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Molecular Docking Studies of Tubulosine against Multidrug-Resistant Tuberculosis

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ABSTRACT

Tuberculosis (TB) causes considerable morbidity among millions of people each year worldwide and ranks as the second leading cause of death from an infectious disease. The present anti-TB drugs produce side-effects primarily liver damage and other adverse reactions like nausea, vomiting, and anorexia. The increase in multi-drug resistant strains of M. tuberculosis has decreased the effectiveness of current standard Tuberculosis treatment options. Thus, the discovery of anti-tuberculosis agents that target new pathways with novel mechanisms of action is crucial for effective short-term tuberculosis therapy that will limit the development of resistance. Tubulosine, an isoquinoline alkaloid from the stem bark of Alangium lamarckii reported to exhibit antiplasmodic activity. The present study was designed to perform molecular docking analysis of Tubulosine for find the binding conformation, affinity and orientation of Tubulosine in the active sites of Mycobacterium tuberculosis DNA gyrase, Isocitrate lyase, Thymidine monophosphate kinase which can inhibit the Tuberculosis. The crystal structure of M. tuberculosis DNA gyrase (4G3N), Isocitratelyase (1F61), Thymidine monophosphate kinase (1W2G) were obtained from the Protein Data Bank (RCSB PDB). The PDB file was prepared using the software UCSF Chimera. The Molecular docking analysis has shown Tubulosine to potentially inhibit thymidine monophosphate kinase (-9.36kcal/mol) than DNA gyrase and Isocitratelyase at a very minimal concentration of 138.37nM. Thus this study offers a route for the development of Tubulosine against multidrug resistant tuberculosis and need further in-vitro investigations to confirm their efficacy and drug ability.

Key words: Antituberculosis, Tubulosine, Molecular Docking, DNA gyrase, Isocitratelyase, Thymidine monophosphate kinase.

INTRODUCTION

The deadly infectious disease tuberculosis is caused by several species of Mycobacteria including *Mycobacterium tuberculosis, M. bovis, M. africanum, M. microti, M. avium* and *M. leprae* that are intracellular, Gram-positive, non-motile, and rod-shaped obligate aerobic pathogens of higher vertebrates [1]. Tuberculosis (TB) causes considerable morbidity among millions of people each year worldwide and ranks as the second leading cause of death from an infectious disease, after the human immunodeficiency virus (HIV). About 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5–10% of the US population test positive [2]. In addition, the emergence of drug-resistant strains of *M. tuberculosis* has led to increased pressure on current

chemotherapy regimes. Current anti-tuberculosis treatments process a long course of a combination of antibiotics and toxic side effects and lead to poor patient compliance. There is now a need to discover and develop new safe and herbal antituberculosis drugs particularly to target drug resistance and improve the treatment of chronic tuberculosis by targeting tubercle bacilli.

The increase in multi-drug resistant strains of *M. tuberculosis* has decreased the effectiveness of current standard Tuberculosis treatment options. Thus, the discovery of anti-tuberculosis agents that target new pathways with novel mechanisms of action is crucial for effective short-term tuberculosis therapy that will limit the development of resistance. Computational (*In silico*) methods have been developed and widely applied to pharmacology hypothesis development and testing. These *in silico* methods include database searching, quantitative structure-activity relationships, similarity searching, pharmacophore identification, computational modeling and docking. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization.

The recent researches focused on natural products have shown a useful way to obtain a potentially rich source of drug candidates, where alkaloids have been found more effective. Anti-tuberculosis compounds from natural sources have an enormous potential for the development of new drugs, which have shown not only antimicrobial activity but also inhibition of the mechanism of resistance (e.g. efflux pumps) or modulation of the immune response (e.g. macrophage stimulation) [3]. Tubulosine, an isoquinoline alkaloid from the stem bark of *Alangium lamarckii* reported to exhibit antiplasmodic with an IC_{50} value of 0.012mM against a chloroquine-sensitive strain of *Plasmodium falciparum* and with an IC_{50} value of 0.023mM against a resistant strain [4]. It showed antitumour activity against the Lu1 human lung cancer cell line (ED50 < 0.001 mg/mL) [5] and amoebicidal activity [6]. The alkaloid tubulosine shows cytotoxic activity by interfering with protein synthesis by blocking the elongation-factor-2-dependent step of translocation and elongation of peptide chain [7].

