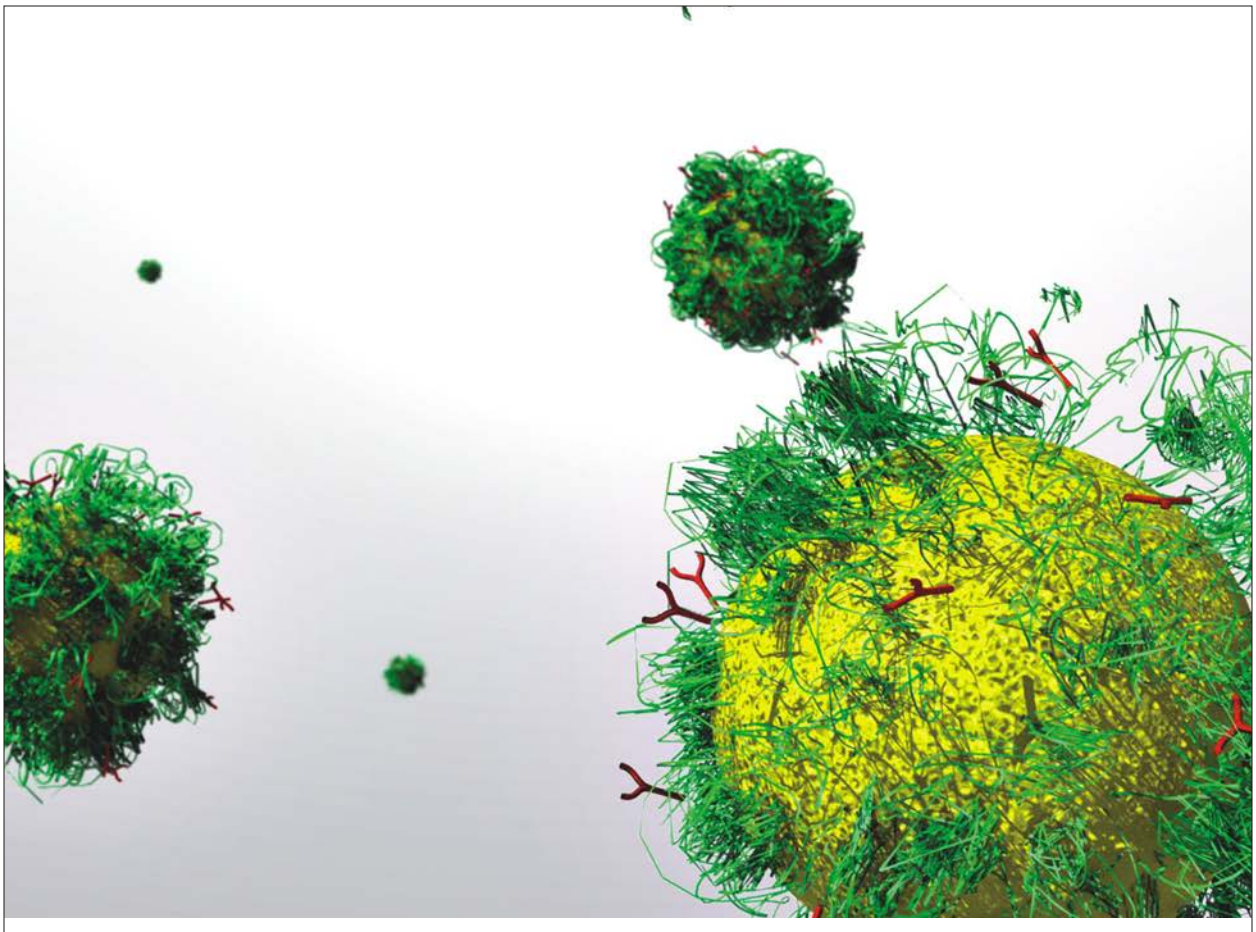

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Interaction of 2, 5-di-tert-butyl-1, 4-Benzoquinone with Selected Antibacterial Drug Target Enzymes by *In silico* Molecular Docking Studies

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ABSTRACT

Currently the criteria used for selecting optimal new antibacterial drug candidates include inhibitors of nucleic acid synthesis, fatty acid biosynthesis and folic acid pathway. The aim of the present study was to study the interaction of the isolated anti-bacterial compound, 2, 5-Di-tert-butyl-1, 4-benzoquinone (DTBBQ) from *Streptomyces* sp. VITVSK1 with 4 selected antibacterial drug target enzymes by *in silico* molecular docking approach. The compound DTBBQ showed minimum binding energy of -3.91 kcal mol^{-1} with Topoisomerase II, with Topoisomerase IV-3.24 kcal mol^{-1} with, Enoyl ACP reductase -4.51 kcal mol^{-1} and 5.82 kcal mol^{-1} with Dihydrofolate reductase protein. The compound DTBBQ interacted with several amino acid residues, of which lysine was found to be common among all the target enzymes. The results of our study suggest that DTBBQ could be used for antibacterial activity by targeting bacterial proteins of drug resistant strains.

