Chapter 2

Chemotherapeutic Agents Used for Tuberculosis

2.1 History

Discovery of broad spectrum antimicrobials is perhaps one of the greatest achievements of scientific community in the 20th century. It is very difficult to imagine today's "relatively" healthy society with an average life expectancy of more than 65 years without these wonder drugs. Tuberculosis appeared invincible throughout human history. Attempts made by several generations of scientists, doctors and spiritual heads and their success or failure stories not only labelled TB as unique, but also helped evolve several aspects of antimicrobial chemotherapy from diagnosis to drug design&discovery.

Pre-Antibiotic ERA

It is evident from the history and primordial manuscripts that, consumptive disorders co-evolved with human race. One of the oldest ancient medical scripture "Ayurveda" written in Sanskrit (~2000 BC), mentioned about "Yakshma", a consumptive disorder with typical manifestations comparable to tuberculosis. There, concoctions of various herbal extracts containing Aswagandha, Asparagus, Pepper, Opium and Allium were suggested for the disease management. The therapy included using highly nutritious food, large quantities of milk, various types of meat and relaxation. Taking care of the patient to be in good humour is also necessary as depression was found to be an aggravating factor for TB.[1]

The role of rest in recuperating the tubercular lungs is well established. By 1930's, several sanatoriums were established throughout the world in serene environments to offer healthy air. The patients have also received sun bath therapy as sun rays were believed to cure this ailment, especially the skin lesions.[2] Cod liver oil rich in vitamin D, once sold as "liquid sun" was also used

in this treatment. In the early 20th century, usage of metals like gold, arsenic were suggested for treating infectious diseases. Surgical interventions to modulate lung function or removal of the severely damaged part proved successful in improving the health condition of many patients and are still in practice as an adjuvant therapy, especially in chronic MDR-TB cases.[3]

Throughout the history, several illustrated medical men including Susruta, Hippocrates and Galen pronounced that TB is incurable, infectious patients are to be isolated. But, 20th century saw the birth of "Chemotherapy" out of prudent thinking coupled with serendipity and luck to bring phenomenal changes in the management of TB.

2.2 Post Antibiotic ERA

Early observations of antimicrobial activity in chemical dyes by Paul Ehrlich, laid foundations for the development of synthetic antibacterials in the modern era. Domagk synthesized first ever antibacterial "prontosil". Further optimization of sulfonamide chemistry accidentally gave isonicotinic acid hydrazide, the most effective first line antitubercular agent ever discovered.[4] Later, structure activity relationship studies on nicotinamide derivatives resulted in another successful anti TB drug, pyrazinamide.[5] Careful observation of rate of oxygen uptake in tubercle bacillus under the influence of benzoates and salycilates by Berheim paved way for the discovery of another drug p-aminosalycilic acid (PAS). Lehmann of Sweden screened several salycilic acid derivatives and found PAS as a very effective anti-TB drug against "actively replicating" mycobacteria. This drug is nontoxic and soon became a very popular clinical agent.[6]

Accidental discovery of the antibiotic Penicillin from a fungus ascertained the idea of effective "chemical communication" by the microbes for protection of

their own turf from the invaders. This has led many academicians and pharmaceutical companies to explore the potential of microbes as a resource for bioactive secondary metabolites and thus began the golden era of antibiotics.[7] Mycobacterium, unfortunately is not sensitive to penicillin and many other natural or synthetic antibiotics. Selman Walksman of Rutgers University took microbial screening to a whole new level and obtained first ever antitubercular antibiotic Streptomycin from a soil sample.[8] Development of resistance to monotherapy of most of these drugs by early 1950's necessitated search for more effective anti-TB agents and optimized multi drug regimens. Though, many antibiotics possessed feeble anti-TB activity, none were found to be clinically useful. It is during 1960's, another remarkable anti tubercular antibiotic rifampicin entered into clinics. Discovery of important class of synthetic antibiotics, fluoroquinolones, in the early 1980's sealed the fate of TB forever. The uniqueness of challenges offered by anti-TB drug discovery is clearly evident from availability of a sparse 20 odd drugs for clinical use.

Chemotherapeutic agents for TB may be divided into two main classes, first line agents (isoniazid, rifampicin, streptomycin, pyrazinamide and ethambutol) and second line agents (ethionamide, *p*-aminosalicylic acid, cycloserine, rifapentine, clarithromycin, kanamycin, amikacin, ofloxacin, ciprofloxacin, viomycin and capreomycin). Current TB drugs and their targets were given in (Table 2.1).

