International Journal of Pharma and Bio Sciences

REVIEW ON CURRENT TRENDS AND ADVANCEMENT IN DRUGS TRENDS AND DRUG TARGETS FOR TUBERCULOSIS THERAPY.

*SUNNY JALHAN, ANIL JINDAL, SHIKHA AGGARWAL, AVNEET GUPTA AND HEMRAJ.

Department of pharmacy, SOLAN-173223, HIMACHAL PRADESH, INDIA

ABSTRACT

Tuberculosis is today amongst the worldwide health threats. As resistant strains of Mycobacterium tuberculosis have slowly emerged, the failure of treatment is too often a fact, especially in countries lacking the necessary health care organization to provide the long and costly treatment have adapted by the patients. Because of lack of treatment or lack of adapted treatment, at least two million people are going to die this year. Due to this concern, this infectious disease was the focus of renewed scientific interest in the last decade. Regimens were optimized and much was learnt on the mechanisms of action of the antituberculosis drugs used. Despite advances in chemotherapy and the BCG (Bacillus Calmette–Guérin) vaccine, tuberculosis remains significant infectious disease. Although it can be cured the therapy takes at least 6–9 months and the laborious and lengthy treatment brings with it dangers of noncompliance, significant toxicity and drug resistance. The increasing emergence of drug resistance and the problem of mycobacterium persistence highlight the need to develop novel TB drugs that are active against drug resistant bacteria but, more importantly, kill persistent bacteria and shorten the length of treatment. Recent new and exciting developments in tuberculosis drug discovery show good promise of a possible revolution in the chemotherapy of tuberculosis.

KEY WORDS: Antituberculor drugs, Chemotherapy, Mycobacterium, Clinical trials, Drug resistance.

SUNNY JALHAN
Department of pharmacy, SOLAN-173223, HIMACHAL PRADESH, INDIA

Corresponding author

1. INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is a tenacious and remarkably successful pathogen that has latently infected a third of the world population. Each year there are eight millions of new TB cases and two million deaths. The increasing emergence of drug resistant TB, and HIV infection, which compromises host defense and allows latent infection to reactivate or render individuals more susceptible to TB, pose further challenges for effective control of the disease [1,2]. In the last 10 years, the research on M. tuberculosis, the causative agent of tuberculosis, has undergone much progress. Regimens were optimized along with the implementation of the directly observed therapy short course (DOTS) initiative. At the laboratory level, the genome of M. tuberculosis was unraveled [3] and much work provided insights into the mechanisms of action of the antituberculosis drugs currently used. Renewed screenings for antimycobacterial substances were made leading to many original growth inhibitors [4, 5]. TB can be cured with chemotherapy; the treatment is exceedingly lengthy and takes 6–9 months [6]. Apart from significant toxicity, the lengthy therapy also creates poor patient compliance, which is a frequent cause for selection of drug resistant and often deadly multidrug resistant TB (MDR-TB) bacteria. Currently, TB chemotherapy is made up of a cocktail of first-line drugs, isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB), given for six months. If the treatment fails as a result of bacterial drug resistance, or intolerance to one or more drugs, second-line drugs are used, such as para-aminosalicylate (PAS), kanamycin, fluoroquinolones, capreomycin, ethionamide and cycloserine, that are generally either less effective or more toxic with serious side effects. Treatment is made quite difficult by the presence of metabolically silent, persistent or dormant bacteria within host lesions, which are not susceptible to antimycobacterial drugs that usually kill growing bacteria but not persistent bacteria [7]. During the interaction between mycobacteria and host cells, a cyclic reinfection of host macrophages by tubercle bacillus can occur, allowing for the prolonged survival and persistence of the bacilli [8] .Thus, it is almost impossible to achieve complete sterilization of lesions. It is this unique ability of the bacilli to withstand chemotherapeutic and host immune attack and to survive for decades before reactivation that makes tuberculosis so difficult to treat and eradicate. Due to the heterogeneous bacterial populations in the tuberculosis lesions and perhaps also to insufficient host immunity, treatment with a combination of drugs must be given for extended periods of time to prevent reactivation of disease by persisting bacilli. Much research effort focuses on understanding the biology of persistence and developing therapies that kill persistent bacilli more efficiently.
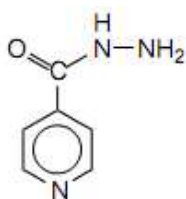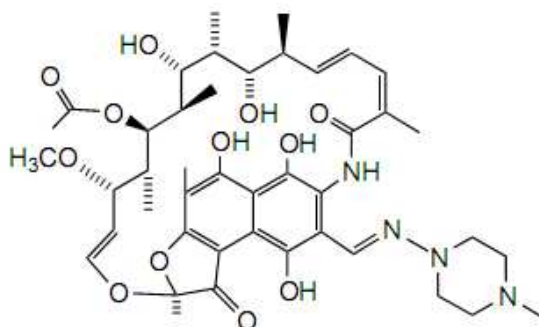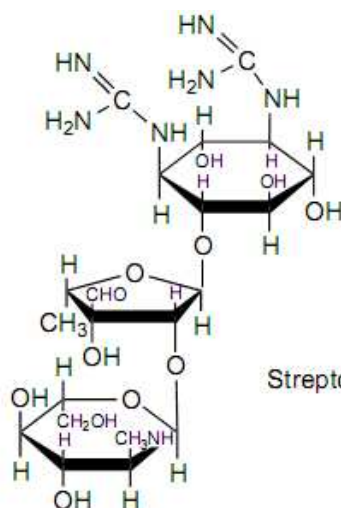
Isoniazid
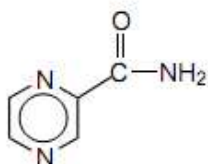
Pyrazinamide
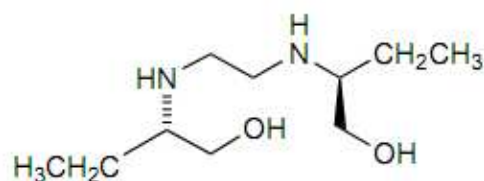
Ethambutol

