Chapter VII

Summary

In this study, the researcher aimed to design potential inhibitors against three different proteins of the *Mycobacterium* cell wall. Efforts are underway to address proteins in three distinct layers of the Mtb cell wall. Specifically, targeting *MmpL* from the outermost mycolic layer membrane, *GlfT2* from the middle arabinogalactan layer, and *MurB* from the innermost peptidoglycan layer is being explored. The researchers used an *in-silico* Structure-based design to screen various chemical databases, including ChemSpider, Drug Bank, and Zinc database, to determine affinity and binding mode.

After a successful virtual screening of 30,417 compounds, a total of 153 compounds were identified, out of which fifty-one compounds were discovered against each target protein. Further screening based on strong binding affinity led to the identifications of thirteen compounds against *MmpL*, thirty against *GlfT2*, and thirty-two against *MurB*. The researcher then evaluated these compounds for drug-like properties, pharmacokinetics, and physiochemical characteristics.

A total of fourteen candidates with the maximum binding affinity and drug-likeness characteristics were selected for molecular dynamic simulation studies, out of which seven compounds each against *GlfT2* and *MurB* were chosen. A total of seven compounds were selected for evaluation against *GlfT2*. Two of the compounds were obtained from the ChemSpider database with the chemical abstracts services (CAS) identifiers CSID541554 and CSID67239. Two additional compounds were obtained from the Drug Bank database with the identifiers DB12983 and DB12424. Finally,

three compounds were obtained from the ZINC databases with the identifiers ZINC000043293371, ZINC000063933734, and ZINC000095092808.

Similarly, a total of seven compounds were selected to assess their potential activity against *MurB*. Two of the compounds were obtained from the ChemSpider database with the CAS identifiers CSID1438694 and CSID2166135. Two additional compounds were obtained from the drug bank database with the identifiers DB15688 and DB12983. Lastly, three compounds were obtained from the ZINC database with the identifiers ZINC003975327, ZINC254071113, and ZINC084726167.

The selection of these compounds was based on their potential efficacy against *GlfT2* and *MurB*, both pivotal targets in ongoing research. Utilizing molecular dynamics (MD) simulation, we analyzed molecular interactions and scrutinized conformational shifts of the ligand alongside the active site residues. Finally, the researcher identified four compounds with the most stable and consistent interaction with the target proteins that showed potential as inhibitors, which could be further developed for therapeutic use against *Mycobacterium tuberculosis*. Among the evaluated compounds, ZINC000095092808 and DB12424 showed the most stable interactions with their respective target proteins, *GlfT2*. while ZINC254071113 and DB15688 demonstrated the most favorable binding profiles against *MurB*.

To further validate the inhibitory activity of the identified compounds, ZINC000095092808, DB12424, ZINC254071113, and DB15688, *in vitro* studies were conducted using the antimicrobial susceptibility testing (AST) method with BACTEC radiometric detection. The compounds ZINC000095092808 and DB12424

exhibited promising inhibitory activity against *GlfT2*, while Compounds ZINC254071113 and DB15688 exhibited promising inhibitory activity against *MurB*. The *in-vitro* validation of the identified compounds provided further evidence of their inhibitory activity against Mtb stains with defined drug resistance mutations. These findings provide support for the prospect of utilizing these compounds as lead candidates in the development of innovative anti-tubercular inhibitors.

In summary, this study identified four compounds with promising inhibitory activity against *GlfT2* and *MurB*. Compounds ZINC000095092808 and DB12424 while ZINC254071113 and DB15688 demonstrated the highest potential as novel inhibitors of *GlfT2* and *MurB,* respectively. The identified compounds showed promising interaction residues and could be used as starting points for further development as lead compounds in medicinal chemistry. The present study provides significant insight into the metabolic pathways of *Mycobacterium* cell walls by investigating the role of key enzymes. These findings offer valuable contributions to our understanding of the biochemistry of *Mycobacterium* and can aid in the development of new therapeutic approaches without the need for the arduous process of developing new antibiotics. However, the study has some limitations, such as the lack of animal models and clinical trials.

Nevertheless, this research demonstrates the significance of utilizing computational biology approaches alongside laboratory experiments to elucidate the molecular mechanisms underlying biochemical pathways. The synergy between these approaches can enhance our understanding of the intricate relationships between enzymes and their potential inhibitors. Thus, it is imperative to continue investigating

the relationship between computational and *in-vitro* approaches in the identification of probable inhibitors.

It is noteworthy that the insights from this study can be leveraged to design targeted interventions that aim to develop novel inhibitors against *Mycobacterium tuberculosis* (MTB). However, further investigations are necessary to establish a greater degree of accuracy and precision in such approaches. Future research should focus on refining the computational models and experimental designs used to identify potential inhibitors, and subsequently evaluate their efficacy in animal models and clinical trials.

In conclusion, this study underscores the significance of a multidisciplinary approach to address the challenges posed by MTB resistance. The findings of this study can serve as a valuable resource for the development of novel therapeutic interventions aimed at combating MTB infections. Nonetheless, continued efforts are necessary to bridge the gap between computational models and experimental results, and to further refine our understanding of the molecular mechanism underlying the metabolism of *Mycobacterium* cell wall.