HHS Public Access

Author manuscript

J Med Chem. Author manuscript; available in PMC 2018 November 28.

Published in final edited form as:

J Med Chem. 2008 May 08; 51(9): 2606–2612. doi:10.1021/jm070719i.

Natural Products, Small Molecules, and Genetics in Tuberculosis Drug Development

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Abstract

The impact of natural products on the well being of mankind has been enormous, and their study continues to influence research in the fields of chemistry, biology, and ecology. Historically, the majority of our medicines originate from natural products and their synthetic derivatives, many of which have taught us valuable lessons about biology. While advances in synthetic and combinatorial chemistry have given rise to notable successes in the development of new drugs, the perceived value of natural products has not waned when it comes to treating infectious diseases. In this Miniperspective, we review the role natural products have played in the treatment of tuberculosis (TB[a]), their value and limitations as chemical probes, the challenges associated with TB drug development, and the current status of natural product and synthetic small molecules as new TB drug leads.

Background

This year marks the 125th anniversary of Robert Koch's discovery of the tubercle bacillus *Mycobacterium tuberculosis* (Mtb), the organism that causes TB.[1] Fast forward to 2007 and over one-third of the world population is infected with Mtb, and 10% of those infected will progress to active TB disease during their lifetime. The burden TB places on global health has been known for decades. Prior to the explosive spread of HIV, Mtb held the distinction of being the single most deadly pathogen for the better part of the 20th century. By the time HIV surpassed Mtb for this distinction in the late 1990s, the synergy among these two pathogens was apparent: HIV-infected people exposed to both drug-susceptible and drug-resistant Mtb progress rapidly to active TB disease, and today's estimates place TB as the leading cause of death among HIV infected people.[2,3] So although the number of TB-related deaths has steadied at about 2 million per year, the number of new infections continues to rise, in large part due to the spread of HIV.

TB, like most infectious and tropical diseases, is a disease with close socioeconomic ties. TB can be cured in most cases, but major impediments to stopping the disease remain. These include limited access to diagnosis and treatment in developing countries and drug regimens that are impractically long (6–12 months of multidrug therapy).[4] The latter sets off the vicious cycle of poor compliance that results in the emergence of multiple drug resistant

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(MDR-TB) strains and, more recently, extensively drug resistant (XDR-TB) strains that are virtually untreatable (vide infra).

Tuberculosis Chemotherapy. Past, Present, and Future

Discovery.

Fleming's serendipitous discovery of penicillin,[5] a fungal natural product, marked the beginning of the antibiotic era. For decades to come, the search for new antibiotics was dominated by the discovery of natural products from bacteria and fungi.[6] This trend extended to Mtb where the first antibiotic that inhibited its growth in vitro was the peptide actinomycin, a streptomycete-derived derived natural product.[7] As a potent inhibitor of DNA elongation and replication, actinomycin proved too toxic for treatment of TB in animals and humans but is still used today as a research tool. Discovery of the aminoglycoside streptomycin (**7**) shortly thereafter was a far greater success.[8] In 1943 it was shown to inhibit Mtb in vivo in test animals with little sign of toxicity and within the year was used to treat a critically ill TB patient providing the first example of antitubercular drug therapy.[9]

In the ensuing decade, both natural products and synthetic compounds comprised the half-dozen or so anti-TB drugs to be identified. The discovery of *p*-aminosalicylic acid (PSA, **10**) as an anti-TB agent in 1949[10] was an extension of an earlier finding that Mtb rapidly metabolized the natural product salicylic acid. The first successful clinical trial of an anti-TB agent, namely, isoniazid (isonicotinic acid hydrazide, INH, **2**), was announced in 1952.[11] Interestingly, reports that a related natural product nicotinamide exhibited anti-TB activity spurred rein-vestigation of INH, which was synthesized in 1912, as an antimycobacterial. Similarly the synthetic molecule pyrazinamide (PZA, **3**), an isostere of INH, was synthesized in the 1930s and found to inhibit Mtb in guinea pigs.[12] Ethambutol (EMB, **4**), a synthetic derivative of ethylenediamine discovered at Lederle Laboratories,[13] and the natural product cycloserine (**12**),[14] an inhibitor of cell wall biosynthesis, are yet two more examples of other new classes of anti-TB drugs discovered in the 1950s. Among the most important natural products to be discovered as anti-TB drug leads was the macrolide rifamycin, a polyketide natural product first isolated from the soil bacterium *Amycolatopsis mediterranei*.[15] Rifamycin provided the lead for development of rifampin (**1**, rifampicin, RIF), a semisynthetic derivative used today as a first line anti-TB drug.