Rifampin

Streptomycin

2. Current anti-tuberculosis regimen and compounds under clinical trials.

2.1 Antituberculosis drugs

As described recently [9], the chemotherapy of tuberculosis has much evolved along the years since it started with the introduction of streptomycin 1946. By 1955, the combination of streptomycin, p-aminosalicylic acid and isoniazid was adopted as a standard treatment by the western world. The minutes of a congress held in 1968 illustrate quite well what the clinical research concerns were at that time [10]. It will never be emphasized enough that the full observance of the treatment is probably at least as important as the level of effciency of the drugs administered for a proper cure. Even worse, the lack of treatment observance is likely to become the main cause of the occurrence and spread of multi drug resistant strains of M. tuberculosis. Two regimens emerged from the many trials [11] and involve first a two-month long treatment with four drugs; either: streptomycin, isoniazid, rifampin and pyrazinamide or: isoniazid, rifampin, pyrazinamide and ethambutol. This is then followed by four months of isoniazid and rifampin. Side effects, especially hepatotoxicity, are an issue which in some cases forces an untimely treatment termination [12]. Patients following this regimen become non-infectious after the first few weeks but the remaining months are crucial to eradicate a slow growing fraction of the bacilli and also to allow time for the host immune system action to achieve a clinical cure.

p-Aminosalicylic acid (2)

Isoniazid (3)

Streptomycin (1)

Pyrazinamide (5)

R =

Rifampin (4)

Ethambutol (6)

R =
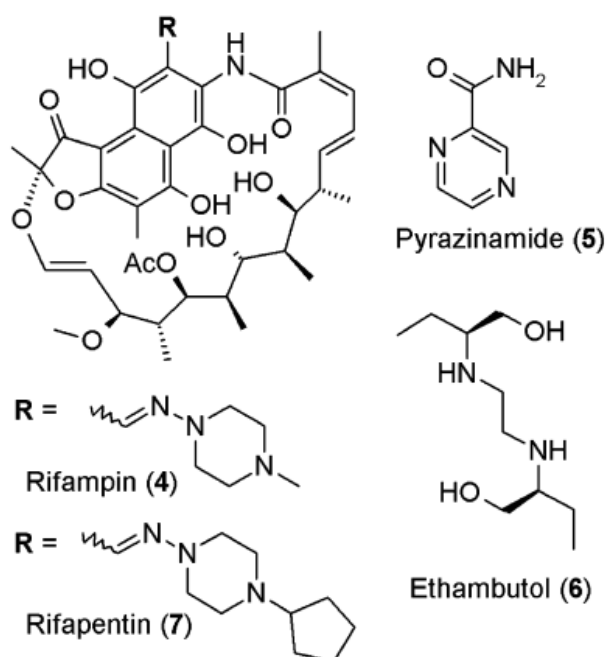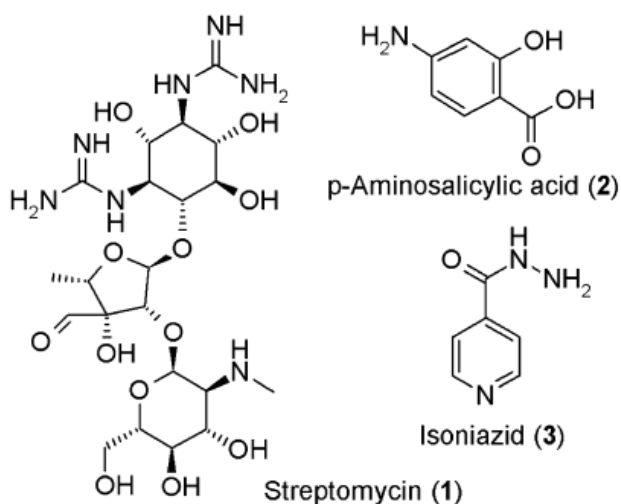
Rifapentin (7)

