CHAPTER 1

LITERATURE REVIEW: INTRODUCTION

1.1. Introduction

1.1.1. History of herbal medicine and plant derived drugs

Herbal medicine, sometimes referred to as herbalism or botanical medicine, is the use of plants for their therapeutic or medicinal value and has been used by all cultures throughout history (Duke, 2002). Plants produce and contain a variety of chemical substances that act upon the body. Herbalists use the leaves, flowers, stems, berries and roots of plants to prevent, relieve, and treat illnesses (Wijesekera, 1991). About 25% of the prescription drugs dispensed in the United States contain, at least, one active ingredient derived from plant material, some are made from plant extracts and others are synthesized to mimic a natural plant compound (Balick, 1990). A number of herbal plants and their compounds have been used, and have served as models for modern medicine (Farnsworth, 1984). Many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from 'white willow bark' and the 'meadowsweet plant' (Gurib-Fakim, 2006). Well known examples of plant derived drugs include the antimalarial 'quinine', extracted from the bark of the 'Cinchona' species. 'Vincristine', which is being used to treat certain types of cancer, comes from the 'Madagascar periwinkle' (*Catharanthis roseus*). In 1819, the isolation of the analgesic morphine, codeine and paregoric laid down the foundation for the purification of pharmacologically active compounds for the treatment of diarrhoea (Potterat *et al*., 2008). 'Laudanum', a tincture of the 'opium poppy', was the favoured tranquilliser in Victorian times. Even today, morphine, the most important alkaloid of the opium poppy, remains the standard against which new synthetic analgesic drugs are measured (Phillipson, 2001; De Smet, 1997).

1.1.2. Use of medicinal plants

The use of plants as the source of remedies for the treatment of many diseases dates back to prehistory and people of all continents have this old tradition. Plants continue to be a major source of medicines, as they have been throughout human history (van Wyk *et al*., 1997). Up to 80% of the world population, South Africa population use medicinal plants as remedies. Plant species serve as a rich source of many novel biologically active compounds, although very few have been thoroughly investigated for their medicinal properties (Heinrich and Gibbons, 2001). Apart of the 30% and 40% used in today's conventional drugs, other plants are used as herbal supplements, botanicals and teas (Kirby, 1996; Hostettmann and Marston, 2002). The World Health Organization (WHO) estimates that 4 billion people, or 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and is a common element in ayurvedic, homeopathic, naturopathic, traditional Oriental and Native American Indian medicines. WHO notes that of the 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures (Shulz *et al*., 2001). The uses of some medicinal plants vary a lot according to regional and cultural aspects. Their use is often associated with witchcraft and superstition, because people do not have the scientific insight to explain or predict the curative action of plants. One example of such an irrational concept is the Doctrine of Signatures (elements of which are found in many of the healing cultures of the world), which is based on the assumption that the appearance of plants may give clues to their medicinal properties (van Wyk and Wink, 2004).

Africa has a long history of people-plant interaction. The continent is characterised by rich ethnic and biotic mosaics that represent 13% of the earth's human population and has one of the largest continental floras of which estimates range from between 50 000 and 70 000 plant taxa (Nigro *et al*., 2004; Klopper *et al*., 2002; Smith and van Wyk, 2002). The African flora is remarkable not only for its diversity but its distinctiveness: as many as 88% of its species are endemic. High levels of endemism

indicate that many of the continent's plant resources are uniquely African and are used in agriculture, horticulture, medicine, forestry, etc (Nigro *et a*l., 2004; Davis *et al*., 1994). African traditional medicine is one of the oldest and the most diverse of all medicinal systems. More than 70% of the population refers to traditional healers concerning health issues. The healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines to treat the symptoms (van Wyk and Wink, 2004). Famous African medicinal plants include *Acacia senegal* (gum Arabic), *Agathosma betulina* (buchu), *Aloe ferox* (Cape aloes), *Artemisia afra* (African wormwood), *Boswellia sacra* (frankincense), *Catha edulis* (khat), *Commiphora myrrha* (myrrh), *Hibiscus sabdariffa* (hibiscus), *Hypoxis hemerocallidea* (African potato) and *Prunus africana* (red stinkwood) (van Wyk and Wink, 2004). The Khoi-San people of southern Africa, nowadays considered to be the most ancient of all cultures, have a remarkable *Materia medica* (medicinal plants and other materials) which typically includes general tonics, fever remedies, sedatives, laxatives and numerous wound healing plants.

There are an estimated 200 000 indigenous traditional healers in South Africa, and more than 60% of South Africans consult these healers, usually in addition to using modern biomedical services and commonly used medicinal plants (Table 1.1). Traditional healers in South Africa are most commonly known as "inyanga" (Zulu) and "tin'anga" (Xitsonga) (van Wyk *et al*., 1997). Traditional medicines are well recognised and different communities use a wide variety of plants to treat gastrointestinal disorders such as diarrhoea, tuberculosis, malaria, sexual transmitted diseases and other infections which are particularly prevalent in rural area (McGraw *et al*., 2000; Yelne *et al*., 2001). Although South Africa contains about 10% of the earth's plant diversity, relatively little work has been done on medicinal plants from this region. South Africa's rich heritage of indigenous medicines coupled with biodiversity, form extremely valuable resources.

Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or synergy to improve health. A single plant may contain bitter substances that stimulate digestion, anti-inflammatory compounds that reduce

Table 1.1. Commonly used medicinal plants (Yelne *et al*., 2000)

Achillea millefolium (yarrow)	*Chicorium intybus* (chicory)	*Leonurus cardiaca* (motherwoth)	*Pimpinella anisum* (anise)
Acourtia runcinata (peonia)	*Cinnamomum camphora* (camphor)	*Lavandula angustiolia* (lavender)	*Pinus sylvestris* (scots pine)
Aloe vera (aloe)	*Cinnamomum zeylanicum* (cinnamon)	*Majorana hortensis* (marjoram)	*Pogostemon cablin* (patchoul)
Allium sativum (garlic)	*Citrus aurantium* (bitter orange)	*Malva parviflora* (malva)	*Rauvolfia serpentina* (rauvolfia)
Amarylis belladonna (belladonna)	*Coriadrum sativum* (coriander)	*Maranta arundinacea* (arrowroot)	*Rhamnus cathartica* (buckthorn)
Angelica antropurpurea (angelica)	*Elettaria cardamomum* (cardamon)	*Marrubiun vulgare* (horehound)	*Rheum rhabarbarum* (rhubarb)
Anethum graveolens (dill)	*Eucalyptus globus* (eucalyptus)	*Melissa officinalis* (balm)	*Rubus fruticosus* (blackberry)
Annona squamosa (apple)	*Eugenia caryophyllata* (clove)	*Mentha arvensis* (mint)	*Ruta graveolens* (rue)
Apium graveolens (celery)	*Foeniculum vulgare* (fennel)	*Monarda fistulosa* (bergamot)	*Sabal palmetto* (palmetto)
Arnica alpina (arnica)	*Gentiana lutea* (gentiana)	*Ocimum basilicum* (basil)	*Sesamum indicum* (sesame)
Artemisia spp. (artemisia)	*Gingko biloba* (gingko)	*Olea europaea* (olive)	*Turnera diffusa* (damiana)
Calendula officinalis (calendula)	*Grindelia robusta* (gumplant)	*Origanum vulgare* (oregano)	*Taraxacum officinale* (dandelion)
Cammiphora myrrha (myrrh)	*Hypericum perforatum* (St John's wort)	*Panax quinquefolia* (ginseng)	*Urtica* spp. (nettle)

swelling and pain, phenolic compounds that act as antioxidants and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste product and toxins and alkaloids that enhance mood and give a sense of well-being (van Wyk and Wink, 2004). The importance of plants lies not only on their chemotherapeutic effect, but also in their role as a source of model compounds for drug development. In addition to plant constituents being used directly as therapeutic agents, they can be utilized as starting material or templates for drug synthesis (Eloff, 1998). In addition to active ingredients, the plant's bioflavanoids and other substances are important in supporting its medicinal properties. These elements also provide an important natural safeguard. Isolated or synthesized active compounds can become toxic in relatively small doses; unlike a whole plant which reaches a toxic level only when taken in large quantities (Eloff, 1998).

Medicinal plants are also important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Mukherjee, 2003). Major pharmaceutical companies are currently conducting extensive research on plant materials, gathered from forests and other habitats, for their potential medicinal value. Rather than using a whole plant, scientists identify, isolate, extract, and synthesize individual components, thus capturing the active compounds. There are over 750,000 plant species on earth, but relatively speaking, only a very few of the healing plants have been studied scientifically. Because modern pharmacology looks for one active ingredient and seeks to isolate it to the exclusion of all the others, most of the research that is done on plants continues to focus on identifying and isolating active ingredients, rather than studying the medicinal properties of the whole plants. Herbalists, however, consider that the power of a plant lies in the interaction of all its ingredients. Plants used as medicines offer synergistic interactions between ingredients both known and unknown (Mabogo, 1990). Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Newman *et al*., 2003).

1.1.3. Selection of medicinal plants and plant-parts used in South Africa

In order to know how to select the most appropriate medicinal plants, the therapeutic specifics must be understood. Choice of dosages must be based on the needs of the patient, the method of preparation which is affected by the chemistry of the plant constituents considered to be responsible for the therapeutic effects and the administration of plant-based drugs are very important. The part of the plant used varies among species, traditional healers and also depends on the nature and state of the disease (Mabogo, 1990). Different parts of a plant (leaves, roots, bark, fruit and seeds) often contain quite different active ingredients, so that one part may be toxic and another one quite harmless (van Wyk and Wink, 2004). The VhaVendas in the Venda region of Lipompo province of South Africa most often prepare a decoction of the plant part in soft porridge (Arnold and Gulumian, 1984). Babies for example, are generally given a soft porridge called 'tshiunza', made from flour mixed with a number of medicinal plants. The immune system of the child is expected to be strengthened by this preparation. Other forms of dosage employed in traditional preparation of medicinal plants include: maceration, juice, syrup, tincture, medicinal ointments, infusion, decoction, digestion and percolation. There are also medicinal products that are mixtures and contain two or more herbs that act individually, additively or even synergistically to restore or maintain health (van Wyk and Wink, 2004).

Traditional healers use medicinal plants for a variety of illness such as chest pains, tuberculosis (TB), malaria, diarrhoea, appetite suppressant, arthritis, asthma, etc (Cragg and Newman, 2005). A few of these plants have been scientifically investigated and valuable products (either in the form of herbal supplements or novel drugs in the form of isolated compounds) are currently going through clinical trials. Examples are: *Hoodia gordonii* which grows naturally in South Africa and Namibia, is used as appetite suppressant. *Catharanthus roseus*, endemic to Madagascar, is used for treatment of diabetes and menorrhagia. *Combretum caffrum*, indigenous to South Africa, is used for cancer. *Pelargonium sidoides*, widely distributed in South Africa, is used for respiratory problems including bronchitis and TB. Examples of plants that

are being used to treat tuberculosis in South Africa are: *Cryptocarya latifolia*, *Chenopodium ambrosioides*, *Euclea natalensis*, *Ekebergia capensis*, *Helichrysum melanacme*, *Nidorella anomala*, *Polygala myrtifolia* and *Thymus vulgaris* (Lall and Meyer, 1999).