Treatment Regimens.

Current short-course TB therapy used to treat drug-susceptible Mtb consists of 2 months' treatment with four so-called first-line drugs (Chart 1, Figure 1) including rifampin (RIF, **1**), isoniazid (INH, **2**), pyrazinamide (PZA, **3**), and ethambutol (EMB, **4**), followed by 4 months' treatment with RIF and INH (Figures 1, **2**).[4] Infection by MDR-TB strains requires treatment with second-line drugs such as kanamycin (**5**), amikacin (**6**), capreomycin (**8**), *p*-aminosalicylate (PAS, **10**), fluoroquinolones (e.g., levofloxacin, **9**), ethionamide (**11**), and cycloserine (**12**) where treatments often extend for as long 2 years.

Challenges to Eradicating Tuberculosis in the Future.

TB is a complex disease, and factors such as latency and drug resistance make treatment and cures particularly challenging. Mtb has the ability to remain dormant or metabolically silent within host lesions for years to decades.[16] This condition can foil attempts at eradication for several reasons: our understanding of the replicative and metabolic state of latent Mtb is limited, making us naive about which strategies to take. Harboring of the bacillus in granulomas or lesions during dormancy leaves the bacteria largely inaccessible to chemotherapeutics, and because many of the cellular processes that antibiotics typically inhibit are not active during dormancy, most of the approved anti-TB drugs are ineffective toward latent TB or persistent infections.[16,17] Of the currently approved antituberculars, only RIF and PZA show activity toward these so-called persisters.[18]

With the recent jolt caused by a South African TB outbreak where 52 of 53 villagers succumbed to XDR-TB within 16 days of diagnosis,[19] an arguably more timely problem is the growing incidence of infections by these drug resistant strains. To put things in perspective, the World Health Organization defines MDR-TB as resistance to the two most effective first-line drugs, RIF and INH, and defines XDR-TB as resistance to first-line drugs RIF and INH together with resistance to second-line drugs of the fluoroquinolone class and at least one of the injectable drugs capreomycin, kanamycin or amikacin.[4] Given the number of drugs available to treat TB (Chart 1, Figure 1), it comes as no surprise that XDR-TB is virtually untreatable.

Toward New Therapies. A Mixed Bag

The incidence of MDR- and XDR-TB demands renewed efforts in the development of new classes of anti-TB drugs. Once antimycobacterial drug regimens were established and proven to be effective, the resulting decline in the incidence of TB (at least in industrialized nations) all but halted development of new antituberculars by the pharmaceutical industry; it has been over 40 years since the last new TB drug was developed.[20] Our current understanding of TB and the relatively limited TB drug pipeline will call for an interdisciplinary approach to developing new therapies. In addition to the standard goal of drug discovery this should include studies to identify new biological targets as well as probes to study TB biology. Recent advances in these areas are described next.

Identification of New Targets.

Part of the challenge of identifying new classes of anti-TB agents lies in the paucity of new targets that have been validated. The genome of Mtb H37Rv was sequenced in 1998[21] and reannotated in 2002,[22] providing the sequences of all and the functions of most Mtb genes. However, with that information comes the challenge of identifying which genes will be suitable targets for new antibiotics. As with any drug discovery effort, characteristics of Mtb genes that would make good targets include essentiality, susceptibility to inhibition, and lack of human homologues.[23] Whether the target is druggable and whether it is amenable to scaled up production to facilitate screening and structural efforts are also important factors.

