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Molecular Docking Simulation of Small Diverse Chemical Molecules Based Virtual Screening for Treatment of Tuberculosis

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Molecular docking simulation based virtual screening with ligand library having benzotriazole derivatives have been performed to identify possible lead molecules to inhibit cytochrome P450 14α-sterol demethylase (CYP51) enzyme for treatment of tuberculor infection. Further these virtually screened lead molecules having best binding energy are tested for absorption, distribution, metabolism, elimination and toxicity (ADMET) profiling for design of novel lead inhibitors for treatment of tuberculosis infection by using phenomenon of bioisoesterism.

Keywords: Molecular docking simulation, Virtual Screening, Infectious disease, Benzotriazole, Antimicrobial.

INTRODUCTION

Infectious diseases are of prime concern now a days as the infectious diseases are responsible for more than 9.5 million deaths people around the world. Lethal infectious diseases like tuberculosis, swine flu, acquired immune definciency syndrome (AIDS), hepatitis, pneumonia *etc*. are responsible for maximum number of deaths and causing pandemic situation around different continents of the world. Co-infection is also a matter of great concern as people infected already is susceptible to other diseases [1]. Treatment of these infections with rapid development of resistance in organisms has added fuel to worsened situation. Some viral infections like dengue, hepatitis, Japanese viral and West Nile viral infections cause large scale deaths every year as epidemics.

The literature study of investigations on synthesis and antimicrobial screening of benzotriazole derivatives in the past two decades showed antimicrobial activities like antibacterial, antifungal, antiviral, antiprotozoal and anthelmentic action. These reports of investigations suggest the possibility of emerging a lead compound of benzotriazole having a potential antimicrobial activity [2].

Benzotriazoles are found to possess various biological activities like analgesic, antimicrobial, anticonvalsant, anti-inflammatory and antitumour. Several derivatives of benzotriazoles are reported as agonists of peroxisome proliferator activated receptors [3]. In view of the above biological importance, we now report the synthesis of some of 1-(1H-benzotriazol-1-yl)-1-substituted phenyl methanamine and its antibacterial and antifungal activities.

Now as per the available literature benzotriazole found to be an active heterocyclic nucleous for antifungal compounds with benefit of little toxicity, elevated oral bioavilability and broad spectrum activity.

The most active antitubercular drug till date is isoniazid (INH). It mainly inhibit tyrosine residue an essential component of cell wall of bacteria which interfering mycolic acid synthesis. Rifampicin targets DNA-dependent RNA polymerase enzyme for bacterial inhibition which is also one of the potent broad spectrum antibiotic. A prodrug pyrazin-amide interferes with fatty acid synthesis in bacteria. Resistance is major problem allied with all the above antitubercular drugs targeting various non-homologous bacterial proteins [4]. Mutational changes are the major cause for resistance in the target protein, by affecting the binding of the drug to the target protein or by creating some biochemical changes in the bacterial life-cycle. The development of resistance of *Mycobacterium tuberculosis* bacteria against the existing antitubercular drugs is major problem now days in controlling TB disease from spreading. The tuberculosis bacteria resistant to at least isoniazid and rifampicin are categorized under multi drug resistant tuberculosis (MDR-TB) [5]. Second-line injectable agent, such as amikacin, kanamycin and/or capreomycin also are resistant such as fluoroquinolone which cause multi drug-resistant tuberculosis (MDR-TB) this leads to so called extensively drug-resistant tuberculosis (XDR-TB). Tuberculosis

infection is cured by longer time duration of drug therapy which is another major problem for drug resistant. Tuberculosis drug therapy requires a minimum 2 months to 24 months for complete treatment or even more. Thus, a new drug targets and drug development is needed to overcome the problems associated with tuberculosis drug therapy.

EXPERIMENTAL

All the molecular docking simulations were carried out utilizing softwares Raccoon, AutoGrid4 and AutoDock 4 [6]. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus.

Selection and preparation of protein: The cytochrome P450 14α-sterol demethylase (CYP51) of *Mycobacterium tuberculosis* bound with inhibitor fluconazole (pdb id-1EA1) was obtained from RCSB protein data bank. The crystal structure of the protein consisting of receptor associated with bound ligand is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (1EA1.pdb) registered in the Protein data bank was used. The bound ligand fluconazole is found within the receptor [7].

The X-ray crystal structure (Fig. 1) of (pdb id: 1EA1) was obtained from Brookhaven PDB (http://www.rcsb.org/pdb). Chimera has selected Chain A of the protein structure for docking. All waters of crystallization has been removed from protein structure for refining except HOH2173, HOH 2087 and HOH2174. Hydrogen's were added to the receptor and finally gasteiger charges were assigned [8].

