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PREDICTION OF BINDING ENERGIES/INTERACTIONS BETWEEN DIOSPYRIN AND DIFFERENT TARGET PROTEINS OF *Mycobacterium tuberculosis* BY *IN SILICO* MOLECULAR DOCKING STUDIES

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ABSTRACT

Diospyrin, the compound under research binds to a novel site on well known enzyme DNA Gyrase of Mycobacterium tuberculosis and inhibits the enzyme. The aim of the present study was to predict the binding energies/interactions between Diospyrin compound and different target proteins of *Mycobacterium tuberculosis*. The following target proteins with their Protein Data Bank (PDB) ID were selected, NADH-dependent enoyl- ACP reductase (InhA) - 2NSD, Adenosine kinase (Adok) - 2PKK, Mycolic acid synthase (PcaA) - 1L1E, Lysine N-acetyltransferase (MbtK) - 1YK3, Thymidylate synthase X (ThyX) - 3GWC, Thymidylate kinase (TmK) – 1G3U, Serine/threonine-protein kinase (PknG) – 2PZI, beta-ketoacyl-ACP synthase III (FabH) – 1HZP, Arabinosyl indolyl acetyl inositol synthase (EmbC) – 3PTY, dTDP-rhamnose synthase (RmlD) – 1KC3, Cyclopropane fatty acid synthase (CmaA2) – 3HEM, Diaminopimelate decarboxylase (LysA) – 1HKV, L,D-Transpeptidase type 2 – 3VAE, Gyrase type IIA topoisomerase C-terminal domain – 3UC1 and Topoisomerase IV (E.coli) – 3FV5 to study their susceptibility to Diospyrin compound. The 3D and 2D structures of target proteins are downloaded from PDB database. The extent of binding positions and affinity of the Diospyrin with the selected target proteins were predicted based upon the scoring functions - Glide score (G Score) of Glide software. The Cyclopropane fatty acid synthase (CmaA2) was identified as potential target enzyme through our molecular docking studies. Structure-based drug design is now becoming the effective tool with the potential to identify the lead molecule to the development of new anti-tubercular agents, effective against persistent and resistant Mycobacterium tuberculosis infections.

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INTRODUCTION

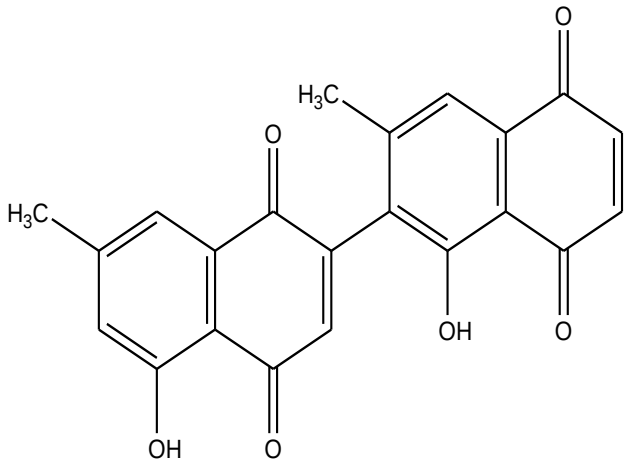
Diospyrin an active compound from the South African toothbrush tree inactivates a drug target for tuberculosis in a previously unseen way. Pharmacological studies indicated that *Salvodora persica* L., the tooth brush tree possess anti-microbial, anti-plaque, aphrodisiac, alexiteric, analgesic, anti-inflammatory, anti-pyretic, astringent, diuretic and bitter stomachic activities[1,2]. The Antimycobacterial activity of diospyrin derivatives against *Mycobacterium tuberculosis in vitro* were also reported [3]. DNA gyrase, a DNA topoisomerase that is present in bacteria and plants, but not animals, and has been widely exploited as a target for antimicrobial chemotherapy. DNA topoisomerases are enzymes that catalyze changes in the topology of DNA and are essential to all cells [4]. The uniqueness of gyrase has made it a successful target for antibacterial agents. Fluoroquinolones (e.g. ciprofloxacin, moxifloxacin) target gyrase and are highly-successful clinical agents that have been used against tuberculosis. The recent study indicates that the Diospyrin compound inhibits the DNA Gyrase of M.tb a novel mechanism of action. [5] Molecular docking is a key tool and is an interface between structural biology and Computer Assisted Drug Design. The ultimate goal of ligand-protein docking is to predict the binding model(s) of the ligand with 3D protein structure. It clearly states that, computational techniques can support and help the design of novel, potent inhibitors by revealing the mechanism of drug-receptor interactions [6]. In our study the molecular docking results showed that Diospyrin compound's binding potential towards **CmaA2**- Cyclopropane fatty acid synthase (critical target involved in cell wall biosynthesis) was more prominent with the G-Score of **--11.24 kcal/mol**. This shows that Diospyrin also has a major role in inhibiting cell wall biosynthesis of Mycobacterium tuberculosis. Isoniazid (cell wall biosynthesis inhibitor) the known Antitubercular drug against **CmaA2** enzyme gives the G-Score of **-5.80 kcal/mol**.

