6. Summary and Conclusion

Tuberculosis (TB) is a most rampant disease of the world over the ages of human history. Even now, it remains formidable by claiming millions of lives through development of resistance. The lack of novel drugs in countering the resistance is a serious threat and this further worsened the clinical presentation. The neuroleptic chlorpromazine has been reported for antitubercular activity, but the associated antipsychotic activity restricted its clinical use. Novel phenothiazine, carbazole and biphenyl derivatives having structural similarity with chlorpromazine were designed by removing/ modifying the group/ ring/ pharmacophore essential in producing antipsychotic activity, in an attempt to reduce the associated side effects, while improving the antitubercular activity. Prior to synthesis, the designed molecules were virtually screened and filtered against the off-targets (dopamine-D2 & D3) to avoid the unwanted antipsychotic effect. The hit molecules obtained from such filtering were later synthesized and screened for antitubercular and antibacterial activities. The blood brain barrier (BBB) permeability and mammalian cell (VERO) cytotoxicity (CC_{50}) were examined to determine the safety of compounds. I also, tried to elucidate the molecular mechanism of the lead molecules by performing molecular docking and *in-vitro* enzyme screening against type-2 NADH dehydrogenase and ATP synthase.

Antitubercular screening

The synthesized compounds were assessed for anti-mycobacterial activity against *M. tuberculosis* H37Rv using microplate alamar blue assay (MABA). The minimum concentration that prevented the color change from blue to pink was recorded as MIC. Compounds **15b** and **16b** were the most active with MIC of 0.78µg/mL. Compounds **14p, 15p, 16p, 14c, 15c, 16c, 17c, 14b** and **17b** were active at MIC of 1.56µg/mL.

The activity of these compounds were better than the template molecule chlorpromazine (12.5µg/mL), standard drugs ciprofloxacin (3.13µg/mL), and pyrazinamide (3.13µg/mL). Different electron withdrawing substitutions on the phenyl ring altered the activity of the compounds. Introduction of nitro group on the phenyl ring significantly increased the activity in comparison to halogens. Strong electron withdrawing nature of nitro group might have influenced the biological activity of the compounds. The number of halogen substitution seems to play a significant role, as the di-substituted halogen compounds (**10p, 11p, 12p, 13p, 10c, 12c, 13c, 14c, 10b, 11b, 12b,** and **13b**) produced better activity in comparison to mono-substituted compounds (**3p-9p**; **3c-9c**; **3b-9b**). A drastic reduction in activity was observed in compounds **19p, 20p, 19c, 20c, 19b** and **20b**, where the acyl linker was replaced with piperazinyl type linker. Among the cycloalkane compounds, **S9** and **S10** were the most active with MIC of 6.25µg/mL.

Antibacterial screening

All the synthesized compounds were further screened against a *gram*-positive (*S. aureus*) and a *gram*-negative (*E. coli*) bacterial strains to evaluate the spectrum of activity. The screening was done through agar plate disc diffusion method. Compound **16b** was the most active against *S. aureus* with MIC of 0.49µg/mL and it produced 7.81µg/mL activity against *E. coli*. It was followed by compounds **14p, 15p, 16p, 14c, 15c, 16c, 17p**, **14b, 15b and 17b** with MIC of 0.98 and 7.81µg/mL against *S. aureus* and *E. coli* respectively. The activity of the compounds against *S. aureus* was better over chlorpromazine (7.81µg/mL) and ciprofloxacin (1.95µg/mL). The activity of the compounds against *E. coli* was also better over chlorpromazine (15.63µg/mL) and ciprofloxacin (3.91µg/mL). Among cycloalkane derivatives, **S4, S10** and **S16** produced MIC of 3.90µg/mL against *S. aureus* and 31.25µg/mL against *E. coli*. The overall

activity was found comparatively good against *S. aureus* than *E. coli*. This may be due to increased permeability of the compounds through the cell membrane of *gram*-positive *S. aureus* than the *gram*-negative *E. coli.*

Blood brain barrier (BBB) permeability screening

The compounds were screened for BBB permeability by Parallel artificial membrane permeability assay (PAMPA). The permeability of the compounds was compared with commercial drugs and classified as high permeable (CNS+), low permeable (CNS-) and permeable uncertain (CNS+/-). The effective permeability (Pe) of chlorpromazine was found as 6.1×10^{-6} cms^{-1}. The test compounds produced effective permeability (Pe) in the range from 2.1×10^{-6} to 5.2×10^{-6} cms^{-1}. Compounds in biphenyl series produced reduced permeability in comparison to phenothiazine and carbazole derivatives. Compounds **6b** and **7b** were found to be least permeable among all compounds in the study, with effective permeability (Pe) of 2.1×10^{-6} cms^{-1}. The reduced BBB permeability of the compounds in comparison to chlorpromazine could reduce the unwanted CNS side effects.

In-vitro *cytotoxicity screening*

The compounds were tested against kidney epithelial (VERO) cells to check their toxicity. The test compounds produced cytotoxicity (CC_{50}) in the range from 84.71 to 222.53μg/mL. The compounds with strong growth inhibition against the whole *Mtb* H37Rv were found non-toxic with CC_{50} >90μg/mL. The compounds should be selective towards desired activity to avoid unwanted side effects. Here, the compounds should kill the microorganisms but not the normal human cells. Hence, the selectivity index (SI) was calculated by dividing the obtained cytotoxicity (CC_{50}) with the antitubercular MIC and found that the compounds were selective in producing

antitubercular activity. Compounds **16b** showed highest selectivity with selectivity index (SI) of 77.8, followed by **14b**, **17b** and **15b** with SI of 77.0, 73.8, 73.2 respectively. The selectivity index was found comparatively higher among biphenyl compounds than phenothiazine and carbazole compounds.