The 14th edition of the Merck index lists 30 different tuberculostatics. Many of them are analogues or pro drugs of the first line drugs mentioned above and others fell out of use. Among strifamycin derivatives, rifapentin [13] is note worthy as it is currently investigated as an alternative to rifampin with a longer serum half life which would simplify treatments especially when following protocols requiring the direct observation of patient compliance [14]. However, the occurrence of multidrug resistant (MDR) strains of M. tuberculosis, at least resistant to the action of isoniazid and rifampin, implies

recourse to other compounds. In year 2000, an estimated 3.2% of all the tuberculosis new cases (between 185,000 and 414,000) were multiresistant [15]. Treatment guidelines for these cases have much less easily been established and are complicated by drug side effects and co-infections with HIV-1 [16]. The clinical isolates cross resistance monitoring provide insights in designing regimen strategies [17]. A recent survey of cohort studies, or more recent clinical data, [18] has led to some recommendations although, as mentioned by these authors, a full comparison of all the clinical data is not

possible. This survey recommends the use of a regimen of at least five adequate antituberculosis drugs. The choice of these drugs will be driven by the actual or presumed (in view of past failed treatment) resistance characteristic of the strains of M.tuberculosis considered. In order of preference they can be chosen from the following.

(i) In any case, the first line agents still active on the patient: isoniazid, rifampin, pyrazinamide and ethambutol.

(ii) This is followed by the group of injectable drugs: streptomycin, kanamycin, amikacin, capreomycin or viomycin/tuberactinomycin B and the related tuberactinomycins A, N and O.

R = H: Kanamycin (8)
R = COCH(OH)CH$_2$CH$_2$NH$_2$: Amikacin (9)

Capreomycin (10)

Viomycin (11)
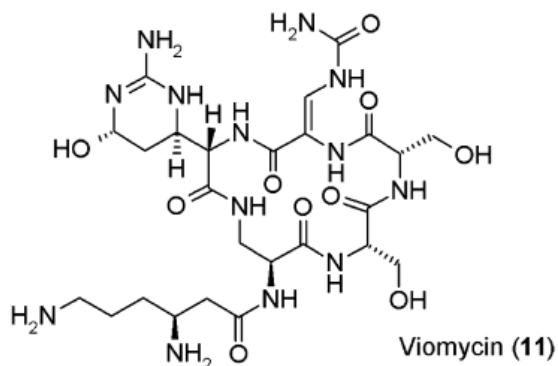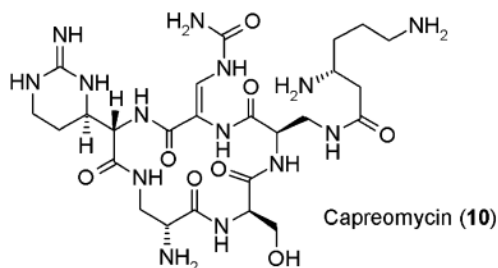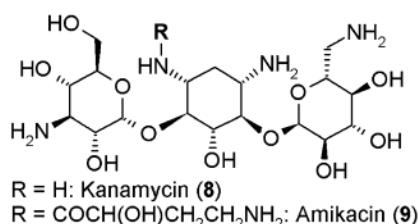
(iii) One of the many related antibacterial fluoroquinolones such as ciprofloxacin, ofloxacin, levofloxacin, or the more recent sparfloxacin, gatifloxacin, moxifloxacin and sitafloxacin should be included in the regimen. This class of antibiotics has now been proven as indispensable treatment for MDR tuberculosis [19]. Moreover, some of these drugs may leads to shorter antituberculosis regimens although their use in immunotherapy also leads to the occurrence of fluoroquinone-resistant strains of M.tuberculosis [20]. An actual preference between fluoroquinolones especially between the latest generations, for the specific treatment of MDR tuberculosis is still the matter of preclinical and clinical research [21].

Ciprofloxacin (12) R/S : Ofloxacin (13a) S : Levofloxacin (13b) Sparfloxacin (14)

Gatifloxacin (15) Moxifloxacin (16) Sitafloxacin (17)
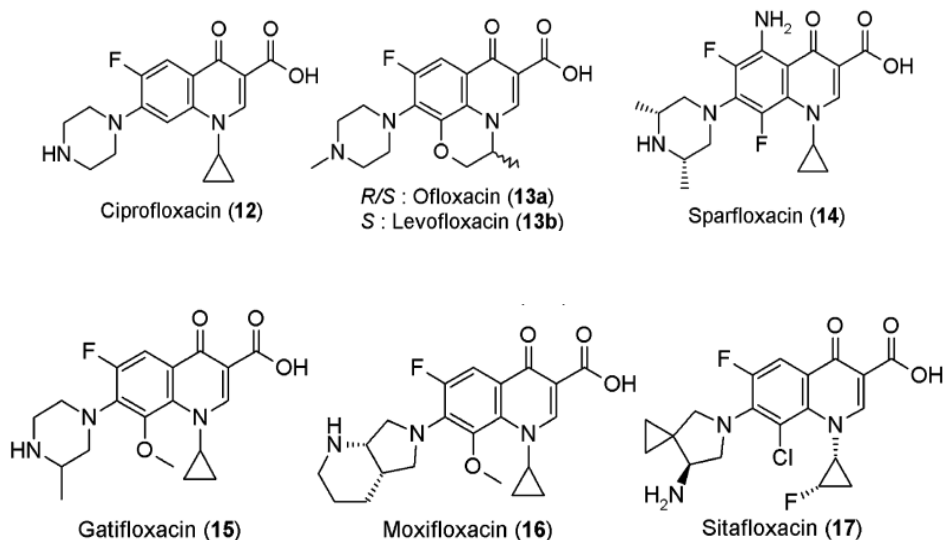
*(iv)*Second line bacteriostatics, with established clinical efficacy, usually have more important side effects [22]. They are p - aminosalicylic acid, ethionamide (the propyl analogue prothionamide is also used) and cycloserine.

