Targeting Tuberculosis: A Glimpse of Promising Drug Targets

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Abstract: Tuberculosis caused by *Mycobacterium tuberculosis* has emerged as the biggest curse of our time causing significant morbidity and mortality. Increasing resistance in mycobacterium to existing drugs calls for exploration of metabolic pathways for finding novel drug targets and also for prioritization of known drug targets. Recent advances in molecular biology, bioinformatics and structural biology coupled with availability of *M. tuberculosis* genome sequence have provided much needed boost to drug discovery process. This review provides a glimpse of attractive drug targets for development of anti-mycobacterial drug development.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, multi-drug resistance, current therapy, novel drug targets.

INTRODUCTION

"If the importance of a disease for mankind is measured from the number of fatalities which are due to it, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera, and the like. Statistics have shown that 1/7 of all humans die of tuberculosis."

—*Die Ätiologie der Tuberculose*, Robert Koch (1882) [1]

The saying remains true till date. Once perceived as conquered disease and lost in oblivion, recent data on the cases of Tuberculosis has taken the researchers and epidemiologists aback who considered it as a disease of poor and developing nations. Reemergence and resurgence of tuberculosis have necessitated a serious reevaluation of current strategy for fighting this pernicious infection. TB, one of the deadliest diseases affecting human, records back to antiquity. Tuberculosis causes high morbidity and mortality worldwide [2, 3] and this pinch is felt more in low and middle income countries of Sub-African and South-East Asia [4]. Recent survey on global prevalence of Tuberculosis indicates that about 9.2 million people become victims of this noxious disease and 1.7 million succumb to it [5]. Incidence of TB has gone up to colossal proportions in nations with high HIV infection rates. Though BCG was initially successful in reducing the number of TB cases but soon after the honeymoon period, it was realized that dependence on only BCG vaccine can not provide riddance from such mighty pathogen. Tuberculosis affects people in most productive age group and imposes huge economic burden in terms of lost DALYS (Disability Adjusted Life Years), cost of prevention, treatment and control [6]. TB has been declared a global health emergency by WHO in the wake of its increasing incidence. Though the disease dates back to 5000 B.C., it was Robert Koch who succeeded in identifying the disease causing organism in 1882 [1]. Tuberculosis is a contagious disease caused by slow-growing pathogen *M. tuberculosis* and *M. africanum* in human and *M. bovis* in cattles. The infection occurs by inhalation of droplets caused by a sneeze or cough. The asymptomatic or latent phase can continue for years, while it is the second stage when bacteria replicates and increases in number and TB manifests its presence by symptoms like persistent cough, chest pain, slow fever, loss of appetite and constant fatigue along with weight loss [7]. Mycobacterium has emerged as one of the most successful pathogens of our times owing to its remarkable tendency of modulating its metabolism to undergo a latent phase, while facing starvation or immune stress during which it demonstrates resistance to many antibiotics. This persistent state necessitates the long treatment regime of antibiotics. Current chemotherapy has the problem of toxic effects and development of drug resistance due to its long duration. It was not before 1940 that a definite treatment of TB was known. Though we have come a long way since the discovery of Streptomycin as anti-tubercular agent, yet we are unable to find proper cure for TB. Initial trials with streptomycin led to the reduction of bacteria in patients but it was not found to be very effective in the long run and led to the development of resistance [8-10]. Combination therapy was advocated when it was found that para-amino salicylic acid (PAS) along with streptomycin demonstrated high success rate. In 1952, recognition of Isoniazid as an effective drug for TB revolutionized the treatment and gained popularity. Emergence of INH-resistance necessitated the need of combination therapy with para- amino salicylic acid (PAS) and Sm. Despite all the advances in the development of chemotherapeutic agents, no strategy has proved successful in reducing prolonged therapy and controlling relapse. The enthusiasm and hope of controlling TB soon faded with the emergence of HIV pandemic and staggering mortality rate which triggered a wave of panic.

One of the most important bottlenecks of TB control programmes was the emergence of multi-drug resistance

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strains of Mycobacterium during early 1990s. The drugs once considered "magic bullets" are now denounced as double edged sword as the requirement for long treatment plan often results in the development of resistant stains [11]. Multidrug resistance occurs due to non-adherence to doses by patients, improper prescription by practitioners, counterfeit/poor quality drugs or irregularity in supply of drugs [12]. It was observed that multi-drug resistance has emerged as a consequence of operational weakness and not due to adoption of new mechanism. 0.5 million cases of multi-drug resistant TB (MDR-TB) were reported in 2007 [13]. The countries which receive the maximum grant of TB are India, China, The Russian Federation, South Africa and Bangladesh [13]. Multidrug resistance has led to many outbreaks in the past. The problem of drug resistance is of high magnitude in developing countries when compared to the developed countries [14]. The problem is of global concern as TB patients showing resistance to conventional drugs face a high risk of death [15-19]. The problem of drug resistance is equally challenging both to public health workers who are treating tuberculosis patients as well as researchers busy in studying mechanism of drug resistance. The biology of Mycobacterium remains poorly understood owing to various hurdles arising due to technical difficulties and its extremely slow growth rate coupled with the requirement of BSL3(Core Biosafety Level 3) facility for manipulation which often limits the efforts.

