-epidemic regions.

DEDICATION

This article is dedicated to Professor Hamao Umezawa in honor of his profound contributions to basic science and the improvement of human health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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