Chapter 1

Introduction

1.1 Tuberculosis Overview

Tuberculosis or TB is an infectious systemic chronic granulomatous disease caused by *Mycobacterium Tuberculosis* (MTB). During the 17th and 18th centuries, TB caused up to 25% of all deaths in Europe. TB mainly spreads through the respiratory system. The usual site for tuberculosis is the lungs but other organs may be involved. It can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin. Other mycobacteria such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, and *Mycobacterium microti* also cause TB, but these species are less common (Parthiban, Prabhu, Muthuraj, Elavazhagan, & Manupriya, 2009).

TB is a global disease which is not only specific to humans. There are variants of the TB bacterium that infect cattle (milk was known to transmit the disease from cattle to humans before heat treatment pasteurization), birds, fish, turtles and frogs (Shih-Ching, Hung-Yuan, & Chun-Yen, 2010).

TB is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel TB germs, known as bacilli, into the air. A person needs only to inhale a small number of these to be infected ("Tuberculosis," 2010).

Persons who infected with tuberculosis harbor the bacteria without developing symptoms is called latent TB infection. In this case, the bacteria are inactive and kept in check by the body's immune defense system. The person does not feel sick. However, the disease may become active in the future. It is estimated that one-third of all living beings are latently infected ("Tuberculosis," 2011).

More than 90% of TB cases occur in developing countries that have poor hygiene and health-care resources and high numbers of people infected with

HIV/AIDS. People with HIV/AIDS are particularly vulnerable to reactivation of latent TB infection to active disease. This is because the HIV-infected individual has weakened immune system. About one-third of the more than 40 million HIV/AIDS patients are co-infected with TB bacteria. TB is actually the cause of death in many HIV-infected individuals. In fact, anything which weakens the body's immune defense system can cause latent TB infection to become active disease (Tapiero & Lamarre, 2003).

1.2 Causes of Tuberculosis

TB was discovered by Robert Koch, a German physician and scientist, in 1882 and established TB as an infectious disease. *Mycobacterium Tuberculosis* (MTB) is a fairly large nonmotile rod-shaped bacterium related to the *Actinomycetes* [1]. The rods are 2 to 4 μm in length and 0.2 to 0.5 μm in width. It divides every 16 to 20 hours that an extremely slow rate when compared with other bacteria. MTB is not classified as either Gram-positive or Gram-negative because it does not have the chemical characteristics of either. They are classified as acid-fast Gram-positive bacteria (AFB) due to their lack of an outer cell membrane and their fatty cell walls prevent the cells from being decolorized by acid solutions after staining during diagnostic tests. The cell wall structure of MTB deserves special attention because it is unique among prokaryotes. It is a major determinant of virulence for the bacterium. This cell wall appears to allow MTB to survive in its preferred environment. The acid-fast cell wall of *Mycobacterium* contains a large amount of glycolipids, especially mycolic acids. The peptidoglycan layer is linked to arabinogalactan (D-arabinose and D-galactose) which is then linked to high-molecular weight mycolic acids. The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidyinositol mannosides (PIM).

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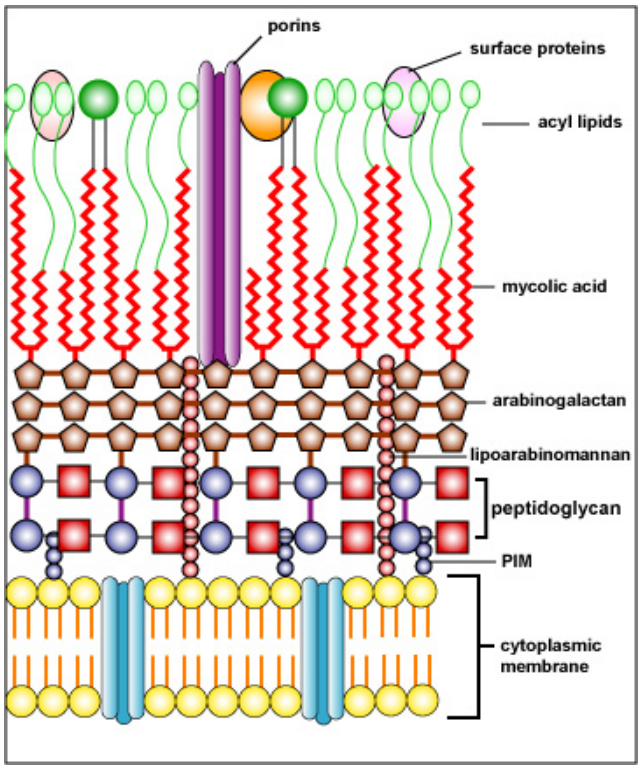
[1] ***Actinomycetes*** Any of various filamentous or rod-shaped, often pathogenic microorganisms of the order Actinomycetales that are found in soil and resemble bacteria and fungi.