1.2. Medicinal plants with antimycobacterial activity

Over the past decade there has been a proliferation of literature on the antibacterial, antituberculosis, antifungal and antiviral properties of plant extracts. Screening plant extracts for antimycobacterial activity is usually carried out using mycobacteria cultured in various types of broth and agar based media (Newton *et al*., 2000). There are several reports on *in vitro* inhibition of mycobacterium species by medicinal plants and the bioassay-guided research for antimycobacterial properties from plants has shown signs of success. Major review articles have appeared on antimycobacterial natural products in the last eight years. Although no marketable products for the treatment of TB have been isolated from plants, some lead compounds have been identified (Cantrell *et al*., 1999). Cantrell *et al*., (2001) isolated norditerpenoid, '12 demethylmulticauline', from the roots of *Salvia multicaulis*, which was more active than the first line TB drugs ethambutol (EMB) and nearly as active as rifampicin (RIF) *in vitro*. Mitscher and Baker discussed plant-derived compounds (berberine, lichoisoflavone, erygibisoflavone, phaseollidin, erythrabyssin II and tryptanthrin) as potential antituberculosis agents (Mitscher and Baker, 1998). Newton *et al*., 2000 reviewed plant-derived antimycobacterial natural products, describing the activity of extracts and compounds from 123 plants species (Newton *et al*., 2000).

Reports of 88 naturally occurring compounds and synthetic analogues from plants, fungi and marine organisms, that demonstrated significant activity in the *in vitro* bioassays against *M. tuberculosis* and other mycobacterial species, have been described (Okunade *et al*., 2004). Recent developments in mycobacteriology and innovative natural products chemistry tools and their potential to impact on the early steps of the TB drug discovery process have been reviewed (Pauli *et al*., 2005).

Gautam *et al*., 2007 described 70% (255 of 365) of Indian medicinal plant species, from a wide range of families, which have shown antimycobacterial activity. Interestingly, when tested preliminary in the *in vitro* screening, 149 species have shown positive ethnomedicinal uses in correlation with the traditional knowledge for TB or related diseases (Gautam *et al*., 2007).

Ten of the 408 ethanolic extracts of plants such as *Actaea spicata*, *Angustura vera*, *Cinnamonium camphora*, *Piper cubeba*, *Guauacum officinale*, *Ipomea purga*, *Rhamnus cathartica* inhibited growth of *M. tuberculosis* H37Rv at dilutions of 1 in 160 to 1280 and a high proportion of the other extracts inhibited growth at lower dilutions (Grange and Dawey, 1990). It was found that *M. tuberculosis* was also sensitive towards *Pentas longifolia*, *Tetradenia riparia* and *Bidens pilosa*, medicinal plants used in Rwanda. The active compound isolated from the leaves of *T. riparia* was tested against *M. tuberculosis* which showed activity at 100 µg/mL (van Puyvelde *et al*., 1994). *Hydrocotyle asiaticum* inhibited growth of *M. tuberculosis* at a dilution of 1:20 (Grange and Dawey, 1990). Organic extracts of *Helichrysum crispum* inhibited the growth of *M. tuberculosis* (Salie *et al*., 1996). Lall and Meyer (2001) demonstrated the inhibition of drug-sensitive and drug-resistant strains of *M. tuberculosis* by diospyrin isolated from the roots of *Euclea natalensis* AD. The phytochemical and biological studies of *E. natalensis* and isolated compounds from this plant indicated *in vitro* activity against *M. tuberculosis*. In structure-activity related studies against *M. tuberculosis* different research groups have found activity using a variety of natural products with no definite trend towards a specific group of compounds (Houghton *et al*., 1999).

In South Africa, it has been reported that people smoke dried flower and seed of *Helichrysum krausii* in a pipe for the relief of coughs and as a remedy for pulmonary TB (Watt and Breyer- Brandwijk, 1962). Chelerythrine isolated from methanolic root extracts of *Sanguinaria canadensis* was found to be the most active isolated compound against *M. smegmatis* at 29.0 µg /mL (Newton *et al*., 2002).

A number of plants have been cited in the literature as being used for medication against various bacterial and viral infections or as containing biologically active compounds. Research conducted by Noristan, Pretoria, suggests that from a total number of about 300 plants screened, at least 31% show marked analgesic, anti-inflammatory and anti-infective properties (Theunis *et al*., 1992).

Plants have been endowed with therapeutic virtues both in legend and in scientific literature and are being used in treating various ailments such as coughs, colds, other pathogenic bacterial and viral infections. The use of antimicrobials from the natural vegetation has a great impact in human health care in undeveloped countries. Herbal medicine has been used for centuries in rural areas by local healers and has been improved in industrialized countries. A number of substances used in modern medicine for the treatment of serious diseases have originated from research on medicinal plants (Theunis *et al*., 1992). In the present study plants used for TB-related symptoms were scientifically investigated for antimycobacterial activity.

1.3. Scope of thesis

1.3.1. Antimycobacterial activity of selected medicinal plants

In this study, the antimycobacterial activity of selected South African medicinal plants, that have been used in the treatment of TB symptoms, was investigated.

Ethanol crude extracts were screened against non-pathogenic strain of mycobacteria, '*M. smegmatis*'and a pathogenic strain, '*M. tuberculosis*'.

1.3.2. Cytotoxicity of the crude extracts

Cytotoxicity evaluation of the plant extracts using African monkey kidney vero cells were carried out with the intention of choosing a plant for the isolation of the active compounds with anti-TB activity and low toxicity.

1.3.3. Bioassay guided fractionation of *Galenia africana*

It was found that *G. africana* possessed high antimycobacterial activity and has less toxicity than the other plants investigated. Our objective was to isolate the active compound(s) and evaluate the minimal inhibitory concentration (MIC) against *M. tuberculosis*. Through the bioassay guided fractionation of the ethanol extract of *G. africana*, four compounds were isolated and identified.

1.3.4. Synergistic activity of the isolated compounds

Synergistic inhibitory activity of the isolated compounds was also investigated against *M. tuberculosis* using the radiometric BACTEC method.

1.3.5. Intracellular antimycobacterial activity of selected samples

It has been reported that *M. tuberculosis* may survive in macrophages by various mechanisms and that anti-TB drugs effective on macrophages are therefore needed for the treatment of TB. It was therefore decided to investigate the cytotoxicity of the ethanol extract of *G. africana*, the isolated compounds, (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-2',4'-dihydroxychalcone on U937 cell lines and their intracellular activities against U937 cells which were infected with *M. tuberculosis*.

1.3.6. Mechanism of action

Mycothiol (MSH) or 1d-*myo*-inosityl 2-(*N*-acetyl-l-cysteinyl)amido-2-deoxy-*α*-d-glucopyranoside, is an unusual conjugate of *N*-acetylcysteine (AcCys) with 1d-*myo*-inosityl 2-acetamido-2-deoxy-α-d-glucopyranoside (GlcN-Ins), and is the major low-molecular-mass thiol in mycobacteria. *M. tuberculosis* lacks glutathione, but instead maintains millimolar concentrations of the structurally distinct low molecular weight thiol MSH. MSH has antioxidant activity as well as the ability to detoxify a variety of toxic compounds. Because of these activities, MSH is a candidate for protecting *M. tuberculosis* from inactivation by the host during infections as well as for resisting

antituberculosis drugs. In order to define the protective role of MSH for *M. tuberculosis*, we investigated the inhibitory activity of selected antituberculosis extracts / compounds against mycothiol reductase, an enzyme responsible for the production of mycothiol.

1.4. Structure of thesis

The contents of each chapter are as follows:

Chapter 1: Literature on the potential of medicinal plants for antimycobacterial activity for various ailments.

Chapter 2: The epidemiology, prevention and treatment of TB and the anti-TB drugs which are in clinical trials.

Chapter 3: The selection, description and phytochemical constituents of selected South African medicinal plant species.

Chapter 4: The antituberculosis activity of selected South African medicinal plants against *M. smegmatis* and *M. tuberculosis*.

Chapter 5: Cytotoxic activity of selected South African medicinal plants.

Chapter 6: The bioassay-guided fractionation of the ethanol extract of *G. africana* and the identification of the bioactive compounds.

Chapter 7: The antimycobacterial activity of the fractions and isolated compounds from the ethanol extract of *G. africana*.

Chapter 8: The synergistic effect, cytotoxicity and intracellular antimycobacterial activity of the ethanol extract and isolated compounds from *G. africana*. Mechanism

of action of selected candidates is also discussed in this chapter.

Chapter 9: General discussion, conclusion and summary of the entire research and the importance of medicinal plants as traditional medicines.

Chapter 10: Summary.

Chapter 11: References.

Chapter 12: Acknowledgements.

Chapter 13: Appendices - Publications

CHAPTER 2
EPIDEMIOLOGY, PREVENTION AND TREATMENT OF TUBERCULOSIS

2.1. Introduction

2.1.1. History of tuberculosis

Tuberculosis (TB) is a disease known since antiquity and evidence of spinal TB in the form of fossil bones dates back to around 8000 BC (Ayyazian, 1993; Basel, 1998). TB occurred as an endemic disease among animals long before it affected humans (Steele and Ranney, 1958). The first confirmed instance of TB in humans was noted in the deformities of the skeletal and muscular remains of the Egyptian mummies of around 2400 BC (Haas, 1996). However, it could not be determined whether the disease was due to *M. bovis* or *M. tuberculosis*. In the 1700s and early 1800s, TB prevalence peaked in Western Europe and the United States and was undoubtedly the largest cause of death. Hundred to 200 years later, it had spread in full force to Eastern Europe, Asia, Africa and South America (Bloom and Murray, 1992).

2.1.2. Mycobacterium species

The genus *Mycobacterium* (order Actinomycetales, family Mycobacteriaceae) consists of about 50 acid-fast, aerobic, non-motile and non-spore-forming bacterial species. Most of these species are environmental saprophytes, existing in various substrate including soil, water, plants, mammals and birds. The genus is divided into the fast-growing and the slow-growing species. The fast-growing species are usually not pathogenic but some may cause opportunistic infections in animals and humans (Grange and Yates, 1986). The pathogenic species are obligate parasites and cause TB in humans and animals (McGaw *et al*., 2008).

2.1.2.1. *Mycobacterium tuberculosis*

M. tuberculosis was described on the 24th of March 1882 by Robert Koch, who in 1905 received the Novel Prize in physiology or medicine for this discovery (Newton *et al*., 2000). *M. tuberculosis* is also known as Koch's bacillus and it is a member of the "tuberculosis complex", a group of closely related mycobacterial pathogens, which include *M. bovis* (which infects cattle and may also infect humans), *M. microti*, *M. africanum* (which causes TB in West Africa), *M. avium*, *M. intracellulare*, *M. leprae* (causes leprosy in man), *M. lepraemurium* infection in rats and cats and *M. scrofulaceum* (causing opportunistic infectious disease in patients with AIDS, Hourne, 1996).

M. tuberculosis the causative agent of TB in humans is a fairly large nonmotile rod-shaped bacterium distantly related to the actinomycetes. The rods are 2-4 um in length and 0.2-0.5 um in width (Figure 2.1). The major components of the cell wall structure of the bacteria consist of peptidoglycan and lipids. Mycolic acids, which are α-branched lipids in cell walls, make up 50% of the dry weight of the mycobacterium cell envelope and are very strong hydrophobic molecules that form a lipid shell around the organism (Goren, 1990). Cord factor is a glycolipid (trehalose dimycolate) found in the cell wall that induces replication *in vitro*, resulting in serpentine cords of organisms. The role of the cord factor in the pathogenesis of TB is still under investigation, however, it is thought to be important because it inhibits and induces secretion of TNF-alpha by macrophages (Brennan, 1998).

