## 7.1 Summary

This thesis entitled "Computational tools for designing peptide and small molecule based therapeutic agents against *Mycobacterium*" comprises the work done in during the past five years. It is a well-known fact that, TB is a global menace for mankind and WHO releases a "Global TB report" since 1997 and the data in past few years is very much alarming and threatening. Besides this, other species of genus *Mycobacterium* is also critically dangerous and responsible for several deadly diseases like leprosy, Buruli ulcer etc. Keeping these hazards in mind, we had tried our best to contribute in the immense research field related to *Mycobacterium*. Our effort might be like a drop in the ocean, but we believe that this work will increase the understanding of therapeutic entities.

The failure or limitations of conventional antibiotics lead to a shift in paradigm towards peptide-based therapies in last two decades and researchers have already explored various peptides for their antimycobacterial activities. But the information regarding antimycobacterial or antitubercular peptides are scattered in the literature; therefore, first of all, we have collected and compiled information and built a knowledgebase, AntiTbPdb (http://webs.iiitd.edu.in/raghava/antitbpdb/). In summary, AntiTbPdb is a unique knowledgebase, consisting 1010 entries and providing comprehensive information about 542 unique experimentally verified antimycobacterial or antitubercular peptides. The primary information was manually curated from 10652 research articles and 35 patents and secondary information like physiochemical properties, amino acid frequency and compositions of antitubercular peptides were calculated by in-house PERL scripts. Besides this, structural information was also provided by using I-TASSER and PEPstrMOD. We have utilized this data to develop an *in silico* tool to design antitubercular peptides. In this study, the positive dataset was prepared from AntiTbPdb, and two negative datasets were prepared from DBAASP and Swiss Prot. We have developed machine learning based models using different sequence features, which can easily differentiate antitubercular peptides from antibacterial peptides as well as non-antibacterial peptides. Ensemble classifiers developed by combining models based on AAC and N5C5 binary pattern achieves highest accuracy of 73.20% and 75.62% with AUROC of 0.80 and 0.83 on main and random dataset, respectively. In addition to ensemble classifiers, we have also provided hybrid models, which achieves accuracy almost in the same range, in a user friendly webserver, AntiTbPred ( https://webs.iiitd.edu.in/raghava/antitbpred/).

The properties possessed by the antitubercular compounds are highly distinctive as compare to the other class of drugs. Thus, keeping that in mind, there is a pressing need of proposing the new prediction models, discriminating the antitubercular compounds from the other classes. Therefore, in the next study, we have developed a computational method which can predict the small molecule based antitubercular therapeutics. We have downloaded 1938 antitubercular compounds from PubChem with IC-50 values. We have considered them positive, i.e. inhibitor if IC-50 is ≤ 10 nM and rest as negative or non-inhibitor. Several features, such as 2D and 3D descriptors as well as fingerprints, were used for the development of prediction models based on machine learning techniques. Besides this, we have also implemented an innovative algorithm 'MCC-based approach' for the optimization and selection of the descriptors. After this, we have selected the top-50 features and develop models.

In the next study, we have demonstrated the antimycobacterial activity of a cell penetrating peptide IMT-P8 and its conjugate IMT-P8-KLA. First of all, we have precited the antimycobacterial property of these peptides using AntiTbPred, and significant positive score motivated us to experimentally validate its antimycobacterial property. Both IMT-P8 and IMT-P8-KLA shows significant antimycobacterial activity and exhibit MIC of 12.5 μM and 6.25 μM, respectively. IMT-P8-KLA was cytotoxic to the mammalian cells, hence it was dropped from further experiments but we have proceeded with IMT-P8 to *in vivo* experiments and achieved significant success in showing its intracellular antimycobacterial activity, i.e. around 40% intracellular bacteria were killed by using 12.5 μM of IMT-P8. Confocal microscopic studies were performed to elucidate antimycobacterial mechanism, and images suggest that IMT-P8 was able to comprise the cell membrane integrity and result in pore formation. Moving a step ahead, we have shown the synergistic effect of IMT-P8 with streptomycin, kanamycin as well as rifampicin, all of these three antibiotics are already established anti-TB drugs and currently given in DOTS. Besides this, we have also designed a short study about the repurposing of an antimalarial drug, fosmidomycin. Fosmidomycin is a potent inhibitor of an enzyme, 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR), which catalyses the second reaction of the DOXP pathway. DXR is essential for the survival of *Mycobacterium,* but species is resistant to fosmidomycin due to lack of its uptake. By REMA and growth curve studies, we have shown that IMT-P8 having cell penetrating property, could help fosmidomycin in crossing the cell wall barrier of *M. smegmatis*, and thus exhibiting its bactericidal activity.

