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Research Article

IN SILICO DRUG DESIGNING APPROACH FOR BIOTIN PROTEIN LIGASE OF *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Tuberculosis is a most dreadful disease causing nearly 2million infection a year. Biotin protein ligase, a key enzyme, which participates in bacterial fatty acid synthesis is indespensible for the existence of *Mycobacterium tuberculosis.* Targeting biotin protein ligase of Mycobacterium will affect its fatty acid synthesis leads to death of the organism. Molecular docking study on this enzyme was undertaken using ten phyto-ligands from *Gloriosa superba.* Drug like properties of these ligand were calculated by ADME calculations. Based on the molecular docking results and ADME values Gloriosal was confirmed as a promising lead compound. The present study should therefore play a guiding role in the experimental design and development of Gloriosal as an Antimycobacterial drug.

Keywords:*Staphylococcus aureus*, Biotin protein ligase, *Gloriosa superba,* Docking, ADME

INTRODUCTION

Tuberculosis is a most dreadful disease caused by *Mycobacterium tuberculosis*. *Mycobacterium tuberculosis* is a gram positive Organism spread through persons to persons through Air. It is the second leading cause of death worldwide. Yearly 2 million of people are infected by this organism. [1] M. tuberculosis is a host pathogen which cannot survive outside the environment. This infection mainly spread through the aerosolized droplets of the infected people. It normally enters into the body through the mucosal via lungs after inhalation of infected droplets from the infected individual. This inhaled droplet will start to get multiply inside the alveolar macrophages. Primary infection of this organism takes place within the lungs. Primary infection is almost invariably asymptomatic and maintains latency. Reactivation of the latent M.tuberculosis occurs most commonly in lungs but can involve any organ [2].

TB can be cured by antibiotics in most cases but the emergence of multidrug resistance strain becomes a most serious problem in the society [3]. There are varieties of reason for the bacterial drug resistance includes slow growth, dormancy, complex cell envelope, intracellular pathogenesis and genetic homogeneity[4]. M.tuberculosis has the capacity to remain viable inside the host for a prolonged time. This makes the antibiotic to be partial effective due to the impermeable nature of the mycobacterial cell wall [5]. The toughness of this cell wall is due to its complex lipid bilayer formed by mycolic acids, glycolipids, lipoprotein etc. Fatty acids are indispensable constituents of mycolic acids that impart toughness & permeability barrier to the cell envelope. Hence there is a need to design a drug which target the protein involved in fatty acid synthesis. Biotin protein ligase is the target recently identified for the inhibition of fatty acid synthesis. The foremost step in the fatty acid synthesis is the production of malonyl-COA. Malonyl-COA is produced from the carboxylation of acetyl CoA by the catalytic action of Acetyl-CoA carboxylase. Acetyl-CoA carboxylase is a multi-subunit enzyme having three functionally dissimilar domains: biotin carboxyl carrier protein (BCCP), biotin carboxylase component, and transcarboxylase component. Active holo BCCP plays a major role in promoting fatty acid initiation and elongation. Biotin protein ligase catalyzes the transfer of biotin to BCCP. ApoBCCP is biotinylated by Biotin Protein Ligase to form holoBCCP [6]. Hence targeting this Bpl will ultimately stops the synthesis of fatty acids which leads to death of the organisms.

The search for new active phytocompounds from natural source is of great interest. Phytocompounds are produced in a biological environment, they are usually able to interact with a variety of macromolecular targets with fairly good binding affinity and selectivity, as compared to their synthetic compounds [7]. Numerous *in vitro* reports suggest the aqueous petroleum extracts and methanol extract of Gloriosa plant sp. possesses potential bactericidal activity against Gram positive and Gram negative bacteria.

