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An in Silico Approach for Prioritizing Drug Targets in Metabolic Pathway of Mycobacterium Tuberculosis

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***Abstract*—**There is an urgent need to develop novel *Mycobacterium tuberculosis* (Mtb) drugs that are active against drug resistant bacteria but, more importantly, kill persistent bacteria. Our study structured based on integrated analysis of metabolic pathways, small molecule screening and similarity Search in PubChem Database. Metabolic analysis approaches based on Unified weighted used for potent target selection. Our results suggest that pantothenate synthetase (*panC*) and and 3-methyl-2-oxobutanoate hydroxymethyl transferase (*panB*) as a appropriate drug targets. In our study, we used pantothenate synthetase because of existence inhibitors. We have reported the discovery of new antitubercular compounds through ligand based approaches using computational tools.

Keywords*—In Silico*, Ligand-based Virtual Screening, Metabolic Pathways, *Mycobacterium tuberculosis*

I. Introduction

MYCOBACTERIUM TUBERCULOSIS, the causing agent of tuberculosis (TB), remains a world-wide health crisis. Drug treatment is a laborious and lengthy process with high risk for non compliance, cytotoxicity and drug resistance. Recently, there has been an alarming rise of multi-drug resistant and drug resistant TB world-wide, making development of new drugs crucial [1]-[3]. Novelty and potency of drug target is key step in rational drug design [3]. It was noted that existing drugs for *M. tuberculosis* tend to target information-processing enzymes (DNA and RNA polymerase, DNA gyrase) rather than metabolic enzymes, so searches for new drugs more feasible with pay attention to metabolic pathways as a drug target pool [4].

In this study, we used a broad range of weighted criteria that cover multiple levels of target to identify plausible drug targets. The output from numerous freely available bioinformatics tools and experimentally published studied can be easily used to assay of weighted criteria. Only those targets with all of the desired traits are selected. Then, by searching on more potent drug targets, it would be possible to find inhibitors of them. Finally, we used a ligand based approach, based on the use of known active compounds, to develop new antitubercular compounds.

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II. Material And Methods

A. Prioritizing Drug Targets via Unified Weighted

Since experimental investigations of possible drug targets are time-consuming and expensive, our study achieves more benefit from *in silico* analyses. These analyses consider criteria investigated to antituberculosis drug target design, including essentiality, known function, rate limiting steps in metabolic network, expression under various condition, non-homology, similarity to anti-targets, similarity to gut flora proteins, Involvement in Persistence and virulence (Table 1). Data sources for all of these criteria inferred from available bioinformatics tools and experimentally published studied. Then prioritization drug targets have done via Unified weighted, in which only those targets of metabolic pathways with all of the desired criteria are selected.

B. Active Compounds Preparation

Active compounds against pantothenate synthetase enzyme (*panC*) were collected from the literature search with IC50 values between 100 to 61000 nM [5]. To identify the new antituberculosis compounds from the NIH PubChem Compound Database, we selected best compound based on two distinct parameters: a) antituberculosis activity; b) Lipinski's rule of 5, including (i) no more than 5 H-bond donors, (ii) no more than 10 H-bond acceptors, (iii) molecular weight no higher than 500, and (iv) calculated primary predictive index of lipophilicity (octanol/water partition) (xlogP) between 5 and -5. In this study, the molecular descriptors for active compounds were calculated using ChemAxon (v. 5.0.0.0, 2009) program.

Known antituberculosis drug generally follow lipinski's rule of five except high lipophilicity, the maximum value allowed for logP was raised from 5 to 6 which presumably can more easily penetrate the cell wall, and high molecular weight (> 500) [6], [7].

C. Ligand-based Similarity Search

We have performed a similarity search of highly active compound, directly binding to pantothenate synthetase, from the NIH PubChem Compound Database. In similarity search of highly active compound against *M. tuberculosis*, the Tanimoto similarity indices for the reference compounds were measured using PubChem dictionary-based binary fingerprint. This fingerprint consists of series of chemical substructure "keys". Each key denotes the presence or absence of a

particular substructure in a molecule. The fingerprint does not consider variation in stereochemical or isotopic information. Collectively, these binary keys provide a "fingerprint" of a particular chemical structure valence-bond form. A Tanimoto score greater than 0.7 is indicative of two molecules having high structural similarity and probably similar bioactivity. In our study, similar compounds search has done using pre-specified (70% - 80%) similarity thresholds, equal to 0.76 – 0.9 Tanimoto that it increase possibility of selecting a subset with same bioactivity.

