

CHAPTER 1- Biopharmaceutic and Pharmacokinetic considerations in current tuberculosis chemotherapy

Abbreviations

AcpM -acyl carrier protein

AIDS- acquired immune deficiency syndrome

ARTs- antiretrovirals

AUC- area under the concentration-time curve

BCS- biopharmaceutic classification system

CNS -nervous system

$C_{\max}$ - maximum plasma concentration

DRIF- 25-desacetyl rifampicin

embCAB -transferase-encoding gene for ETB

ETB- ethambutol

FAS -fatty acid synthase

FDC- fixed dose combination

FPE- first-pass effect

GAT- gatifloxacin

GIT -gastrointestinal tract

gyrA -DNA gyrase, subunit A

HIV- human immune deficiency virus

HYD- isonicotinylhydrazone

INH- isoniazid

katG -catalase-peroxidase

kasA -ketoacyl protein synthetase

MBC- minimum bactericidal concentration

MDR-TB- multi-drug resistant TB

MIC- minimum inhibitory concentration

MXF- moxifloxacin

M.tb. - *Mycobacterium tuberculosis*

NNRTIs- non-nucleoside transcriptase inhibitors

PAE- post-antibiotic effect

PIs- protease inhibitors

PD- pharmacodynamics

PK- Pharmacokinetics

PZA- pyrazinamide

RIF- rifampicin

rpob -RNA polymerase β subunit

rpsl -ribosomal protein, small

TB- tuberculosis

$T_{\max}$ - time taken to reach this maximum concentration

WHO- World Health Organization

XDR-TB- extensively drug-resistant TB

1.1 Introduction

Approximately 70 years ago, tuberculosis (TB) was considered virtually incurable. It was with the discovery and market entry of streptomycin in 1944 and later the rifamycins in 1964, that the mortality rate of TB was no longer considered a crisis (Sanjay *et al.* 2004:315). However, the emergence of the acquired immune deficiency syndrome (AIDS) caused by the human immune deficiency virus (HIV) led to the resurgence of this opportunistic disease, TB (Friedland, 2007:252). TB persists in HIV positive individuals due to a compromised immune system (Masur *et al.* 2002:435). With the incidence of HIV/AIDS being the highest amongst adults between the ages of 18-25 (Charles *et al.* 2008:970), co-infection with TB has become the leading killer of young adults on a global scale, affecting almost one third of the global population. HIV-infection is of particular concern in patients infected with TB since significant pharmacokinetic interactions exists between anti-TB drugs and drugs used in the treatment of HIV. South Africa ranks as one of the 22 high burden countries contributing to approximately 80% of all TB cases globally. In 2007, the country had the seventh highest incidence of TB in the world (WHO, 2007:24). In 2010, South Africa was reported to have the third highest incidence of TB globally (range 0.40 million- 0.59 million). The global TB incidence rates are summarised in Figure 1.1 (WHO, 2011:11). The high burden areas are seen in the southern parts of Africa.

The primary reasons for the increase in TB cases are the high incidence of poor TB patient compliance caused mainly by drug side effects and inconsistent drug dosing, i.e. skipping prescribed doses. This leads to the emergence of drug resistant strains such as multi-drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) (Raviglione, 2006:1185). This appears to be especially relevant in poverty stricken developing countries, which are enormously affected by this disease (Zager & McNerney 2008:10). In 2007, there were a reported 0.5 million cases of MDR-TB with the largest incidence in India. XDR-TB has been reported from 55 countries (Vashishtha, 2009:2). Addressing these challenges worldwide are some of the key objectives of the TB Global Alliance, World Health Organization (WHO) and the Stop TB Partnership.

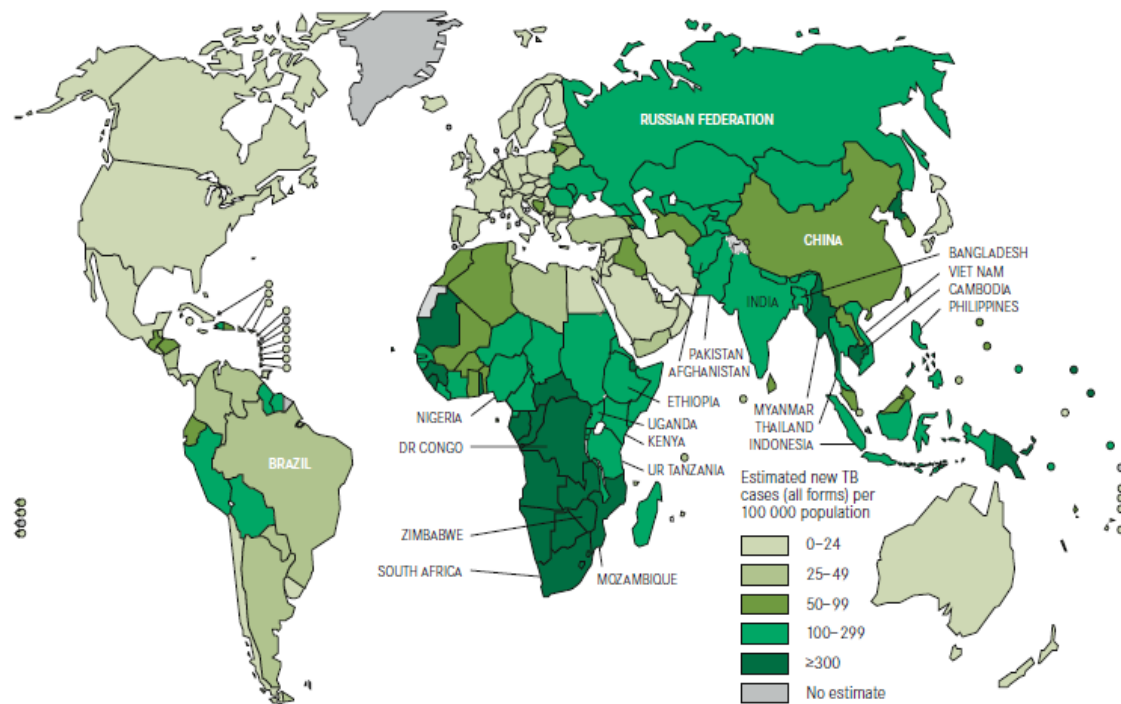


Figure 1.1 Global TB incidence rates in 2010 as reported in the WHO Global TB report. The shades of green assigned to each region illustrates the estimated new TB cases per 100 000 population. In the southern parts of Africa more than 300 per 100 000 population new TB cases have been reported (WHO, 2011:14).

Research into ways of combating this life threatening disease is currently underway. This includes the development of many novel drug candidates, antibiotics previously used for other bacterial infections as well as novel drug delivery systems are being explored. These novel drugs candidates are currently undergoing clinical trials (TB Alliance, 2007:1). All avenues of research are aimed at combating tuberculosis by developing therapeutic compounds that are low in toxicity and will reduce dose frequency and/or treatment duration and in so doing improve patient compliance. Poor patient compliance is ascribed to the rigorous and lengthy therapeutic regimen, which involves at least four antibiotics for a minimum for 6 months (Duncan, 2003:207).

1.2 Pathogenesis of TB

TB is a common and deadly bacterial disease caused by *Mycobacterium tuberculosis* (*M.tb*). Man is the primary host for *M.tb* and transmission occurs via airborne dissemination of aerosol droplets 0.5 to 5 μm in diameter. A single sneeze, for example, can release up to 40000 droplets. A person with active but untreated tuberculosis can infect 10-15 other people per year. About 90% of people infected have asymptomatic or minimal clinical

manifestations with only a 10% chance of disease progression or manifestation (Saunders & Britton 2007:104). When TB is latent or dormant, the person is unable to infect others. Patients with latent TB have a death rate of 50% if untreated (Saunders & Britton 2007:104).

TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within alveolar macrophages. The primary site of infection in the lungs is called the Ghon focus (Saunders & Britton 2007:105). Bacteria are picked up by dendritic cells, which do not allow replication, although these cells can transport the bacilli to local lymph nodes. Further spread is through the bloodstream to the more distant tissues and organs where secondary TB lesions can develop in lung apices, peripheral lymph nodes, kidneys, brain and bone (Van Dyck *et al.* 2003:1772). TB is classified as one of the granulomatous inflammatory conditions. Macrophages, T lymphocytes, B lymphocytes and fibroblasts are among the cells that aggregate to form a granuloma, with lymphocytes surrounding the infected macrophages. The granuloma functions not only to prevent dissemination of the mycobacteria, but also provides a local environment for communication of cells of the immune system (Saunders & Britton 2007). Within the granuloma, T_{helper} lymphocytes (CD4⁺) secrete cytokines such as interferon gamma (INF γ), which activates macrophages to destroy the bacteria with which they are infected. T_{cytotoxic} lymphocytes (CD8⁺) can also directly kill infected cells (Saunders & Britton 2007).

Understanding the pathogenesis of *M.tb* forms an important platform for targeting drugs to the desired site or pathway. One of the targeted cells are the macrophages since *M.tb* uses them as a “safe haven” as they concentrate and replicate within the macrophage. Hence, research in the field of drug carrier systems that are specifically targeted to infected macrophages is on-going (Ahsan *et al.* 2002:29). Drug targeting is discussed in further detail in chapter 2.

1.3 Challenges in TB chemotherapy

Mycobacteria are slowly growing organisms (Van Dyck *et al.* 2003:1772), making them relatively resistant to antibiotics, the activity of which depends on how rapidly the cells are dividing. Mycobacterial cells can also be dormant, resistant to many drugs or killed very slowly by the few drugs to which they are sensitive (Chambers, 2001:803). The lipid-rich mycobacterial cell wall is impermeable to many agents rendering these bacteria resistant. Furthermore, a substantial portion of mycobacterial organisms is intracellular, residing within

macrophages, and inaccessible to drugs that penetrate poorly. Thus, the use of a single drug is not an option due to resistance. Therefore, combination therapy remains the norm (Chambers, 2001:803).

Currently, the four first-line drugs administered to TB patients are isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (ETB), which involves an intensive phase with all four drugs for two months and a continuation phase of RIF and INH for the remaining four months of therapy (Chambers, 2001:803). The challenges in TB chemotherapy have been widely attributed to the emergence of drug resistant strains, patient non-compliance as well as the lengthy durations of therapy, thus resulting in the current high prevalence of TB.

With the emergence of MDR-TB (defined as strains of *M.tb* that are resistant to INH and RIF (Zager & McNerney 2008), concerted efforts to fight these pathogens have been employed, necessitating treatment of patients with second-line drugs, such as streptomycin and amikacin. These second line drugs have the disadvantage of being more costly, having increased toxic effects compared to first-line drugs and thus poor patient compliance. Data from genetic and molecular evaluations suggest that bacilli also acquire resistance either by altering the drug target as a function of gene mutation or by up regulation of the target gene. The gene loci involved in the conference of drug resistance in *M.tb* for RIF are RNA polymerase β subunit (*rpob*), catalase-peroxidase (*katG*) for INH, *pnca* gene encoding pyrazinamidase for PZA, transferase-encoding gene, *embCAB* for ETB, DNA gyrase, subunit A (*gyrA*) for the fluoroquinolones and ribosomal protein, small (*rpsL*) for the streptomycins (Rattan *et al.* 1998:196). The accumulation of the mutations within a patient at the drug targets, have been reported to lead to MDR- and XDR-TB (Rattan *et al.* 1998). In 2006, strains of *M.tb* which demonstrated lack of susceptibility to first-line drugs as well as moxifloxacin, ofloxacin, gatifloxacin and ciprofloxacin and at least three parenteral second-line drugs, kanamycin, amikacin and capreomycin was identified as XDR-TB (Zager & McNerney 2008).

