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Chapter 16

Research and Development of New Drugs Against Tuberculosis

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54278

1. Introduction

Tuberculosis (TB) is a contagious infectious disease caused byspecies belonging to the *Mycobacterium tuberculosis* complex. At present, it is a re-emerging disease, due to co-infection with the Human Immunodeficiency Virus (HIV), but also to global bacterial resistance, and lack of adequate treatment in some places in the world. Approximately one third of the world's population is infected with *M. tuberculosis*, and out of these people, about 1.1 million people die every year of TB [1], making this disease the main cause of bacterial infectious death in adolescents and adults all around the world. In 2010 there was an estimation of 8.8 million incident cases and 12.0 million prevalent cases of TB worldwide. *M. tuberculosis* drug-resistant isolates have appeared giving origin to multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. XDR-TB has been identified in every continent of the planet. By 2010, the World Health Organization (WHO) was notified of the existence of 53.018 cases of multi-drug resistant TB (MDR-TB) worldwide; figure that only represents 18% of the TB-MDR estimated cases among reported pulmonary TB cases around the world [1]. Currently, there is global alarm since the infection with these strains is cured only in 66% of MDR cases and in 60% of the XDR cases [2].

More than sixty years ago, the introduction of the first anti-TB drugs for the treatment of TB (streptomycin (STR), *p*-aminosalcylic acid (PAS), isoniazid (INH) and then later ethambutol (EMB) and rifampicin (RIF)) gave optimism to the medical community, and it was believed that the disease would be completely eradicated soon. After a 30-year halt of anti-TB drug Research & Development pipeline, the Global Alliance for TB Drug Development (TB Alliance) started to fill the gap between the existing chemotherapeutics and the clinical need. Despite the efforts carried out with candidates in clinical trials such as PA-824 and bedaquiline, there is an urgent need of in-depth medicinal chemistry discovery studies for assuring enough leads

and candidates feeding the pipeline within the next decade[3]. Emerging chemical entities must shorten the time of treatment, be potent and safe while effective facing resistant strains and non-replicative, latent forms, and not interfere in the antiretroviral therapy [4]. In this review, we explore why we require to work continuously on the development of novel anti-TB agents, the stages necessary for the development of new anti-TB agents, breakthroughs in the discovery of new active principles and targets, the preclinical and clinical development of drugs, as well as the new approaches for the search of anti-TB active principles.

2. Targets and action mode of active principles currently used in the treatment of TB

Current TB chemotherapy is based on the combination of four anti-TB drugs which inhibit the bacterial metabolism, particularly the cell wall synthesis [5]. During the therapy, the goal of this drug combination strategy is to prevent effectively the mutational events [6]. According to their action mode, first and second line anti-TB drugs are grouped into cell wall inhibitors (INH, EMB, ethionamide (ETH), and cycloserine (DCS)), protein synthesis inhibitors (RIF, fluoroquinolones, STR, kanamycin (KAN)), and membrane energy metabolism inhibitors (PZA).

Current chemotherapy principally inhibits cell processes such as cell wall biosynthesis and DNA replication, and they only turn to be active regarding bacteria in active growth [5]. This implies that the chemotherapeutic agents in use are efficient bactericides but are poor sterilizers, not able to kill "dormant" *M. tuberculosis* which persists in macrophages after the death of the active bacteria [5]. RIF and PZAhave a partial sterilizing activity and they play an important role in the decrease of therapy from 18 to 6 months, even though there is a persistent population surviving these two agents. Consequently the current therapy ensures a clinical cure but fails to obtain a bacteriological cure [5].

3. Why we need new active anti-TB agents?

Whereas it is true that TB can be cured with the current active principles, treatment is complex and long, involving four drugs for two months and two drugs for four months more as a minimum.

	Active principle (year of discovery)	Source	MIC (μM)	Action mechanism	Target site	Genes involved in the resistance
First Line	Isoniazid (1952)	Synthetic	0.182	Mycolic acids synthesis inhibition, multiple effects on DNA, lipids and carbohydrates	Enoylreductase (InhA)	katG, inhA, ndh

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	Active principle (year of discovery)	Source	MIC (μM)	Action mechanism	Target site	Genes involved in the resistance
	Rifampicin (1966)	Semi-synthetic	0.486	RNA synthesis inhibition	RNA polymerase β sub-unit	rpoB
	Pyrazinamide (1952)	Synthetic	490 pH 5.5	Breakage of transport membrane and energetic depletion	Membrane energy metabolism	pncA
	Ethambutol (1961)	Synthetic	2.45	Aarabinogalactanbiosynthesisinhibition	Arabinosyltransferase	embCAB
	Streptomycin (1944)	Natural	1.72	Protein synthesis inhibition	rRNA ribosomal proteins S12 and 16S	*rpsL, rrs*
Second Line	Kanamycin (1957)	Natural	3.43	Protein synthesis inhibition	rRNA ribosomal proteins S12 and 16S	*rpsL, rrs*
	Amikacin (1972)	Semi-synthetic	0.85-1.7	Protein synthesis inhibition	rRNA ribosomal proteins S12 and 16S	rpsL, rrs
	Fluoroquinolones (1980s)	Synthetic	0.6-1.4	DNA replication and transcription inhibition	DNA gyrase	gyrA, gyrB
	Ethionamide (1956)	Synthetic	1.5	Mycolic acid biosynthesis inhibition	Enoylreductase (InhA)	inhA, etaA/ethA
	Prothionamide	Synthetic	2.77	Mycolic acid biosynthesis inhibition	Enoylreductase (InhA)	*inhA, etaA/ethA*
	p-aminosalicilic acid (1946)	Synthetic	1.9-6.5	Inhibition of thymidilate synthase and iron acquisition	Thymidilate synthase	thyA
	Cycloserine (1952)	Natural	245	Peptidoglycan synthesis inhibition	D-alanine racemase	alrA,ddl

Table 1. Reported MIC and molecular targets drugs of first and second-line drugs used in the treatment of TB [7].

Since the start of chemotherapeutic era, physicians have realized the slowness and difficulty of achieving effective cure. McDermott et al proved in 1956 that the *in vitro*efficacy of first-line TB drugsdo not correlate to their *in vivo* efficacy [5]. Cultures of *M. tuberculosis* in exponential growth are sterilized *in vitro* in a few days by firstline agents such as INH and RIF, while the same combination requires months to achieve the same result in host tissue. It has been stated that mycobacterial persistency is due to the physiologic heterogeneity of bacillus in the tissues, the existence of subpopulations with completely different rate-determining factors. Despite an urgent need for new therapies targeting persistent bacteria, our knowledge of bacterial metabolism throughout the course of infection remains rudimentary [8].Mitchison and colleagues proposed in 1979 that, in lesions, *M. tuberculosis*exists under at least four different population stages listed below [9] and showed in Figure 1:

1. Bacteria in active growth, susceptible to INH.
2. Bacteria with intermittent metabolism period, susceptible to RIF.
3. Low metabolic activity bacteria residing in acidic pH, susceptible to PZA.
4. "Dormant" or "persistent" bacteria, non susceptible to any current active principle.