MATERIALS AND METHODS

SELECTION OF LIGANDS

The antitubercular compound Diospyrin[4] was selected from Pubchem database based on their reported antimycobacterial activity. The selected ligand was converted to 3D structures using ISIS Draw software.

STRUCTURE OF DIOSPYRIN:



LIGAND PREPARATION:

The process of ligand preparation is carried out in Ligprep tool of Maestro 9.1 version.

MOLECULAR DOCKING:

Docking is a process of calculating the binding energy of particular conformational shape of ligand with the receptor. Accessing the affinity of varying ligand poses with the particular receptor is carried out by glide in XP (Extra precision mode). XP scoring function was utilized to rank (order) the compounds.

Diospyrin was docked into receptor site by using Glide[7]. The crystal structures of the enzymes (with their respective PDB codes) were used. The ligand with all water molecules was deleted. And charges were assigned. The structure was then minimized using initial optimization, with OPLS-2005 force field. The ligand was preprocessed before docking calculations.

SELECTION OF TARGET ENZYMES:

Table 1: The following target proteins were selected from the Protein Data Bank and the informations of the proteins are given below.

Name of the Enzyme(PDB ID)	Functional category / Mechanism
InhA- Enoyl acyl carrier protein reductase(2NSD[8])	This isozyme is involved in mycolic acid biosynthesis the second reductive step in fatty acid biosynthesis and also responsible for resistance against the antituberculosis drugs isoniazid and ethionamide.
Adok- Adenosine kinase(2PKK[9])	Adenosine phosphorylation
PcaA- Mycolic acid synthase(1L1E[10])	Involved in the mycolic acid modification or synthesis; essential for the cyclopropanation function. Required for cording and mycolic acid cyclopropane ring synthesis in the cell wall.
MbtK- Lysine N-acetyltransferase(1YK3[11])	Involved in the biogenesis of the hydroxyphenyloxazoline-containing siderophore mycobactins
ThyX- Thymidylate synthase X(3GWC[12])	Catalyzes the formation of dTMP and tetrahydrofolate from dUMP and methylenetetrahydrofolate
TmK- Thymidylate kinase(1G3U[13])	Phosphorylation of dTMP to form dTDP in both de novo and salvage pathways of dTTP synthesis
PknG- Serine/threonine-protein kinase(2PZI[14])	Involved in signal transduction (via phosphorylation). Thought to regulate amino-acid uptake and stationary-phase metabolism.
FabH- beta-ketoacyl-ACP synthase III(1HZP[15])	Involved in fatty acid biosynthesis. Catalyzes the condensation reaction of fatty acid synthesis by the addition to an acyl acceptor of two carbons from malonyl-ACP.
EmbC- Arabinosyl indolyl acetyl inositol synthase(3PTY[22])	Involved in the biosynthesis of the mycobacterial cell wall arabinan and resistance to ethambutol
RmlD- dTDP-rhamnose synthase(1KC3[16])	Involved in dTDP-L-rhamnose biosynthesis: converts dTDP-6-deoxy-L-lyxo-4-hexulose to dTDP-L-rhamnose with the concomitant oxidation of NADPH to NADP+
CmaA2- Cyclopropane fatty acid synthase(3HEM[17])	Essential for the cyclopropanation function. Transfers a methylene group from S-adenosyl-L-methionine to the cis double bond of an unsaturated fatty acid chain resulting in the replacement of the double bond with a methylene bridge.
LysA- Diaminopimelate decarboxylase(1HKV[18])	Involved in biosynthesis of lysine
L,D-Transpeptidase type 2(3VAE[19]	biosynthesis and maintenance of their peptidoglycan layer
Gyrase type IIA topoisomerase C-terminal domain(3UC1[21])	Gyrase, a prokaryotic type IIA topoisomerase, consumes ATP to introduce negative supercoils through a strand passage mechanism.
Topoisomerase IV (E.coli)(3FV5[20])	Cleaves and rejoins one strand of double-stranded DNA to relax the negatively supercoiled DNA.