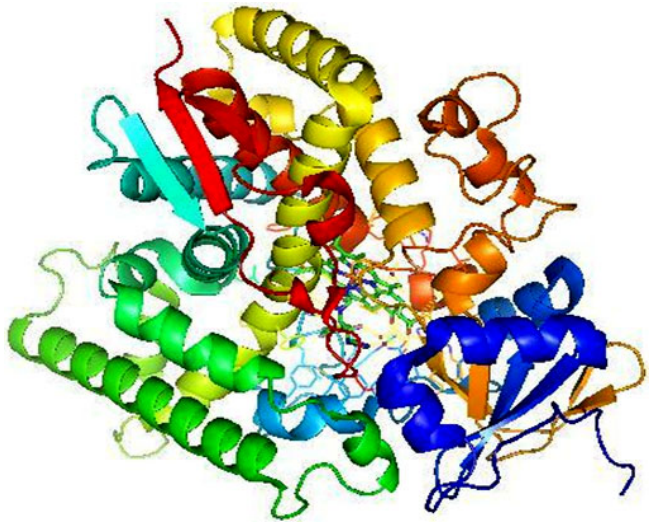


Fig. 1. Crystal structure of 1EA1.pdb

Ligand preparation: The ligand fluconazole is separated from the receptor 1EA1.pdb by the means of Chimera software. The ligand is processed by manually adding the number of rotatable, un-rotatable and non-rotatable bonds in the ligand fluconazole mentioned in the 2D and 3D conformation of bound ligand fluconazole (Fig. 2).

Grid parameters: AutoDock suite facilitate Autogrid utility to prepare map files for different atom types in ligands and receptor *viz.* A, C, OA, HD, N, NA, SA, S. These map files are in turn taken up by AutoDock for carrying docking

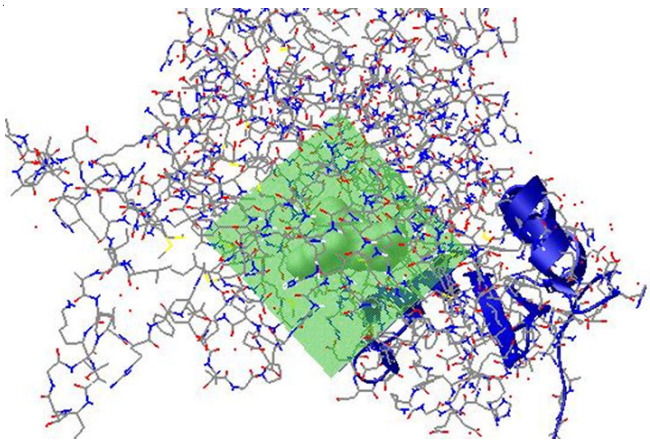


Fig. 2. 2D and 3D conformation of bound ligand fluconazole

simulations (Fig. 3). The binding pocket in the receptor was defined by grid box grid points: 44x, 44y, 46z; spacing: 0.37; grid center: -17.924x, -2.675y, 66.879z. The box is fitted with the extended conformations of ligands. The grid box is kept in the center of the ligand to ensure complete fit model.

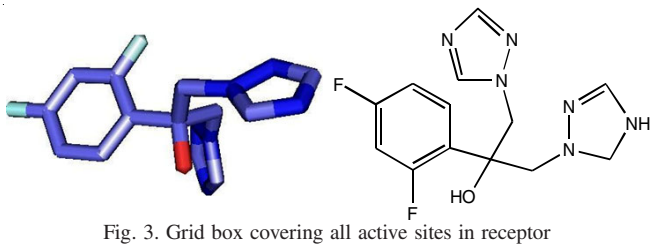


Fig. 3. Grid box covering all active sites in receptor

Docking parameters: Docking parameter file was prepared for each ligand using the following parameters: GA runs 150; maximum number of evaluations (short)-250000; maximum number of generations-27000; GA runs-10 and rate of gene mutation-0.02 [6].

Docking process: The docking methods and parameters used were validated by redocking individually crystallized fluconazole over cytochrome P450 14α-sterol demethylase (CYP51) of *Mycobacterium tuberculosis*. An rms value was calculated by overlaying their docked and crystallized chemical structures [4].

To validate the process of docking of particular ligand with particular macromolecule is done by following parameters

Chemical resemblance: The docking methods and parameters used were validated by redocking the crystallized DCKA and observing the chemical interactions of the docked and crystallized DCKA chemical structures [9].