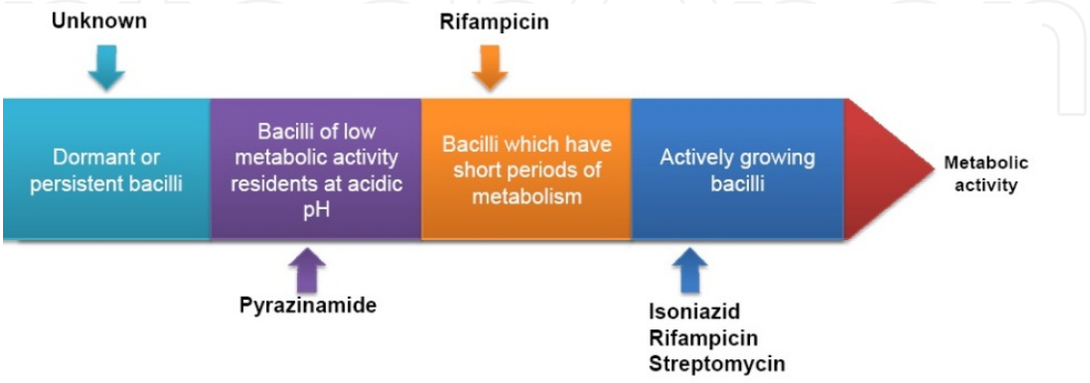

Figure 1. Spectrum of *M. tuberculosis* physiology. Extent of variation of physiological cell subpopulations of *M. tuberculosis* on an *in vivo* environment. Notice that first-line drugs mainly inhibit actively dividing bacteria, while there is not a single agent targeting the lower physiologically active stages.

During the initial chemotherapy phase (2 months), actively dividing bacilli rapidly die mostly because of INH bactericidal activity. Thereafter bacilli of low metabolic activity suffer from a slow death under the effects of RIF and PZA. There is evidence that persistentbacillarpopulation existing in the lesions usually determines the duration oftherapy [9]. Therefore efforts need to be made to target every physiological state of *M. tuberculosis* thus shortening the time of therapy and the appearance of drug resistance.

That brings us to the second reason why we need new anti-TB drugs. Drug resistance has emerged as a phantom from the dark, threatening today every corner of the world. RIF-resistance often correlates to MDR category (resistant to INH and RIF). XDR *M. tuberculosis* is an MDR strain also resistant to any fluoroquinolone and at least one injectable agent. Prognosis is less favorable for patients harboring XDR-bacilly compared to patients with MDR, with five times higher risk of death, require longer hospitalization or treatment times. However it has been shown that within an aggressive treatment, XDR-TB patients have been successfully cured in 60% [10,11]. Treatment of M/XDR-TB usually takes more than two years, and requires the use of more toxic, less effective and more expensive drugs. In resource-limiting countries, supplies of second-line drugs cannot be guaranteed. In an attempt to improve the conditions for millions of patients, Jim Yong Kim and Paul Farmer from Partners in Health brought down the price of second-line drugs has by more than 80%. Unfortunately the latest reports from Italy, India and Iran, facing the extremely (XXDR) or totally (TDR) super-bug, have made imperious the essential necessity of new drugs targeting novel mechanisms of action [12].

TB infection in immune-compromisedpopulation leads tosevere cases,possibly affecting other parts of the body, such as the pleura, meninges, the lymphatic system, the genitourinary

http://dx.doi.org/10.5772/54278

system, and the bones [13]. It has been estimated that HIV infected patients are 100 times more likely to develop TB [14]. Although the studies support a decrease of mortality for TB patients after the introduction of antiretroviral therapy, evidence of the existence of interactions between Highly active antiretroviral therapy (HAART)and TB chemotherapy. HAAR is based on a combination therapynormally involving two reverse-transcriptase inhibitors and a non-inhibitor [15]. P450 Cytochrometypicallymetabolizes reverse-transcriptase inhibitors,however this cytochrome is also induced by RIF. TB chemotherapy may reduce significantly the concentrations of anti-retroviral drugs which may lead to treatment failure or resistance. An increase of the nevirapine dose to compensate for this interaction increases the risk of toxic effects and hepatotoxicity in patients who already present a low body mass index and high level of CD4 lymphocytes [16]. Physicians prefer to avoid the concomitant use of nevirapine and RIF; consequently there is a clinical need for mycobactericidal agents devoid of P450 catabolism.

4. A 50-year wait

Antibiotic discovery began in the early 1930s when different classes were discovered [17]. At the end of the 1950 decade, the combined regime was established and was thought to eradicate the disease completely. In the following thirty years after the introduction of the last first-line anti-TB drug, RIF, the regimen remained unchanged. The landscape changed in 1993 when the WHO declared TB a global health emergency [18]. Until recently, research in development of new anti-TB drugs was poor. These days, the TB Alliance has emerged as a non-profit organization promoting and funding anti-TB drug development by creating consortia over a defined project involving oftenbig pharmacompanies, institutes of research, and universities. Interest in drug discovery has placed on both phenotypic and target-based approaches to set in motion strong pipeline. With the joint effort of the Working Group on New TB Drugs, Stop TB Partnership and other societies.gatifloxacin, delamanib, PA824, rifapentine, sutezolid, SQ-109, bedaquiline and linezolid are candidatesin clinical trial [19]. There are other promising compounds (CPZEN-45, BTZ043, AZD5847, DC-159a and others), but a handful ofscientists believe that the gap is large and there is no certainty whether there will be a full new regimen in the next decade [3].

Neglected diseases affect mostly the poorest population on Earth, predominantly those who live in remote, rural areas, in depressed urban settings, or in regions of conflict. Together with malaria, leishmaniasis,filariasis and Chagas disease, TB makes part of the high impact neglected diseases, which unfortunately represent an insufficient market to attract enough investment on research by the pharma industry [20]. Whereas the most advanced societies have increased their life expectation thanks to technological development of medicine, in developing countries these diseases (some of which are preventable, treatable, and curable) still devastate the frailest populations. However, governments, multilateral organizations, and foundations spend billions of dollars in the procurement of treatments; and with the current situation of the disease, world health care organisms applaud recent efforts to develop new anti-TB drugs, even though the panorama is not that promising yet [3,21].

5. Platform for the development of active principles in the treatment of TB

Both basic and clinical pharmacology have contributed to the progress in the discovery of drugs applying their experience to the development and validation of hypotheses of new action targets in order to produce novel drugs. In this sense, researchers need to be innovative and they must have a wide vision over the interpretation of the results [20]. The choice of a therapeutic candidate is probably the most important decision to make in the discovery and development of a medication. The chemical structure of a drug confers its biologic, pharmacokinetic, physicochemical, and toxicological properties [22]. On the other hand, the discovery and development of new drugs is a complex and costly process requiring large amount of resources and time. The cost of launching a new drug to the market ranges from US$ 800 million and 1000 million, and it may take between 8 and 17 years depending on the disease and the treatment (Figure 2) [23].

