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Antitubercular *In Vitro* Drug Discovery: Tools for Begin the Search

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1. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* continues being a big public health problem around the world. The total number of cases of TB worldwide in 2009 was 9.4 million of which 1.8 million died of this disease, reported as the higher in history (Lawn & Zumla, 2011), World Health Organization (WHO) estimates that the one third of global population is infected latently by *M. tuberculosis* (LTBI), however 10% will develop active disease (Zumla et al., 2011). Although several strategies and programs have been implemented and anti TB drugs have been available for 50 years, many TB patients are not diagnosed and treated at time (Ghanashyam, 2011; Sosnik et al., 2010). These mismanaged patients, with non-optimal treatments are the principal source of multidrugresistant TB (MDR-TB), which is resistant to the first line drugs isoniazid and rifampicin, as well as extensive drug resistant TB (XDR-TB), that in addition of isoniazid and rifampicin is resistant to any fluoroquinolone and any aminoglycoside second line anti TB injectable drugs (Koul et al., 2011). Other aspect that aggravates the situation is the coinfection with Human Immunodeficiency Virus (HIV) disease, which increases the TB incidence rates three to five times and affected 1.1 million of TB cases in 2009 (Lawn & Zumla, 2011).

The most important control measures in TB are the prevention and chemotherapy. The current TB therapy has difficulties in controlling effectively the disease, due to inadequate adherence to treatment course caused by the length of time of medication and adverse reactions (Ginsberg & Spigelman, 2007). New antitubercular drugs should comply with following characteristics with the aim of reduce the low adherence that induce therapeutic failure and resistance: be active against MDR and XDR isolates, be active in less time to shorten the therapy, not interact with antiviral drugs, effectivity against latent TB infection, low toxicity and high bioavailability (Mitchinson & Fourie, 2010; Sosnik et al., 2010).

For those reasons the design of an antitubercular drug discovery initiative should have a strong *in vitro* screening program with the ability of optimize the current process and to identify in high degree chemical scaffolds with potent *in vivo* activity for clinical development. The aim of this chapter is offer different tools to perform a rational search for new anti TB drugs improving *in vitro* screening as a powerful source of selection of new compounds.

1.1 Antitubercular drug resistance versus the discovery and development of new antitubercular agents

The drug resistant TB (DR-TB) emergence and spread is a multifactorial problem produced by health mismanagement attention; inadequate therapy courses, antibiotic misuse, insufficient socioeconomic conditions, presence of immunodeficiency disorders and low patient compliance (Haydel, 2010). In addition, coinfection TB-VIH complicates the current treatment regimen because: decrease compliance and increase drug interactions producing toxic side effects (Koul et al., 2011). The need for more effective and less toxic anti TB drugs is really urgent, but the antibiotic drug discovery and development is a long and expensive process with very few compounds making it to the market (Vaddady et al., 2010). The current anti-TB drugs were developed since 1950s until 1980s which represented a missed period in TB drug research that contributed greatly to new challenges for improving treatments for DR-TB and prevent LTBI (Ginsberg, 2010). Actually, the biggest challenge for discover and develop a new era of TB medicines is prevention of drug resistance, which is necessary for treat the patients under ineffective therapeutic regimens (Ginsberg, 2010). Because of this, all efforts between sponsors, TB drug researchers, regulators and funders should be directed to the development of new and optimized portfolio of multidrug treatments.

1.2 Antitubercular *in vitro* drug discovery program design

In vitro experiments seeking to assess the interaction between the drug and the bacteria, which validates the selection of candidate compounds and the determination of the target drug concentrations for further testing (Vaddady et al., 2010). Is a fact that drug candidates fail in the stage of clinical development, in the Tuberculosis Antimicrobial Acquisition and Coordinating Facility Program (TAACF) were evaluated 88601 compounds and finally were selected five potential leads (Lenaerts et al., 2008), which is a high cost drug discovery program. An *in vitro* antitubercular drug screening strategy should consider and integrate several aspects as whole cell screening; single enzyme targets, toxicity testing and the inclusion of *in vitro* pharmacological tests for optimize the selection of promissory new drugs and predicts their clinical behaviour (Koul et al., 2011). In Fig. 1. is shown the design of an *in vitro* drug discovery program with the major phases looking for evaluate and select the largest possible number of novel antitubercular molecules.

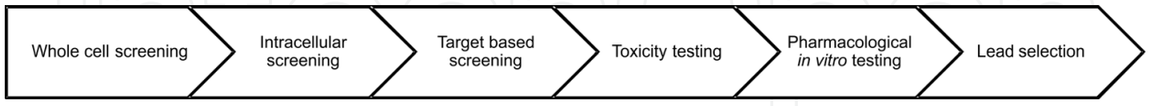

Fig. 1. *In vitro* anti TB drug discovery program components, each phase is an important step in the selection of promising anti TB drugs

2. Screening methods

In the 1950s, Canetti et al. described the first Drug Susceptibility Testing (DST) method for *M. tuberculosis*, which was a agar dilution method, involving the preparation of a concentration series of drugs against *M. tuberculosis* complex in Lowenstein-Jensen medium, inoculation of the bacterial cultures on the slants, and reading of the inhibition of growth by drugs at different concentrations (Canetti et al., 1963). The agar dilution tests permit to

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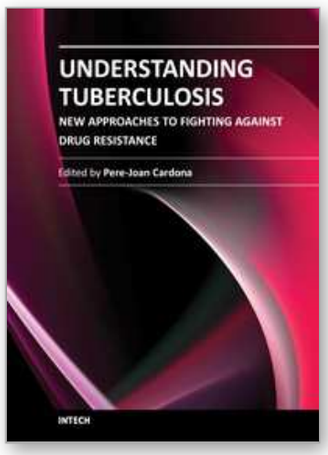

Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance

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In 1957, a Streptomyces strain, the ME/83 (S.mediterranei), was isolated in the Lepetit Research Laboratories from a soil sample collected at a pine arboretum near Saint Raphael, France. This drug was the base for the chemotherapy with Streptomicine. The euphoria generated by the success of this regimen lead to the idea that TB eradication would be possible by the year 2000. Thus, any further drug development against TB was stopped. Unfortunately, the lack of an accurate administration of these drugs originated the irruption of the drug resistance in Mycobacterium tuberculosis. Once the global emergency was declared in 1993, seeking out new drugs became urgent. In this book, diverse authors focus on the development and the activity of the new drug families.

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