The emergence of resistant strains has also been associated with patient non-compliance. Patients initiate therapy exposing the pathogen to the drugs and before sterility is achieved, patients stop treatment. Because of the pathogenicity of the bacteria, short exposure periods enable the bacteria to develop resistance mechanisms (Maartens & Wilkinson 2007:2031). In

addition, patients stop therapy when symptoms subside as the drugs exert their pharmacological effect. The dosage form's organoleptic properties are also not always favourable. Another consideration is that adverse side effects are often dose dependant and may differ on an individual basis; therefore certain patients end treatment when these effects, such as drug-induced hepatitis, become unbearable (Duncan, 2003). This then eventually leads to treatment failure.

Another challenge contributing to treatment failure is the duration of therapy. The pathogenesis of *M.tb* allows it to achieve a non-replicating or latent state within the host (Van Helden *et al.* 2006). Only once the host's immune system is compromised, such as is the case with HIV-infection, does reactivation occur (Manabe and Bishai 2000). However, the efficacy of most antibiotics is dependent on the replicating state of bacteria. This period of latency has been reported to be the mechanism that *M.tb* utilises to reach a state of phenotypic resistance to otherwise bactericidal antibiotics (Connolly *et al.* 2007:438). TB drug treatment data has shown that the time to reach complete cure correlates with the overall bacterial burden (Connolly *et al.* 2007:435). For this reason a longer duration of therapy is necessary to ensure that both active and dormant bacilli are eradicated.

1.4 Pharmacokinetic and pharmacodynamic factors of anti-TB drug therapy

Pharmacokinetics (PK) is defined as the study of the kinetics of drug absorption, distribution, metabolism and elimination. Pharmacodynamics (PD) is defined as the time course for the drug effect and the relationship between the drug concentration and the observed therapeutic effects (Hedaya, 2007:2-4). These are two of the most important considerations in drug development and therapy.

Another important factor is the bioavailability of a drug, which is the fraction of administered dose that reaches the systemic circulation. The route of administration is a major contributing factor. For instance, a drug administered via the intravenous route is 100% bioavailable since it is administered directly into the systemic circulation. Absorption of a drug determines the amount of drug which becomes available at the site of action and is not equal to the amount of drug administered since the metabolic pathway of drugs are affected by the contents in the

stomach and ‘first pass’ through the liver. The fraction of the drug eventually reaching the systemic circulation determines the bioavailability of the drug (Hedaya, 2007:1).

Drug distribution is important because it determines whether the drug reaches its site of action and drug elimination is important because the rate of elimination and factors influencing elimination are important for dose frequency and toxicity considerations (Hedaya, 2007:24). Understanding the pathway that a drug follows from the point of administration to the site of action would allow a calculated prediction of the extent and duration of drug effect. All of these parameters are crucial to successful drug and product development. They form a vital part of all clinical research in drug development (Mahato, 2007:3).

The PK and PD parameters of currently available tuberculosis drugs are variable (Davies & Nuermberger 2008) and a comprehensive understanding is needed to ensure accurate pharmacokinetic modelling during drug analysis with regards to bioavailability and efficacy of the drug in any new form of delivery vehicle or dosage form. Figure 1.2 illustrates the various factors influencing the biopharmaceutics of drugs and the correlation between pharmacokinetics and pharmacodynamics.

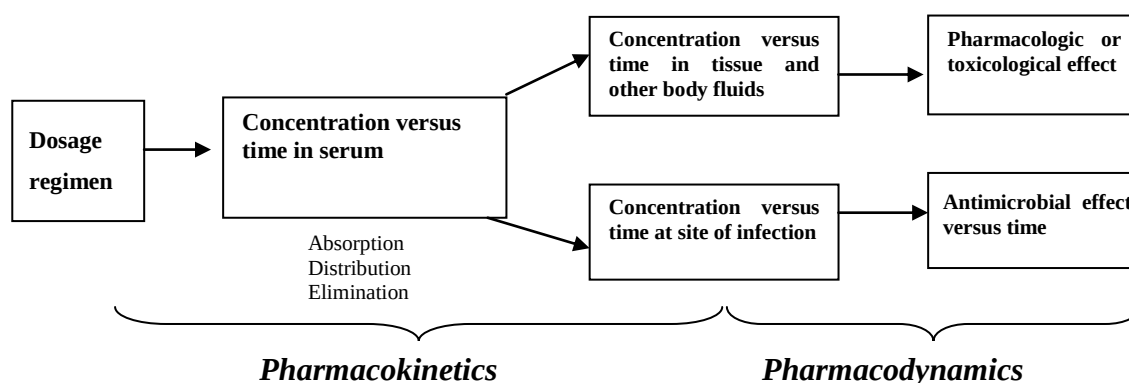


Figure 1.2 Classification of the relationship between pharmacokinetics (drug in serum) and pharmacodynamics (drug effect) following drug administration, adapted from (Craig, 1998:2).

1.4.1 PK/PD correlations with regards to bactericidal activity

Pharmacokinetic parameters include maximum plasma concentration ($C_{\max}$), which is the highest concentration reached or estimated in the compartment of reference. If $C_{\max}$ is high it is indicative of a high absorption rate. $C_{\max}$ is also an indication of the intensity of a therapeutic effect for drugs in which there is a direct relationship between plasma

concentration and the pharmacological response. The time taken to reach this maximum concentration is termed T_{max} . The area under the concentration-time curve (AUC), which is the area under the time vs. concentration curve (Hedaya, 2007:65; Mouton *et al.* 2002), has a value that is directly proportional to the amount of administered drug that reaches the systemic circulation in the same individual. This is also a useful parameter for measuring or comparing the extent of absorption from different formulations of the same active ingredient (Hedaya, 2007). Minimum inhibitory concentration (MIC) is the minimum concentration required to cause inhibition of bacterial activity. This is a pharmacodynamic parameter and combining MIC with any one of the above mentioned pharmacokinetic parameters will give an indication of the ratio and type of bactericidal killing the drug will exert (Hedaya, 2007).

Bactericidal activity can be classified as either concentration- or time-dependant (also termed concentration-independent) (Graham & McLeod 1999:135, Craig, 1998:1). Anti-mycobacterial drugs such as the aminoglycosides, fluoroquinolones and rifamycins, which have intracellular targets, are known for exhibiting concentration-dependant killing, which means that the rate of bactericidal killing increases with increasing concentration in which case the C_{max}/MIC and the AUC/MIC ratios determine the rate of killing. This has also been termed as non-saturable killing (Graham & McLeod 1999:135). As for drugs active against the cell wall exhibiting time or concentration- independent killing (also termed saturable-killing) (Graham & McLeod 1999:135), the rate of killing depends on the time at which the drug levels is maintained above the MIC, thus T_{MIC} is the rate limiting factor. The drug level has to be maintained above the MIC for as long as possible since increasing the concentration does not significantly increase the killing (Craig 1998:1; Graham & McLeod 1999:135; Li *et al.* 1999). For the latter, dosing frequency may have to be increased to prolong the time above the MIC as observed with current anti-tuberculosis drugs.

Based on early bactericidal activity studies in TB patients, as well as studies that show low 2-hour peak plasma concentrations in TB patients treated with 10mg/kg RIF (Mitchison, 2000:796), Ruslami *et al* (2007:2547) performed a randomised Phase II clinical trial to test the hypothesis that increasing the standard dose of 450 mg (10 mg/kg standard dose used for test group in Indonesia) to a higher dose of 600 mg in addition to the other first-line drugs, could increase the efficacy of RIF, which would result in a decrease in dosing frequency. An improved pharmacokinetic profile was seen for the increased dose with only mild hepatotoxicity observed. There is insufficient data to conclude that this would allow for a

decrease in dosing frequency of RIF and further studies are warranted (Ruslami *et al.* 2007:2548).

1.4.2 Persistent growth inhibition

The appearance of persistent growth inhibition, commonly known as the ‘post-antibiotic effect’ (PAE) is an important factor in the PK/PD relationship of antimicrobials. This effect can be observed *in vitro* as a period of growth inhibition after a defined period of exposure to an antibacterial compound and may be representative of the time required for the organism to recover from cellular injury (Graham & McLeod 1999:135), or for the dissociation of the antimicrobial from its receptors or both (Li *et al.* 1999). This holds potential significance since dose intervals may be prolonged because bacterial re-growth continues to be inhibited when serum concentrations fall below the MIC.

It has been suggested that the PAE requires prolonged exposure to the antimicrobial agent to exert a maximal effect (Graham & McLeod 1999:135), thus since current first-line treatment of tuberculosis requires combination therapy of drugs displaying varying pharmacokinetic and pharmacodynamic properties, it could also be suggested that this would enhance the PAE and leave room for further investigation into dose frequency reduction. TB drugs such as INH, RIF, streptomycin and ETB have been established as displaying PAE against *M.tb* (Duncan, 2003:202). This effect, however, is highly dependent on the antimicrobial-bacterial combination (Li *et al.* 1999). Although PAE is a very positive finding its total efficacy and significance has not been fully explored. There are several factors contributing to reliable prediction of the PAE since most documented studies in this regard are based on *in vitro* models (Chan *et al.* 2004; Fursted 1997). *M.tb* has the characteristics of a prolonged doubling time, possibility of intracellular replication and a capacity for dormancy also termed as latency. Thus, the dynamics of PAE may not be as easily applied to the treatment of the mycobacteria.

The pharmacokinetic parameters mentioned earlier differ for different host species and microbial susceptibility. However, the pharmacodynamic parameters are able to correct these differences. Also, the magnitude of these parameters necessary to achieve efficacy are probably similar for host species, susceptible and resistant strains. Thus, using results from a suitable animal model to predict effect on human infections would be considered acceptable (Craig 1998; Nuermberger & Grosset 2004:243).

1.5 First-line TB drug regimens

The use of chemotherapy for the treatment of TB was started in 1940. This came as a result of the discovery of a number of compounds that were found to be active against *M.tb*. Most of these agents were discovered following a broad random screening that was conducted due to the lack of knowledge about the biochemistry of *M.tb* at the time (Tripathi *et al.* 2005:5). Thus, for the past six and a half decades, extensive research has gone into the development and enhancement of these agents. These drugs have achieved great success in TB chemotherapy and of these drugs, INH and RIF has been considered the keystones in modern chemotherapy (Tripathi *et al.* 2005:5).

Extensive studies into the mechanisms of tuberculosis drugs, alone and in combination are still on-going. RIF and INH are the most widely investigated (Benator *et al.* 2002; Brooks & Orme 1998; Dhillon & Mitchison 1992; Ellard, 1999; Grosset & Leventis 1983; Gurumurthy *et al.* 2004; Maggi *et al.* 1966; Pähkla *et al.* 1999; Panchagnula & Agrawal 2004; Shishoo *et al.* 1999; Takayama *et al.* 1972) and most effective anti-tuberculosis agents, but with a high incidence in drug resistance. Both of these drugs have significant characteristics in the mechanism of action with regards to pharmacokinetic and pharmacodynamic considerations. The PK/PD characteristics of each these drugs along with some of the other recommended tuberculosis drugs will be evaluated in the following sections.

