RESEARCH ARTICLE

Bioactivity, Molecular Docking, and Pharmacophore Modeling of *Mycobacterium tuberculosis*: A Study Targeting the Microarray Data of the Microbe

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Abstract

Aim: *Mycobacterium tuberculosis* (TB) is the causative agent of tuberculosis (TB) and is responsible for more than eight million new infections worldwide and about two million deaths each year. New chemotherapeutics are required to treat the emerging threat of multidrug-resistant and extensively drug-resistant strains. **Materials and Methods:** Microarray data analysis techniques used to find novel gene target. In the present study, it was found that four novel genes named as MetZ (amino acid biosynthesis), aceAB (respiration system), relE (virulence activity), and kdaP (cell transport system) can be targeted that are inclusive of unique and important function in cell metabolism of organism. Discontinuing the function of these genes might kill the mycobacterium and prominently the specified relE gene, which plays a significant role in virulence effect, by inhibiting this gene, an individual with TB can be saved from TB disease if diagnosed and prognosis will be done at an early stage. Pharmacophore techniques are used in the present study to screen out lacs of molecule. Molecules downloaded from ZINC database are run through pharmacophore screening and docking procedure. **Results:** Finally, it was observed that top five (on the bases of binding energy) molecules from docking procedure gave improved result in ADME and toxicity analysis.

Key words: ADMET, AutoDock, drug discovery, microarray data analysis, *Mycobacterium tuberculosis*, scaffold hopping

INTRODUCTION

Mycobacterium genus consisted of 120 members,[1] which were acknowledged and categorized founded on the sequence similarity of 94.3% in the 16S ribosomal RNA.[2] These microbes are structurally distinguishable by an exceptionally composite cell wall envelope, which can be stained by the Ziehl–Neelsen acid-fast stain for its microscopic identification and morphological appearance. The bacterium genus is divided into few groups for the reason of diagnosis and treatment. The *Mycobacterium tuberculosis* (Mtb) complex abbreviated as MTBC entails of a closely related cultivable members, which can cause tuberculosis (TB) in their corresponding hosts, for example, *Mycobacterium bovis* in bovine (cattle), *Mycobacterium pinnipedii* in marine mammals, and Mtb in humans.[3]

Mtb is the etiological agent of TB, an older disease that has plagued human civilization since its emergence. Today, the World Health Organization (WHO) calculated that a third of the global population is infects with Mtb and reported a total 8.7 million new TB cases and 1.4 million TB deaths in 2011. The situation is also compound by coinfection with human immunodeficiency virus (HIV), with 13% of the new cases and approximately half a million deaths that are HIV-associated.[4]

Diabetes prevalence also saw a significant increase in the large number of people with TB.[5] Complacency and

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non-compliance due to the long drug treatment regimen has worsened the global situation, with the reemergence of TB and rapid spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB strains[6] and the recently identified totally drug-resistant-TB strains.[7,8]

TB infection starts with the inhalation of a small bacterial load in aerosolized precipitations into the lung cavity, and presents as two clinical outcome that is active and latent TB. Within the infected population, 90% of the individuals are capable to control and contain the infection without any sign of symptoms.[9] Pulmonary TB is the primary manifestation of the pathogen, where susceptible patients exhibit classical clinical symptoms including chronic cough, appetite loss, sputum production, weight loss, night sweats, fever, and hemoptysis. Extrapulmonary TB can also occur and accounts for around 10% of new TB cases, with a high prevalence in HIV-infected patients.[10]

Tuberculosis is a treatable disease if the standard TB drug treatment regimen is faithfully administered for 6 months (a combination of rifampicin [RIF], ethambutol, isoniazid [INH], and pyrazinamide for 2 months, followed by a 4-month continuation phase of RIF and INH) on early and accurate diagnosis.[11] Non-compliance to the long treatment period has controlled to the emergence of MDR-TB. To halt the spread of MDR-TB, the WHO recommends pre-therapy drug susceptibility testing before initiating a 20-month treatment entailing appropriate second-line drugs, which are regularly associated with multiple (and sometimes serious) side effects and lower cure rates. With the same drugs prescribed for HIV-TB coinfections, their efficacy and tolerability has been affected by the connections between anti-TB and antiretroviral therapies.[12]

The 2016 WHO report on the worldwide incidence of TB6 indicates that countless millions of people have died from TB. In 2015, data show that there were an estimated 10.4 million new TB cases worldwide, around 480,000 new cases of MDR-TB and an additional 100,000 people with rifampicin-resistant TB who were also newly eligible for MDR-TB treatment. The most recent treatment outcome data show a treatment success rate of 83% for TB, 52% for MDRTB, and 28% for extensively drug-resistant TB.[13]

Several attempts to develop novel drugs for infectious diseases have employed a target-based strategy, for example, conducting high-throughput assays of large compound libraries for inhibition of a critical enzyme/protein.[14]

METHODS AND MATERIALS

Data selection

Microarray data were used for analysis of thousands genes simultaneously which saved a lot of time. Microarray data were selected from NCBI database named as DNA repair mechanisms in mycobacteria (GDS326).[15]

Microarray data and analysis

The major aim of the current microarray procedure is to deliver a base of measurement for each gene that is functional in an organism's nucleic acid content. Microarray experiments are providing unprecedented quantities of genome-wide data on gene-expression patterns. Study of microarray data is dependent on finding cluster of similar genes depending onto the findings and thus grouping those genes that are "close" to each other.[16]

In the present study, at first normalization process was carried out, it was used to remove systemic errors or bias in a microarray experiment; normalization techniques are applied to the data. In normalization, one of the part log transformation normalization process was used.[17]

Second, the filtration was carried out to filter highly fluctuated genes. Filtration removes undesirable genes from further analysis. Highly fluctuated genes are the genes with very high standard deviation or ones that show high variation after treatment. This method extracts user-defined number of highly fluctuating genes present in the data.[17] After applying the procedure, from all the fluctuated genes, only 50 highly fluctuated genes were left. These genes were analyzed through Kyoto Encyclopedia of Genes and Genomes (KEGG).

