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MOLECULAR DOCKING STUDIES OF POTENTIAL CHEMICAL INHIBITORS ON MULTI-DRUG RESISTANCE GENES IN *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Resistance to antimicrobial agents among bacteria and fungi is a persistent problem complicating the management of critically ill patients. To understand the issues involved in resistance in critical care, it is essential to understand the epidemiology and mechanisms of resistance. This present *Insilico* research aims at finding out the potential multi drug resistance protein target present in *Mycobacterium tuberculosis*. In this work, we performed a complete molecular modeling and visualization of the Multidrug Resistance Gene Coded Proteins, *emrB* (MTB). Drug designing and development involves ligand selection, protein target identification, protein modeling and molecular docking studies by using online software like Marvin sketch, Microbial genome database, CPH 3.0 model server, Patch dock and Discovery studio. Then the designed chemical molecules were introduced and validated using advanced Cheminformatics software and tools. Finally, the docking studies were performed on the designed chemical compounds with the pathogen target of *Mycobacterium tuberculosis* (emrB) based on the drug – protein docking score value. Among the 80 designed chemical compounds, only eighteen compounds docked with emrB had high docking score. The docking results clearly show that the designed chemical compounds act as potential chemical inhibitors with the pathogen targets of *Mycobacterium tuberculosis* (emrB) based on the drug – protein docking score value. The drug with emrB had high docking score. These chemical agents are potential inhibitors for Human pathogens. These chemicals are eligible candidates for the chemical compound synthesis in Drug designing and development studies. So finally we conclude that the compounds act as excellent therapeutic agents against the human pathogen, *Mycobacterium tuberculosis* compared to existing drugs like Isoniazid and Pyrazinamide.

KEY WORDS: Marvin Sketch, *Mycobacterium tuberculosis*, docking studies, Patch dock, Discovery studio.

INTRODUCTION

Tuberculosis (TB) is a bacterial infectious disease caused by the obligate human pathogen, Mycobacterium tuberculosis. Multidrug-resistant tuberculosis (MDR-TB) is a global reality that threatens tuberculosis control. Tuberculosis caused 1.3 million deaths among HIV-negative people and 0.38 million deaths among HIV-positive people in 2009. An estimated 250,000 TB patients were notified in 2009, to have multi-drug resistant TB (MDR-TB) [1]. In 2010, there were 8.8 million new cases of TB diagnosed, and 1.45 million deaths, most of these occurring in developing countries TB, caused by the genus *Mycobacterium tuberculosis*, is a stubborn and lethal infectious disease [2, 3]. It spreads widely by airborne microscopic droplets from people in active TB phase when they are coughing, sneezing or talking. The main targeted organ of tuberculosis is lung, but it can also damage many other parts of the body like bones, lymph node, joints, central nervous system and genitourinary. The appearance

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of tuberculosis can be traced back to more than 5000 years ago. The battle between human beings and TB has also been around for a long time. During the next decades, chemotherapy, general public health improvement, and the use of BCG vaccine greatly controlled the spread of TB. However, the emergence of drug-resistant TB and HIV co-infected TB announced the new round TB outbreaks. *M. tuberculosis* makes a lethal combination with HIV, both catalyzing the progression of AIDS and TB respectively. Since the WHO launched the surveillance project to monitor the anti-tuberculosis drug resistance trends in 1994, the multi-drug resistant TB (MDR-TB) cases have been reported in every country and the extensively drug-resistant TB (XDR-TB) cases have been found in more than 50 counties. Because of the ineffectiveness of the standard six-month treatment, the treatment for drug resistant TB is an inefficient, time-consuming and expensive process. WHO recommended the minimum length of treatment for drug-resistant TB is 20 months and the treatment cost for a MDR-TB patient is 10 times higher than that for a drug-susceptible TB patient [4]. Human immunodeficiency virus (HIV), a lentivirus, is able to cause the progressive failure to the carrier's immune system. The rapid growth of HIV infection is accelerating the emergence and spread of drug-resistant TB. Meanwhile MDR-TB and XDR-TB lead to a much higher mortality rates among TB/HIV confected patients. India is the highest TB burden country with World Health Organization (WHO) statistics for 2011 giving an estimated incidence of 2.2 million cases of TB for India out of a global incidence of 8.7 million cases. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB. While more and more countries, research institutions and health organizations are focusing on both global and their local TB situation, the progress of TB treatment is moving slowly. A breakthrough in TB treatment is always desired and essential. There is hence an urgent need for novel therapeutics for combating these diseases. The genome of *M. tuberculosis* codes for about 4000 proteins and identification of an ideal target is the most critical task in the process of drug discovery [5].

