TARGETING CYCLOPROPANE SYNTHASE (CmaA1) IN *MYCOBACTERIUM TUBERCULOSIS*: *IN SILICO* APPROACHES FOR IDENTIFYING POTENTIAL DRUG CANDIDATES

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Abstract. There is an urgent need for new and better drugs to treat tuberculosis. As there is complex treatment regimens and a rising problem of drug resistance. New targets and drug candidates are being explored by researchers across the globe. This will fulfil the urgent need of new drugs for tuberculosis. Using computational method, old targets are also being explored. Recent study shows, cyclopropane synthase (CmaA1) in *Mycobacterium tuberculosis* is potential drug target. In this *in silico* study, we have used online drug discovery web server named DrugRep for target based Virtual Screening and docking against experimental drug library (5935 drugs) from the DrugBank database. Further, molecule with least minimum docking energy were considered for simulation study.

Key words: Docking, simulation, *Mycobacterium tuberculosis,* cyclopropane synthase (CmaA1).

INTRODUCTION

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, continues to pose one of the most pressing global health challenges [11]. Despite significant advancements in medical science, TB remains a leading cause of mortality, particularly in low- and middle-income countries where access to effective treatments is limited [2]. The current standard of care for TB involves prolonged treatment regimens spanning several months, which can be burdensome for patients

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and healthcare systems alike [13]. Moreover, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* has further complicated efforts to control the disease, rendering many first-line and second-line treatments ineffective [3].

In the face of these challenges, there is a growing consensus among researchers and clinicians that new therapeutic strategies are urgently needed. The development of novel drugs targeting critical pathways in *M. tuberculosis* has become a key focus of TB research [5]. Advances in computational biology and in silico methodologies have provided powerful tools to identify and evaluate potential drug targets more efficiently and cost-effectively compared to traditional experimental approaches [12].

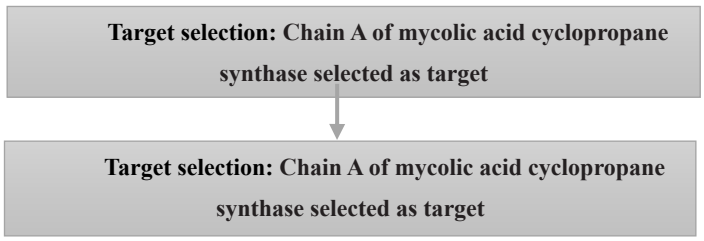
This study investigates cyclopropane synthase (CmaA1), an enzyme essential for the biosynthesis of mycolic acids, as a promising drug target. Mycolic acids are crucial components of the mycobacterial cell wall, conferring structural integrity and resistance to host immune responses. By disrupting the activity of CmaA1, it may be possible to compromise the bacterium's survival and pathogenicity, making this enzyme an attractive target for therapeutic intervention.

The research employs a combination of target-based virtual screening and molecular docking to identify candidate compounds capable of inhibiting CmaA1. Using an extensive library of experimental drugs sourced from the DrugBank database, potential inhibitors are screened for their binding affinity to the enzyme. The most promising candidates are then subjected to molecular dynamics simulations to assess the stability and physical behavior of protein-ligand complexes, providing a comprehensive evaluation of their potential efficacy.

Through these in silico approaches, the study not only identifies promising drug candidates but also underscores the role of computational techniques in accelerating drug discovery [12]. This approach aligns with the growing emphasis on utilizing computational methods to streamline the drug development process [5]. The findings hold significant promise for addressing the urgent need for new and effective TB treatments, contributing to the global fight against this enduring health crisis [11].

MATERIALS AND METHODS

In this *in silico* study, major steps are outlined in (Fig.1). In this figure both for molecular docking and simulation were outlined.



Target selection: Chain A of mycolic acid cyclopropane synthase selected as target

Fig. 1. An outline for the main steps and options of the applied both for molecular docking and simulation.

PROTEIN STRUCTURE PREPARATION

In this *in silico* study, we obtained the Fig. 2. crystal structure of mycolic acid cyclopropane synthase CmaA1 (1KP9).

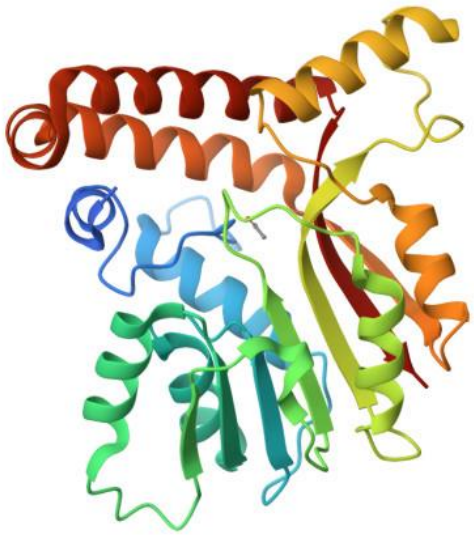


Fig. 2. Structure chain A of mycolic acid cyclopropane synthase [4].