Figure 2.1. Rods of *M. tuberculosis*, magnification x 6.250 (based on a 35 mm slide *image* of 24 mm in the narrow dimension (Courtesy: SEM/ 97229A)

M. tuberculosis is not classified as either a Gram-negative or Gram-positive bacteria because it does not have the biochemical characteristics of either (Camus *et al.*, 2002). If a Gram stain is performed on *M. tuberculosis*, it stains very weakly Gram-positive or not at all. *Mycobacterium* species, along with members of a related genus *Norcardia*, are classified as acid-fast bacteria due to their impermeability by certain dyes and stains. One acid-fast staining method for *M. tuberculosis* is staining with carbon-fuchsin (a pink dye) and decolourising with acid alcohol. The smear is counterstained with methylene blue or certain other dyes on different media such as Lowenstein-Jensen (Figure 2.2; Fadda and Roe, 1984).

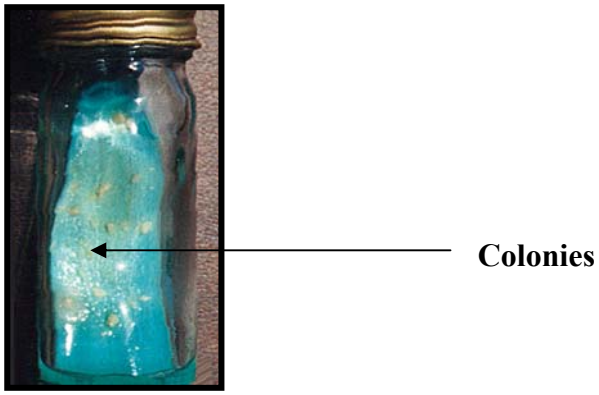


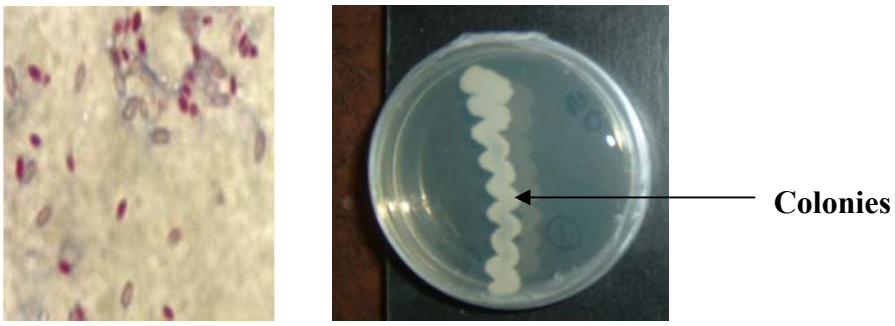
Figure 2.2. Colonies of *M. tuberculosis* on Lowenstein-Jensen medium

M. tuberculosis is a slow-growing bacillus which is transmitted primarily by the respiratory route. Infection with *M. tuberculosis* occurs by inhalation of small (1 – 10 microns) droplets containing only a few live tubercle bacilli. The primary focus of infection is usually the middle or lower zones of the lung. The bacilli are readily taken up by lung macrophages but can survive and grow to form the primary focus of infection and from there, enter the local lymphatic system and then move throughout the body via the blood and lymphatic system. This stage (the local lymphatic system), of disease is usually clinically silent or associated with mild fever and in most cases immunity develops within a few weeks and the patient becomes tuberculin positive (Girling, 1989). A key characteristic of *M. tuberculosis* infection is that the bacterium multiplies intracellularly, primarily in macrophages and in this way evading many host defence mechanisms (Banki *et al*., 1999; Velasco-Velazquez *et al*., 2003).

M. tuberculosis infects about 32% of the world's population. Every year, approximately 8 million of these infected people develop active tuberculosis and almost 2 million of these will die from the disease (WHO, 2006).

2.1.2.2. *Mycobacterium smegmatis*

M. smegmatis is a gram positive bacteria, belonging to the family Mycobacteriaceae and the genus *Mycobacterium* (Megehee and Lundrigan, 2007). The bacterial species is found in the soil, water and plants. It is acid-fast staining (Figure 2.3a) that shares many features with the pathogenic *M. tuberculosis*. It was first reported in November 1884 by Lustgarten who found a bacillus with the staining appearance of tubercle bacilli in syphilitic chancres. *M. smegmatis* is generally considered a fast growing non-pathogenic microorganism that can be cultured in any laboratory and the culture smear is grown on media such as Middlebrook 7H11 agar (Figure 2.3b), however, in some cases it can cause disease, mainly in animals (Niederweis *et al*., 1999). *M. smegmatis* possess a limited degree of similarity to *M. tuberculosis* with regard to drug susceptibility (Gautam *et al*., 2007).



(a) (b)

Figure 2.3. ***M. smegmatis***

(a) Rods of *M. smegmatis*: acid-fast stained red (due to carbon fuchsin dye), magnification x 1000, taken in a general microbiology lab (slide *image* of 21 mm in the narrow dimension (Courtesy: SEM/ 97229A)

(b) Colonies of *M. smegmatis* on Middlebrook 7H11 agar medium

2.1.3. Epidemiology

In the late 1980s, TB began re-emerging and now globally, kills more than 2 million people each year. It is thought that as many as 2 billion people have been exposed to the TB bacillus and are therefore at risk of developing the active disease (Gutierrez-Lugo *et al*., 2005). According to WHO (2001), TB is known to be the largest cause of death of the human species. There has been a resurgence of the disease over the last two decades with currently eight million new cases and about 200,000 deaths annually. It is estimated that between 2000 and 2020, nearly one billion people will become infected, 200 million will acquire the disease and 35 million will die from TB (WHO, 2000), in contrast to the 1.6 million deaths from TB in 2005. Both the highest number of deaths and the highest mortality rate are in the Africa region. The two essential factors for the rapid spread of TB are; crowded living conditions, which favour airborne transmission and a population with little natural resistance. TB in populations can be attributed to three distinct factors:

- Infection of an individual in the community with tubercle bacilli within a given time period.
- Development of the disease shortly after such infection.
- The disease developing long after the original infection, owing to the reactivation of latent bacilli (Raviglone *et al*., 1995; Bloom, 1994).

Today, TB is the leading cause of death worldwide from a single human pathogen, claiming more lives than diseases such as Human Immunodeficiency Virus / Acquired Immune deficiency Syndrome (HIV/AIDS), malaria, diarrhoea, leprosy and all the other tropical diseases combined (Zumla and Grange, 1998). The pandemic of HIV/AIDS infection and the evidence of an association with TB, have caused marked increases in the incidence of the disease in some countries (Bloom, 1994). Because of its ability to destroy the immune system, HIV has emerged as the most significant risk factor for progression of dormant TB infection to clinical disease (Selwyn *et al*., 1989). The Global Programme on AIDS of the WHO estimated that in 1992 at least 13 million adults and 1 million children had been infected with HIV worldwide

(WHO, 1999). The impact of HIV/AIDS infection on the TB situation is greatest in those populations where the prevalence of TB infection in young adults is very high (Bloom, 1994).

The number of cases worldwide is now increasing rapidly due to multi-drug resistant strains of *M. tuberculosis* as a result of patient non-compliance and also due to an increase in patients with HIV/AIDS (Collins, 1998; Zumla and Grange, 1998). About 450 000 multi-drug resistant tuberculosis (MDR-TB) cases are estimated to occur every year, the highest rates are in countries of the former Soviet Union and China (WHO, 2006). There are a number of countries that have made remarkable progress in expanding population coverage with cure rates, whereas South Africa battles with more than 188 000 new TB cases per year (Bloom, 2002). South Africa is burdened by one of the worst TB epidemics in the world, with the disease rates more than double of those observed in other developing countries and up to 60 times higher than those currently seen in developed countries (Bapela, 2005).

The TB problem in South Africa is largely as a result of historical negligence and poor management systems, compounded by the legacy of fragmented health services. In South Africa, a high proportion of the population lives under poor condition and this may lead to the disease becoming uncontrollable (Fourie and Weyer, 2000). South Africa has by far the worst TB prevalence rate in the world, with 998 South Africans out of every 100 000, living with TB (WHO, 2008). More than 280 000 cases of TB were reported in different South African provinces in 2006, which is an increase of 98% since 2001 when just over 120 000 TB cases were reported. In 2006, there were more than 131 000 new infectious cases and a 57% increase, since 2001 of spreading the disease to others (Figure 2.4., Tuberculosis Fact Sheet, 2007). The breakdown of TB patients reported from 2001 - 2006 in South Africa increased yearly, and the primary aim of the South African National TB Control Programme is to cure all new smear positive patients first time around (Table 2.1). The South African Medical Research Council (MRC) estimated 273 365 new cases of TB in the year 2000, of which 113 945 were infectious and 46,7% were HIV related.

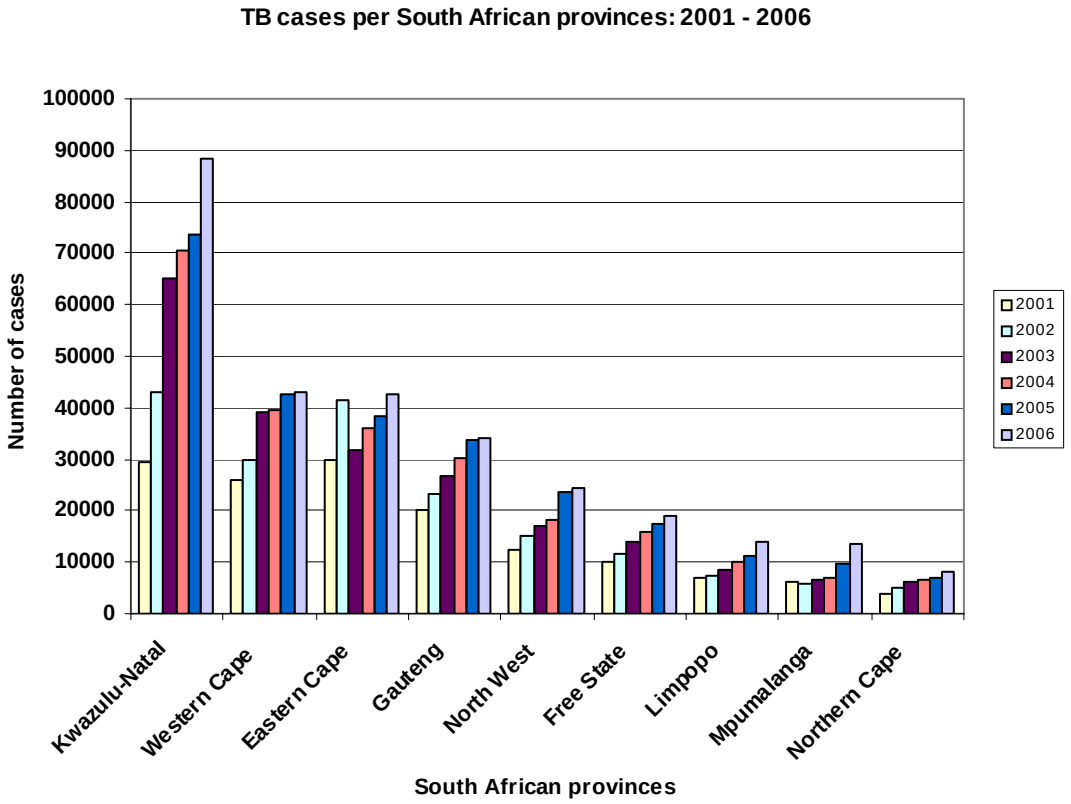


Figure 2.4. The increase of reported TB cases per South African provinces from 2001 - 2006 (Tuberculosis Fact Sheet, DoH -RSA, 2007)

Table 2.1. Breakdown of TB patients reported from 2001 - 2006 in South Africa (Tuberculosis Fact Sheet, DoH -RSA, 2007)

Cases	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
TB	188 695	224 420	255 422	279 260	302 467	342 315
Pulmonary TB	144 910	182 583	215 154	234 213	257 604	287 440
New smear positive pulmonary TB	83 808	98 800	116 337	117 971	125 460	131 619
Re-treatment pulmonary TB	20 686	25 091	30 331	32 882	37 541	40 736

TB is not distributed evenly, throughout South Africa and the rates vary considerable among the nine provinces (Table 2.2). Current strategies for the control of TB centres around treatment with multi-drug regimes based on the very effective combination of isoniazid (INH., Figure 2.5) and rifampicin (RIF., Figure 2.6). In endemic areas, the

diagnosis and treatment of smear positive patients are emphasized in order to interrupt the spread of the disease within the community. Obstacles to the success of this strategy are the difficulties of early diagnosis and operational problems associated with delivery of a treatment that involves administration of multiple drugs over a period of at least six months (Young and Duncan, 1995).