(v) Other drugs are also considered. Their use is the subject of debate and only time and proper observations will provide the necessary data. Clofazimine is among these compounds and is also used against Mycobacterium leprae. The combination of amoxicillin and the penicillinase inhibitor clavulanic acid has an antimycobacterial effect in vitro. The same is true for clarithromycin although its clinical efficacy remains to be established. The use of rifabutin is a matter of debate as, although in vitro, some M.tuberculosis strains HIV-1 bearing patients. Moreover, cross-resistance is observed with ethionamide [23].

Promising new drug candidates
Some promising TB drug candidates are described in the following paragraphs.

Diarylquinoline.
The recent discovery of diarylquinoline as a promising TB drug that can shorten therapy has caused a lot of excitement. Andries et al.[24] identified diarylquinoline compounds that are highly active against mycobacteria in an in vitro

drug screen using fast-growing Mycobacterium smegmatis. Modification of the diarylquinolines led to the identification of diarylquinoline R207910 (J compound) as the most active agent, with minimum inhibitory concentration (MIC) of 0.003 μ g/ml for M. smegmatis and 0.030 μ g/ml M. tuberculosis. J compound is much less active against other bacterial species, such as Escherichia coli and Staphylococcus aureus (MIC >32 μ g/ml). M. tuberculosis and M. smegmatis could develop resistance to diarylquinoline at a frequency of 1 × 10^{-7} to 1 × 10. Diarylquinoline resistant M. smegmatis and M. tuberculosis strains were found to harbor mutations in the subunit c encoded by atpE (D32V for M. smegmatis and A63P for M. tuberculosis) in the F0 moiety of mycobacterial F1F0 proton ATP synthase, which is a key enzyme for ATP synthesis and membrane-potential generation. Complementation studies confirmed that the mutations in atpE are responsible for resistance to diarylquinoline. The target for diarylquinoline was proposed to be the mycobacterial F1F0 proton ATP synthase, which is a new drug target in mycobacteria. Indeed, the J compound is also active against MDR-TB strains. Based on transposon mutagenesis analysis, F1F0 ATP synthase seems to be an essential enzyme in M. tuberculosis, although the enzyme is not essential for E. coli because mutants of F1F0

were viable but grew at a reduced rate and were attenuated for virulence in mice [25]. The J compound was more active than INH and RIF in the mouse model and could shorten TB therapy from four months to two months in mice with established infection. Of particular interest is the synergy between diarylquinoline and PZA, which seems to be the most effective drug combination in sterilizing infected spleens and lungs. This finding is consistent with the previous observation that N,N'-dicyclohexylcarbodiimide (DCCD) – which also inhibits the same c chain of the F0 moiety of F1F0 ATPase as diarylquinoline – has synergy with PZA against M. tuberculosis [26] . Thus, the observed synergy of diarylquinoline with PZA could be explained in the same way as the synergy of DCCD with PZA. The J compound had excellent early and late bactericidal activity, good pharmacokinetic and pharmacodynamic properties with a long half life, absence of significant toxicity in mouse and preliminary human safety testing, raising the hope that diarylquinoline might be used for shortening TB therapy in humans. However, there are several unusual features of the J compound that require further explanation. First, antimycobacterial drugs usually do not show the same degree of activity against fast and slow growing mycobacteria. Drugs like

INH, RIF, and PZA are more active against slow growing M. tuberculosis but less active against fast growers like M. smegmatis, which has higher efflux activity and is better able to maintain its energy status compared with M. tuberculosis [27]. However, in this case, diarylquinolines are even more active against fast growing M. smegmatis than against M. tuberculosis, which is quite unusual. Second, the high early and late bactericidal activity in mice is unusual because other TB drugs show either early or late sterilizing activity but not both. Third, the selective activity of diarylquinolines against the mycobacterial enzyme F1F0 ATPase (present in all mycobacteria, but also in host cell mitochondria) without apparent toxicity is quite remarkable. Fourth, mycobacteria would be expected to have alternative means, such as the electron transport chain, to produce energy or ATP without F1F0 ATPase, and thus the inhibition of F1F0 ATPase by diarylquinoline would not be lethal unless the J compound also interferes with other drug targets in the mycobacterium. The J compound is currently under clinical testing. It remains to be seen whether diarylquinoline can be used as a new TB drug for more effective treatment of TB in humans.
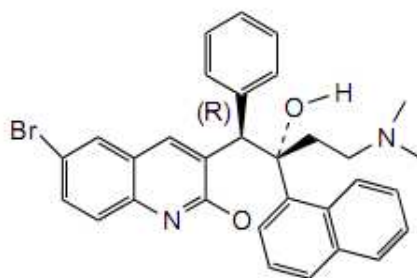
Diarylquinoline