Through KEGG database analysis, it was analysed that specificity of the genes in the metabolic pathways was observed that which gene has its active role (active role in important metabolic pathway) at which place of the metabolic pathway, and thus the importance of pathway is decided [Figure 1].

The objective of the present study was to target these genes, which will stop the effect and activity of whole organism.

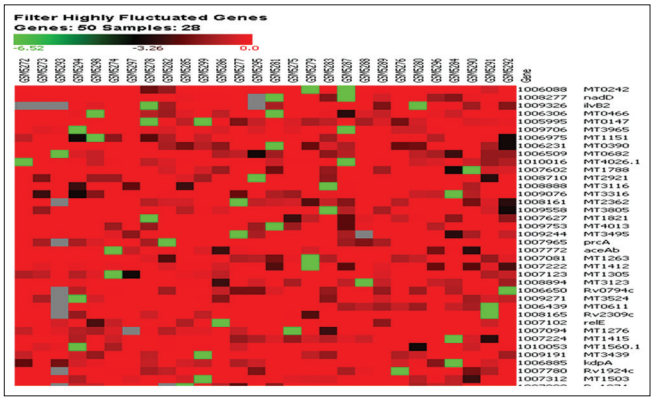

Figure 1: Gene selection by filtration (highly fluctuated gene) process by Genowiz software[17]

Analysis of the entire 50 genes was done with a final retrieval of four effective genes, which were playing an important role.

Homology model and validation

Homology modeling calculates the 3D structure of a target protein built on the sequence alignment through one or more template proteins of known structure. Homology modeling process consists of four steps:

1. Selects homologous template proteins of known structure,
2. Pick the best template or set of templates,
3. Optimizing the multiple sequence alignment between query and template protein sequences, and
4. Creating the homology model for the query sequence that resembles as closely as possible the structures of the templates, accommodating for deletions and insertions of query residues with respect to the template structures.

The present study aligned multitemplate modeling approach with the popular MODELLER homology modeling software in our free HHpred server http://toolkit.tuebingen.mpg.de/hhpred that in all offered open source software for running MODELLER with the new restraints at https://bitbucket.org/soedinglab/hh-suite.[18]

A key goal of protein engineering is the enhancement of protein stability. The models were analyzed by means of a Ramachandran plot (RAMPAGE, de Bakker and Lovell, http://raven.bioc.cam.ac.uk/rampage.php).[19]

Pocket finding

The protein binding to several molecules occurring at different binding pockets of a protein's surface that represents its several biochemical functions. Binding pockets for ligands are usually clefts or cavities of a protein. There are various site detection 16 and pocket search 17 (PS) methods available to accomplish this task.[20] SiteHound-web online server was used in the present study to discover the pocket. In SiteHound grid, maps are calculated for the probes covering the entire proteins with 1 and 0.9 A° spacing, respectively. SiteHound was test with both carbon and phosphate probes. SiteHound and Q-SiteFinder ranks the results according to the TIE, which is the sum of non-bonded interaction energy of all probe points with the protein atoms in the detected binding site.[21]

Molecular docking

The mechanism of binding of drug with the target protein is called docking.[22] Docking can be used to find inhibitors for specific target proteins and thus to design new stable drugs from docking results.[23] Docking can be calculated by binding energy (energy release during protein and ligand interaction). In this project, AutoDock software was used for docking. AutoDock is a suite of free open-source software for the computational docking and virtual screening of small molecules to macromolecular receptors. The suite presently includes several complementary tools, in which have used AutoDock[16,24-26] (a computational docking program based on an empirical free-energy force field and rapid Lamarckian genetic algorithm search method[27]) and Raccoon (an interactive graphical tool for virtual screening and analysis[28]) tool. In the discussed study, four protein from Mtb as a receptor was used. Natural molecules library approximately 200 molecules and ZINC database molecules approximately nine lac molecules as ligands were used for docking.

Pharmacophore model preparation

Pharmacophore designing is the initial step before starting the screening. The pharmacophore modal was created using our LigandScout software.[29] LigandScout software can support to screen out molecule from the pharmacophore model. LigandScout starts with a macromolecule/ligand complex and automatically detects bound ligands generating a standard residue around the non-standard residues.[30] For creating a pharmacophore model, use the best three small molecules on the bases of binding energy with target. Ligand–ligand pharmacophore used to make pharmacophore model. Further generating the pharmacophore model, this same tool used to perform virtual screening of the pharmacophore against already created ligand library of approximately nine lakh ligand molecules each obtained from ZINC database. We got 7384 ligands from aceAB protein, 11522 ligands from relE protein, 3359 ligands from kdpA protein, and 1105 ligands from metZ protein from virtual screening again these ligands subjected to docking with Mtb protein. From that, the ligands with the best minimum binding energies were selected for the further studies.

ADME-Tox analysis

Once the docking was completed, ADMET analysis and toxicity prediction were done. An important step remains in the drug discovery method, mostly in the advanced stages of lead discovery, is analysis of the ADME and over toxicity properties of drug candidates. Over 50% of the molecules were unsuccessful due to ADMET deficiencies during development. To evade this failure at the development, a set of *in vitro* ADME screens has been implemented in most pharmaceutical companies with the purpose of removal compounds in the discovery stage that are likely to fail further down the line. PreADMET and FAFDrug4 is suitable for high throughput screening and combinatorial chemistry library design considering the Lipinski's rule or lead-like rule, drug absorption, and water solubility.[31] We used the PreADMET (http://preadmet.bmdrc.kr/) tool[17,32] and FAFDrug4 (http://fafdrugs3.mti.univ-paris-diderot.fr/).[33] The PreADMET program provides rapid and reliable data of drug-likeness and ADME properties.[24]

Scaffold hopping

The aim of scaffold hopping is to discover structurally novel compounds starting from known active compounds by modifying the central core structure of the molecule. This approach requires the availability of a template – a chemical structure displaying the desired biological activity, and it is based on the assumption that the same biological activity can be exerted by other compounds that maintain some essential features of the template but are structurally different otherwise.[34] Scaffold hopping done with online server mcule 1-click-scaffold-hop (https://mcule.com/apps/1-click-scaffold-hop/).[35]

RESULTS

Microarray data analysis

Microarray data analysis was done by Genowiz software. *Mycobacterium* sp. data were taken from GEO database (NCBI-DataSet Record GDS326) to focus on the DNA repair mechanisms in the bacterial species. In this dataset, molecular analysis of DNA repair mechanisms was taken into consideration to hinder the metabolic activities of the bacterial species and around 28 samples were considered to target 4417 gene.