Binding energy: Binding energy of the docked ligand should be in the range between -5 to -15 Kcal/mol.

Overlay: The docked conformation of ligand should be perfectly overlayed with the crystal structure of the downloaded protein to validate that the actual binding process happening inside human body is exactly simulated by using molecular docking simulation technique. The re-docking of this ligand was performed to get finer results.

Selection of chemical libraries: The molecular libraries containing 1,540 benzotriazole derived molecules are obtained from the portal of ZINC database. All the ligands present in the molecular library is filtered by applying Lipinsky's rule of

five, *i.e.* Clog P < 5; H bond donor < 5; H bond acceptor < 10; Molecular weight < 500 [10]. All compounds of the selected molecular library is screened by using following acceptable limits of the various physico-chemical properties mentioned in Table-1.

TABLE-1
ACCEPTABLE LIMITS OF THE VARIOUS PHYSICO-CHEMICAL PROPERTIES

S. No.	Property	Acceptable limits
1	Molecular weight	300
2	log P	-1 to 3
3	Net charge	0
4	Rotatable bonds	0 to 10
5	Polar surface area	0 to 120
6	Hydrogen donor	0 to 5
7	Hydrogen acceptor	0 to 10
8	Polar salvation	-400 to 1.00
9	Polar desolvation	-100 to 1.00

Virtual screening process: Virtual screening (VS) study is carried out on following target sites present in the 1EA1. All the necessary files for the virtual screening study is prepared by RACCOON (virtual screening preparation tools). All the compounds employed in virtual screening study were down-loaded from ZINC portals (Table-2). About 4500 compounds were subjected to virtual screening on target sites present in the receptor. Out of these compounds, the 10 best compounds were selected on the basis of their binding energy for each target site. The reference compound was also included in the virtual screening study as control. The predicted binding affinity is often closely proportional to the number of atoms in the ligand and ligand efficiency is designed to counteract the strong bias of virtual screening towards large compounds [9,11].

TABLE-2
MOLECULAR LIBRARY USED FOR VIRTUAL SCREENING OF 1EA1

Molecular library	No. of molecules	Source
Zinc molecule	1592	ZINC

Result analysis of docking simulation: After the screening was over, a script *summarize_results.py* from Scripps Research Institute was utilized to sort the binding energies of the docked ligand and select the best hits. The results of all dockings were evaluated based on hydrophobic and polar interactions between ligand and protein active site residues and the binding energy which must come in the empirical range -5 to -15 kcal/mol. Binding affinity is calculated from the formula [12]:

$$K_i = e^{[\Delta G/(RT)]}$$

where ΔG = change in free energy upon binding, R = gas constant and T = temperature.

Preparation of necessary files for virtual screening: Software RACCOON is used for preparation of all necessary files required for virtual screening.

Virtual screening: Actual docking of each ligand on the binding site of the receptor is done by software Autodock Tools™ and Raccoon. Raccoon can convert multiple-molecule ligand files into AutoDock format, by splitting them and filter them by using common criteria. For checking the presence of non-standard atom types and ensuring that parameters, like input filenames and grid maps having a coherent format, a validation check of the input files is performed at every step [13-17].

Analyzing virtual screening results: After the docking calculations are terminated, the docking results are recorded in the form of their binding energy in *.dlg file. The docking results summarized and ranked by lowest binding energy, to proceed to inspect the most promising results. The top 20 ligands selected after virtual screening of 1EA1 protein for antifungal activity were individually docked many times for refining of result and their various physio-chemical properties were also studied in order to improve their pharmacokinetic profile [18].

Prediction of ADME & toxicity of lead compounds: OSIRIS online program for toxicity and ADME properties prediction is used to evaluate toxicity of the screened top 5 lead molecules. Toxicities such as mutagenicity, tumorigenicity, irritant effect and reproductive effects were checked by this program to ensure toxicity effect of the lead molecules as the presence of functional group in chemical structure varies the effect of the lead molecule. Physic-chemical properties is taken as the basis to calculates drug-likeness and drug score of the lead molecule by this program [19].

RESULTS AND DISCUSSION

Grid parameters: The regions of interest used by AUTODOCK were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions [20]. The binding pocket in the receptor was defined by a grid box for three proteins (Table-3).

Validation of docking process

Chemical resemblance: Similar chemical interactions between the docked ligand and the receptor should be observed after docking, to that of the interactions present in the crystallized structure of protein. The interaction is shown in Fig. 4 between the 1EA1 ligand with RSCB PDB databank.