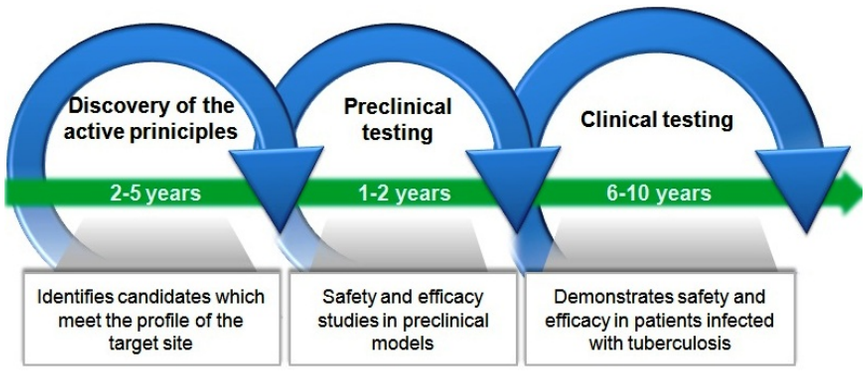

Figure 2. TB drug pipeline. From the discovery bench through preclinical and clinical studies for novel anti-TB agents, a process that could last more than 15 years.

The term "hit" is used to describe a small number of structurally related molecules possessing an established biologic (antituberculosis) activity [24,25]. The term "lead" is defined as a molecule belonging to a series, which shows a substantial structure – activity improvement around a determined *"hit"*, and from which other important factors have been obtained such as evidence of selectivity and pharmacokinetic data or *in vivo* activity [24].

Once these terms are defined it is important to knowthe biochemical target on which a certain structural type of a chemical compound exerts a biological action. The determination of the mechanism can be carried out *in vitro* by generating drug-resistant mutants which are examined on their whole-genome sequences analysis. The transcriptional profile using cutting edge mycobacterial microarrays or *q*PCR) can be interrogated among the whole transcriptome for potentially distinguishing a defined set of genes involved in the response against chemical injury. Once a determined protein or receptor has been identified, cloning, over-expressing and purifying the proteins is usually performed with the aim of examining its biochemistry and its possibility of affinity or interaction in the tube test is always possible option. Gene deletions and over-expressing systems in *Mycobacterium* are also used for confirming the mechanism of action of a defined candidate [26].Ideally, an antibacterial agent must show bactericidal activity often impeding an essential function for the survival of the microorganism.

Another more classical possibility is monitoring of microbiologic parameters such as growth rate, CFU counting and chemical analysis of metabolites in the treatment with sib MIC, MIC and over-MIC values of the agent. Currently, many active principles are identified as the result of a rational design, supported by genomics inspired hypotheses or from another perspective, by automated high-throughput screening (HTS) using compounds libraries [23].

6. Discovery of active compounds

The parameter most commonly determined to examine the in vitro antibacterial activity of a specific moleculeis the minimum inhibitory concentration (MIC) which represents the concentration required to inhibit 99.9% of the growth of bacilli The main limitations of these trials is that do not describe the percentage of dead bacteria (which critically depends on cell density) or the metabolic state of the bacteria, if we aim to examine the persistent antimicrobial effects of a certain drug [27]. Most publications include at least a compound with a MIC lower than 6.25 mg/Ll [24,26]. It is recommended that active compounds under a colorimetric assay (Resazurin, Alamar Blue, MTT) are reconfirmed usingagar-based techniques or MGIT. A simple and easy to use, agar-based method using Middlebrook 7H10 was introduced in 2004 by Bhakta et al for measuring MIC values [28,29]. The spot culture growth inhibition assay (SPOTi) has now being used to screen more at least more than 1000 compounds. Simultaneously, the cytotoxicity in different type of mammalian and/or macrophages is carried out. The selectivity index (SI) is determined by dividing the growth inhibitory concentration 50 (GIC_{50}) corresponding to the concentration of compound capable of killing half of the mammalian cellsby the MIC using the same concentration units. If the SI is larger than 10, infection of a macrophage with a selected strain of mycobacteria and treating with the drug candidate can help to determine its intracellular potential (Figure3)[26].

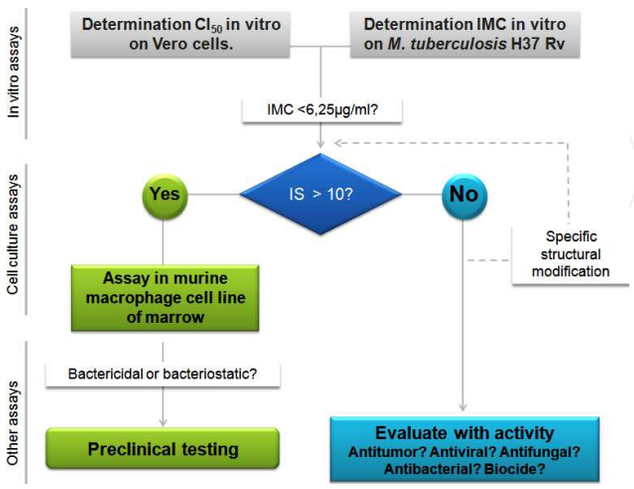

Figure 3. Research and development of new TB active compounds. In an attempt to promote quickly pre-clinical studies of early leads, Orme propose this rapid diagram based on the selectivity ration between a bacteria and mammalian cell line [26].

The macrophage infectionmodel offers the possibility of evaluating the compound in a physiologically challenging intracellular space. By plotting a viability curve fordifferent concentrations of the active principle, The $EC_{90,}$ EC_{99} and $EC_{99.9}$values of are determined verifying the concentration that is able to reduce the bacterial load by 1, 2 and 3 logarithmic units. MIC is most usually defined as EC_{99}.(or EC_{95}). Bactericidal compounds are generally associated with a 3-fold reduction in CFU logarithmic units. In addition the infection assay determines the activity of a compound in an intracellular medium which does not always correlate with *in vitro* media-based inhibition measurements. For instance, transport mechanisms in the cell may influence the intracellular concentration of the drug regardless of the external fluid concentration [30].

The success of a discovery program of antibacterial principles is founded on three factors: identification of key elements contributing to pathogenicity of the microorganism, the understanding of the existing relationships between the microbe and the host, and importantly, the properties of the chemical compound [30]. Two pathways have been traced with the aim of discovering active principles. One is the empirical pathway, mainly based on chemistry and phenotypic screening; and the more modern is the mechanistic, based on genomics, biochemistry and molecular biology. The former begins with the identification of an active principle with potent antimicrobial activity on *in vitro* conditions. The active principle is discovered by chance or by random screening. Then, it is subject to trial on rigoroustoxicological assays before using animal models. Some candidates may eventually beselected for human trials. The limitation of the empirical pathway is the lack of information on the specific target or the action mode, sometimes this lack of understanding can led to high failure rates mostly for toxicity problems [30]. On the other hand,the mechanistic pathway started with the age of molecular biology and genomics which allowed the identification of specific targets of the microbe, absent or structurally different in human hosts. This strategy can be upgradeto high-throughput screening (HTS) platform and to evaluate a large amount a substances in little time. Crystallization of the target proteins and X-ray diffraction spectroscopy, together with an analysis of the active site in the presence of the natural substrate and inhibitors allow the detailed study of the crucial structural interactions.