Many well known anti-TB drugs are known to target the biosynthetic pathways that involve the production of macromolecules such as proteins, nucleic acids, or cell wall polymers. Many of the inhibitors of protein synthesis like tetracycline, chloramphenicol and macrolides do not show activity against M. tuberculosis. The new targets to be specific to Mycobacteria to limit the transfer of resistance factors from other bacteria.

Nucleotide biosynthesis has been reported to be a good target particularly for TB in HIV cases [8]. In this regard, thymidine monophosphate kinase (dTMKase) has been suggested as validated target to develop new anti-tubercular agents particularly for the treatment of MDR TB and TB in HIV infected patients [9]. This enzyme is an essential enzyme of nucleotide metabolism that catalyzes the reversible phosphorylation of thymidine monophosphate (dTMP) to thymidine diphosphate (dTDP) [10].

Another promising target is DNA topoisomerases particularly DNA gyrase, a type II topoisomerase. DNA gyrase is involved in many reactions including ATP-dependent negative supercoiling of closed circular double stranded DNA. Many quinolone drugs act by inhibiting DNA gyrase [11].

During latent infection, *M. tuberculosis* bacteria are thought to be in a slow-growing or non-growing state and are resistant to the treatment of many conventional drugs. The bacterium shifts its metabolic priorities and turns on the glyoxylate cycle - presumably to adapt to an inhospitable environment where carbohydrates are limiting and lipids are more abundant. An enzyme isocitrate lyase (absent in mammals) responsible for the conversion of isocitrate to glyoxylate is a very hot molecule and is considered to be a promising target for new drug development for TB [12].

MATERIALS AND METHODS

Macromolecule Preparation

The crystal structure of *M. tuberculosis* DNA gyrase **(4G3N)**, *M. tuberculosis* Isocitratelyase **(1F61)**, *M. tuberculosis* Thymidine monophosphate kinase **(1W2G)** was obtained from the Protein Data Bank (RCSB PDB, http://www.pdb.org). The PDB file was prepared using the software UCSF Chimera (Pettersen et al. 2004). All bound substances (ligands and cofactors) and solvent molecules were removed from the model. Since the protein structure contains two identical domains, only one **(Domain A)** was used in the docking experiment.

Active site prediction

The ligand binding domains of *Mycobacterium tuberculosis* DNA gyrase, Isocitratelyase, Thymidine monophosphate kinase were predicted using the Site Finder module of Molecular Operating Environment.

Molecular Docking Analysis

In order to understand binding conformation, affinity and orientation of Tubulosine in the active sites of *Mycobacterium tuberculosis* DNA gyrase, Isocitratelyase, Thymidine monophosphate kinase. Tubulosine was docked to the active site of the three enzymes. All the three targets and ligand were prepared by addition of hydrogen's and gasteiger charges. A grid defining the active site was constructed before running the docking simulation. Genetic algorithm was adopted for conformer search while docking.

