NOVEL NON-NEUROLEPTIC PHENOTHIAZINES INHIBIT *MYCOBACTERIUM TUBERCULOSIS*

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Background
Phenothiazines are commercially available and in clinical use for the treatment of psychosis. In addition to their neuroleptic properties, these psychotropic drugs were found to be bactericidal against laboratory and clinical strains of *Mycobacterium tuberculosis*, but produce undesirable side effects at clinically relevant doses. Studies have shown a diverse range of bioactivity with minimal structural remodelling based on the versatility of the phenothiazine pharmacophore. Thus, this study aimed to evaluate four rationally modified phenothiazines (PTZ3, PTZ4, PTZ31 and PTZ32) as antimycobacterial drug candidates without neuroleptic effects.

Methods
Radioligand binding assays were used to evaluate the modified phenothiazines' neuroleptic properties, i.e. their ability to bind to dopamine and serotonin receptors. The *in vitro* bactericidal activities of the modified phenothiazines were screened using green fluorescent protein (GFP) microplate assays against H37Rv-GFP. The *in vitro* cytotoxicity of the compounds was evaluated in primary bone marrow derived macrophages and the CellTiter® Blue assay. The efficacy of intracellular bacilli killing was also evaluated by cfu enumeration in bone marrow derived macrophages. The *in vivo* toxicity profiles of the modified phenothiazines were evaluated in C57Bl/6 mice via oral gavage over a period of 14 days. Single dose (acute) and repeat dose (daily dose) toxicity studies were performed.

Results
Radioligand binding assay results demonstrated that these phenothiazine derivatives did not exhibit dopamine nor serotonin receptor binding, except PTZ31 which displayed marginal serotonergic activity. The modified phenothiazines displayed minimum inhibitory concentrations of 12.5 – 25mg/L. None of the phenothiazine derivatives displayed cytotoxicity compared with thioridazine. The modified phenothiazines were able to inhibit intracellular bacilli replication without displaying cytotoxicity to infected macrophages between 12.5 – 100mg/L. In the single dose studies to test for the effect of acute exposure, there were no mortalities or significant weight loss in the mice treated with 100mg/kg of a modified phenothiazine. To test the effect of repeat exposure, the mice were then treated daily with 100mg/kg of the modified phenothiazine. The PTZ32 treated group resulted in a 55% mortality rate and biochemical analysis indicate to possible liver and kidney toxicity. PTZ31 treatment resulted in 20% mortality with no toxicity evident in biochemical or histological analysis. PTZ3 and PTZ4 treated groups had no mortalities and displayed no signs of toxicity.

Conclusion
These modified phenothiazines demonstrated their ability to inhibit *Mycobacterium tuberculosis* without undesirable neuroleptic effects. The *in vivo* toxicity results illustrate the potential of these compounds as antimycobacterial drugs.