In the mechanistic approach discovery usually involves firstly the identification and validation of amycobacterial target macromolecule to be inhibited or interrupted. Obtaining the small molecules which inhibit such a target is another story. Large collections of compounds can be screened directly against the protein if a high-throughput method of assay is available. Alternatively if there is structural information it is possible to computationally interrogate the target against a defined set of computer-based compounds (docking). The preferred targets are generally the ones ocurring in *M. tuberculosis* and not represented in the human genome. By means of comparative genomics, the targets are present in the human genome. For example, nicotinamide adenine dinucleotide (NAD)is generated in humans either by *de novo*biosynthesis, or by DNA and RNA degradation. However*M. tuberculosis*can only synthesize NAD using the*de novo* synthesis. This allows to rationally explorequinolinatephosphoribosyltransferase (QAPRTase) inhibition (*de novo* pathway) for the developing of microbial selective inhibitors [30].

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Targets existing in *M. tuberculosis*whileabsent in other bacteria would seem ideal since active compounds against this target will be harmless to bacteria beneficial for the human being. However, selecting targets complying with this requisite leads to restrict extensively the likely targets: for the most of it only the biosynthesis of the mycobacterial cell- wall or those implied in specific mycobacterial process (virulence, detoxification, others).

The validation of a target, involves the examination of bacterial viability when decreasingthe protein expression. If reducing the enzyme level,led to lose in bacterial viability, then the target is known as "vulnerable", and it is meant to be attacked [30]. The elimination or knockout of the gene that codifies an essential protein is difficult (or impossible) to produce by homologous recombination if the gene is essential in the conditions of growth, and therefore inducible promoters are a better chance to show the effect of tightly reducing its expression. Over-expression of the target is also possible, the growth of the over-expressed mutant being rescued under higher concentrations of inhibitor. These studied have led to many targets that have been identified and validated. The studies of Sasettiet al identified which enzymes were essential in vitro and in vivo using a transposon site hybridization analysis (TraSH) using both in vitro or in vivo [31,32].

Another approach is related to the genomics of virulence. Some mycobacterial genes are only expressed in granulomabut not inside the macrophages. Isocitratelyase enzyme is fundamental in the persistence of bacilli in chronic infection in mice and its function is related to obtaining carbon during its persistence in the host [8,33]. The extracellular repetition protein (Erp) is another essential protein involved in *M. tuberculosis* virulence that was the first discovered virulence factor. The mutant Δ-erp that does not express correctly the extracellular repetition protein does not show any alteration in standard *in vitro* culture, but maintains an essential function for *in vivo* survival [34,35]. This protein is also a potential target for the development of anti-TB active principles. Two independent proteins (fadD28 and mmpL7) have been identified contributing to the early growth of *M. tuberculosis* in mice lungs and are related to the synthesis and transport of a complex lipid associated to the cell wall, i.e. phthioceroldi-mycocerosate (PDIM) [34]. Although the function of this lipid is unknown, it is suspected that itplays a role in the decrease of the host's immune response. There is no doubt about the remarkable progress that the sequencing of the*M. tuberculosis*genomehas brought to the anti-TB drug discovery area of research. The functional annotation of all these genes remains a considerable amount of experimental work. Sequencing of other related organisms such as *M. marinum*, *M. leprae*, *M. aurum*and othersoffer often clues about the essentiality of specific set of genes and operon distribution.

7. Target or compound type in discovery stage

Analogues of thiolactomycin: Thiolactomycinwas the first natural thiolactonedisplaying antibiotic activity. The compound showed moderate *in vitro* activity of 56 μM against *M. tuberculosis*[36]. Thiolactomycin analogues have been synthesized and some hits were found [5]. Analogues of thiolactomycin seem to inhibit mycolatesynthetase, an enzyme involved in the cell wall biosynthesis.

Nitrofuranilamides:*M. tuberculosis*has been found to be susceptible to compounds containing a nitro group. Nitrofuranilamide was identified in a screening for inhibitors of UDP-galactosemutase [5]. A set of compounds structurally related to nitrofuranilamides was synthesized and tested for antimicrobial activity. All resulted active both to sensitive and resistant strains with a MIC ranging from 0.0004 – 0.05 mg/L [37]. Four nitrofuranilamide type compounds showed significant activity in the tuberculous infection in mice models [37].

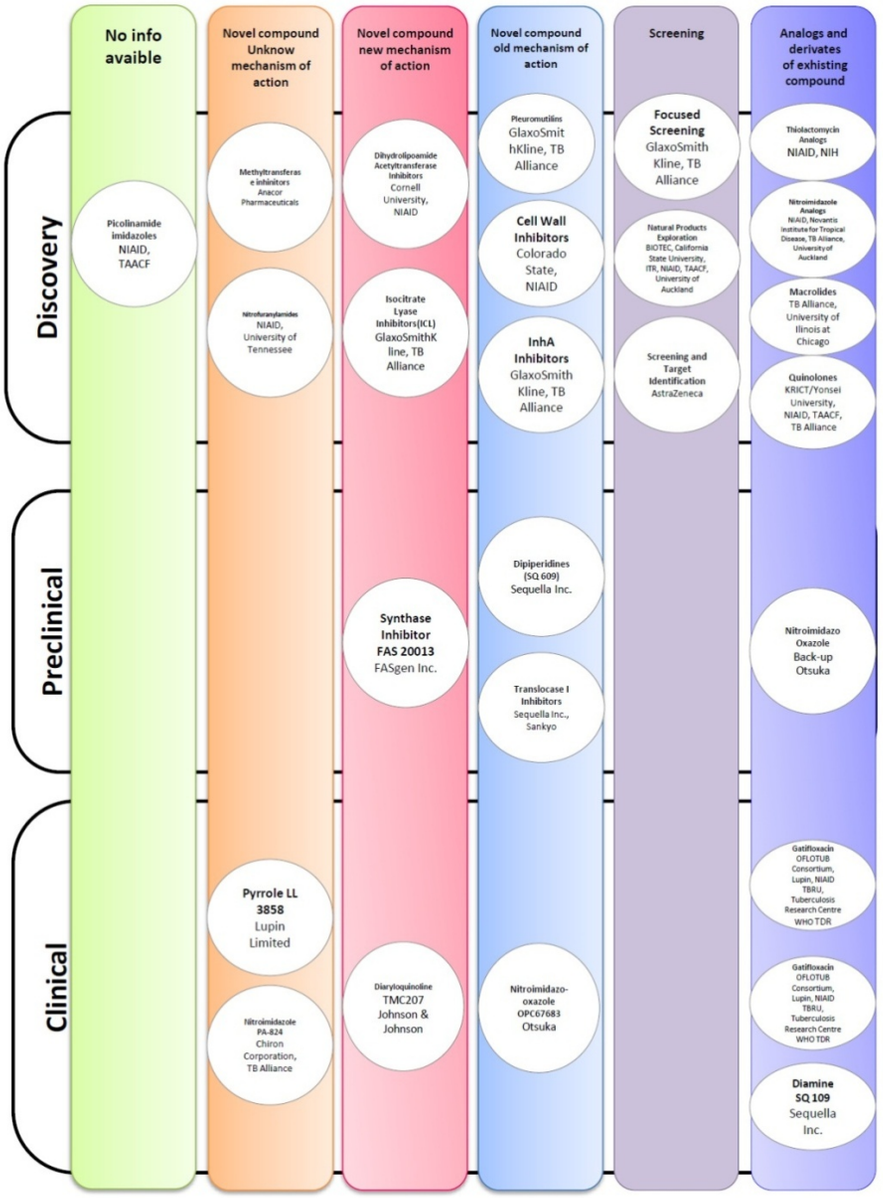

Figure 4. Development of new active compounds targeting *M. tuberculosis*

Analogues of nitroimidazole: While the PA-824 product is developed, the TB Alliance started a project to maximize the potential of this class, by identifying the improved versions of PA-824 [38,39].