Like the outer membrane of the gram-negative cell wall, porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall **(Figure 1)**.



Source: From http://student.ccbcmd.edu/courses/bio141/lecguide/unit1/prostruct/u1fig11.html

Figure 1. The Mycobacterial cell wall structure

Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of MTB's cell wall consists of three major components such as mycolic acids, cord factor, and wax-D (Todar, 2011).

Mycolic acids are high-molecular weight fatty acid. They are complex hydroxylated branched-chain fatty acids with elevated carbon numbers (60-90). They may also contain diverse functional groups such as methoxy, keto, epoxy ester group and cyclopropane ring. Their common structure was elucidated in 1950 by Asselineau J and shown to be formed of a β-hydroxy-α-alkyl branched chain

(Figure 2). R_1 may or may not contain another oxygenated group, such as hydroxyl, methoxyl, keto, or carboxyl group (Asselineau & Lederer, 1950).

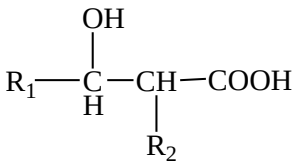
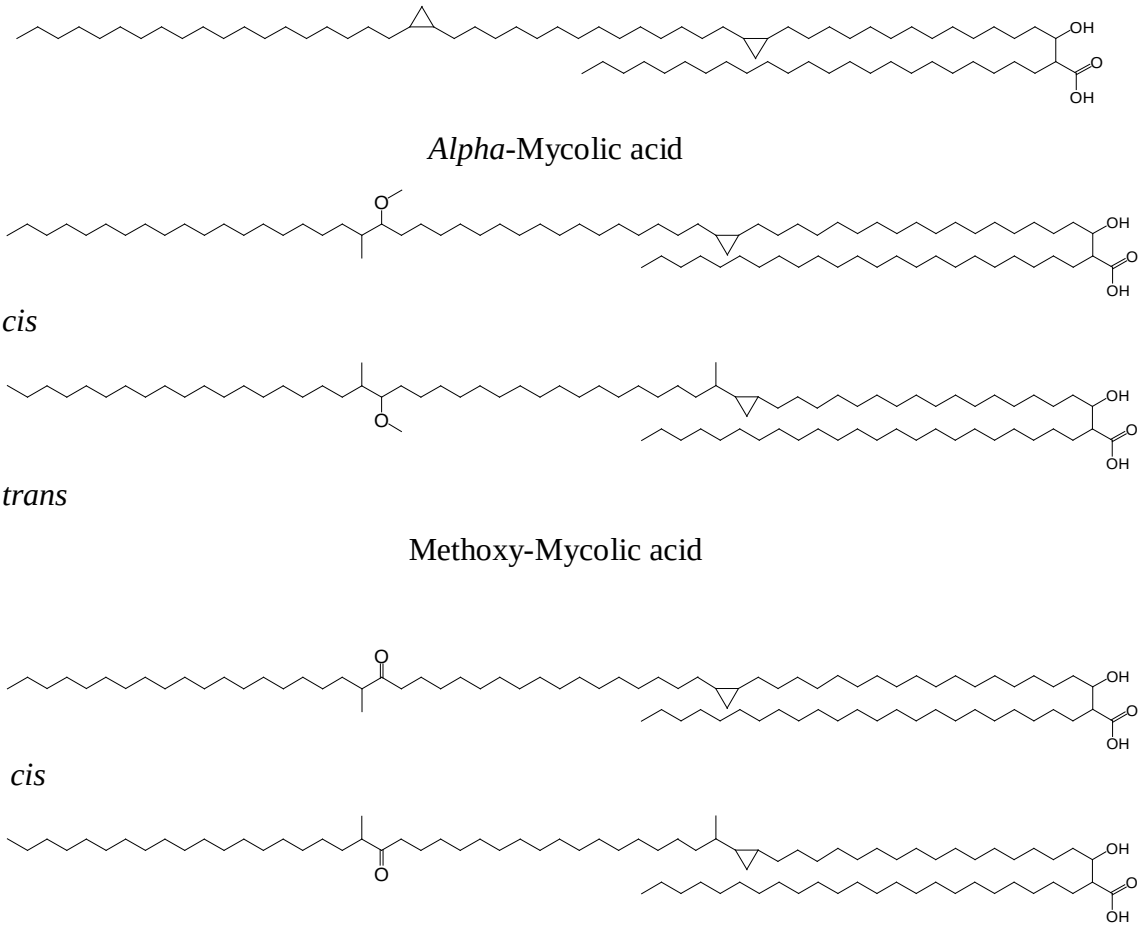


