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Synthesis, Characterization and Evaluation of 1- (phenyl(4-phenyl-1H-pyrrol-3yl)methyl)-1H-imidazole Derivatives as Potent Anti-Microbial Agents

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Abstract

Development of new drug molecule is expensive and time consuming. Improving safety efficacy ratio of “old” drugs has been attempted using different methods such as individualizing drug therapy, dose titration and therapeutic drug monitoring. Delivering drug at controlled rate, slow delivery, targeted delivery are other very attractive methods and have been pursued vigorously. Numerous animal and human investigations have provided an increased understanding of the pharmacokinetic and pharmacodynamic principles that govern the action and disposition of potent opioid analgesics, inhalation anaesthetic agents, sedative/hypnotics, and muscle relaxants. These studies suggest that skin, buccal and nasal mucous membranes may have use as alternate routes of analgesic and anesthetic delivery. Similar developments with other compounds have produced a plethora of new devices, concepts and techniques that have together been termed controlled-release technology (CRT). Equally important, these advances may appear attractive relative to the costs of new drug development. Rising research and development costs, alternative investment opportunities for drug firms, fewer firms conducting pharmaceutical research and erosion of effective patent life have resulted in a decline in the introduction of new chemical entities since the late 1950s. Bringing a new drug through discovery, clinical testing, development, and regulatory approval is currently estimated to take a decade and cost well over $ 120 million. Novel drug delivery systems may account for as much as 40% of US marketed drug products by 2000.

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Introduction

Tuberculosis:

India is the country with the highest burden of TB. The World Health Organisation (WHO) statistics for 2015 give an estimated incidence figure of 2.5 million cases of TB for India out of a global incidence of 9.6 million. The latest preliminary TB statistics for the United States show that in 2015 there were 9,563 TB cases reported. In 2014 there were 9,412 new cases of active TB disease. This was a 2.2% decline from 2013. About 480 000 people worldwide developed MDR-TB in 2015. In addition, around 100 000 people developed resistance to Rifampicin (the most effective first-line medicine) and needed MDR-TB treatment. The MDR-TB burden largely falls on 3 countries – China, India, and the Russian Federation – which together account for nearly half of the global cases. About 9.5% of MDR-TB cases had XDR-TB in 2015.Worldwide; only 52% of MDR-TB patients and 28% of XDR-TB are currently successfully treated. Patients with

XDR-TB or resistance to second-line anti-TB drugs cannot use this regimen, however, and need to be put on longer MDR-TB regimens to which 1 of the new drugs (bedquiline and delamanid) may be added. By the end of 2015, 70 countries had introduced bedaquiline and 39 countries had introduced delamanid, in an effort to improve the effectiveness of MDR-TB treatment regimens.

In 1839, Johann Schönlein labelled the disease "tuberculosis." In 1882, the tubercle bacillus was discovered by Robert Koch, the German physician who pioneered the science of bacteriology.5, 6

Etiology of TB:

The main cause of TB is Mycobacterium tuberculosis, a small, aerobic, non motile bacillus. The high lipid content of this

pathogen accounts for many of its unique characteristics. The bacterium is carried in airborne particles. Mycobacterium tuberculosis primarily invades the host through the pulmonary tract. These Infectious droplet nuclei are generated when persons with pulmonary or laryngeal TB disease cough, sneeze, shout, or sing.Once the bacteria invade the lungs, the body's immune system sends out white blood cells which build walls of fibres around the bacteria to keep them confined, forming small, hard lumps known as "tubercles." Once the body has formed tubercles to encapsulate the bacteria, the primary infection may be contained and although the person will always test positive for the TB bacteria, the disease itself may not develop. Later in life, if the walls containing the germs are broken down, the lungs once again become infected.

If the immune system is initially unsuccessful in walling off the germs, a full case of TB develops, new bacilli grow and multiply, and then the lung tissue actually dies and becomes soft. Liquid from the tissue is coughed up leaving a cavity in the lung. Cavities may have already formed before a person even notices symptoms such as a cough or fever. Eventually, however, coughing becomes painful and brings up blood with the lung tissue. By this time, the case is well advanced. If large areas of the lungs are damaged, breathing becomes difficult and the body fails to deliver the necessary oxygen to tissues. The bacilli may spread to other tissues of the body causing secondary infections and complications. If untreated, the person will die. If treated with antibiotics and other drugs, the patient may recover, usually over a period of time.

