ABSTARCT

Current epidemiological evidence suggests that one-third of the world`s population is infected with *Mycobacterium tuberculosis* (*M.tb*), 8 million new cases emerge annually, 3 million deaths occur per year. This problem is further complicated by a dramatic increase in multidrug-resistant (MDR) strains of *M.tb* . An additional factor is the prevalence of human immunodeficiency virus (HIV), which has significantly increased the incidence of tuberculosis (TB) in sub-Saharan Africa and Asia .Therefore new antiTB drugs are urgently needed which can hopefully improve the management of TB by

1) shortening the total duration of therapy
2) improving the treatment success of MDR-TB and
3) provide a more effective treatment of latent TB infection.

Many groups are trying to develop compounds matching these criteria. Some of these are There are some promising new agents, such as the longer-acting rifamycins, fluoroquinolones, oxazolidinones, and nitroimidazopyrans. Oxazolidinones are a new class of antibacterial agents which inhibit early step of protein synthesis. The only marketed oxazolidinone, linezolid (LNZ) has shown activity against mycobacteria .

The oxazolidinone program of Ranbaxy Research Laboratories led to the identification of RBx 7644 as a clinical candidate for nosocomial infections. Modification in the heterocycle ring of RBx 7644 and the linker led to the discovery of RBx 8700 which is a second generation oxazolidinone with activity against respiratory pathogens. The aim of the study is to evaluate and compare the antiTB potential of oxazolidinone class of compounds.

Also there now there is a trend towards natural product research due to its diversity and chemical novelty. Cyanobacteria (blue green algae) are an ancient and diverse group of photosynthetic microorganisms and have shown anticancer and antimicrobial activity. The curacins and the dolastatins from *Lyngya majuscula* have shown great pharmaceutical potential. Cyanovirin N from Nostoc has antiHIV activity. Cyanobacteria are exciting and potentially productive area of investigation.

Therefore, this study examines cyanobacteria for antiTB activity which is a novel unexplored area.

The objectives of the thesis were

- **Determination of MIC of NCEs and Cyanobacterial extracts by**

Agar incorporation and dilution method
Microplate Alamar Blue Assay (MABA)
Flow cytometry assay

- **Determination of Mode of action of RBx 8700, the active compound by in vitro time kill kinetics**

Against log phase bacteria
Against stationary phase bacteria

- **Determination of intracellular activity of RBx 8700 in J774 A.1 cell line**

Against H37Rv
MAC

- **Determination of in vitro and intracellular synergy of the compound with the existing antiTB drugs**

In susceptibilty testing by **agar dilution assay**, the activities of RBx 7644 and LNZ were moderate with MIC50 of 4 μg/ml against both *M.tb* monoresistant and MDR strains and 1-2 μg/ml against sensitive strains. Excellent activity was observed with RBx 8700 with MIC an MIC range of 0.06 to 0.25 μg/ml against all strains. Against MAC the MIC50 of RBx 7644 and RBx 8700 was determined to be 8 and 0.25 μg/ml respectively as compared to 8 μg/ml of CLAR.

MABA MICs showed good agreement with the agar dilution. Results were obtained in a short time frame of 8 days for *M.tb* and 5 days for MAC strains. This should be used for susceptibility testing of MAC cultures.

Flow cytometry gave results in 24 h and NCEs tested displayed comparable MICs with the above methods. The method can be used for screening compounds at few concentrations to differentiate active/inactive.

The determination of MIC by different methods showed that RBx 8700 is the most potent compound with good activity against the MDR strains. Its activity against

MAC and fluoroquinolone resistant strains show that it has a unique spectrum in comparison to the existing antiTB drugs.

A concentration dependent bactericidal activity, similar to RIF was observed with RBx 8700 against all the strains. Reduction in CFU was observed with 0.5 to 1μg/ml as early as 48 h against the sensitive strains.

RBx 8700 showed complete clearance at 0.125 μg/ml at day 3 against INH-r strain as well as MDR strain.

RBx 8700 displayed similar activity on the bacteria in the stationary phase of growth. The same was observed with RIF but INH did not eliminate the bacteria completely.

This part of the study concluded that RBx 8700 has bactericidal activity against both the actively multiplying and dormant bacilli. This indicates its potential against the latent mycobacteria present in human tubercular lesions.

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Study of cyanobacterial extracts indicated that organic extracts of few cyanobacterial strains exhibit antimycobacterial activity. An active fraction was identified from the methanol extract of Hapalosiphon.

Although the MICs are high, the activity may be enhanced, by separating the active moiety by physiological methods.

Cyanobacteria have the potential of antimicrobial activity and is worthy of investigation.