D. Virtual Library Design

Library design was also applied as a *in silico* (virtual) screening, by using the evaluation of specific biological properties of molecules to filter similar compounds in PubChem Database. This consists of the following two filters: 1) Lipinski's rule of five (logP=6). 2) *Mycobacterium* Bioassay: The PubChem Bioassay Database provides ability to screen of similar compounds (70% - 80%) for *Mycobacterium* bioassay. It also provides searchable descriptions of each bioassay, including descriptions of the conditions and readouts specific to a screening protocol. Only not tested compounds in *Mycobacterium* bioassay are retained.

In general, virtual screening has become attractive for the computational filtering of large databases of compounds, in order to evaluate their properties and thus identify preferred compounds and eliminate those having undesired features [6].

E. Assay for Antitubercular Activity

The minimal inhibitory concentration of each compound against Bacillus Calmette–Guérin (BCG) was determined by the microplate alamar blue assay. Ethambutol, used in the assay as positive control drugs, were solubilized at DMSO to yield concentration of 100 µg/ml. Microbial suspension of BCG (1173P2) was diluted 1:10 in deionized water till its standard concentration reached to 0.5 Mcfarland. Initial compound solution was prepared in dimethylsulfoxide at concentration of 2000 µg/ml, and twofold serial dilutions were made in 7H9 broth in the microplates (duplicate). The microbial suspension of BCG was diluted 1:50 in 7H9 and inoculated. The plates containing compounds dilutions and BCG were incubated at 37 °C for 4 days, and then 20µl of 0.01% alamar blue solution with 12µl of 10% Tween 80 was added to each well. The color change from blue to pink was observed after 24 h, and 48h incubation, and the minimal inhibitory concentration (MIC) was defined as the lowest concentration of compounds that inhibited a color change.

III. Results

A. Prioritized Drug Targets

We used Unified weighted, set of many of the criteria covered in Table 1, to identification and validation of *Mycobacterium tuberculosis* drug targets. From these criteria a list of prioritized targets is generated (Table 1). As shown in Table 1, only two targets of metabolic pathways, contain all of the desired criteria. Our results suggest that pantothenate synthetase (*panC*) and 3-methyl-2-oxobutanoate hydroxymethyl transferase (*panB*) as a possible potent drug targets.

TABLE I
PRIORITIZED TARGETS LIST

Criteria	Advantageous Role of Criteria on Drug Design	PanB	PanC	Reference
Essentiality	Vital for bacterial growth and viability	+	+	Database of essential gene (http://tubic.tju.edu.cn/deg/)
Known Function	Easy to study	+	+	KEGG (Kyoto encyclopedia of genes and genomes) Database
Rate Limiting Steps in Metabolic Network	Not compensated by alternative pathways in Mtb	+	+	[2]
Expression under Various Condition	Important for growth and persistent state	+	+	[8]-[10]
Non-homology	Lead to adverse effects	+	+	[11]
Similarity to Anti-targets	Lead to adverse effects	+	+	[12]
Similarity to Gut Flora Proteins	Lead to adverse effects	+	+	[12], [13]
Involvement in Persistence	Important for persistent or latent bacilli	+	+	TDR Targets Database (TDRtargets.org)
Involvement in Virulence	Important for pathogenesis	+	+	[14]

B. Selected Active Compounds

We can only find inhibitors to pantothenate synthetase by searching on the list of prioritized targets and we structured our study based on their inhibitors. In this step, Lipinski's Rule of Five as a method for selecting drug-like compounds was used. When 15 active compounds were filtered with the Rule of Five, all of them succeeded. In spite of all of active compounds follow Lipinski's rules, three of them were discarded because of their high molecular weight (>500). The high molecular weight is related to hard and expensive synthesis that is not suitable for our study. Finally, antituberculosis activity as a second filtering method was applied and as a result one compound (Fig. 1) was selected from the 12 active compounds because of best antituberculosis activity, which has IC50 = 90 nM.

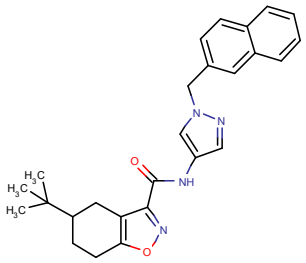


Fig. 1 Compound structure used for similarity search analysis

C. Similar Compounds

The strategy for the similarity search was using the functional class fingerprints based weighting using Tanimoto coefficient which is commonly used for binary data, as the quantitative measure of bit similarity [15]. In highly active compound against *panC* were used to screen the PubChem small molecule database consisting of 57916 similar compounds. The hits with pairwise Tanimoto coefficient values greater than 0.76 and smaller than 0.9 were selected.