Dihydrolipoamideacetyltransferase inhibitors: Dihydrolipoamideacetyltransferase (dlaT) enzyme of *M. tuberculosis* is a potential target for TBdrug discovery [5]. This enzyme is a component of the pyruvate dehydrogenase sub-unit, an enzyme catalyzing acetyl-CoA synthesis and also contributes to peroxinitritereductase, adefense enzyme against oxidative/ nitrosative stress. Some heterocyclic compounds have been found to be inhibitors of the dlaT enzyme, displaying non-replicative bacterial killing [40].

"Focused Screening": TB Alliance is helping to develop a set of projects identify chemical compounds which are active against specific molecular targets including DNA gyrase inhibitors (fluoroquinolones targets), peptidedeformylase inhibitors, and quinone-analogous electron transport inhibitors [5].

InhA inhibitors: InhA is the well-known enoyl-reductase of *M. tuberculosis*being an essential-biocatalyst forlong chain fatty acids biosynthesis (FAS-II) [41]. INH resistance is mainly mediated by mutations on KatG, the enzyme activating the prodrug. Consequently, InhA inhibitors that do not require activation by KatGcould be interesting candidates. The main goal of the screening is directInhA inhibition. Some compounds of the biphenylether type have proven to be inhibitory of InhAin a degree correlatingwith*in vitro* growth inhibition [42]. A possible limitation of this class is the possibility of cross resistance with INH and potentially with ETH [5].

Isocitratelyase inhibitors: The isocitratelyase enzyme (ICL) has been found to be essential for the long term persistence of *M. tuberculosis* in mice but not in culture medium or under hypoxia conditions. McKinney and colleagues have proved recently that inhibition of the ICL1 and ICL2 isoforms block bacterial growth and survival in macrophages [8]. The absence of orthologues in mammals for this enzyme, makes it a good target for the development of inhibitors [5]. A screening of more than 900,000 compounds has been performed without satisfactory results. The potential of traditional Chinese medicine has also been researched in obtaining specific inhibitors of this enzyme [43].

Pleuromutilins: Pleuromutilins represent a new kind of antibiotics derived from pleuromutiline, a bioactive diterpene initially isolated from edible *Clitopilusscyphoides*fungus [44]. These molecules interfere with protein synthesis associating to the 23S rRNA unit. Despite the structural novelty of these compounds, recent studies have pointed out cross-resistance among pleuromutilins and oxazolidinones[5]. Pleuromutilins have proved to inhibit *M. tuberculosis* growth of *in vitro.*

Macrolides: This project aimed to optimize the anti-TB activity of the macrolide class through the synthesis of modified derivates of erythromycin [5]. Derivatives of erythromycin such as 11, 12-diol, 11, 12-carbamates, and 11, 12-carbazates have been found to be to most promissory [45].

Quinolones and DNA gyrase inhibitors. The goal of this projectwasto synthesize and assess the potential of novel quinolones trying to decrease the time of treatment. More than 450 compounds were synthesized and assessed [5]. The 2-pyridones class has proven to be active DNA gyrase of *M. tuberculosis*, being KR1-10018 an interesting lead for the development of anti-TBdrugs [46].

Survey of natural products: Natural products represent an alternative for the search of new compounds. Different research institutescontinuously carry out screening of natural products (products from plants, fungi, and bacteria) with the hope of identifying compounds with anti-TB activity [5]. Some natural substances have shown significant anti-TB activity: saringosterol 24-epimers, esgosterol-5,8-endoperoxide, micromolide, ascididemin, the manzamines, and engelhardione, among others; however, there is lack of more research regarding selectivity and toxicity [47-50].

Plants: Drugs based on plants extracts have been used worldwide for the treatment of several diseases from ancient times.A great interest in phytomedicine and natural product structures are screened in order to measure their pharmacologic activity. In Colombia, there has been a resurgent interest in the discovery of novel natural anti-TB drugs [50-54].

Natural sea products: oceans are outstanding sources of natural products, not only in invertebrate species such as sponges, mollusks, bryozoos, but also in marine bacteria and marine sediments. The alkaloid (+)-8-hydroxymanzamine A was initially isolated from the *Pachypellina*sp sponge [55]. In the same way, irciniol A was found in sponges from the Indian Pacific proving to be a good candidate for further studies [56]. Aerothiononine isolated from the marine sponge *Aplysinagerardogreeni*marine sponge was active against clinical isolates of MDR-TB, despite of the resistance patterns, with MIC from 6.5 to 25 mg/L [57]. The alkaloid (+)-8-hydroxymanzamine A alkaloid showed potent inhibitory activity against *M. tuberculosis* H37Rv [58].

Insects. The immune system of invertebrates and vertebrates is made up by cytolitic peptides which act as antimicrobial agents during the invasion of eukaryotes and prokaryotes microorganisms. Poison from arachnids (spiders and scorpions) contains toxic peptides of high molecular weight (2 – 12 kDa) with high specificity against prokaryote cells [59]. This type of compounds may be very promising as a drug in the treatment of tuberculosis.

Microorganisms. Most of the major antibiotics drugs have been isolated frommicroorganisms. Streptomycin,the first effective anti-TB drug was identified in *Streptomyces griseus*. Besides streptomycin, aminogyicosides kanamycin, amikacin, and capreomycinhave been very important therapeutically as second-line agents [59]. Other important anti-TB drugs in TB treatment are the rifamycins, which constitute a group of semi-synthetic antibiotics isolated from *Streptomyces mediterrani*[59].

8. Preclinical and clinical development for new anti-tuberculosis drugs

8.1. Preclinical development of anti-TB active principles

Preclinical tests involve the use of animal models to prove the efficacy and safety of a given candidate before being tested in humans. Because of its management in terms of size, offer, maintenance, strength, and reproducibility, the mouse constitutes the preferred animal model for *in vivo* research of the TB infection [60]. Other possible animal models include rats, guinea pigs, and macaques. The amounts of viable mycobacteria and mortality and the possibility of organomegaly in the pulmonary tissue are evaluated during therapy, at the end of therapy

and in the post-therapy period. Post antibiotic effect, relapses, and resistance development are examined. Antagonists, additives, or synergistic effects are also evaluated when the compound is administered in combination with other active principles, as well as its capability to sterilize lesions in experimentally infected animals. Finally, toxicological studies, which must be highly controlled and documented, are carried out forthe determination of the safety window in order to perform the subsequent clinical trials in humans [60].