Figure 2. Mycolic acid structure

Mycolic acids isolated from *Mycobacterium* are called mycolic acids (or eumycolic acids), they have 60-90 carbon atoms, while those from other species (*Corynobacterium, Nocardia*) which are shorter are named corynomycolic (22-36 carbons) or nocardomycolic (44-60 carbons) acids (Minnikin, Minnikin, Parlett, Goodfellow, & Magnusson, 1984).

MTB produces three main types of mycolic acids including *alpha*-, methoxy-, and keto- **(Figure 3)**.



Source: From http://en.wikipedia.org/wiki/Mycolic_acid

Figure 3. Mycolic acids in *Mycobacterium tuberculosis*

The unsaturation and cyclopropanes may be either *cis* or *trans* (with adjacent methyl branch), they are also known as α-mycolic acids. Mycolic acids containing a methoxy group with double bond or cyclopropane ring are known as methoxymycolic acids. mycolic acids containing an a-methyl-branched ketone are known as keto-mycolic acids.

The presence of mycolic acids gives MTB many characteristics that defy medical treatment. They lend the organism increased resistance to chemical damage and dehydration, and prevent the effective activity of hydrophobic antibiotics (Barry, et al., 1998).

Cord factor is a molecule generated from trehalose dimycolate **(Figure 4)** by *Mycobacterium tuberculosis*. It was found in the cell walls of mycobacteria. It is toxic to mammalian cells. Also, it is most abundantly produced in virulent strains of MTB.