However, the scenario is changing with the availability of new tools for dissecting metabolic pathways. Unveiling of *M. tuberculosis* genome sequence has opened a window of opportunity for understanding host-pathogen interaction and is expected to contribute towards the rational drug design. Recent advances in structural biology and high throughput methods have facilitated elucidation of drug targets, rapid development of drug candidates and vaccine candidates to control the exorbitant rise in TB incidence. The dream that pervades the pharmacists is to find a magical drug that can shorten the treatment regimen and capable of treating MDR-TB and latent phase of bacterium. It is very unfortunate that all pharma majors are shying away from investing in tuberculosis research due to the low profit margin and this comes as a rude shock that only 3 new anti-tuberculosis drugs came to market between 1975 and 2004 [20]. The major challenge is to develop an effective and affordable drug that can control active as well as latent mycobacterium to eliminate the risk of reactivation. This review is an effort to summarize the potential drug targets.

Lines of Treatment

TB has emerged as a major health problem in recent times [21, 22] and the foremost challenge which remains the control of transmission and forestalling drug resistance [23]. A first-line drug is capable of sterilizing and reducing treatment duration [24]. Current treatment regimen known as DOTS (directly observed treatment, short-course) recommended by WHO includes administration of a cocktail of four first line drugs i.e. isoniazid, rifampicin, pyrazinamide and ethambutol or streptomycin for initial two months which is then followed by four months of treatment with INH and RIF alone. DOTS considered as the best therapy for TB with a high cure rate of 95% has its own pitfalls [25]. When the first line of treatment fails, practitioners prescribe second line drugs. Second line drugs are known to be less effective even in high dosages and result in more side effects and pronounced toxicity [26]. Some of the commonly used second line drugs include capreomycin, ethionamide [ETA], para-aminosalicylic acid, kanamycin and cycloserine. Structures of some important drugs used for the treatment of tuberculosis are shown in Fig. (**1**).

More and more effective drugs are needed to combat this problem but the cost and time required for bringing one drug in market is the main bottleneck in controlling this disease. The existing "Innovation gap" in drug discovery process can be reduced by utilizing new and inexpensive methodologies for identification of druggable targets. Target identification is considered the foremost and crucial step and is the cornerstone of all drug development programs. Desirable characteristics of a drug target are its crucial involvement in survival, metabolism and growth of Mycobacterium and absence of its homolog in host. Enzymes involved in establishing infection and resistance to antibiotics are also considered good drug targets. Enzymes involved in cell wall biosynthesis, metabolism, persistence, virulence, signal transduction considered as the attractive targets for new drugs are described below.

TARGETING CELL WALL BIOSYNTHESIS

Cell envelope of Mycobacterium that distinguishes it from other bacterial species comprises of plasma membrane, cell wall and outer coat [27]. Mechanistic separation of cell wall and plasma membrane has facilitated the study of their inherent properties. Mere knowledge of Mycobacterial cell wall chemistry is not sufficient to comprehend the barrier properties as these properties are functions of physical organization of cell wall [28]. Mycobacterium cell wall tends to protect the pathogen from the immune response generated by the host and acts as a formidable barrier to drugs due to low permeability [29-31] which warrants long therapy duration. As the formation of cell wall is vital to Mycobacterium survival and its growth in host, enzymes involved in cell wall synthesis and assembly have drawn attention in the recent times [32]. Mycobacterium cell wall structure basically comprises of peptidoglycan, arabino-galactan and β-hydroxy long-chain (C70–C90) fatty acids known as mycolic acid which are linked by covalent bonds and form "cell wall core" [33,34]. It is now known that mycolic acid constitutes about 60% weight of the cell wall dry mass [35]. High lipid content and hydrophobic character of other compounds that are known to be associated with cell membrane by non covalent bonds like lipoarabinomannan (LAM), trehalose dimycolate, and phthiocerol dimycocerate also hold prime importance in virulence along with lipids like cord factor [36-39]. One of the integral and main components of cell wall, LAM, is immunomodulatory in nature [40] and is instrumental in inducing the release of TNF from macrophages which leads to bacterial killing [41]. Past few decades have witnessed a sudden focus on understanding the cell wall biosynthetic pathways. Absence of homologs of enzymes responsible for the synthesis of lipids crucial for cell wall biosynthesis in mammalian system, makes the enzymes of cell wall synthesis pathway lucrative drug targets for development of anti-tubercular