Mycobacterium tuberculosis:

The MTB cell envelope differs substantially from the cell wall structures of both Gram-negative and Gram-positive bacteria. This unique cell wall structure accounts for its unusual low permeability and resistance towards common antibiotics. The mycobacterial cell envelope is composed of a core of three macromolecules covalently linked to each other. They are peptidoglycan (PG), arabinogalactan (AG), and mycolic acids (long fatty acids i.e. C60-C90) and a lipopolysaccharide, lipoarabinomannan (LAM), which is thought to be anchored to the plasma membrane. The AG is a cross-linked network of PG in which some of the muramic acid residues are replaced with a complex polysaccharide and in turn is acylated at its distal end to PG with mycolic acids. The entire complex is abbreviated as the mAGP (mycolylarabinogalactan–peptidoglycan) and is essential for viability in M. tuberculosis and other mycobacteria. The gross structural features of the mAGP complex and LAM (lipoarabinomannan) including aspects of its biosynthesis were thoroughly studied.7

MTB is an intracellular pathogen that establishes infection in oxygen-rich alveolar macrophages of the lung.The outer membrane of the mycobacterial cell wall is an important targets for anti-mycobacterial agents, in particular the biosynthesis of cell wall components. The mycobacterial cell wall is very hydrophobic, resulting in an efficient barrier to a range of antimycobacterial agents.8

Uptake of any drugs through the outer membrane requires the drugs to be lipophilic in nature although there is evidence of the presence of porin channels in the mycobacterial cell envelope through which both nutrients and drugs could diffuse.9,10 Currently TB is treated with agents that target mycolic acid biosynthesis including isoniazid (INH), inhibitors of nucleic acid biosynthesis such as rifampicin (RIF) which binds and inhibits mycobacterial DNA-dependent RNA polymerase, and the aminoglycoside antibiotic streptomycin (SM) which targets protein synthesis.11

MTB complex:

The M.tuberculosis complex includes four other TB-causing mycobacteria, M.bovis, M.africanum, M.canetti, and M.microti. The M. tuberculosis complex consists of human and animal pathogens that are acid-alcohol fast bacilli. M. tuberculosis found in dogs, cats, pigs12 and some wild animals infects human and non-human primates.13 Mycobacterium bovis is an agent that causes bovine tuberculosis which infects a wide range of domestic and wild hosts. BCG (Bacillus Calmette-Guerin) is a vaccine strain obtained by M. bovis. Other strains include M.africanum infects cattle, pigs. M.microti found in small rodents but infection is also recorded in cats, llamas and pigs.

Symptoms: [14, 15]

The symptoms of pulmonary TB are as follows:

1. A cough that lasts for more than 2-3 weeks
2. Coughing up blood or sputum
3. Weakness or extreme tiredness
4. Loss of appetite
5. Weight loss
6. Night sweats
7. Fever
8. Pain in the chest

Risk factors:

TB mostly affects people whose immunity is compromised. E.g.: Infants, old people, pregnant women, women who have recently delivered, people living in unsanitary conditions, uncontrolled diabetes patients, cancer patients, HIV positive people. Also, close contact with people suffering from TB, alcoholics, people using illegal drugs and narcotics and people who weigh10% lower than the body weight are also predisposed to develop TB.16

TB diagnosis:

Chest X rays, sputum smears and culture examinations can show the presence of tuberculosis. Tuberculosis is nearly always diagnosed by most common tuberculin skin test and the Mantoux test. Small amount of tuberculin, a purified protein taken from the tuberculosis bacilli, is injected into the forearm. Reddening and swelling of the area after 24- 72 hrs signals the presence of TB, but not necessarily active TB disease. However, a negative result may not necessarily exclude a diagnosis of TB.Recently using ELISPOT (enzyme linked immunospot) assay both active and latent TB can be diagnosed, but not on regular practice. ELISPOT assay is a blood test that can detect specific response to MTBover night17. This is identified by immune response to infection by

MTB that causes sensitization of individual's T- lymphocytes to MTB proteins.