Dataset was projected on Genowiz software; further, highly fluctuated genes were selected. Normalization and filtration of the genes was done with the aid of same platform, in which the genes showing peculiar and highest variations were considered.

Four genes were found potent to be targeted and these are as follows:

1. MetZ gene (o-succinylhomoserine sulfhydrylase)

KEGG pathway ID - Rv0391.

Involved in pathway - Mtu00270 cysteine and methionine metabolism.

MetZ gene is involved in amino acid biosynthesis specifically in cysteine and methionine metabolic pathways. If this gene targeted than amino acid synthesis can be hindered that will directly affect the formation of protein, in turn inhibiting the production of protein that has cysteine and methionine as key components.

2. AceAB gene (isocitrate lyase)

KEGG pathway ID - Rv1916

This gene involves in the respiration system of the organism and also on the carbon uptake mechanism. If the function of this gene will blocked, bacterial growth can be controlled.

3. RelE gene (Toxin relE)

KEGG pathway ID - Rv1916

This gene is involved in the virulence activity of the bacterium that as a result is affecting the host body. Virulence is the degree of damage caused by microbes to its host. If the function of gene is slowed down/blocked than host body can overcome with the damage caused by the bacterial species and further medications might help in the removal/killing of the Mtb.

4. kdpA gene (potassium-transporting ATPase ATPase A chain)

KEGG pathway ID - Rv1916

This gene is involved in the cell transportation process. Potassium-transporting chain transports the essential molecule inside and outside the cell through the potassium-transporting ATPase. If this is targeted than molecule transportation will be hindered/stop that can affect the metabolic process in the cell, forcing the cell to collapse [Table 1].

Homology model and validation

Four gene selected through microarray data and 3 out of 4 genes selected to design a 3D structure of protein, one gene aceAB 3D structure already on PDB (PDB id- 1F8I). For homology modeling HHpred online server used, it is a fast server for remote protein homology detection and structure prediction and is the first to implement pair-wise comparison of profile hidden Markov models. It allows searching a wide choice of databases, such as the PDB, SCOP, Pfam, SMART, COGs, and CDD. It accepts a single query sequence or a multiple alignment as input.

Validating gene 3D structure was analyzed through Ramachandran plot. Ramachandran plot was analyzed through RAMPAGE[Table 2].[19]

All four-proteins came under favored region so it means it is showing high stability and thus it can be used as target.

Pocket finding

After designing 3D structure of protein next important part is pocket finding, where molecule will attach with the protein. The identification of ligand binding sites can also

Table 1: Similarity find with **Homo sapiens**

Gene	***Similarity to Homo sapiens***
aceAB	No significant similarity found
relE	No significant similarity found
kdpA	Color alignment<40, identity=48%
metZ	Color alignment 50-80, Identity=36%

Table 2: Ramachandran plot analysis through RAMPAGE

Genes	Number of residues in favored region	Number of residues in allowed region	Number of residues in outlier region
aceAB	97.4%	2.6%	0.0%
relE	96.8%	3.2%	0.0%
kdpA	95 (96.0%)	4 (4.0%)	0.0%
MetZ	99.3%	0.7%	0.0%

be an important part of the drug discovery process. Knowing the location of binding sites facilitates virtual screening for hits, lead optimization, and identification of features that influence the selectivity of binding. Here, it was described that the SiteHound-web server for identification of ligand binding sites in protein structures. It is used as an energy-based approach to identify regions with high potential for interaction with ligands.

A unique feature of SiteHound-web is that it implements the use of different probes to characterize a protein structure, which enables not only the identification of different types of binding sites but also a preliminary description of its interaction properties [Table 3].

Table 3: Binding site of 1st rank result in SiteHoundx for all 4 genes

Genes	Binding site
aceAB	THR 5, PRO 6, THR 7, ASP 8, ASN 10, LEU 11, GLN 13, THR 14, LYS 18, ASN 27, GLU 32, ILE 33, VAL 35
RelE	ASN 51, ASP 52, LEU 53, GLU 54, LEU 56, ARG 90, PRO 93, CYS 94, PRO 96, ARG 97
MetZ	SER 37, ARG 40, VAL 41, PHE 44, LEU 101, ARG 102, LEU 103, ILE 104, SER 115, ILE 117, SER 145, PRO 146, SER 147, GLY 148, ARG 150, THR 151, PRO 152, THR 153, THR 154
kdpA	THR 19, VAL 42, PHE 43, GLY 44, VAL 56, ASP 57, PRO 58, GLY 59, GLU 61, GLN 62, ARG 63, THR 66, LEU 69

Molecular docking with naturally occurring small molecules

From the literature cited, it was found that natural protein molecules are used as ligands. In certain aspects, drug candidates can be provided from large libraries of synthetic or natural compounds (e.g. pharmaceutical small molecule compounds and/or peptides). One example is an FDA approved library of compounds that can be used by humans. Several commercial libraries can immediately be used in the screens. Such libraries can include the analogs of naturally occurring or synthetic small molecules. Non-limiting examples of naturally occurring small molecules include alkaloids, glycoside, lipids, phenazines, phenols, polyketide, terpenes, or tetrapyrroles.[36] Approximately 200 molecule library was created to dock the targets [Table 4].