Binding energy: The binding energy of the bound ligand is -6.71 Kcal/mol, which is found to be in the optimum range of -5 to -15 Kcal/mol.

Overlay: The docked conformation of ligand should be perfectly overlayed with the crystal structure of the downloaded protein (Fig. 5). This testing is completed successful and the docked confirmation of the fluconazole is perfectly super-imposed with reference structure of fluconazole used by the

TABLE-3
COORDINATES OF GRID BOX FOR THE THREE PROTEINS

Proteins	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
1EA1	44	44	46	0.375	-17.924	-2.675	66.879

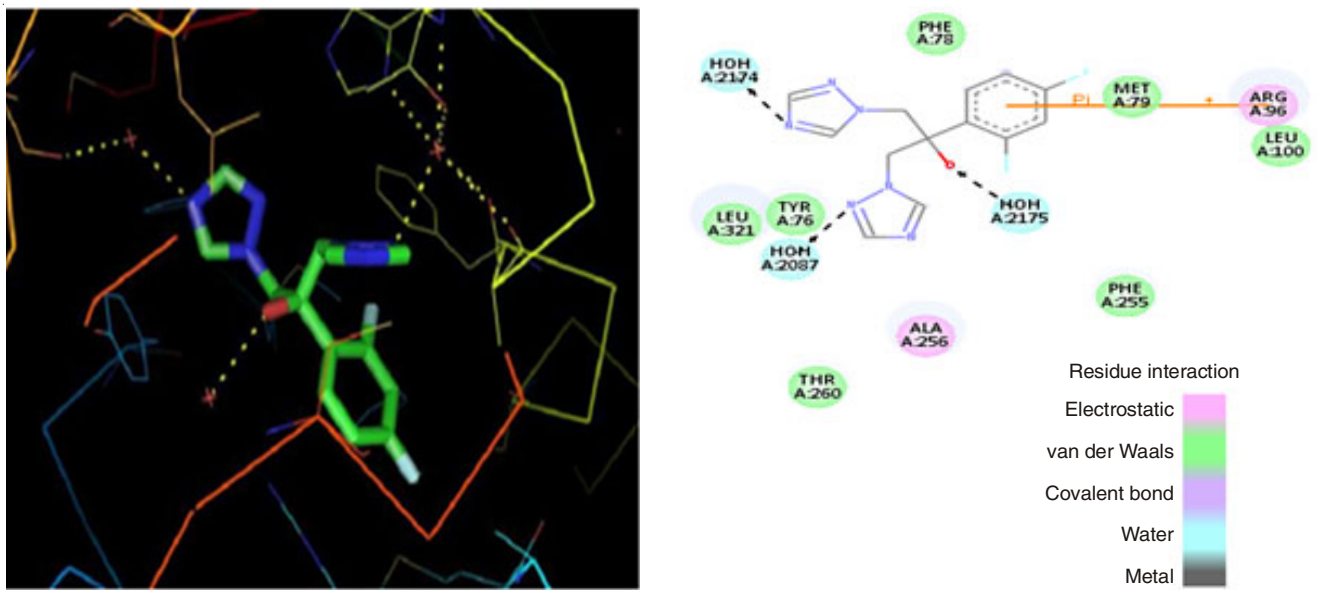
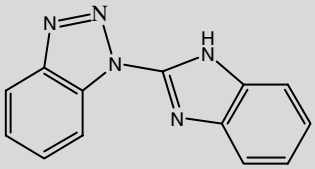
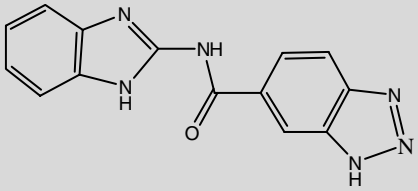
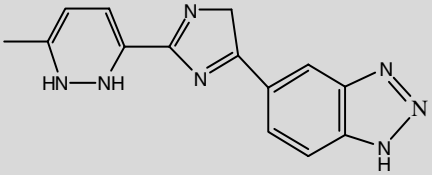
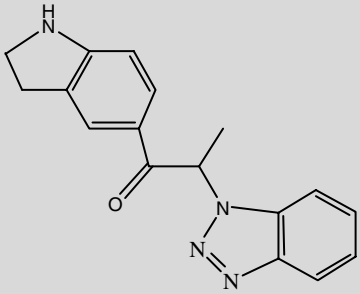
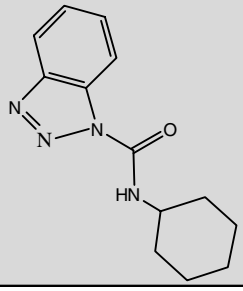