New Fluoroquinolones

Fluoroquinolones are broad-spectrum antibiotics currently used as second-line drugs in tuberculosis therapy or as stand-ins for drugs made unfeasible by drug resistance. The new C-8-methoxy-FQ moxifloxacin (MXF) and

gatifloxacin (GATI) have a longer half life and are more active against M. tuberculosis than ofloxacin and ciprofloxacin [28]. MXF has been shown to kill a subpopulation of tubercle bacilli not killed by RIF. In a mouse model, MXF was as active against M. tuberculosis as INH. MXF

in combination with RIF and PZA killed tubercle bacilli in mice more effectively than the standard regimen of INH+ RIF+ PZA and could achieve stable cure in 4 months with no relapse. These promising studies raise the hope that MXF might replace INH+RIF+PZA to shorten TB therapy in humans. MXF has early bactericidal activity against tubercle bacilli comparable to INH and was shown to be well tolerated in a preliminary human study. Combination therapy with MXF seems to be as effective as current standard drug combinations. MXF and GATI are currently being evaluated in clinical trials in combination with RIF and PZA (R. Chaisson, personal communication). Both agents could have the potential to be used as first-line drugs for improved treatment of TB.

Moxifloxacin

Rifamycin derivatives

Rifamycin derivatives, such as rifapentine, rifabutin and rifalazil (RLZ, also known as KRM1648 or benzoxazinorifamycin), have been synthesized to improve antimycobacterial activity and prolong half life. Rifapentine was approved by the FDA in 1998 for the treatment of TB. Rifapentine appears to be safe and well-tolerated at once-weekly dosing and is currently being evaluated in Phase III efficacy trials for treatment of latent tuberculosis [29]. RLZ is a new semisynthetic rifamycin derivative with a long half life, which is highly active against a range of intracellular bacteria including M. tuberculosis, Mycobacterium avium, Chlamydia trachomatis, Chlamydia pneumoniae, and Helicobacter pylori. RLZ is more active than RIF or rifabutin against M. tuberculosis in mice both in vitro and in vivo. RIF-resistant strains confer cross-resistance to all rifamycins, including RLZ, limiting the use of RLZ in the treatment of RIF-resistant TB. A preliminary safety study in humans showed that although RLZ at doses of 10 mg and 25 mg was safe, a dose of >100 mg produced flu-like symptoms and a transient dose-dependent decrease in white blood cell and platelet counts, and did not show better efficacy than RIF [30]. However, no information is available for clinical trial of RLZ for the treatment of mycobacterial infections.

Oxazolidinones

Oxazolidinones, discovered at DuPont in the 1970s and later sold to Pharmacia Upjohn, are a new class of compounds that are active against a variety of Gram-positive bacteria, including M. tuberculosis. Oxazolidinones inhibit protein synthesis at an early stage by binding to 23S rRNA of the 50S ribosomal subunit [31]. Oxazolidinones had significant activity against M. tuberculosis with an MIC of 2–4 µ g/ml and were also active against tubercle bacilli in mice. It has been recently demonstrated the antimycobacterial activity of 3-(1H-pyrrol-1-yl)-2-oxazolidinone. Two novel oxazolidinones, RBx7644 and RBx8700, were active against MDR-TB and tubercle bacilli inside macrophages. Linezolid is the first oxazolidinone to be developed and approved by the FDA to treat single- or multiple-resistant Gram-positive bacterial infections. In two recent clinical studies, most MDR-TB patients were successfully treated with linezolid in combination with other drugs, but prolonged use of linezolid in the treatment of MDR-TB

frequently caused significant toxicity, including anemia and peripheral neuropathy. Although oxazolidinones have promising potential for the treatment of MDR-TB, more extensive clinical studies are needed to evaluate their efficacy and toxicity, as well as the degree of resistance development by mycobacteria.

Nitroimidazopyran PA824

A particularly promising candidate for TB treatment is nitroimidazopyran PA824, derived from 5-nitroimidazoles. PA824 is highly active with MIC as low as 0.015–0.250 µg/ml against M. tuberculosis and MDR-TB [39]. PA824 is a prodrug that requires activation by a bacterial F420-depedent glucose-6-phosphate dehydrogenase (Fgd) and nitroreductase to activate components that then inhibit bacterial mycolic acid and protein synthesis [32]. However, the active moiety of activated PA824 remains to be identified. In complementation experiments in F420-negative mutants resistant to the drug, Fgd could restore drug sensitivity in M. tuberculosis and Mycobacterium bovis. In animal models, PA824 was active against non-growing bacilli even in microaerophilic conditions (which might have some sterilizing action for non-growing persistent bacilli in the anaerobic environment of granulomas) and its activity is comparable to INH, RIF and moxifloxacin. PA824 had bactericidal activity in the mouse model in the initial two months of treatment and also in the continuation phase of treatment, which indicates significant activity against non growing persistent bacilli in vivo. Additional studies are needed to evaluate PA824 in terms of its ability to shorten TB therapy in combination with other drugs without significant relapse, drug resistance or toxicity. PA824 is currently being evaluated in clinical trials by the Global Alliance for TB Drug Development.
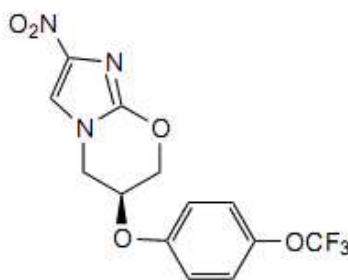
Nitroimida zolepyran (PA824)

