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Review

Anti-tubercular drug discovery: *in silico* implications and challenges

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ABSTRACT

Tuberculosis (TB) has been reported as a major public health concern, especially in the developing countries. WHO report on tuberculosis 2016 shows a high mortality rate caused by TB leading to 1.8 million deaths worldwide (including deaths due to TB in HIV positive individuals), which is one of the top 10 causes of mortality in 2015. However, the main therapy used for the treatment of TB is still the Direct Observed Therapy Short-course (DOTS) that consists of four main first-line drugs. Due to the prolonged and unorganized use of these drugs, *Mycobacterium tuberculosis* (Mtb) has developed drug-resistance against them. To overcome this drug-resistance, efforts are continuously being made to develop new therapeutics. New drug-targets of Mtb are pursued by the researchers to develop their inhibitors. For this, new methodologies that comprise of the computational drug designing techniques are vigorously applied. A major limitation that is found with these techniques is the inability of the newly identified target-based inhibitors to inhibit the whole cell bacteria. A foremost factor for this limitation is the inability of these inhibitors to penetrate the bacterial cell wall. In this regard, various strategies to overcome this limitation have been discussed in detail in this review, along with new targets and new methodologies. A bunch of *in silico* tools available for the prediction of physicochemical properties that need to be explored to deal with the permeability issue of the Mtb inhibitors has also been discussed.

1. Introduction

Tuberculosis (TB) is a contagious bacterial infection caused by a group of bacteria known as *Mycobacterium tuberculosis* complex, which comprises *Mycobacterium tuberculosis* (Mtb), *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium africanum*, *Mycobacterium caprae*, *Mycobacterium canettii* and *Mycobacterium pinnipedii* (Zumla et al., 2013a, 2013b; Grange, 2009). TB infections spread from the patients suffering from active TB. However, in many individuals infected with Mtb, TB is asymptomatic; such form of TB is known as latent TB infection (LTBI). In patients with weak immune response (immuno-compromised patients), the risk of TB is more (Barnes et al., 1991). In HIV-positive individuals, TB is the main cause the death of these patients (Kwan and Ernst, 2011). People suffering from HIV infections are 20 to 30 times at higher risk of developing active TB (WHO, 2015).

According to the World Health Organization (WHO) report (WHO, 2016) on global tuberculosis, about 10.4 million new cases of TB were estimated in 2015, and 1.4 million deaths occurred from this disease (WHO, 2016). In addition, 4,00,000 deaths of the individuals suffering from HIV occurred from TB. However, the death rate due to TB declined to nearly 22% between 2000 and 2015. 60% of the new TB cases took place in six countries *viz.* India, China, Indonesia, Pakistan, Nigeria and South Africa (WHO, 2016). Multidrug-resistant TB (MDR-TB) has spread worldwide that causes the ineffectiveness of the current TB therapies in use (WHO, 2014; WHO, 2015; WHO, 2016). In MDR-TB, the bacteria show resistance to at least two main first-line drugs *viz.* rifampicin and isoniazid (Falzon et al., 2012). It has been estimated that 4,80,000 people suffered MDR-TB in 2015, and 100,000 individuals additionally reported with rifampicin-resistant (WHO, 2016) also required MDR-TB therapy. Further, a new risk to TB therapies has occurred with the emergence of the extensively drug-resistant TB (XDR-TB) that has been found in 117 member states of WHO by the end of 2015. It has been found that about 9.5% of individuals suffering from MDR-TB experienced XDR (WHO, 2016).