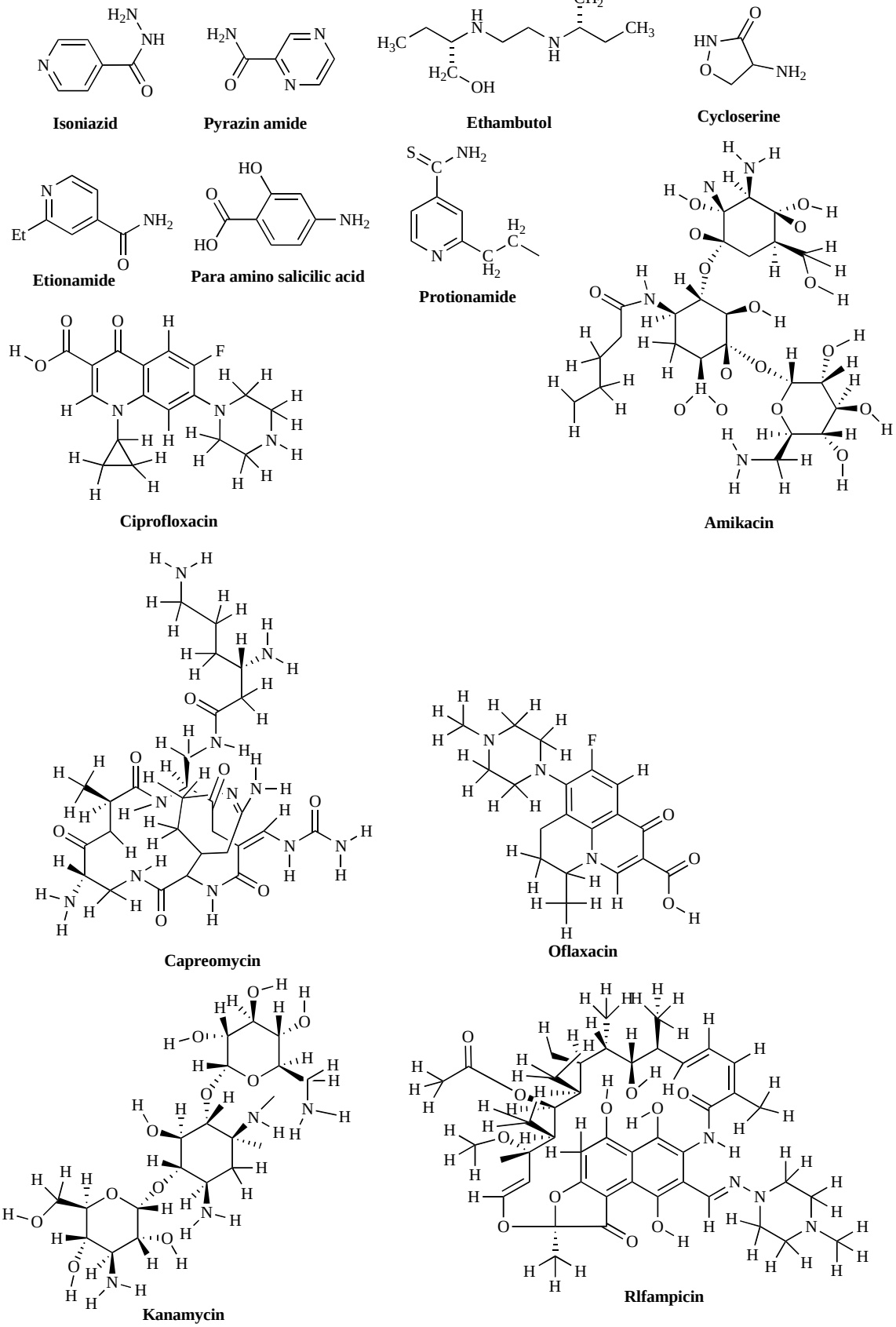

Fig. (1). Important TB drugs.

agents [42, 43]. Enzymes involved in biosynthesis of mycolic acid, LAM, PDIM (PDIM transferase) and membrane transporter which serves as vehicle for PDIM in its transit from the cell membrane to the cell surface (MmpL7) are excellent drug targets and have been reviewed in the past [44].

Many important TB drugs like isoniazid, ethionamide, ethambutol and cycloserine target cell wall biosynthetic pathway [45-47]. Several enzymes (RmlA, RmlB, RmlC and RmlD) involved in the synthesis of dTDP-rhamnose, an important structural moiety of the cell wall, have been used for *in vitro* screening of a huge chemical library of lead compounds [48]. Thiolactomycin is active against two β -ketoacyl-acyl-carrier protein synthases, KasA and KasB enzymes belonging to fatty acid biosynthetic pathway and mycolic acid biosynthesis [49,50]. TLM as well as many of its derivatives are active against multi-drug resistant clinical isolates. Cerulenin has also emerged as an effective inhibitor of lipid synthesis in Mycobacterium and was found to inhibit KasA involved in Mycolic acid biosynthesis with an MIC of 1.5–12.5 μg/ml [51]. Recent studies have indicated that N-octanesulfonylacetamide (OSA) hampers fatty acid and mycolic acid biosynthesis and is active against *M. tuberculosis* as well as multi-drug resistant strains with an MIC of 6.25–12.5 μg/ml [52]. These studies suggest that fatty acid and mycolic acid synthesis pathway can be exploited as good source of drug targets but their worth as drug targets in persisting dormant slow growing Mycobacterium poses a question on their possible use in reducing the treatment duration.

TARGETING FATTY ACID BIOSYNTHESIS

Ubiquitous presence of fatty acid biosynthesis pathway supports the hypothesis of its antiquity. Presence of 2 types of fatty acid biosynthesis pathways (FAS I and FASII) in Mycobacterium makes it interesting as this is quite unique compared to other microbes which possess only one fatty acid biosynthesis pathway (FAS I) [53,54]. Even the presence of surprisingly high number of enzymes involved in fatty acid metabolism in Mycobacterium when compared to microbial genomes of similar size is intriguing and underscores the importance of this pathway for Mycobacterium survival [55,56]. The products of this pathway are crucial components of protective lipid layer in mycobacterial cell wall. Suitability of fatty acid biosynthesis pathway II for antimicrobial drug development has been reviewed in past by several groups as enzymes of this pathway are targets of common anti-bacterial compounds like INH, diazoborines [57], triclosan [58], and thiolactomycin [59,60]. These 2 pathways differ from each other in respect of extent of fatty acid chain elongation as de novo synthesis of C16-C26 fatty acids is carried out by FAS I pathway, while extension up to C56 is carried out by FAS II [60-62]. FAS II comprises of many enzymes while all FAS I enzymes work together as monofunctional enzyme unit with multiple catalytic activities [63,64]. FAS I pathway is akin to mammalian fatty acid biosynthetic pathway while FAS II is found only in microbes, thus making the enzymes of this pathway attractive targets [65]. Despite initial setbacks due to difficulty in culturing Mycobacterium, several enzymes of FAS II pathway have been characterized. Among them, enoyl acyl carrier protein reductase (ENR) catalyzing the ultimate step of this pathway involving conversion of trans-2-enoyl-ACP to acyl-ACP in a NADH-dependent reaction is targeted by Isoniazid. Methyltransferases of a gene cluster coding for 4 such enzymes have been successfully targeted by analogs of S-Adenosyl Methionine. Enzymes involved in the synthesis of substrate for ligase have been implicated as essential for growth of *Mycobacterium tuberculosis*. Polyketide synthase 13 (Pks13), a critical enzyme for Mycobacterium growth and survival, brings about the final condensation step in mycolic acid synthesis in FAS-II [66]. Though its structure has not been determined, yet the availability of structure of its homolog has greatly facilitated us in obtaining knowledge about its reaction mechanism. Inhibitors of Acyl-AMP ligase, FadD32, AccD4-containing acyl-coenzyme A (CoA) carboxylase, FabH, MabA, InhA successfully deter the growth of *M. tuberculosis* [67].