Table 2.1 *Current Anti Tb Drugs and Their Targets.*

Drug (year of discovery)	Target	Effect
Group 1 TB drugs: First Line Oral Agents		
Isoniazid (1952)	Multiple targets including Acyl carrier protein reductase (InhA) and β-ketoacyl synthase (KasA)	Inhibits mycolic acid synthesis
Pyrazinamide (1954)	Disruption of membrane function and energy metabolism, Inhibition of fatty acid synthesis	Disruption of member function and energy metabolism, Inhibition of fatty acid synthesis, acidifies cytoplasm
Ethambutol (1961)	Arabinosyltransferases	Inhibits arabinogalactan biosynthesis
Rifampicin (1963)		
Rifabutin (1975), Rifapentin 1965)	RNA polymerase, beta subunit	Inhibits transcription
Group 2 TB drugs: Injectable Agents		
Streptomycin (1944)	S12 and 16S rRNA componentsof 30S ribosomal subunit	Inhibits protein synthesis
Kanamycin (1957)	30S ribosomal subunit	Inhibits protein synthesis
Capreomycin (1963)	Interbridge B2a between 30Sand 50S ribosomal subunits	Inhibits protein synthesis
Amikacin (1972)	30S ribosomal subunit	Inhibits protein synthesis
Group 3 TB drugs: Fluoroquinolones		
Ofloxacin (1980), levofloxacin (1983), moxifloxacin (1999)	DNA gyrase and DNA topoisomerase	Inhibits DNA supercoiling
Group 4 TB drugs: Oral Bacteriostatic Second Line Agents		
Para–aminosalicylic acid (1946)	Dihydropteroate synthase	Inhibits folate biosynthesis
Cycloserine (1952)	D-alanine racemase and ligase	Inhibits peptidoglycan synthesis
Terizidone (1952)	L-alanine racemase and D-alanine ligase	Inhibits peptidoglycan synthesis
Ethionamide (1956)	Enoyl-[acyl-carrier-protein] reductase	Inhibits mycolic acid biosynthesis
Protionamide (1956)		
Group 5 TB drugs: Agents with an unclear role in the treatment of drug resistant TB		
Clofazimine (1952)	Multiple mechanisms. Membrane destabilization, redox cycling	Membrane disruption, DNA damage
Linezolid (2000)	P site of the 50S ribosomal subunit	Inhibits protein synthesis
Amoxicillin/Clavulanate	Penicillin binding protein/β-lactamase	Inhibits cell wall synthesis
Thioacetazone (1952)	Cyclopropanemycolic acid synthases	Inhibits mycolic acid synthesis
Imipenem/cilastatin	Penicillin binding proteins/ renal dihydropeptidase.	Blocks cell wall synthesis/Cilastatin blocks imipenem metabolism
High dose isoniazid	Same as INH	Same as INH
Clarithromycin (1991)	50S ribosomal subunit, inhibits transfer of peptidyl t-RNA from A site to P site.	Inhibits protein synthesis

2.2.1 Streptomycin (SM) and Other Aminoglycoside Antibiotics

The first clinically used antitubercular antibiotic, streptomycin (Fig. 2.1), was isolated from the microbe *Streptomyces griseus*, by Albert Schatz and Selman Walksman in the year 1944, in their intense search for an antibiotic against gram –ve and other clinically important microbes. The general structure of the aminoglycosides is characterized by an aminocyclitol ring connected to one or more amino sugars by a glycosidic connection. This drug inhibits the translation of mRNA *via* interaction with the 30S ribosomal subunit. Mutations on the genes coding for the 16S rRNA and ribosomal protein S_{12} confers streptomycin resistance.[9, 10]

Resistance to streptomycin has become less common due to the wider use of ethambutol as the fourth drug in WHO standard treatment schedule. One of its derivatives, dihydrostreptomycin also showed anti-TB activities. It has an MIC value of 1 μg/mL with 50–60% plasma protein bound and a half-life of 5–7 hr. It penetrates the inner membrane of *M. tuberculosis* and binds to the 30S subunit of the ribosome.[11] Variety of synthetic derivatives of streptomycin have been synthesized and evaluated against *M. tuberculosis*.[12] Due to unwarranted toxic effects streptomycin and its derivativesare now largely replaced by other aminoglycoside antibiotics like kanamycin and amikacin used in anti-TB therapy as second-line agents.[13, 14]

