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# ELECTRON SINK AS A POTENTIAL DRUG TARGET: IN SILICO DRUG DEVELOPMENT AGAINST MYCOBACTERIUM TUBERCULOSIS

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**Abstract -** *Latency of Mycobacterium tuberculosis and prolonged therapy regimen with emergence of MDR (multi-drug resistance) has mandated new therapy and it is prudent new antimicrobials be developed. Taking one of the potent mechanisms of actions of most of the antibiotics of generating reactive oxygen species (ROS) which the resistance bugs tend to mitigate in exhibiting resistance, one of the areas to develop antibiotics would be disruption of pathogens' ROS evading mechanism and prevention of entry to low metabolic state. In this regard, glutathione and mycothiol, two electron sink thiols, biosynthetic pathway were chosen as target metabolic network. Based on Flux Balance Analysis (FBA) and OGEE (Online Gene Essentiality) results six proteins, MshA, MshC, CysM, CysK, MetH and Mrx-1, were taken as lead targets. NCI database II was taken as ligand database and top ten ligands with higher binding potential were screened for each proteins. They were further screened for ADME/T and druglikeness from which two ligands: ZINC16951320 and ZINC00990239 with higher binding energy towards all six enzymes as multi-drug target molecules were narrowed down. It is suggested that these molecules be further pursued for additional works on drug development as drug candidate leads and their antimicrobial potential be explored in other pathogens than Mycobacterium to develop novel antimicrobials that have broad spectrum.*

***Key Words***: MDR, ROS, electron sink thiols, multi-target drug, broad spectrum

## 1. INTRODUCTION

The efficacy of antibiotics has been endangered by the rapid and widespread emergence of resistant bacterial strains towards therapeutics, forming a league of multi-drug resistant superbugs. And similar is the case in *M. tuberculosis*, a causative agent of (TB), which is already multidrug-resistant (MDR) [4]. In 2017, WHO marked TB as the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. Metabolically diverse population of *M. tuberculosis* within the human host requiring 6–9 months of chemotherapy with a combination of frontline antibiotics, slow clearance rate[23], latent/persistent infection[22] make the TB treatment difficult. Besides *M. tuberculosis* strains, resistant to four or more of the front-line treatments i.e., extremely drug-resistant strains (XDR) have appeared and spread rapidly in the last decade or so [17]. And now there are TDR (totally drug resistant strains), compromising TB therapy throughout the world [20]. This rise in resistance has limited our repertoire of effective antimicrobials [8] which ensures the need for continual cycles of discovery and development of new antibiotics.

The generation of reactive oxygen species (ROS) is the central and common mechanism of antibiotics mediated lethality in bacterial cell and mitigating this allows bacteria to develop resistance against the antibiotics. It is done through the augmentation of cellular respiration followed by Fenton catalyzed Haber-Weiss reaction [5].

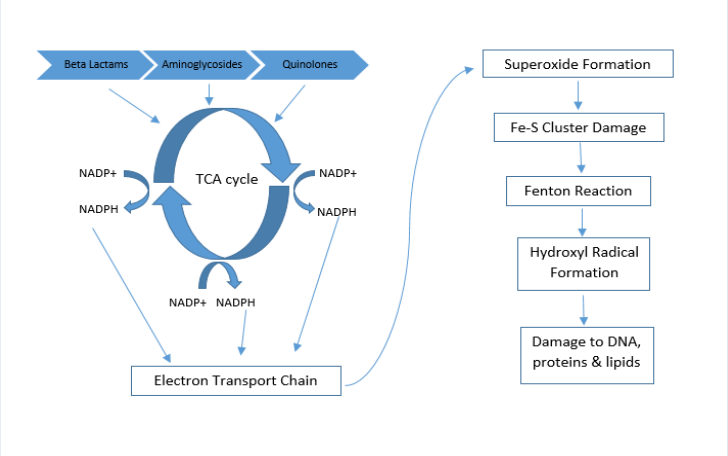


**Figure 1:** Schematic of Hydroxyl radical hypothesis of antibiotic action [8]

Sulfur assimilation pathway, particularly cysteine mediated through $H_2S$ production [11] and glutathione/mycothiol mediated electron sink [21], is found to be involved in antibiotics resistance through ROS mitigation. Genes involved in sulfur metabolism have consistently been identified as up-regulated in response to oxidative stress, nutrient starvation and dormancy adaptation and during macrophage infection [7]. Mycothiol is a principal low molecular thiol compound in actinobacteria, which acts as a redox buffer and is essential for the cellular defence against oxidative stress and antibiotics [12]. Hence, one of the areas for drug development could be impeding ROS mitigating mechanism.