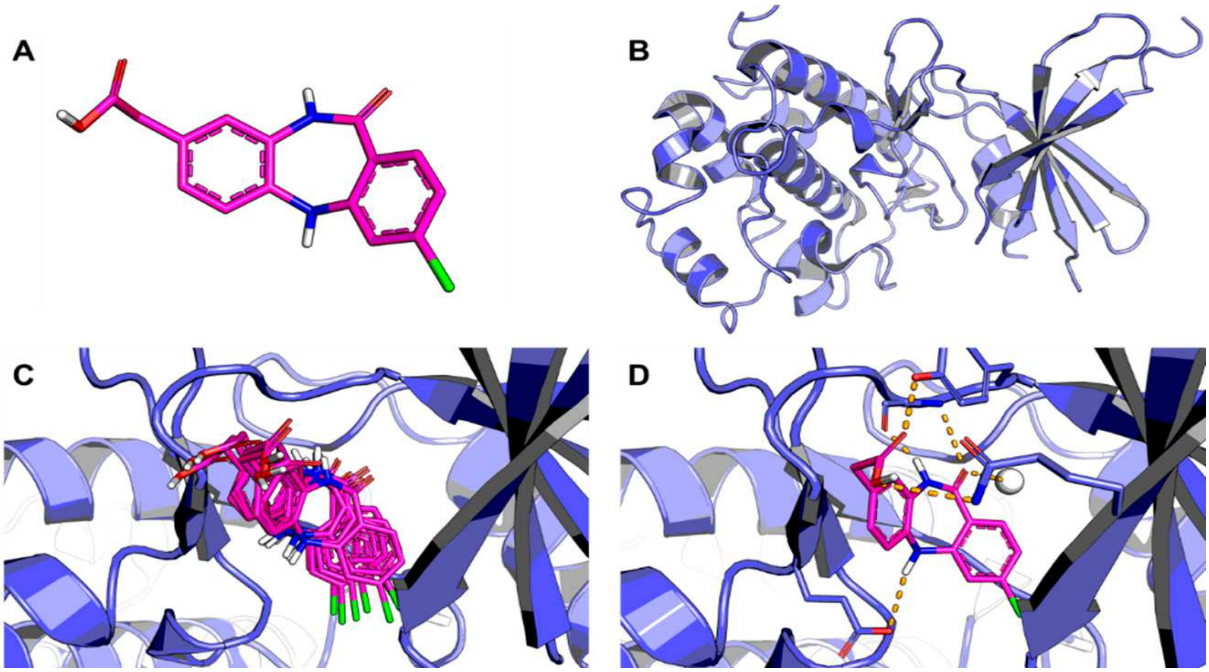
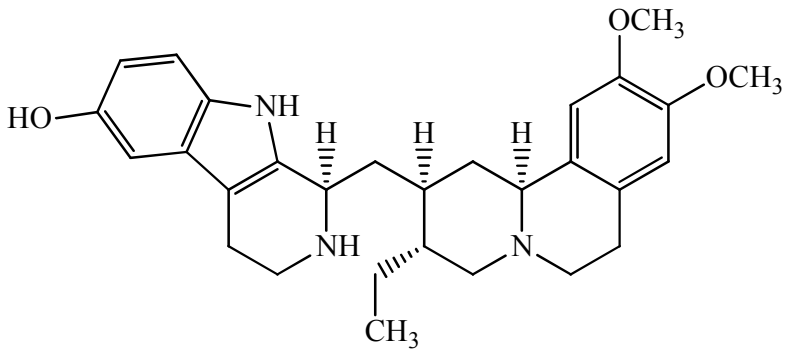


Fig 1: Outline of the molecular docking process. (A) Three-dimensional structure of the ligand; (B) Three-dimensional structure of the receptor; (C) The ligand is docked into the binding cavity of the receptor and the putative conformations are explored; (D) The most likely binding conformation and the corresponding intermolecular interactions are identified. The protein backbone is represented as a cartoon. The ligand (carbon in magenta) and active site residues (carbon in blue) are shown in stick representation. Water is shown as a white sphere and hydrogen bonds are indicated as dashed lines



Tubulosine (β-carboline-benzoquinolizidine)

Tubulosine is a β-carboline-benzoquinolizidine alkaloid derivative, bearing methoxy groups at positions 10 and 11 and a hydroxy group at the 8' position.