Table 2.2. Occurrence of TB in different provinces of South Africa during the year 2000: 600 total cases per 1000 000 population; 250 smear-positive cases per 100 000 population (Fourie and Weyer, 2000)

Name of Province	Total TB cases	Proportion HIV+
Eastern Cape	56 495	40.0%
Gauteng	45 498	44.8%
Free State	14 654	51.7%
Kwazulu Natal	65 695	64.6%
Limpopo	23 338	36.3%
Mpumalanga	15 657	59.1%
Northern Cape	4 649	33.2%
North West	15 549	45.5%
Western Cape	34 211	31.6%
South Africa	**273 365**	**46.7%**

2.1.4. Transmission of tuberculosis

The principal risk method of being infected with TB is by inhaling contaminated air containing microbes that cause this disease (Rom and Garay, 1996). TB microbes can be present in sufficient concentrations in the air to cause infections and the disease. Once in air, water evaporates from the surface of a particle, decreasing its size and concentrating its contents of microbes. These particle forms a droplet nuclei in which evaporation continues until the vapour pressure of the droplet equals to the atmospheric pressure. The droplet nuclei are very stable, settle very slowly and remain suspended in the air for very long periods. Droplet nuclei are produced when a

patient with active pulmonary or laryngeal TB coughs, speaks, sneezes or sings. Coughing can produce 3000 infectious droplet nuclei, talking for 5 minutes an equal number and sneezing can produce over a million particles with a diameter of less than 100nm (Bloom, 1994). When inhaled, droplet nuclei usually travel through the airway until they reach the alveoli. Larger particles that are deposited on the way are removed through normal mechanism of airway clearance (Dannenberg, 1989).

2.1.5. Immunology of tuberculosis

TB is a prototype infection that requires control by the cellular immune response. In the first few weeks the host has almost no immune defence against infection by the bacteria causing TB. Small inhalation inocula multiply freely in the alveolar space or within alveolar macrophages. Unrestrained bacterial multiplication proceeds until the development of tissue hypersensitivity and cellular immunity intervene. The organism causing TB adheres to alveolar macrophages via multiple complements and might be destroyed in the phagosome (Beyers, 1999). The intracellular mechanisms for killing or inhibiting the growth of the bacteria in alveolar macrophages include the production of nitric oxide and reactive oxygen intermediates. Alveolar macrophages can also participate, in a broader context of cellular immunity, through the process of antigen presentation and recruitment of T-lymphocytes, which are the white blood cells produced in the bone marrow but which mature in the thymus. These cells are important in the body's defence against certain bacteria and fungi (Beyers, 1999).

Macrophages which are antigens are processed in phagosomes via Major Histocompatibility Complex (MHC) class II molecules to CD4 T-lymphocytes which are the major effector cells in cell-mediated immunity. The antigens bind to T-cell receptors on the surface of the T-lymphocytes. These CD4 T-lymphocytes tend to polarize into either Th1 cells (these are essential in controlling intracellular pathogens), producing predominantly interferon gamma (IFN-γ) and interleukin 2 (IL-2) or Th2 cells producing predominantly cytokines interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 10 (IL-10) and interleukin 13 (IL-13). In mice, immunity correlates with a Th1 response. Macrophages infected with *M. tuberculosis*

secrete interleukin 12 (IL-12), which induces the secretion of IFN- γ by CD4 cells and natural killer cells. The IFN- γ enhances the activation of macrophages and improves their ability to prevent the spread of *M. tuberculosis* (Orme, 1993). However, *M. tuberculosis* is not defenceless. It can produce ammonia to counteract phagosomal acidification. Its lypogly can actively scavenge toxic radicals reduced against it by the macrophage (Orme, 1993; Bapela, 2005; Banki *et al*., 1999).

2.1.6. Prevention of tuberculosis

The outcome of mycobacterial infection depends on the host immune response. In most individuals, infection with *M. tuberculosis* induces an immune response sufficient for the protection against progression to the primary disease (Bapela, 2005). Bacille Calmette-Guérin (BCG) vaccine reproduces minimal infection but does not impose a disease risk. BCG vaccine, which is derived from a strain of *M. bovis* attenuated through years of serial passage in culture, was first used in 1921 to protect against TB in humans (Young and Duncan, 1995). Many BCG vaccines are currently administered to 100 million young children each year throughout the world. These vaccines are derived from the original strain but vary in cultural characteristics and ability to induce sensitization to tuberculin. There are differences in techniques and methods of producing them as well as various routes of vaccine administration (Young, 1994).

2.1.7. Infection and symptoms of tuberculosis

TB infection means that the TB germ can be found in the host body, but it cannot always make a person feel sick. This is because of the health immune system which cannot destroy the TB germ by itself, but it can keep the TB germ trapped in the lungs and prevent it from spreading further. Because the TB germ is strong and protects itself with a thick coating, it can remain in an inactive state (dormant) in the body for many years. People with TB infection whose immune systems are weakened and those that have some other kinds of lung disease are more likely to develop TB disease (WHO, 2007). Depending on where in the body the TB bacteria are

multiplying, symptoms of TB disease can vary. Coughing up blood, chest pains, bad cough lasting longer than 2 weeks, weight loss, chills, fever and night sweats are common symptoms of TB disease. Sometimes people experience joint pain like arthritis if the TB is in their bones. Without treatment, a person who has TB disease will infect an average of 10 - 15 people with TB every year (Davies, 2003).

2.1.8. Treatment of tuberculosis

Before effective drugs were available, half of the patients with active pulmonary TB died within 2 years, and only a quarter were cured. With the advent of anti-TB chemotherapy, protracted bed rest and lengthy isolation became unnecessary, and in theory at least, successful treatment was a reasonable goal in all adults (Bartmann, 1988). *Mycobacterium* is naturally resistant to most common antibiotics and chemotherapy agents. This is probably due to their highly hydrophobic cell envelope acting as an efficient permeability barrier. Due to the discovery of the effective antimycobacetrial agents ethambutol (EMB., Figure 2.7), INH, pyrazinamide (PZA., Figure 2.8), RIF and streptomycin (STR., Figure 2.9) between 1950 and 1970s, and reduction in poverty, there was a drastic decrease in the number of TB cases especially in developed countries. However, since 1980s, the number of TB cases throughout the world has been increasing rapidly due to the emergence of MDR-TB (Chan and Iseman, 2002).

The MDR forms of the disease, defined as forms resistant to two or more existing TB-drugs, are often fatal and are difficult and expensive to treat (Basso and Blanchard, 1998; Bastian and Colebunders, 1999). The situation has recently been complicated by the association of TB with HIV in sub-Saharan Africa and many developing countries (Corbett *et al*., 2003; Lurie *et al*., 2004). The situation is exacerbated by the increasing emergence of extensively drug-resistant (XDR) TB (Core Curriculum on Tuberculosis, 2006). Reliable treatment therapy for TB treatment takes a period of 6 - 9 months with the first line TB drugs (EMB, INH, PZA, RIF and STR). In the case of acquired drug resistance only second-line drugs (capreomycin, cycloserine, kanamycin and ethionamide) can be used and these have significant side effects with

approximately 50% cure rate (Gautam *et al*., 2007; Heym and Cole, 1997). The current therapies reduce the pulmonary bacterial burden but the treatment periods of 6 months for non-immune suppressed individuals and at least 9 months for immune suppressed patients are required for reliable treatment efficacy (Bapela, 2005; Quenelle *et al*., 2001). However, floroquinolones such as ofloxacin, norfloxacin can be used which are safer than the above-mentioned second-line drugs but have the disadvantage of being very expensive (Tripathi *et al*., 2005). Emergence of drug-resistant mycobacterial strains is alarming these days. This occurs when a single drug is given alone and when the viable bacterial population in the lesions is large. The occurrence of drug resistance is widely thought to be due to the overgrowth of sensitive organisms by mutant resistant bacilli present in wild strains before they were ever in contact with the drug concerned (Mitchison, 1984). There have been no new anti-TB drugs introduced in the past 30 years. Thus, there is an urgent need to search for and develop new effective and affordable anti-TB drugs (Gautam *et al*., 2007).

2.2. Targets, mode of action of first-line 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 (Zhang, 2005). According to their mode of action, first and second line drugs can be grouped as cell wall inhibitors (INH, EMB, ethionamide, cycloserine), nucleic acid synthesis inhibitors (RIF, quinolones), protein synthesis inhibitors (STR, kanamycin) and inhibitors of membrane energy metabolism (PZA) (Mitchison, 1980). Targets and mechanisms of action of current TB drugs are summarised in Table 2.3.

Existing TB drugs are therefore only able to target actively growing bacteria through the inhibition of cell processes such as cell wall biogenesis and DNA replication. This implies that current chemotherapy is characterised by an efficient bactericidal activity but an extremely weak sterilising activity (defined as the ability to kill the slowly growing or metabolising bacteria that persist after most of the growing bacteria have

Table 2.3. Commonly used first and second line TB drugs and their targets (Zhang, 2005)

Drug (year of discovery)	**MIC (µg/mL)**	**Effect on bacterial cell**	**Mechanisms of action**	**Targets**	**Genes involved in resistance**
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)	*katG*; *inhA*, *ndh*
Rifampicin (1966)	0.05 - 0.5	Bactericidal	Inhibition of RNA synthesis	RNA polymerase β subunit	*rpoB*
Pyrazinamide (1952)	20.0 - 100.0	Bactericidal	Disruption of membrane transport and energy depletion	Membrane energy metabolism	*pncA*
Ethambutol (1961)	1.0 - 5.0	Bactericidal	Inhibition of cell wall arabinogalactan synthesis	Arabinosyl tranferase	*emb*CAB
Streptomycin (1944)	2.0 - 8.0	Bacteriostatic	Inhibition of protein synthesis	Ribosomal S12 protein and 16S rRNA	*rps*L; *rrs* (operon)
Kanamycin (1957)	1.0 - 8.0	Bactericidal	Inhibition of protein synthesis	Ribosomal S12 protein and 16S rRNA	*rps*L*; rrs* (operon)
Quinolones (1963)	0.2 - 4.0	Bactericidal	Inhibition of DNA replication and transcription	DNA gyrase	*gyrA*; *gyr*B
Ethionammide (1956)	0.6 - 2.5	Bacteriostatic	Inhibition of mycolic acid synthesis	Acyl carrier protein reductase (inhA)	*inh*A; *eta*A/*ethA*
Para-aminosalicylic acid (1946)	1.0 - 8.0	Bacteriostatic	Inhibition of folic acid and iron metabolism	Unknown	Unknown
Cycloserine (1952)	5.0 - 20.0	Bacteriostatic	Inhibition of peptidoglycan synthesis	D-alanine racemase	*alr*A; *Ddl*

been killed by bactericidal drug). Sterilising activity also describes the ability to eliminate latent or dormant bacteria that survive inside the host macrophages (Mitchison, 1980). Although achieving a clinical cure, the current TB chemotherapy does not achieve a bacteriological-eradication of all bacilli in the lesions (McCune and Tompsett, 1956).