Tuberculosis management:

Treatment of TB uses antibiotics to kill the bacteria. Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which hinders the entry of drugs and makes many antibiotics ineffective.18 The two antibiotics most commonly used are isoniazid and rifampicin, and treatments can be prolonged, taking several months. Latent TB treatment usually employs a single antibiotic, 19 while active TB is treated with combination of antibiotics to reduce the risk of bacteria developing antibiotic resistance. The recommended treatment of new-onset pulmonary tuberculosis, as of 2010, is six months of a combination of antibiotics containing rifampicin, isoniazid, pyrazinamide and ethambutol for the first two months, and only rifampicin and isoniazid for the last four months. Where resistance to isoniazid is high, ethambutol may be added for the last four months as an alternative.

Table 1.1 Current Anti TB Drugs and their Targets

Drugs	Mechanisms of action	Targets
Isoniazid	Inhibition of cell wall mycolic acid synthesis, and other potential multiple effects on lipids, carbohydrates, and NAD metabolism	Multiple targets including Acyl carrier protein reductase(InhA) β-ketoacyl synthase(KasA)
Rifampicin	Inhibition of RNA synthesis	RNA polymerase β subunit
Pyrazinamide	Disruption of member function and energy metabolism, Inhibition of fatty acid synthesis	Membrane function energy metabolism
Ethambutol	Inhibition of cell arabinogalactan synthesis	Arabinoyltransferase
Streptomycin	Inhibition of protein synthesis	Ribosomal S12 protein and 16S rRna
Amikacin/ Kanamycin/ Capreomycin	Inhibition of protein synthesis	16S rRna
Fluoroquinolones	+ Inhibition of DNA gyrase	DNA gyrase
Ethionamide	Inhibition of mycolic synthesis	Acyl carrier protein redutase (InhA)
Cycloserine	Inhibition of peptidoglycan synthesis	D-alanine racemase /synthase
PAS	Inhibiton of folic acid synthesis and iron uptake	Unknown

Resistance in tuberculosis:

Unlike many drug resistant baceria, TB does not acquire drug resistance through exchange of plasmids. Instead, normal error rates of DNA replication in M. tuberculosis ensure that spontaneous mutations that arise cause resistance in the absence of antibiotic exposure. Spontaneous mutation to INH resistance, for example, occurs in about 1 in 106 bacteria; resistance rates for streptomycin and ethambutol are similar. About 1 in 108 bacteria is rifampicin-resistant. If the bacterial load is large enough, treating a patient with a single drug will lead to the suppression of susceptible bacteria and the selection for growth of a drug resistant strain. Avoidance of selection for resistance is, in part, the reason for treating TB with multiple drugs. The probability of resistance arising when rifampicin and isoniazid are used in combination is only 1 in 1014, sufficiently low to prevent resistance for either drug.

Drug - Resistant Tuberculosis (MDR-TB and XDR-TB):

In drug resistant TB, the bacteria are resistant to one or more anti tubercular drugs.

Multi-Drug Resistant Tuberculosis (MDR-TB):

MDR-TB is caused by organisms that are resistant to at least the two most effective anti-TB drugs, isoniazid (INH) and rifampicin (RIF).21 It is usually found in patients after failed treatment regimens and represents a significant proportion of tuberculosis patients with acquired resistance

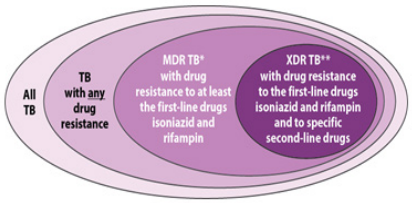


Figure 1 Resistance in Tuberculosis

Extensive-Drug Resistant Tuberculosis (XDR-TB):

XDR-TB or Extensively Drug Resistant TB (also referred to as Extremely Drug Resistance) is a form of TB caused by organisms that are resistant to INH and RIF(i.e. MDR-