MATERIALS AND METHODS

1) In the present research work, the multi-drug resistant gene sequences of emrB were retrieved using Microbial genome database in order to perform protein modeling studies. MBGD is a database for comparative analysis of completely sequenced microbial genomes, the number of which is now growing rapidly. 2) The tertiary structure prediction was done using an automated protein modeling server called CPH 3.07. The target protein sequence was converted into 3D structure based on this server. After modeling, we view the structure with the help of discovery Studio Software, Molegro Molecular Viewer and Molsoft. 3) The next step is Chemical Compound Selection. The NCBI PUBCHEM chemical compound was used to select the chemical compound. 4) Following this step is Drug designing. Drug designing and validation was done using Molinspiration [6] and Chemaxon [7] in order to identify the molecular properties of the drug and perform combination studies. 5) The final step is Molecular drug docking. Molecular drug docking was done using an automated drug docking server called Patchdock server.

Protein Sequence Analysis

The amino acid sequence of emrB in FASTA format was retrieved from Microbial genome database. The fasta sequence converted to 3D structure by Cph server. A typical amino acid structure file consists of heavy atoms, water molecules, cofactors, metal ions and it can be polymeric form. The structure generally has no information about bond orders, topologies, or formal atomic charges. So, the raw PDB structure should be prepared in a suitable manner for docking studies. The Protein Preparation Wizard was used to prepare the protein. During protein preparation, water molecules and peptide substrate (NAG) were deleted.

emrB Enzyme 3D-Structure Prediction (Homology Modeling)

As the 3D structure for *Mycobacterium tuberculosis* H37Rv emrB [8] protein is not available in structural databases, its 3D structure was generated using the Swiss-Pdb Viewer 4.0 homology modeling software. *Mycobacterium* emrB amino acid sequence was loaded in Swiss PDB Viewer and the predicted 3D structure of *Mycobacterium* emrB was obtained.

Preparation of quinazoline derivatives

Afatinib, Balaglitazone, Dacomitiniband Cediranib (about 25 clinically used drugs are quinazoline derivatives) have been reported to have a possible effect on various proteins. Quinazoline derivative drugs have already been demonstrated to have anti-fungal activity [9] and anti-tubercular activity [10, 11]. Structural analogs of eighty quinazoline derivative molecules were designed. All these molecules were screened *in silico* for their inhibitory activity against H37Rv. Hence only side chain modifications were performed to prepare the library of ligands for docking with the receptor emrB enzyme.

Automated Molecular Docking

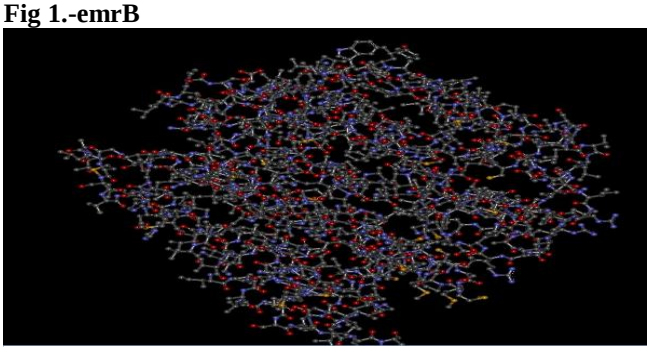
The ligand-receptor interactions were studied for Quinazoline derivatives and emrB enzyme using Patch dock software. Receptor emrB was docked with the standard drugs like Isoniazid and Pyrazinamide structure and then with the library of Quinazoline derivatives. Default parameters were used for the docking process and Energy values (E values) of each docking were obtained.