1. Thymidine Monophosphate Kinase
Target: *Mycobacterium tuberculosis* - **Thymidine Monophosphate Kinase**
Target PDB id: 1W2G
Method: *GENETIC ALGORITHM

Ligand Binding Data

PARAMETER	PREDICTED VALUES
Binding Energy	-9.36
Inhibitory Constant	138.37nM
Intermolecular Energy	-11.15
Internal Energy	-0.55
Torsional Energy	1.79
Electrostatic energy	-0.9
Unbound Extended Energy	-0.55
Hydrogen Bonding Interactions	Present – 1
Interacting Residues	ARG 74
Vanderwaal's Hydrogen Bond Desolvation Energy	-10.25

2. Gyrase Type II A Topoisomerase C-terminal domain
Target: *Mycobacterium tuberculosis* - **DNA Gyrase subunit A**
Target PDB id: 4G3N
Method: *GENETIC ALGORITHM

Ligand Binding Data

PARAMETER	PREDICTED VALUES
Binding Energy	-8.66
Inhibitory Constant	447.68nM
Intermolecular Energy	-10.45
Internal Energy	-0.13
Torsional Energy	1.79
Electrostatic energy	-1.08
Unbound Extended Energy	-0.13
Hydrogen Bonding Interactions	Present – 3
Interacting Residues	ALA 765, ASN 715, ILE 608
Vanderwaal's Hydrogen Bond Desolvation Energy	-9.37

3. Isocitrate lyase
Target: *Mycobacterium tuberculosis* - **Isocitrate lyase**
Target PDB id: 1F61
Method: *GENETIC ALGORITHM

Ligand Binding Data

PARAMETER	PREDICTED VALUES
Binding Energy	-7.37
Inhibitory Constant	3.98Um
Intermolecular Energy	-9.16
Internal Energy	-0.4
Torsional Energy	1.79
Electrostatic energy	-2.06
Unbound Extended Energy	-0.4
Hydrogen Bonding Interactions	Present – 1
Interacting Residues	ASP 220
Vanderwaal's Hydrogen Bond Desolvation Energy	-7.1

RESULTS AND DISCUSSION

Molecular Docking continues to hold great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. As a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Number of

reports citing successful application of CADD in developing specific drugs in different therapeutic areas is expanding rapidly. It is estimated that docking programs currently dock 70 – 80 % of ligands correctly [13].

To gain better insight for the interactions between *Mycobacterium tuberculosis* DNA gyrase, Isocitratelyase, Thymidine monophosphate kinase and Tubulosine, molecular docking studies were carried out. The interactions of the ligands with the active site residues of the target were analyzed in terms of the following parameters: Binding energy, number of hydrogen bonds established by the ligand with residues of the active site, π-π interactions, conformation oriented by the ligand within the active site and root mean square deviation (RMSD) of the active site residues. The dock score of Autodock is reported in kcal/mol. Autodock uses the following empirical formula to calculate the free energy of binding:

Binding energy (ΔG) = Intermolecular energy + Vanderwaal's hydrogen bond desolvation energy + Electrostatic energy + Total internal energy + Torsional energy – Unbound energy of the system.
Desolvation energy is a prime parameter that decides a molecules interaction with its pharmacodynamic target. In the biological environment, all drug binding pockets of a target protein remain solvated and hence a ligand cannot as such occupy the active site unless it dislodges the water molecules. The similarity of docked structures is measured by computing the root mean square deviation and clusters are created based on the comparison of conformations and estimated RMSD values. The docking score of Tubulosine with *Mycobacterium tuberculosis* DNA gyrase, Isocitratelyase, Thymidine monophosphate kinase are shown in **Table.1**