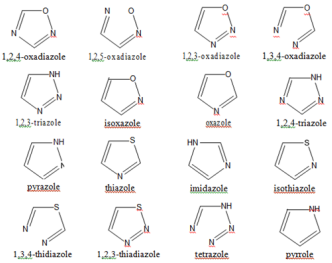
TB) as well as Fluoroquinolone and any of the second line anti-TB injectable drugs (Amikacin, Kanamycin or Capreomycin).21

Totally drug-resistant TB is resistant to all currently used drugs. It was first observed in 2003 in Italy, but not widely reported until 2012, 26 and has also been found in Iran and India.27Bedaqiline is tentatively supported for use in multi-drug resistant TB.28

New drugs to treat TB are urgently required, specifically for use in shorter treatment regimens than are possible with the current agents and which can be employed to treat multidrug-resistant and latent disease.

New TB drugs are needed because of the complexity and toxicity of the current TB drug regimens and the major problem of TB drug resistance. This together with the problem of the interactions of the current TB drugs with the antiretroviral drugs taken by HIV positive people, means that there is an urgent need for new TB drugs.

AZOLES AS ANTIMICROBIAL AGENTS



Heterocyclic chemistry includes a large class of compounds, azoles being one among them. Azoles are five-membered heterocyclic compounds containing nitrogen atom and at least one other non-carbon atom of nitrogen, sulphur, or oxygen29. It includes the following heterocyclic rings.

1 nitrogen (only includes one nitrogen and no other heteroatom)

- Pyrrole

1 nitrogen atom and 1 oxygen atom

- Oxazole
- Isoxazole

1 nitrogen atom and 1 sulfur atom

- Thiazole
- Isothiazole

2 or more nitrogen atoms

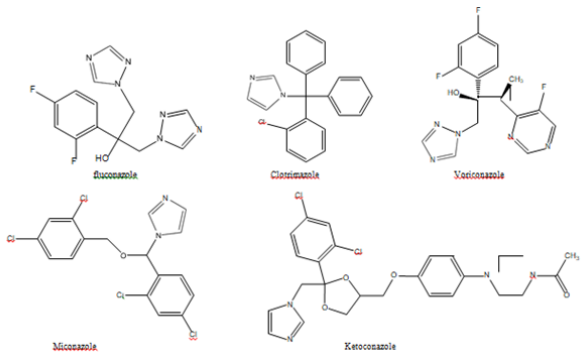
- Pyrazole
- Imidazole, included in histidine
- Triazole, included in Ribavirin
- Tetrazole
- Pentazole

Being main ingredients in many drugs, azoles are known for their broad spectrum of biological activities including antimicrobial, anti-inflammatory, analgesic, anti-mitotic, anti convulsive, diuretic and many other uses30-36. They play an important role against skin diseases and secondary symptoms of AIDS. They are also used in the protection of plants and in industry (leather, wool, fibres). The rapid development in this field affords a comprehensive handbook about their uses and applications.

Reported activities of azoles

Azole drugs showing antifungal activity:

The first report of antifungal activity o f an azole compound, benzimidazole, was described in 1944, it was however only after the introduction of topical chlormidazole in 1958 that researchers became interested in the antifungal activity of azole compounds37. In the late 1960s, three new topical compounds were introduced clotrimazole, developed by Bayer Ag, Germany, miconazole and econazole, both developed by Janssen Pharmaceuticals, Belgium38-40.



Miconazole, a phenethyl imidazole synthesized in 1969, was the first azole available for parenteral administration. Like other azoles, it interferes with the biosynthesis of fungal ergosterol, but at high concentrations. Miconazole may also cause direct membrane damage that result in leakage of cell constituents. It has been recently withdrawn from the markets because of toxicity. In 1981, Food and Drug Administration (FDA) approved the systemic use of ketoconazole, an imidazole derivative synthesized and developed by Janssen Pharmaceuticals, Belgium41.

However, the poor response rates and frequent recurrences o f major fungal infections, as well as the toxicity associated with ketoconazole therapy, led to the search for a second chemical group of azole derivatives, namely the triazoles. In general the triazoles demonstrate a broader spectrum of antifungal activity with reduced toxicity in comparison to imidazole antifungals. The serum half-life allows once-daily dosing of triazole based antifungals agents viz. fluconazole. In contrast to ketoconazole, renal clearance is the major route o f elimination o f fluconazole, with 70-80% of unchanged drug excreted in the urine42.