RESULTS

The aim of MBGD is to facilitate comparative genomics from various points of view such as Ortholog identification, Paralog clustering, motif analysis and gene order comparison.

The protein modeling studies were done using an advanced automated modeling server called PHYRE. The sequence of emrB was applied into PHYRE server. After modeling, the structure was viewed with the help of Molecular visualization tools such as Discovery studio software.

Fig 1.-emrB



Drug designing studies were done using CHEMAXON software in order to validate the chemical structure and the drug likeness properties. The structures of 80 chemical compounds were predicted using Chemaxon software in order to perform molecular docking studies.

Chemaxon is a software company specializing in application programming interfaces and end user applications for cheminformatics and life science research with headquarters in Budapest, Hungary and Cambridge.
The company's main customer base consists of pharmaceutical, agrochemical and biotechnology companies, as well as academic research groups and third parties wishing to integrate cheminformatic functionalities in their products and services.

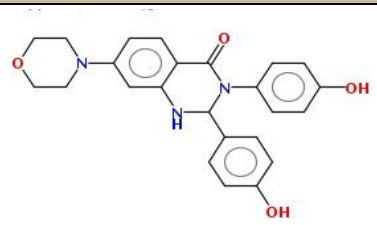
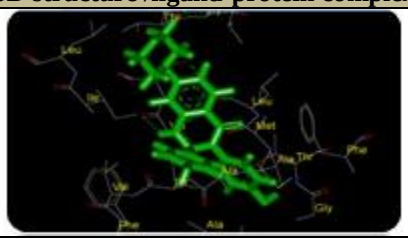
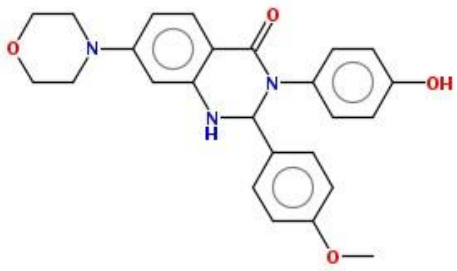
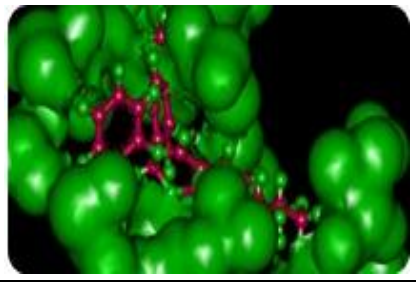
Molecular docking studies were done using an automated drug docking server called Patch dock. In these docking studies, the results are validated based on the binding energy value. Best negative values indicate more affinity between the ligand and receptor. After docking, the results were viewed with the help of molecular visualization tools such as Discovery studio software. Out of eighty compounds, eighteen compounds showed best docking results are shown in table 1.