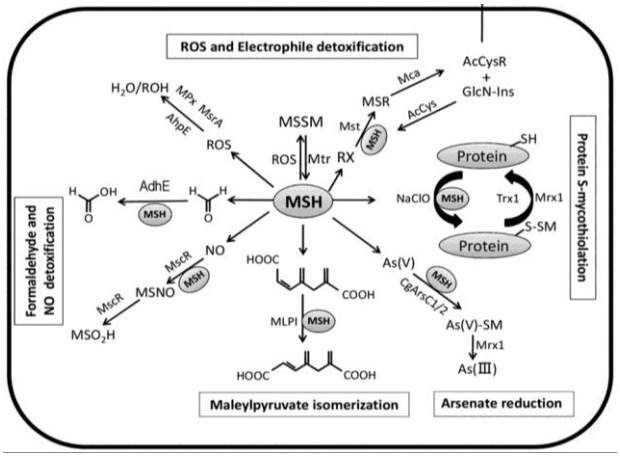


**Figure 2:** Functions of Mycothiol [18]

In silico method could be one of the tools to expedite drug discovery process, mainly through lead protein target identification, ligand identification from chemical library and predicting their drugability [19]. In the present study sulfur assimilating enzymes CysK, CysM, MetH, mycothiol biosynthetic enzymes: MshA , MshC , along with mycothiol reducing Mrx-1 [15] and rate limiting enzyme of glutathione biosynthetic pathway gshA were narrowed from Constraint Based Reconstruction and Analysis (COBRA) tool box and Online Gene Essentiality (OGEE).

## 2. EXPERIMENTAL PROCEDURE

### 2.1 Metabolic Reconstruction Analysis

#### 2.1.1 BiGG Database for COBRA modeling

BiGG - a knowledgebase of Biochemically, Genetically and Genomically structured genome-scale metabolic network reconstructions contains more than 75 high quality, manually-curated genome scale metabolic models. The metabolic capabilities of organisms are analyzed using genome-scale metabolic reconstructions under the COBRA framework [16]. The reconstructed metabolic network and biomass composition of *Mycobacterium tuberculosis* H37Rv were obtained from its latest reconstruction, called iNJ661, which contains 661 genes, 1025 network reactions and 825 metabolites. COBRA approach to genome-scale models is applied with a motive of creating a system that enables a mechanistic description of metabolic physiology when certain metabolites or substrates are limited in a given growth conditions.

#### 2.1.2 Flux Balance Analysis (FBA)

FBA was performed for predicting growth through the calculation of biomass production by obtaining reaction stoichiometry in defining biological relevant objective function and addition of other biochemical constraints for optimization [13].

Initially, six genes were selected for analysis where reactions of sulfur reduction pathway (sulfur assimilation pathway) were chosen. The conditions were modified in anaerobic and aerobic conditions in order to stimulate altered oxygen availability. The reactions for which the biomass production is zero, the associated genes were listed as essential genes.

#### 2.1.3 Online Gene Essentiality (OGEE)

OGEE is a database to test essentiality of genes. The genes tested for essentiality were inserted where genes are organized into the data sets according to their source. Those genes which have variable essentiality status across data sets are tagged as conditionally essential, illustrating the complex interplay between gene functions and environments. It not only tests experimentally tested essential and non-essential genes but also associated gene features such as expression profile, duplication status, conservation across species, evolutionary origins and involvement in embryonic development are collected in the database [3].

### 2.2 Docking

#### 2.2.1 Identification of binding site

The 3D structures of the proteins were taken from RCSB PDB server. The ligand binding sites were determined by Discover Studio Visualizer 2016 molecular viewer. The native ligand was removed and redock to confirm the binding site.

#### 2.2.2 Preparation of ligand database

The virtual ligands of NCI diversity set-II containing 1880 different synthetic compounds were obtained from Zinc database. Using Lipinski's rule of five which states drug-like compounds should have: Molecular weight < 500, Lipophilicity (log P) < 5, Hydrogen bonds < 5, Hydrogen bond acceptors < 10 [10], ligands were pre-screened. Then the structural-based virtual screening was carried using AutoDock-Vina from which top 10 molecules with higher binding energy with protein receptor than the natural ligand were narrowed.

### 2.3 ADME/Tox screening for Druglikeness

The top ten ligands were subjected to OSRIS based ADME/T analysis to analyze toxicity and other parameters. It is estimated from the molecular structure before the substance is even synthesized and tested. Absorption, distribution, metabolism, excretion and toxicity (ADME/Tox) describe the kinetics of drug exposure to the tissue and pharmacological activity of the compound.