Glorisa superb Linn commonly called as Glory lilly is a medicinal plant used in our traditional medicine for various ailments. It belongs to the family Colchicaceae and it is native to tropical Asia and Africa. Phytochemical analysis of this plant showed that colchicine and colchicoside are the major constituents of this plant. [8, 9]. It is the national flower of Zimbabwe and state flower of Tamilnadu [10]. Phytoligands reported from this plant were used for docking studies.

In this study we tried to design an inhibitor for the Biotin protein ligase of *Mycobacterium tuberculosis* through *In silco* studies. The binding interaction between the phytoligands and protein were studied using Autodock Vina software.

MATERIALS AND METHODS

Hardware and Software

Docking calculation was carried out on HP PC running on windows Xp as the operating system, with Intel 3 GHz core i3 and 4GB memory hardware. MGL tools was used for docking preparation and Autodock Vina was used for binding energy calculations. Virtual analysis of docking site was analyzed by Pymol.

Preparation of protein

The 3D structure of protein 3RUX was obtained from protein databank. All heteroatoms including water molecules, and definitions for symmetry were excluded from the file. Polar hydrogens were added to each protein and it was minimized by applying Kollman's partial atomic charges. Minimized structure was saved in PDBQT file format that contains a protein structure with hydrogen's in all polar residues.

Ligand Preparation

Coordinates for BS5 was extracted from PDB file 3RUX. Phytocompounds details present in *Gloriosa superba* were obtained from Dr. Duke database (http://www.arsgrin.gov/duke/). The 2D structure of these phytocompounds were searched against pubchem database and then with the help of open babel [11, 12] these 2D structures were converted to 3D structures. Ten ligands from Gloriosa plant were prepared using MGL tools by adding hydrogen atom to check the valencies of the heavy atoms. Ligands were minimized by computing gasteiger charges and saved in PDBQT

Analysis of Binding

The binding sites for docking were designed such that the entire ligand binding region was included within the GRID. Ligand binding

region of the protein was selected by using Autodock tools. The dimensions of the Grid were 50.45071, 19.03953, 20.61726. Docking analysis of Biotin protein ligase with the ligands were carried out using Autodock Vina with AMBER force field and Monte Carlo simulated annealing [13]. Throughout the docking studies the protein molecule was kept as rigid and drug molecules as flexible.

Structure Validation

In order to perform docking Native ligands present in the protein structure was removed. In order to check the conformation RMSD value was calculated between the original structure and the ligand deleted structure.

ADME Calculations

With the intention of find out the drug like properties for the phytocompounds ADME calculation was performed by using Accord for Excel's ADME add-on. Structure of the ligands was directly introduced into software by using introducing the edit chemistry module. Using function module from this software, blood brain penetration level, aqueous solubility, Cytochrome 450 binding, Hepatotoxicity, and Plasma protein Level were calculated.

RESULT AND DISCUSSION

In order to find a suitable inhibitors for bpl of *Mycobacterium tuberculosis* docking studies was performed by using Auto dock vina. Docking is a computational method attempt to predict the noncovalent interaction between macromolecule and the drug. Autodock Vina which was used for docking is a recently developed software shows more accuracy than other softwares. It uses genetic algorithm for the docking calculation. A small change in the structure of a protein can abolish binding [14]. In order to find whether deletion of native ligand causes conformational changes, RMSD calculation were carried out. The structure was valid if the root mean square deviation is less than 2·A from original structure [15]. The RMSD calculation between these two conformations was 0.00 A°. So we have concluded that deletion of ligand did not alter structure of protein. Hence this structure was more accurate to perform docking with other phyto ligands.