One of the highest impact studies aimed at identifying genes essential to growth of Mtb, or the so-called “survivasome”, was carried out by Sassetti et al.[24] Building upon random (or transposon) mutagenesis techniques that produce large pools of mutants in a single experiment, transposon site hybridization (TraSH) goes a step further by mapping DNA adjacent to transposon insertions using whole genome microarrays. Because only viable mutants are detected by the microarray, absence of hybridization reveals genes that are considered to be essential. By use of this method, approximately 600 genes were found to be essential for growth under standard aerobic conditions. These included genes involved in biosynthetic and metabolic pathways such as the synthesis of amino acids, nucleic acids, and cofactors as well as genes that play roles in fundamental cellular processes including replication, transcription, protein synthesis, and cell division. TraSH is now being used to investigate growth of Mtb in macrophages,[25] studies that should help to prioritize these essential genes as possible drug targets.

The biosynthetic enzymes responsible for synthesis of the small molecular weight thiol mycothiol have been implicated as possible therapeutic targets.[26] Using a reverse genetics or targeted gene knockout approach,[27,28] Buchmeier, Fahey, and co-workers have targeted individually each of the four mycothiol biosynthesis genes to demonstrate that MshA and MshC are essential for growth of Mtb Erdman.[29,30] Complementation of these genes restored growth as well as mycothiol biosynthesis. In addition to establishing the essentiality of these enzymes, those studies showed mycothiol itself to be essential for growth of Mtb. These enzymes also were among proteins identified by Sassetti et al.[24]

Conditional mutagenesis is another approach being used to categorize essential versus nonessential Mtb genes. A recent study illustrating the value of this technique involved the β-ketoacyl-acyl carrier protein synthases, KasA and KasB, key enzymes of mycolic acid biosynthesis. Using this approach, the Jacobs group was able to show that depletion mutants of KasB but not KasA could be generated in *M. smegmatis*.[31] Further, KasA depletion resulted in cell lysis, suggesting KasA and more generally cell wall biosynthesis to be targets for new antimy-cobacterials.

The identification of Mtb genes that are associated with specific growth conditions (e.g., oxygen depletion), environmental pressures, and disease states is a priority in TB research.[17,23,32] Access to complete and partial sequences of the genomes of Mtb and other mycobacteria permits construction of whole genome microarrays that can be used to construct gene expression profiles on a genome-wide scale. These types of comparative analyses have proven especially useful in identifying genes that are nonessential for growth of Mtb under normal conditions but are required during other growth conditions or environments, such as those associated with latency.[33–36] For example, transcriptional analyses of Mtb in oxygen depletion conditions showed that genes encoding for glycine dehydrogenase, nitrate reductase, and α crystalline, as well as proteins associated with fatty acid metabolism and genes of the dormancy regulon (DosR), were up-regulated in this nonreplicating state.[33]

In a separate study using transcriptional profiling and microarrays, Boshoff et al. characterized the gene expression profiles of Mtb when subjected to 75 different growth and

environmental conditions.[37] To name just a few, these included starvation, growth on alternative carbon sources, treatment with individual anti-TB antibiotics with multiple examples of each drug class (such as inhibitors of transcription and cell wall biosynthesis), low pH, and impaired iron uptake. Unbiased grouping of these profiles not only clustered profiles generated from drugs with the same known mechanisms of action, for example, but could be used to accurately predict mechanism of action for unknown drugs. Moreover, a signature subset of genes could be used to classify all known agents by mechanism of action, making this technique a potentially powerful method for drug discovery.

In addition to transcriptional profiling, proteomic analyses have been used to identify proteins that are differentially expressed in low oxygen models that simulate nonreplication persistence (NRP). In one study, expression of proteins involved in small molecule metabolism increased in NRP-1 (microaerophilic conditions) while expression of proteins involved in energy metabolism increased in NRP-2 (anaerobic conditions). This result suggests that building blocks are generated in NRP-1 and later used for energy metabolism to maintain viability under NRP-2. Proteins involved in adaptation and maintenance of these stages may represent a new area for targeting latent Mtb.[38]

Structural Genomics and Bioinformatics.