Table 4: Top 3 docking result with naturally occurring small molecules

Gene id	Zinc database id	Binding energy (kcl/mol)
aceAB	zinc64624093	−7.91
	zinc64624174	−7.65
	zinc64624173	−7.34
relE	zinc18210358	−7.54
	zinc1532734	−7.35
	zinc1883067	−6.99
kdpA	zinc64624093	−8.44
	zinc64624173	−8.19
	zinc59789263	−7.63
metZ	zinc64624173	−9.55
	zinc64624174	−9.09
	zinc3875383	−7.96

Pharmacophore designing

Pharmacophore sites (site points) of the ligands were defined by a set of six pharmacophore features: H-bond donor (D), H-bond acceptor (A), hydrophobic group (H), negatively charged group (N), positively charged group (P), and aromatic ring (R).

Each pattern is associated with a geometric representation (point, group, or vector) and additional flags for hydrogen bond acceptors and donors.[37] Pharmacophore model designed by the best three molecules on basis of binding energy. From this pharmacophore model, 9-lac molecules were retrieved from ZINC database to screen out, finally, the resulted molecules were used to dock with the main target. Pharmacophore model helps to saves time so as to dock 9-lac molecules with target molecules [Figure 2 and Table 5].

These all molecules were identified by pharmacophore process and were docked with main targets, to get the result.

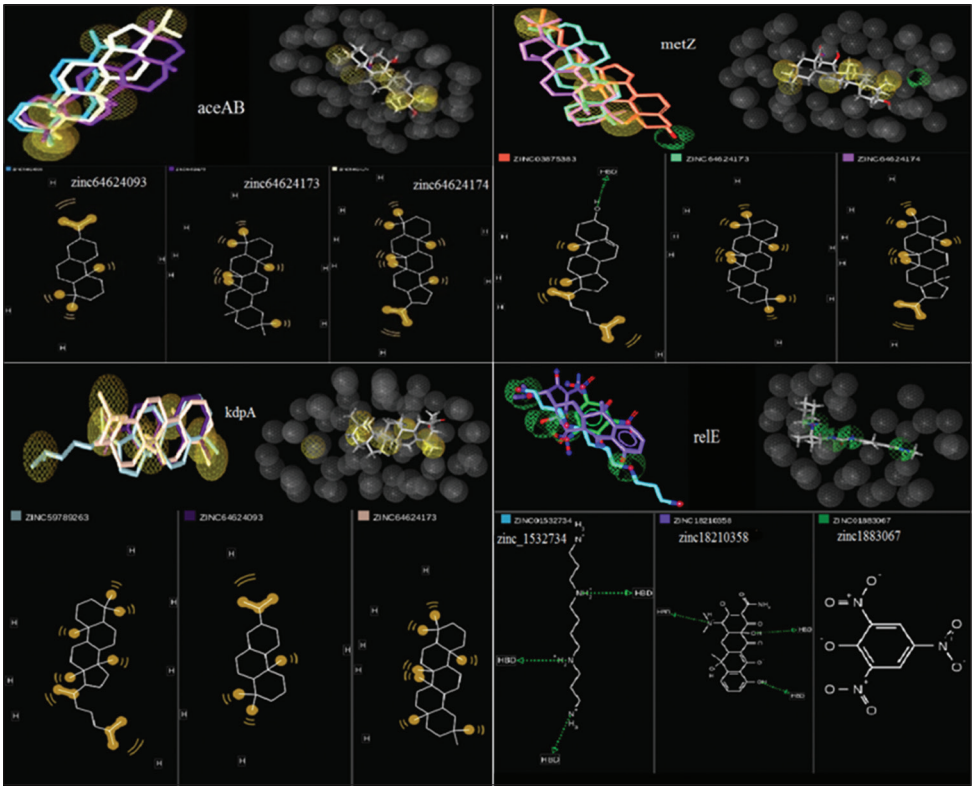

Figure 2: Pharmacophore of all target docking results

Table 5: Pharmacophore result of molecules

Gene id	ZINC database id	Pharmacophore result
aceAB	zinc64624093	7834 molecules
	zinc64624174	
	zinc64624173	
relE	zinc18210358	11522 molecules
	zinc1532734	
	zinc1883067	
kdpA	zinc64624093	3359 molecules
	zinc64624173	
	zinc59789263	
metZ	zinc64624173	1105 molecules
	zinc64624174	
	zinc3875383	

Docking

Automated docking is widely used for the prediction of biomolecular complexes in structure/function analysis and in molecular design. For docking process, AutoDock software[38] was used. AutoDock combines an empirical free energy force field with a Lamarckian genetic algorithm, providing fast prediction of bound conformations with predicted free energies of association.[39] Docking experiments were performed with AutoDock4, and all the molecules were docked with the target on the same pocket on which the natural molecules were docked [Tables 6 and 7].

The above 5 molecules were selected take for checking the ADMET properties so that which molecules are suitable as drug for further process and in the clinical trials.

ADMET

There is no doubt that ADME/Tox drug properties, absorption, distribution, metabolism, elimination, and toxicity, are properties crucial to the final clinical success of a drug candidate. It has been estimated that nearly 50% of drugs fail because of unacceptable efficacy, which includes poor bioavailability because of ineffective intestinal absorption and undesirable metabolic stability 1. For ADMET/TOX and drug-likeness analysis, online free tool FAFDrug4[40] and PreADMET[41] was used. An ADMET profiling using a traffic lights representation: An oral bioavailability evaluation considering Lipinski, Veber, Egan, and Bayer rules. A drug safety profiling considering the GSK 4/400 rule according to Gleeson *et al.*,[42] the Pfizer 3/75 rule,[43] a phospholipidosis inducing estimation according to Przybylak *et al.*,[44] and finally the Lilly MedChem Rules rating.[45] A compound positioning within the Pfizer 3/75 rule,[46] according to Hughes *et al*. [Table 8].[43]