The drugs regime must be administered for several months, using commonly between 100 – 150 mice per test, therefore requiring large amounts of space and resources for maintaining the animals. Model in mice is more effective regarding the cost-benefit relation, and most of the data obtained can be reproduced in clinical studies. The model of infection by TB in mice has served to predict the sterilizing potential of new compounds, the effectiveness of the combination of drugs, success in intermittent therapy and the duration of therapy necessary to avoid relapse. The effectiveness of the active principle is measured mainly the reduction of the colony forming units (CFU) in the lung and spleen. Several varieties of mice have been used in laboratories conducting this type of test and, to this date, no comparisons have been reported [61].

Genetically modified mice have been used in the in bioassay of compounds with anti-mycobacterial activity [62]. A mouse that does not express the interferon-γ gene (knock-out) is incapable of producing cytokine Th1 and therefore suffers a more acute infection. Bioassay with this mouse allows determining the initial efficacy of a chemical compound in six days. Because of their statistical power, substances with low antimycobacterial activity can be detected by a small decrease in the CFU count. The model has great usefulness in initial trials, when there is a limited amount of the chemical compound. Another model, still under development, has been proposed to study relapse. An animal that cannot produce the granulocyte-macrophage-colony stimulation factor (GM-CSF) is used.

Wayne's model, which indicates the effect of compounds against persistent bacilli, has also been used. Bacilli under anoxia conditions are used and they are directly inoculated in the mouse. The guinea pig model also allows observing the destruction of tissue by caseous necrosis where there is not oxygen contribution and bacteria go into a hypoxia state [61].

Pharmacokinetics and pharmacodynamics range from *in vitro* tests, *in vivo* tests in animal models, and finally clinical trials in humans [57]. The simplest pharmacodynamic measure is determining the MIC *in vitro,* used widely in the primary discovery of active principles. This measure can be roughly related to the maximum cut point of the active principle concentration in plasma (C_{max}) and can aid in the prediction of *in vivo* pharmacodynamics among a series of structurally related agents. However, it does not represent the concentration at which the growth ceases, and, as we have already seen, does not allow distinguishing between bactericide and bacteriostatic activity. Moreover, it does not allow obtaining information regarding the dynamic relationships *in vivo* either, since the growth conditions do not represent the ones of persistent organisms in the living tissue.

Animal models enable to evaluate the *in vivo* efficacy of novel active principles regimens. Protection experiments using monotherapyfor a certain amount of time and then performing

lethal intravenous or aerosol inoculation can prove the efficacy and selection of a preliminary dose. Studies on the short term using colonies count in different homogenized organs allow estimating the bactericide capability of a medication or a combination of drugs, as well as the likely appearance of resistance [57]. However, in order to describe the sterilizing activity of a given compound, a larger study time is required as well as other techniques since negative cultures finalizing the therapy do not necessarily indicate that there was sterilization. Three months are required after the end of treatment to determine a durable cure and success of the sterilization. Cornell's mouse model uses an intensive therapy in order to obtain negative cultures and then evaluate the ability of individual active principles or their combinations to prevent relapse when the mouse is left untreated or when it is maintained immunocompromised[57].

The following are the PK and PD parameters which are calculated in the trial with mice: the C/MIC quotient, defined as the ratio of the serum maximum concentration (C_{max}) over the MIC; the AUC/MIC quotient, defined as the ratio of the area below the concentration-time curve (AUC) over the MIC in the serum during the total time of treatment(144 h) divided by 6 in order to obtain a daily value (AUC_{24}/MIC); and the percentage of time above the MIC (T > MIC) estimated by the first order kinetic equation $C = C_0 e^{-kt}$, where C_0 is the concentration to time 0, k is the constant and t the time, and it is defined as the percentage of the 144-hour time in which the medication concentration surpasses the MIC in the serum [63].

Recent studies of the PK and PD parameters for INH, RIF, and fluoroquinolones have improved the understanding of PK and PD properties of these drugs. Although the PK and PD parameters are characterized for antibacterial agents, a clear description of the efficacy is still lacking [63]. The parameter that best describes the bactericidal activity of anti-TB drugs in the mice model is AUC_{24} / MIC, with a correlation of 0.83. For INH when the value of AUC_{24} / MIC reaches 500, the maximum effect is observed with a decrease of 1.3 log CFU per mouse lung. In other words, the INH effect was the same when the total doses were administered into 6, 12, or 18 doses divided equally during one week [63]. Mitchison observed that the administration of a single total dose of INH in infected guinea pigs had the same effect than if administered daily, every other day, or every four days during a six-week period. Therefore, the efficacy of INHwas dependent on the size of the doses but not on the regime [63]. Preclinical trials that establish pharmacodynamic and pharmacokinetic properties enable to obtain information regarding the optimal doses and regimens.

Despite of the large use of the mouse model,this rodentdoes not develop the typical human lesions observed in pulmonary TB such as caseous necrosis or cavitations[57]. Also, one has to be very prudent conducting escalation in the doses of the agents between the mouse and the human due to the metabolic differences and possible pharmacokinetic interactions. The histological characteristics of guinea pigs in a TB infection are more similar to human pathology; but there is little experience in the chemotherapeutical use of this model. Preliminary studies suggest that the guinea pig model is capable of differentiating the sterilizing activities of INH and RIF [57].

A good model to study latent forms of TB is the cynomolgus macaque (*Macacafascicularis*) [61, 64]. All primates infected by bronchial instillation developed the infection, based on the tuberculin test and immune responses to *M. tuberculosis* antigens. Differences in the progres-

http://dx.doi.org/10.5772/54278

sion of the disease for the 17 macaques studied were observed. Between 50 – 60% of infected primates developed active and chronic infection, characterized by clear signs of disease in thoracic x-rays and other tests. Approximately 40% of the initially infected macaques did not develop the disease in the 20 months of study. These primates showed clinical signs of latent TB. In summary, the study proves that it is possible to use the cynomolgus macaque in infection by *M. tuberculosis* because it presents the complete spectrum of infection in humans (rapid and lethal infection, chronic infection, and latent infection). This animal model is the only one that enables to study the latent forms of the infection. Its great advantage is the high pathologic similarity of the infection in macaques and humans, whereas the disadvantages are the cost and maintenance of the animals, particularly since they require facilities with Biosafety Level 3 [64]. This model has been proposed in final preclinical trials for the development of active principles for latent forms of TB [61].The following are examples of promising compounds in preclinical phase. Some of these substances are protected by patents and therefore access to information is restricted.

The following are examples of promising compounds in preclinical phase. Some of these substances are protected by patents and therefore access to information is restricted.

SQ609 dipiperidine: this compound is a completely novel anti-TB active principle. It acts by interrupting the biosynthesis ofcell wall, but its specific mechanism is unknown [5]. It demonstratedantymycobacterial activity in an*in vivo* mouse model.

FAS20013 synthetase ATP inhibitor: Inhibition of bacterial fatty acids synthesis (FASII) still represents a valid, target for the discovery of anti-TBdrugs. However, this novel compound was identified by Fasgen and it has as action target the inhibition of enzymes for biosynthesis of fatty acids in *Mycobacterium* [65]. It belongs to the β-sulfonylcarboxamides class.

Translocase inhibitors SQ641: The pharmaindustryis developing a series of translocase inhibitors for the treatment of TB. The mycobacterial translocase I is an enzyme required for the biosynthesis of the cell wall, and the SQ641 compound has been reported as a selective inhibitor of this enzyme [66,67].