Streptomycin was the first antibiotic that was put in use for treating TB in 1944 (Schatz et al., 1944; Wassersug, 1946). Subsequently, several more anti-TB drugs were discovered in 1950s that included isoniazid, kanamycin, para-amino salicylic acid, cycloserine and pyr-

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azinamide. This helped in the use of a combination therapy for TB, which was initially of 18 months or more. However, with the discovery of rifampicin as an anti-TB drug and its inclusion in a combination regimen in 1960s, the duration of the therapy was reduced to 9 months. Further, when pyrazinamide was included in a regimen, the duration of the treatment was reduced to 6 months. This resulted in the formulation of a combination regimen that is currently in use for drug susceptible TB as direct observed therapy short-course (DOTS) (Zumla et al., 2013b; Yew et al., 2011; TBCTA, 2009; WHO, 2010). When the patient suffering from drug-susceptible TB is treated under DOTS, the success rate of > 95% is achieved (Zumla et al., 2013b). Since the approval of the current 6 months DOTS regimen in 1970s, no new anti-TB drug has been introduced for the drug-susceptible TB. There exists a pressing requirement to identify new anti-TB drugs and to shorten the DOTS therapy period. In the past years, several significant efforts have taken place worldwide for the treatment of TB, as a result of which two new drugs *viz*. bedaquiline (TMC207 or R207910) and delamanid (OPC67683) have been approved for MDR-TB (Zumla et al., 2013b; Andries et al., 2005; EMA, 2013; Ryan and Lo, 2014).; Despite of all the current efforts in anti-TB drug development, the fact is that only two new anti-TB drugs have been approved and that too after a period of approximately 40 years. Further, these drugs are only recommended for MDR-TB and when other treatment options are not available. In addition, the drug resistance problem is continuously rising that further hikes the risk of ineffectiveness of the current therapeutic interventions. Therefore, pace to the anti-TB drug discovery is required because of the serious health concerns caused by Mtb. With the availability of the new validated targets of TB, and rational application of the latest tools and techniques of computer-aided drug design (CADD) on these targets, the pace of the anti-TB drug discovery process can be increased. Cole et al. (1998) sequenced the genome of Mtb in 1998 that presented an opportunity to gain an insight into the Mtb genome and study the essential genes of Mtb. Various other researchers continued their work for the identification of the genes and proteins that are essential for the survival of Mtb and validated them as anti-TB targets (Sassetti et al., 2003; Sassetti and Rubin, 2003; McKinney et al., 2000; Marrero et al., 2010; Venugopal et al., 2011; Shi and Ehrt, 2006; Gandotra et al., 2007; Darwin et al., 2003; Parish and Stocker, 2002; Senaratne et al., 2006; Bhave et al., 2007; Park et al., 2003; Walburger et al., 2004; Wolff et al., 2009; Hutton et al., 2007; Cox et al., 2000; Pavelka and Jacobs, 1996; Fernandez et al., 2006; De Voss et al., 2000). These newly identified ant-TB targets have been explored by the computational chemists and new methodologies are being applied on these targets for the identification of new anti-TB compounds. Here in this review, validated new targets of TB, new approaches to the anti-TB drug discovery including various challenges, and techniques to overcome these challenges have been discussed.