Table 1: Molecular Docking Analysis

TARGET	DNA Gyrase	Isocitrate Lyase	Thymidine Kinase
Binding Energy	-8.66	-7.37	-9.36
Inhibitory Constant	447.68nM	3.98uM	138.37nM
Intermolecular Energy	-10.45	-9.16	-11.15
Internal Energy	-0.13	-0.4	-0.55
Torsional Energy	1.79	1.79	1.79
Electrostatic energy	-1.08	-2.06	-0.9
Unbound Extended Energy	-0.13	-0.4	-.055
Hydrogen Bonding Interactions	Present – 3	Present – 1	Present – 1
Interacting Residues	ALA 765, ASN 715, ILE 608	ASP 220	ARG 74
Vanderwaal's Hydrogen Bond Desolvation Energy	-9.37	-7.1	-10.25

Molecular docking analysis has shown Tubulosine to potentially inhibit thymidine monophosphate kinase (-9.36kcal/mol) than DNA gyrase and Isocitratelyase. Inhibition of *Mycobacterium tuberculosis* thymidine monophosphate kinase by Tubulosine has been predicted to occur at a very minimal concentration of 138.37nM.

Fig 1: Docked Conformation of Tubulosine with Isocitrate lyase

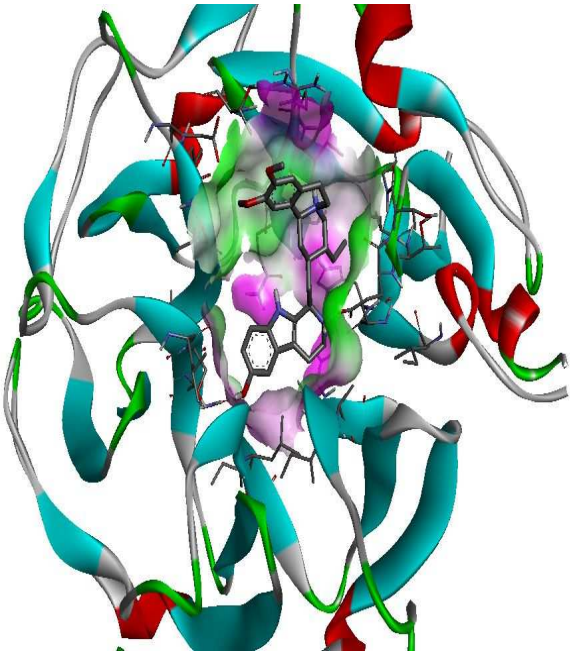


Fig 2: Docked Conformation of Tubulosine with DNA gyrase

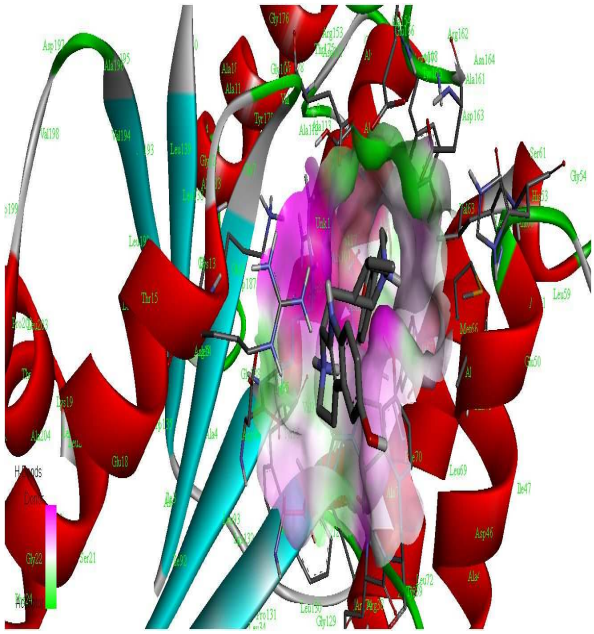


