4. CONCLUDING REMARKS

Pyrazinamidase sequences of various species of *Mycobacterium* have been retrieved from protein primary database. The three-dimensional structures of pyrazinamidases from *M. abscessus, M. avium, M. kansassi, M. bovis, M. marinum, and M. smegmatis* have been homology modeled and the modeled structures have also been thoroughly validated using SAVES meta server. All the pyrazinamidases structures have been subjected to molecular dynamic simulations at close quarters of physiological conditions (pH 7.0, 310 K and 0.1M NaCl) and the resultant dynamic structures were used for analysing structural architectures and also for predicting surface grooves of the enzymes. The binding sites of the pyrazinamidases have been predicted using an array of online and offline computational tools. The binding sites were then used for setting up grids for docking studies performed between PZA and PZases. However, the binding energies obtained for PZA – PZase from *M. tuberculosis*, which is susceptible to PZA and for PZA - PZases from other six PZA-resistant species were found to be comparable to each other. Comprehensive analyses on overall volumes, average depths and structural architectures of the binding sites of the PZases were also inconclusive to figure-out the mechanism of the PZases resistance towards PZA. It seems that the sizes and shapes of active sites of the enzymes have no or negligible effect on their activities. Interestingly, either bereft of 'GATE' region or 'ill-formed GATE' regions of PZases as demonstrated in the present study may act as probable structural factors for the differential binding affinities of the pyraziamidase variants towards the interaction with pyrazinamide and as well PZA-resistant properties of the enzymes.

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