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COMPUTER-AIDED REVERSE DOCKING STUDIES ON BIOACTIVE BENZOFURANS AS POTENTIAL ANTITUBERCULAR AGENTS

MAHENDAR GANTALA[1], DR. MAHESH REDDY GHANTA[2], DR. VASANTHA MITTAPELLI[3]

1. Department of Chemistry, Pacific University, Pacific Hills, Airport Road, Pratap Nagar Extension, Debari, Udaipur, Rajasthan, India
2. Process Research Laboratory, Brundavan Laboratories private Limited, Survey No.60-62, Yellagiri Village, Choutuppal Mandal, Yadadri Bhongir Dist, Telangana-508 252, India
3. Department of Chemistry, Mahatma Gandhi University, Nalagonda, Telangana-508 254, India

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Abstract: Computer-aided reverse docking study (CARD) was performed on two bioactive benzofuran derivatives against ten selected antitubercular drug targets to establish the preliminary putative binding target for each of the selected bioactive benzofuran derivatives. The docking technique was applied to selected bioactive compounds within the active site regions of selected drug target using iGmedock v 2.1. For these compounds, the binding free energy (kcal/mol) was determined. The results avail to understand the type of mechanisms by which these compounds could have exhibit the in vitro antitubercular activity.

Keywords: Chalcone, Benzofuan, iGemdock v 2.1, antitubercular activity

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Corresponding Author: MR. MAHENDAR GANTALA

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INTRODUCTION

Computer-aided reverse docking (CARD) approach has been used as a useful tool in facilitating drug design. In this approach, docking single or multiple small molecules in single or multiple conformations to a receptor site is attempted to find putative ligands. A number of flexible docking algorithms have been introduced. These include multiple-conformer shape matching, genetic algorithm, evolutionary programming, simulated annealing, fragment-based docking, and other novel algorithms. Testing results have shown that these algorithms are capable of finding ligands and binding conformations at a receptor site close to experimentally determined structures. Because of their capability in identifying potential ligands and binding conformations, these algorithms are expected to be equally applicable to an inverse-docking process for finding multiple putative protein targets to which a small molecule can bind or weakly bind. This may be applied to the identification of unknown and secondary therapeutic targets of drugs, drug leads, natural products and other ligands [1-20]. The CARD approach is now applied to the database of compounds synthesized in the present study for finding 'best fit' (hit identification) against selected antitubercular protein drug targets. The compound with least binding energy against each individual target protein is considered scope for further study. By these means, it is possible to understand how the compounds interact with the target protein. The results emerging out of these studies can be used to identify new active ligands by using the knowledge obtained from the *in silico* established secondary protein targets [21-40].

MATERIALS AND METHODS

COMPUTATIONAL SOFTWARE

Computer aided drug discovery softwares along with graphical user interface (GUI) were utilized for molecular modeling, energy minimization, molecular docking and virtual screening protocols. [41-45].

Table 1. Softwares used in the present study

S.No	Activity (will be performed)	Software (will be used)	License type (will be obtained)	Source
1).	Molecular modeling (2D-Drawing)	Accelrys Draw	Academic License GPU (General Public User) License	Open Source
2).	Molecular modeling	Open Babel	Academic License	Open

	(2D-3D Conversion)		GPU (General Public User) License	Source
3).	Molecular modeling (Molecular Mechanics & Energy Minimization)	ArgusLab v 4.0	Academic License GPU (General Public User) License	Open Source
4).	Molecular Docking & Virtual screening	iGemdock v 2.1	Academic License	Open Source

COMPUTATIONAL HARDWARE

The minimum central hardware system configuration include Intel (R) Core (TM) 2Duo Central Processing Unit (CPU), 2.5 GHz, 1 TB hard disk, 2 KV Power Backup, WinXP or higher operating system was used for running all the selected computer aided drug discovery softwares. All softwares were well compatible with the selected system configuration.

LIGAND PREPRATION

The chemical structures of the most active compounds (Chapter 9) synthesized in the present study such as **chalcone 2a** (MIC: 6.25 µg/mL) and **benzothiazepine 7b** (MIC: 6.25 µg/mL)were initially modeled as 2D chemical structures using Accelrys Draw software and transformed into 3D chemical structures using Open Babel software and subjected for energy minimization using ArgusLab v 4.0 software. The minimization was executed until the root mean square gradient value reached a value smaller than 0.0001 kcal/mol. Such energy minimized structures were considered for molecular docking studies using iGemdock v 2.1 software. The corresponding docking engine compatible 'MDL MOL' file format has been adapted to ligand by using integral option (save as /MDL MOL) [46-50].