Table2:The Docking results of our study are given below:

Enzyme Name	PDB i.d	G-Score (Kcal/mol)	Glide Energy	AminoAcid residue/residues giving H-Bond Interaction
InhA	2NSD	-2.99	-25.92	No interaction
Adok	2PKK	-5.79	-41.84	SER8, GLN172, GLN173
PcaA	1L1E	-6.65	-44.50	No interaction
MbtK	1YK3	-3.11	-24.96	LYS138, TRP59, ARG62
ThyX	3GWC	-4.65	-41.40	HIP194, MET198, ARG95
TmK	1G3U	-3.13	-38.65	ASP94
PknG	2PZI	-6.71	-46.64	VAL235, SER239
FabH	1HZP	-3.93	-30.69	ARG36, GLY152
RmlD	1KC3	-6.11	-54.63	TRH601
CmaA2	**3HEM**	**-11.24**	**-50.61**	**TYR247**
LysA	1HKV	-2.57	-39.52	GLN351
Ldt(Mt2)	3VAE	-2.51	-25.56	TRP324, ASP323
Topoisomerase IV(E.coli)	3FV5	-4.13	-34.86	ARG132
Gyrase type IIA CTD(M.tb)	3UC1	-1.94	-35.28	ASP810, LEU549, VAL783
EmbC	3PTY	-3.57	-36.90	ASN740, ASP1052, ARG1055

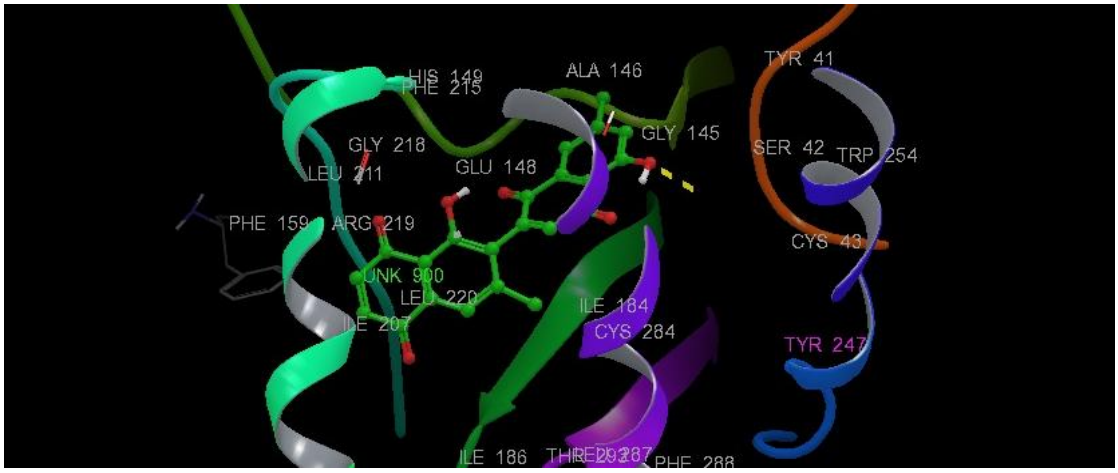


Fig-1 Cyclopropane fatty acid synthase (3HEM) WITH DIOSPYRIN (Highest Score)