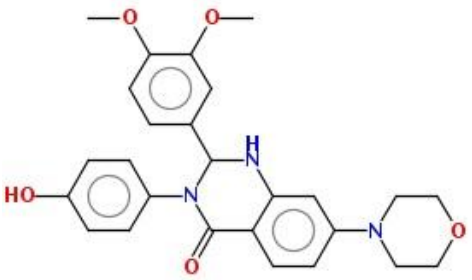
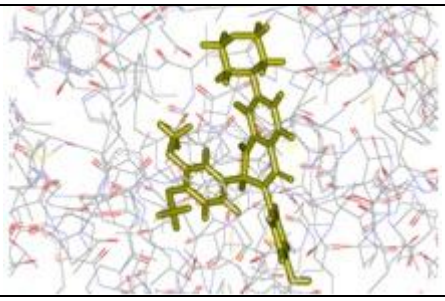
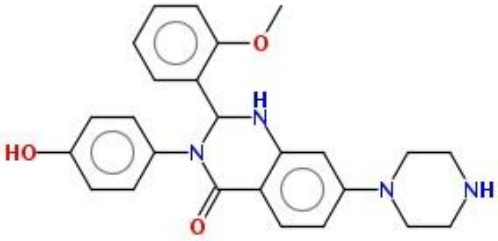
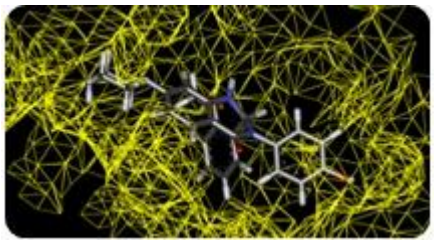
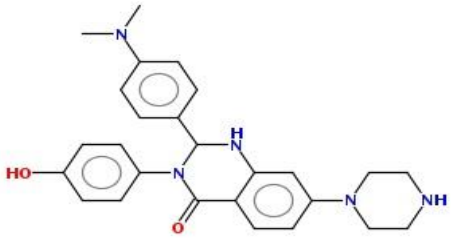
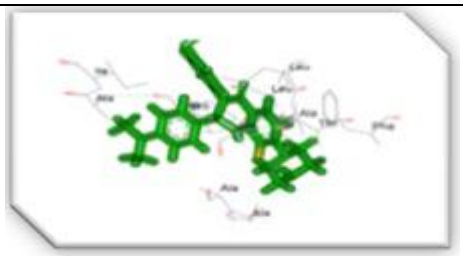
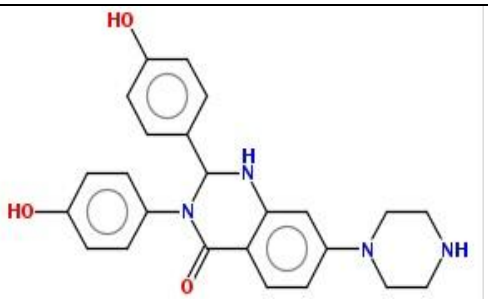
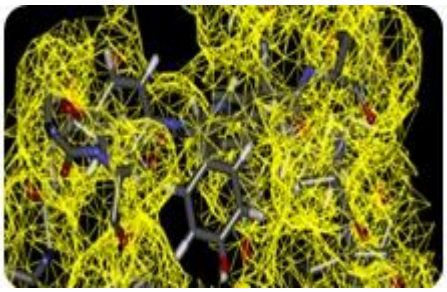
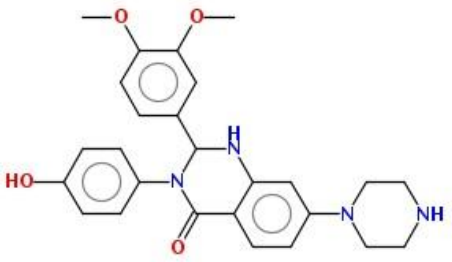
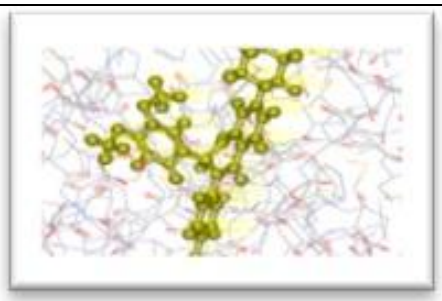
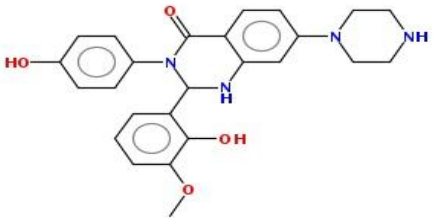
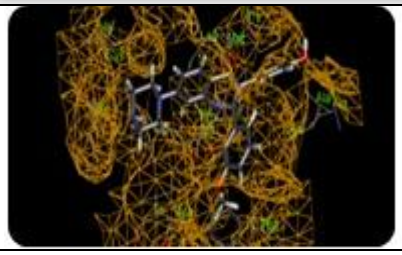
DISCUSSION AND CONCLUSION