Mechanism of action of azoles against fungal infection:

Azoles inhibit the enzyme lanosterol 14 a-demethylase which is necessary to convert lanosterol to ergosterol. Diminution o f ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition o f fungal growth. Moreover, a number of secondary effects such as inhibition of the morphogenetic transformation of yeasts to the mycelia form, decreased fungal adherence and direct toxic effects on membrane phospholipids have also been reported43, 44.

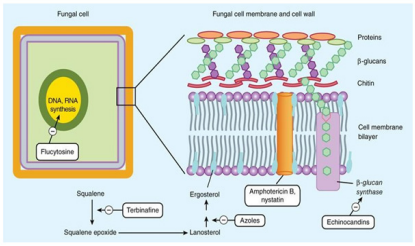


Figure 1 Resistance in Tuberculosis

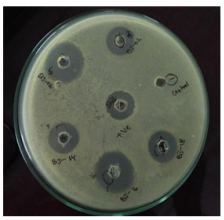


Fig.3.1 *S.aureus*(50µg/ml)

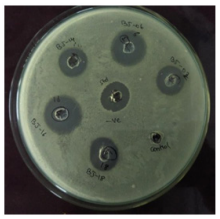


Fig.3.2 *E. coli* (50µg/ml)

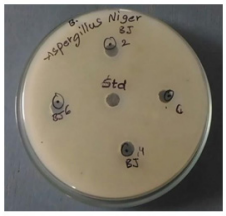
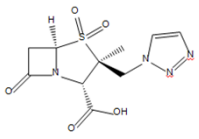


Fig.3.3

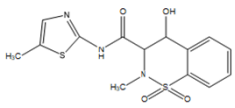
Fig.3.4

Azoles as antibacterial agents:

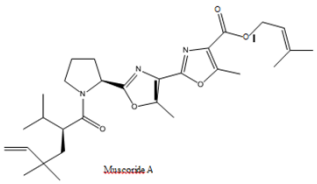
The azole pharmacophore is still considered a viable lead structure for the synthesis of more efficacious and broad spectrum antimicrobial agents. Potential antibacterial activities have been encountered with some azoles. Some of the potent azoles as antibacterial agents are as follows.



Tazobactum



Meloxicam

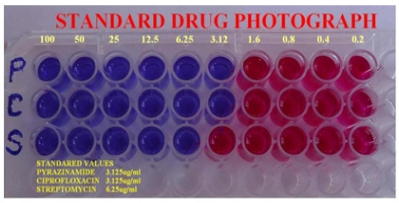


Muscoride A

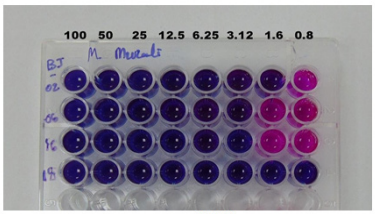
RESULTS

RESULTS OF ANTI-MICROBIAL ACTIVITIES

MIC RESULTS



RESULT OF ANTI-TB ACTIVITY



RESULT AND DISCUSSION

- Synthesised compounds were (50µg/50µl) screened for anti-microbial activity by cup plate method against the organisms S.aureus, E.coli, A.niger.
- When compared to the standard drug (Rifampicin) compounds BJ-02, BJ-06, BJ-14, BJ-16, BJ-18 were found to exhibit moderate anti-bacterial activity.
- Compounds which posses electron withdrawing groups as substituent show potent anti bacterial activity than electron donating substituents.
- Compound BJ-18 has shown antifungal activity almost equal to standard drug Fluconazole.
- All the synthesised compounds were to found to possess potent anti-tubercular activity (MIC-3.12µg/ml) when compared to standard drug streptomycin.
- Compounds BJ-02 and BJ-18 were found to possess potent anti-tubercular activity with MIC-1.6µg/ml and 0.8µg/ml.
- Compounds which posses electron donating group as substituent show potent anti tubercular activity than electron with drawing substituent.

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