TB structural genomics efforts in the U.S. and Europe have been established with the aim of solving structures of Mtb proteins that are of interest to the TB research community. Coordinates for over 165 unique Mtb proteins have been deposited to the Protein Data Bank (corresponding to more than 250 PDB entries), most of which were deposited by TB consortium members. Continued deposition of new structures of TB proteins is expected to facilitate in silico screening and molecular docking studies aimed at identifying inhibitors of a given target protein. Successful applications of this approach include identification of inhibitors of AccD5 and inhA.[39–44] In silico screening of the National Cancer Institute's compound library and the University of California, Irvine's ChemDB against AccD5, an essential acyl-CoA carboxylase carboxyltransferase domain involved in lipid biosynthesis, resulted in identification of one inhibitor with a K_i of 13 μM.[39] Using structure based drug design for development of inibitors of InhA, the enoyl reductase enzyme in the Mtb fatty acid biosynthesis pathway, Sullivan et al. developed a series of alkyl diphenyl ethers that are noncompetitive inhibitors of InhA, the most potent of which (8PP) has a K_i value of 1 nM. Unlike INH, 8PP does not require activation by the mycobacterial KatG enzyme and may circumvent the known mechanisms of resistance to INH.[41]

Comparative genomics, a bioinformatics approach wherein whole genome comparisons are made within and between species, is another method that is likely to find use in TB drug development. By use of this approach, genes that have over time been concomitantly inherited or lost among species can be identified.[32] The complete genome sequences of several pathogenic as well as nondeleterious mycobacterium species make such comparisons possible for Mtb.[45–47] Because genes that are conserved among species are likely to be essential, this method has the potential to reveal drug targets in an independent manner. Comparison of the genomes of laboratory and clinical strains of Mtb with genomes of *M. leprae*[45] and other mycobacteria species uncovered a core of 219 genes that are conserved

among these species.[47] Genomic information may further be used to identify virulence functions,[48] to cluster genes participating in the same or related pathways, and to validate surrogate models or organisms.[32]

Target Prioritization.

An ideal new TB drug would shorten current treatment regimens (which in turn could facilitate improved patient compliance and reduce the development of acquired drug resistance), would be active against latent TB and drug-resistant strains, and would be compatible with currently prescribed antimycobacterial and antiretroviral drugs. Target prioritization should take into consideration the importance of each of these factors as well as those that might increase the probability of a compound to advance in the drug discovery process. Toward this end, Hasan et al. of the Novartis Institute for Tropical Diseases developed the program AssessDrugTarget to integrate published data on potential TB drug targets with factors such as essentiality, druggability of its protein domains, epidemiology, metabolic signatures unique to Mtb, and sequence and structural similarities to other mycobacteria and human proteins.[49] Three sets of targets based on different prioritization criteria were thus generated where targets included genes associated with critical metabolic pathways, genes specific to Mtb, and genes associated with persistence. As an example, high ranking genes associated with persistence included the iron dependent repressor and activator *IdeR*, isocitrate lyase *icl*, the transcriptional regulatory protein *devR*, the sensor histidine kinase *devS*, and a cytochrome P450 *cyp51* that is involved in sterol biosynthesis. Similarly, by analyzing combined data sets comprising mutagenesis results and gene expression profiles for Mtb grown under model dormancy conditions, Murphy and Brown of GlaxoSmithKline pinpointed genomic patterns that included consistent up- or down-regulation of specific sets of genes.[50] Examples included regulatory elements *devR/devS, relA*, and *mprAVB*; enzymes involved in redox balance, sulfur transport, and respiration; and enzymes involved in synthesis of panthothenate, isoprene, and NAD; any of which would represent new drug targets. Balganesh and Furr of AstraZeneca made use of an evaluation matrix to prioritize targets in terms of factors such as the level of confidence of their essentiality and their vulnerability relative to designated risk factors.[51] If a target of interest is essential for Mtb and its essentiality is demonstrated through a specific inhibitor (for example, rifampicin), there is little risk in initiating an HTS and lead generation program. On the contrary, if a target is essential in some bacteria and yet has multiple homologues in other mycobacteria or its essentiality is unknown, the risk of initiating a drug discovery program around it is very high and should be avoided. These approaches offer starting points for the selection and prioritization of new targets.

Small Molecules and Natural Products as Chemical Probes and Drug Leads.

In addition to their long standing value as drugs and therapeutics, natural and synthetic products have found great utility as chemical probes for identifying protein targets, unraveling mechanisms of action, and studying biological systems on a global scale. It is worth mentioning, however, that thus far the newer approaches (e.g., chemical genetics) have been put to use far more in studies of eukaryotic than prokaryotic systems. This bias can be attributed to the availability of robust bacterial genetic tools (vide supra), the relative ease with which prokaryotic genomes can be altered (in comparison to human and other

mammalian cell types), and the rapid dividing time of most bacteria which greatly facilitates their study. That is not to say that genetic approaches are not without their own limitations. Several of the examples described below reemphasize the value of small molecules in the discovery process.