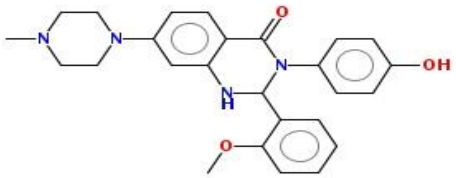
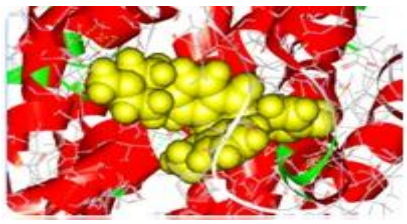
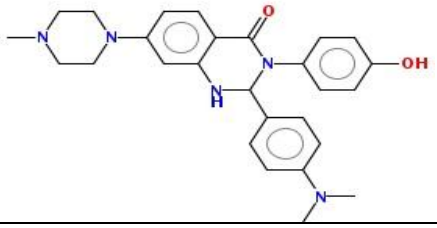
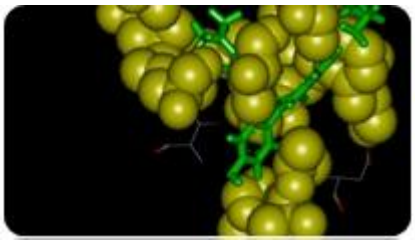
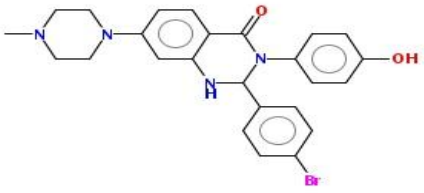
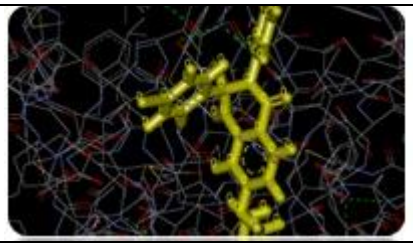
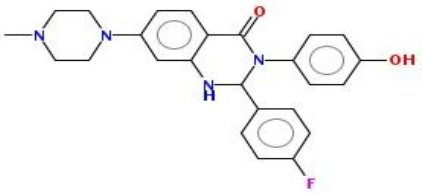
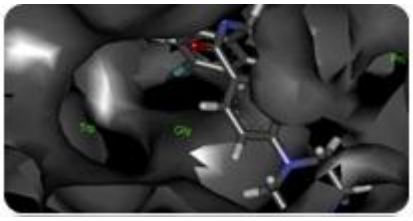
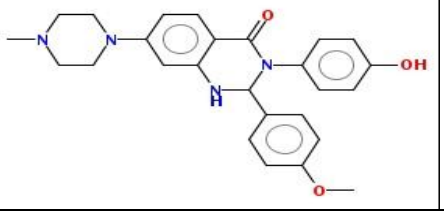
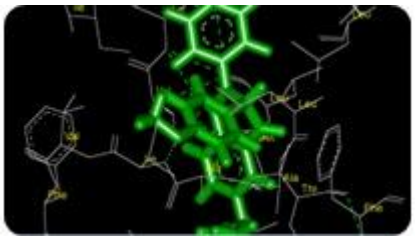
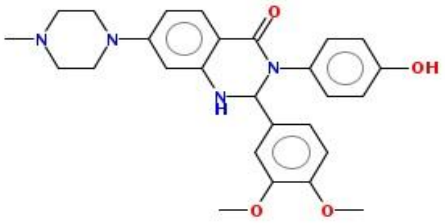
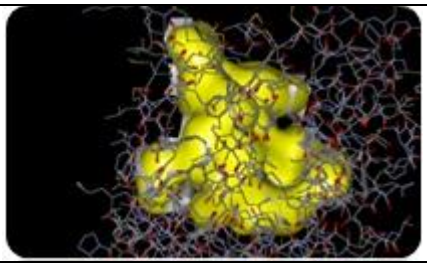
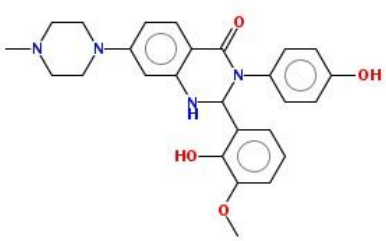
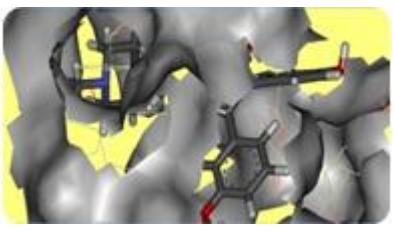
This research study clearly shows the potential targets of Human pathogens. The identified protein target play a key role in structure based drug designing. Hence, they act as best candidates for drug docking studies. The selected chemical molecules (80 molecules) were docked individually with multi drug resistant gene responsible protein (emrB) in Mycobacterium.