Key words: 2, 5-di-tert-butyl-1, 4-benzoquinone, topoisomerase II, topoisomerase IV, enoyl ACP reductase, dihydrofolate reductase

INTRODUCTION

There is an increasing incidence of bacterial infections both in developing and developed countries (Keane *et al*., 2001). Diseases commonly result from exposure to gram positive bacteria such as *Bacillus* species and gram negative bacteria like *Helicobacter pylori* (Bakheet and Doig, 2010). Organisms belonging to the genus *Bacillus* cause some serious diseases that include Endocarditis, bacteremia, pneumonia, visceral abscess and tissue necrosis (Sliman *et al*., 1987). *Helicobacter pylori* is responsible for causing gastric lymphomas, small intestine diseases and cancer (Parsonnet, 1995). Currently there is a serious concern due to the increase in resistant bacteria worldwide and considerable decrease in the development of newer drugs that has serious health and economic issues (Spellberg *et al*., 2004). The antibacterial agents that are currently available for treating bacterial infections include penicillins, α-lactam-inhibitors, aminoglycosides, oxazolidinones, fluoroquinolones, macrolides, and tetracyclines (Stevens *et al*., 2005). Bacterial pathogens develop resistance to existing drugs; due to prolonged use of antibiotics, therefore it is essential to identify and to develop an effective antibiotic therapy. One of the ways in which this can be dealt by identifying bacterial proteins which can serve as targets for new classes of antibiotics (Sakharkar *et al*., 2008). Certain class of antibacterial agents block DNA synthesis by

inhibiting DNA gyrase and topoisomerase IV, that are responsible for super coiling and required for replication (Maxwell, 1997). There are other criteria for selection of new drug candidates that act as inhibitors for fatty acid synthesizing enzymes such as enoyl ACP reductase (Moir, 2005) and some block the metabolism of bacteria by inhibiting the enzymes needed for the synthesis of folic acid like dihydrofolate reductase (Zuccotto *et al.*, 1998). The use of computational tools, simulation methods and *in silico* pharmacology tools would be useful for predictions and this would increase the overall market of pharmaceutical industry (Ortega *et al.*, 2012). Virtual screening methods have been reported to be very effective for the discovery of new inhibitors and drug molecules for the advancement of therapeutics (Cosconati *et al.*, 2010).

The present study evaluated the interaction of the pigmented compound 2, 5-di-tert-butyl-1, 4-benzoquinone extracted from *Streptomyces* sp.VITVSK1 with selected antibacterial drug target enzymes.

MATERIALS AND METHODS

Ligand: *Streptomyces* sp., VITVSK1 was isolated from the Cheyyur salt pan region, Tamil Nadu. India. The antibacterial compound was extracted from VITVSK1 was characterized as 2, 5-di-tert-butyl-1, 4-benzoquinone (DTBBQ) by means of spectral analysis such as UV, FT-IR, ^{1}H and ^{13}C NMR and GCMS (Gopal *et al.*, 2013). The structure of the DTBBQ compound was generated using chemdraw ultra 10 version software. The 3D structure of DTBBQ was used as ligand for the docking study.

Target enzymes: Topoisiomerase II, Topoisomerase IV, Enoyl ACP reductase and Dihydrofolate reductase were chosen as drug targets. The structure of the target enzymes were retrieved from the protein data bank (PDB ID-1AB4, 2N0V, 1D8A and 3JW5). The A chain of above proteins was used as receptor. The water molecules, HETATM and ANISOU sequences were removed prior to docking.

Active site prediction: The active site of the enzyme was predicted using CASTp (Computer Atlas of Surface Topography of proteins) server (http://sts.bioengr.uic.edu/castp/calculation.php). This tool was used to identify functional site residues involved for ligand binding.