The first step to a newly diagnosed TB case, which is based on a smear-positive test for *M.tb*, would be an intense initial therapy of INH, RIF, PZA and ETB or INH, RIF, PZA and streptomycin for a period of two months followed by four months of only INH and RIF (Chambers, 2001:803). Table 1.1 shows the recommended adult dosages worldwide (Chambers, 2001:804). Following the initial two-month therapy, the patient is tested again and if the sputum has converted then the patient progresses to the 4-month continuation phase. However, if the sputum is still smear-positive, then a follow-up is carried out and a decision tree followed (Van Helden *et al.* 2006). The decision tree is based on a model developed to assist health care workers and medical professional in the step-wise treatment of patients with unresponsive or unexpected treatment outcomes (Keeler *et al.* 2006:51). The mechanism of action of the first-line drugs along with compounds active against *M.tb* under development is illustrated in Figure 1.3. These mechanisms will be further elaborated in sections 1.5.1-1.5.5.

Table 1.1 Recommended daily dosages of first-line drugs in the treatment of TB (Chambers 2001:804).

Drug	Typical adult dosage
INH	300 mg/d
RIF	600 mg/d
PZA	25 mg/kg/d
ETB	15-25 mg/kg/d
Streptomycin	15 mg/kg/d

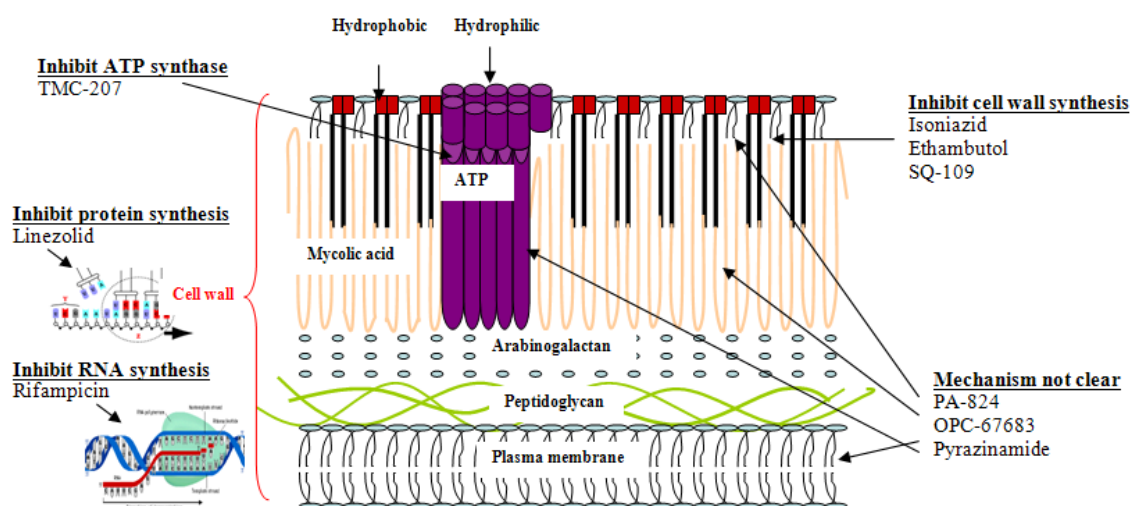


Figure 1.3 Illustration of the mycobacterial cell wall indicated the mechanism by which various anti-tuberculosis drugs exert their pharmacological effect adapted from (Brennan, 2011).

1.5.1 INH

INH (Figure 1.4) is a prodrug (Chambers, 2001:804; Tripathi *et al.* 2005:13) that is activated by the mycobacterial catalase-peroxidase (*katG*) to form isonicotinic acyl anion. Resistance mechanisms to INH occur as a result of mutation within the *katG* gene, which leads to the up regulation of the genes responsible for the type II fatty acid synthase (FAS) system. This occurs because of short exposure of the drug to the target site (Slayden *et al.* 2000). INH is the most active drug against susceptible strains of tuberculosis (Brennan, 2011; Chambers 2001:803).

It is the only bactericidal drug against actively dividing *M.tb*. However, it is bacteriostatic in the presence of semi-dormant organisms, thus having a less sterilizing effect than RIF and PZA (Graham & McLeod 1999:129). In its activated form, the lethal effect is exerted by the formation of a covalent complex with an acyl carrier protein (*AcpM*) and a beta-ketoacyl protein synthetase (*kasA*), which then blocks mycolic acid (MA) synthesis (Chambers 2001:804). MAs are essential components of mycobacterial cell walls. Takayama *et al* (1972), investigated the effect of INH on the *in vitro* synthesis of mycolic acids in *M.tb* H₃₇R_a using ¹⁴C-acetate as the metabolite and found that at a concentration of 0.5 µg/ml, INH began to reduce the cells' ability to synthesize mycolic acids (Takayama *et al.* 1972).

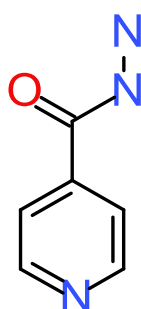


Figure 1.4 Chemical structure of INH also known as isonicotinylhydrazine with the chemical formula C₆H₇N₃O.

1.5.1.1 Pharmacokinetics

INH is readily absorbed, but the absorption and therefore the bioavailability, is affected by the intake of food due to a delay in gastric emptying time which decreases the window for absorption through the gastrointestinal tract (GIT) (Chambers, 2001:804). The adult dose of 300 mg/d and the children's dose of 5mg/kg results in peak plasma concentrations of 3-5 µg/ml within 1-2 hours. INH has a very low minimum inhibitory concentration (MIC) against the *M.tb* complex (*M. tuberculosis*, *M. bovis*, *M. articularum* and *M. microti*) with values ranging between 0.02 and 0.06 µg/ml (Tripathi *et al.* 2005:14).

In general, pharmacokinetics of drugs differs between individuals. In the case of INH, its half-life is influenced by the patient acetyltransferase activity, which is an enzyme found in the liver and the small intestines. These patients are referred to as either rapid or slow acetylators. The possible detrimental result of this is that for rapid acetylators the elimination half-life is 50% faster than for slow acetylators and the former may display poor therapeutic results. On the other hand, slow acetylators have an increased incidence of toxicity due to

drug accumulation. INH diffuses readily into all body fluids and tissues (Chambers, 2001:803).

1.5.1.2 Adverse effects

The most frequently observed toxic effect of INH is INH-induced hepatitis. This toxic effect is said to be idiosyncratic, i.e. the adverse effect is not related to the pharmacological drug properties, but rather dose dependant in susceptible individuals (thus having no effect at any dose in non-susceptible patients) (Lee, 2003; Tostmann *et al.* 2008:193). Therefore a dosage adjustment can be made without influencing therapeutic efficacy. The side effects include loss of appetite, nausea, vomiting and jaundice. Patients (1%) receiving INH may also experience right upper quadrant pain and this can be fatal (Chambers, 2001:805). This adverse effect appears to be largely dependent on age with an occurrence of 0.3% of those aged 21-35, 1.2% of those aged 36-50, 2.3% for patients above 50 and a negligible occurrence in patients under 20 (Chambers, 2001:806; Graham & McLeod 1999:132).

An incidence of 10-20% of patients present with peripheral neuropathy when given in doses greater than 5 mg/kg/d. The likelihood of occurrence is higher in slow acetylators and patients with conditions such as malnutrition, alcoholism, diabetes and AIDS. This effect is due to a pyridoxine (Vitamin B6) deficiency due to the promotion of increased pyridoxine excretion by INH. Thus, the adverse effect can be avoided or reversed with the concomitant use of pyridoxine supplement. In patients treated with phenytoin for epilepsy, INH increases phenytoin toxicity by reducing its metabolism, therefore limiting the use of INH in TB patients suffering from this condition (Chambers, 2001:806).

1.5.1.3 Drug interactions

Various drugs or drug classes exhibit adverse interactions with concomitant use of anti-tuberculosis drugs. For INH, the drugs or drug types and their interactions with INH are presented in Table 1.2.

Table 1.2 Drug-drug interactions to be considered when INH is administered whilst patient is being treated for other illnesses

Drug or group of drugs	Drug-drug interaction
Acetaminophen	Increased toxicity caused by unfavourable metabolites
Antacids	Decreases INH absorption from the gastrointestinal tract
Anticoagulants (oral)	Increases the anticoagulant effect
Benzodiazepines	Increases the toxicity of benzodiazepines
Carbamazepines	Increases the toxicity of both drugs possibly due to decrease in elimination
Cycloserine	Increases the central nervous system (CNS) effects of Cycloserine
Disulfiram	May cause severe psychotic episodes. Absolute contraindication
Enflurane	Increased nephrotoxicity
Haloperidol	Increased haloperidol toxicity
Ketoconazole	Decreases the effect of ketoconazole
Phenytoin	Increases phenytoin toxicity as already mentioned
Theophylline	Increases theophylline toxicity
Valproate	Increases hepatic and central nervous system (CNS) toxicity

INH has significant interactions with RIF and antiretrovirals. This is discussed in detail in section 1.5.2.4.

1.5.2 RIF

RIF is a large, complex semi-synthetic derivative of rifamycin B. These two compounds are similar in structure except for the aromatic structure that occurs on the third carbon of the naphthoquinone of the RIF structure (Gianniosis, 2006:7). RIF (Fig 1.5a) binds strongly to the β subunit of bacterial DNA-dependant RNA polymerase (a complex oligomer consisting of various subunits encoded with *rpoA*, *rpoB*, *rpoC*, and *rpoD*) and thereby hindering transcription and consequently inhibits RNA synthesis (Chambers, 2001:806). The mutation in the *rpoB* gene results in conformational changes, which lead to defective binding of the drug and subsequent development of resistance (Rattan *et al.* 1998:200). When semi-dormant

tubercle bacilli undergo sporadic bursts of metabolism and growth RIF has the unique ability to kill the bacilli (Ellard & Fourie 1999:S301). It has poor aqueous solubility and it has also been established to be dose-dependent in its bacterial sterilizing action. It is active *in vitro* against gram-positive and gram-negative cocci, some enteric bacteria, mycobacteria and Chlamydia. Susceptible organisms are inhibited by less than 1 µg/ml.

1.5.2.1 Pharmacokinetics

RIF is bactericidal for mycobacteria with an MIC ranging from 0.1- 0.2 µg/ml (Tripathi *et al.* 2005:14). It readily penetrates most tissues and phagocytic cells. It can kill intracellular organisms and those sequestered in abscesses and lung cavities. It is well absorbed after oral administration and results in serum levels of 5-7 µg/ml (Chambers, 2001:806). A therapeutic concentration is reached 2-4 hours after oral administration (Graham & McLeod 1999) and is well distributed throughout the body tissues. RIF is a potent enzyme inducer, stimulates its own metabolism and is thus able to decrease its plasma half-life by as much as 3 hours. Plasma half-life for RIF is between 2.3 and 5 hours (Graham & McLeod 1999:129).

Studies have shown that rifampicin has high permeation from the small intestine and colon and thus has excellent oral bioavailability of about 92% (Agrawal & Panchagnula 2005). It is also a potent hepatic enzyme inducer and could thus stimulate the metabolism of other drugs passing the hepatic circulation. This is an important consideration in drug-drug interaction when a patient is on TB medication.

The elimination of RIF is affected by ‘the first-pass effect’ (FPE). The FPE occurs following oral administration and is described as the concentration of the drug reduced through elimination before it reaches the systemic circulation. After a drug is administered orally, it is absorbed by the digestive system and transported to the hepatic circulation. It is then transferred via the portal vein for ‘first-pass’ metabolism through the liver where it is metabolized. The fraction of drug eventually reaching the systemic circulation is referred to as the percentage bioavailability of the drug (Chambers, 2001:806; Holford, 2001:43). During this first elimination, RIF is progressively deacylated into its microbiologically active metabolite, 25-desacetyl rifampicin (DRIF), which is less absorbable than its parent drug (Ellard, 1999:S322; Ellard & Fourie 1999:S302; Panchagnula *et al.* 1999:1013). Modifications were made to the core structure of RIF to produce possible compounds with increased pharmacokinetics such as rifapentine shown in Fig 1.5c (Janin, 2008:2494).