Table 6: Top 5 binding results of pharmacophore molecules with targets

aceAB (zinc id with binding energy)	kdpA (zinc id with binding energy)	metZ (zinc id with binding energy)	relE (zinc id with binding energy)
ZINC08385540–8.72	ZINC05483016–8.56	ZINC08385536–9.91	ZINC91252717–9.85
ZINC04915551–8.58	ZINC35480325–8.47	ZINC17185454–9.07	ZINC75977032–9.09
ZINC04915379–8.51	ZINC59506386–8.37	ZINC86860236–8.97	ZINC72147438–9.08
ZINC04915349–8.48	ZINC04544548–8.36	ZINC65239465–8.71	ZINC91683995–8.85
ZINC04915370–8.36	ZINC02846570–8.26	ZINC17111855–8.66	ZINC08266355–8.82

Table 7: Final result after comparison between natural molecules and molecules from pharmacophore select high binding energy molecules

aceAB (zinc id with binding energy)	kdpA (zinc id with binding energy)	metZ (zinc id with binding energy)	relE (zinc id with binding energy)
ZINC08385540–8.72	ZINC05483016–8.56	ZINC08385536–9.91	ZINC91252717–9.85
ZINC04915551–8.58	ZINC35480325–8.47	ZINC64624173–9.55	ZINC75977032–9.09
ZINC04915379–8.51	ZINC64624093–8.44	ZINC64624174–9.09	ZINC72147438–9.08
ZINC04915349–8.48	ZINC59506386–8.37	ZINC17185454–9.07	ZINC91683995–8.85
ZINC04915370–8.36	ZINC04544548–8.36	ZINC86860236–8.97	ZINC08266355–8.82

Table 8a: ADME/tox analysis of molecules from FAFDRUG4 online free ADME/tox filtering tool.[40] For oral absorption, these zones are obtained with the following descriptors ranges: LogP (–2 to 5), molecular weight (150 to 500), tPSA (20 to 150), rotatable bonds (0 to 10), H-bonds acceptors (0 to 10), and donors (0 to 5)

I.D.	tPSA	logP	H-bond acceptor	H-bond doner	Solubility	Veber rule	Egan rule	Phospholipidosis	Fsp3	State
aceAB										
zinc4915349	37.38	4.94	3	0	1770.62	Good	Good	Non-inducer	0.64	accepted
zinc4915370	37.38	4.94	3	0	1770.62	Good	Good	Non-inducer	0.64	accepted
zinc4915379	37.38	4.94	3	0	1770.62	Good	Good	Non-inducer	0.64	accepted
zinc4915551	55.84	4.75	5	0	1955.19	Good	Good	Non-inducer	0.60	accepted
zinc8385540	35.53	7.47	3	0	285.47	Good	Good	Non-inducer	0.97	accepted
relE										
zinc91252717	34.37	0.02	5	4	74808.5	Good	Good	Non-inducer	1.00	accepted
zinc75977032	50.01	2.96	5	4	7185.44	Good	Good	inducer	0.68	accepted
zinc72147438	52.31	2.51	3	4	11320.5	Good	Good	Non-inducer	0.70	accepted
zinc91683995	75.76	3.40	7	3	5430.24	Good	Good	Inducer	0.41	accepted
zinc8266355	62.64	3.16	5	3	9217.92	Good	Good	Non-inducer	0.88	accepted
kdpA										
zinc5483016	63.99	4.45	5	1	2244.88	Good	Good	Non-inducer	0.06	accepted
zinc35480325	90.65	3.27	6	2	5232.48	Good	Good	Non-inducer	0.39	accepted
zinc64624093	0.00	8.08	0	0	384.24	Good	Good	Non-inducer	0.06	accepted
zinc59506386	37.38	6.12	3	0	710.52	Good	Good	Non-inducer	0.26	accepted
zinc4544548	99.78	5.05	5	1	1645.61	Good	Good	Non-inducer	0.06	accepted
metZ										
zinc8385536	35.01	7.88	3	0	192.69	Good	Good	Non-inducer	0.88	accepted
zinc64624173	0.00	11.6	0	0	24.96	Good	Good	Non-inducer	1.00	accepted
zinc64624174	0.00	11.6	0	0	29.12	Good	Good	Non-inducer	1.00	accepted
zinc17185454	35.53	7.47	3	0	285.47	Good	Good	Non-inducer	0.97	accepted
zinc86860236	80.59	6.88	4	3	414.50	Good	Good	Non-inducer	0.06	accepted

Table 8b: ADME analysis

I.D.	BBB	Buffer solubility (mg/L)	CaCO2	HIA	MDCK	Pure water solubility (mg/L)	Skin permeability
zinc4915349	0.526623	19.6617	34.6666	98.33	17.9498	0.622172	−2.74082
zinc4915370	0.526623	19.6617	34.6666	98.33	17.9498	0.622172	−2.74082
zinc4915379	0.526623	19.6617	34.6666	98.33	17.9498	0.622172	−2.74082
zinc4915551	0.151445	386.945	29.1976	97.68	8.7421	2.01242	−2.71028
zinc8385540	11.5458	32.4211	55.755	98.67	0.04447	0.0246501	−2.84229
zinc91252717	0.146789	5.6357	23.4621	84.61	0.538258	2.17122	−5.21528
zinc75977032	0.508811	124.954	20.4032	90.13	0.69959	1022.24	−4.78173
zinc72147438	0.362891	77.955	9.07612	90.98	2.97542	2939.1	−4.75565
zinc91683995	0.287537	1424.42	38.7737	89.94	3.56106	246.778	−4.5368
zinc8266355	0.845602	700.753	17.3146	78.98	0.741002	1145.53	−4.94216
zinc5483016	0.348582	0.09647	25.073	96.56	19.8321	0.842235	−3.0675
zinc35480325	1.59357	5748.82	17.4525	92.27	1.00255	22.8981	−3.69418
zinc64624093	17.4862	93.0812	22.2014	100	67.1758	0.0434132	−0.950175
zinc59506386	0.562999	5.74451	41.325	98.72	0.431511	0.0453475	−2.06574
zinc4544548	0.361824	19386.6	35.7872	96.79	0.202134	0.0297085	−2.92086
zinc8385536	8.62423	337.594	57.2745	98.94	0.043936	0.0177384	−3.37574
zinc64624173	26.6379	20.359	22.2014	100	68.0161	0.0001354	−1.3749
zinc64624174	22.1063	18.254	22.2014	100	68.0181*	9.85275	−1.16741
zinc17185454	11.5458	32.4211	55.755	98.67	0.044470	0.0246501	−2.84229
zinc86860236	2.28775	1536.19	21.4036	94.27	0.044001	0.141937	−3.40489