TARGETING TERPENOID BIOSYNTHESIS

Isoprenoids belong to a major class of naturally occurring compounds known to perform a gamut of functions ranging from signal transduction, growth regulators, carriers for electron transport chain and many more making them indispensable for growth and survival of an organism [68-78]. Despite all the heterogeneity in the function, all isoprenoids have the same basic structure with monomer of 5-carbon isoprene [79].

Till recently, it was believed that precursors of isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) can be synthesized only *via* Acetate/Mevalonate (MVA) pathway [80-82]. This notion lost ground with the discovery of an alternate pathway called Non-mevalonate pathway/2C-methyl-D-erythritol-4-phosphate (MEP) pathway /Rohmer pathway or 1-deoxy-D-xylulose-5-phosphate (DXP) pathway in eubacteria [83]. MEP pathway is functional in most eubacteria, lower plants but is absent in animals, fungi, archaebacteria and certain bacterial species [84]. In Mycobacterium, isoprenoids play crucial role in the synthesis of many cellular components [85]. The presence of MEP pathway in *M. tuberculosis* has been well established using biochemical techniques. The differences in route for isoprenoid biosynthesis in human and *M. tuberculosis* make the enzymes involved in MEP pathway a good source of attractive targets for chemotherapeutics development [86]. Non-mevalonate pathway involves a cascade of reactions involving seven steps catalyzed by IspC-H enzymes [87]. First step involves the condensation of pyruvate and glyceraldehyde-3-phosphate to form DXP [88] which in turn gets transformed to MEP in the presence of DXP synthase and DXP reductoisomerase. 2C-methyl-D-erythritol-4-phosphate cytidylyltransferase catalyzes the formation of CDP methylerythritol (CDP-ME) and pyrophosphate from MEP and cytosine 5′-triphosphate (CTP). CDP-ME kinase leads to phosphorylation of CDP-ME at the 2-hydroxy group after which 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate is formed. This reaction culminates in the intramolecular elimination of the diphosphate of ME cyclodiphosphate and may include two reductases and two dehydratases as well [89]. Three dimensional structures of 4 enzymes of the pathway are now

available which will enlighten our path to drug discovery using this unique pathway. Various groups have reported successful targeting of this pathway using inhibitors like fosmidomycin in other organisms [90].

TARGETS IN AMINO ACID BIOSYNTHESIS

Most of the microorganisms rely on amino acid biosynthesis for their growth. This is especially important for *M. tuberculosis* when it can not derive sufficient nutrients from infected host. This indicates that unique enzymes in amino acid biosynthesis pathways can be used as possible targets for novel drugs. Mutants in the biosynthesis of lysine (lysA), proline (proC), tryptophan (trpD) and leucine (leuD) have been described [61] and structures of LeuA (α-isopropylmalate synthase) and LeuB (β -isopropylmalate dehydrogenase) from leucine biosynthesis pathway and LysA (meso-diaminopimelate decarboxylase, DAPDC) from the lysine biosynthetic pathway and HisG (ATP phosphoribosyltransferase) from the pathway for histidine biosynthesis have been elucidated [91]. The enzyme dihydrodipicolinate reductase in lysine biosynthesis represents an excellent drug target [92]. Lysine biosynthesis pathway holds much significance as besides the need of lysine in protein synthesis, its precursor meso-diamino-pimelic acid is incorporated into peptidoglycan [93-95]. Arginine biosynthesis is crucial in *M. tuberculosis* as the dependence of the bacilli on exogenous resource for its growth showed a marked decrease in virulence [96]. Branched chain amino acids are important for the growth of bacteria and enzyme Acetolactate synthase (ALS) catalyzes the first step in the biosynthesis of branched-chain amino acids. Mammals lack the biosynthetic pathways for branched chain amino acids, so the enzymes of the pathway hold promise as suitable drug targets owing to their specificity.

TARGETS IN SHIKIMATE PATHWAY

Aromatic compounds are synthesized *via* shikimate pathway in algae, higher plants, bacteria, apicomplexan parasites and fungi [97-100] but absent in animals which rely exclusively on an exogenous source for these aromatic compounds. Shikimate pathway is essential for Mycobacterium [101]. Shikimate pathway is a series of seven biochemical reactions, which involves condensation of phosphoenolpyruvate and erythrose-4-phosphate to yield Chorismic acid [102]. Seven enzymes catalyzing the steps of pathway are AroA, AroB, AroC, AroE, AroG, AroK and AroQ and some of them have been studied in great details [103,104]. Chorismate synthase is considered an important choke point enzyme as Chorismate is the substrate for subsequent biosynthesis of folates, ubiquinone, napthoquinones and the aromatic amino acids, tryptophan, tyrosine and phenylalanine [105]. Structures of AroC , AroK and Aro Q in complex with shikimic acid in *M. tuberculosis* have been determined and provided a great deal of insight on the functional groups and residues involved in binding substrate [106,107]. Enzymes of Shikimate pathway have generated a great deal of interest and are considered excellent drug targets for development of new chemotherapeutic agents owing to their absence in mammals [108-110]. Inhibitors of *Mtu*DAH7PS have been synthesized recently using modeling approach [111].