Rifapentine has already been approved by the FDA for the use in tuberculosis therapy. It has similar inhibitory activity as rifampicin, but has a 25-to 50-fold greater accumulation in macrophages (Dhillon & Mitchison 1992). However, it has not been fully established whether it is a better compound than RIF (Janin 2008). Rifapentine has a longer serum half-life of 10-15 hours versus 2-5 hours for RIF (Benator *et al.* 2002) and thus has a possibility for once a week use instead of RIF's twice a week treatment. However, the overall efficacy is still reduced (Benator *et al.* 2002).

Phase I clinical trial results for another new compound, rifametane shown in Figure 1.5d, was published in 1999 (Potkar *et al.*, 1999:153) and has since advanced to Phase II (Janin, 2008:2494). It has a bactericidal spectrum and potency similar to that of RIF, but with much better pharmacokinetic properties. In a comparative study with RIF it was observed that MIC₉₀ values were the same for 20 strains of *M.tb*, but rifametane was more effective orally (Hudson *et al.* 2003:33). The elimination half-life for rifametane was 10.58 hours compared to 1.89 hours for RIF. The mean residence time was 18.05 hours for rifametane compared to 3.93 hours for RIF. During a phase I trial for rifametane serum drug levels above the MIC for *M.tb* were maintained for up to 48 hours after administration. The success of this compound has thus far demonstrated great potential as replacement drug for RIF.

Rifabutin Figure 1.4b is another derivative that has been considered as an alternative agent in tuberculosis therapy. However, Rifabutin presents a high level of toxicity *in vivo* and its use is limited (Barluenga *et al.* 2006:5717).

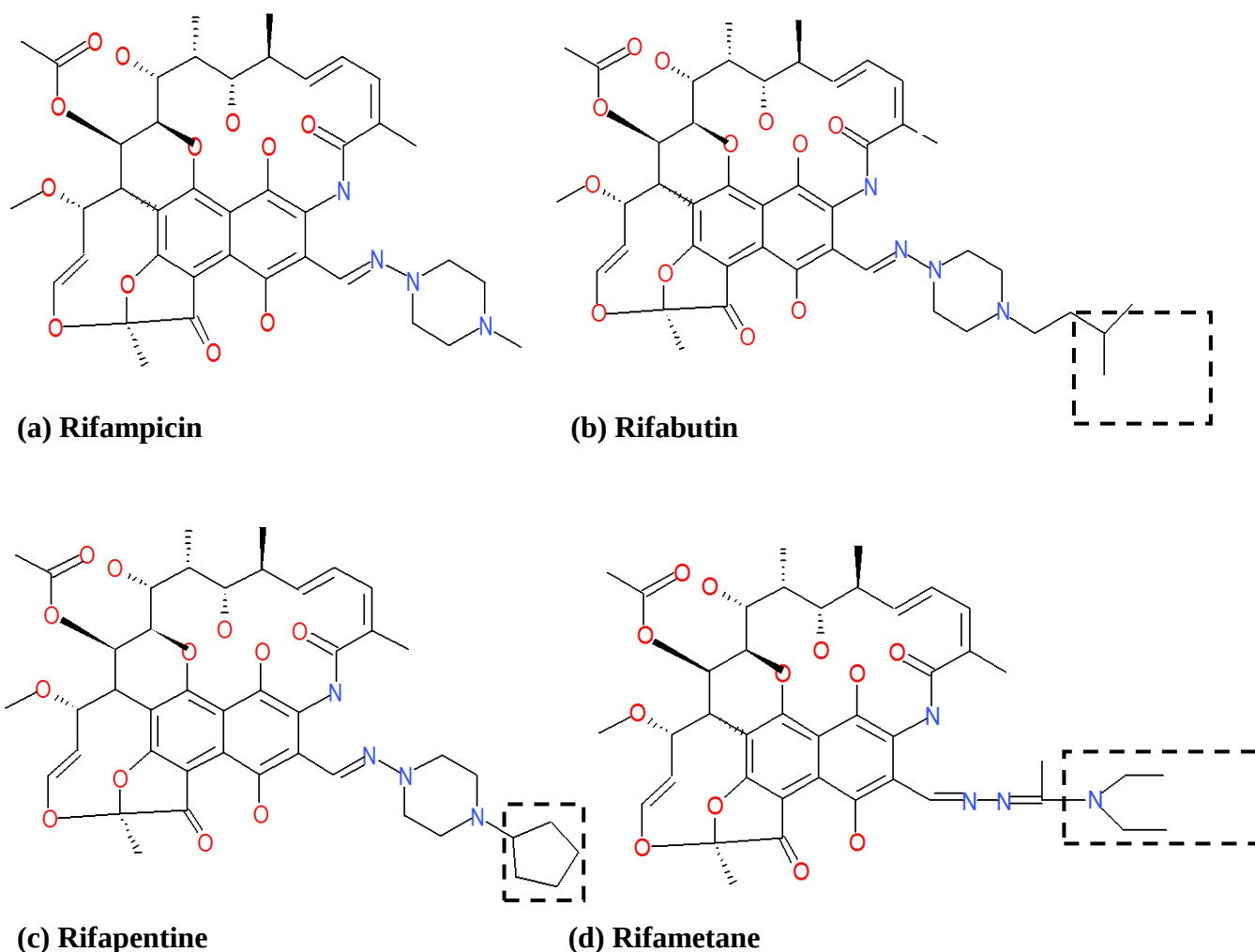


Figure 1.5(a) The chemical structure of RIF and the analogues (b) rifabutin, (c) rifapentine and (d) rifametane derived from the RIF structure (Hudson *et al.* 2003, Li *et al.* 1997). The dashed boxes indicate the structural differences that occur in the RIF analogues.

1.5.2.2 Adverse effects

Rifampicin colours the urine, tears, sweat and contact lenses orange. This is however a harmless side effect. Rifampicin induces the microsomal enzyme cytochrome P450, which is responsible for the elimination of numerous drugs such as methadone, anticoagulants, some of the anti-convulsants, protease inhibitors and contraceptives. These are important considerations since reduced serum levels of these drugs could have serious repercussions. Concomitant use of rifampicin with ketoconazole reduces rifampicin concentrations (Chambers, 2001:806).

1.5.2.3 Rifampicin bioavailability in fixed dose combinations (FDCs)

A Fixed Dose Combination (FDC) is the formulation of two or more active ingredients in a single dosage form (Bangalore *et al.* 2007) to limit the number of tablets/capsules per intake thus improving patient compliance. FDCs for anti-tuberculosis drugs have been formulated in either a tablet or capsule dosage form. The WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) encourage the use of FDCs for TB treatment. However, the poor bioavailability of the drugs in FDCs is the cause of great concern. Due to RIF's bacterial sterilizing activity being dose-dependent it is critical that TB control programmes should only use rifampicin-containing FDCs with proven rifampicin bioavailability (Ellard, 1999:S323). Pillai *et al* (1999:S310) conducted bioequivalence studies using 10 different RIF containing FDC formulations. The formulation consisted of either different concentrations of drug per tablet or different combination of the first-line TB drugs in each formulation, or both. The results show only three (one two-drug and two three-drug) of the 10 formulations being bioequivalent. All of the other seven were not bioequivalent to the reference standard, which consisted of separate drug formulations. The therapeutic margin of RIF is low and any reduction in bioavailability could be detrimental to patient therapy (Pillai *et al.* 1999).

Rifampicin is the only hydrophobic component in the FDC belonging to class II of the biopharmaceutical classification system (BCS) (low solubility and high permeability). The BCS provides a mechanistic approach for exploring the problem of oral bioavailability through drug classification. The other TB drugs namely, INH, PZA and ETB all belong to BCS class I (highly soluble and highly permeable) and thus demonstrate no bioavailability problems (Van Helden *et al.* 2006). RIF may thus be affected by various factors such as raw material characteristics, excipients, manufacturing and/or process variables. Probable variables illustrated in Figure 1.6 were suggested by Panchagnula and Agrawal (2004:3) as being causes for the variable bioavailability of RIF.

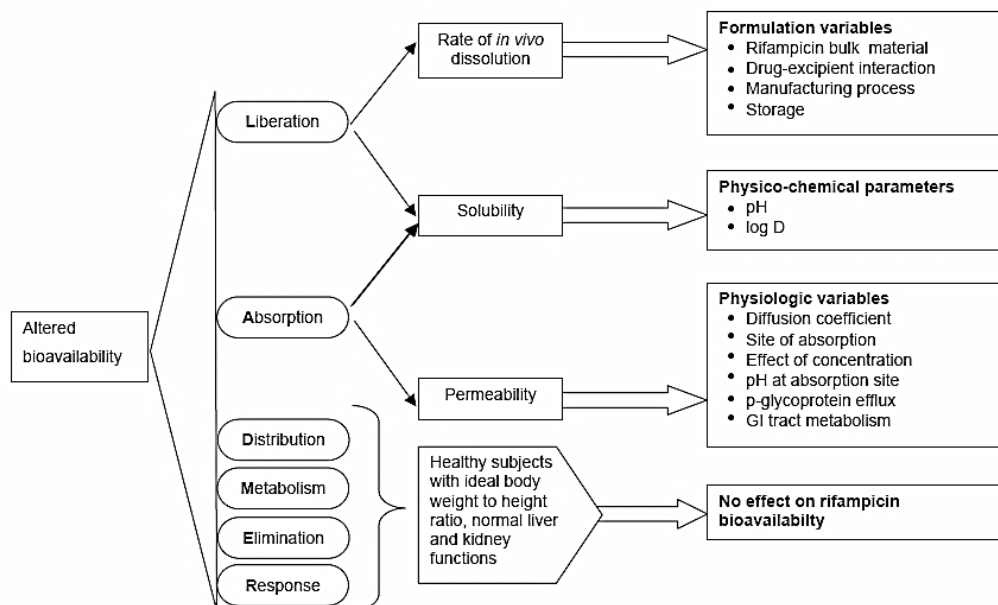


Figure 1.6 Probable explanations for the variable bioavailability of rifampicin. Diagram taken from (Panchagnula & Agrawal 2004:3) with permission.

Variables illustrated above may affect the bioavailability of RIF in the following ways as documented by Agrawal and Panchagnula (2005:322):

- During various manufacturing procedures such as grinding, mixing, granulation and compression, the crystalline nature or particle size of RIF may be affected, thus altering its absorption;
- Pharmaceutical excipients used during formulation such as the binders, glidants e.g. bentonite, kaolin and talc, rapidly and strongly adsorb to RIF and reduce its gastrointestinal absorption;
- Inadequate drug dissolution from the formulation resulting in insufficient drug delivery to the absorptive sites in the gastrointestinal (GI) tract;
- Decomposition of the drug to a non-absorbable complex in the GI tract;
- Metabolism and/or efflux of the drug *en route* to the systemic circulation.

These may all be very possible factors for poor bioavailability of RIF, but no comprehensive or systematic study exists to assess the aforementioned variables on the *in vivo* bioavailability of RIF (Agrawal & Panchagnula 2005:322).

Gurumurthy *et al* (1999) found that the pharmacokinetic properties of RIF, INH and PZA, as assessed after individual and combined administration, are not modified when the same drugs are combined in a single preparation (Gurumurthy *et al.* 1999). A similar study conducted by Zwolska *et al* (1998:826) using FDC capsules containing RIF, INH and PZA, displayed comparable results.