PROTEIN SELECTION AND PREPARATION

The selection of protein target for molecular docking studies is based upon several factors i.e. structure should be determined by X-ray diffraction, and resolution should be between 2.5-3.0 A^{o}, it should contain a co-crystallized ligand; the selected protein should not have any protein breaks in their 3D structure. On the other hand, we considered Ramachandran plot statistics as the important filter for protein selection with none of the residues present in disallowed region. Finally the resultant protein target was prepared for molecular docking simulation in such a way

that all heteroatoms (i.e., nonreceptor atoms such as water, ions, etc.) were removed. Kollmann charges were assigned [51-62].

SOFTWARE VALIDATION

iGEMDOCK v 2.1 software validation was performed by using X-ray structures (Table 2) deposited with co-crystallized ligand was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The Root Mean Square Deviation (RMSD) between the X-ray co-crystallized ligands and docked conformations were < 2 A^{o} indicated that the parameters for docking simulation was good in reproducing X-ray crystal structure.

GENERAL PROCEDURE FOR THE MOLECULAR DOCKING

CARD technique was employed to dock the bioactives 2a and 7b against 4KD7 using iGEMDOCK to locate the interaction between 2a, 7b and drug targets. iGEMDOCK requires the receptor and ligand coordinates in either Mol2 or PDB format. Non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. Molecular docking was performed using standard protein-ligand docking protocol. The binding site was defined by crystallographic ligand of selected crystallographic structures. Default settings were used for all the calculations and docking run was performed.

REVIEW ON SELECTED ANTIUBERCULAR DRUG TARGETS

SHIKIMATE KINASE (SK)

Shikimate kinase (SK) and other enzymes in the shikimate pathway are potential targets for developing non-toxic antimicrobial agents, herbicides, and anti-parasite drugs, because the pathway is essential in the above species but is absent from mammals. The shikimate pathway is the biosynthetic route that converts erythrose-4-phosphate to chorismic acid in seven steps. Chorismic acid is an essential intermediate for the synthesis of aromatic compounds, such as aromatic amino acids, *p*-aminobenzoic acid, folate, and ubiquinone. The shikimate pathway is essential for algae, higher plants, bacteria, and fungi, whereas it is absent from mammals. This makes the enzymes in the pathway potential targets for the development of non-toxic antimicrobial agents, Shikimate kinase (EC 2.7.1.71), and the fifth enzyme in the shikimate biosynthetic pathway, from *Mycobacterium tuberculosis* is obviously an excellent target for developing novel anti *Mycobacterium tuberculosis* agents [63,64].

CHORISMATE SYNTHETASE (CS)

The enzymes of shikimate pathway are good candidates for development of new therapies against TB. Enzymes from this metabolic pathway have been submitted to intensive structural

studies. The last enzyme from this pathway is the chorismate synthase (CS), which catalyzes the conversion of the 5-enol-pyruvylshikimate-3-phosphate (EPSP) to chorismate. It is the only enzymatic reaction known of such transformation in biological systems, making the CS a unique enzyme in the nature. The CS requires reduced flavin mononucleotide (FMN), an essential cofactor typically found in many biological redox reactions. Surprisingly, the reaction catalyzed by CS does not involve an overall change in redox state. The reduced FMN donates an electron to EPSP to facilitate the loss of the phosphate and receive it back after the reaction. So, only flavin in its reduced form is functional and it is not consumed during the reaction [65].

ISOCITRATELYASE (ICL)

The strategy for survival during chronic stages of infection entails a metabolic shift in the bacteria's carbon source to C2 substrates generated by β-oxidation of fatty acids. Under these conditions, glycolysis is decreased and the glyoxylate shunt is significantly up regulated to allow anaplerotic maintenance of the tricarboxylic acid (TCA) cycle and assimilation of carbon *via* gluconeogenesis. The glyoxylate shunt accomplishes this by converting isocitrate to succinate and glyoxylate by isocitrate lyase (EC 4.1.3.1), followed by addition of acetyl-CoA to glyoxylate to form malate by malate synthase. The carbon conserving glyoxylate pathway is present in most prokaryotes, lower eukaryotes and plants, but has not been observed in vertebrates [66-68].