VIRTUAL SCREENING AND MOLECULAR DOCKING

A target-based virtual screening and molecular docking were performed using an online drug discovery web server named DrugRep. Virtual screening was performed using an experimental drug library (5935 drugs) from the DrugBank

database [10] against chain A of mycolic acid cyclopropane synthase. Chain A of mycolic acid cyclopropane synthase was extracted using USCF Chimera [8] and uploaded to the DrugRep server to perform target-based virtual screening. As the DrugRep server can also perform docking using AutoDockTools version 1.5.6 and AutoDock Vina version 1.1.2 [7], this study performed docking using the DrugRep default docking coordinates (*x*: 4.9, *y*: –4.9, *z*: 39.0) with grid box dimensions of 171920 Angstroms.

SIMULATION STUDY

A simulation study was performed for the docked complex with the least energy using the iMODS server [6], which is used to evaluate the stability and physical movements of the docked complexes. Normal mode analysis (NMA) was implemented to investigate the slow dynamics of the docked complexes and to demonstrate their large-amplitude conformational fluctuations [9]. This approach aligns with the goal of understanding the intrinsic flexibility and dynamic stability of the docked structure.

RESULTS AND DISCUSSION

An overview for the results of molecular docking for the best 10 drugs can be observed in (Table 1). Based on findings in this table, the docking energy for these drugs is ranging between –13.3 and –11.5. kcal/mol.

Table 1

Results of molecular docking for the best 10 drugs with different seven parameters such as docking energy, HBD (hydrogen bond donor), HBA (hydrogen bond accepter), RB (rotatable bonds), NOA (number of atoms), ring and Log *P* (partition coefficient).

Compound ID	Docking energy (Kcal/mol)	HBD	HBA	RB	NOA	Rings	Log *P*
DB02729	–13.3	4	7	16	13	9	6.7
DB04289	–12.6	0	1	3	4	6	4.2
DB02112	–12,0	1	0	6	6	6	5.2
DB14067	–11.9	1	3	10	6	5	4.1
DB02226	–11.8	2	3	12	8	8	9.5
DB02009	–11.7	4	4	16	9	6	4.3
DB03642	–11.5	2	6	10	10	4	3.5
DB04708	–11.5	4	4	15	9	6	4.3
DB06925	–11.5	2	3	4	5	4	4.9
DB07853	–11.5	1	4	5	7	5	3.4

DB02729 compound with significant docking energy –13.3 kcal/mol is SD146 (generic name) as compared to other nine compounds (Table 1). The other docking parameters with HBD (hydrogen bond donor) value 4 suggests significant solubility

and interaction with biological targets (Table 1). HBA (hydrogen bond accepter) value 7 signifies polarity for suitable interaction with interacting molecules in docking (Table 1). RB (rotatable bonds) value 16 contributing for molecular flexibility, impacting significant target CmaA1 protein binding (Table 1). NOA (number of atoms) higher value 13, predicting suitable molecular behavior. Other structural parameter Ring count value 9, significantly improving biological activity and stability (Table 1). The lipid solubility is provided by Log *P* (partition coefficient) value 6.7 signifies the better membrane permeability (Table 1). These all-comparative compounds belong to the class of organic compounds known as benzimidazoles(Table 1). These are organic compounds containing a benzene ring fused to an imidazole ring (Table 1). Below (Fig. 3) are docked complex of chain A of mycolic acid cyclopropane synthase with DB02729 compound.

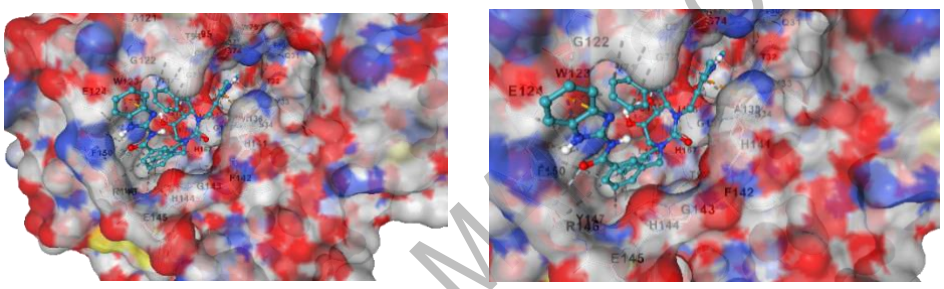


Fig. 3. Docked complex of chain A of mycolic acid cyclopropane synthase with DB02729 compound.

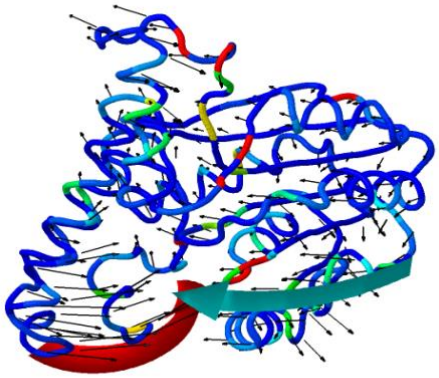


Fig. 4. Molecular mobility evaluated by NMA of the docked complex.