1.5.2.4 The interaction of rifampicin and isoniazid after oral administration

INH and RIF have been documented as displaying drug-drug interactions following oral administration on an empty stomach, with pH ranges between 1.4 and 2.1, yielding isonicotinylhydrazone (HYD). This may account for the reduction of the bioavailability of RIF. In a study with formulations containing all four first-line drugs i.e. INH, RIF, PZA and ETB, it was found that the presence of PZA and ETB accelerated the reaction between INH and RIF (Bhutani *et al.* 2005:897) with ETB having a greater effect.

Bhutani *et al* (2005) postulated that this reaction was catalysed by PZA and ETB, involving intra-molecular proton transfer during a reaction between RIF and INH. Since the reaction between INH and RIF is also associated with the conditions in the stomach (Bhutani *et al.* 2005) enterically coating one of the drugs to be released at different sites in the gastrointestinal tract should be considered during the manufacture of FDC dosage forms. The task would thus remain in obtaining a thorough understanding of the absorption kinetics of the two drugs.

Mariappan and Singh (2003:800) conducted a study to establish the permeability of INH and RIF in various parts of the gastrointestinal (GI) tract of rats. The results show that RIF was more readily absorbed in the stomach and duodenum with highest absorption at pH 1. INH had an opposite behaviour showing less permeation in the stomach and more in the intestines (Mariappan & Singh 2003). In another study, Shishoo *et al* (1999:113) demonstrated that RIF should be formulated to be absorbed in the upper part of the intestine instead of the stomach (Shishoo *et al.* 1999). The study was based on previous reports, which indicated that in acidic medium RIF is hydrolysed into 3-Formyl rifamycin SV (3-FRSV) (Maggi *et al.* 1966; Sensi *et al.* 1966). This poorly absorbed derivative shows antimicrobial activity *in vitro* but is inactive *in vivo*. This may be an important factor affecting the bioavailability of RIF. Furthermore, in the acidic environment of the stomach, INH is postulated to increase the degradation of RIF into 3-FRSV via the reversible binding of the isonicotinyl hydrazone of 3-

FRSV with INH (Singh *et al.* 2000:405). Figure 1.7 illustrates the possible mechanisms for this reaction. Figure 1.7 (a) shows a possible Schiff's reaction where the carbonyl groups and amine groups may rearrange to form an iminium ion. The C-4 hydroxy group causes enhanced complex formation by the possible formation of a hydrogen bond with the hydrogen atom and nitrogen. In Figure 1.7 (b) a possible condensation reaction between the carboxylic acids and alcohols of the two compounds is observed and Figure 1.7 (c) illustrates this reaction continuing to further undergo Fischer esterification since the hydroxyl groups of RIF is readily able to react with the aqueous carboxylic acid degradants yielded by INH (du Toit *et al.* 2006:129).

Considering these results, it may seem plausible to formulate a fixed-dose combination (FDC) in which absorption of INH and RIF is controlled in the different sites of the GI tract where their permeability is favoured and their interaction is limited.

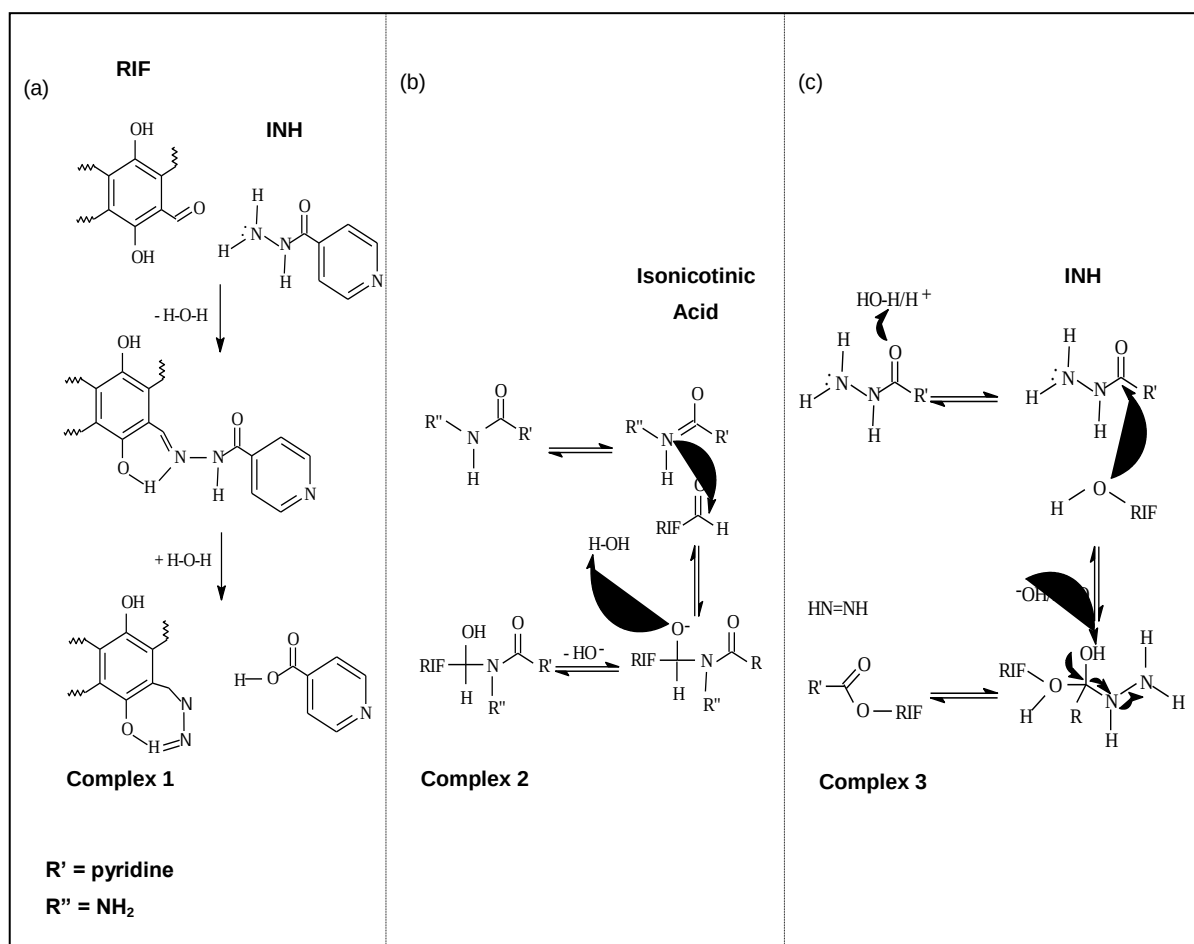


Figure 1.7 Proposed mechanisms for RIF-INH interaction (a) Schiff's reaction; (b) Carbonyl condensation reaction and (c) Fischer's esterification reaction (du Toit *et al.* 2006:129) with permission.

Variable data thus exists with many investigations being divided on the optimal absorption site for RIF (Ellard & Fourie 1999; Pähkla *et al.* 1999; Panchagnula & Agrawal 2004; Zwolska *et al.* 1998).

1.5.2.5 Other RIF drug interactions

RIF and its analogues interact adversely with many other drugs used for treating various other conditions as illustrated in Table 1.3. Besides the drug interactions listed below, the interaction of rifamycin drugs (rifampicin, rifabutin and rifapentine) that deserves special attention is the interaction with antiretroviral (ARTs) drugs used in the treatment of HIV and AIDS since many TB patients are HIV positive. These are discussed in further detail in the section 1.4.2.6.

Table 1.3 Drug-drug interactions to be considered when INH is administered whilst patient is being treated for other illnesses (Self, 1999:304).

Drug or group of drugs	Drug-drug interaction
Aminosalicylic acid	Decrease in rifampicin absorption, thus decreased bioavailability
Anticoagulants (oral)	Decreases the anticoagulant effect
Antidepressants (tricyclic, barbiturates, benzodiazepines)	Decreases antidepressant effect
Most of the beta-adrenergic blockers	Decreases the blocking mechanism on beta receptors
Metoprolol	Possibly increases the blocking action on beta receptors
Chloramphenicol	Decreases the effect of chloramphenicol
Clofibrate	Decreases the effect of clofibrate
Oral contraceptives	Decreases contraceptive activity
Corticosteroids	Significant decrease in corticosteroid effect
Dapsone	Potential decrease in dapsone efficacy
Depo-Provera	Decreased contraceptive effect
Digitoxin	Decreased digitoxin effect

The rifamycins are inducers of the cytochrome P3A family (CYP3A) of enzymes, including CYP3A4. Of the groups of ARTs, the protease inhibitors (PIs) and the non-nucleoside transcriptase inhibitors (NNRTIs) are metabolized by CYP3A, specifically CYP3A4 (Davies & Nuermberger 2008). Thus, the maximal drug levels and total exposure times expressed as C_{max} and AUC, respectively, of the ARTs will be reduced with co-administration of RIF. The virus will thus not be suppressed and the risk of treatment failure is eminent. RIF is the most potent inducer of CYP3A and rifapentine the least potent, but with increased chance of resistance developing for the rifamycin group (Munsiff *et al.* 2007:3).

Other anti-tuberculosis drugs do not have clinically important interactions with ARTs. A combination adjustment of ARTs proves useful for avoiding these interactions. Thus, it is important that the prescriber is thoroughly informed about all possible treatment regimens. It has been documented that HIV infection in itself in the absence of ARTs also affects the absorption of anti-tuberculosis drugs resulting in low serum concentrations (Gurumurthy *et al.* 2004).

Current TB treatment takes into account the HIV status of a TB patient and treatment is initiated on the basis of the patient's cluster of differentiation 4 ($CD4^+$) count. This count of the patients $CD4^+$ lymphocytes is an indication of the status of the patients' immunity (Khomanani-Campaign South Africa Department of Health, 2004). Provisions for HIV-infected TB patients are as follows:

- **$CD4^+$ count above 200-** TB treatment takes preference and is initiated for 6 months after which the physician will make a decision regarding initiation of ARTs.
- **$CD4^+$ count below 200-** TB treatment is initiated for 2 months before ARTs are started. The TB treatment will continue for the 6 month course concurrently with ARTs.
- **$CD4^+$ count below 50-** TB treatment for 2 weeks followed by ART initiation. TB treatment is continued for 6 months.
- Patients diagnosed with TB after starting treatment with ARTs will have to be given regimen adjustments to make provision for the interactions described above (Khomanani-Campaign South Africa Department of Health, 2004).

TB treatment has to be taken for the full 6 months to avoid the emergence of resistant strains. Thus, a prescriber will have to ensure that this is possible for HIV-infected TB patients.

1.5.3 ETB

ETB, depicted in Figure 1.8 is a synthetic, water-soluble heat-stable compound. Susceptible strains are inhibited by ETB at 1-5 µg/ml serum level. The mechanism of action of ETB has not been fully established, but much research into its effect has been documented (Mikusova *et al.* 1995; Silve *et al.* 1993). Takayama and Kilburn (1989:1494) documented the primary mode of action of ETB as the inhibition of mycobacterial arabinosyltransferases that are involved in the polymerization reaction of arabinoglycan, an essential component of the mycobacterial cell wall (Takayama & Kilburn 1989). The role of arabinosyltransferase in ETB's mechanism provided clarity to the resistance mechanism of ETB. The transformation of the *emb* locus resulted in resistance as a function of the copy number of the gene which can be interpreted as resistance due to target over expression (Rattan *et al.* 1998:201). In a follow up study, Deng *et al.* (1995:699) presented data that not only confirmed the study by Takayama and Kilburn (1989), but also was also consistent with the idea that ETB inhibits the biosynthesis of the entire mycolylarabinogalactan-peptidoglycan cell wall core. They did, however, conclude that this inhibition occurs as a function of inadequate formation of arabinan in the cell wall core caused by ETB (Deng *et al.* 1995:698). Disruption of arabinoglycan synthesis alters the cell barrier (Deng *et al.* 1995) enhancing the activity of lipophilic drugs such as RIF and ofloxacin that cross the cell wall primarily in lipid domains of this structure (Chambers, 2001:807).