TARGETING ATP BIOSYNTHESIS

Although the Mycobacterium remains in the macrophages in apparently dormant phase, it still requires some energy for survival even after major reshuffling of its metabolic pathways [112]. Respiratory chain enzymes are essential for meeting its requirement of maintaining basal metabolic processes in both dormant and replicating phases. Recently published data on inhibitors of ATP producing machinery underscores the importance of this pathway for chemotherapeutics development [113-115]. There is an absolute requirement of ATP synthase in Mycobacterium for survival unlike many other bacteria which can generate energy from substrate-level phospho-rylation [116]. Out of the two types of NADH dehydro-genases present in mycobacteria for NADH oxidation, NDH2 is an essential component of respiratory chain and is up-regulated during dormant phase [117-119]. NDH2 can serve as a good target for anti-tuberculosis drug development owing to absence of its homologs in humans. Phenothiazines and its analogs are lethal to *M. tuberculosis* and effectively stall the activity of both homologs of NDH-2 [120,114] by blocking an intermediate species [121]. Inhibition of ATP synthase causes death of the mycobacterium even in the dormant phase. Diarylquinolines have been reported to effectively block ATP synthase activity [113,122] and possess the ability to target both active as well as dormant bacilli.

TARGETING MYCOTHIOL SYNTHESIS

Mycothiol (MSH), a conjugate of *N*-acetylcysteine (AcCys) with 1D-myo-inosityl 2-acetamido-2-deoxy-D-glucopyranoside (GlcNAc-Ins) is a major low-molecular mass thiol found in many pathogenic bacteria including mycobacteria. Mycothiol protects *M. tuberculosis* from the oxidative stress in lungs, toxic oxidants and action of antibiotics [123-129]. Synthesis of MSh proceeds in a multi-step fashion that has 4 designated reactions catalyzed by MshA, MshB, MshC and MshD. Essentiality of MshC has been confirmed through targeted disruption of the enzyme [123]. Structures of MshB and MshD are now known [130-133]. Absence of this pathway in human makes it a lucrative drug target for developing anti-infective agents for tuberculosis.

TARGETING MENAQUINONE SYNTHESIS

Availability of *M. tuberculosis* genome has provided insights on many functional aspects of the pathogen lifecycle and led to the elucidation of essential genes and pathways involved. There exists a possibility of occurrence of different mechanisms acting as electron transport system in varying conditions which enable the mycobacterium to survive in hostile environment in host [134]. The fact that respiratory chain of Mycobacterium offers many good drug targets stimulated many studies that culminated in its characterization. NADH:menaquinone oxidoreductase has been proposed as a rewarding target for anti-tubercular agents [114]. Researchers have pointed out the modular nature of ETS in mycobacterium [135,119]. Quinones ferry electrons across the membrane in the ETS [136]. Mammals utilize membrane soluble form of lipoquinone known as ubiquinone whereas bacterial ETS possess both

menaquinones as well as ubiquinone [137]. Major lipoquinones of mycobacteria are Menaquinones [138]. Mammals depend on exogenous supply of menaquinone. Therefore, menaquinone biosynthesis pathway is advocated as attractive drug target for anti-mycobacterial drug development [139]. Biosynthesis of menaquinone from chorismate and 2-ketogluatarate occurs by the concerted action of 6 genes i.e. *men-A*, *-B*, *-C*, *-D*, *-E*, and *–F* [140]. Complete reliance of Mycobacterium on MenD as the sole electron acceptor for its survival in hypoxic conditions has generated much interest on targeting its synthesis for curbing tuberculosis. Structural analogs of the substrate or products can be exploited to block the reaction pathway. As DHNA (1, 4-dihydroxy-2-naphthoate), an intermediate of this pathway has a napthoquinone moiety in its structure, napthooquinone derivates like Plumbagin derivative, have been tested to determine their effect on Mycobacterium [137]. Crotonate plumbagin inhibits *M. tuberculosis* H37Rv in the bioassay. Another group after designing and testing a library of 100 synthesized Demethylmenaquinone (DMMK) analogs on mycobacterium showed that allylaminomethanone-and phenethylaminomethanone-A effectively stalled growth of multi drug resistant Mycobacterium species [141].

TARGETING TRANSCRIPTION

Life revolves around the central theme of gene expression and regulation of gene expression plays a pivotal role in normal development and functions in all living beings. Maintenance of DNA topology is crucial for processing of genetic information and enzymes catalyzing topological changes play important part in replication, transcription and recombination [142]. Mycobacterial genome is maintained in negatively supercoiled state. Supercoiling of the genome is known to influence the extent of recombination and complexity of the product. Type II DNA topoisomerases, DNA gyrase and Topoisomerase IV which act in ATP dependent manner for facilitating important DNA transaction processes are targets of many anti-bacterial drugs [143,144]. DNA gyrase, an enzyme found only in prokaryotes, is a unique enzyme as it negatively supercoils DNA in the presence of ATP and relaxes negatively supercoil DNA in the absence of ATP [145-147]. This enzyme represents an important drug target being essential to many vital processes in Mycobacterium [148]. Broad spectrum antibiotics like fluoroquinones target bacterial DNA gyrase and topoisomerase IV in many bacterial species including *M. tuberculosis* [149,150]. Mechanism of action and resistance and data pertaining to clinical efficacy of inhibitors like pyrazoles, coumarins, novobiocin against *M. tuberculosis* DNA gyrase have been documented and subject of many review articles [151].