Molecular docking: The possible docking modes between the ligand (DTBBQ) and the bacterial drug target enzyme were studied using Autodock 4.0 suite molecular-docking tool (Morris *et al.*, 2009). The docking tool requires ligand and the receptor as input in PDB format. The antibacterial compound DTBBQ was manually docked into functional sites of the target proteins individually. Each docking experiment consisted of 10 docking runs with population size 150 and 25,000 energy evaluations. Autogrid was run by using the grid box and followed by Autodock in which the output was generated by using Lamarckian genetic algorithm. The Autodock results indicates the binding position and bound conformation of the ligand-receptor complex, as well as a rough estimate of its interaction. The docked conformation which had the minimum binding energy and close to the active site region was selected to analyze the mode of binding. All the docking runs were performed in Intel Pentium® D CPU at 3.20 GHz of Lenovo desktop. The molecular docking simulation was run under Linux operating system.

RESULTS

Docking of DTBBQ (ligand) with the active site of bacterial drug target enzymes resulted in 10 docked confirmations. The docking results are provided in Table 1. The 3D structure of the

Table 1: Binding pattern of DTBBQ with target bacterial proteins

Bacterial target enzyme	PDB ID	Binding energy (kcal mol^{-1})	Binding pocket	Hydrogen bond acceptor
Topoisomerase II	1AB4	-3.91	60th	LYS 129
Topoisomerase IV	2N0V	-3.24	70th	GLU 125
Enoyl ACP reductase	1D8A	-4.51	34th	LYS163
Dihydrofolate reductase	3JW5	-5.82	16th	ALA8

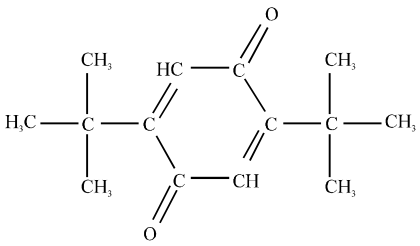

Fig. 1: Structure of 2,5-di-tert-butyl-1, 4-benzoquinone modelled using chemdraw ultra 10 software

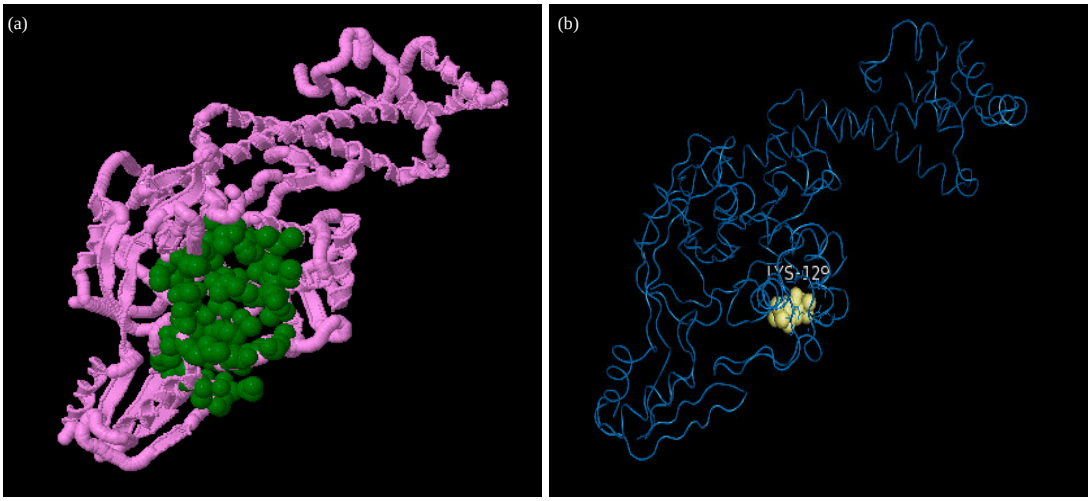

Fig. 2(a-b): *In silico* binding of DTBBQ with 1AB4 protein, (a) Binding pocket 60th generated using CastP and (b) Hydrogen bond interaction involving LYS 129 residue