1.5.3.1 Pharmacokinetics

ETB is well absorbed with a blood level peak of 2-5 µg/ml in 2-4 hours (Chambers, 2001:807). Nearly all strains of mycobacteria are sensitive to ETB and it has no effect on other bacteria (gram-positive). ETB (75%) is excreted unchanged in the urine making it useful in treating TB patients with liver disease (Graham & McLeod 1999). Jia *et al.* (2005:794) tested the pharmacokinetic properties of different ETB analogues in mice in an attempt to develop a 'better ethambutol' with regards to improving toxicity and improving its potency (Jia *et al.* 2005). The chemical structures of these analogues comparable with ETB are illustrated in Figure 1.8.

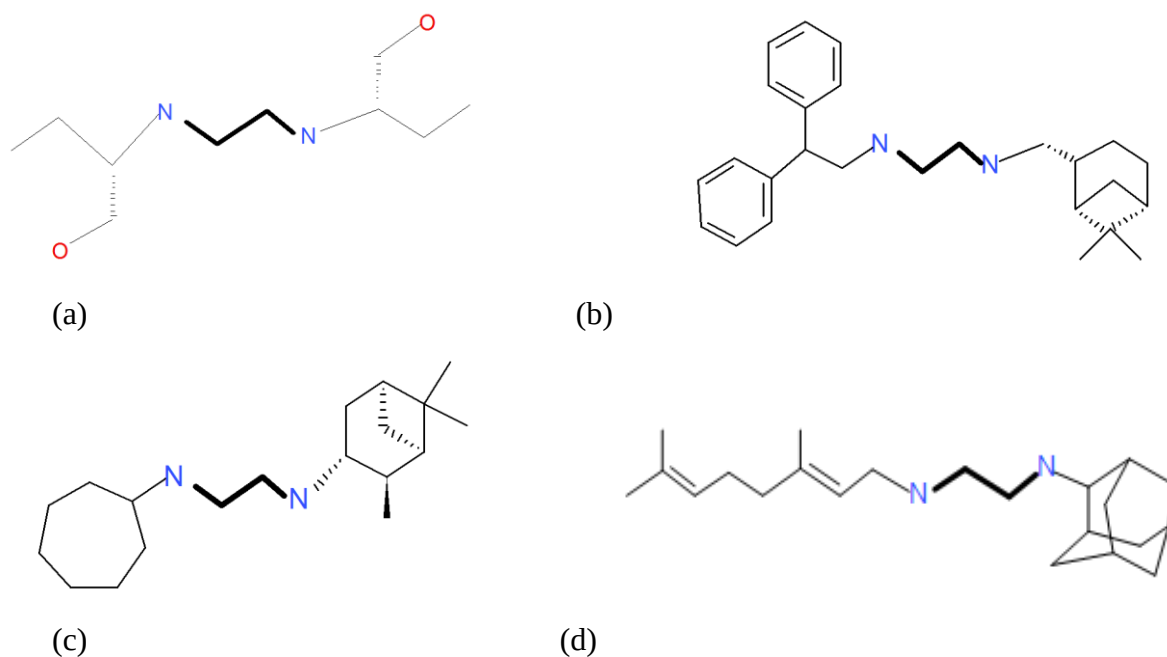


Figure 1.8 Chemical structures of (a) ethambutol and its analogues, (b) SQ37, (c) SQ59 and (d) SQ109. Adapted from (Jia *et al.* 2005:794) with permission.

These were the three most promising analogues out of 26 isolates that demonstrated anti-tubercular activity *in vitro* that was equal to or better than that of ETB. Documented studies have shown the MIC of ETB to be 1-5 $\mu\text{g/ml}$ (Chambers, 2001:807; Franzblau *et al.* 1998:364). However, ETB as for many other antimycobacterial drugs, demonstrate variable MIC depending on the method of determination and the strain of *M.tb* (Tripathi *et al.* 2005; Barluenga *et al.* 2006). *In vitro* studies revealed the MIC required to produce 90% inhibition (MIC_{90}) for SQ37, SQ59 and SQ109 to be 1.25, 12.5 and 0.63 $\mu\text{g/ml}$, respectively. Figure 1.8, SQ109 proved to be the most potent analogue (Jia *et al.* 2005:799). The development of analogues may be a step towards improving PK/PD properties of TB drugs by decreasing drug toxicity and enhancing therapeutic outcomes.

1.5.3.2 Adverse effects

Hypersensitivity to ETB is uncommon (Chambers, 2001:807). The most frequent, serious adverse event is retrobulbar neuritis, which causes loss of visual acuity and red-green colour blindness. This effect is, however, dose related occurring in patients who receive 25 mg/kg/d for several months. At dosages of 15 mg/kg/d, these effects are rare. Due to this possible serious visual disturbance, ETB is relatively contraindicated in children (Chambers, 2001:807). A noteworthy drug interaction with ETB is aluminium- hydroxide containing

antacids, which has demonstrated a decrease in the oral absorption of ETB (Chambers, 2001:807).

1.5.4 PZA

PZA (Figure 1.9) is also a prodrug and is only active when enzymatically converted to pyrazinoic acid by the enzyme pyrazinamidase (*Pzase*). Zhang *et al* (2003:791) hypothesised that pyrazinoic acid, as a weak acid, could potentially inhibit membrane transport function, resulting in the postulated mechanisms of action (Zhang *et al.* 2003). Mycobacterial strains which are naturally resistant, such as *M.bovis*, lack the enzyme *Pzase*, thus PZA cannot be activated. *Pzase* of *M.tb* has both pyrazinamidase and nicotinamidase activities. Isolation of the *pncA* gene, which codes for the amidases revealed a single point mutation, which results in the production of a defective *Pzase* therefore, conferring resistance. These mutations of the *pncA* gene include missense alterations, nucleotide insertions or deletions and termination mutations from PZA-resistant *M.tb* isolates (Scorpio *et al.* 1997:541). PZA plays a key role in combination therapy with INH and RIF and contributes largely to the reduction of treatment time from 9-12 months to 6 months (Boshoff *et al.* 2002; Somoskovi *et al.* 2004). This is due to its ability to kill bacterial populations with a low metabolic activity residing in acidic environments. It is also active against semi-dormant or old non-growing bacilli (Somoskovi *et al.* 2004).

A study by Hu *et al* (2006:320) reported that as metabolic bacillary rates decreases, the activity of PZA increases (Hu *et al.* 2006). This is contrary to what has been described for the other three first-line drugs, which demonstrates decreased drug activity with a decrease in metabolic activity.

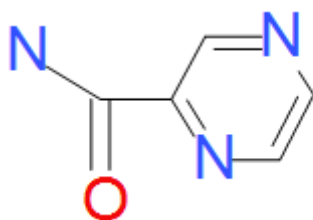


Figure 1.9 Chemical structure of PZA, the pyrazine analogue of nicotinamide. Chemical formula C₅H₅N₃O.

1.5.4.1 Pharmacokinetics

PZA is a stable, slightly water compound, which is inactive at neutral pH, but at pH 5.5 exhibits inhibition of tubercle bacilli at concentrations of approximately 20 µg/ml (Chambers,

2001:807). Other authors have documented that at pH 5.5-6.0, killing of *M.tb* is both incomplete and ineffective, killing only 76% of the bacterial population at a concentration of 1000 µg/L. This concentration is 10-20 times higher than the MIC, which is 50-100 µg/ml (Zhang *et al.* 2003; Somoskovi *et al.* 2004). Similar studies have demonstrated that for 21 PZA-susceptible strains of *M.tb*, the MIC's for 90% of the strains were 50 µg/ml at pH 5.5-5.7, 100µg/ml at pH 5.8 and 200 µg/ml at pH 5.95 (Salfinger & Heifets 1988:1003).

Serum concentrations of 30-50 µg/ml are reached at 1-2 hours after oral administration. It is well absorbed and widely distributed with a half-life of 8-11 hours (Chambers, 2001:807). Conte *et al* (1999:1332) has demonstrated that the absorption of PZA is not affected by gender or the presence of AIDS when administered orally (Conte *et al.* 1999). PZA was also found in a group of test subjects in the epithelial lining fluid following a bronchoscopy and broncho-alveolar lavages, which could be a partial contribution to its efficacy in pulmonary tuberculosis.

The exact mechanism of action for this drug is not known, but according to Conte *et al* (1999:1331) it is taken up by the macrophages and is active intracellularly. Heifets *et al* (2000:493) found that PZA is not effective against *M.tb* residing in resting or activated human-monocyte derived macrophages (Heifets *et al.* 2000). It has no significant bactericidal activity, but is said to have a sterilizing effect (Tripathi *et al.* 2005). A study by Somoskovi *et al* (2004), demonstrated that ferrous sulphate enhanced the activity of pyrazinoic acid at concentrations of 0.15-0.6 mM (Somoskovi *et al.* 2004).

1.5.4.2 Adverse effects

Hepatotoxicity is seen in 1-5% of patients. Minor side effects may occur such as nausea, vomiting, drug fever and hyperuricemia, but it's not severe enough to halt therapy. The hyperuricemia may provoke acute gouty arthritis (Chambers, 2001:808). The only noteworthy drug interaction of PZA is allopurinol since it causes allopurinol to be ineffective in decreasing serum uric acid levels (Chambers, 2001:808).

1.5.5 Streptomycin

Streptomycin (Figure 1.10), first discovered in 1944, is an aminoglycoside antibiotic listed as a first-line drug due to the fact that it was the first effective drug against TB (Tripathi *et al.* 2005:13). Now, with the discovery of more effective first-line drugs, it is mainly used as

second-line therapy (Chambers, 2001:808). Streptomycin is administered intramuscularly due to its poor absorption from the gastrointestinal tract. Streptomycin penetrates into cells poorly and consequently it is active mainly against extracellular tubercle bacilli. The mode of action of streptomycin is penetration of the inner membrane of *M.tb* and binding to the 30S ribosomal subunit (Tripathi *et al.* 2005:13) preventing initiation of protein synthesis (Chopra & Brennan 1998:90).

1.5.5.1 Pharmacokinetics

It is suggested that aminoglycosides display concentration-dependent killing. Thus their activity relies largely on the $C_{\max}$: MIC ratio. Streptomycin has an MIC of 1 µg/ml with 50-60% plasma protein bound and a half-life of 5-7 hours (Tripathi *et al.* 2005). This would suggest that streptomycin has a bioavailability of approximately 55%. These drugs are also largely documented for their PAE (Graham & McLeod 1999). Since this finding was established, the dosage was decreased to once daily and has been proven just as effective as multiple daily doses.