BBB: *In vivo* blood–brain barrier penetration (C.brain/C.blood). Buffer solubility mg/L: Calculated water solubility value in buffer system by SK atomic types (mg/L). CaCO2: *In vitro* CaCO2 cell permeability (Human colorectal carcinoma). HIA: Human intestinal absorption (HIA, %). MDCK: *In vitro* MDCK cell permeability (Madin–Darby Canine Kidney). Pure water solubility mg/L: Calculated water solubility in pure water by SK atomic types (mg/L). Skin permeability: *In vitro* skin permeability (transdermal delivery)

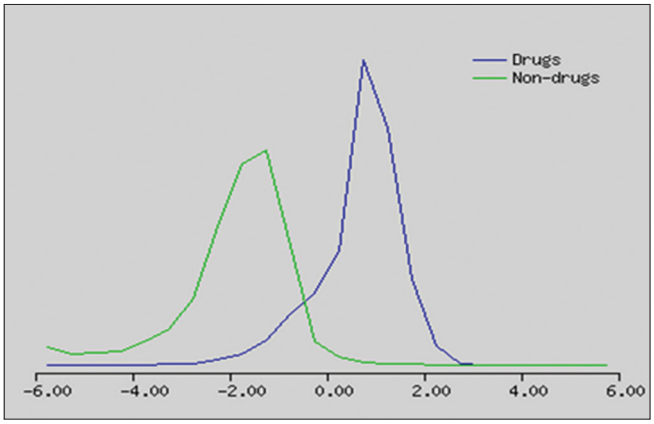

Figure 3: Reference graph for druglikeness. Range should be from −1.00 to 2.20 for passing drug conditions

Bioactivity and druglikeness

Bioactivity of the molecule calculate by Molinspiration Chemoinformatics[51] web tool and druglikeness calculated by Molsoft online tool[Table 9].[52]

Bioactivity of all selected agents was evaluated against six different protein structures. Biological activity is measured by bioactivity score that are categorized under three different ranges[Figure 3]:

1. If bioactivity score is more than 0.00, having considerable biological activity.
2. If bioactivity score is −0.5 to 0.00, having moderately activity.
3. If bioactivity score is less than −0.50, having inactivity.[53]

Scaffold hopping

All best molecules which got from docking take for scaffold hopping to find out more molecules that are similar. Scaffold hopping done with online server mcule 1-click-scaffold-hop [Table 10].

Got another 5 molecule by scaffold hopping by base molecules show high similarity with base molecules. These molecules also can use as inhibitor for genes. Therefore, 25 molecules for 1 genes which can use for further analysis.

Table 8c: Drug-likeness properties of molecules

I.D.	CMC-like Rule	Lead-like Rule	MDDR-like Rule	Rule of Five	WDI-like Rule
zinc4915349	Qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc4915370	Qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc4915379	Qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc4915551	Qualified	Violated	Mid-structure	Suitable	In 90% cutoff
zinc8385540	Not qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc91252717	Failed	Violated	Mid-structure	Suitable	Failed
zinc75977032	Failed	Violated	Mid-structure	Suitable	Failed
zinc72147438	Failed	Violated	Mid-structure	Suitable	Failed
zinc91683995	Failed	Violated	Mid-structure	Suitable	Failed
zinc8266355	Failed	Violated	Mid-structure	Suitable	Failed
zinc5483016	Qualified	Violated	Mid-structure	Suitable	In 90% cutoff
zinc35480325	Qualified	Violated	Mid-structure	Suitable	In 90% cutoff
zinc64624093	Not qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc59506386	Not qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc4544548	Qualified	Violated	Mid-structure	Suitable	In 90% cutoff
zinc8385536	Not qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc64624173	Not qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc64624174	Not qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc17185454	Not qualified	Violated	Mid-structure	Suitable	Out of 90% cutoff
zinc86860236	Failed	Violated	Mid-structure	Suitable	Failed

CMC-like Rule: CMC-like rule: Qualified/not qualified. Lead-like Rule: ???. MDDR-like Rule: MDDR-like rule: Non-drug-like/drug-like/mid-structure. Rule of Five: Lipinski's Rule, so-called (Rule of Five), is published by Christopher A. Lipinski *et al.* in Pfizer Central Research (Groton, NJ, USA). They selected a subset of 2245 compounds from WDI database and defined drug-like character through this subset.[31,47] WDI-like Rule: WDI-like rule: In 90% cutoff/out of 90% cutoff. WDI: World Drug Index

Table 8d: Toxicity

I.D.	Ames test	Carcino Mouse	Carcino Rat	Acute daphnia toxicity	hERG inhibition
zinc4915349	Mutagen	Negative	Negative	0.017828	Low risk
zinc4915370	Mutagen	Positive	Negative	0.017828	Low risk
zinc4915379	Mutagen	Positive	Negative	0.017828	Low risk
zinc4915551	Mutagen	Positive	Negative	0.0352271	Low risk
zinc8385540	Non-mutagen	Negative	Positive	0.0139707	Medium risk
zinc91252717	Mutagen	Negative	Positive	87.0801	Medium risk
zinc75977032	Mutagen	Negative	Negative	2.04882	Ambiguous
zinc72147438	Non-mutagen	Negative	Negative	4.88176	Ambiguous
zinc91683995	Mutagen	Negative	Negative	0.370616	High risk
zinc8266355	Mutagen	Negative	Negative	1.17981	Low risk
zinc5483016	Mutagen	Positive	Negative	0.00925519	Medium risk
zinc35480325	Mutagen	Negative	Negative	0.0579144	Medium risk
zinc64624093	Non-mutagen	Negative	Positive	0.0125506	Medium risk
zinc59506386	Non-mutagen	Negative	Negative	0.00188314	Low risk
zinc4544548	Mutagen	Negative	Negative	0.0039737	Medium risk
zinc8385536	Non-mutagen	Negative	Positive	0.0111318	Low risk
zinc64624173	Non-mutagen	Negative	Positive	0.00276071	Medium risk

(Contd..)