2.2.1. Isoniazid (INH)

INH (Figure 2.5) is the synthetic hydrazide of isonicotic acid discovered in 1952 and the first-line antituberculosis medication used in the prevention and treatment of TB. INH is the cornerstone of the therapy and should be included in all regimens unless a high degree of INH resistance exists. This drug is never used on its own to treat TB because resistance develops quickly. INH is highly selective and acts almost exclusively against *M. tuberculosis*, *M. bovis* and *M. africanum*. This remarkable selectivity in its action is thought to be mediated by the bacterial enzyme catalase peroxidase which catalyses the reaction converting INH to a potent bactericidal derivative. INH is bactericidal at MIC levels of less than 0.1 µg/mL for 80% of susceptible strains of *M. tuberculosis* (Reichman and Hershfield, 2000). INH is available in tablet, syrup and injectable forms (given intramuscularly and intravenously).

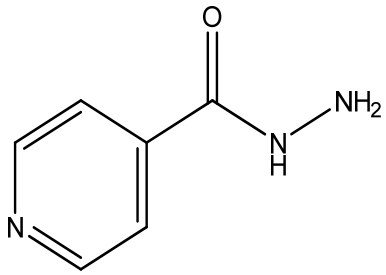


Figure 2.5. Chemical structure of isoniazid (INH)

2.2.1.1. Mechanism of action

The exact mechanism of the action of INH has not been fully elucidated, but several

mechanisms including interference with the metabolism of bacterial proteins, nucleic acids, carbohydrates and lipids have been proposed. INH is a prodrug and must be activated by bacterial catalase. It is activated by catalase-peroxidase enzyme katG to form isonicotinic acyl anion or radical. These forms will then react with a NADH radical to form isonicotinic acyl-NADH complex. This complex will then binds tightly to ketoenoylreductase known as InhA and prevent access of the natural enoyl-AcpM substrate. This mechanism inhibits the synthesis of mycolic acid in the mycobacterial cell wall. INH combines with an enzyme which interferes with the cell metabolism of the bacteria. As a result of the disruption in its metabolism and without a cell wall the bacteria die (Bapela, 2005). INH reaches therapeutic concentration in serum, cerebrospinal fluid (CSF) and within caseous granulomas and metabolised in the liver via acetylation. INH is bactericidal to rapidly-dividing mycobacteria, but is bacteriostatic if the *Mycobacterium* is slow-growing. Susceptible bacteria may undergo 1 or 2 divisions before multiplication is arrested (Bartmann, 1988).

2.2.1.2. Resistance to INH

INH inhibits the biosynthesis of mycolic acids present in the cell wall of *M. tuberculosis*. This renders the mycobacterial cell wall defective, thereby penetratable to toxic oxygen. KatG is the only enzyme in *M. tuberculosis* capable of activating INH. Expression of either the KatG or an alkyl hydroperoxidase AhpC is considered sufficient to protect the bacilli against toxic peroxides (Wilson and Collins, 1996).

2.2.1.3. Side effects and toxicity

Adverse reactions include rash, abnormal liver functions, hepatitis, sideroblastic anemia, peripheral neuropathy, mild central nervous system (CNS) effects and drug interactions resulting in increased phenytoin (dilantin) or disulfiran (antabuse) levels. Peripheral neuropathy and CNS effects are associated with the use of INH and are due to pyridoxine (vitamin B6) depletion. Headache, poor concentration, poor memory and depression have all been associated with INH use. The frequency of these side effects is not known and the association with INH is not well validated. The presence

of these symptoms is not frequently disabling and is not a reason to stop treatment with INH and the patients are strongly encouraged to continue treatment despite these symptoms (Holdiness, 1984).

The hepatoxicity associated with INH results from the toxic effect of an intermediate product produced by N-hydroxylation of monoacetylhydrazine, one of the metabolites of INH, by the liver cytochrome P-450 mixed function oxidase system. Hepatoxicity, nausea, vomiting, abdominal pains and appetite loss can be avoided with close clinical monitoring of the patient (Holdiness, 1984).

2.2.2. Rifampicin (RIF)

RIF (Figure 2.6) is the second major antituberculosis agent which is used in conjunction with other antituberulosis agents in the treatment of TB. RIF is a semi synthetic derivative of one of a group of structurally similar, complex macrocyclic antibiotics produced by *Streptomyces mediterranei* (Bartmann, 1988).

RIF inhibits the growth of most Gram-positive bacteria as well as many Gram-negative bacteria. RIF inhibits *M. tuberculosis* at concentrations ranging from 0.005 – 0.2 µg/mL *in vitro* (Bapela, 2005; Duman *et al*., 2004). RIF is soluble in organic solvents and in water at acidic pH (Bapela, 2005).

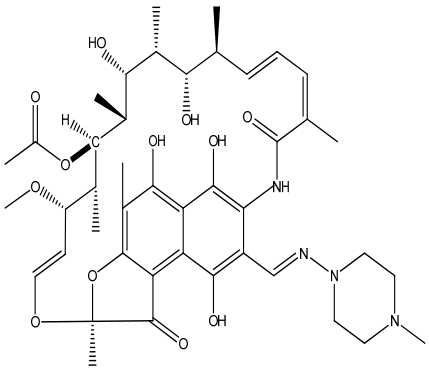


Figure 2.6. Chemical structure of rifampicin (RIF)

2.2.2.1. Mechanism of action

RIF may be bacteriostatic or bactericidal in action, depending on the concentration of the drug attained at the site of infection and the susceptibility of the infecting organism. RIF inhibits dioxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA)-polymerase of the *Mycobacterium* by forming a stable drug-enzyme complex, leading to the suppression of the initiation of chain formation in RNA synthesis (Bapela, 2005). More specifically, the β–subunit of this complex enzyme is the site of the action of the drug, although RIF binds only to the holoenzyme (Bartmann, 1988).

2.2.2.2. Resistance to RIF

Mutation in RNA polymerase beta subunit gene (rpoB) is the major mechanism of resistance to RIF with high frequencies of 90% or more (Taniguchi, 2000). Evaluation of the relationship between RIF's susceptibility and genetic alteration in rpoB gene also showed that 95% of the RIF-resistant *M. tuberculosis* isolates involved genetic alterations in an 81-base pair core region of rpoB gene. This region is called the rifampicin resistance-determining region (Ramaswammy and Musser, 1998). Moreover, these genetic alterations in the rpoB gene are suspected as being the resistance mechanisms to RIF (Taniguchi, 2000).

2.2.2.3. Side effects and toxicity

In humans, acute overdose with RIF, i.e. up to 12g has not been fatal, however one fatality has been reported following ingestion of a single 60g dose of the drug (AHFS Drug Information, 2000). The lethal dose (LD_{50}) of RIF in mice is 0.885 g/kg. The most important complication of RIF is liver toxicity, which occurs 4 times more frequently in regimens containing both INH and RIF than in those containing INH alone (Bartmann, 1988., Bapela, 2005).

2.2.3. Ethambutol (EMB)

EMB (Figure 2.7) is a synthetic antituberculosis agent prescribed to treat TB. It is active *in vitro* and *in vivo* against *M. tuberculosis*, *M. bovis*, *M. marinum*, *M. avium* and *M. intracellulare*. EMB is usually given in combination with other drugs such as INH, RIF and PZA, at a daily dose of 25 mg/kg to humans during the first 2 months of well-supervised therapy and at 15 mg/kg for longer, often less well supervised periods (Bartmann, 1988).

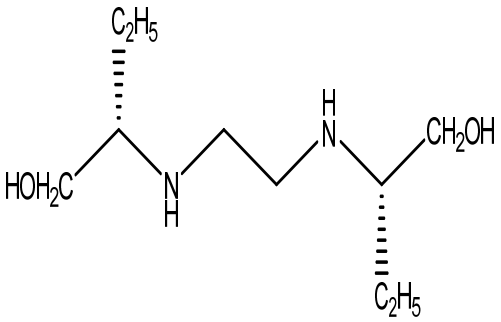


Figure 2.7. Chemical structure of ethambutol (EMB)

2.2.3.1. Mechanism of action

EMB may be bacteriostatic or bactericidal in action, depending on the concentration of the drug attained at the site of infection and the susceptibility of the organism. Although the exact mechanism has not yet been fully determined, the drug appears to inhibit the synthesis of one or more metabolites in susceptible bacteria resulting in the impairment of cellular metabolism, arrest of multiplication, and cell death. EMB is active against susceptible bacteria only when they are undergoing cell division (AHFS Drug Information, 2000).

2.2.3.2. Resistance to EMB

EMB has been shown to inhibit the incorporation of mycolic acids into the cell wall. It has also been shown to inhibit the transfer of arabinogalactan into the cell wall of

mycobacteria (Ramaswamy and Musser, 1998). Only the dextroisomer of EMB is biologically active, an observation consistent with the idea that the drug binds to a specific drug target, which is assumed to be arabinosyl transferase (Ramaswammy and Musser, 1998). The EmbB gene, encoding arabinosyl transferase which catalyses cell wall synthesis, is mutated in EMB-resistant strains (Taniguchi, 2000).

2.2.3.3. Side effects and toxicity

A single administration of EMB has low toxicity in mice (Diermeier *et al*., 1966). In humans the adverse effects of EMB include dermatitis, pruritis, headache, dizziness, fever and mental confusion.

2.2.4. Pyrazinamide (PZA)

PZA (Figure 2.8) is a derivative of niacinamide and is a synthetic antituberculosis drug used to treat TB in patients (AHFS Drug Information, 2000). Currently, PZA is considered as a first line drug, and only used in combination with other drugs such as INH and RIF in the treatment of *M. tuberculosis*. PZA has no other medical uses and is not used to treat other mycobacteria (*M. bovis* and *M. leprae*) which are resistant to the drug. PZA is used in the first two to four months of treatment to reduce the duration of treatment required.

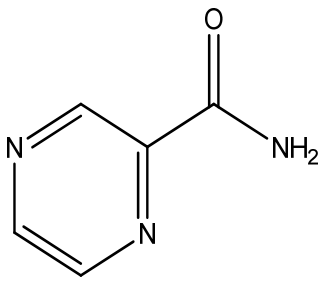


Figure 2.8. Chemical structure of pyrazinamide (PZA)

2.2.4.1. Mechanism of action

PZA may be bacteriostatic or bactericidal in action, depending on the concentration of the drug attained at the site of infection and the susceptibility of the organism (Bapela, 2005). The drug is active *in vitro* and *in vivo* at a slightly acidic pH. PZA stops the growth of *M. tuberculosis*, which has an enzyme pyrazinamidase that is only active at acidic pH. PZA converts the enzyme to the active form, pyrazinoic acid (POA). POA inhibits the enzyme fatty acid synthetase 1, which is required by the bacterium to synthesise fatty acids.

In addition, POA lowers the pH of the environment below a certain level which is optimal for *M. tuberculosis* growth. Mutations of the pyrazinamidase gene (*pncA*), are responsible for PZA resistance in *M. tuberculosis*. This appears to contribute to the drug's antimycobacterial activity *in vitro* (Bartmann,1988).