Novel drug targets

Desirable targets should be involved in vital aspects of bacterial growth, metabolism and viability, whose inactivation will lead to bacterial death or inability to persist. The availability of the M.tuberculosis genome sequence and mycobacterial genetic tools, such as transposon mutagenesis, gene knockout and gene transfer, greatly facilitate target identification. Moreover, targets involved in the pathogenesis of the disease process should also be considered for drug development [33]. For example, liquefaction from solid necrotic lesion to cavity formation is a key step in the spread of infection to other individuals. If the liquefaction and cavity formation could be interrupted, the bacilli in the lesion would not be coughed up and spread to others. Thus, inhibition of host liquefaction process represents a novel approach to the design of new drugs that stop the transmission of the disease. It is increasingly acknowledged that new drugs should not only be active against drug resistant TB, but should also kill persisters and shorten the lengthy TB treatment, which underlies the problem of drug resistance due to poor compliance to the length of therapy. Current TB drugs inhibit

particular targets in DNA synthesis, RNA synthesis, and cell wall synthesis and energy metabolism pathways. Enzymes in these metabolic pathways that are not inhibited by current TB drugs could also be good targets. Mycobacterial two-component systems, sigma factors and virulence factors have also been proposed as targets for TB drug development and will not be detailed here. Novel drug targets for which new drugs can be developed are discussed below.

Essential genes

Transposon mutagenesis and signature-tagged mutagenesis have been used to identify genes essential for M. tuberculosis growth in vitro and survival in vivo [34]. In a recent study, 614 genes, about one-sixth of the total number of genes in M. tuberculosis, were found to be essential for in vitro growth, whereas 194 genes were demonstrated to be essential for in vivo survival in mice. The genes that are essential for survival in vitro and in vivo are grouped into the following categories: lipid metabolism; carbohydrate and amino acid transport and metabolism; inorganic ion transport and metabolism; nucleotide transport and metabolism; energy production and conversion; secretion; cell envelope biogenesis; cell division; DNA replication; recombination and repair; transcription and translation; post-translational modification; chaperones; coenzyme metabolism; and signal transduction. However, the function of a significant number of essential genes is unknown. Besides systematic analysis of essential genes by transposon mutagenesis, targeted knockout of specific genes is also a valuable approach to identifying essential genes, in other words, those whose disruption leads to non-viability of the bacilli. These essential mycobacterial genes should be good targets for TB drug development.

Persistence targets

Mycobacterial persistence refers to the ability of tubercle bacillus to survive in the face of chemotherapy and/or immunity [35]. The nature of the persistent bacteria is unclear but might consist of stationary phase bacteria, post-chemotherapy residual survivors and/or dormant bacteria that do not form colonies upon plating. The presence of such persistent bacteria is considered to be the major reason for lengthy therapy. A lot of research activity is currently aimed at understanding the biology of persistence of the tubercle bacillus and developing new drugs that target the persister bacteria. Gene products involved in mycobacterial persistence, such as isocitrate lyase (ICL), PcaA (methyl transferase involved in the modification of mycolic acid), RelA (ppGpp synthase), and DosR (controlling a 48-gene regulon involved in mycobacterial survival under hypoxic conditions), have been identified and could be good targets for the development of drugs that target persistent bacilli.

Toxin–antitoxin modules

Studies – primarily in E. coli – have identified toxin and antitoxin pairs (called TA modules), such as MazEF, HigAB, ParDE, Phd/Doc, RelBE, VapBC, that are involved in bacterial cell death and persistence [36]. Inappropriate or uncontrolled expression of the toxin or a decrease in the expression of antitoxin can cause bacterial cell death. In E. coli, some antibiotics (e.g. rifampin, chloramphenicol and spectinomycin) that inhibit transcription and translation, respectively, and also sulfa drugs that cause thymine starvation, kill bacteria by inducing the toxin MazF. It is interesting to note that the M. tuberculosis genome has recently been found to contain at least 38 TA modules including three relBE and nine mazEF loci. The TA modules are attractive targets in M. tuberculosis for designing drugs that either induce the production of the toxin or inhibit the expression of the antitoxin.

Energy production pathways

All bacteria require energy to remain viable. Although the energy production pathways in M. tuberculosis are not well characterized, their importance as drug targets is demonstrated by the recent finding that PZA (a frontline TB drug

that is more active against non-growing persistent bacilli than growing bacilli and shortens TB therapy) acts by disrupting membrane potential and depleting energy in M. tuberculosis. This study implies that energy production or maintenance is important for the viability of persistent non-growing tubercle bacilli in vivo. The recent discovery of the highly effective TB drug diarylquinoline also highlights the importance of energy production pathways for mycobacteria. It is likely that energy production pathways, such as the electron transport chain, glycolytic pathways (like the Embden–Meyerhof pathway) and fermentation pathways, could be good targets for TB drug development.