The more negative binding energy values corresponding to the RMSD value of zero were considered as the binding affinity value of the ligands for each docking. These values and their interaction with the protein were tabulated. (table 2)

Table 1: ADME results of the phytocompounds

Ligands	Aqueous solubility	Blood brain Penetration	CYP456	HEPATATOXICITY	Plasma proteinlevel
Benzoic acid	4	2	0	0	2
Choline	4	4	0	0	0
Salicyclic acid	4	3	0	1	0
Colchicine	3	3	0	1	2
Chrysophanic acid	3	2	0	1	2
2-demethylcolchicine	3	3	0	1	0
Sitosterol	0	4	0	1	2
3-desmethylcolchicine	3	3	0	1	2
Stigmasterol	1	4	0	1	2
Glorisol	4	4	0	0	2

Phytocompounds Sitosterol and Stigmasterol showed more negative binding energy value compared to other ligands. Stigmasterol and sitosterol are steroids present in most plants. (16) They both have more or less similar structure but they only differ in the presence of C22=C23 double bond in Stigmasterol and C22-C23 single bond in Sitosterol (17). It showed one hydrogen bond interaction with the amino acid Alanine 209 (FIG 1).

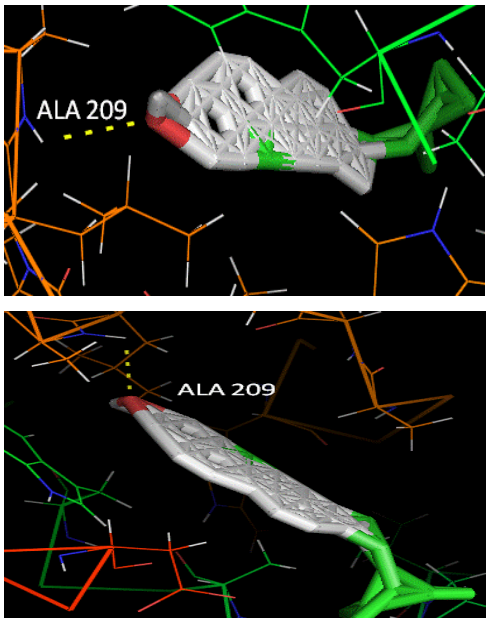

Fig 1: Hydrogen bond interaction in Sitosterol & Stigmasterol

The second highest score was shown by chrysophanic acid with one hydrogen bond interaction with trp 201 (FIG 2). Glorisal, the key component of this plant also shows good binding score with more number of hydrogen bond interactions. It shows 8 eight bond interactions with the protein (FIG 3). Compound like Salicyclic acid, Choline, and Benzoic acid showed low binding score and showed hydrogen bond interaction with ALA 209, ASN 205, GLN 200 (FIG 4, 5, 6).