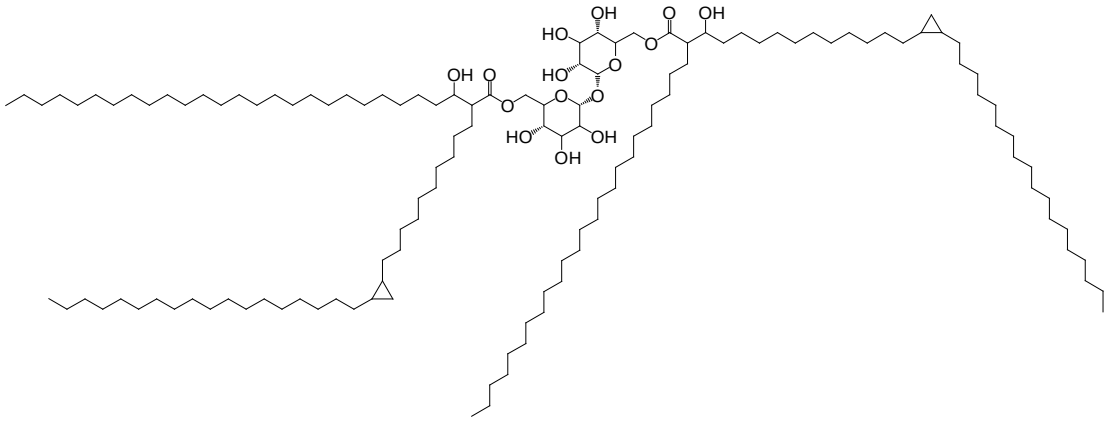


Figure 4. Trehalose dimycolate

Wax-D is macromolecular peptidoglycolipids **(Figure 5)** with important biological properties such as Freund's adjuvant effect [1] and the induction of adjuvant arthritis [2] (Jollès, Migliore, & Bonhomme, 1968).

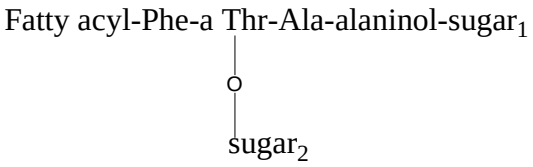


Figure 5. Common structure of acetylated peptidoglycolypid

[1] **Freund's adjuvant** is an antigen solution that is emulsified in mineral oil and is used as a booster of the immune.

[2] **Adjuvant arthritis** is an experimental immunopathy that is thought to share many features of human rheumatoid arthritis and, as such, is one of the most widely used models for studying the anti-inflammatory properties of compounds.

The high concentration of lipids in the cell wall of MTB have been associated with properties of the bacterium such as impermeability to stains and dyes, resistance to many antibiotics, resistance to be killed by acidic and alkaline compounds, resistance to cytolysis [1] *via* complement deposition and resistance to lethal oxidations and survival inside of macrophages, etc.

1.3 Tuberculosis problems

Since World Health Organization (WHO) declared TB as a global emergency in 1993. TB eradication has become a matter of greater concern among the national, international and local health authorities. Over the past decade, at least 30 million individuals have died from the disease and estimated that one-third of the world's population has been infected with latent MTB and 95% which occured in developing countries. The highest incidence and burden of disease is observed in India, China, Indonesia, Nigeria, and Bangladesh, etc. Moreover, WHO estimated that between 2000 and 2020, about 1 billion people will be newly infected, 200 million people will get sick, and 35 million will die from TB.

Although TB can be cured with current drugs treatment, it is complex and long-lasting, involving four drugs for two months and two drugs for at least another 4 months. This makes compliance difficult. Drug resistant tuberculosis is transmitted in the same way as regular TB. Primary resistance occurs in persons who are infected with a resistant strain of TB. A patient with fully susceptible TB develops secondary resistance during TB therapy because of inadequate treatment, not taking the prescribed regimen appropriately, or using low quality medication. Multi-drug-resistant TB (MDR-TB) is defined as resistance to the two most effective first-line TB drugs. Extensively drug-resistant TB (XDR-TB) is also resistant to three or more of the six classes of second-line drugs. Moreover, HIV has dramatically increased the risk of developing active tuberculosis and HIV co-infection makes tuberculosis more difficult to diagnose (due to more complicated presentations) and treat (due to

[1] **Cytolysis** or osmoticlysis occurs when a cell bursts due to an osmotic imbalance that has caused excess water to move into the cell.

interactions and side-effects). The increasing emergence of multi-drug resistant TB (MDR-TB) and the recalcitrant nature of persistent infections pose additional challenges to treatment with conventional anti-TB drugs (Casenghi, 2006).

Several studies have shown that MDR-TB can be cured by a combination of second-line drugs under DOTS-Plus [1] to improve compliance for the difficult and long regimen that need to be addressed in areas where there is high prevalence of MDR-TB. Thus, DOTS-Plus works as a supplement to the standard DOTS strategy. By definition, it is impossible to conduct DOTS-Plus in an area without having an effective DOTS-based TB control program in place. ("Tuberculosis (TB) ", 2003)

The treatment strategy was proposed by the WHO to address the management of MDR-TB in settings with good control programs. However, these drugs are expensive and have to be taken for long periods and can caused adverse reactions. Despite the need for better TB therapies, no new TB drugs have been introduced over the last 40 years. Due to this concern, this infectious disease was the focus of renewed scientific interest in the last decade.