Gene regulation basically operates at the level of transcription and to some extent at the post transcription level. Such transcription regulators have attracted researchers since their discovery and have been explored as possible drug targets. The boom in genomics and high throughput methodology has enhanced our knowledge about these transcription regulators manifolds and enabled us to explore the possibility of exploiting transcription regulators as possible drug targets. Dormant form of bacilli gets reactivated on the onset of favorable environmental conditions. This involves a series of changes including change of forms mediated through various transcription regulators. The cue of signals like hypoxia, starvation is perceived by cyclic AMP (cAMP) receptor protein CRP/FNR class of transcriptional regulators [152]. Deletion of CRP/FNR homolog is reported to create growth defects in *Mycobacterum tuberculosis* [153]. Structure of CRP/FNR of *M. tuberculosis* has been determined. It is also of common knowledge that various sigma factors are involved in regulation of gene expression and are crucial for Mycobacterium, as they aid the pathogen in adjusting in dynamic environmental situations [55, 154]. Various groups have explored and established the role of these sigma factors in *M. tuberculosis* life cycle [155]. Table **1** provides a brief description of roles of important sigma factors in Mycobacterium based on data extracted from literature.

TARGETING DNA DAMAGE RESPONSE

DNA repair is necessary for maintaining genome integrity, which in turn ensures proper segregation of homologous chromosomes during meiosis and necessitates the DNA repair. It becomes more significant in Mycobacterium which spends a substantial time in host macrophages where it encounters reactive oxygen and reactive nitrogen intermediates capable of damaging DNA. Response to DNA damage occurs by concerted expression of a number of genes in SOS regulon. RecA is a highly conserved enzyme which forms the main component of SOS response to DNA damage in bacteria [169]. It plays an important role in DNA repair by forming nucleoprotein filament on single stranded DNA substrate and brings about homologous pairing and strand exchange [170]. Along with LexA, it regulates the expression of other genes that facilitate the survival of bacteria on DNA damage. It is considered a potential drug target as it permits survival of bacteria in the event of metabolic stress resulting from various anti-bacterials and is implicated in the transmission of antibiotic resistance genes. Inhibition of RecA can limit evolution of resistance in Mycobacterial genome [171,172]. It is anticipated that the inhibitors to recA can be developed as adjuvants for tackling the problem of antibiotic resistance in *M. tuberculosis* [173].

Single stranded DNA binding proteins (SSBs) belong to an important class of proteins crucial for DNA metabolism such as DNA replication, repair and homologous recombination [174]. SSBs exist in various oligomeric forms ranging from monomeric to tetrameric in various organisms and display different binding affinities towards DNA [175-179]. SSBs protect single-stranded DNA from various actions of nucleases as well as prevent the formation of anomalous secondary structure [180,181]. Availability of MtuSSB structure and knowledge of its biochemical properties along with its cognate RecA has raised the possibility of its use as drug target [182]. Differences in mechanism of action of mycobacterial and *E.coli* single-stranded DNA binding proteins can be exploited for drug development.

TARGETING LIPOLYATION

Lipoic acid is an essential cofactor that is covalently attached through an amide bond to complex multi-

Table 1. Role of Important Sigma Factors in *M. tuberculosis*

S. No.	Sigma factor	Role	Reference
1	Sig A	Indispensable for growth, Virulence factor, also control several house keeping genes	[156,157,158]
2	Sig B	Essential for growth, required for adaptation to multiple environmental conditions , Induced in latent phase	[159,160,161]
3	Sig E	Involved in oxidative stress and heat stress on exposure to SDS and survival in macrophages	[162,163]
4	Sig F	Control the expression of Acr protein, which is induced in the macrophages. Induced on exposure to several antibiotics, anaerobiosis, cold shock, oxidative stress, nutrient depletion and on entry in stationary phase. It enables the persistence of *M. tuberculosis* in macrophages.	[164, 165, 166]
5	Sig H	Level elevated when pathogen is inside macrophages	[167,168]

component enzyme systems during posttranslational modifications [183]. These complex systems are known to catalyze many key events and metabolic processes. The event of lipoylation is mediated by 2 distinct enzymes lipoyl protein ligase A or LipB. While LipA requires an exogenous supply of lipoic acid, LipB circumvents the requirement by synthesizing lipoate attachment group from endogenous octanoic acid moieties by transferring them to phosphopantetheine cofactor of acyl carrier protein (ACP) onto lipoyl domain [184]. LipA then converts these octonylated domains into lipolyated derivatives. LipB, being the key mediator for activation of cellular machines, is important for Mycobacterium metabolism. Expression of LipB is up-regulated as much as 70 times in acutely infected cells in lungs of patients suffering from pulmonary multidrug resistant tuberculosis [185]. This altered expression during pathogenesis has established LipB as an interesting drug target for developing anti-tuberculosis drugs. Availability of LipB structure has accelerated efforts in this direction.