Fig 3: Docked Conformation of Tubulosine with Thymidine Kinase

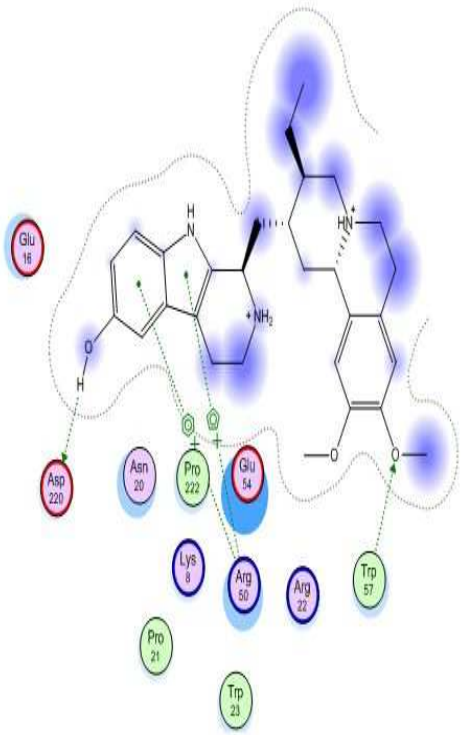


Fig 4a. Isocitrate lyase

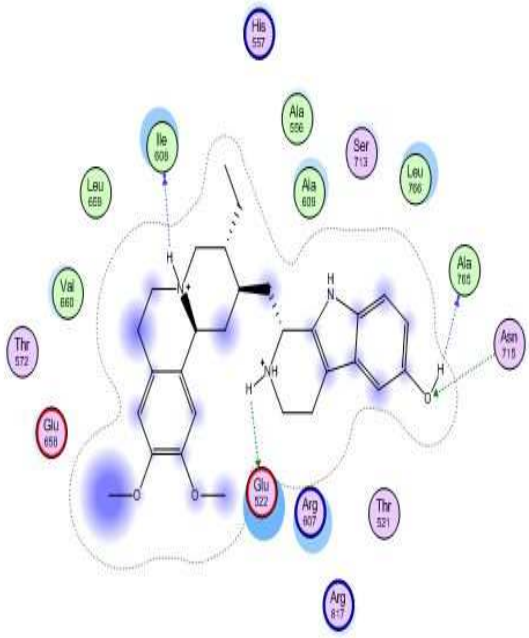


Fig 4b. DNA gyrase

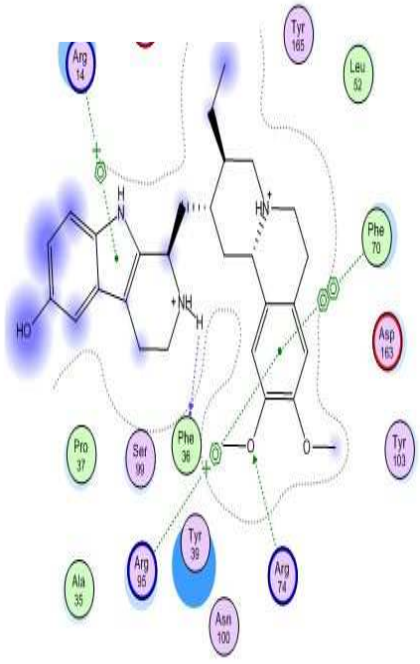


Fig 4c. Thymidine kinase

Fig 4: 2D-Ligand Interaction Maps of Tubulosine with 4a. Isocitrate lyase, 4b. DNA gyrase, 4c. Thymidine kinase

CONCLUSION

The docking analysis of potential phytocompound tubulosine derived from the stem bark of *Alangium lamarckii* into *Mycobacterium tuberculosis* DNA gyrase, Isocitratelyase, Thymidine monophosphate kinase active site was done to determine the probable binding site against tuberculosis using commercial tool UCSF Chimera. The Molecular docking analysis has shown that Tubulosine potentially inhibit thymidine monophosphate kinase (-9.36kcal/mol). It is be concluded from this study that Tubulosine could serve as anti-tuberculosis drug and need further *in-vitro* investigations to confirm their actual therapeutic potential efficacy and drug ability towards the disease.

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