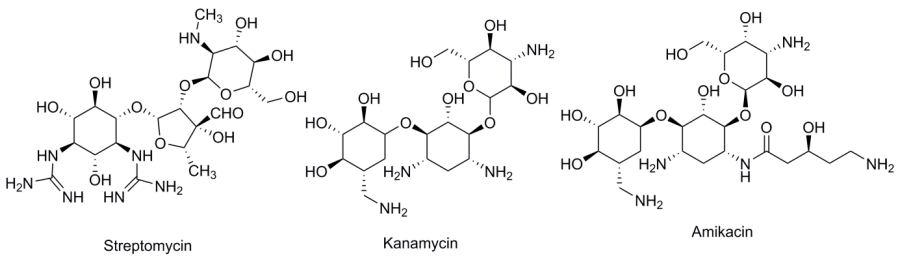

Fig. 2.1 *Clinically important aminoglycoside antibiotics.*

2.2.2 Isoxyl (Thiocarlide) & Thiacetazone

A number of diacylthioureas have shown significant anti Tb activity in experimental models. One such agent, 4, 4´-diisoamyloxydiphenylthiourea (4, 4´-diisoamyloxy diphenylthiocarbanitide, isoxyl, thiocarlide) [15-17] has proved to be clinically useful. In a study involving exposure of *M. bovis* to this drug revealed the mode of action of to be the inhibition of mycolic acid biosynthesis. Thiacetazone was discovered to have antitubercular activity in 1940s and was used as an antitubercular agent despite its toxic side effects.[18, 19]

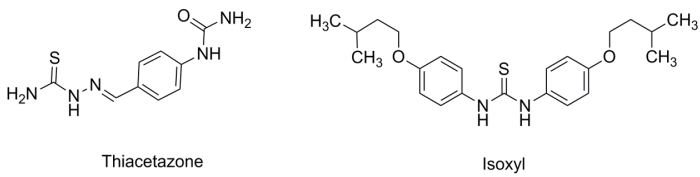

Fig. 2.2 *Thiourea containing Antitubercular drugs.*

Thiacetazone, similar to the thioisonicotinamides, is activated by EthA resulting in a reactive intermediate that inhibits mycolic acid oxygenation as well as cyclopropanation.[20, 21] Thiacetazone causes gastrointestinal disturbances and particularly in HIV-infected patients, can cause severe life-threatening skin reactions known as Stevens–Johnson syndrome.[22]

2.2.3 Isoniazid (INH)

Antitubercular activity of isoniazid (isonicotinicacidhydrazide, INH) was discovered accidentally in the year 1952, while screening synthetic products obtained from SAR studies of an anti-TB agent, thiacetazone (Fig. 2.3). INH revolutionized the TB treatment after its entry in the year 1952 and is still considered the best synthetic anti-TB drug ever discovered. This agent is orally effective, inexpensive, free from toxicity and highly active in both acidic and

basic conditions. INH is a specific antimycobacterial agent as it lacks inhibitory activity on other microbes. It acts by selectively inhibiting the synthesis of mycolic acids essential for mycobacterial cell wall. INH is a prodrug and gets activated by mycobacterial catalase-peroxidase (*katG*), which transforms the drug into a nucleophilic radical. Reaction of this radical, with the cofactor NAD^+, yields a potent inhibitor of enoyl- ACP (acyl carrying protein) reductase. This particular enzyme is essential for the mycolic acid synthesis of bacterial cell wall. The enormous class of mutations in INH-resistant *M. tuberculosis* map to a gene which encodes the catalase-peroxidase.[23] INH is orally active and shows bacteriostatic action on resting bacilli and is highly active against the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. microti* and *M. africanum*,). It has very low MICs (0.02–0.06 μg/mL) against these pathogens.[24] INH enters the organism by diffusion and oxygen-dependent active transport, and this diffusion and active transport was reported to have an effect on almost every aspect of mycobacterial metabolism.[25] A large number of compounds related to INH have been synthesized and evaluated against *M. tuberculosis* $H_{37}Rv$. Anti-TB drugs like Ethionamide and PZA are the result of these research works. This drug has a few limitations including its ineffectiveness against dormant bacteria and some unwanted effects like peripheral neuritis and liver problems.

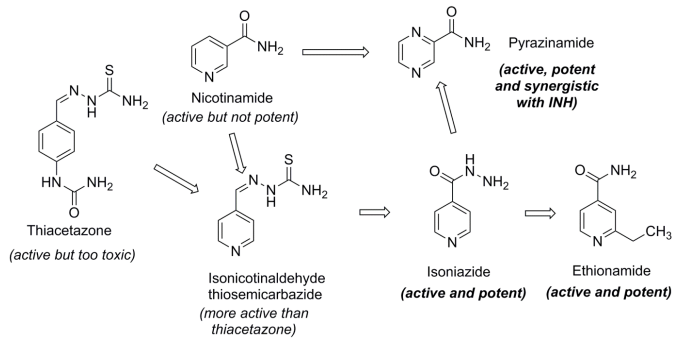

Fig. 2.3 *Development of INH and pyrazinamide.*

2.2.4 Pyrazinamide (PZA)

This, a structural analogue of nicotinamide, is a first-line drug of short course TB therapy. It is also active against semi-dormant bacilli not affected by any other drug. It has strong synergy with INH and RMP and shortens the therapy period to 6 months.[26, 27] PZA likely kills MTB by intracellular acidification following hydrolysis by Mtb-nicotinamidase/pyrazinamidase,[28] although inhibition of fatty acid synthase has also been proposed as a mechanism.[29-31] The activity of PZA depends on the presence of bacterial amidase that converts PZA to pyrazinoic acid, which is an active anti-TB molecule; this pyrazinoic acid conversion is highly prcise to *M. tuberculosis*. Mutation in the *pncA* gene is responsible for the production of pyrazinamidase and is shown to be the reason for resistance against this drug.[26, 32, 33] Interestingly, some pyrazinoic esters were also reported to possess good antitubercular activities.[34] Extensive SAR studies were conducted on this drug to ultimately find that the structural features like pyrazine ring, amide group, position of amide group are essential for bioactivity. Any change in these may either significantly reduce or nullify bioactivity.[35-38]