8.2. Clinical development of anti-TB active principles

Identifying new anti-TB is a complex and highly regulated process carried out around a critical moment: when the new compound is tested in humans [5]. Currently, clinical images offer a support method for generation of drugs which enables to establish information about the biodistribution of the molecule, interaction of the target, and pharmacokinetics[68]. Clinical development of a promising substance is usually divided into four phases. The first phase is carried out in healthy human beings and it provides information regarding the chemical compound pharmacokinetic profile, and some preliminary information regarding safety [69]. Phase I trials are conducted in a small size, usually 15 to 30 subjects, and can be of single or multiple doses. Besides the phase I trials, researchers may consider incorporating the pharmacokinetics and safety studies to a wider population size (200 to 300 subjects). Phase II studies are conducted on patients diagnosed with active TB. The efficacy in monotherapy and

combination therapy is evaluated. One of the objectives of trials in phase I/II is to determine the optimal dose for the phase III studies.

Early Bactericidal Activity (EBA) is one of the fundamental parameters to determine the clinical efficacy of active principles [70]. It consists on a large trial conducted on patients recently diagnosed with pulmonary TB who are treated with active principles or combinations for a period of 2 to 14 days. Patients must not have used anti-TB drugs previously. During the treatment period, the amount of viable bacilli appearing in sputum samples is determined quantitatively. The traditional EBA unit is the logarithmic decrease of colony forming units (CFU/mL sputum/day during the first 48 hours). EBA studies have shown that there are differences between the fall of viable bacteria counts in the first two days of treatment in comparison with the following twelve [71]. Differences among several treatments were also more significant during the first two days. In the early therapy, the activity of INH was superior and dominant regarding the other active principles administered in combination. Any addition of INH to a regime leads to an increase of EBA but never higher than INH on its own. The addition of PZA to a regime of STR, INH, and RIF increased 0-2 days EBA from 0.415 to 0.472 [71]. The greatest disadvantage of determining the EBA is its inability to detect sterilizing activity. Some researchers have concluded that extended EBA trials (2 to 14 days) do not correlate to the sterilizing activity [72]. For example, the potent sterilizing activity of PZA was not detected in an extended EBA trial. STR showed potent activity in extended EBA, and it is known it has a very low sterilizing activity in randomized clinical trials. In extended EBA, EMB appears as antagonist; however, there is no clinical evidence that this drug interferes with the sterilizing effects of RIF and PZA [72].

In order to determine the sterilizing activity of the anti-TB active principles, and 8-week study has been proposed, and the ratio of patients whose sputum be negative is determined; this parameter correlates to the ratio of patients who suffer relapse after the treatment [73]. In these studies, frequent counts of the number of viable bacilli are carried out being known as “serial sputum CFU counts” or SSCC. This method enables to distinguish between differences in the organisms that divide rapidly from the persistent ones.These studies turn out to be appropriate to determine the possibility of a regime to decrease the time of treatment [73].

Phase III clinical trials are carried out at large scale; they are randomized and they are conducted to demonstrate the improved or equivalent efficacy of a new treatment against standard treatments [60]. Around 1000 patients are enrolled per study for TB and the cure on treated patients is bacteriologically observed during certain amount of time as well as the ratio of relapse. The accepted end point to demonstrate efficacy is 2 years. The experimental design of Phase III trials must be designed cautiously clearly definingcritical primary and secondary end points, the size of the sample, the intervalsof confidence, and the statistical methods that will be used to obtain the data [60]. It is fundamental that microbiologic assessments are being conducted during an appropriate time, with the aim of determining the real activity of the researched agent. To ensure a sufficient population in Phase III studies, trials may be conducted in countries with high incidence rate of TB. Countries possessing a robust and expansive TB control program that provides essential information such as annual incidence (location of the disease, comorbility, resistance) are preferred. A reference laboratory is required for most of

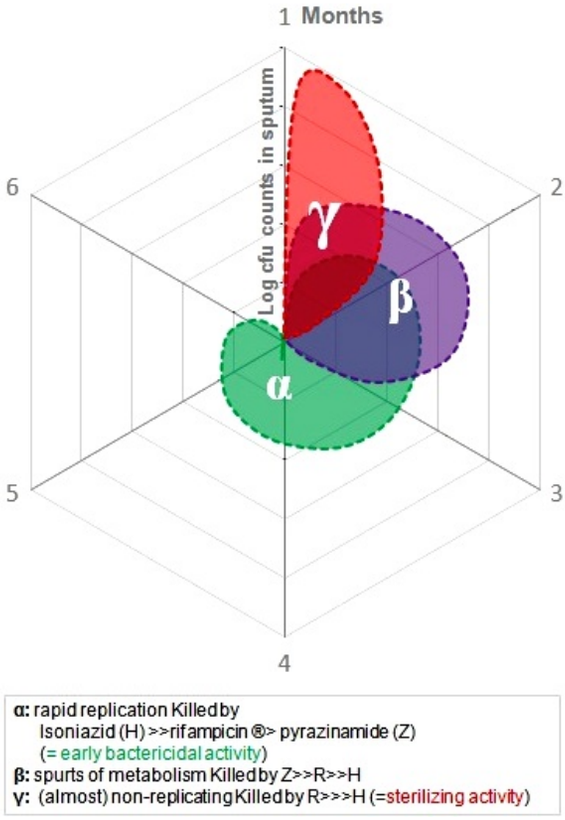

Figure 5. Pharmacological activity of RIF, INH and PZA targeting *M. tuberculosis* subpopulations. Differential population of *M. tuberculosis* in the lesions, observed after 6 months of treatment.as log CFU in sputum

the trials, but there is also need to extend duplicates to local laboratories. Finally, validated relapse markers, which provide evidence of the sterilizing activity of an active principle or regime, are used. To this end, the most used method is determining the ratio of patients who have a negative sputum culture, 8 weeks after the beginning of the treatment compared to the standard treatment. Molecular methods using relapse markers require greater study and validation in order to be employed successfully [60].

Phase IV studies include product development efforts such as patents, description of biologic activity, toxicity, safety profile in humans and demonstrated clinical efficacy. Best manufacturing practices studies are conducted, as well as laboratory and clinical practice to ensure the marketing needs of the product. Post-marketing studies duringPhase IV are typically assessment of new regimens in comparison to thenormally used, and surveillance of likely adverse effects, including the development of resistance. The acceptance for use of the new active principle must be subscribed by the patient, and the economic benefits of the new drug must also be established [60].

SQ109 diamine: The lead was identified in a screening conducted in 2003 using a combinatory library based on EMB as pharmacophore. It shows an MIC value of 0.11 μg/ml. t remains equally active as EMB at 100 mg/kg when administered in the mouse model at a dose of 1 mg/kg. However, SQ109 did not increase its effectiveness at higher doses (10 mg/kg, 25 mg/kg) and it was clearly less effective than INH[5]. Its effectiveness has been proved against MDR

strains. Preclinical toxicology studies have been completed and further phase II clinical studies are underway [67].