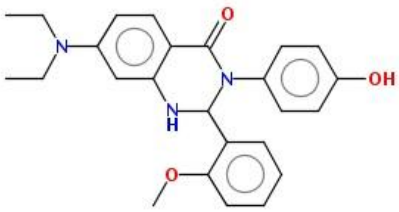
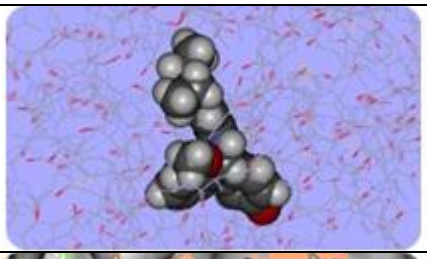
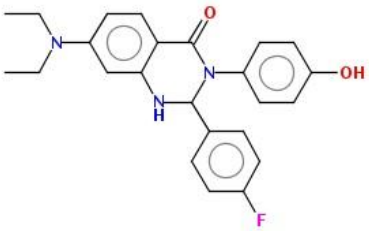
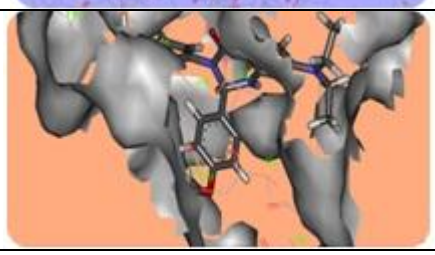
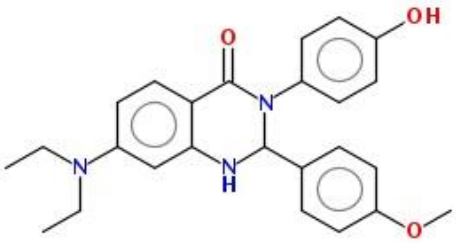
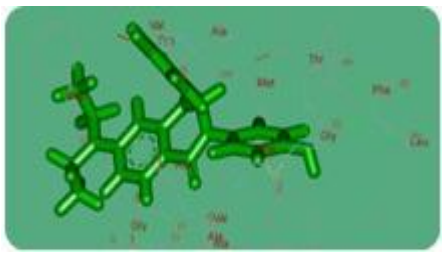
The docking results will be validated based on the binding values between the drug and the receptor. Among the 80 designed chemical compounds, 18 compounds showed good activity and obeyed Lipinski's rule of five. From the above 18 compound 2, 3, 8, 9 and 13 showed higher binding energy values with emrB. These chemical agents are potential inhibitors for Human pathogens. These five chemicals are eligible candidates for chemical compound synthesis in Drug designing and development studies. So finally, we conclude that the five compounds are excellent therapeutic agents against human pathogen, *Mycobacterium tuberculosis*.

Table 1. Molecular docking results

Compounds	Molecular formula /2D structure	3D structure /ligand-protein complex	Binding values
1.			-494.28
2.			-503.65

3.			-528.42
4.			-487.72
5.			-464.47
6.			-466.67
7.			-462.33
8.			-525.56

9.			-515.62
10.			-470.05
11.			-466.73
12.			-450.38
13.			-512.62
14.			-476.58
15.			-455.66

16.			-450.88
17.			-453.12
18.			-475.10

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