Besides target identification and validation, recent efforts have focused on discovery of new classes of antibiotics and improving the pharmacologic properties of those already in use to shorten treatment duration and expand efficacy to MDR-TB and XDR-TB. A variety of approaches have been taken in attempts to identify new drug classes; the successful outcomes of some of these studies have in turn provided new chemical probes for further study of Mtb.

Two related nitroimidazoles **18** (PA-824)[52] and **19** (OPC-67863)[53] are among the most promising of the current anti-TB leads. Both compounds have been shown to be prodrugs requiring activation by the same F420-dependent enzyme (Rv3547)[53,54] and to inhibit the growth of Mtb and MDR-TB by inhibiting mycolic acid biosynthesis and protein synthesis. The active species and ultimate targets of each remain unknown. Two findings pertaining to these inhibitors are especially significant. First, **18** was shown in animal models to be active against nondividing bacilli living under microaerophilic conditions, a model that approximates the dormant state of Mtb. Second, the combination of **18** or **19** with RIF and PZA resulted in reduced eradication times (by at least 2 months) of viable TB bacilli in the lung in comparison to the standard regimen of RIF, INH, EB, and PZA. Both **18** and **19** are currently being evaluated in phase II clinical trials by the Global Alliance for TB Drug Development and Otsuka Pharmaceutical, respectively (Chart 2).

The discovery of diarylquinoline **20** (TMC207)[55] made headlines recently not only because it is a potent inhibitor of Mtb, *M. smegmatis*, and MDR-TB but because its discovery revealed an unexpected target, namely, ATP synthase, that appears to be essential to mycobacteria.[55] Compound **20**, identified from a library of diarylquinolines using a whole cell screen on *M. smegmatis*, appears to be more potent than both INH and RIF and shows no cross resistance with other antimycobacterials. In mouse studies, combinations of **20** with any two of the drugs INH, RIF and PZA was more effective than the standard combination of INH, RIF, and PZA suggesting that substitution of one of them by **20** has the potential to shorten current TB therapies. Diarylquinoline **20** is unusual in that it shows activity against fast and slow growing mycobacteria (*M. smegmatis* vs Mtb) and is active whether administered to Mtb infected mice in early or late stages. Because ATP synthase is not unique to mycobacteria and is indeed present in host cell mitochondria, it is puzzling that the compound shows no overt toxicity. Compound **20** is currently in phase II development.[56,57]

Compound **17** (LL-3858 or Sudoterb)[58] represents a new class of anti-TB compound being developed by Lupin Limited (India). Related to pyrrole alkaloid natural products frequently isolated from plants of the genus *Lupinus*, **17** was reported to be active against both sensitive and drug-resistant Mtb, suggesting a new mechanism of action. In mice studies, **17** showed similar activity as INH and in combination with INH, RIF, and PZA was effective in eradicating sensitive and resistant Mtb within two months. Compound **17** is now in multidose phase I clinical development.[59]

There are several examples of derivatives of currently used antibiotics that are showing varying degrees of promise. Gatifloxacin (GAT, **21**) and moxifloxacin (MXF, **22**) are new fluoroquinolone DNA gyrase inhibitors that offer advantages over currently used second line fluoroquinolines ofloxacin and ciprofloxacin. Compound **22**, which displays anti-TB activity comparable to INH in a mouse model, has been shown to kill rifampin-resistant Mtb populations and when administered with INH, RIF, and PZA, to be more effective at killing Mtb than the 3-drug treatment on its own.[60] Fluoroquinolines **21** and **22**, currently in phase III, are the most advanced anti-TB compounds in clinical development showing promise to be the first new anti-TB drugs in nearly 30 years. Rifapentin, rifabutin, rifalazil, and rifametane, all semisynthetic derivatives of the natural product rifamycin, are in various phases of clinical trials showing enhanced activity toward Mtb and improved pharmacokinetic properties.[61,62]

