Chapter VII

Summary and Conclusion

Summary and Conclusion Chapter 7

TB has become one of the most dangerous infectious diseases of the modern times with the epidemic of acquired immune deficiency syndrome (AIDS) in the 1980s. The emergence of drug resistant strains of MTB along with some other factors has resulted in multidrug-resistant (MDR), extensively drug-resistant (XDR), or more recently, totally drug-resistant (TDR-TB). This has rendered the presently available anti-tubercular drug regimen inadequate to address the many inherent and emerging challenges of treatment. These factors initiate the need for the development of newer, safer and more effective drugs which can reduce the TB treatment duration drastically. Hence we designed the compounds emphasizing molecular hybridisation approach to merit cost effective and reduced treatment time. Active core of existing antitubercular molecules were identified and made an attempt to tailor them in a single entity anticipating improved features. All compounds were designed with reported active core and varied with triazoles. All synthesized novel compounds were characterized by spectral data (IR, NMR and MS), elemental analysis and few compounds were confirmed by single crystal XRD. All compounds were evaluated for their antimycobacterial activity and most active compounds were evaluated for cytotoxicity studies in normal cell line.

In **chapter 3**, novel 1-((1-(substituted)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*,3-diphenyl-6,7-dihydro-1*H*-pyrazolo[4,3-*c*]pyridine-5(4*H*)-carboxamide derivatives designed and synthesized by molecular hybridization approach using reported MTB PS inhibitor and substituted 1*H*-1,2,3-triazole antitubercular compounds. Twenty six compounds were synthesized and evaluated for their antimycobacterial activity against MTB H_{37}Rv, MTB *Spec.* 192 and MTB *Spec.* 210 strains and pantothenate synthetase enzyme studies was also carried out. Amongst, the synthesized compounds, **7c** exhibited 99% inhibition of MTB H_{37}Rv strain with MIC 49.64 µM. Compound **7b** was significantly active against MTB with MIC 25.53 µM**.** Compound **7d** exhibited good activity with MIC 24.72 µM. Seven compounds (**7b, 7d, 7h, 7p, 7r, 7s** & **7v**) inhibited MTB PS with IC_{50} <2.00 µM. Compounds **7d** and **7s** emerged as the most active compounds with IC_{50} 1.01±0.32 and 0.91±0.32 µM respectively. The active compounds **7b, 7c** and **7d** were evaluated for cytotoxicity (RAW264.7 cell line). These compounds showed low cytotoxicity.

The anti-tubercular SAR profile suggests that tailoring methoxy and triazole group by means of appropriate substituent or functional group might provide an insight to obtain the lead compound.

In **chapter 4,** novel 9*H*-fluorenone analogues were synthesized with three modifications of these three groups -NH-, -S- and -SO_2-. In **scheme 4.1**, *N*-((1-substituted phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-9*H*-fluoren-9-amine analogues and in **scheme 4.**2 *N*-4-(((9*H*-fluoren-9-yl)thio)methyl)-1-substituted phenyl-1*H*-1,2,3-triazole & 4-(((9*H*-fluoren-9-yl) sulfonyl) methyl)-1-substituted phenyl-1*H*-1,2,3-triazole) analogues were synthesized**,** evaluated for their antimycobacterial activity against MTB. In this fifty compounds, *N*-4-(((9*H*-fluoren-9-yl)thio)methyl)-1-substituted phenyl-1*H*-1,2,3-triazole analogues showed good activity. **17p** showed excellent MTB activity with MIC 52.35 μM. Out of fifty compounds synthesized, InhA activity was studied for fifteen compounds. Amongst these compounds, **17f** & **17p** showed >73% of inhibition at 50 μM. The most active compounds did not exhibit cytotoxicity against HEK 293 cell line at 50 μM.

From these **schemes 4.1** & **4.2**, preliminary anti-tubercular screening results drive us to engineer the chemical structure of 9*H*-fluorenone derivative to generate essential pharmacophoric features that could lead to the synthesis of a promising candidate to develop anti-tubercular agent. We discovered that incorporation of sulfonyl group on the 9*H*-fluorenone in the moiety plays a pivotal role in the activity profile.

In **chapter 5**, thirty six sulfonamide tetrazole and 1,2,4, triazole containing derivatives were synthesized and evaluated for their antimycobacterial activity. *In vitro* anti tubercular screening results indicate that ten compounds *viz.*, **25a, 25d, 25e, 25h, 26b, 27a, 27d, 27i, 28b** and **28f** showed moderate activity (MIC = 3.12 μg/mL). Eight compounds, **25c, 25f, 26g, 27e, 27g, 27h, 28d** and **28h** displayed good anti-TB activity (MIC = 1.56 μg/mL). Compound **26c** exhibited excellent anti-TB activity (MIC = 0.78μg/mL). Most of the compounds did not show toxicity when screened in CHO-K1 cell line (SI value >13).