2.2.4.2. Resistance to PZA

The mechanism of action and the resistance of *M. tuberculosis* to PZA have also been partially identified. PZA is crucial for achieving sterilization by killing persisting semi-dormant bacilli in the lungs. Its activity depends on the presence of a bacterial amidase, which converts PZA to POA, which is the active antibacterial molecule (Mitchison and Selkon, 1996). PZA-resistant bacilli lack this amidase activity. The pncA gene encoding for this has been identified and the mutation to this pncA gene has been associated with resistance to PZA (Scopio and Zhang, 1996).

2.2.4.3. Side effects and toxicity

The most frequent adverse effect of PZA is hepatoxicity. Hepatoxicity becomes a problem when PZA is given in large doses and for long periods. Hepatoxicity may appear at any time during therapy. When PZA is used in short-course therapy no increase in the incidence of hepatotoxicity is noted (Bapela, 2005). The original dose for PZA was 40 – 70 mg/kg and the incidence of drug-induced hepatoxicity has fallen

significantly since the recommended dose has been reduced. In the standard four-drug regime (INH, RIF, PZA and EMB), PZA is the most common cause of drug-induced hepatitis. It is not possible to clinically distinguish pyrazinamide-induced hepatitis from hepatitis caused by INH or RIF.

Another common side effect of PZA is joint pains (arthralgia), which can be distressing to patients, but never harmful. Other side effects include nausea and vomiting, anorexia, sideroblastic anemia, skin rash, urticaria, pruritus, hyperuricemia, dysuria, interstitial nephritis, malaise, rarely porphyria and fever (British Thoracic Society, 1984). In mice, PZA has a LD_{50} of 3.4 g/kg when administered orally (Robinson *et al*., 1954).

2.2.5. Streptomycin (STR)

STR (Figure 2.9) is an aminoglycoside antibiotic which is particularly active against *M. tuberculosis* as well as against many Gram-negative bacteria. STR is bactericidal for the tubercle bacillus *in vitro*. Concentrations as low as 0.4 µg/mL inhibit growth of the tubercle bacillus *in vitro*. STR is an alternative to EMB in the four-drug protocols for the treatment of TB. STR is easily soluble in water (Bapela, 2005).

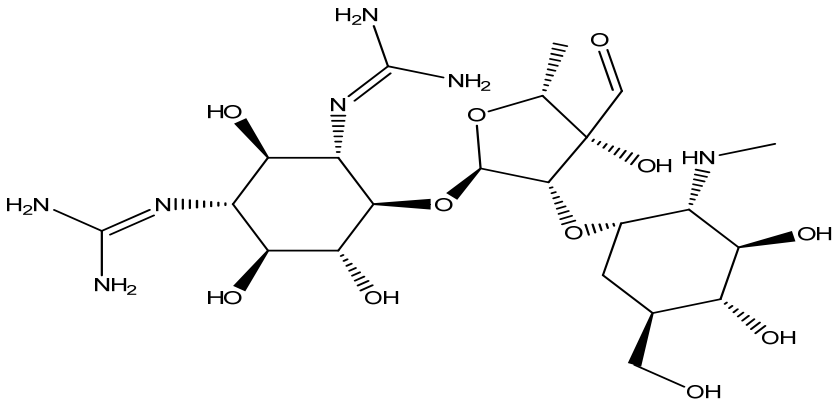


Figure 2.9. Chemical structure of streptomycin (STR)

2.2.5.1. Mechanism of action

STR, like other aminoglycosides, is actively transported across the bacterial cell membrane by an oxygen-dependent system. Once inside the bacteria, it binds to the polysomes and inhibits the synthesis of proteins. The drug binds to the 30S subunit of the bacterial ribosome which consists of 21 proteins and a single 16S molecule of RNA. Protein synthesis in the bacteria is blocked by inhibiting the movement of the peptidyl-Trna associated with translocation and this stimulates tRNA errors (Bapela, 2005).

2.2.5.2. Resistance to STR

Most STR resistance strains have a mutation on the rrs and rspL genes encoding a 16S rRNA and a 12S ribosomal subunit protein respectively (Taniguchi, 2000). In contrast to other bacteria, which have multiple copies of rRNA genes, *M. tuberculosis* complex members have only one copy. Hence, single nucleotide changes can potentially produce antibiotic resistance (Ramaswammy and Musser, 1998).

2.2.5.3. Side effects and toxicity

The toxic effects of STR are manifested mainly on vestibular rather than auditory function in human beings. An acute toxic effect following intracisternal injection in animals is clonic-convulsion. Other acute toxic effects following cutaneous or intravenous injections are nausea, vomiting and ataxia (Holdiness, 1984).

2.3. Why are new TB drugs needed?

HIV/AIDS has dramatically increased the risk of developing active TB and HIV co-infection makes TB more difficult to diagnose and treat. The increasing emergence of MDR-TB and the nature of persistent infections pose additional challenges to the treatment with conventional anti-TB drugs. Although TB can be treated with current

drugs, treatment is complex and long involving 4 drugs for 2 months and 2 more drugs for at least another 4 months. Direct Observed Treatment (DOT) as promoted by the WHO to improve compliance for the difficult and long regimen can improve cure rates, but is demanding for patients and labour intensive for health staff (O'Brien and Nunn, 2001).

In pioneering studies McDermott and colleagues showed that the efficacy of drugs against *M. tuberculosis in vitro* was not matched by their efficiency *in vivo* (McCune and Tompsett, 1956). The exponentially growing cultures of *M. tuberculosis* can be sterilised using frontline bactericidal drugs such as INH and RIF, yet the same drug combination requires months to achieve similar effects against bacteria living in host tissues. This is because of the failure of the drugs to achieve optimal levels within TB lesions, but there is evidence that drug availability is not a limiting factor (Barclay *et al.*, 1953; Clark, 1985). It has been proposed that persistence of tubercle bacilli, which consists of 4 different populations, in the chemotherapy treatment might be attributable to physiologic heterogeneity of the bacteria in the tissues (Mitchison, 1979).

These populations are:

- Bacteria that are actively growing are killed primarily by INH.
- Bacteria that have spurts of metabolism are killed by RIF.
- Bacteria that are characterised by low metabolic activity and reside in acid pH environment are killed by PZA.
- Bacteria that are dormant or persisters are not killed by any current TB drug.

This idea was supported by the long established observation that slow and non-growing bacteria are phenotypically resistant or tolerant to killing by antimicrobials (Handwerger and Tomasz, 1985).

During the initial phase of chemotherapy treatment, which lasts about 2 days, the bacilli are killed exponentially at a rapid rate, followed by a further lengthy period of

much slower exponential killing. It is assumed that those bacilli that are killed in the first 2 days are actively multiplying, while those in the succeeding period are persisters killed by the slower sterilising activities of the drugs (Jindani *et al*., 2003). In an *in vitro* model of drug action, a 30-day static culture has been extensively used for the last 60 years and has been taken to resemble the persister population in its response to the drugs (Mitchison and Selkon, 1956; Mitchison, 1992; Herbert *et al*., 1996). The drugs added to this static culture have the same slow sterilising actions that are responsible for the prolongation of therapy. This evidence suggests that activity against the population of persistent bacilli ultimately determines the duration of therapy necessary to provide a stable cure of the host (Grosset and Ji, 1998).

Evidently there is an urgent need to develop new and more effective TB drugs that are not only active against MDR-TB but also shorten the length of treatment and target the non-replicating persistent bacilli.

2.4. New TB drugs in the pipeline

Drug development for TB and other diseases has been at a standstill for decades. Today thanks to, the Global Alliance for TB Drug Development (TB Alliance), which was created in 2000 and funded by the Bill and Melinda Gates Foundation, the TB drug pipeline is richer than it has been in the last 40 years. This TB alliance focuses on both pre-clinical and clinical development of candidate compounds for TB chemotherapy and is associated with projects aimed to identify compounds currently being developed. In addition to this, increased public awareness on the lack of research and development for neglected diseases have led in recent years to some multinational pharmaceutical companies up setting Research and Development (R&D) institutes on a ‘no-profit-no-loss’ basis for drug development for TB, malaria and leishmaniasis. Among the multinational pharmaceutical companies currently involved in anti-TB drug R&D are: Novartis, AstraZeneca and GlaxoSmithKline (GSK). Smaller pharmaceutical companies have also engaged in neglected disease R&D on a commercial basis and with some success as two of the anti-TB candidate

drugs currently in clinical trials have been developed by medium-size pharmaceutical companies such as Lupin Limited (India) and Otsuka Pharmaceuticals (Japan., Moran *et al*., 2005).

The Global TB drug pipeline as reported by the Stop TB partnership working group on new TB drugs is summarised in Figure 2.10 and Table 2.4. This is an overview of all drug candidates in the pipeline, belonging to different entities and not only the TB Alliance. In order to analyse the pipeline, the drug candidates are grouped in two main categories: novel chemical entities and compounds originating from existing families of drugs where novel chemistry is used to optimise the compounds.

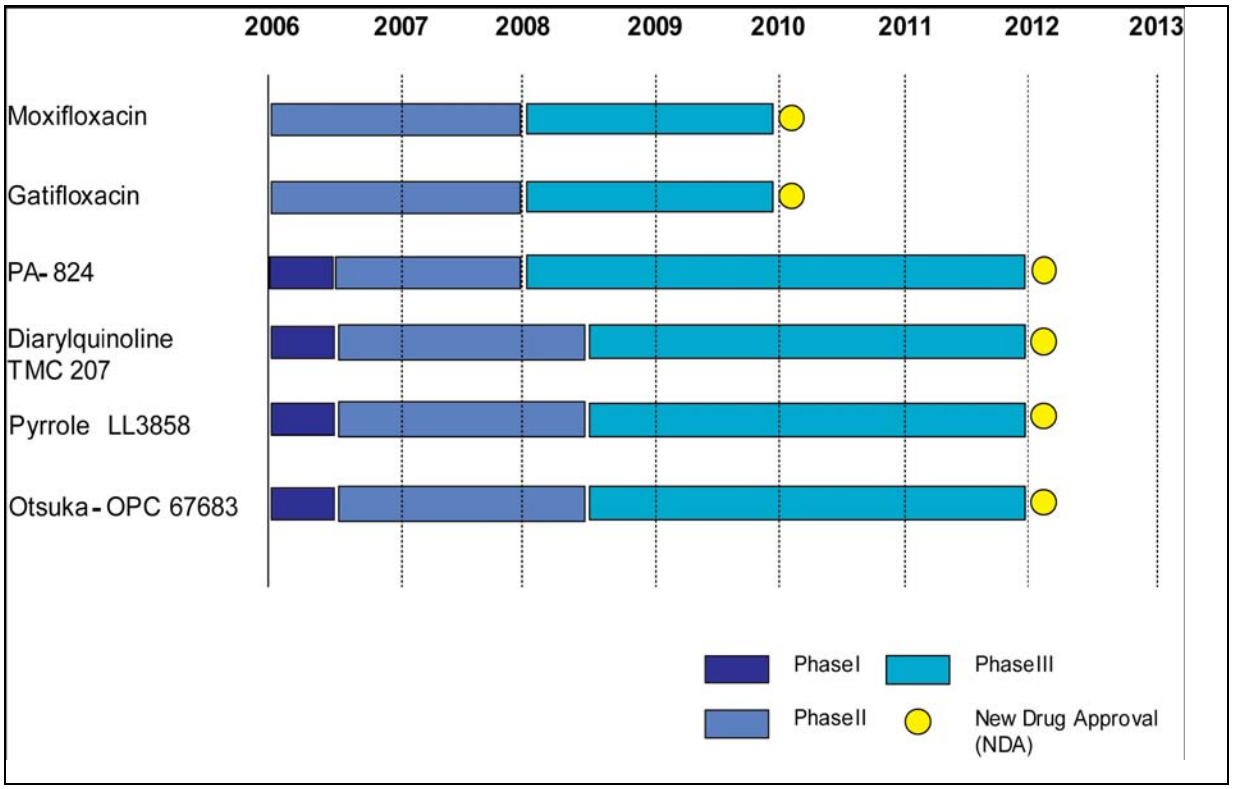


Figure 2.10. Expected time lines towards approval of candidate drugs currently in clinical stages of development (Global TB Alliance Annual report 2004 - 2005)