The estimation of protein-ligand complex stability was performed using MD (molecular dyanamics) simulations for docked complex of chain A of mycolic acid cyclopropane synthase with DB02729 compound. The MD simulation provides critical insights into the stability and dynamic behavior of the docked complex between chain A of *mycolic acid cyclopropane synthase* and the compound DB02729. The results from the IMODS server indicate that DB02729 forms a stable and potentially effective interaction with the target protein.MD simulations are able to assess ligand-induced alterations in the protein structure. MD simulations performed using IMODS sever. In Fig. 4 and Fig. 5 are shown the results of MD simulation performed using IMODS server. These results support DB02729 forms a stable and potentially effective interaction with the target protein.

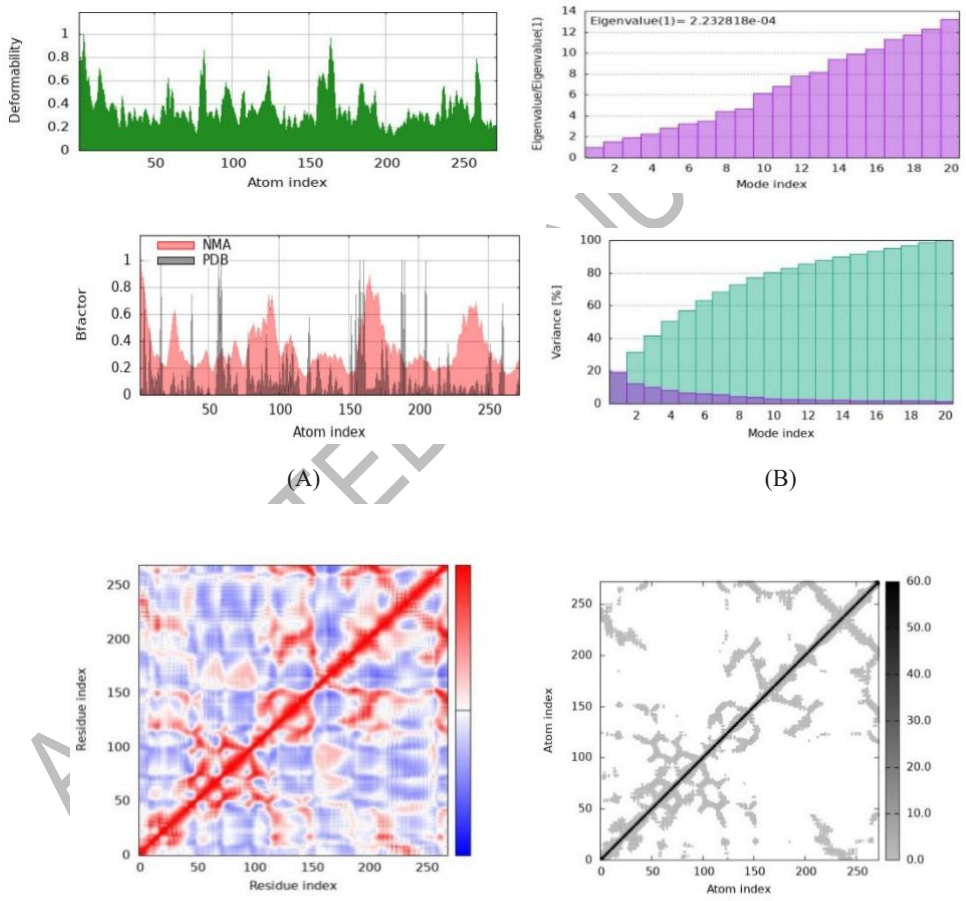


Fig. 5. Outputs of molecular dynamics simulations in iMODS (A) deformability

and B-factor plot; (B) eigen value and variance plot; (C) elastic network model; and (D) covariance map.

Figure 5 shows the deformability and B-factor give the mobility profile of the docked protein. The deformability and B-factors of docked protein illustrate the peaks corresponding to the regions in the proteins with deformability, where the highest peaks represent the regions of high deformability. The B-factor graphs provide a comparison between the NMA and the PDB field signify essentially contrasting predicted and experimentally derived flexibility or mobility data of the protein complex (Fig.5).

The eigenvalue and the variance are inversely linked to each normal mode. The eigenvalue and variance graphs of docked complex are described in Fig.5 respectively. The variance graph of docked compound with the target protein indicates the individual variance with purple-shaded bars, whereas cumulative variance is represented by green-shaded bars (Fig.5).

CONCLUSION

This study highlights the potential of cyclopropane synthase (CmaA1) in *Mycobacterium tuberculosis* as a promising drug target for combating tuberculosis, particularly in the context of rising drug resistance. Using ***in silico*** techniques, the research successfully identified several compounds with strong binding affinities through virtual screening and molecular docking. Among the screened drugs, SD146 (DB02729), a benzimidazole derivative, demonstrated the lowest docking energy, suggesting high potential as an effective inhibitor.

Molecular dynamics simulations further validated the stability and physical movements of the protein-ligand complexes, confirming the viability of the identified compounds for further experimental investigation. These findings contribute to the growing body of research leveraging computational methods to accelerate drug discovery and development for tuberculosis treatment. Future experimental validation and optimization of the identified compounds will be essential to translate these promising results into clinical applications.

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