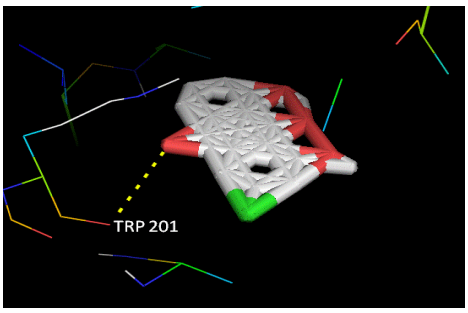

Fig 2: Hydrogen bond interaction in Chrysophanic acid

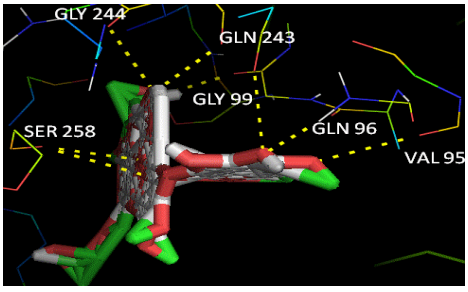

Fig 3: Hydrogen bond interaction in Gloriosal

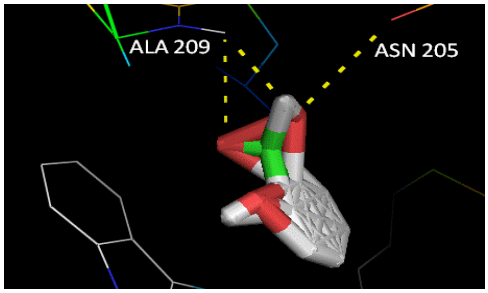

Fig 4: Hydrogen bond interaction in Salicyclic acid

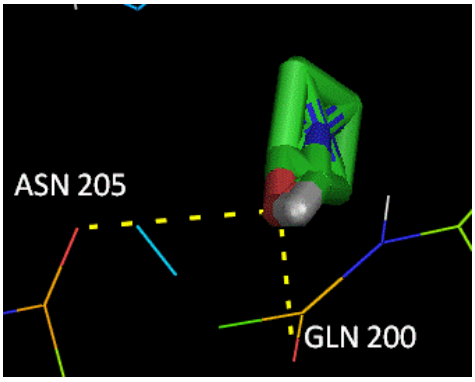

Fig 5: Hydrogen bond interaction in choline

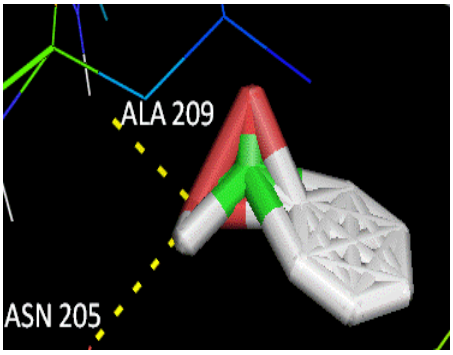

Fig 6: Hydrogen bond interaction in Benzoic acid

Compounds such as 2- Demethylcolchicine, Colchicine, 3-Demethylcolchicine showed good binding score but it showed no Hydrogen bond interaction with target protein. Hence these compounds can't be an inhibitor of BPL.

All the phytocompounds were evaluated for their druglike behavior through analysis of pharmacokinetic parameters required for absorption, distribution, metabolism, excretion, and toxicity (ADMET) by using Accord Excel. ADME prediction is the most crucial step in drug discovery. Drugs which satisfy these properties only will survive in the Phase 1 clinical trial (18). All the calculated ADME parameters of phytocompounds were tabulated in table 1.

Table 2: Docking result of Phytocompounds

Compound Name	Binding Energy	H Bond	Amino Acid	Atom in amino acid	Atom in Ligand
2-Demethylcolchicine	-9.1	Nil	-	-	-
3-DESMETHYLCOLCHICINE	-8.8	Nil	-	-	-
			ALA 209	HN	O
BENZOIC_ACID	-5.8	2	ASN 205	O	H
			GLN 200	O	O
CHOLINE	-3.4	2	ASN 205	O	
CHRYSOPHANIC-ACID	-9.3	1	TRP 201	O	O
COLCHICINE	-8.8	Nil	-	-	-
			GLY 244	O	O
			SER 258	O	O
			GLN 243	OE	O
			GLN 96	HE	O
			GLY 99	O	H
GLORISOL	-8.3	8	VAL 95	O	O
			ALA 209	HN	O
SALICYLIC-ACID	-5.8	3	ASN 205	O	O
SITOSTEROL	-9.6	1	ALA 209	HN	O
STIGMASTEROL	-9.6	1	ALA 209	HN	O

Oral administration is the most common route of drug administration. Most drugs in marketplace are administered via the oral and it is one of the convenient and cost effective routes of administration [19, 20].

Low and good solubility is detrimental to good and complete oral absorption, and so the early measurement of this property was great importance in drug discovery [21]. Aqueous solubility was used to predict the solubility of compound in water at 25·C and it has eight different levels from 0-6. Aqueous solubility levels ranging from 0-2 indicates low solubility, level 3 indicates good solubility, level 4 indicates optimal solubility and level 5 indicates high solubility [22].Therefore phytocompounds Glorisol, Choline, Benzoic acid, Salicyclic acid, shows good solubility range compared with other compound. Hence these compound can be administered as a oral drug