1.4 Tuberculosis treatment

1.4.1 Antituberculosis drugs

The chemotherapy of tuberculosis has much evolved along the years since it started with the introduction of streptomycin **5** in 1946. By 1955, the combination of streptomycin **5**, *p*-aminosalicylic acid **6** and isoniazid **1** was adopted as a standard treatment by the western world.

It will never be emphasized enough that the full observance of the treatment is probably at least as important as the level of efficiency of the drugs administered for a proper cure. The lack of treatment observance is likely to become the main cause of the occurrence and spread of multi drug resistant strains of MTB. Two regimens

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[1] **DOTS-Plus** (Directly Observed Treatment-Plus) a comprehensive management strategy under development and testing that includes the five tenets of the DOTS strategy. DOTS-Plus takes into account specific issues such as the use of second-line anti-TB drugs

emerged from the many trials and involve first a two-month long treatment with four drugs; either: isoniazid **1**, rifampin **2,** pyrazinamide **4** and streptomycin **5** or isoniazid **1**, rifampin **2**, ethambutol **3** and pyrazinamide **4**. This is then followed by four months of isoniazid **10** and rifampin **11**. Side effects, especially hepatotoxicity, are an issue which in some cases forces an untimely treatment termination. However, the occurrence of multidrug resistant (MDR) strains of MTB, at least resistant to the action of isoniazid **1** and rifampin **2**, implies recourse to other compounds. Treatment guidelines for these cases have much less easily been established and are complicated by drug side effects and co-infections with HIV-1 (Costa, et al., 2006).

The first-line drugs are the most effective and have lowest toxicity **(Figure 6)**.