2.2.5 *P*-Aminosalicylic Acid (PAS)

The anti-mycobacterial activity of PAS was first reported in 1946, although it was synthesized long before.[39] It is a highly specific and effective inhibitor of *M. tuberculosis*.[40] Follow up of DOTS (Directly Observed Treatment, Shortcourse), is hardly ever used today. However, it is seldom used in the regimens for the treatment of TB caused by MDR-TB. The mechanism of action of this drug is still unclear, but it was suggested that it interferes with the salicylate-dependent biosynthesis of the iron chelating mycobactins involved in iron assimilation.[41]

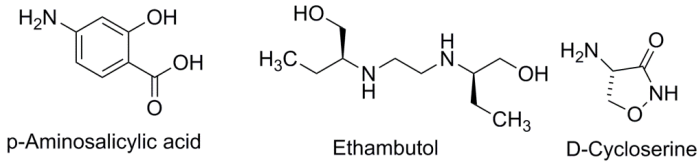

Fig. 2.4 *Structures of PAS, EMB and cycloserine.*

2.2.6 Ethambutol (EMB)

Significant anti TB activity was first observed in diisopropylethylenediamine, while screening an assortment of chemical compounds for antimicrobial activity at Lederle Laboratories in the early 1950s. Structure modification studies of this compound resulted in EMB as a potential antitubercular agent in the year 1961. It is a synthetic amino alcohol (ethylene diamino-di-1-butanol), orally effective bacteriostatic agent that is active against most strains of mycobacterium.[42-44] Structural requirements for antitubercular activity of EMB are very rigid. Several QSAR studies conducted on EMB confirmed that the ethylene-diamine unit is the minimum pharmacophore required for antitubercular activity. Any alterations in the linker region of the molecule including lengthening, incorporation of heteroatoms, or branching of the ethylene linker led to reduced activity.[45] The nitrogens must be replaced very carefully, as any change in the basicity of either amino group led to decreased antimycobacterial activity.[46, 47]

The proposed site of action of EMB is ranged from trehalosedimycolate, mycolate and glucose metabolism to spermidine biosynthesis. The critical target for EMB lies in the pathway for the biosynthesis of cell wall arabinogalactan. It inhibits arabinosyltransferase, responsible for the polymerisation of arabinose into the arabinan of arabinogalactan. Disturbing the biosynthesis of arabinogalactan would destroy the macromolecular assembly of the mycolyl-arabinogalactan-peptidoglycan complex ofthe cell wall, permitting

drugs with intracellular targets (such as rifampicin) to enter the cell without any hardships. EMB resistant *M. tuberculosis* strains carry mutations in one certain part of the gene encoding for arabinosyltransferase. In the case of MDR-TB, if there is still a consistent susceptibility, EMB might be a valuable drug for preventing the emergence of resistance with other active drugs.[48, 49]

2.2.7 Cycloserine

It is a structural analogue of the amino acid D-alanine, produced by *Streptomyces* sp. Cycloserine possesses activity against a wide range of bacteria [50] and inhibits *M. tuberculosis*at concentrations of 5–20 μg/mL. It obstructs peptidoglycan biosynthesis by inhibiting the enzymes D-alanine racemase by forming an irreversible isoxazole-pyridoxal adduct.[51] It also inhibits D-alaninyl alanine synthetase involved in synthesis of the terminal D-alanine–D-alanine of the peptidoglycan UDP-N-acetylmuramyl-pentapeptide.[52] When cyloserine was used in treating microorganisms, they accumulate a muramic-uridine -nucleotide-peptide, which differs from that produced by mycobacteria in the absence of terminal D-alanine dipeptide.[54, 54] As the bioactivity is highly conserved in the chemistry and stereochemistry of cycloserine, no novel synthetic derivative could be found with better activity.[55, 56] Cycloserine produces side effects in the central nervous system that can also generate psychotic states with suicidal tendencies and epileptic convulsions and hence used mostly in lower concentration as a second line anti-TB drug.