Table 8d: (*Continued*)

zinc64624174	Non-mutagen	Negative	Positive	0.00104795	Medium risk
zinc17185454	Non-mutagen	Negative	Positive	0.0139707	Medium risk
zinc86860236	Non-mutagen	Positive	Positive	0.0132798	Low risk

Ames test: Ames test is a simple method to test mutagenicity of a compound, which is suggested by Dr. Ames. It uses several strains of the bacterium *Salmonella typhimurium* that carry mutations in genes involved in histidine synthesis so that they require histidine for growth. The variables being tested is the mutagens ability to cause a reversion to growth on a histidine-free medium.[48] Carcinogens as Frameshift Mutagens: Metabolites and Derivatives of 2-acetylaminofluorene and other Aromatic Amine Carcinogens. PNAS 69: 3128-213].[43,49,50] Carcino Mouse: 2 years carcinogenicity bioassay in mouse. Carcino Rat: 2 years carcinogenicity bioassay in rat\. daphnia_at: Acute daphnia toxicity, hERG_inhibition: *In vitro* human ether-a-go-go-related gene channel inhibition

Table 9: Bioactivity and druglikeness

I.D.	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor	Druglikeness	Accepted/not accepted
zinc4915349	−0.11	−0.19	−0.61	−0.13	−0.16	−0.16	0.47	Accepted
zinc4915370	−0.11	−0.19	−0.61	−0.13	−0.16	−0.16	0.47	Accepted
zinc4915379	−0.11	−0.19	−0.61	−0.13	−0.16	−0.16	0.47	Accepted
zinc4915551	−0.12	−0.16	−0.52	−0.17	−0.07	−0.16	0.58	Accepted
zinc8385540	0.14	0.04	−0.32	0.39	0.12	0.39	−0.26	Accepted
zinc91252717	−0.25	0.13	−0.44	−0.71	−0.50	−0.07	−0.78	Accepted
zinc75977032	0.11	0.02	−0.22	−0.24	−0.04	0.03	1.00	Accepted
zinc72147438	0.57	0.25	−0.08	0.10	0.18	0.18	0.94	Accepted
zinc91683995	0.23	0.20	−0.09	−0.86	−0.05	0.04	1.06	Accepted
zinc8266355	0.01	−0.00	−0.29	−0.38	0.21	−0.01	−0.03	Accepted
zinc5483016	−0.57	−0.76	−0.49	−0.78	−0.53	−0.69	−0.61	Accepted
zinc35480325	−0.37	−0.67	−0.34	−0.60	−0.36	−0.46	1.00	Accepted
zinc64624093	0.08	0.18	−0.34	0.37	−0.06	0.31	−0.97	Accepted
zinc59506386	−0.17	−0.23	−0.34	−0.07	−0.25	−0.28	0.44	Accepted
zinc4544548	−0.96	−1.45	−0.77	−1.03	−1.28	−0.64	−0.46	Accepted
zinc8385536	0.22	−0.12	−0.17	0.41	0.04	0.31	0.01	Accepted
zinc64624173	0.10	0.00	−0.23	0.35	−0.05	0.26	−0.57	Accepted
zinc64624174	0.15	0.03	−0.22	0.41	0.06	0.29	−0.40	Accepted
zinc17185454	0.14	0.04	−0.32	0.39	0.12	0.39	−0.26	Accepted
zinc86860236	0.18	−0.12	−0.32	0.61	0.12	0.51	0.16	Accepted

Table 10: Scaffold Hopping result molecules

I.D.	MOLECULE 1 mcule ID - Score	MOLECULE 2 mcule ID - Score	MOLECULE 3 mcule ID - Score	MOLECULE 4 mcule ID - Score	MOLECULE 5 mcule ID - Score
aceAB					
zinc4915349	MCULE-71872 56574-0 0.8777	MCULE-29420 51831-0 0.8759	MCULE-18693 50907-0 0.8687	MCULE-2175 556590-0 0.8568	MCULE-1055 326046-0 0.8556
zinc4915370	MCULE-7187 256574-0 0.8777	MCULE-29420 51831-0 0.8759	MCULE-18693 50907-0 0.8687	MCULE-21755 56590-0 0.8568	MCULE-10553 26046-0 0.8556
zinc4915379	MCULE-71872 56574-0 0.8777	MCULE-29420 51831-0 0.8759	MCULE-18693 50907-0 0.8687	MCULE-21755 56590-0 0.8568	MCULE-10553 26046-0 0.8556

(Contd...)