The blood-brain barrier (BBB) is a complex cellular system helps to maintain the homeostasis of the central nervous system (CNS) by separating the brain from the systemic blood circulation. Drugs acting at central nervous system should have the capacity to cross these barriers whereas other drugs crossing these barriers will cause unwanted side effects. [23]. The level 0&1 shows high penetration, level 2 shows medium penetration, level 3 shows low penetration and level 4 shows undefined penetration level. Blood - brain barrier penetration levels of the phytocompounds were predicted in order to find out whether these compounds are having their activity in the CNS. Benzoic acid and chrysophanic acid shows medium penetration. So these drugs have the possibility to cross the blood brain barrier. The other compounds like Salicyclic acid, Colchicine, 2-demethylcolchicine, 3-desmethylcolchicine have low penetration level. Choline, Sitosterol, Stigmasterol, Glorisol has undefined penetration level to cross blood–brain barrier. Hence for these compounds the chances of CNS side affect are low or absent.

Cytochrome 450 are the enzymes that catalyze the oxidation of organic substance. These are the major enzymes involved in drug metabolism [24]. Most of the drugs undergo metabolism via the cytochrome P450(CYP) enzymes [25]. Cytochrome 450(CYP450) predicts CYP2D6 enzyme inhibition using 2D chemical structure and it has 2 levels namely 0 for non-inhibitor and 1 for inhibitor. [26] CYPs often have distinct roles in xenobiotic metabolism with active sites that enable broad and overlapping substrate specificity [27]. All the 10 phytocompounds has falls in level 0 and these phytocompounds were non-inhibitor and unfavourable to inhibit CYP2D6 enzyme.

Drugs continue to be pulled from the market with disturbing regularity because of late discovery of hepatotoxicity. The liver synthesizes, concentrates, and secretes bile acids and excretes other toxicants, such as bilirubin. Drug-induced injury to hepatocytes and bile duct cells can lead to cholestasis. Cholestasis, in turn, causes intrahepatic accumulation of toxic bile acids and excretion products, which promotes further hepatic injury. [28]. Hepatotoxicity predicts potential organ toxicity for a wide range of structurally diverse compounds and it has 2 levels namely 0 for non-toxic and 1 for toxic [29]. The phytocompounds Benzoic acid, choline, and Gloriosal have non-toxic effect and unfavourable to cause Liver injury. Other compounds such as Salicyclic acid, Colchicine, Chrysophanic acid, 2-demethylcolchicine, Sitosterol, 3-desmethylcolchicine, Stigmasterol have toxic effect and favourable to cause dose-dependent liver injuries.

Plasma binding protein helps to identify the binding of the inhibitors to the carrier protein in the blood. It is generally assume that only free drug can cross membrane and bind to the intended molecular target therefore it is important to find the plasma protein binding [30, 31]. Drug can cross membranes and bind to the intended molecular target, and it is therefore important to estimate the fraction of drug bound to plasma proteins.

Plasma protein has three levels of binding capacity namely level 0 has <90%, level 1 has >=90% and level 2 has >=95%. The phytocompound Benzoic acid, Colchicine, Chrysophanic acid, Sitosterol, 3-desmethylcolchicine, Stigmasterol, Gloriosal have binding capacity of >=95% to cross the membrane and bound to plasma protein. While the other phytocompounds Choline, Salicyclic acid, 2-demethylcolchicine have binding capacity of <90% to cross the membrane and bound to the plasma protein.

CONCLUSION

Gloriosa superba linn is the most common plant used in traditional medicine for various ailments. The chemical constituents of this plant were analyzed for its antimycobacterial action through docking studies. Our studies conclusively revealed Gloriosal as a potent lead compound better than other ligands based on best values of docking energy and HB interactions. This compounds also satisfies ADME properties. Hence further analysis through invitro and invivo studies will prove the action of this compound. Hence we concluded that compound Gloriosal from this plant render antimicrobial action against *Mycobacterium tuberculosis*, and it may be used as drug through future studies.

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