## 3. RESULTS AND DISCUSSIONS

Microbial sulfur metabolic pathways are largely absent in humans and therefore, represent unique targets for therapeutic intervention. Small molecule inhibitors of these pathways represent valuable chemical tools that can be used to investigate the role of sulfur metabolism throughout the *Mycobacterial* lifecycle and may also represent new leads for drug development.

### 3.1 Single Gene Deletion Analysis using FBA

Using MATLAB based COBRA tools for FBA analysis of single gene deletion assay involving cysteine, methionine, glutathione and mycothiol biosynthetic pathway revealed that CysK/CysM and MetH are critical for survival in both aerobic and anaerobic conditions. Thus inactivation of CysM/CysK and MetH could potentially be fatal for bacterial survival. CysM/CysK involves in alternative cysteine biosynthesis pathway [1], MetH complete the final step of methionine biosynthesis and methionine is essential for all organism [6].

**Table 1:** Results of gene deletion analysis on iNj661 model after deletion of individual reactions of sulfur assimilation (+ = growth, - = no growth)

| Gene involved in sulfur assimilation pathways | Amount of glucose & oxygen Glucose = -18, 0xygen = -100 (Aerobic) and 0 (Anaerobic) | |
|---|---|---|
| **Genes** | Aerobic Condition | Anaerobic Condition |
| mshA | + | - |
| mshC | + | - |
| mshD | + | - |
| cysM/cysK | - | - |
| metH | - | - |
| gshA | + | - |
| gshB | + | - |

The associated reactions for each gene of sulfur assimilation pathway were disabled (vmin, i = vmax, i = 0 mmol.gDW-1.hr-1) and the ability of model to produce biomass was assessed, i.e., the biomass reaction was chosen as the objective function and maximized [2]. The reactions for which the biomass production is zero, the associated genes were listed as essential genes.

### 3.2 Gene essentiality test

**Table 2:** Results of gene essentiality analysis of various genes in Mycobacterium tuberculosis H37Rv using OGEE online database

| Genes | Essentiality |
|---|---|
| MshA (Rv0486) | Conditional |
| MshC (Rv2130c) | Essential |
| CysK1 (Rv2325) CysK2 (Rv848) | Non-essential |
| Cys M (Rv1336) | Non-essential |
| MetH (Rv2124) | Conditional |
| Mrx-1* | Novel mycothiol-dependent reductase |

MshC could be an essential gene which catalyzes the ATP-dependent condensation of GlcN-Ins and L-cysteine to form L-Cys-GlcN-Ins. Furthermore, MshC could be novel drug targets in *Mycobacterium tuberculosis* [9]. Mycothiol prevents *Mycobacterium tuberculosis* from toxic xenobiotics so, it is essential for growth during oxidative stress [15].

### 3.3 Virtual screening of NCI Diversity set- II

Based on results of two models of gene essentiality six potential targets MshA, MshC, CysM, CysK, MetH and Mrx-1 were chosen and docked with ligands of NCI diversity set-II. The cancer cells that have high rate of metabolism for growth rarely succumb to ROS mediated fatality indicating that the cells could be using electron sink mechanism through glutathione. So, it was presumed that some of the anticancer agents could be acting on the proteins involved in glutathione or mycothiol biosynthesis [14].

Then top 10 molecules exhibiting higher binding energy than the natural ligand of proteins were narrowed with the presumption that the higher binding energy could be indicative of competitive inhibition by these molecules to the target binding sites than the native ligand. This could then potentially block biosynthesis pathway.

### 3.4 Druglikeness

High cLogP value or high lipophilicity means that the drug cannot be absorbed properly [10]. The preliminary drug candidacy showed that most of the high-affinity ligands had negative drug-likeness values. Also, those who had positive drug-likeness score also showed high lipophilicity. Considering a positive drug-likeness score range -5 to 5 and a cLogP score less than 5, the molecules were further screened for their toxicity such as mutagenicity, tumorogenicity and other parameters. Based on all these molecules were narrowed as potential lead drug candidates against the respective protein investigated

### 3.3 Multi-target inhibition potential of the drug leads

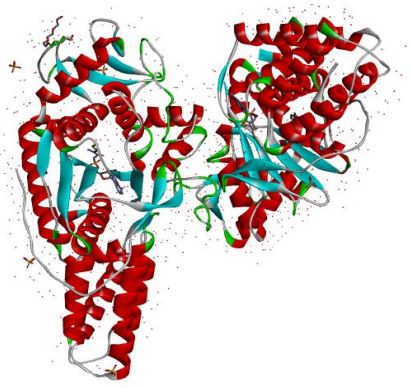

**Figure 3:** In silico 3D crystal structure of MshC

Specific to mycobacteria MshC (ligase) is a rate limiting enzyme in mycothiol biosynthesis and it could be a novel drug target for management of multiple drug resistant Mycoabacteria. However, the bacteria could survive partially because of the glutathione. Hence glutathione also be taken as a target**.** Thus, potential inhibitors of MshC further docked with other targets, which also have effort on multiple drug resistant development in bacteria.