ligand obtained using chemdraw software is given in Fig. 1. Different enzymes were selected on the basis of their mode of action. DTBBQ showed binding energy of -3.91 kcal mol^{-1} with Topoisomerase II (PDB ID: 1AB4) and -3.24 kcal mol^{-1} with Topoisomerase IV (PDB ID: 2NOV). Target receptor proteins and the binding pocket residues chosen were 60th for Topo II (Fig. 2a) and 70th for Topo IV (Fig. 3a). The ligand showed hydrogen bond formation with LYS129 residue of Topo II (Fig. 2b) and GLU125 residue of Topo IV enzymes (Fig. 3b). Enoyl ACP reductase (PDB ID: 1D8A) showed binding energy of -4.51 kcal mol^{-1} and maximum interaction with the enzyme responsible for inhibiting the fatty acid biosynthesis pathway in bacteria leading to cell death. The compound DTBBQ interacted with the active site residue LYS163 (Fig. 4a) within 34th functional pocket of the target protein as predicted by CastP software (Fig. 4b). The

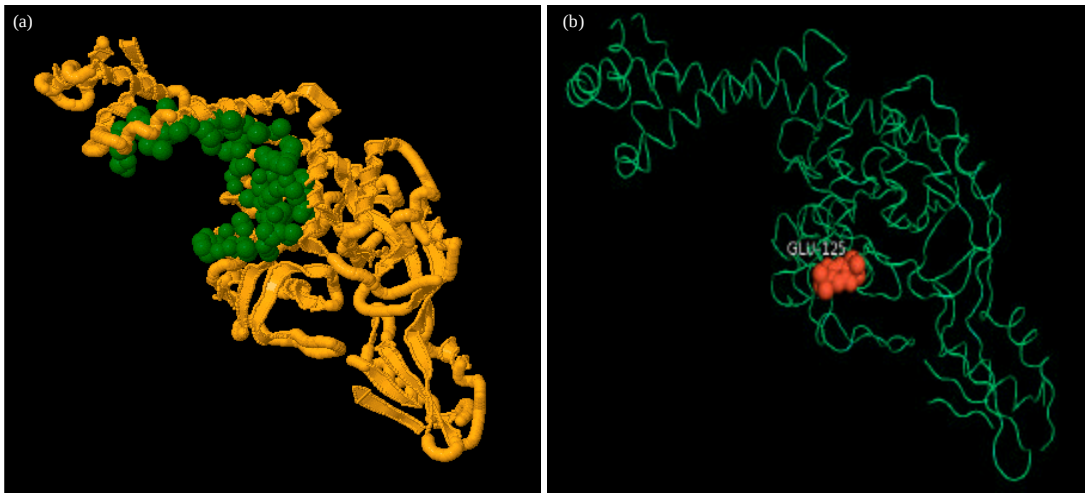

Fig. 3(a-b): Functinal site prediction and *in silico* binding of DTBBQ with 2NOV protein (a) Binding pocket 70th generated using CastP and (b) Hydrogen bond interaction involving GLU 125 residue

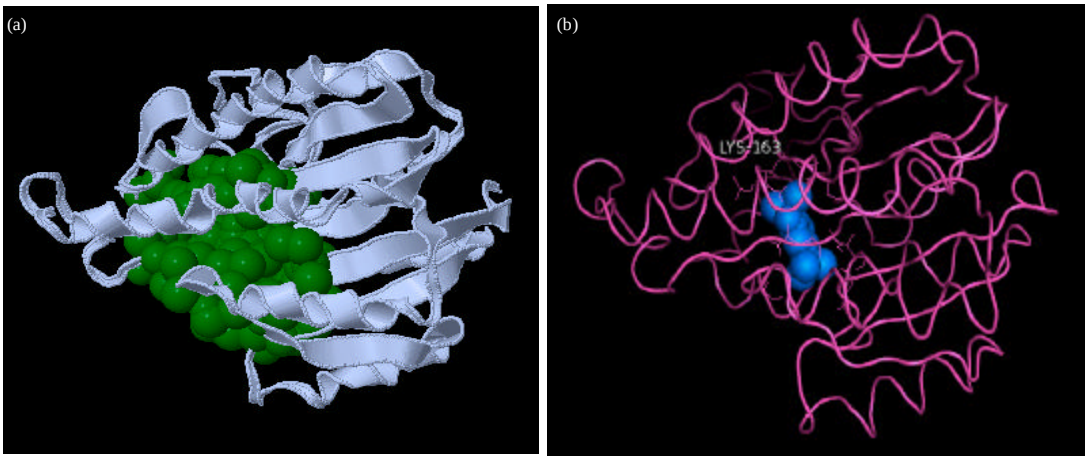

Fig. 4(a-b): *In silico* binding of DTBBQ with 1D8A protein (a) Binding pocket 34th generated using CastP and (b) Hydrogen bond interaction involving LYS 163 residue

Dihydrofolate reductase (DHFR) protein also showed interaction with ligand and formed hydrogen bond involving ALA8 residue (Fig. 5a) in the 16th functional pocket as predicted by CastP analysis (Fig. 5b). The results of docking study showed that the active sites of enzymes can hold DTBBQ within or close to the active region, adopting a variety of binding modes and interactions.