PANTOTHENATE SYNTHETASE (PS)

Pantothenate (vitamin B5) is a key precursor of the 4-phosphopantetheine moiety of coenzyme A (CoA) and the acyl carrier protein (ACP). Both CoA and ACP are necessary cofactors for cell growth and are involved in essential biosynthetic pathways. Pantothenate is biosynthesized in micro-organisms, plants, and fungi, but not in animals, and the enzymes of the pantothenate pathway are considered to be potential herbicide and antimicrobial targets. The pathway to pantothenate is best understood in *Escherichia coli*, where it comprises four enzymatic reactions. The final transformation, to produce pantothenate, is catalyzed by pantothenate synthetase (EC 6.3.2.1), encoded by the *panC* gene. Pantothenate is biosynthesized by the condensation of D-pantoate and β-alanine [69-73].

ENOYL-[ACYL-CARRIER PROTEIN] REDUCTASE (InhA)

Two enzymes, InhA and KasA, have been proposed as targets for INH. Both are members of the type II dissociated fatty acid biosynthesis pathway (FASII) in *Mycobacterium tuberculosis*, consistent with the observation that INH interferes with the biosynthesis of mycolic acids, very long chain fatty acid components of the mycobacterial cell wall. InhA, an enoyl reductase that catalyzes the NADH-dependent reduction of long chain *trans*-2-enoyl-acyl carrier proteins

(ACPs), was first identified as a target by Jacobs and coworkers. InhA is inhibited by INH, Barry and coworkers have also proposed that KasA, one of three ketoacyl synthases in the FASII pathway, is a target for INH *in vivo* [74, 75].

3-OXOACYL-[ACYL-CARRIER PROTEIN] REDUCTASE (MabA)

The fatty acid elongation system FAS-II is involved in the biosynthesis of mycolic acids, which are major and specific long-chain fatty acids of the cell envelope of *Mycobacterium tuberculosis* and other mycobacteria, including *Mycobacterium smegmatis*. The protein Maba, also named FabG1, has been shown recently to be part of FAS-II and to catalyze the NADPH specific reduction of long chain β-ketoacyl derivatives. This activity corresponds to the second step of an FAS-II elongation round. FAS-II is inhibited by the antituberculous drug isoniazid through the inhibition of the 2-trans-enoyl-acyl carrier protein reductase InhA. Thus, the other enzymes making up this enzymatic complex represent potential targets for designing new antitubercular drugs [76].

ORNITHINE ACETYLTRANSFERASE (OAT)

Mycobacterium tuberculosis ornithine acetyltransferase (*Mtb* OAT; E.C. 2.3.1.35) is a key enzyme of the acetyl recycling pathway during arginine biosynthesis. It reversibly catalyzes the transfer of the acetyl group from N-acetylornithine (NAORN) to L-glutamate. *Mtb* OAT is a member of the N-terminal nucleophile fold family of enzymes. The three-dimensional structure of *Mtb* OAT will provide crucial information for elucidating the mechanism of OAT-catalyzed reaction and structure-based drug design [77].

LUMAZINE SYNTHETASE (LS)

The enzymes involved in endogenous riboflavin biosynthesis pathways are not present in the human or animal host, they are promising candidates for the inhibition of bacterial growth. Two enzymes, lumazine synthase (EC 2.5.1.9; LS) and riboflavin synthase (RS), are catalyzing the penultimate and the last step of riboflavin biosynthesis. LS from *Mycobacterium tuberculosis*, which has shown the homopentameric state as well. The LS monomer shows some folding similarity to bacterial flavodoxins and is constructed from a central four-stranded β-sheet flanked on both sides by two and three α-helices, respectively. Lumazine synthase inhibitors can be considered as potential lead compounds for the design of therapeutically useful antibiotics [78-79].

QUINOLINATE PHOSPHORIBOSYL TRANSFERASE (QAPRT)