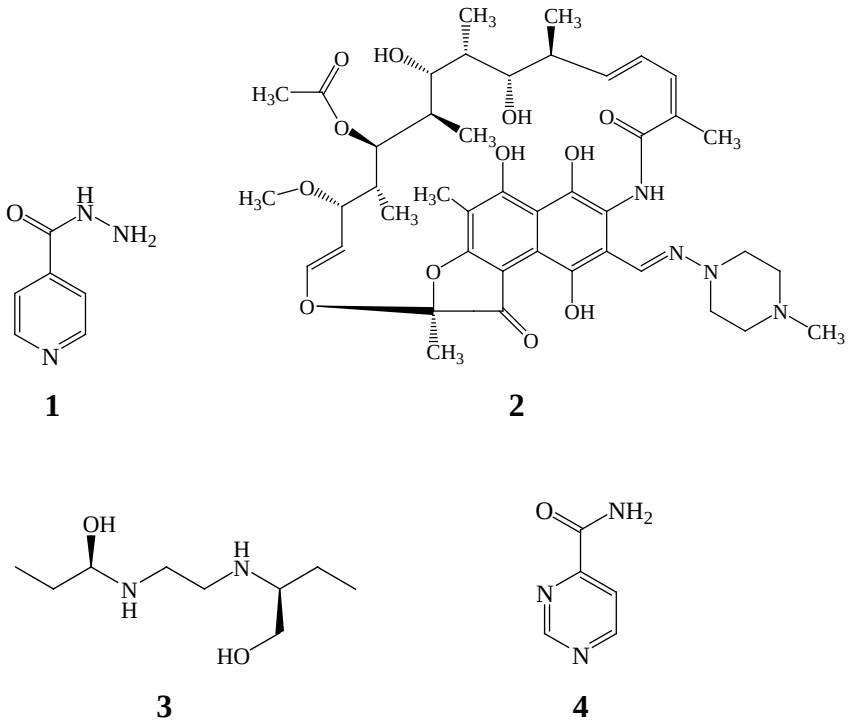
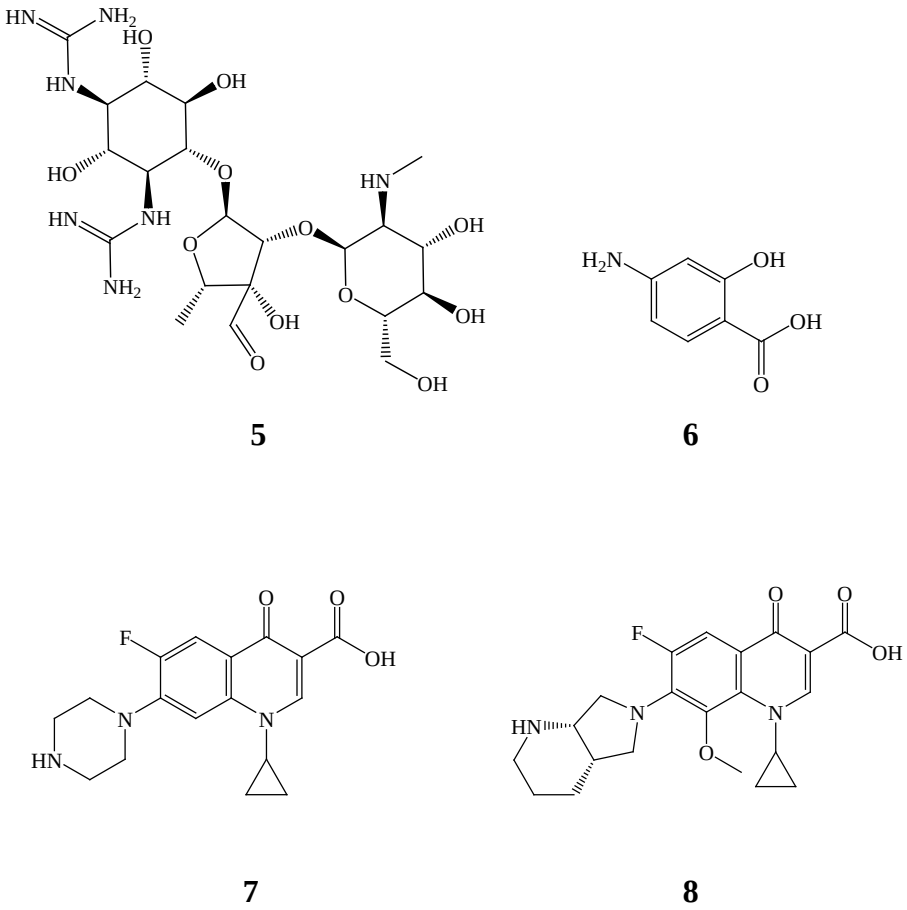


Figure 6. The first-line tuberculosis drugs

The second-line drugs are less effective and more toxic effects than first-line drugs. These drugs may be unavailable in many developing countries. They include streptomycin **5**, *p*-amino salicylic acid **6**, ciprofloxacin **7** and moxifloxacin **8** **(Figure 7)**.



5 **6**

7 **8**

Figure 7. The second-line tuberculosis drugs

The third-line drugs are least effective and most toxic. They include amikacin **9**, linezolid **10**, clarithromycin **11**, kanamycin **12,** cycloserine **13**, R207910 **14**, capreomycin **15,** and viomycin **16.** These drugs were considered as "third-line drugs" because they are not very effective or because their efficacy has not been proven **(Figure 8)**.