**Table 3:** Comparison of Binding Affinity (kcal/mol) Potential Drug Candidates of MshC with MshA, GsHA, MetH, CysK, CysM, Mrx-1 and Grx-1 *HBE = Highest Binding Energy among NCI Diversity set II

| Ligands | MshC | MshA | GshA | MetH | CysK | CysM | Mrx-1 | Grx1 |
|---|---|---|---|---|---|---|---|---|
| ZINC18057104_1 | -9.9 | -6.2 | -0.7 | -9.4 | -7.5 | -7.4 | -5.2 | -5.3 |
| **ZINC16951320_2** | **-9.6** | **-10.3** | **-8.7** | **-9.4** | **-6.6** | **-7.8** | **-5.2** | **-6.5** |
| **ZINC00990239** | **-9.5** | **-9.6** | **-10** | **-10.1** | **-10.7** | **-8.0** | **-5.6** | **-5.7** |
| CONTROL | -7.9 | -9.4 | -7.8 | *HBE -11.8 | -5.1 | -6.1 | -3.8 | -4.6 |

From the cross docking results, ZINC16951320_2 and ZINC00990239 could be the lead molecule that have multi-target potential as inhibitors of L-cysteine:1D-myo-inositol 2-amino-2-deoxy-alpha-D-glucopyranoside ligase (MshC), D-inositol 3-phosphate glycosyltransferase (MshA), glutamate cysteine ligase (GshA), methione syntheses (MetH), O-acetylserine sulfhydrylase (CysK)**,** cysteine Synthase B (CysM)**,** mycoredoxin-1 (Mrx-1) and Glutaredoxin-1 (Grx-1)**.** Thus, co-administration of presently prescribed antibiotics with identified ligands could be a new strategy to kill multi-drug resistant *M. tuberculosis.*

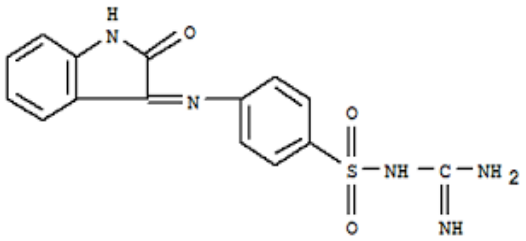

**Figure 4:** Benzenesulfonamide, N-(aminoiminomethyl)-4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino] (ZINC16951320_2)

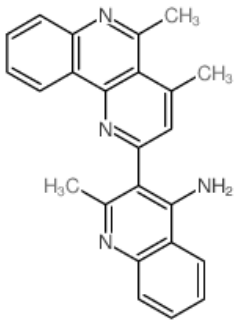

**Figure 5:** 3-(4,5-Dimethylbenzo(h)-1,6-naphthyridin-2-yl)-2-methylquinolin-4-amine (ZINC00990239)

## 4. CONCLUSIONS AND RECOMMENDATIONS

Multi-drug resistant tuberculosis is becoming more pervasive and is a major health issue facing in today's global scenario. The multi-drug resistance continues to emerge and spread due to the mismanagement of TB treatment, inadequate chemotherapy, not directly observed treatment, shortage of effective anti-tuberculosis, interruption of therapy and person-to-person transmission. The scientific communities need to work seriously in managing resistance, designing rapid diagnostics and discovery of new antimicrobials. The present study focused on designing a protocol for identification of the lead molecule to research as the potential anti-mycobacterial substance that could potentially work in managing the multidrug resistant. The work mainly focused on the study of sulfur assimilation pathway, ROS scavangening mechanism and transpeptidase activity. Using computational biology, sulfur assimilation pathways were identified as targets with therapeutics potentials and two multi-potential lead molecules were discovered using high throughput virtual screening which was then analyzed for their drug-likeness and toxicity. It is suggested that these molecules could be tested experimentally for protein inhibition assay and possible riboswitch mechanism to illuminate its efficacy in further designing experimental models. Antibiotics induced ROS production along with the inhibitions of redox buffer could be lethal for multiple drug resistant mycobacteria.

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