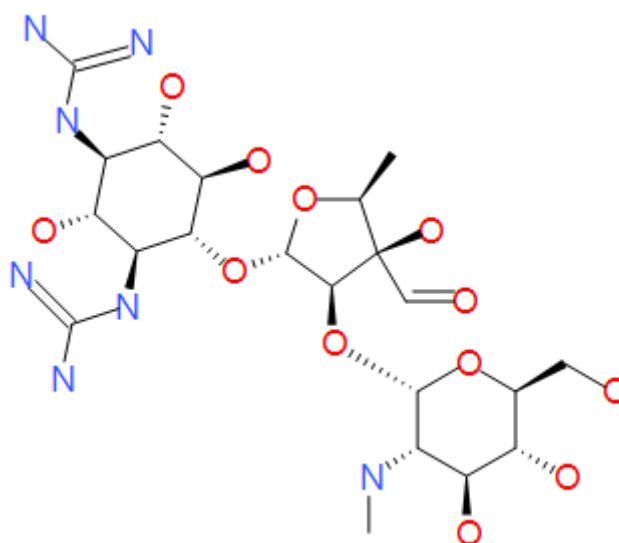


Figure 1.10 Chemical structure of streptomycin, a member of the aminoglycoside group of drugs. Chemical formula of streptomycin is $C_{21}H_{39}N_7O_{12}$.

1.5.5.2 Adverse effects

Streptomycin is ototoxic and neurotoxic causing vertigo and hearing loss, which may be permanent. These are the most common side effects, but are mostly dose related and accentuated in the elderly. As with all aminoglycosides dosage adjustment is necessary in

renal insufficiency (Chambers, 2001:808). Due to these adverse effects, streptomycin is not the most popular choice in therapy (Tripathi *et al.* 2005).

1.6 Second line TB drugs

The antibiotic classes indicated in Table 1.4 are also used as second line TB drugs. The use of this regimen is considered only when: (1) there is resistance to the first-line drugs which as mentioned earlier, is a major problem at this stage; (2) failure of clinical response to conventional therapy occurs and (3) expert guidance is available to deal with toxic effects. For many of these drugs on-going investigations are underway to fully establish the justification for use. Hence, these are not the drugs of choice. One specific area of concern is that toxicity and development of resistance caused by long-term therapy of these drugs has not been established (Chambers, 2001:808).

Table 1.4 Adult doses and subsequent mechanism of action following administration of second line agents adapted from (Chambers, 2001:808).

Drugs	Adult dosage	Mechanism of action
Amikacin	15mg/kg/d	Irreversible inhibitor of protein synthesis
Amino salicylic acid	8-12g/d	Folate synthesis antagonist that is active almost exclusively against <i>M. tuberculosis</i>
Capreomycin	15mg/kg/d	Peptide protein synthesis inhibitor
Levofloxacin	500mg/d	Inhibit bacterial DNA synthesis
Ciprofloxacin	1500mg/d, divided	Inhibit bacterial DNA synthesis
Clofazimine	200mg/d	Mechanism of action not known, but may involve DNA binding
Cycloserine	500-1000mg/d, divided	Inhibits cell wall synthesis
Ethionamide	500-750mg/d	Chemically related to INH. Blocks mycolic acid synthesis

1.7 New trends in anti-tuberculosis drug research

Various avenues of improving treatment of TB are being explored. These include investigating new FDC; new drug analogues that will result in reduced doses and treatment time optimisation and enhancement of drug delivery systems, which will be described in further detail in Chapter 2. The resurgence of resistant strains of *M.tb* across the scope of

current treatments has caused an increasing need for new drug development. This has been achieved by development of novel compounds, by producing derivatives from already established drugs with potential for mycobactericidal activity and also exploring drugs not commonly used in the treatment of TB, but already approved and utilized for the treatment of other bacterial infections. Some compounds have been proven to be active against *M.tb* with a number of them currently undergoing clinical trials. These are summarised in Table 1.5. Some of these are further described in sections 1.7.1- 1.7.6.

Table 1.5 Summary of drugs being explored for TB chemotherapy

Drug compound/analogue/derivative	Mechanism of action	Stage of development	References
Linezolid	Orally active anti-bacterial agents acting through inhibition of protein synthesis. Explored for treatment of MDR, it was the first antibiotic of its class to be approved	Pre-clinical development	(Janin 2008;TB Alliance 2012;Tripathi <i>et al.</i> 2005)
TMC-207	Inhibits ATP synthase	Phase II human trials	(TB Alliance 2007;TB Alliance 2012)
Moxifloxacin	Inhibition of ATP-dependant DNA-gyrase. Efficacy in pulmonary TB has already been reported. It has the advantage of not interacting with anti-retroviral therapy	Pivotal large scale phase III clinical trials in TB patients	(Gosling <i>et al.</i> 2003, Johnson <i>et al.</i> 2006, Miyazaki <i>et al.</i> 1999, Pletz <i>et al.</i> 2004)
PA-824	A prodrug that requires activation by a bacterial F420 dependant glucose-6-phosphate dehydrogenase and nitroreductase to activate components that then inhibit bacterial mycolic acid and protein synthesis. Active against MDR-TB	Phase II clinical trials	(TB Alliance 2012;Tripathi <i>et al.</i> 2005;Zhang <i>et al.</i> 2006)
OPC-67683	Chemically related to PA-824 with similar mechanism. Mechanism has not been fully elucidated	Clinical trials	(Janin 2008;Zhang <i>et al.</i> 2006)
Riminophenazines	Thought to inhibit energy metabolism, which is a required process in both active and latent <i>M.tb</i>	Lead optimisation	(TB Alliance 2012)
Multifunctional molecules	Multiple TB drugs are chemically linked to function as one, ensuring simultaneous optimal concentrations at the site of action	Lead optimisation	(TB Alliance 2007)
Quinolone analogues	Inhibition of DNA-gyrase. These 600 synthesized quinolones are being tested for an optimal quinolone	Preclinical	(TB Alliance 2007)
InhA inhibitors	Resistance to INH occurs as a result of KatG, the enzyme needed to activate INH, becoming non-functional. This new group of inhibitors will not need KatG, thus potentially eliminating INH-resistant strains	Lead optimisation	(TB Alliance 2007)
Mycobacterial gyrase inhibitors	Novel inhibitor of DNA-gyrase. May be effective against fluoroquinolone-resistant strains	Lead optimisation	(TB Alliance 2007;TB Alliance 2012)
Pleuromutilins	New class of antibiotics able to selectively inhibit multiple steps in bacterial protein synthesis	Lead optimisation	(TB Alliance 2007)
Malate synthase inhibitors	Malate synthase thought to be a key enzyme used by <i>M.tb</i> to convert its food source to maintain its slow-growing, latent state. Inhibition may starve persisters and shorten therapy	Lead identification	(TB Alliance 2007)

1.7.1 Oxazolidinones

Oxazolidinones are synthetic, orally active anti-bacterial agents acting through inhibition of protein synthesis (Tripathi *et al.* 2005:19). This group represents the first completely new class of antibacterials to be marketed in 25 years (Janin, 2008:2492). Linezolid (Figure 1.11) a member of the oxazolidinone class of drugs was studied for the treatment of MDR-TB and was in fact the first antibiotic of its class to be approved (Janin, 2008:2492). This class of antibiotics function by inhibition of protein synthesis at an early stage by binding to 23S rRNA of the 50S ribosomal subunit and are active against a variety of Gram-positive bacteria. Oxazolidinones show MIC of 2-4µg/ml and patients have successfully been treated with linezolid by inhibiting activity against MDR-TB at MIC of 2µg/ml (Hudson *et al.* 2003:25). However, the significant toxicity such as anaemia and peripheral neuropathy still remains a factor and further studies are needed.

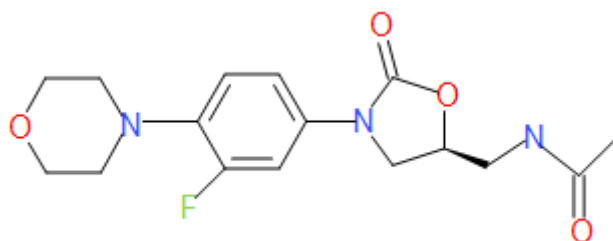


Figure 1.11 Chemical structure of linezolid. A chemical “template” which is essential for antimicrobial activity has a 1, 3-oxazolidin-2-one moiety with an aryl and an S-methyl group (Brickner 1996). Chemical formula of linezolid is C₁₆H₂₀FN₃O₄.

1.7.2 Diarylquinolones

The new compound Bedaquilline (formerly TMC-207 and R207910) illustrated in Figure 1.12 has also received much attention in the past seven years. Its anti-tuberculosis mechanism of action is by the inhibition of the mycobacterial proton pump F₀F₁H-ATPase. *In vitro* studies demonstrated potential inhibition of both drug-sensitive and drug-resistant *M.tb* with an MIC of 0.06µg/ml (Andries *et al.* 2005:223). Bedaquilline demonstrated higher activity than INH and RIF, which could potentially contribute to a reduction in dosing frequency. This drug is currently undergoing Phase II human trials (TB Alliance 2012:1).

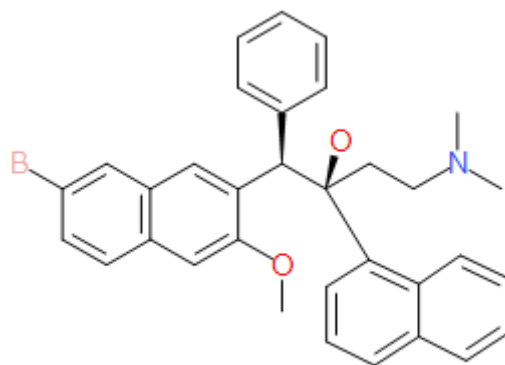


Figure 1.12 The chemical structure of the experimental drug Bedaquilline. The chemical formula for Bedaquilline is $C_{32}H_{31}BrN_2O_2$.

1.7.3 Fluoroquinolones

These broad spectrum antibiotics have been identified as alternative additional treatment in the category of second-line anti-tuberculosis drugs. Fluoroquinolones' mode of action is by dual inhibition of an ATP-dependant DNA gyrase (topoisomerase II) and in most gram positive bacteria, ATP-dependent topoisomerase IV (Janin, 2008:2494). Their effect, however, on mycobacteria would be inhibition of topoisomerase II since no topoisomerase IV exists in this class of bacteria (Janin, 2008:2494). A finding that received much attention was the new C-8-methoxy-FQ moxifloxacin (MXF) and gatifloxacin (GAT), which have a longer half-life and is more active against *M.tb* than other fluoroquinolones (Li *et al.* 1999).

Certain fluoroquinolones have been reported as having excellent early bactericidal activity (EBA) (Johnson *et al.* 2006, Gosling *et al.* 2003). EBA is the rate at which the drug kills tubercle bacilli in the sputum during the first two days of therapy. This test is used to compare activities of new drugs to old drugs and to establish the best effective dose for a new drug (Johnson *et al.* 2006). Johnson *et al.* (2006:607) found that moxifloxacin, gatifloxacin and high doses of levofloxacin have an excellent EBA, proving to be only slightly less than that of INH (Johnson *et al.* 2006). Other fluoroquinolones, such as ciprofloxacin and ofloxacin, have also shown potential in the treatment of *M.tb*, but due to the increased risk of resistance with prolonged use, their potential use is limited (Johnson *et al.* 2006:609).