Next objective was based on the fact that *Mycobacterium* infection, is accompanied by activation of components of innate immune response, such as release of certain pro-inflammatory cytokines, leading to an intense local inflammation, and excessive inflammation is responsible for increase tuberculosis susceptibility. In order to reduce the *Mycobacterium* burden, immune system must be suppressed to a certain extent to reduce the inflammation. Immunosuppressive peptide are more safer and promising approach, due to its low toxicity and inherent targeted molecular action as compared to small molecule based anti-inflammatory drugs which exhibit severe side effects such as cardiotoxicity and nephrotoxicity. First of all, we have compiled all the immunosuppressive peptides reported in the literature and developed a resource named as ImmunoSPdb (https://webs.iiitd.edu.in/raghava/immunospdb/). In summary, ImmunoSPdb is a comprehensive, manually curated database of around 500 experimentally verified immunosuppressive peptides compiled from 79 research article and 32 patents, comprising of 553 entries. The annotated tertiary structure by using I-TASSER and PEPstrMOD as well as other secondary information like physiochemical properties, amino acid frequency and compositions etc. were also stored in the database. Further, we have developed an *in silico* tool to predict and design immunosuppressive peptides. ImmunoSPdb was used to prepare positive dataset, while negative dataset was prepared by SPdb, HMRBase and PIP-EL. Various features such as AAC, DPC, terminus composition and binary profiles of peptides were generated and used for developing models based on machine learning. To facilitate scientific community in designing of immunosuppressive peptides, we implemented best models in a user friendly webserver (https://webs.iiitd.edu.in/raghava/immunosppred/).

## 7.2 Future prospective

Despite the tremendous research in the field of *Mycobacterium* and TB, it is still a major concern for mankind. The emergence of drug resistant strains and failure or limitation of BCG vaccine in immunocompromised individuals, make the scenario more worst. It is the need of hour to look for alternative approaches and peptides have emerged as a more safer and better therapeutic candidate in much diversified range from antimycobacterial to anticancerous. Despite the considerable progress in computational peptidology field, much work needs to be done in the future. *In vivo* stability, short half-life and oral bioavailability are the major obstacles in using antitubercular peptides. A possible way of conquering could be by structural constrain and introduction of non-natural amino acids as well as chemical modifications.

In the field of machine learning, especially developing prediction tools concerning biological importance, the non-availability of negative data remains a major problem. We have tried to overcome this inadequacy by our assumptions in generating the negative data, but availability of experimentally verified non-antitubercular and non-immunosuppressive peptide must have guaranteed more robust tools. These flaws must be conquered in the future and this could only be possible, if experimental biologist start reporting negative findings also. In both of the *in silico* tool, AntiTbPred and ImmunoSPpred, we have utilised limited dataset comprising of natural amino acids only. Inclusion of other novel natural as well as modified peptides will certainly provide a chance to improve the method.

IMT-P8, a novel arginine-rich CPP has antimycobacterial activity as well as exhibited synergism with rifampicin, streptomycin and kanamycin. Its combinatorial effect with other newly approved antitubercular drugs such as bedaquline can be explored in future. Its efficacy within animal models should be explored and in case of significant performance, it may lead as therapeutic candidate.