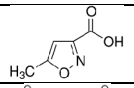
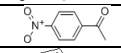
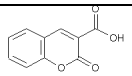
D. Virtual Library Design

In this first step of screening, 2239 hits were obtained by applying drug like and tested bioactiviy filter to identify more potent and efficient antitubercular compounds. We used defined biological outcomes as a mean for next step of filtration against *Mycobacterium* bioassay. The selected compounds from previous step, in which some contained *Mycobacterium* bioassay and others did not, were subjected to the further virtual screening. The PubChem Bioassay Database provides ability to screen of 2239 hits for *Mycobacterium* bioassay that 650 hits have tested in this bioassay. The raw screening results was obtained based on now publicly available bioassay on PubChem. Only not tested compounds in *Mycobacterium* bioassay, 1589 hits, are retained.

E. Assay for Antitubercular Activity

Unfortunately, none of selected compounds were available from vendors for purchasing. Therefore, we used commercial substructures in our study (essentially reduced to the time required for testing against MTB). The minimum concentrations of the hits for the complete inhibition of the bacterial growth per spot (minimum inhibitory concentration, MIC) were determined and reported in Table 2. Ethambutol was used as a reference compound and it showed the MIC value of 38.226 µM. Among the selected hits, two hits exhibited good biological activity, which have MIC at 47.29 µM and 65.53 µM, that near MIC of Ethambutol. The antitubercular activities of other hits are summarized in Table 2. This provided biological data useful for assessing the effectiveness and reliability of our protocol before undertaking the time-consuming and costly step of synthesis.

TABLE II
ANTITUBERCULAR ACTIVITY OF NEWLY IDENTIFIED COMPOUNDS IN THE WHOLE BACTERIUM ASSAY

Compound Name	Structure	MIC (µM) BCG
5-methylisoxazole-3-carboxylic acid		245.86
4-Nitroacetophenone		47.29
Indoline		65.53
Indole GR for analysis		266.72
Coumarin-3-carboxylic acid		164.13

IV. Discussion

The modern approach to drug design is based on the use of validated molecular targets. In recent years, many researchers are concentrated in determination and validation of drug targets [2], [8]-[14] but unfortunately, most of current drugs do not use these strategies for the development. In view of this, it seems, the current limiting factor is not anymore the availability of new drug targets [12], [16]. Thus, under these circumstances, different approaches are required to select, establish, and characterize a promising drug target [17]. Accordingly, we used multi-criteria as a unified prioritize drug targets. In multi-criteria searches, there is a high risk for Boolean intersection of the criteria so that only those targets with all of the desired traits are selected. However, a target may lack one or more preferred properties and still be the appropriate drug [4].

The increasing emergence of persistent bacteria highlights the need to develop novel TB drugs that kill them and shorten the length of treatment [3]. Unfortunately, most of current drugs are interesting only because of their activity against growing *Mycobacterium tuberculosis*. Further effort must be made to identify compounds acting on key targets that are essential for persistence [18]. In this study, we note to persistence and virulence as a critical criteria in prioritizing drug targets to obtain probability ability to combat with persistent bacteria.

The computer-aided drug design is widely used in workflow by many researchers because of speed and low cost. This approach was used as a useful tool to virtual screening. In general, virtual screening has become attractive for the computational filtering of large library of compounds, in order to evaluate their properties and thus design of virtual library that contain preferred compounds [6]. In this study, we used ligand-based virtual screening approach to identify the relatively novel compound with improved antibacterial activity against MTB. These compounds broadly derive from Pubchem Database, possibly providing the seeds for novel antitubercular compounds.

Previous gap analysis for computational methods in TB drug discovery by Ekins et al. 2010 [7] has showed limited use

of filtering of the library input or resulting hit lists for drug likeness or lead likeness. Early consideration of such factors has been advocated to improve ultimate success in drug identification. Many current combinatorial libraries do not discriminate against compounds that may have inadequate oral activity [7], [19].

V. Conclusion

In the present study, we seek to apply various *in silico* techniques with the aim of avoiding the time-consuming and costly step. A similarity search-based virtual screening approach has allowed the identification of compounds exhibiting inhibitory activity toward MTB from chemical Pubchem Database. Both Lipinski's rule of five and bioassay against *Mycobacterium* were used to optimize the efficiency of virtual screening and result in identifying more potent and efficient antitubercular compounds. Search of databases of commercially available compounds allowed the identification of putative hits. These were purchased and submitted to biological testing. In next step after biological assay, we have identified the two antitubercular agents with their MIC, 47.29 µM and 65.53 µM from pubchem database which is near MIC of Ethambutol as a reference compound. Therefore we believe that this compound is helpful to identify the new hits with better activity profile against *M. tuberculosis*.

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