TARGEING COFACTOR BIOSYNTHESIS

Cofactor biosynthesis has generated much interest in researchers owing to its essentiality in metabolism. One of the important cofactors is NAD involved in maintaining redox mechanism and energy metabolism and is known to be implicated in the activity of the NAD-dependent DNA ligase in prokaryotes, protein ADP-ribosylases, protein deacetylation, as a substrate in cobalamin biosynthesis and for calcium homeostasis. Being an essential cofactor in the synthesis of lipid, enzymes involved in the biosynthesis of CoA are considered as good drug targets. Structure of Pantothenate kinase (PanK), an enzyme catalyzing the first step of CoA biosynthesis, along with its feedback inhibitor is now available [186]. There is a growing evidence that riboflavin and pantothenate are indispensable for *M. tuberculosis* and thus, enzymes like Pan B-E and lumazine synthase involved in their synthesis represent attractive drug targets [187-189]. Availability of crystal structures of these enzymes has greatly facilitated the drug discovery process [187,190-192].

TARGETING VIRULENCE GENES

Availability of advanced bioinformatics techniques and comparative genomics approaches have made the identification of virulence genes a child's play. Some such genes have been identified in *M. tuberculosis* genome. Among them, ERP (Extracellular Repeat Protein) which is essential for proliferation of the bacilli in mouse deserves to be mentioned as it does not have any homolog in man, thus making it an attractive drug target [155,193]. Researchers have also identified 2 gene clusters required for growth of mycobacterium in lungs during the early infection and has a special role in bringing about the synthesis and export of phthiocerol dimycocerosate [192]. Inhibitors of such genes or their products, if used judiciously along with other drugs can be very effective in curbing the disease [193].

TARGETING SIGNAL TRANSDUCTION

When seen in new light, host-pathogen interaction can provide many clues for targeting the pathogen effectively. Survival of *Mycobacterium* within host relies to a great extent on its ability to adapt and manipulate its key processes and components *via* signal transduction in response to the environmental cues and aid the pathogen in overcoming the hostile environment and the signal transduction mechanism is a fine-tuned process mediated by a myriad of kinases and phosphatases [170]. These enzymes modify host protein and thus signify an important step in establishing infection. For example, Lipoarabinan (LAM) is reported to modify host signaling pathways essential for the survival of Mycobacterium by phosphorylating an apoptotic protein (Bad) in phosphatidylinositol 3-kinase (PI-3K)-dependent pathway [194]. Protein kinases are tractable targets for small drug molecules and have been exploited to provide solution to unmet therapeutic needs in a number of diseases [195].

Serine/Threonine Protein Kinases

Serine/Threonine protein kinases (STPKs) are conserved during the course of evolution and play cardinal role in signal transductions by reversible phosphorylation and regulate diverse cellular processes and functions. *M.*

tuberculosis encodes 11 eukaryotic type STPKs and most of them have been biochemically characterized and are implicated in Mycobacterium survival and virulence [196-198]. But little is known about the activators, modulators and substrates of these STPKs. Mycobacterial STPKs are considered good drug targets owing to their involvement in important metabolic processes of bacteria [199,200]. Comparative genomics studies have established that only 3 orthologs (*pknA, pknB*, and *pknG)* of 4STPKs retained in downsized genome of *M. leprae* are essential for growth of *M. tuberculosis*. Mutations in PknG and PknH were found to affect the survival of bacillus *in vitro*. PknB is responsible for bringing about key processes in life cycle of mycobacterium and is essential for its growth [199]. Majority of kinase inhibitors are non-specific as they bind to ATP binding site which is common and evolutionarily conserved in all kinases. This issue can be addressed by selecting appropriate screens and parameters such as toxicity. AX20017 (tetrahydrobenzothiophene), AX33510 and AX14585 (derivatives of AX20017) were identified as PknG inhibitory compounds in a screening experiment involving 1000 compounds [201]. 1-(5-isoquinolinesulphonyl)-2-methylpiperazine, a sulphonyl compound inhibits the kinase activity of the PknB in *M. tuberculosis* [202]. Owing to low sequence similarity shared between the mycobacterial STPKs and the human counterparts, they have emerged as good targets for rational design of antituberculosis drugs. Availability of crystal structure of PknB and PstP will pave a way for development of effective inhibitor molecules to curb the menace of this dreaded disease [203,204].

Tyrosine Kinases and Phosphotases

Mycobacterium encodes protein tyrosine kinase PtK, a member of HAD family, lacking kinase signature sequence [205]. *M. tuberculosis* genome encodes genes for eukaryotic type PstP; PtpA and PtpB. PtpA and PtpB are secretory proteins involved in dephosphorylation of host proteins. Deletion of ptpB gene in *M. tuberculosis* resulted in attenuation of pathogen in lung and spleen of diseased animals. It is presumed that mutation in ptpB affects survival of mutant strains in macrophages activated with IFN-α clearly indicating its role in dephosphorylation of host proteins in IFN-α signaling route [206,207].

Two-Component Systems

Role of 12 two-component systems homologs with 8 response regulators in *M. tuberculosis* has not been elucidated till date [55]. Disruption studies on *mtrA-mtrB,* devR-devS, PhoP/PhoR TCS have established that these are important for survival of Mycobacterium [208-210]. These findings clearly suggest that genes of 2 component systems are also proposed to be important drug targets.

TARGETING DORMANCY AND PERSISTENCE

The unique property of Mycobacterium to lie dormant enables it to survive the chemotherapy and has become a cause of concern to researchers across the globe [211]. These persistent forms necessitate long therapy duration [212]. Despite all the advances in the modern biology, our knowledge of mechanisms involved in survival strategies of Mycobacterium is poor. This has initiated a hunt for new drug candidates targeting these persistent forms [213].