In **chapter 6**, thirty one, quinoxaline 1,4-dioxide tethered 1,2,3 triazole analogues were synthesized. In this chapter quinoxaline 1,4-dioxide was varied with chloro at 6,7 positions. .*In vitro* anti tubercular screening results indicate that 6-chloro-3-(((1-(substituted phenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)carbonyl)-2-methylquinoxaline 1,4-dioxide derivatives showed good activity. Two compounds **36a** and **37d** displayed excellent anti-TB activity with respect MIC 30.35, 47.60 µM. Cytotoxicity assay of **36a, 36e, 36i, 36l, 37b, 37c** & **37d** was determined against HEK 293 cell line. These compounds did not exhibit cytotoxicity against HEK 293 cell line.

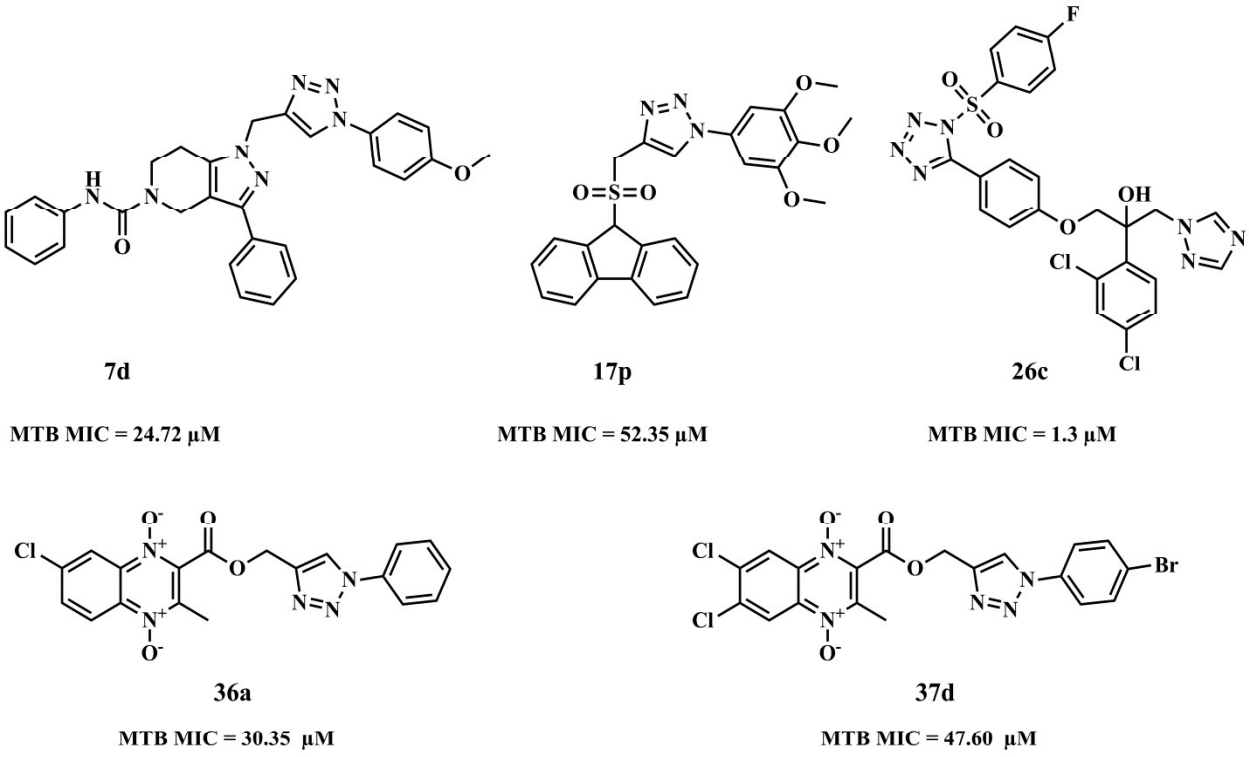

Figure 7.1: Most active compounds amongst the synthesized compounds

In conclusion, the most active compounds from the synthesized derivatives are depicted in **figure 7.1.** The class of compounds described here besets a collection of promising lead compounds for further optimization and development to yield best novel drugs aimed to combat ever-present and ever-increasing mycobacterial infections. The study also provides the basis for further chemical optimization of these potent inhibitors as potential anti-TB agents.

Future perspectives

- The present thesis described development of series of molecules as potential anti-tubercular agents, pantothenate synthetase enzyme inhibitors and InhA inhibitors. The molecules reported herein displayed considerable in-vitro enzyme inhibition and potency against *Mycobacterium tuberculosis* H37Rv strain. Although these results were encouraging further lead optimization can be carried out.

- The progression of any of the candidate molecules presented in this thesis along a drug development track would require a substantial investment in medicinal chemistry, preclinical and clinical studies.

- Extensive side effect profile of all the synthesized compounds may be studied. Sub-acute and acute toxicological screening of novel chemical entities has to be carried out.

- Extensive pharmacodynamic and pharmacokinetic studies of the safer compounds have to be undertaken in higher animal models.

- Based on the pharmacophore model proposed, various substituents which lead to activity proposed could be incorporated into the compounds synthesized and study further in various animal models.

Further, the viability, reduce the therapy period, cost effectiveness and reproducibility of synthesizing these compounds in big scale has to be attempted.