1.7.3.1 MXF

MXF (Figure 1.13) is a Bayer-manufactured quinolone (BAY12-8039) and the newest member of this 4th generation class of antibiotics to progress so rapidly through clinical development (Hudson *et al.* 2003:29). It has shown *in vitro* and *in vivo* activity against *M.tb*

with an MIC of approximately 0.25 µg/ml and an elimination half-life of 12 hours (Hudson *et al.* 2003; Ji *et al.* 1998). An *in vivo* study in mice demonstrated that MXF in combination with RIF and PZA killed tubercle bacilli more effectively than the standard regimen of INH+RIF+PZA and could achieve stable cure in four months with no relapse (Nueremberger *et al.* 2004:424). In a similar study, a highly virulent clinical strain of *M.tb* (CSU93) demonstrated susceptibility to MXF at a MIC of 0.25 µg/ml (Miyazaki *et al.* 1999:86). MXF has already been approved by the FDA for use in the treatment of other lung infections such as pneumonia and bronchitis (CenterWatch, 2004). MXF is currently undergoing large scale, pivotal Phase III clinical trial in TB patients. The clinical trials, with six sites in Africa, were aimed at determining whether a four-drug combination therapy including MXF could shorten therapy time from 6 months to four months (CenterWatch, 2004; TB Alliance Pipeline, 2012). Potential efficacy of MXF in pulmonary tuberculosis has already been reported (Pletz *et al.* 2004:781). All the studies thus far, demonstrate the potential of including MXF in the first-line TB regimen (Gosling *et al.* 2003; Ji *et al.* 1998; Johnson *et al.* 2006; Pletz *et al.* 2004).

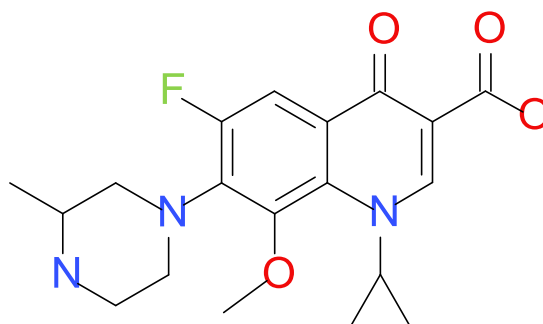


Figure 1.13 Chemical structure of MXF, a fourth generation fluoroquinolone synthetic agent. Each fluoroquinolone subset has a fluorine atom attached to the central ring system. The chemical formula for MXF is C₂₁H₂₄FN₃O₄.

1.7.3.2 GAT

GAT (Figure 1.14) falls in the same 4th generation class of fluoroquinolones as MXF with a similar mode of action. The potential use of GAT alone and in combination with other first-line anti-tuberculosis drugs has demonstrated *in vitro* activity against *M.tb*. (Alvarez-Freites *et al.* 2002:1023). The study concluded that the results gave merit to further evaluation into the possibility that GAT could be used for TB treatment.

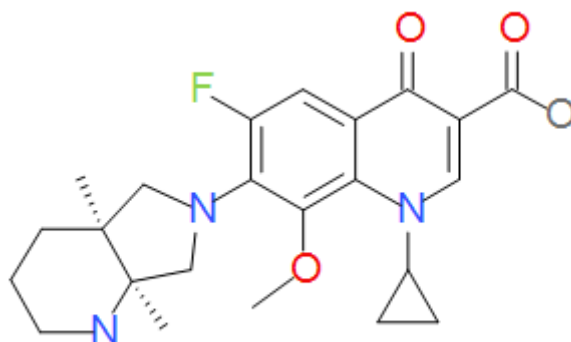


Figure 1.14 Chemical structure of GAT. The chemical formula is $C_{19}H_{22}FN_3O_4$.

1.7.4 Nitroimidazopyran

Nitro-bearing imidazoles were first reported in the late 1970's. Imidazoles are commonly used as anaerobic antibiotic, but their effect on *M.tb* was soon recognized and this led to the identification of PA-824 (Figure 1.15). PA-824 is the first novel TB drug candidate developed by a non-profit organization clinical trial. The first test in TB patients was performed in Cape Town, South Africa in 2008. This compound is highly active with an MIC of 0.015-0.250 $\mu\text{g/ml}$ against *M.tb* (both replicating and latent) and MDR-TB (Tripathi *et al.* 2005). Multi-drug resistant strains of *M.tb* were susceptible, which indicates no cross-resistance with other drugs (Tripathi *et al.* 2005). PA-824 is a prodrug that requires activation by a bacterial F420-dependant glucose-6-phosphate dehydrogenase and nitroreductase to activate components that then inhibit bacterial mycolic acid and protein synthesis. PA-824 is currently undergoing clinical trials (TB Alliance 2012:1).

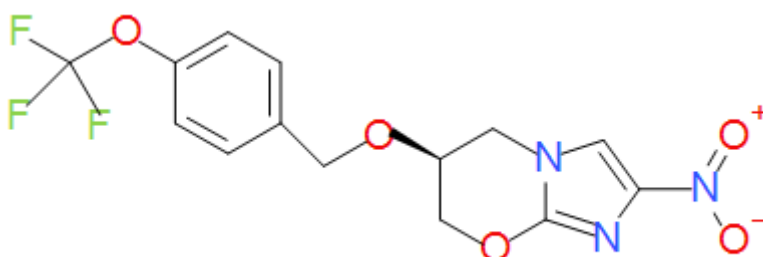


Figure 1.15 Chemical structure of the Nitroimidazopyran (PA-824). The chemical formula for PA-824 is $C_{14}H_{12}F_3N_3O_5$.

1.7.5 Azole drugs

The potential use of azole drugs stemmed from the discovery of genomic sequencing in *M.tb* that showed similarities to fungi (Guardiola-Diaz *et al.* 2001, McLean *et al.* 2002). Ahmad *et al.* (2005:21) have evaluated the potential activity of antifungal azole drugs against *M.tb* and

they were also the first to report activity of these drugs against MDR-TB (Ahmad *et al.* 2005). They conducted an *in vitro- ex vivo* study for clotrimazole and econazole based on the finding that a protein similar to that found in mycobacteria, involved in sterol synthesis which is present in fungi and inhibited by azole compounds and their derivatives, may also have the same effect on mycobacteria (Ahmad *et al.* 2005:22). Each drug demonstrated significant potential achieving 90% inhibition (MIC_{90}) at 0.12 $\mu\text{g/ml}$. Further studies led to the claim that azoles may very well replace the most potent frontline TB drugs. A continued study into the activity of azoles on MDR-TB was performed. Econazole (Figure 1.16) was found to inhibit the growth of all MDR strains of *M.tb*. The MIC_{90} and minimum bactericidal concentration (MBC)>99.99 of econazole, were found to be in the ranges 0.120- 0.125 $\mu\text{g/ml}$ and 0.125-0.15 $\mu\text{g/ml}$, respectively. The *M.tb* strain H₃₇Ra has demonstrated susceptibility to other azole drugs such as miconazole, clotrimazole and 2-nitro-imidazole with a MIC of 2-5 $\mu\text{g/ml}$ (Sun & Zhang 1999:320). Ahmad *et al* (2007:240) evaluated the chemotherapeutic potential of alginate nanoparticle-encapsulated econazole and anti-tubercular drugs (INH, RIF, PZA and ETB) against murine TB. They detected the anti-tubercular drugs above the MIC for as long as 15 days. The formulation also presented favourable tissue distribution with econazole being detected above the MIC until the eighth day of the study. They concluded that the results obtained for a murine TB model were sufficient to confirm that econazole would be a suitable replacement for RIF and INH in TB chemotherapy (Ahmad *et al.* 2007).

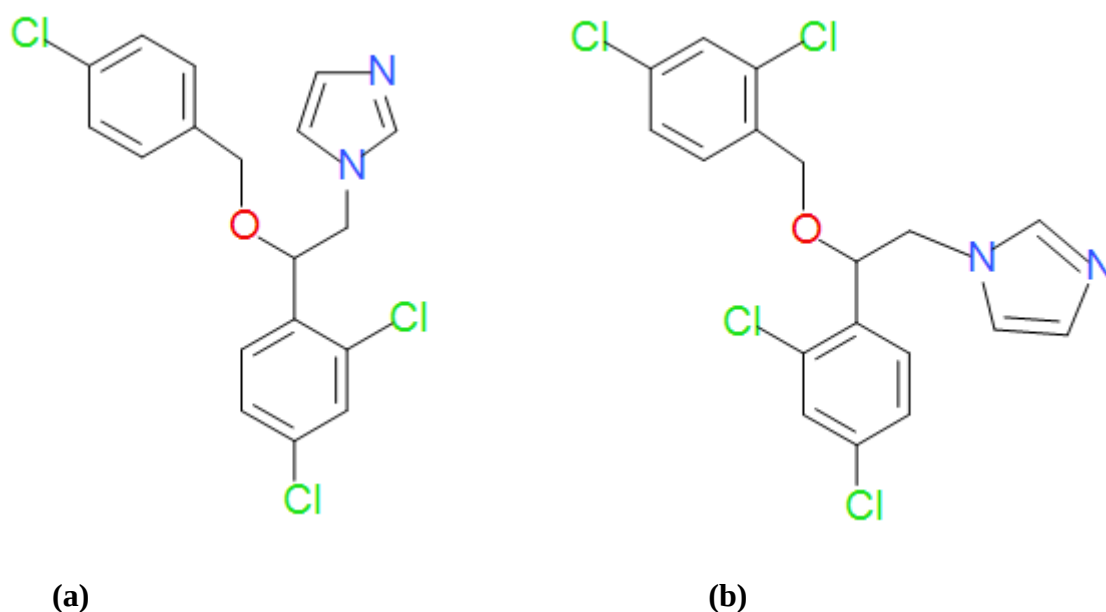


Figure 1.16 Chemical structures of azole drugs, (a) econazole (chemical formula $C_{18}H_{15}Cl_3N_2O$) and (b) miconazole (chemical formula $C_{18}H_{14}Cl_4N_2O$). The difference

between the two structures is observed by the fourth chlorine group in the econazole structure.

1.7.6 Phenothiazines

For the past few years Amaral *et al* (2001) has been involved in the identification and modification of phenothiazines for their potential use as alternative anti-tuberculosis drug treatment. The unlikelihood of these compounds being studied for use in antimycobacterial use is based on the fact that these drugs are used as scheduled antipsychotic medication with no history of use as antibiotics. Furthermore, the potential toxic effects that accompany long term use of antipsychotics do not motivate their use (Amaral *et al.* 2001:505). However, the results obtained from these studies warranted further consideration of these drugs. Chlorpromazine and thioridazine are the least potent of this group of drugs in terms of toxic effects and thus also the focus of their study. According to their review, these compounds inhibit *in vitro* growth of *M.tb* at concentrations that are significantly higher than those that can safely be achieved in a patient harbouring these infections. However, chlorpromazine concentration in human macrophages was 10- 100 times more than in plasma and had activity against mycobacteria that have been phagocytosed by these cells. Therefore, it may be possible to consider the use of this group of drugs as an adjunct to conventional therapy during the period when susceptibility test results are still pending (Amaral *et al.* 2001:511). Phenothiazines have shown activity against MDR-TB with complete inhibition of respiration at a concentration of 12µg/ml. Since inhibition of respiration usually requires concentrations that exceed those required for inhibition of replication, one can assume that inhibition of replication will result before MIC is reached (Bettencourt *et al.* 2000:70).

1.8 Conclusion

The global TB situation is a long way away from no longer being viewed as critical. Despite the high burden of this disease in many countries today, the absolute number of TB cases has been falling since 2006 (WHO, 2007; WHO, 2011). Therefore, the various campaigns to improve treatment outcomes are gradually demonstrating results. However, the research community are still hard-pressed to focus all avenues into developing and/or enhancing current treatment regimens. This may be achieved by developing novel compounds for TB treatment or evaluating existing drugs for potential in TB treatment. Furthermore, new and innovative mechanisms of drug delivery are also under constant investigation to address the challenges in TB chemotherapy discussed previously. These have been the focus of many research groups globally over the past few years.

In the following chapter, the various drug delivery systems currently in use for TB drugs are reviewed. This includes the emergence of the application of nanotechnology in drug delivery systems.

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