Table 2.4. Global TB drug pipeline, March 2006 (provided by Stop TB Partnership working on new TB drugs)

Discovery		Preclinical	Clinical
Thiolectomycin Analog NIAID, NIH	**Nitrofuranylamides** NIAID, University of Tennessee	**Diamine SQ-109** Sequella Inc	**Diarylquinoline TMC207** Johnson & Johnson
Cell Wall Inhibitors Colorado State University, NIAID	**Nitroamidazole Analogs** NIAID, Novartis Institute for Tropical Diseases TR Alliance (University of Auckland)	**Dipiperidines SQ-609** Sequella Inc.	**Gatifloxacin** OFLOTUB Consortium, Lupin, NIAID TBRU, Tuberculosis Research Centre, WHO-TDR
Dihydrolipoamide Acyltransferase Inhibitors Cornell University, IAID	**Focused Screening** GlaxoSmithKline, TB Alliance	**Nitroimidazo-oxazole** Otsuka	**Moxifloxacin** Bayer Pharmaceuticals, CDC TBTC, Johns Hopkins University, NIAID TBRU, TB Alliance
InhA Inhibitors GlaxoSmithKline, TB Alliance	**Picolinamide Imidazoles** NIAID, TAACF	**Synthase Inhibitor FAS20013** FasGEN Inc.	**Nitroimidazole PA-824** Chiron Corporation, TB Alliance
Isocitrate Lyase Inhibitors (ICL) GlaxoSmithKline, TB Alliance	**Pleuromutilins** GlaxoSmithKline, TB Alliance	**Translocaase Inhibitors** Sequella Inc. Sankyo	**Nitroimidazo-axazole OPC-67883** Otsuka
Macrolides TB Alliance, University of Illinois	**Quinolones** KRICT/Yonsel University, NIAID, TAACF, TB Alliance	**Non-Fluorinated Quinolones** TaiGen	**Pyrrole LL-3858** Lupin Limited
Methyltransferase Inhibitors Anacor Pharmaceuticals	**Screening and Target identification** AstraZeneeca		
Natural Products Exploration BIOTEC, California State University, ITR, NIAID			

2.4.1. Novel chemical entities

2.4.1.1. Diarylquinoline TMC_{207}

The diarylquinoline TMC_{207} (Table 2.4, Figure 2.11), an extremely promising member of a new class of anti-mycobacterial agents, has a potent early and late bactericidal activity in the non-established infection in murine TB model exceeding that of INH. The substitution of RIF, INH or PZA with diarylquinoline TMC_{207} accelerated activity leading to complete culture conversion after 2 months of treatment in some combinations.

The diarylquinoline-isoniazid-pyrazinamide with diarylquinoline-rifampicin-pyrazinamide combinations cleared the lungs of TB in all mice after 2 months. Diarylquinoline TMC_{207} also has been tested in various combinations with the second line drugs such as amikacin, PZA, moxifloxacin and ethionamide in mice infected with the drug-susceptible virulent *M. tuberculosis* H_{37}RV strain (Adries *et al*., 2005). The target and mechanism of action of diarylquinoline TMC_{207} is different from those of other anti-TB agents implying low probability of cross-resistance with existing TB drugs (Adries *et al*., 2005).

It is further suggested that diarylquinoline TMC_{207} is able to inhibit bacterial growth, when tested on MDR-TB isolates, by inhibiting ATP synthase leading to ATP depletion and pH imbalance (Adries *et al*., 2005., Petrella *et al*., 2006). About 20 molecules of this agent have been shown to have an MIC of below 0.5 µg/mL against *M. tuberculosis* H_{37}RV strain. Antimicrobial activity was confirmed *in vivo* for three of these molecules. A thorough assessment of diarylquinoline activity against MDR-TB *in vivo* would however require testing of animal models infected with multi-drug resistant bacterial strains rather than with drug-susceptible strains (Lounis *et al*., 2006). Diarylquinoline TMC_{207} is currently in Phase II Clinical Trials (Figure 2.10).

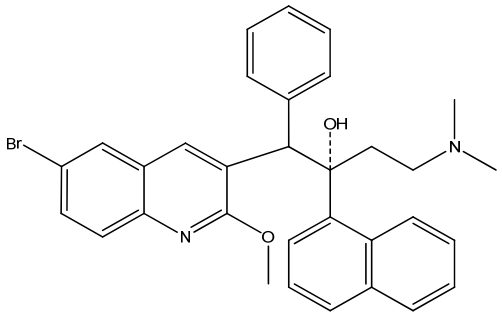


Figure 2.11. Chemical structure of diarylquinoline TMC_{207}

2.4.1.2. Nitroimidazole PA-8_{24}

Nitroimidazole PA-8_{24} (Figure 2.12), is a new nitroimidazole derivative developed by PathoGenesis-Chiron in 1995 and currently being developed by the TB Alliance. The TB Alliance received worldwide exclusive rights to PA-8_{24} and its analogs for the treatment of TB. PA-8_{24} entered Phase I clinical trials in June 2005 (Casenghi, 2006).

In vitro, PA-8_{24} showed high activity against drug-sensitive and drug-resistant *M. tuberculosis* strains, indicating that there is no cross-resistance with current TB drugs (Stover *et al*., 2000). Experiments performed on mice showed that the administration of PA-8_{24} at doses ranging from 25.0 - 100.0 mg/mL produced reductions in the bacterial burden in the spleen and lungs when compared to that produced by INH at 25 mg/mL (Stover *et al*., 2000., Tyagi *et al*., 2005).

Further investigations are required to assess the potentiality of PA-8_{24} to improve the treatment of both drug-susceptible and multi-drug resistant tuberculosis when used in novel combinations with new drug candidates in addition to existing antituberculosis drugs. Nitroimidazole PA-8_{24} is currently in Phase II Clinical Trials (Figure 2.10).

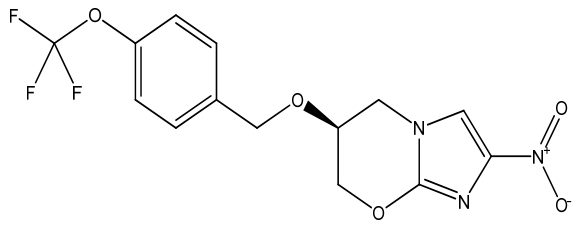


Figure 2.12 Chemical structure of Nitroimidazole PA-8_{24}

2.4.1.3. Nitroimidazole OPC-$6_7 68_3$

Nitroimidazole OPC-$6_7 68_3$ (Figure 2.13) belongs to a subclass of mycolic acid inhibitors, which interferes with the biosynthesis of the mycobacterial cell wall. MIC's of this compound were determined using standard and clinical isolated *M. tuberculosis* strains, including MDR strains. *In vitro*, OPC-$6_7 68_3$ showed high activity against drug-sensitive as well as drug-resistant strains with MIC's ranging from 6.0 - 24.0 mg/mL and also strong intracellular activity against *M. tuberculosis* H_{37}RV strain residing within human macrophages (Casenghi, 2006). Studies in animal models showed that OPC-$6_7 68_3$ is effective against sensitive H_{37}RV and MDR-TB strains *in vivo* starting from a concentration of 0.03125 mg/body (Casenghi, 2006).

The TB Alliance is currently negotiating with Otsuka Pharmaceuticals concerning the further joint development of this compound. OPC-$6_7 68_3$ is in Phase II Clinical Trials (Casenghi, 2006., Table 2.4; Figure 2.10).

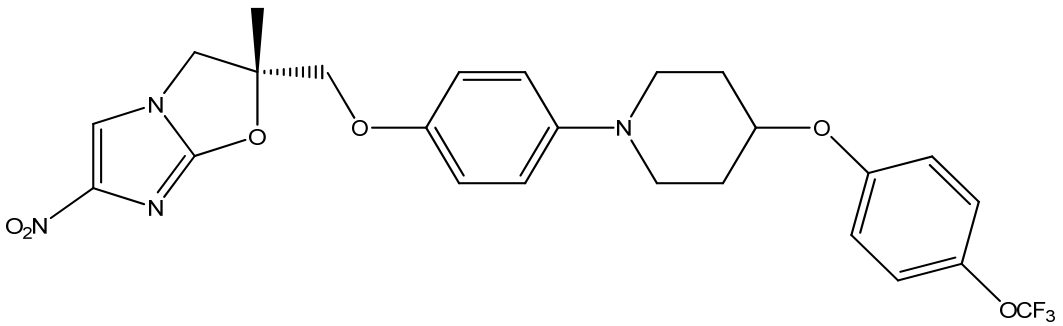


Figure 2.13. Chemical structure of Nitroimidazole OPC-$6_7 68_3$

2.4.1.4. Pyrrole LL- $_{3}8_{5}8$

Very little information on the development of pyrroles as anti-mycobacterial agents is currently available (Casenghi, 2006). Pyrroles derivatives were found to be active against standard and drug-sensitive *M. tuberculosis* strains *in vitro* (Deidda *et al*., 1998., Ragno *et al*., 2000). Lupim Limited reported the identification of a pyrrole derivative (LL-$_{3}8_{5}8$) that showed higher bactericidal activity than INH when administered as monotherapy to infected mice. In mice models, a 12 weeks treatment with LL-$_{3}8_{5}8$ plus INH and RIF, or LL-$_{3}8_{5}8$ plus INH-RIF-PZA, sterilized the lungs of all infected mice. Experiments conducted in mice and dogs showed that the compound is well absorbed, with levels in serum above the MIC. No information is available concerning the molecular mechanisms that mediate LL-$_{3}8_{5}8$'s bacterial activity (Casenghi, 2006). Pyrrole LL- $_{3}8_{5}8$ is in Phase II Clinical Trial (Figure 2.10).

2.4.1.5. Pleuromutilins

The pleuromutilins represent a novel class of antibiotics derived from a natural product. They interfere with protein synthesis by binding to the $_{23}$S rRNA and therefore inhibiting the peptide bond formation (Schlunzen *et al*., 2004). Recent studies showed that cross-resistance might occur among pleuromutilins and oxazolidinones (Long *et al*., 2006). Pleuromutilins have also showed to inhibit the growth of *M. tuberculosis in vitro* (Casenghi, 2006)

2.4.1.6. Dipiperidine SQ-6_{09}

Dipiperidine SQ-6_{09} is a novel compound structurally unrelated to existing anti-TB drugs. It kills *M. tuberculosis* by interfering with cell wall biosynthesise. Antimicrobial activity has been demonstrated *in vivo* in mice models (Nikonenko *et al*., 2004).