Table 10: (*Continued*)

I.D.	MOLECULE 1 mcule ID - Score	MOLECULE 2 mcule ID - Score	MOLECULE 3 mcule ID - Score	MOLECULE 4 mcule ID - Score	MOLECULE 5 mcule ID - Score
zinc4915551	MCULE-9383050417-0 0.8470	MCULE-9184143140-0 0.8447	MCULE-6640197758-0 0.8400	MCULE-3269456781-0 0.8301	MCULE-7187256574-0 0.8293
zinc8385540	MCULE-8094535579-0 0.8328	MCULE-1823233726-0 0.8275	MCULE-8814403403-0 0.8197	MCULE-5615873580-0 0.8196	MCULE-8189159141-0 0.8153
relE					
zinc91252717	MCULE-8674319594-0 0.8912	MCULE-9391343164-0 0.8628	MCULE-8069641396-0 0.8567	MCULE-3971913353-0 0.8560	MCULE-1828460427-0 0.8396
zinc75977032	MCULE-7139116397-0 0.8575	MCULE-4090849415-0 0.8520	MCULE-2115514505-0 0.8438	MCULE-4219051159-0 0.8429	MCULE-5567053350-0 0.8407
zinc72147438	MCULE-4811115522-0 0.8754	MCULE-2589945220-0 0.8691	MCULE-3634413421-0 0.8670	MCULE-3543259564-0 0.8547	MCULE-2115514505-0 0.8373
zinc91683995	MCULE-8069827554-0 0.8818	MCULE-8746547068-0 0.8797	MCULE-6656749706-0 0.8779	MCULE-8322086378-0 0.8685	MCULE-1126042927-0 0.8677
zinc8266355	MCULE-3551556048-0 0.8592	MCULE-4025187583-0 0.8534	MCULE-3957648827-0 0.8444	MCULE-8934375828-0 0.8435	MCULE-1948460838-0 0.8273
kdpA					
zinc5483016	MCULE-8869050757-0 0.9270	MCULE-1061657898-0 0.9247	MCULE-1634868698-0 0.9135	MCULE-9258411681-0 0.9133	MCULE-3905291011-0 0.9123
zinc35480325	MCULE-8011013126-0 0.9117	MCULE-8871678910-0 0.8962	MCULE-9191003523-0 0.8921	MCULE-1944663181-0 0.8717	MCULE-4335441442-0 0.8649
zinc64624093	MCULE-4138183274-0 0.9168	MCULE-3098166722-0 0.8809	MCULE-1823233726-0 0.8685	MCULE-7831325076-0 0.8604	MCULE-4090849415-0 0.8536
zinc59506386	MCULE-4553592953-0 0.8763	MCULE-3776059174-0 0.8609	MCULE-8715812417-0 0.8567	MCULE-8885796253-0 0.8502	MCULE-4139914727-0 0.8497
zinc4544548	MCULE-5059420198-0 0.9011	MCULE-1392357455-0 0.8980	MCULE-8509437124-0 0.8974	MCULE-1665567712-0 0.8952	MCULE-1819084205-0 0.8941
metZ					
zinc8385536	MCULE-1823233726-0 0.8385	MCULE-8094535579-0 0.8226	MCULE-8189159141-0 0.8155	MCULE-1994866798-0 0.8118	MCULE-8410351029-0 0.8114
zinc64624173	MCULE-1581197064-0 0.8599	MCULE-8189159141-0 0.8369	MCULE-4138183274-0 0.8317	MCULE-1823233726-0 0.8287	MCULE-5615873580-0 0.8094
zinc64624174	MCULE-1581197064-0 0.8599	MCULE-8189159141-0 0.8485	MCULE-4138183274-0 0.8341	MCULE-1823233726-0 0.8222	MCULE-5615873580-0 0.8173
zinc17185454	MCULE-8094535579-0 0.8328	MCULE-1823233726-0 0.8275	MCULE-8814403403-0 0.8197	MCULE-5615873580-0 0.8196	MCULE-8189159141-0 0.8153
zinc86860236	MCULE-1581197064-0 0.8369	MCULE-4090849415-0 0.8204	MCULE-3984725706-0 0.8175	MCULE-8189159141-0 0.8118	MCULE-3551556048-0 0.8116

CONCLUSION, CHALLENGES, AND DEVELOPMENT

Typical TB drugs are persevered for the previous 50 years, in spite of having limited efficiency in latent TB and MDR/XDR-TB. The prolonged treatment periods and various side effects with poor abilities rising from high dosage and drug–drug interactions on coadministration with other chronic disease treatments such as HIV and diabetes that has complicated the control of comprehensive epidemic globally. An ideal TB drug should, therefore, possess the following criteria:

1. Short treatment duration,
2. Target drug-resistant strains,
3. Simplify treatment by reducing pill burden,
4. Lower dose frequency, and
5. Can be coadministered with HIV or diabetes medication.[54]

In the present study, microarray data analysis was done that has aid in the selection of highly potent genes to target. During the process of data analysis of the datasets, when the complete datasets were subjected to Genowiz software, it was found that 50 highly fluctuated genes should be the next target to achieve the goal. Further, from these highly fluctuated gene 4, potent genes were selected, namely, aceAB, relE, kdpA, and metZ. These genes were subjected as targets and pharmacophore modeling was carried to retrieve the best possible natural molecules to dock with these genes. Once the process of modeling was completed, from lacs of molecules few thousands of molecules were selected to target with the desired genes. This docking resulted into the interaction of receptor and ligand concept in which according to the least binding energy the stability of the molecule was studied.

Besides the metabolic pathway of these were also targeted for the study, predicting that once a desired inhibitor is designed for the anticipated proteins encoded by the targeted genes, the metabolic pathways of the organism can be fully studied, and this will thus aid in the hindrance of metabolism of the microbe to flourish in the host body.

Thus, the key protracting factor in TB drug discovery has been the deprived understanding of the proper interactions between the pathogen and its host, for Mtb is known to employ a diverse line of attack to survive within its host and evade host immune surveillance. Thus, the deficiency of chemical assortment in drug scaffolds[55] collective with *in vitro* target and phenotypic-based screening methods have been insufficiently inefficient, yielding only one US FDA permitted drug candidate, TMC207 or bedaquiline, for the management and treatment of MDR-TB.[55]

Nevertheless, several pharmaceutical companies and non-governmental organizations have also launched a contemporary initiative (Critical Path to New TB Regimens) to advance new drug regimens including different course of therapies which comprises of investigational and untried TB drugs and existing or TB drug candidates, to avoid emerging drugs and thus shortening the developmental timeline.[56]

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