DISCUSSION

Marine *Streptomyces* produce wide range of secondary metabolites that include bioactive compounds and antibiotics. Microbes from extreme environments mainly actinomycetes had gained

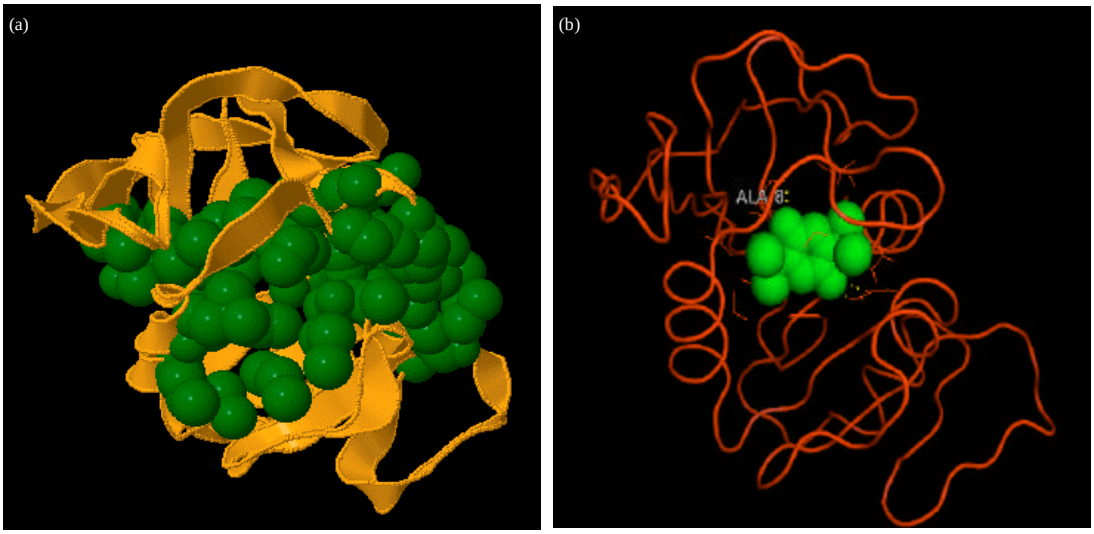

Fig. 5(a-b): *In silico* binding of DTBBQ with 3JW5 protein, (a) Binding pocket 16th generated using CastP and (b) Hydrogen bond interaction involving ALA 8 residue

significant attention in recent years because of biological applications and also for the production of novel bioactive compounds that are of clinical significance (Stackebrandt *et al*., 1997). *In silico* tools are essential to determine the various properties of the compound necessary to be a lead compound. Most of the existing bacterial drugs are enzyme inhibitors which bind to the enzymes and inhibit the activity. The results of this study highlights the significance of using computational and molecular simulation studies to access the binding efficiency of DTBBQ with the antibacterial drug target proteins. De-oxyribo nucleic acid gyrase is a type II DNA topoisomerase from bacteria that introduces supercoils into DNA. Topoisomerases help in unwinding the DNA during replication, blocking these enzymes would inhibit nucleic acid synthesis. Dihydrofolate reductase is involved in folic acid synthesis pathway essential for the bacterial growth and survival. Inhibiting DHFR would block folic acid synthesis pathway and thereby suppress bacterial survival. Quinolone antibiotics bind in and around the active site residue TYR 122 and catalyses the breakage of a DNA duplex (Cabral *et al*., 1997). A similar binding pattern mechanism occurs in topoisomerase IV of *S. pneumoniae* (Laponogov *et al*., 2007). Triclosan is the known antibacterial antibiotic and blocks lipid biosynthesis by specifically inhibiting the enzyme enoyl-acyl carrier protein reductase (Levy *et al*., 1999). The binding pattern of triclosan complexed with Enoyl ACP protein had closely related interactions with DTBBQ. All those enzymes chosen for this study would serve as potential target for antibacterial study.

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