Type-2 NADH dehydrogenase (NDH-2) *inhibition screening*

The molecules in the study were docked against *Mtb* NDH-2 developed through homology modelling. Docking score was found in the range between -6.1926 and -1.5277 Kcal/mol. Compounds with strong interaction with NDH-2 in docking were selected for *in-vitro* enzyme study. Percentage inhibition of residual NADH oxidation activity was measured at 50μM concentration. Compounds **16p, 15p, 13p, 15c** and **16b** produced 30.09, 28.42, 26.85, 25.80 and 22.55 percent inhibitions respectively. Rest of the compounds produced < 20 percent inhibitions. It was observed that all the phenothiazine, carbazole and biphenyl derivatives produced < 50 percent inhibitions at 50μM concentration. Therefore, it was concluded that the IC_{50} of these compounds could be >50μM.

ATP synthase inhibition screening

All the designed molecules were docked against ATP synthase (PDB code:4V1F). The cycloalkane derivatives **S3, S4, S9, S10** and **S16** produced docking score of -4.9711, -5.1939, -5.6423, -5.8029 and -4.9137 Kcal/mol respectively. Then, these compounds were screened *in-vitro* for enzyme activity. Compounds **S9** and **S10** inhibited the function of ATP synthase with IC_{50} of 14 and 10.4μM respectively, while **S3**, **S4** and **S16** produced IC_{50} > 100μM. Compounds **S9** and **S10** were established as ATP synthase inhibitors.

In conclusion, the class of compounds described here seems to be a good starting point for further development as drug candidates. The antitubercular activity of the lead compounds was better over the template molecule chlorpromazine and was comparable with standard drugs, ciprofloxacin and pyrazinamide. *In-silico* virtual screening and filtering provided molecules with reduced binding towards dopamine receptors. Further, the BBB permeability of the compounds was reduced in comparison to chlorpromazine. The *in-vitro* and *in-silico* toxicity screening also showed that the compounds were safe. Hence, our objective to increase antitubercular activity with concurrent reduction in side effects was achieved (**Figure 6-1**). The developed lead compounds can pave new vista in the discovery of antitubercular drugs.
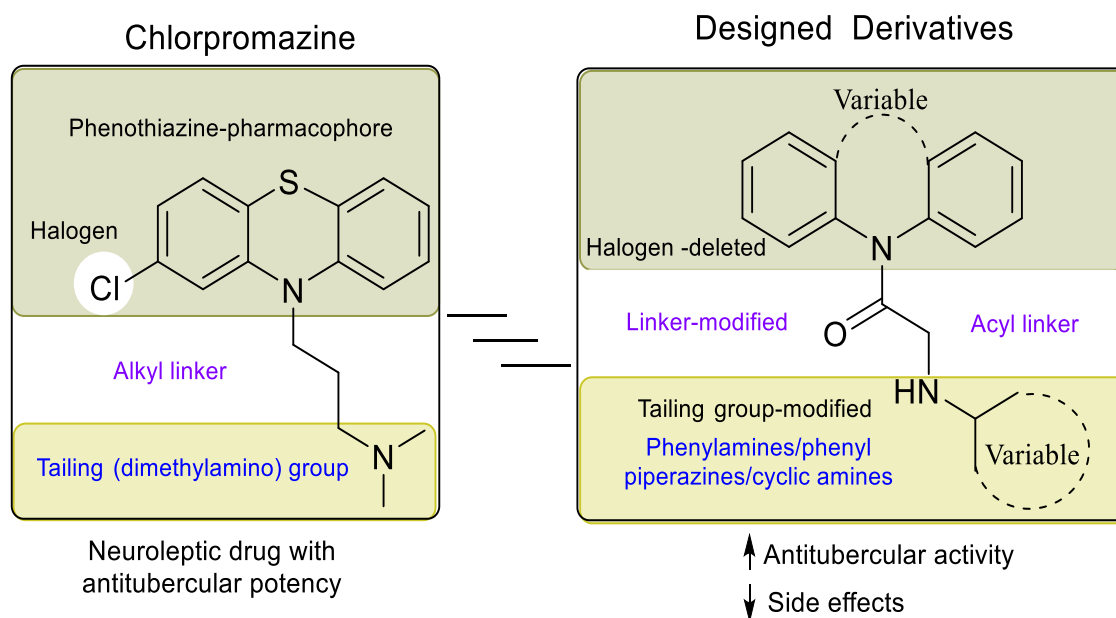
Figure 6-1: Graphical depiction of the present study

Scope for future work

The present work could be extended to study *in-vivo* efficacy of the lead compounds. The pharmacokinetic and pharmacodynamics profile of the lead compounds can be investigated. X-ray crystallography of ATP synthase bound with compounds S9 and S10 can be resolved. NDH-2 and ATP synthase are vulnerable targets in latent/dormant

tuberculosis, therefore, the lead compounds can be screened through nutrient starvation model to assess the effect against latent *Mtb*. Three-dimensional quantitative structure activity relationship (3D-QSAR) can also be performed to optimize the lead molecules for further improvement of antitubercular activity.