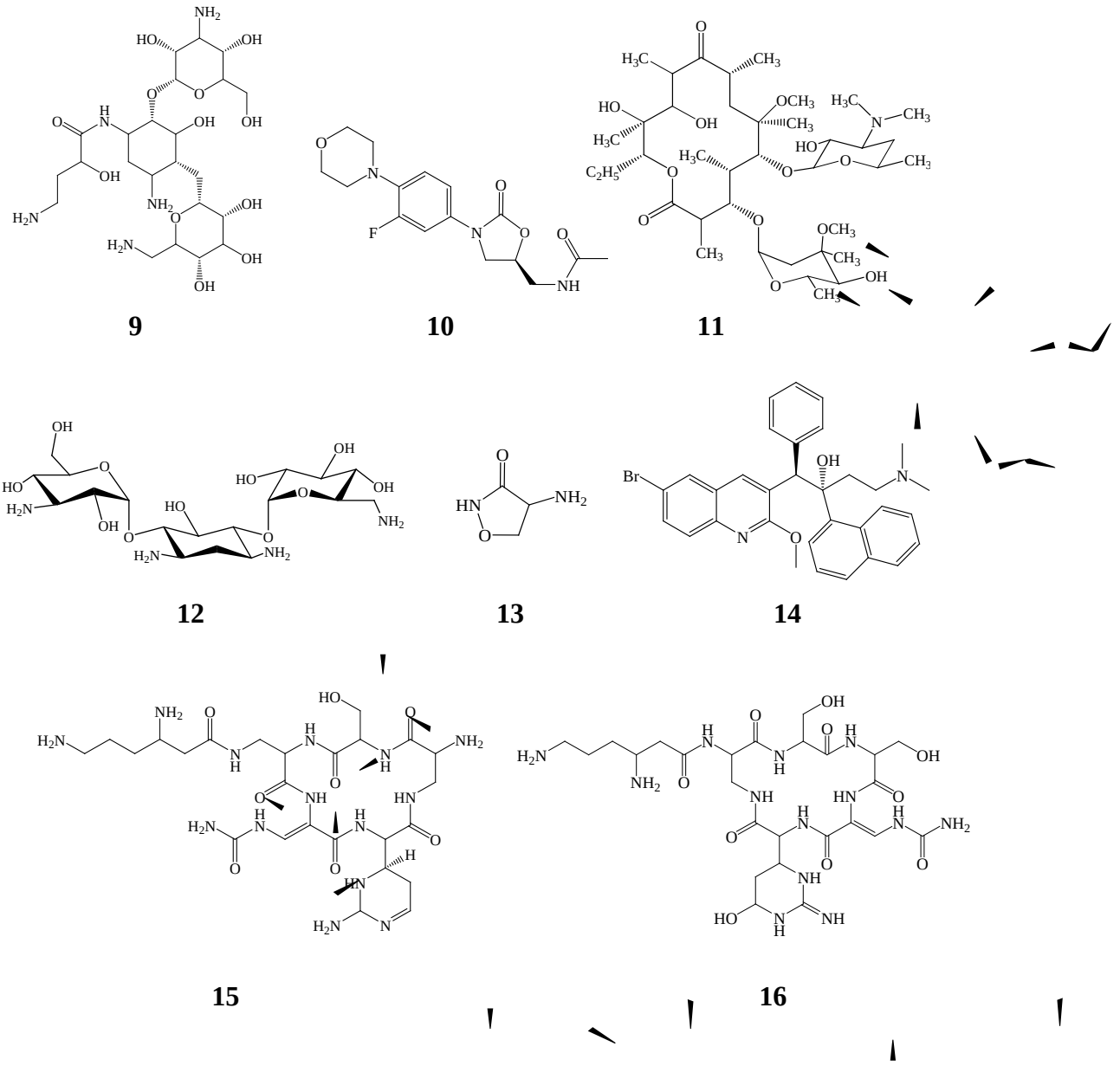


Figure 8. The third-line tuberculosis drugs

The introduction of the antibiotics for TB treatment some 50 years ago led to optimism that the disease could be controlled if not eradicated. These medicaments, coupled with generally increasing standards of health care, caused a rapid decline of tuberculosis in many industrialized countries which produced a climate of indifference to the need for fresh drugs. As a result of this apathy and the perception by the pharmaceutical industry that such agents would be unlikely to generate a suitable return on investment, few new drugs have been introduced in the last 30

years. There is now recognition that new drugs to treat TB are urgently required, specifically for use in shorter treatment regimens than are possible with the current agents and which can be employed to treat multidrug-resistant and latent disease. A variety of new initiatives have been created to tackle these objectives.