2.2.8 Rifampicin (RMP)

Rifamycins comprise of a complex mixture of novel antibiotics isolated from the microbe *Amycolatopsis mediterranei*. These compounds possess a highly unusual ansamycin skeleton containing a hydroxyl naphthalene core and a

19 atom polyketide ring (Fig. 2.5). Extensive QSAR studies were performed on rifampicin and found that the position and substitution of the aliphatic bridge is very critical in stabilizing the overall conformation of the molecule and positioning the phenolic –OH at C-1 and C-8 and the aliphatic –OH at C-21 and C-23 for optimal inhibition of their bacterial target, RNA polymerase.[57] It binds to a pocket of RNA polymerase within the DNA/RNA channel, but greater than 12 A˚ away from the active site. It acts by directly blocking the path of elongating RNA when the transcript becomes 2 to 3 nucleotides in length. RMP is effective against *M. tuberculosis* with MIC ranging from 0.1-0.2 μg/mL. As it diffuses freely into tissues, living cells, and bacteria, it is extremely effective against intracellular pathogens like *M. tuberculosis*. However, bacteria develop resistance to rifampicin with high frequency. Mutations conferring rifampicin resistance map almost exclusively to the *rpo B* gene that encodes the RNA polymerase *β*- subunit.[58] Resistance may also occur through ADP-ribosylation of the alcohol at position C(21).[59] However, a combination of INH and RMP may increase a risk of hepatotoxicity.

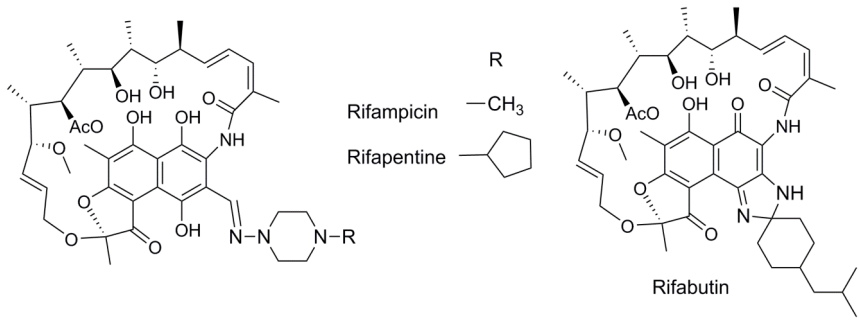

Fig. 2.5 *Rifampicin and its analogues used in anti TB therapy.*

Rifapentine was obtained from rifampicin by replacing the methyl on piperazine by a cyclopentyl unit. This is a first line anti-TB agent recommended by WHO and approved by FDA for pulmonary TB treatment in 1998. Rifapentine is a long-acting derivative of rifampicin and the drug is taken just once or twice

weekly by patients. Adding up, clinical studies also demonstrated that rifapentine could potentially shorten the current six-month treatment regimen for latent TB.[60] In contrast, once-weekly rifapentine and isoniazid treatment administered under direct observation showed a comparable effectiveness and a higher treatment completion rate compared with a 9-month isoniazid therapy regimen for the treatment of latent TB infection.[61] However, a major drawback of rifamycins is that they induce cytochrome P450 enzymes in the liver, which lead to drug–drug interactions with antiHIV agents (particularly protease inhibitors) and other TB drug candidates such as bedaquiline.[62] Hence, there is a significant interest in developing rifamycin-free regimens.[63, 64]

2.2.9 Fluoroquinolones

Serendipitous discovery of antibacterial activity in nalidixic acid, an impurity obtained during synthesis of chloroquine analogues in the year 1962, revolutionized antibacterial chemotherapy.[65, 66] Fluoroquinolones (Fig. 2.6), synthetic derivatives of nalidixic acid, display broad-spectrum antimycobacterial activity.[67-69] Ciprofloxacin, Ofloxacin and Moxifloxacin are used as second line anti- TB agents in the treatment of MDR TB patients. Structural modifications of fluoroquinolones (FQs) to optimize antimycobacterial activity have been extensively carried out to produce candidates that are more efficacious than earlier FQs. Their bactericidal effects involve an interaction of the drugs with DNA-gyrase and DNA-topoisomerase IV leading to altered DNA topology and cell death.[70] But the genome sequence of Mtb was found to be devoid of topoisomerase IV [71] thus making DNA gyrase as the primary target for its antitubercular activity. Excellent oral availability and diffusion of drug into all critical body compartments including CNS (Central Nervous System) made this a valuable drug in treating different forms of tuberculosis. Though these drugs are well tolerated, causing mild side effects that tend to be self-limiting and rarely

require discontinuation or regimen changes.[72] The most frequent adverse events reported include: gastro-intestinal upset, disturbances of the CNS and skin reactions.[73, 74] Some of the serious side effects including tendonitis and tendon rupture due to collagen damage, have been reported for FQs. Though rare, hepatotoxicity, kidney and liver dysfunction, and dysglycemia were also reported.[73] A few combinations with Gatifloxacin and Moxifloxacin with other anti-TB drugs are undergoing clinical trials as an attempt to optimize therapeutic regimen for shortening therapy to less than 4 months.[75]