Novel approaches in drug discovery
Besides the choice of drug targets, there are many different approaches one should consider and use in TB drug development. One is the way drug screens are designed. Current TB drugs were mostly discovered based on their activity against growing bacilli in vitro, with the exception of PZA. However, activity against non-growing persister bacilli is correlated with good sterilizing activity that is responsible for shortening therapy in vivo, as shown by PZA and RIF. Thus, novel drug screens that mimic in vivo conditions in lesions (i.e. acidic pH and hypoxia) and act against old stationary-phase non-growing bacilli could be important for identifying drugs that kill persisters and thereby shortening TB treatment. In addition, drug combination screens could be performed to identify drugs that have synergistic effects. For example, it would be of interest to identify agents that synergize with PZA or RIF and improve the activity of these TB drugs. Along this line of combination screen is the recent interest in the use of systems biology approach for drug discovery [37]. Instead of the conventional reductionist approach of finding a single drug that hits a single target, the systems biology approach proposes using multiple compounds that hit multiple targets in different pathways to achieve the desired outcome. A systems biology approach can be used for identifying novel drug combinations against TB. Another approach is to make use of the growing knowledge of the unique

CONCLUSION

The Drug resistant TB and the HIV pandemic presents major challenges for the effective control of TB. The TB drugs currently in use were developed 40 years ago and there is a great need for a new generation of TB drugs. Current research involves testing new or reformulated drugs, combinations of different drugs to shorten therapy, supplementation and enhancement of existing drugs, development of novel slow-release drug delivery systems that could reduce the frequency and amount of drug necessary during treatment [38], and research into molecular targets. The goal is to find better and more effective drugs that reduce time of treatment, reduce toxicity associated with drugs and provide backup measures in case of drug resistance. The approaches include: chemical modification of existing drugs (such as rifampin, fluoroquinolones and macrolides); the identification of drug targets (such as persistence genes) using microarray analysis and molecular biology tools; structure based drug design and in vitro and in vivo screening to identify new drugs; evaluation of novel drug combinations; and the order of drugs given in treatment. There are currently a number of drug candidates that are being investigated in the laboratory and in clinical trials that show high antituberculosis potential, the most promising are diarylquinoline and PA824because of their high activity against M. tuberculosis and the potential to shorten therapy. Besides chemotherapy, immunotherapeutic approaches such as DNA vaccines and cytokines used in combination with chemotherapy also offer some promising prospect for improved treatment of TB. In searching for novel drug targets, more research must go into understanding the biology of persistence, the factors involved in

tissue liquefaction and cavity formation, and the host immune mechanisms that control latent infection. The outcome of such research will not only provide improved understanding of the disease, but also new relevant biology-based targets for drug intervention. This is a battle that might not be easily won, but given persistent effort and sustained support, it is quite likely that the current endeavors will bear fruit and lead to more effective treatment. After years of neglect, the field of TB drug discovery is finally gaining momentum thanks to the persistence of many dedicated individuals and the establishment of The Global Alliance for TB Drug Development and the support of The Gates Foundation. The race to develop new and more effective TB drugs is on. With several promising drug candidates in the pipeline, there is a fair amount of excitement and even optimism in the field. It is hoped that new more effective TB drugs will be discovered in the not-too-distant future that could shorten the therapy from six months to a few weeks and improve control of TB worldwide.

REFERENCES

1. Corbett EL et al., The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch. Intern. Med. 2003; 163: 1009–1021.
2. Nachega JB and Chaisson RE, Tuberculosis drug resistance: a global threat. Clinc. Infect. Dis. 2003; 36: S24–S30.
3. Camus JC, Pryor MJ, Medigue C, Cole ST, Microbiology, Re-annotation of the genome sequence of Mycobacterium tuberculosis H37Rv. Microbiol. 2002; 148: 2967–2973:
4. Chung GA, Aktar Z, Jackson S, Duncan K, High-throughput screen for detecting antimycobacterial agents. Antimicrob. Agents Chemother. 1995; 39 2235–2238.
5. Orme I, Search for New Drugs for Treatment of Tuberculosis. Antimicrobial Agents Chemother. 2001; 45: 1943– 1946.
6. Blumberg HM et al., American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. Am. J. Respir. Crit. Care Med. 2003; 167: 603–662.
7. Zhang Y, Persistent and dormant tubercle bacilli and latent tuberculosis. Front. Biosci. 2004; 9: 1136–1156.
8. Global Alliance for Tuberculosis Drug Development Executive Summary of the Scientific Blueprint for TB Drug Development. Tuberculosis, 2001; 81: 1–52.
9. Mitchison DA. Modern methods for assessing the drugs used in the chemotherapy of mycobacterial disease. J Appl Bacteriol 1996;81:72S–80S.
10. Freerksen E, Orlwski EH, Thumim JH, Karger S, Antituberculosis drugs: Ten years of research In Antibiot. Chemother. 1970; 16: 536.
11. Datta M, Radhamani M P, Selvaraj R, Paramasivan C N, Gopalan B N, Sudeendra C R, Prabhakar R. Critical assessment of smear-positive pulmonary tuberculosis patients after chemotherapy under the district tuberculosis programme. Tuber. Lung Dis. 1993; 74: 180–186.
12. Forget EJ, Menzies D. Adverse reactions to first-line anti-tuberculosis drugs. Expert Opin Drug Saf, 2006;5:231-249.
13. De Souza MVN. Marine Natural Products Against Tuberculosis. The Scientific World Journal,2006;6,847–861.
14. O'Brien RJ, Spigelman M. New drugs for tuberculosis: current status and future prospects. Clin Chest Med 2005;26:327–340.
15. Dye C, Espinal, M.A., Watt CJ, Mbiaga C, Williams, BG, 2002.Worldwide incidence of multidrug-resistant tuberculosis. J Infect Dis. 185:1197-1202.
16. Mukherjee JS, Rich ML, Socci AR, Joseph JK, Virú FA, Shin SS, et al. Programmes