Quinolinic acid phosphoribosyl transferase (EC 2.4.2.19), encoded by the *nadC* gene, is a key enzyme in *de novo* biosynthesis of NAD. The enzyme carries out the Mg2+ dependent transfer

of the phosphoribosyl moiety from 5-phosphoribosyl-1-pyrophosphate (PRPP) to quinolinic acid (QA) yielding nicotinic acid mononucleotide (NAMN), pyrophosphate and CO_2. In *Mycobacterium tuberculosis*, the three genes encoding the enzymes involved in the *de novo* biosynthesis of NAMN are part of a single operon (*nadABC*). In bacteria, the *nad* operon is transcriptionally regulated by a repressor encoded by the *nadR* gene in response to intracellular levels of nicotinamide mononucleotide (NMN). Alternatively, NAMN can be produced by a salvage pathway that proceeds via the phosphoribosylation of nicotinic acid (NA), generated by the degradation of NAD; this reaction is catalyzed by the enzyme nicotinate phosphoribosyltransferase (NAPRT). Despite the similarity between their enzymatic reactions, QAPRT and NAPRT exhibit exclusive specificity for their respective substrates. In *Mycobacterium tuberculosis*, unlike most organisms, the salvage pathway appears to be disrupted. This is proposed to be a consequence of the lack of detectable NAPRT activity and results in secretion of NA produced by degradation of NAD. Relying entirely on the *de novo* pathway for its NAD requirements, *Mycobacterium tuberculosis* should be extremely vulnerable to drugs targeted against QAPRT [80-85].

GLUCOSAMINE-1-PHOSPHATE-N-ACETYL TRANSFERASE (GLmU)

The glmU gene is essential in *Mycobacterium tuberculosis*, being required for optimal bacterial growth, and has been selected as a possible drug target for structural and functional investigation. The gene glmU has been identified as essential for optimal growth of *Mycobacterium tuberculosis* and is not present in humans; hence, it is of interest as a drug-design target [86,87].

Table 2. Software validation data for selected antitubercular drug targets.

S.No	Name of the target protein	PDB ID	RMSD (A°)
1	Shikimate Kinase (SK)	1L4Y	**1.22**
2	Chorismate Synthetase (CS)	2QHF	**1.00**
3	Isocitratelyase (ICL)	1F8I	**1.05**
4	Pantothenate Synthetase (PS)	1N2H	**1.14**
5	Enoyl-[Acyl-Carrier Protein] Reductase (InhA)	2X22	**0.99**
6	3-Oxoacyl-[Acyl-Carrier Protein] Reductase (MabA)	1UZN	**1.31**

7	Ornithine Acetyltransferase (OAT)	3IT4	**1.22**
8	Lumazine Synthetase (LS)	2C9D	**1.14**
9	Quinolinate Phosphoribosyl Transferase (QAPRT)	1QPN	**1.18**
10	**Glucosamine-1-Phosphate-N-Acetyl Transferase (GLmU)**	**3D8V**	**1.91**

MOLECULAR DOCKING RESULTS

Table 3. Bioactives 2a and 7b with their Gemdock Scores (kcal/mol) against selected antitubercular drug targets.

Drug Target	Compound 2a	Compound 7b
SK	-102.57	-96.8181
CS	**-106.656**	-97.531
ICL	-102.526	-92.6002
PS	-74.0527	-82.3916
InhA	-82.2662	**-107.937**
MabA	-104.579	-97.531
OAT	-88.6987	-87.2604
LS	-91.7312	-101.304
QAPRT	-93.5827	-95.449
GLmU	-99.2442	-85.7546

DISCUSSION ON THE RESULTS

Molecular docking approach has been used as an essential means in facilitating drug-target search. The compound with least binding energy against each target protein is considered as 'Best fit'. By this means, it is possible to understand how the compounds interact with the

receptor. The results emerging out of this study can be used to establish the possible inherent mechanism of action of compounds **2a** and **7b** as potential antitubercular agents. The Molecular docking simulation technique was performed using Gemdock program with **2a** and 7**b** in vitro active compounds having potential antitubercular activity. Each compound was docked into 10 different targets shown in **Table 3**. The lowest energy docked conformation of the most populated cluster (the best cluster) was selected and then taken into account. From the results, **2a** was accomplished best binding efficiency against **CS** with Gemdock score **-106.656 kcal/mol.** Similarly, compound **7b** against **InhA** with Gemdock score **-107.937 kcal/mol.** Among the selected antitubercular targets, **2a** showed good binding efficiency against **CS** protein target was identified on the basis of their Gemdock score (kcal/mol). Likewise **7b** was showed good binding efficiency against **InhA** protein target is identified on the basis of its Gemdock score (kcal/mol). The results were used to understand the status of binding capacity of the in vitro active compounds **2a** and **7b** with established mechanism of action. In conclusion, CARD studies on **2a** and **7b** showed that these computational studies were useful for predefining antitubercular drug targets for the compounds with observed inhibitory activity against *Mycobacterium tuberculosis* H37Rv.

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