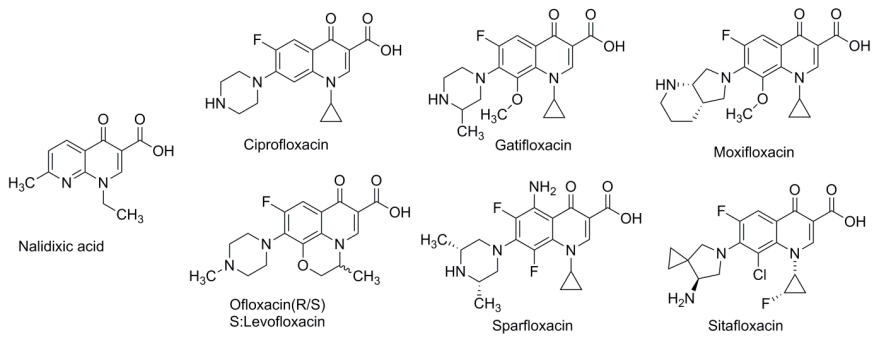

Fig. 2.6 *Clinically useful fluoroquinolones.*

2.3 Conclusions

With its unusually lipid rich cell wall and very quick adaptability makes Mycobacterium a very tough target to aim and hit. Last century saw immense progress in our understanding of this disease, biochemistry of the pathogen and its countless tricks. Though it appeared to be limited by the end of 1980's, but TB resurfaced in the early 1990's due to increase in HIV infection and much graver MDRTB form. Though very strict FDA norms and costlier clinical trials posed a complex picture, sustained efforts by the international community resulted in a few remarkable success stories, which are briefly discussed in the next chapter.

References

[1] Mohan, A.; Sharma S. K. History. In: *Tuberculosis*. 2nd edition. Sharma S. K.; Mohan A., (Editors), Jaypee Brothers Medical Publishers; 2009, 7.

[2] Margaret, C. *Medical History*, 49, 2005, 463.

[3] Cummings, I; O'Grady, J.; Pai, V.; Kolvekar, S. and Zumla, A. *Curr. Opin. Pulm. Med*.: 2012, *18*, 241.

[4] Bernstein, J. W.; Lott, A.; Steinberg, B. A. and Yale, H. L. *Am. Rev. Tuberc*., 1952, *65*, 357.

[5] Zhang, Y. and Mitchison, D. *Int. J. Tuberc. Lung Dis.*, 2003, *7*, 6.

[6] Lehmann. J. *Lancet*, 1946, *I*, 15.

[7] Aminov, R. I. Front. *Microbiol.*, 2010, 1, 1.

[8] Schatz, A.; Bugie, E. and Waksman, S. A. *Proc. Soc. Exp. Biol. Med*., 1944, *55*, 66.

[9] Kotra, L. P.; Haddad, J. and Mobashery, S. *Antimicrob. Agents Chemother*., 2000, *44*, 3249.

[10] Ramaswamy, S. and Musser, J. M. *Tuber Lung Dis*., 1998, *79*, 3.

[11] Blanchard, J. S. *Annu. Rev. Biochem*., 1996, *65*, 215.

[12] Phillips, I. and Shannon, K. P., "Aminoglycosides and aminocyclitols", In: O'Grady F., Lambert H. P., Finch R. G. and Greenwood D. (Editors), Antibiotic and chemotherapy, 7th edition. Edinburgh, Churchill Livingstone, 1997, 164.

[13] Peloquin, C. A.; Berning, S. E.; Nitta, A. T.; Simone, P. M.; Goble, M.; Huitt, G. A.; Iseman, M. D., Cook, J. L. and Curran-Everett, D. *Clin Infect Dis*., 2004, *38*, 1538.

[14] Handbook of anti-tuberculosis agents. Introduction. Patrick J. Brennanm, Douglas B. Young (Eds) Tuberculosis (Edinb) 2008, *88*, 85.

[15] Winder, F. G.; Collins, P. B. and Whelan, D. *J. Gen. Microbiol.*, 1997, *66*, 379.

[16] Phetsuksiri, B.; Jackson, M.; Scherman, H.; McNeil, M.; Besra, G. S.; Baulard,

A. R.; Slayden, R. A.; De Barber, A. E.; Barry C E 3rd, Baird M. S.; Crick, D. C. and Brennan P. J., *J. Biol. Chem.*, 2003, *278*, 53123.

[17] Phetsuksiri, B.; Baulard, A. R.; Cooper A. M.; Minnikin, D. E.; Douglas, J. D.; Besra, G. S. and Brennan, P. J., *Antimicrob. Agents. Chemother.*, 1999, *43*, 1042.