Isocitrate Lyase

During the persistence, Mycobacterium relies on a metabolic shift of carbon source to C2 substrate such as acetate generated as a product of β-oxidation of fatty acids [214-218]. These hypoxic conditions lead to a marked decrease in glycolysis and upregulation of a carbon assimilatory pathway known as glyoxylate shunt, allowing sustained TCA cycle [217]. Isocitrate Lyase (ICL), the gating enzyme of glyoxylate shunt [219] brings about the conversion of isocitrate to succinate and glyoxylate. This is followed by catalysis of another reaction by malate synthase to form malate on addition of acetyl-CoA to glyoxylate [220, 221]. Up-regulation of icl gene expression is observed during infection of macrophages by Mycobacterium [222]. It is interesting to note that ICL is not essential for the survival of Mycobacterium in normal cultures but is required for persistence of bacteria in mice [223-225]. This was demonstrated by inhibition of persistence in mice by disruption of icl gene [217]. ICL is encoded by 2 genes while malate synthase is coded by single gene. Product of smaller gene is ICL1 which is closely related to eubacterial ICL, while ICL2 coded by larger gene shows more homology to eukaryotic enzyme [223]. Isocitrate lyases (ICL1 and ICL2) play a crucial role in completion of 2-methylcitrate acid cycle [223, 226] where they are involved in elimination of toxicity produced by propionyl-CoA buildup [227]. 3–D structure of ICL with its inhibitor 3-nitropropionate and glyoxylate has been solved at high resolution [228]. Although, the implication of ICL in persistence of Mycobacterium in human remains a topic of debate, yet its plausible role in lipid metabolism of the bacterium *in vivo* can not be denied. The enzymes of glyoxylate cycle, ICL and malate synthase are propounded to be alluring drug targets against persistent Mycobacterium owing to their presence in plants, lower eukaryotes and prokaryotes and absence from mammals. Availability of structural information of these enzymes has spurred inhibitor screening studies and many inhibitor targeting ICLs are in the discovery phase.

Proteasome

Proteasomes are large multisubunit complexes found in all archaea and eukaryotes but only in few actinomycetes [229-231]. Proteasome play important roles in regulation of cell cycle, cellular differentiation and the production of antigenic peptides [232, 233]. Mycobacterial proteasome resembles eukaryotic proteasome in organization. Proteasome is responsible for degradation of abnormal, misfolded or oxidized cytosolic proteins, which if accumulated, can prove toxic to cell [230]. Darwin and colleagues pointed out the role of proteasome in implicating resistance to reactive nitrogen intermediates secreted by macrophages during the dormant phase of Mycobacterium by screening mutants hypersensitive to acidified nitrite [234]. The study established the role of proteasome in determining the susceptibility of Mycobacterium to reactive nitrogen intermediates and also identified Mycobacterium proteasomal ATPase(Mpa)and proteasome accessory factor (PafA) believed to be associated with the function of

proteasome. Mutants lacking accessory ATPase associated with Mpa were found to be hypersusceptible to reactive nitrogen intermediates. In another study, loss of mpa in CDC1551 strain was found to impair the growth and virulence. Role of threonine residue in active site of proteasome in persistence was highlighted by the studies on crystal structure of *M. tuberculosis* proteasome. As inhibition of proteasome activity affects virulence and proliferation of Mycobacterium [235], it is being considered as a target for drug development. But the efforts to develop and use compounds inhibiting proteasome activity against Mycobacterium are marred by the high degree of conservation of proteasome between mycobacterium and mammals which can consequently lead to toxicity. Nevertheless, Lin *et al.*, identified oxanthiazol-2 –one compounds capable of specifically targeting mycobacterial proteasome with 1000-fold less toxicity towards human proteasomes [236]. The activity of these compounds is attributed to their ability to modify active site threonine 1 and thus modifying active side environment as well as substrate binding pocket. This irreversible inhibition of proteasome inhibition by these compounds will be particularly useful in curbing Mycobacterium in dormant stages where protein synthesis is blocked during antibiotic treatment [236].

CONCLUSION

Emergence of multi-drug resistance in *M. tuberculosis* and rise in the number of TB cases to the epidemic scale has necessitated the need to look beyond obvious. Despite advances in understanding of biochemistry and host parasite interactions of Mycobacterium, we are far from developing treatment options for taming this deadly parasite. The urgent need for developing a safe, cheap and effective drug can not be met satisfactorily without concerted efforts by scientific community harnessing knowledge emerging from multiple disciplines like genomics, structural biology, genetics, and bioinformatics. Availability of *M. tuberculosis* genome sequence and abundance of information arising from *Mtb* structural genomics projects (http://www.doe-mbi.ucla.edu/ TB/ and http://xmtb.org/), have provided much needed impetus to the field of drug discovery against this parasite. Better understanding of mechanisms underlying resistance and persistence of this parasite will aid in designing strategies for eliminating TB from every corner of the world and will present a window of opportunity for development of anti-mycobacterial drugs. Presently, various antibiotics target very few essential functions of Mycobacterium, it is anticipated that identification of pathways essential for bacterial survival and growth will provide many more drug targets that can serve as starting point for structure based designing of anti-mycobacterial drugs [237]. As more and more structural data is being poured with advances in structural biology, we are entering a new phase where the need of validation and prioritization of these drug targets is now felt more than ever. Prioritization of novel drug targets will reap huge benefits in quenching the thirst of dry pipeline of TB drugs. There is a growing optimism that knowledge of validated drug targets will herald a new era in surmounting the burden of tuberculosis.

CONFLICT OF INTEREST

None declared.

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