2. New TB Targets

The sequencing of the genome sequence of Mtb H37Rv strain in 1998 opened up a new era of target-based designing of anti-TB compounds (Cole et al., 1998). Several genes were identified as important for the optimal growth of Mtb from genome based analyses such as transposon mutagenesis, gene knockout and gene transfer studies (Sassetti et al., 2003; Sassetti and Rubin, 2003). Currently, the targets of many of the TB drugs are known. First-line and second-line drugs of TB mainly inhibit the enzymes of cell wall synthesis, DNA replication, RNA transcription, translation and energy pathways (Zhang, 2005). For example, isoniazid targets InhA (enoyl-[acyl-carrier-protein] reductase), rifampicin targets β-subunit of RNA polymerase, pyrazinamide targets S1 component of 30S ribosomal subunit and ethambutol targets arabinosyl transferase. Similarly, main targets of the second line drugs include DNA gyrase (drug: ofloxacin), DNA topoisomerase (drug: ofloxacin), 30S ribosomal subunit (drugs: amikacin, kanamycin), dihydropteroate synthase (drug: para-amino-salicylic acid), 16S rRNA of the 30S ribosomal subunit (drug: streptomycin) and D-alanine racemase (Drug: cycloserine) (Zumla et al., 2013b). However, with the use of the available therapy, drug resistance has emerged that decreases their efficacy and lengthens the duration of treatment. Therefore, there is a need to identify hits that target a novel enzyme of the key bacterial pathways. The new targets should be vital for the growth of the bacterium and essentially should be required for their persistence (Mdluli and Spigelman, 2006; Duncan, 2004). Proper consideration should be given to the targets that are implicated in the pathogenesis of the bacterium (Zhang et al., 2006; Palomino et al., 2009). Worldwide various public and private sector organizations have evolved their interest on the validation of the new TB targets and considering them as an approach for target-based design of the new anti-TB drugs. Examples of some main organizations and programs include WHO's “Global TB Programme”, “Stop TB Strategy” (2006) and new “End TB Strategy” (2015); TB alliance; Open Source Drug Discovery (OSDD); Stop TB Partnership, National Institute of Allergy and Infectious Diseases (NIAID); Medical Research Council (MRC) TB unit. All these efforts have controlled TB and reduced the number of deaths caused by TB worldwide as revealed by the WHO reports on “Global Tuberculosis” (WHO, 2014; WHO, 2015). For example, Stop TB Partnership works to unite partners together and maintains coordination among them globally. This effort has brought around 1200 partners together and facilitates a platform to set various goals to cure people suffering from TB. The WHO's End TB Strategy focuses to diminish the TB mortality by 95% and to reduce new TB cases by 90% by 2035. Similarly, OSDD, which is an association that focuses on discovery new therapies of neglected tropical diseases, has taken the initiative to control TB by identifying leads against TB targets in an open platform. The targets pursued by OSDD include Rv0129c (Antigen 85 C), Rv2753c (dap A), Rv2773c (dap B), Rv1018c (glmU), Rv0548c (men B), Rv055c (men C), Rv0014c (PknB), Rv1258c, FAAD's, MbtA. A list of some of the new validated targets of Mtb is shown in Table 1, and these targets are discussed below.

2.1. Targets of Cell Wall Biosynthesis

GlmU, which is a bifunctional enzyme having acetyltransferase and uridyltransferase activity, represents a potential target for developing novel Mtb drug candidates (Sassetti et al., 2003; Zhang et al., 2009). Sassetti et al. have identified *glmu* as an essential gene for the growth of Mtb (Sassetti et al., 2003), and due to the absence of the homolog of its acetyltransferase region in human, it represents an interesting target (Zhang et al., 2009). It catalyzes the conversion of glucosamine-1-phosphate (GlcN-1-P) to UDP-*N*-acetyl-glucosamine (UDP-GlcNAc). UDP-GlcNAc, which is the final product of GlmU catalysis, is the substrate for the biosynthesis of peptidoglycan and lipopolysaccharide that form important components of the bacterial cell wall (Zhang et al., 2009).

Decaprenylphosphoryl-D-ribose-2′-epimerase (DprE1) represents as an essential target that catalyzes the isomerization of decaprenylphosphoryl-β-D-ribose to decaprenylphosphoryl-β-D-arabinose thereby helps in the biosynthesis of arabinogalactan (Batt et al., 2012). Arabinogalactan forms an essential structural constituent of the Mtb cell wall (Bhamidi et al., 2009), and therefore, targeting DprE1 causes the inhibition of arabinogalactan biosynthesis. BTZ043 was the first lead compound identified from benzothiazinone (BTZ) series as DprE1 inhibitor (Batt et al., 2012; Makarov et al., 2009; Hoagland et al., 2016). This is a covalent inhibitor of DprE1, which shows a very high potency with MIC in nanomolar concentration (Makarov et al., 2009; Piton et al., 2017). Lead optimization studies on benzothiazinones identified a highly potent inhibitor PBTZ169 (Makarov et al., 2014; Poce et al., 2014), which is now under clinical trials. A range of other covalent DprE1 inhibitors have also been identified which include, dinitrobenzamides (DNBs), nitroquinoxalines (VI-9376), nitroimidazoles (377790) and benzothiazoles (BTO and cBT) (Piton et al., 2017;