[18] Domagk, G. *Am. Rev. Tuberc.*, 1950, *61*, 8.

[19] Behnisch, R.; Mietzsch, F. and Schmidt, H. *Am. Rev. Tuberc.*, 1950, *61*, 1.

[20] Alahari, A.; Trivelli, X.; Guerardel, Y.; Dover, L. G.; Besra, G. S.; Sacchettini, J. C.; Reynolds, R. C.; Coxon, G. D. and Kremer, L. *PLoS ONE*, 2007, *2*, e1343.

[21] Alahari. A.; Alibaud, L.; Trivelli, X.; Gupta, R.; Lamichhane, G.; Reynolds, R. C.; Bishai, W. R.; Guerardel, Y. and Kremer, L. *Mol Microbiol*., 2009, *71*, 1263.

[22] Lawn, S. D.; Frimpong, E. H. and Acheampong J. W. Life-threatening cutaneous reactions to thiacetazone-containing antituberculosis treatment in Kumasi, Ghana. *West Afr. J. Med*., 18, 249.

[23] Heath, R. J.; White, S. W. and Rock, C. O. *Progress in lipid research.*, 2001, *40*, 467.

[24] Zhang, Y.; Heym, B.; Allen, B.; Young, D. and Cole, S. T. *Nature*, 1992, *358*, 591.

[25] Zhang, Y.; Garbe, T. and Young, D. *Mol. Microbiol.*, 1993, *8*, 521.

[26] Scorpio, A. and Zhang, Y. *Nat. Med.*, 1996, *2*, 662.

[27] McCune, R. M.; Feldman, F. M. and Mc Dermat, W. *J. Exp. Med.*, 1966, *123*, 445.

[28] Konno, K.; Feldmann, F. M. and Mc Dermott, W. *Am Rev Resp Dis*, 1967, *95*, 461.

[29] Boshoff, H. I. and Mizrahi, V. *J Bacteriol*., 2000, *182*, 5479.

[30] Boshoff, H. I.; Mizrahi V. and Barry C. E III., *J Bacteriol*., 2002, *184*, 2167.

[31] Zimhony, O.; Cox, J. S.; Welch J. T.; Vilcheze C. and Jacobs, W. R. Jr. *Nat Med*., 2000, *6*, 1043.

[32] Pelin, Yüksel. and Özlem, Tansel., *New Microbiologica*, 2009, *32*, 153.

[33] Mc Dermott, W. and Tompsett, R., Am. Rev. Tuberculosis., 1954, *70*, 748.

[34] Cyanamon, M. H. and Klemmens, S. P., *J. Med. Chem.*, 1982, *35*, 1212.

[35] Rogers, E. F.; Leanza, W. J.; Becker, H. J.; Matzuk, A. R.; O'Neill, R. C.; Basso, A. J.; Stein, G. A.; Solotorovsky, M.; Gregory, F. J. and Pfister, K. III., *Science*, 1952, *116*, 253.

[36] Kushner, S.; Dalalian, H.; Sanjurjo, J. L.; Bach, F. L.; Safir, S. R.; Smith, V. K. and Williams, J. H. *J Am Chem Soc.*, 1952, *74*, 3617.

[37] Felder, E.; Pitre, D. and Tiepolo, U. *Minerva Med.*, 1962, *53*, 1699.

[38] Chung, W. J.; Kornilov, A.; Brodsky, B. H.; Higgins, M.; Sanchez, T.; Heifets, L. B.; Cynamon and M. H. Welch, J., *Tuberculosis*, 2008, *88*, 410.

[39] Offe, H. A. "Historical introduction and chemical characteristics of antituberculosis drugs", In Bartmann K, (Editor). Antituberculosis drugs, Handbook of experimental pharmocology, Vol. 84. Berlin, Springer-Verlag, 1988, 1-30.

[40] Trinka, L. and Mison, P. "Drugs and treatment regimens, *p*-aminosalicylic acid (PAS)", In Bartmann K, editor. Antituberculosis drugs, handbook of experimental pharmocology, Vol. 84. Berlin Springer-Verlag, 1988, 51-68.

[41] Rengarajan, J.; Sassetti, C. M.; Naroditskaya, V.; Sloutsky, A.; Bloom, B. R. and Rubin, E. J. *MolMicrobiol*., 2004, *53*, 275.

[42] Rastogi, N. and Labrousse, V., *Antimicrob. Agents. Chemother*, 1991, *35*, 462.

[43] Inderlied, C. B.; Barbara-Burnham, L.; Wu, M.; Young, L. S. and Bermudez, L. E. M., *Antimicrob. Agents. Chemother.*, 1994, *38*, 1838.

[44] Mikusova, K.; Slayeden, R. A.; Besra, G. S. and Brennan, P. J. *Antimicrob. Agents. Chemother.*, 1995, *39*, 2484.