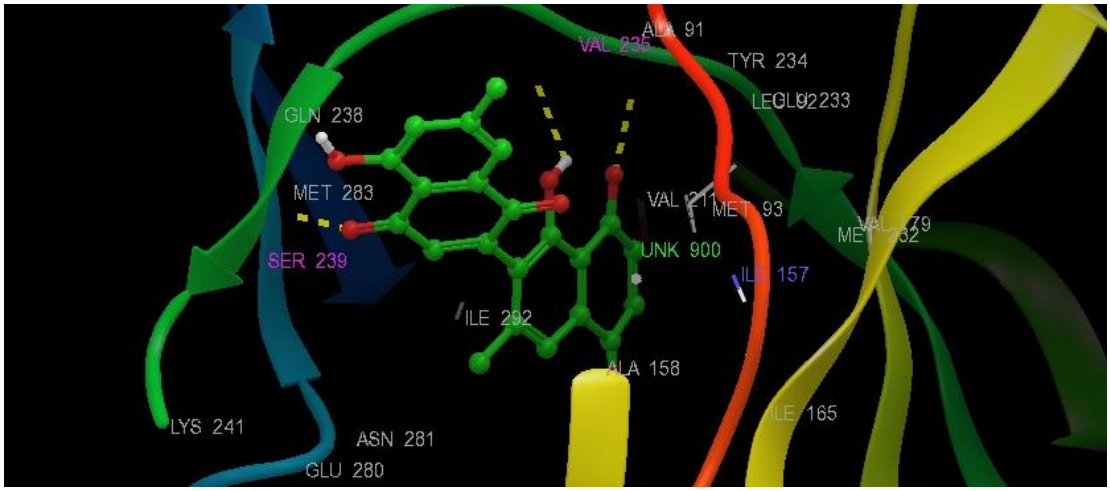


Fig-2 Serine/threonine-protein kinase (2PZI) WITH DIOSPYRIN (Moderate Score)

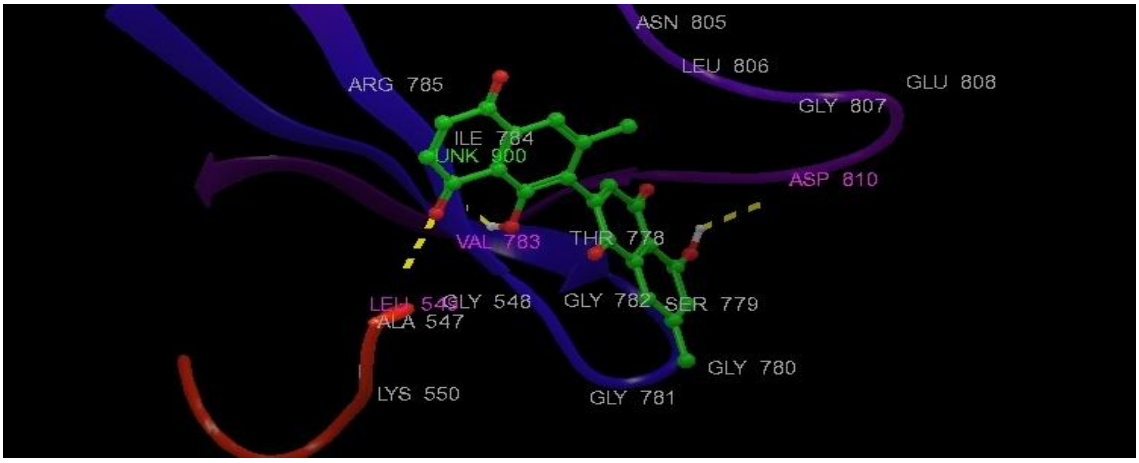


Fig-3 Gyrase type IIA topoisomerase C-terminal domain (3UC1) WITH DIOSPYRIN

RESULTS AND DISCUSSION

CmaA2- Cyclopropane fatty acid synthase, the enzyme which is essential for the cyclopropanation function in the cell wall of Mycobaterium tuberculosis showed best interaction with G-Score of -11.24 Kcal/mol as shown in Fig.1(Refer Table 2). The compound Diospyrin showed hydrogen bond interaction with the amino acid residue Tyr247, which is important for Cyclopropane fatty acid synthase inhibitor recognition via hydrogen bonding mechanisms. The results of our study showed that Diospyrin can also binds with other crucial enzymes of *Mycobacterium tuberculosis* and inhibits other mechanisms.

The results of our study supporting the fact that i*n silico* molecular docking studies are very useful in predicting the extent of interaction and binding between Diospyrin and the other target enzymes of Mycobacterium tuberculosis.

CONCLUSION

The results of our study clearly showed that Diospyrin is capable of making strong interaction with target proteins evidenced by having best Glide scores. Based on the results of our study it can be concluded that the compound Diospyrin is not only acts by inhibiting the DNA Gyrase but also with other target enzymes of Mycobacterium tuberculosis. Cyclopropane synthases of Mycobacterium tuberculosis is considered as a novel class of persistence genes and the need of new inhibitors for the persistent phase of tuberculosis infection and the absence of the cyclopropanated lipids in human results Cyclopropane synthase as an attractive target for the new drug development. However further in-vitro/in-vivo studies are needed to establish its Anti-tubercular potential against variety of Mycobacterium tuberculosis target enzymes based on the predictions of the *insilico* studies. As Diospyrin's novel mechanism of action for anti-tubercular revealed very recently, the re-engineering and repositioning of known scaffolds like will help in our future studies to find a cure for tuberculosis through in-silico docking studies.

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