Fig. 4. 1EA1-ligand interaction present in RSCB PDB databank

TABLE-4
TOP FIVE LEAD MOLECULES AND ADME-T PROFILING SELECTED BY VIRTUAL SCREENING

S. No.	Compound ID	Chemical structure	Binding energy (kcal/mol)	Binding affinity (nM)
1	ZINC26472867		-8.42	649.48
2	ZINC08343238		-8.36	703.23
3	ZINC91829539		-8.34	748.27
4	ZINC41581470		-8.32	791.79
5	ZINC16545658		-8.31	813.12

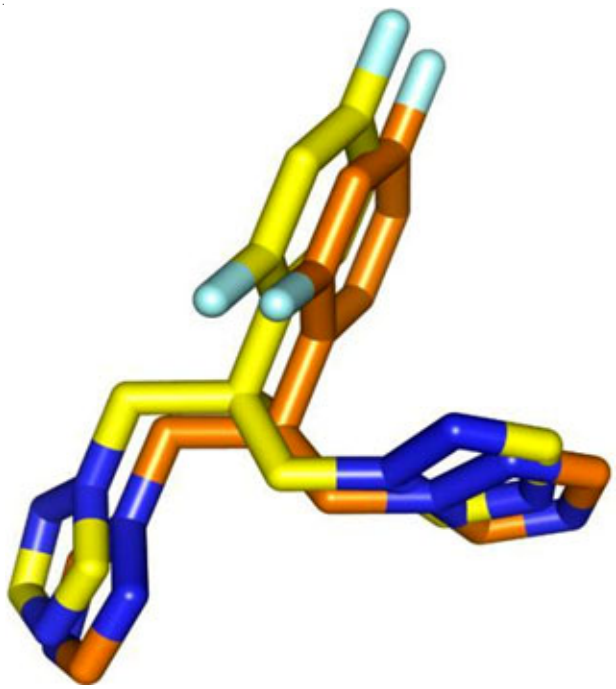


Fig. 5. Docked confirmation of the fluconazole is perfectly superimposed with reference structure of fluconazole, *i.e.* its crystal structure

Autodock docking algorithm [21]. The re-docking of this ligand was successfully achieved to get finer results.

Virtual screening: Following lead molecules in Table-4 are obtained by virtual screening of zinc molecular library containing 1592 benzotriazole derivative small molecules against Cytochrome P450 14α-sterol demethylase (CYP51) (pdb id-1EA1).

ADME and toxicity of lead compounds: The ADME prediction of screened top 5 lead compounds are shown in Table-4. Only one lead compound out of top five screened leads passed the toxicity test with a good drug-likeness score.

The results of ADME and toxicity prediction suggest that only ZINC91829539 lead molecules shows satisfactory results with a good drug-likeness score and without any toxic effects. While remaining four lead molecules ZINC26472867 [22], ZINC08343238, ZINC41581470 and ZINC16545658 have shown the poor drug in Table-5 likeness score as well as the presence of some toxic effects like carcinogenicity, mutagenicity, tumerogenicity, *etc*.

TABLE-5
TOXICITY PREDICTION OF PROPOSED LEAD MOLECULES

Lead compound	Mutagenic	Tumorigenic	Irritant	Reproductive effect
ZINC26472867	High	High	NA	NA
ZINC08343238	High	High	NA	High
ZINC91829539	NA	NA	NA	NA
ZINC41581470	High	High	NA	NA
ZINC16545658	High	High	NA	NA

Conclusion

An *in silico* framework is successfully used for virtually screening 1540 benzotriazole based ligands against cytochrome P450 14α-sterol demethylase (CYP51) receptor of *Mycobacterium tuberculosis* bacteria to identify possible lead molecules for development of novel drug molecules for treatment of tuberculosis. The cytochrome P450 14-α-sterol demethylase (CYP51) protein plays a vital role in the metabolic process in *Mycobacterium tuberculosis* bacteria and its inhibition will lead to the termination of bacteria. These virtually screened inhibitors are also tested for their pharmackokinetic properties and presence of any toxic effects, which shows that the lead molecule ZINC91829539 have shown the best pharmacokinetic properties with very good drug likeness score and no toxic effects are shown by this lead molecule. It is supposed that the designed benzotriazole based lead molecule should having better pharmacokinetic profile and lesser toxicity with potent antimicrobial activity.

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