Derivatives of ethambutol include **16** (SQ109),[63] reported to act synergistically with INH and RIF and to exhibit improved pharmacokinetic profiles. Although **16** is a second generation EMB derivative, it does not appear to inhibit cell wall biosynthesis as does its parent compound. Compound **16** is currently in phase I evaluation.[56]

New classes of anti-TB compounds currently in preclinical testing include derivatives of the natural product capuramycin, oxazolidinones and β-sulfonylcarboxamides. Capuramycins inhibit translocase I, an enzyme involved in the biosynthesis of peptidoglycan, a key component of the cell wall. The most active capuramycin derivative identified to date is **13** (RS-118641).[64] Linezolid[65] (**14**) is a synthetic oxazolidinone that acts by inhibiting protein synthesis. The compound was approved by the FDA and has been used on occasion in patients with MDRTB.[63] Several oxazolidinones derivatives are currently under development.[58] β-Sulfonylcarboxamide **15** (FAS20013),[66] shown to be active against MDR- and latent TB, was designed to be a transition state mimic for β-ketoacyl synthase, the condensing enzyme required for fatty acid biosynthesis. The synthesis was inspired by the activities of the natural products cerulenin and thiolactomycin which inhibit the two-carbon homologation catalyzed by β-ketoacyl synthase. Cerulenin irreversibly inhibits the enzyme itself, while thiolactomycin inhibits the β-ketoacyl carrier protein (ACP) synthase and acetylcoenzyme A/ACP transacylase.[66]

As reviewed recently by Copp and Pearce, eight natural products that may represent useful scaffolds for future development as antituberculars have been identified by screening natural products extracts for antimycobacterial activity.[67] Two of these natural products, pleuromutilin (**23**) and erythromycin (**24**) (Chart 3), are currently under development by the TB Global Alliance.

Perspective

Great strides have been made in the area of Mtb genomics, proteomics, and target identification through recent advances in technology. Infusions of funding[68] and newly made commitments on the parts of a number of industrial, government, and academic organizations have reopened the TB drug pipeline, and an estimated 30 antibiotics are now in various stages of preclinical or clinical development. However, the fact remains that Mtb

is an extremely difficult organism to study. Only very recently have the vast efforts devoted to adapting genetics and various screening technologies to the study of Mtb begun to pay off, starting with the identification of approximately 600 essential genes. With no shortage of potential targets from which to choose, the time is ripe for adopting more integrated approaches for TB drug development. Priorities should continue to include development of robust and transportable models that will facilitate reliable target validation; further identification of processes governing latency and dormancy; and initiation of productive partnerships between chemists, TB biologists, and computational and structural biologists. Ideally, investments in screening each new validated target utilizing chemically diverse libraries containing both natural products and synthetic small molecules will augment the already growing TB drug pipeline and will in turn provide ample opportunities for medicinal chemistry, the value of which is reflected by fluoroquinolone analogues **21** and **22** that have advanced to phase III clinical trials. In the meantime, synthetic and medicinal chemistry efforts devoted to the well publicized and immediately available targets reviewed here will undoubtedly be of great value.

Acknowledgment.

We thank the Intramural AIDS Targetted Antiviral Program, Office of the Director, NIH (C.A.B.), and the Intramural Research Program (NIDDK) of the National Institutes of Health for financial support.

Biographies

Maria-Teresa Gutierrez-Lugo received her Ph.D. in Natural Products Chemistry from Universidad Nacional Autónoma de México in 2001 under the direction of Dr. Rachel Mata. She then moved to the University of Arizona (UA) as a Postdoctoral Research Associate on the International Cooperative Biodiversity Group (ICBG) Project “Bioactive Agents from Dryland Biodiversity of Latin America” at the Center for Phytomedicine Research and the Southwest Center for Natural Products Research and Commercialization, UA. Dr. Gutierrez-Lugo is currently a Research Fellow at the National Institute of Diabetes and Digestive and Kidney Diseases, NIH. Her research interests include natural products chemistry, structural biology, and biochemistry of *Myco-bacterium tuberculosis* enzymes that have potential as new drug targets.