1.4.2 Targets and mode of action of current TB drugs

Current chemotherapy for TB largely relies on drugs that inhibit bacterial metabolism with a heavy emphasis on inhibitors of the cell wall synthesis. According to their mode of action, first and second line TB drugs can be grouped as cell wall inhibitors (isoniazide **1**, ethambutol **3**, ethionammide and cycloserine **13**), nucleic acid synthesis inhibitors (rifampicin **2**, quinolones **7**, **8**), protein synthesis inhibitors (streptomycin **5** and kanamycin **12**) and inhibitors of membrane energy metabolism (pyrazinamide **4**). Targets and mechanisms of action of current TB drugs are summarized in Table 1 (Casenghi, 2006).

Existing TB drugs are therefore only able to target actively growing bacteria through the inhibition of cell processes. For example cell wall biogenesis and DNA replication. This implies that current TB chemotherapy is characterized by an efficient bactericidal activity but an extremely weak sterilizing activity. It can be defined as the ability to kill the slowly growing or slowly metabolising bacteria that persist after the growing bacteria have been killed by bactericidal drugs. Sterilizing activity also describes the ability to eliminate latent or “dormant” bacteria that survive inside the host macrophages. This bias is hardly surprising as anti-TB drugs have traditionally been identified by their ability to suppress or kill replicating cultures of bacteria in *vitro*.

The weak sterilizing property of available TB drugs is one of the major drawbacks for current TB chemotherapy. Although rifampicin **2** and pyrazinamide **4** are partially sterilizing drugs and play an important role in shortening the therapy from 12-18 months to 6 months, there are still populations of persisting bacteria that are not killed by rifampicin **2** and pyrazinamide **4** Thus, although achieving a clinical cure, the current TB chemotherapy does not achieve a bacteriological cure since the therapy cannot completely eradicate all bacilli in the lesions (Casenghi, 2006)

TABLE 1.

COMMONLY USED TB DRUGS AND THEIR TARGETS

Drug (year of discovery)	MIC (μg/ml)	Effect on bacterial cell	Mechanisms of action	Target
Isoniazid (1952)	0.01-0.2	Bactericidal	Inhibition of cell wall mycolic acid and other multiple effects on DNA, lipids, carbohydrates and NAD metabolism	Primarily acyl carrier protein reductase (InhA)
Rifampin (1966)	0.05-0.5	Bactericidal	Inhibition of RNA synthesis	Membrane energy metabolism
Pyrazinamide (1952)	5.5-6.0	Bactericidal	Disruption of membrane transport and energy depletion	Membrane energy metabolism
Ethambutol (1961)	1.0-5.0	Bacteriostatic/ Bactericidal	Inhibition of cell wall arabinogalactan synthesis	Arabinosyl transferase
Streptomycin (1944)	2.0-8.0	Bacteriostatic	Inhibition of protein synthesis	Ribosomal S12 protien and 16S rRNA
Kanamycin (1957)	1.0-8.0	Bactericidal	Inhibition of protein synthesis	Ribosomal S12 protien and 16S rRNA
Quinolones (1963)	0.2-4.0	Bactericidal	Inhibition of DNA replication and transcription	DNA gyrase
Ethionammide (1956)	0.6-2.5	Bacteriostatic	Inhibition of mycolic acid synthesis	Acyl carrier protein reductase (InhA)
p-aminosalicylic acid (1946)	1.0-8.0	Bacteriostatic	Inhibition of folic acid and iron metabolism	Unknown
Cycloserine (1952)	5.0-20	Bacteriostatic	Inhibition of peptidoglycan synthesis	D-alanine racemase

Source: Casenghi, M. (2006). *DEVELOPMENT OF NEW DRUGS FOR TB CHEMOTHERAPY Analysis of the current drug pipeline*: Médecins Sans Frontières.