[45] Shepherd, R. G. and Wilkinson, R. G. *J. Med. Pharm. Chem*., 1962, *91*, 823.

[46] Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M. and Barry, CE, III,. *J. Comb. Chem.*, 2003, *5*, 172.

[47] Hausler, H.; Kawakami, R. P.; Mlaker. E.; Severn, W. B. and Stutz, A. E. *Bioorg. Med. Chem. Lett.*, 2001, *11*, 1679.

[48] Suzuki, Y.; Suzuki, A.; Tamaru, A.; Katsukawa, C. and Oda, H. *J. Clin. Microbiol.*, 2002, 501.

[49] Belanger, A. E,; Besra, G. S.; Ford, M. E.; Mikusova, K.; Belisle, J. T.; Brennan, P. J. and Inamine, J. M. *Proc. Natl. Acad. Sci., USA*, 1996, *93*, 11919.

[50] Otten H., "Experimental evaluation of efficacy VII. Cycloserine (CS) and terizidone (TZ)", In Bartman K, (Editor). Antituberculosis drugs. Handbook of experimental pharmacology, Vol. 44, Berlin, Springer- Verlog; 1988, 158-166.

[51] Peisach, D.; Chipman, D. M.; Van Ophem, P. W.; Manning, J. M. and Ringe, D. *J. Am. Chem. Soc*., 1998, *120*, 2268.

[52] Feng, Z. and Barletta, R. G. *Antimicrob. Agents Chemother.*, 2003, *47*, 283.

[53] David, H. L.; Takayama, K. and Goldman, D. S. *Am. Rev. Resp. Dis.*, 1969, *100*, 579.

[54] Cacers, N. E.; Harris, N. B.; Wellehen, J. F.; Feng, Z.; Kapur, V. and Barletta, R. G. *J. Bacteriol.*, 1997, *179*, 5046.

[55] Kim, M. G.; Strych, U.; Krause, K.; Benedik, M. and Kohn, H. *J. Antibiot.* (Tokyo), 2003, *56*, 160.

[56] Neuhaus, F. D-cycloserine and O-carbamoyl-D-serine. In: Gottlieb, D. and Shaw, P. (Editors) Antibiotics I (mode of action) pp 40-83. 1967 Springer-Verlag, New York.

[57] Lancini, G. and Zanichelli, W. Structure-activity relationships in rifamycins. In: Perlman, D. (Editors) Structure-activity relationships among the semisynthetic antibiotics. 1977 Academic, New York, pp 531-600.

[58] Campbell, E. A.; Korzheva, N.; Mustaev, A.; Murakamin, K.; Nair, S.; Goldfarb, A. and Darst, S. *Cell*, 2001, *104*, 901.

[59] Baysarowich, J.; Koteva, K.; Hughes, D. W.; Ejim, L.; Griffiths, E.; Zhang, K.; Junop, M. and Wright, G. D. *Proc. Natl. Acad. Sci. USA*, 2008, *105*, 4886

[60] Benator, D.; Bhattacharya, M.; Bozeman, L.; et al. *Lancet*, 2002, *360*, 528.

[61] Sterling, T. R.; Villarino, M. E.; Borisov, A. S.; et al. *N. Engl. J. Med*., 2011, *365*, 2155.

[62] Andries, K.; *et al. Science*, 2005, *307*, 223.

[63] Benator, D.; *et al.*, *Lancet*, 2002, *360*, 528.

[64] Steingart, K. R.; *et al. Int. J. Tuber. Lung Dis.*, 2011, *15*, 305.

[65] John, S. W. and David, C. H. *Clin. Microb Rev*, 1989, *2*, 378.

[66] Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H. and Brundage, R. P. *J. Med. Pharm. Chem.*, 1962, *91*, 1063.

[67] Tumbanatham, A. and Vinodkumar, S. *J. Assoc. Physicians. India*., 2000, *48*, 647.

[68] Renau, T. F.; Gage, J. W. and Dever, J. A. *Antimicrob. Agents. Chemother.* 1996, *40*, 2363.

[69] Albrecht, R. *Prog. Drug. Res.*, 1977, *21*, 9.

[70] Wolfson, J. S. and Hooper, D. C., *Antimicrob. Agents. Chemotherp.*, 1985, *28*, 581.

[71] Cole S. T., *et al*. *Nature*, 1998, *393*, 537.

[72] Mandell, L. and Tillotson, G. *Can. J. Infect. Dis*., 2002, *13*, 54.

[73] Mitscher, L. A., *Chem. Rev*., 2005, *105*, 559.

[74] Liu, H. H., *Drug Saf*., 2010, *33*, 353.

[75] Zumla, A.; Nahid, P. and Stewart T. Cole, *Nature Rev. Drug Disc.*, 2013, *12*, 388.