2.4.1.7. ATP Synthase Inhibitor FAS_{20013}

FAS_{20013} is a novel compound identified by Fasgen. It belongs to the class of β-sulphonylcarboxamides. Fasgen claims that FAS_{20013} will kill more organisms in a 4 hour exposure that INH or RIF can during a 12 -14 day exposure (Casenghi, 2006). The compound is very effective in killing MDR-TB organisms that are resistant to the multiple drugs currently in use. A series of recent laboratory experiments indicate the superior effect of FAS_{20013} as compared to current drugs in terms of its ability to sterilize TB lesions and kill latent TB. Therapeutic evaluation of FAS_{20013} has repeatedly shown its effectiveness in mice, but it appears to or has no serious side effects. The compound is up to 100% bio-available when administered orally. To date no dose-limiting toxicity has been encountered, even when doses are 10 times administered. The compound is thought to act through inhibition of ATP synthase, however the available publications assessing the efficacy of this compound are of poor quality (Casenghi, 2006., Jones *et al*., 2000., Parrish *et al*., 2004).

2.4.1.8. Translocase I inhibitor

These are compounds which specifically inhibit mycobacterial translocase I, an enzyme required for bacterial cell wall. Preclinical evaluations of the compounds are planned (Casenghi, 2006).

2.4.1.9. InhA Inhibitors

Frontline drugs such as INH target the enoyl reductase enzyme InhA, found in *M. tuberculosis* which catalyses the last step in the fatty acid synthase pathway (FAS II). Drug resistance to INH results primarily from KatG (the enzyme that activates INH), therefore the InhA inhibitors that do not require activation by KatG are attractive candidates in the search for new drugs. The main purpose is to bypass the activation step and directly inhibit InhA. A possible limitation for this kind of compound is that cross-resistance with INH may easily occur (Casenghi, 2006., Banerjee *et al*., 1994).

2.4.1.10. Isocitrate Lyse Inhibitors

The isocitrate lyase (ICL) enzyme has been shown to be essential for long-term persistence of *M. tuberculosis* in mice, but not required for bacilli viability in normal culture (McKinney *et al*., 2000). McKinney and collaborators have shown that inhibition of ICL1 and ICL2 (the two isoforms of isocytrate lyase present in *M. tuberculosis*), blocks the growth and survival of *M. tuberculosis* in macrophages and in mice at an early and late stage of infection (McKinney *et al*., 2000). GSK planned in 2000 to screen 400 000 ICL inhibitors as potential therapeutic drugs. Up to now 900 000 compounds have been screened but no successful inhibitors have been identified. (Casenghi, 2006). The structure of the ICL active site makes the screening of inhibitors lengthy and the active site of this enzyme appears not to be easily reached by compounds (Casenghi, 2006).

2.4.2. Chemicals originating from existing families of drugs

2.4.2.1. Fluoroquinolones

Fluoroquinolones were introduced into clinical practice in the 1980's. They are characterised by broad spectrum antimicrobial activity and are recommended and widely used for the treatment of bacterial infection of the respiratory, gastrointestinal and urinary tracts (Bartlett *et al*., 2000., Neu, 1987). Fluoroquinolones have been also found to have activity against *M. tuberculosis* and are currently part of the recommended regimen as second line drugs (Grosset, 1992., Tsukamura *et al*., 1985). Since fluoroquinolones share the same molecular targets, it is highly probable that they will trigger the same mechanisms of resistance. The major concern is that widespread use of fluoroquinolones for treatment of other bacteria infections may select for resistant strains of *M. tuberculosis*.

In a study conducted in USA and Canada, among referral samples isolates between 1996 and 2000, resistance to ciprofloxacin was assessed and was found to occur in 1.8% of isolates and 75.8% were also MDR. The authors concluded that despite the

widespread use of fluoroquinolones for treatment of common bacterial infection in USA and Canada, resistance to fluoroquinolones remains rare and occurs mainly in MDR strains (Bozeman *et al*., 2005). In contrast, in a different study conducted by Ginsburg and collaborators between 1998 and 2002, the incidence of *M. tuberculosis* fluoroquinolone resistance in a small sample of patients with newly diagnosed TB was found to be high among patients with prior fluoroquinolone exposure (Ginsburg *et al*., 2003b). The risk of selecting fluoroquinolones-resistant *M. tuberculosis* strains by empirically treating with fluoroquinolones for presumed infections before a diagnosis of TB established is of great concern. For this reason, some investigators in the TB field argue that the use of fluoroquinolones might be better reserved for specific serious infection such as TB rather than becoming the workhorse of antimicrobial treatment, however given the current widespread use of quinolones this might not be realistic (Bozeman *et al*., 2005).

The interest in fluoroquinolones as antituberculosis agents has focused on the new fluoroquinolones moxifloxacin and gatifloxacin. Despite a lack of a comprehensive work comparing the activities of old and new classes of fluoroquinolones in *M. tuberculosis*, what can be inferred from published sources are that moxifloxacin and gatifloxacin are characterised by a higher activity against *M. tuberculosis in vitro* when compared to the old fluoroquinolones ofloxacin and ciprofloxacin (Hu *et al*., 2003; Paramasivan *et al*., 2005; Rodriguez *et al*., 2001; Sulochana *et al*., 2005).

2.4.2.1.1. Gatifloxacin

Gatifloxacin (GAT, Figure 2.14) is a new fluoroquinolone marketed in the U.S. by Bristol-Myers Squibb as Tequin. GAT has been found to have *in vitro* and *in vivo* bactericidal activity against *M. tuberculsosis* (Hu *et al*., 2003). In an *in vivo* study, GAT showed the highest bactericidal activity during the first 2 days but not thereafter (Paramasivan *et al*., 2005). Similar results were obtained when GAT was used in combination with INH or RIF, GAT was able to slightly increase the bactericidal activity of INH or RIF only during the first 2 days (Paramasivan *et al*., 2005). One paper reported that when tested in mice in combination with ethionamide and PZA

(high doses: 450 mg/kg, 5 days per week) GAT was able to clear lungs of infected mice after 2 months of treatment (Cynamon and Sklaney, 2003).

Current available data on GAT does not support the hypothesis that introduction of GAT with first-line drugs will impressively contribute to shorten TB treatment. Further investigation should be done to properly assess the activity of GAT *in vitro* and in animal models. GAT is currently in Phase II Clinical Trials (Figure 2.10), conducted under the supervision of the European Commission Oflotub Consortium (Hu *et al*., 2003). The aim of the trial is to evaluate the efficacy and safety of a four months gatifloxacin-containing regimen for treatment of pulmonary TB (Hu *et al*., 2003).

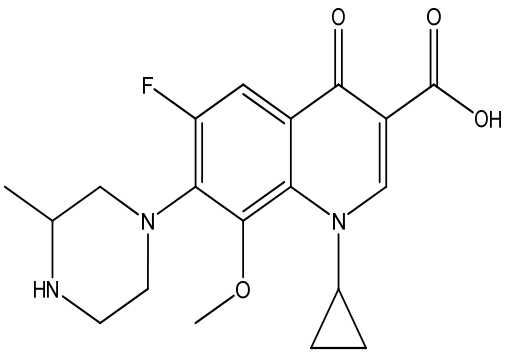


Figure 2.14. Chemical structure of gatifloxacin (GAT)

2.4.2.1.2. Moxifloxacin

Moxifloxacin (MXF, Figure 2.15) is produced by Bayer Pharmaceuticals and marketed as Avelox in the USA. MXF is the most promising of the new fluroquinolones being tested against *M. tuberculosis* (Miyazaki *et al*., 1999). *In vitro*, MXF appeared to kill a subpopulation of tubercle bacilli not killed by RIF, i.e. rifampicin-tolerant persisters, while other fluoroquinoles ciprofloxacin and ofloxacin did not have any significant bactericidal effect on the same subpopulation (Hu *et al*., 2003). One possibility is that MXF interferes with protein synthesis in slowly metabolising bacteria, through a mechanism that differs from that used by RIF.

In mice models, the activity of MXF against tubercle bacilli was comparable to that of INH (Miyazaki *et al*., 1999).When used in combination with MXF and PZA, MXF has been reported to kill the bacilli more effectively that the INH+RIF+PZA combination. In conclusion, *in vitro* and *in vivo* studies suggest that MXF might be a promising candidate drug to shorten TB treatment (Grosset *et al*., 1992). MXF is currently in Phase III Clinical Trials (Figure 2.10). A trial substituting EMB with MXF during intensive phase was initiated before the animal models and showed no advantage over EMB (Miyazaki *et al*., 1999).

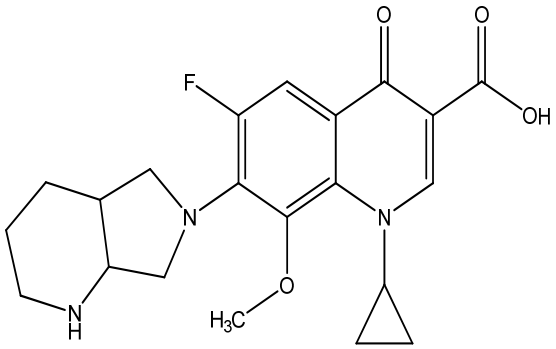


Figure 2.15. Chemical structure of moxifloxacin (MXF)

2.4.2.2. New quinolones

In 2003, the TB Alliance launched a project in collaboration with the Korean Research Institute of Chemical Technology (KRCT) and the Yonsei University aimed to synthesise and evaluate novel and more effective quinolone compounds that could shorten first-line treatment. To date, 450 compounds have been synthesised and tested for their antituberculosis activity (Flamm *et al*., 1995). During the research, the sub-class termed 2-pyridones was identified as being the one showing the most potent activity against *M. tuberculosis* in both its growing and persistent states. This sub-class of compounds was already identified by Abbott in 1998 and found to have activity against drug-susceptible and drug-resistant *M. tuberculosis*. The lead compounds identified so far, showed better activity than GZF and MXF (Oleksijew *et al*., 1998).

2.5. Discussion and Conclusion

According to the WHO Global TB Report of 2006, South Africa has an estimated number of nearly 340,000 new TB cases per year with an incidence rate of 718 cases per 100,000. The TB epidemic in South Africa is exacerbated due to HIV/AIDS with an estimated 60% of adult TB patients being HIV positive. MDR-TB is further exacerbating the epidemic (WHO, 2007 Fact Sheet 104). Early stage drug discovery is a key in the pipeline to find novel drugs for TB. While the existence of a TB drug pipeline, after decades of virtually no new TB drug, is welcome, there are still far too few compounds that represent new chemical classes with novel mechanisms of action and a low probability of encountering pre-existing drug resistance. Of the approximately 40 compounds in the current pipeline, it is unlikely that a useful therapy will emerge, given that only about 1 compound in 20 successfully emerges from an anti-infective drug discovery program. Since new drugs for TB should only be used in combination, to prevent resistance, it would be a responsible act of global leadership to take whatever steps are necessary to attract as many new lead compounds into the pipeline as quickly as possible.

Another possible approach for generating a significant scale-up of TB drug discovery is to improve public sector capacity for running drug discovery programs. Government funding agencies could establish a medicinal chemistry resource center that would work as a core facility offering free lead optimization and ADMET (absorption, distribution, metabolism, excretion, and toxicology) studies in animal models. Such a facility would be directed by scientists with experience in drug discovery, and could also carry out training activities in order to ensure medicinal chemistry expertise in academia. A major challenge, though, would be attracting talented scientists to the not-for-profit medicinal chemistry sector and retaining them in competition with industry. Moreover, to invest in medicinal chemistry for the public sector without the other technologies, resources, and expertise that go into drug development might result in limited success or strategic failure (Casenghi *et al.*, 2007).