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aAbbreviations:

Mtb	Mycobacterium tuberculosis
TB	tuberculosis

HIV	human immunodeficiency virus
MDR	multiple drug resistant
XDR	extensively drug resistant
PAS	*p*-aminosalicylic acid
INH	isoniazid
PZA	pyrazinamide
EMB	ethambutol
RIF	rifampin
TraSH	transposon site hybridization
DosR	dormancy regulon
NRP	nonreplication persistence
PDB	Protein Data Bank
NAD	nicotinamide adenine dinucleotide
HTS	high throughput screening
MXF	moxifloxacin
GAT	gatifloxacin
FDA	Food and Drug Administration
ACP	acyl carrier protein

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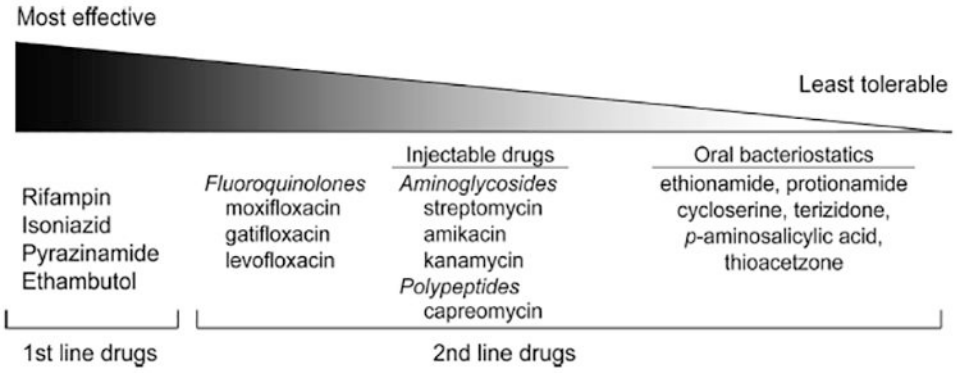


Figure 1.

Currently prescribed antituberculars. First-line drugs are listed at left, and various classes of second-line drugs in descending order of tolerability and potency appear at right.

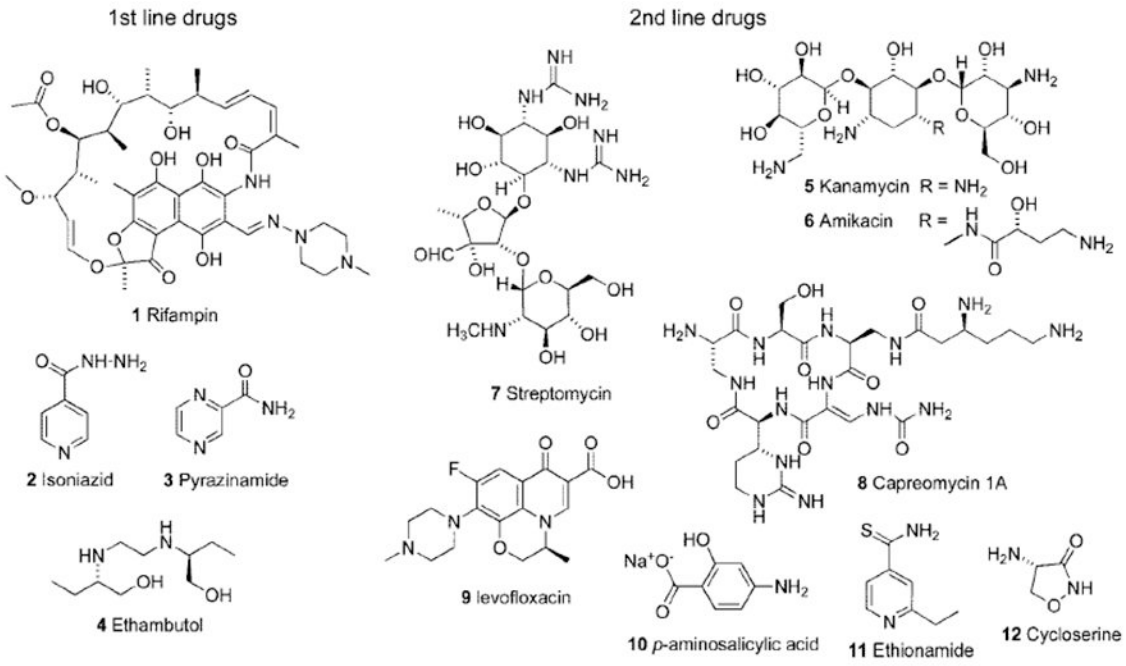


Chart 1.
Structures of Currently Used First-Line and Second-Line Drugs for Treating TB