and principles in treatment for multidrug-resistant tuberculosis. Lancet. 2004;363:474–81.

17. Gupta P, Jadaun GPS, Das R, Gupta UD, Srivastava K, Chauhan A, Sharma VD, Chauhan DS, Katoch VM. Simultaneous ethambutol and isoniazid resistance in clinical isolates of Mycobacterium tuberculosis. Indian J Med Res. 2006;123:125–130.

18. Mirsaeidi SM, Tabarsi P, Khoshnood K, Pooramiri MV, Rowhani-Rahbar A, Mansoori S. D, Masjedi H, Zahirifard S, Mohammadi F, Farnia P, Masjedi MR, Velayati AA. Treatment of multiple drug-resistant tuberculosis (MDR-TB) in Iran. Int. J. Infect. Dis. 2005; 9: 317– 322.

19. Chan ED, Laurel V, Strand MJ, Chan JF, Huynh ML, Goble M, Iseman MD. Treatment and outcome analysis of 2005 patients with multidrug-resistant tuberculosis. Am J Respir Crit Care Med.2004;169:1103–9.

20. Aubry A, Veziris N, Cambau E, Truffot-Pernot C, Jarlier V, Fisher LM. Novel gyrase mutations in quinolone-resistant and -hypersusceptible clinical isolates of Mycobacterium tuberculosis: functional analysis of mutant enzymes Antimicrob. Agents Chemother. 2006; 50: 104–112.

21. Yew WW, Chan CK, Leung CC, Chau CH, Tam CM, Wong PC, J. Comparative roles of levofloxacin and ofloxacin in the treatment of multidrug-resistant tuberculosis. Chest. 2003; 124: 1476–1481.

22. Newton RV. Side effects of drugs used to treat tuberculosis.Scott. Med. J. 1975; 20: 47–49.

23. Eule H, Werner E, Beitr Klin, Erforsch Tuberk, Lungenkr. Antituberculosis drugs: Ten years of researchChem. Abstr. 1967; 66: 247–258.

24. Andries K et al., A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis, Science. 2005; 307: 223–227.

25. Turner AK et al., Contribution of proton-translocating proteins to the virulence of Salmonella enterica serovars Typhimurium, Gallinarum, and Dublin in chickens and mice. Infect. Immun. 2003; 71: 3392–3401.

26. Zhang Y et al., Mode of action of pyrazinamide: disruption of membrane potential and transport by pyrazinamide. J. Antimicrob. Chemother. 2003; 52: 790–795.

27. Zhang Y et al., Role of acid pH and deficient efflux of pyrazinoic acid in the unique susceptibility of Mycobacterium tuberculosis to pyrazinamide. J. Bacteriol. 1999; 181: 2044–2049.

28. Miyazaki E et al., Moxifloxacin (BAY12-8039), a new 8methoxyquinolone, is active in a mouse model of tuberculosis. Antimicrob. Agents Chemother. 1999; 43: 85–89.

29. Rothstein DM et al., Development potential of rifalazil. Expert Opin. Investig. Drugs 2003; 12: 255–271.

30. Dietze R et al., Safety and bactericidal activity of rifalazil in patients with pulmonary tuberculosis. Antimicrob. Agents Chemother. 2001; 45: 1972–1976.

31. Ashtekar DR et al., Oxazolidinones, a new class of synthetic antituberculosis agent. In vitro and in vivo activities of DuP-721 against Mycobacterium tuberculosis. Diagn. Microbiol. Infect. Dis.1991; 14: 465–471.

32. Stover CK et al., A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature. 2000; 405: 962–966.

33. Cole ST et al., Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence, Nature. 1998; 393: 537–544.

34. Lamichhane G et al., Designer arrays for defined mutant analysis to detect genes essential for survival of Mycobacterium tuberculosis in mouse lungs. Infect. Immun. 2005; 73: 2533–2540.

35. McKinney JD et al., Persistence of Mycobacterium tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase, Nature. 2000; 406: 735–738.

36. Engelberg-Kulka H et al., Bacterial programmed cell death systems as targets for antibiotics. Trends Microbiol. 2004; 12: 66–71.

37. Borisy AA et al., Systematic discovery of multicomponent therapeutics. Proc. Natl. Acad. Sci. U. S. A., 2003; 100: 7977–7982.

38. Barrow WW, Microsphere technology for chemotherapy of mycobacterial infections. Curr. Pharm. Des. 2004; 10: 3275–3284.