SUMMARY AND CONCLUSION

The present study was carried out to prioritize FDA-approved small molecule drugs with potential for repurposing for TB. To achieve this goal, first of all, a total of six proteins were prioritized and selected as potential drug targets from the complete genome of *M. tuberculosis* using various bioinformatics tools and databases that was further supported by a comprehensive literature survey. A total of 1554 known FDA-approved small molecule drugs were virtually screened using multiple rounds of docking with the selected six target proteins that resulted in prioritization of 55 known drugs as potential candidates drugs for repurposing for tuberculosis. Ten of these 55 prioritized drugs are previously reported by other researchers to have activity against *M. tuberculosis*; in addition, three of the currently used TB drugs, namely, streptomycin, capreomycin and bedaquiline were also observed to be prioritized in the present study; since about one fourth of the prioritized drugs (23.64%, 13 out of 55) are either reported to have anti-mycobacterial activity or already used for TB, other drugs prioritized in this study gain significance for further evaluation.

Furthermore, these findings also support for the procedure employed in the study, thus, it can be adopted to prioritize drugs for repurposing against any other diseases. Furthermore, a total of 9 prioritized drugs were tested against *M. tuberculosis* *in vitro* using LRP assay. Two drugs, namely, cefpodoxime and lymecycline were identified to inhibit both drug sensitive and MDR strains of *M. tuberculosis*. Cefpodoxime and lymecycline were also observed to have synergistic activity with rifampin and isoniazid against *M. tuberculosis*, which encourage for further examination of these drugs towards repurposing for TB.
A total of eight molecular properties of small molecules that have relevance to their biological activity were selected and analysed for currently used TB drugs, prioritized drugs as well as rest of the known drugs. Significantly higher values observed for hydrogen bond donor count, hydrogen bond acceptor count, clogP and polar surface area among currently used TB drugs and prioritized drugs than the rest of the known drugs. These findings suggest that relaxation in the limits of these molecular properties during the initial screening of small molecules may increase the rate of identification of drugs and drug-like molecules for TB as well as for other diseases.

Since 28 different drugs are used in various combinations in the treatment of TB including different forms drug-resistant TB, and a good number of drugs are being studied in various phases of clinical research, an online database called TB DRUGS (Database of Drugs for Tuberculosis) was developed to provide researchers access to information about these drugs. Later, those known drugs which have been demonstrated to have inhibitory activity against M. tuberculosis were also incorporated in the updated version of the database (TB DRUGS, 2.0) which will be useful for clinical researchers for planning new clinical trials for TB towards repurposing.

Based on the findings observed in the present study, the following conclusions can be arrived: (i) Bioinformatics analysis of M. tuberculosis genome enabled to select potential drugs targets; (ii) a total of 55 known drugs were prioritized as potential drugs for repurposing for TB based on multiple rounds of docking with the selected six target proteins; ten of these 55 drugs are already reported by other researchers to have anti-mycobacterial activity and another three drugs are already being used for TB;
identification of previously reported drugs with activity against *M. tuberculosis* advocate for evaluating other prioritized drugs for biological activity against *M. tuberculosis* (iii) cefpodoxime and lymecycline, which were identified to have activity against both drug sensitive and MDR-strains of *M. tuberculosis* and synergistic activity with rifampicin and isoniazid strongly suggest for further studies in animal models for these two drugs for translational research towards repurposing; (iv) the fact that one fourth of the prioritized drugs are either already reported to have activity against *M. tuberculosis* or already used in the treatment of TB also support for the method employed in this study; hence, this method can be used to prioritize drugs for repurposing for any other diseases; (v) relatively higher value of some of the molecular properties of currently used TB drugs and 55 prioritized drugs for TB (from this study) than other known small molecule drugs with respect to ‘rule of 5’ and ‘Veber rule’; these findings suggest for relaxing the value of molecular properties during the initial large scale screening of small molecules, that may potentially improve the rate of success in the drug discovery; (vi) the tuberculosis-specific drug database, TB DRUGS developed in this study provides useful information about all known TB drugs and novel candidate drugs in research pipeline and has been updated with useful information about those drugs which have been demonstrated to be active against *M. tuberculosis* by others; therefore, the TB DRUGS database may be useful for researchers, particularly, clinical researchers as an educational tool and provides necessary information for planning new clinical trials and development towards repurposing for TB.

The in silico prioritization and subsequent *in vitro* observations of cefpodoxime and lymecycline inhibiting both drug sensitive and MDR-strains of *M. tuberculosis*
strongly encourage to carry out *in vivo* studies in animal models to determine their efficacy against *M. tuberculosis*; such studies may lead to potential clinical applications for these two drugs in the treatment of TB. Since cefpodoxime and lymecyline were observed to synergistically act with rifampin and isoniazid, they may also potentially bring down the time required for the successful treatment of TB in the future. Since 13 of the 55 prioritized drugs are already reported to be active against *M. tuberculosis* or currently used in the TB treatment, and another two of the nine prioritized drugs found to be active against drug sensitive and drug resistant *M. tuberculosis* in this study, efforts can be taken in the future to screen *in vitro* the remaining prioritized drugs against *M. tuberculosis*. The bioinformatics protocol employed in the present study has been useful in prioritization of known drugs and enabled to identify two drugs with anti-mycobacterial activity; hence, the protocol designed in the study can be generically used to prioritize known drugs for repurposing for any other diseases.