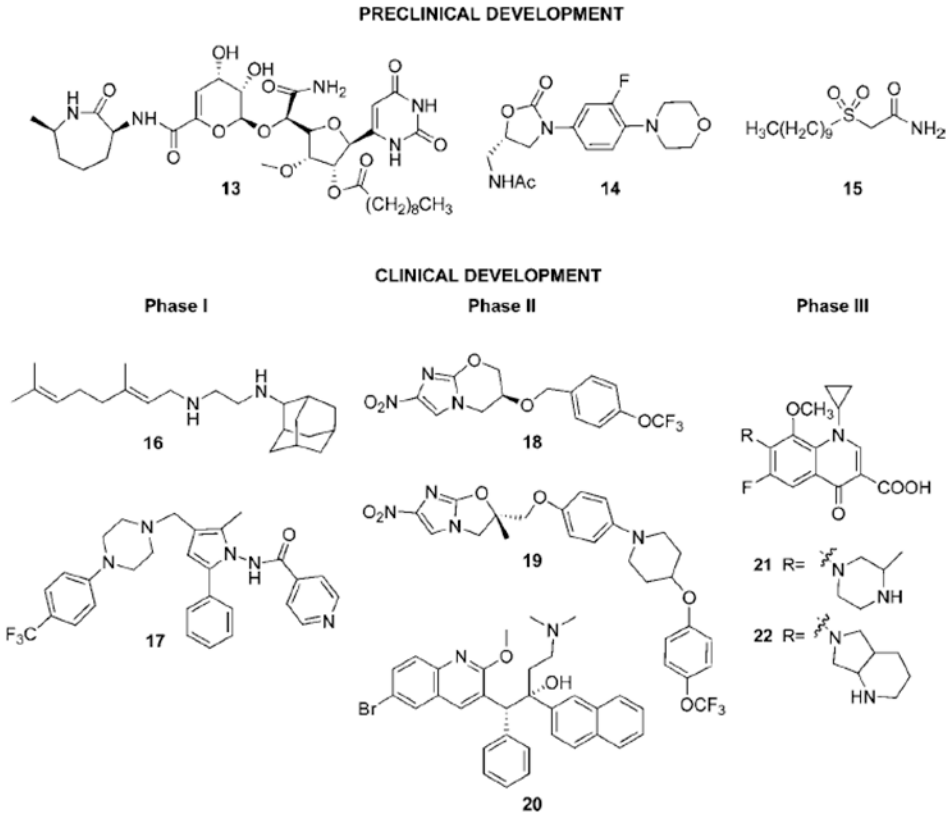
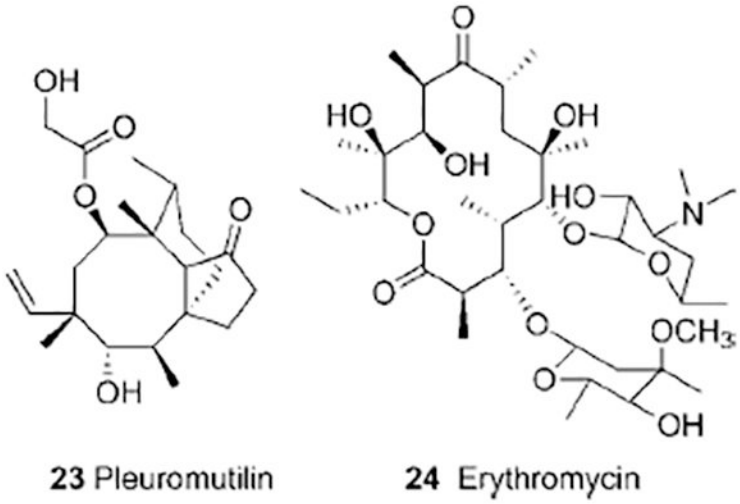


Chart 2.
Structures of Promising Lead Compounds in Various Stages of Development



23 Pleuromutilin **24** Erythromycin

Chart 3.
Structures of Natural Products Currently under Development