TMC-207 diarylquinoline: This agent is a promising agent as a new kind of antimycobacterial agent. Twenty diarylquinolines have been reported on the series with MIC lower than 0.5 mg/L against $H_{37}Rv$. Antimycobacterial activity was confirmed *in vivo* for three compounds of this class. The most effective agent was TMC-207, which had a MIC of 0.06 mg/L against $H_{37}Rv$ and its spectrum was unique in specificity against mycobacteria [74]. TMC-207 inhibits the ATP synthetase leading to a decrease of ATP and a pH imbalance. This compound has a potent EBA in the murine infection model, superior or similar to INH. The combinations TMC-207, INH, and PZA cleansed the bacilli present in the lungs of all mice after two months. Trials have also been conducted in mice with combinations of second line agents. Preliminary studies have proved *in vivo* sterilizing activity in the mice model, and decrease in the treatment time. Currently it is in phase IIa. Therefore, it is the most promising drug candidate in the last 30 years.

Gatifloxacin: It has been reported bactericidal activity *in vitro* and *in vivo* against *M. tuberculosis* to this compound. Its MIC has been reported between 0.25 mg/L and 0.03 mg/L against $H_{37}Rv$ [76]. In an *in vitro* study on bacilli in stationary phase, gatifloxacin showed the greatest bactericidal activity in the first two days, but none afterwards. In mice studies, the combination of gatifloxacin with EMB and PZA cleansed the lungs of infected animals after two months of treatment. Currently, gatifloxacin is under phase III to prove the efficacy and safety of a four-month regime for the treatment of pulmonary TB supervised by the European Commission Oflotub Consortium, WHO-TDR, Tuberculosis Research Unit (TBRU), National Institute of Allergy and Infectious Diseases (NIAID), Tuberculosis Research Centre [5,67].

Moxifloxacin: Moxifloxacin is the most promising fluorquinolone against *M. tuberculosis.* Its activity *in vitro* seems to affect bacilli unaffected by RIF. Its MIC reported *in vitro* is 0.5 mg/L [77]. It seems that moxifloxacin interferes with the protein synthesis on bacteria with low metabolic activity by a mechanism different to the one of RIF. However, the specific action mechanism is unknown. In the mouse model, the effectiveness of moxifloxacin is comparable to INH. When administered in combination with PZA, moxifloxacin has a greater bactericidal activity tan the INH + RIF + PZA regime. In fact, the combination RIF + moxifloxacin + PZA decreases completely bacilli count within four months, whereas the combination RIF + INH + PZA requires 6 months. It is likely that there is synergism among the three drugs, and the alternative regime replacing INH by moxifloxacin has been proposed. Moxifloxacin is under phase III [67]. Clinical studies have not proved a greater sterilizing activity of a regime containing moxifloxacin in comparison with the standard regime; however, it has increased activity in early points [5,75].

Nitroimidazole PA-824: This bicyclical nitroimidazole is under development by the TB Alliance, which has the proprietary rights. The *in vitro* MIC of the PA-824 compound is between 0.15 and 0.3 μg/ml [78].After an activation by the F420 factor of *M. tuberculosis,* the PA-824 compound inhibits biosynthesis of the cell wall components by means of mechanisms still to be established. It has proved bactericidal activity against replicative and static bacilli *in vitro.* Although PA-824 was more efficient than INH or moxifloxacin, during the continuation phase it was not better than the RIF + INHH combination. On the long term, the 12-month treatment

did not achieve total eradication in any of the mice treated. The 6-month regime of PA-824 in combination with RIF, INH, and PZA in mice proved to be superior to the standard regime regarding quickness of eradication and lower relapse rate. This compound has been widely evaluated in animals and humans; currently it is under phase II clinical trials as part of an initial scheme (PA-824, moxifloxacin, and PZA) containing new anti-TB drugs[79].

Nitroimidazole-oxazol OPC67683: There is very little public information regarding this compound. It belongs to a subclass of mycolic acids synthesis inhibitors. It has shown *in vitro* activity against standard and resistant strains, showing a MICof 12 ng/ml [80]. It has not shown cross resistance with any other medication. The compound shown activity against bacilli residing within human macrophages and type II pneumocytes. The chronic TB trial in mice demonstrated an activity 6 – 7 times more effective than the one observed for first line INH and RIFdrugs. Favorable oral absorption and distribution have been reported. Currently it is under phase II clinical trials.

Pyrrole LL-3858: Some pyrroles derivatives have been found to have *in vitro* activity against *M. tuberculosis.* The MIC of pyrrole LL-3858 is between 0.025 and 0.12 μg/ml. The LL-3858 derivate identified by Lupin Limited showed greater bactericidal activity in the lungs of mice infected in monotherapy than INH. The trial of LL-3858 in combination with INH and RIF, or with INH, RIF, and PZA sterilized the totality of mice in 3 months [5]. Currently, the compound is under phase II clinical trials [67].

8.3. New approaches for the development of anti-TB active principles

In the dawn of the 21st century, pathogenesis of the infectious disease appears as a competition between the host and the pathogen involving short term adaptations and co evolution of the genomes [81]. The pathogen and the host constantly exercise selective pressure over each other, making the environment in test tube completely different from that within the host.

In latent tuberculous infection (LTBI), most of bacilli are not replicating, whereas in a phase of active disease most of the population is on active growth. Chemotherapy must take this metabolic adversity to favor the host.A durable cure must eliminate both the replicative and the persistent bacilli.Eradication of the persistent bacilli onchemotherapylasting between 6 – 24 months has been proposed in order to avoid relapse. However, such a long treatment is difficult to sustain and there is always resistance-associated risks in interrupted regimens [81].A philosophy of mycobacterial infection states that the essential genes for infection in mice include genes that are not essential *in vitro.*

The proteasome of *M. tuberculosis* is a set of proteins that provide a quick adaptation to changing conditions [82]. Two genes, *mpa (Mycobacterium* proteosomal ATPase) and *pafA (*proteasome accessory factor) were identified as important in the survival of *M. tuberculosis*to exposure against reactive nitrogen species (RNS) *in vitro* and required for active disease *in vivo* [82]. These genes codify for proteins involved in the bacteria proteasomal function. Proteasomes are barrel-shaped proteases consisting of 14 α units and 14 β units [82]. Mpa is similar to ATPases found in the proteosome of eukaryotes cells, and chemical inhibition of the protease activity of the *M. tuberculosis* proteosome causes sensitization of the wild strain to

reactive nitrogen species (RNS). The PafA protein does not share homology with any protein of known function [82]. Two specific proteasomeinhibitors, epoxomicin and a peptidic-boronate prevented the growth of *M. tuberculosis* and turned out to be bactericidal during the recovery of the mycobacterium against exposure to RNS [81]. The operon that codifies for the proteasome was knocked out by using conditional gene silencing and it was proved that bacteria require it to survive during the chronic infection in mice and its silencing allowed the mouse to be free of the persistent infection [83]. Whereas the proteasome of the mycobacterium is essential for the infection of a host, it is not required to grow in a rich and aerated medium such as Middlebrook 7H9 broth [81].

Unlike other bacteria, *M. tuberculosis* possesses a unique defense system that relates the antioxidant and metabolic activities [81]. The system includes a peroxyredoxin, the C subunit of an alkylhydroperoxy-reductase (AhpC), a thioredoxin type protein (AhpD), dihydrolipoamideacyltransferase (DlaT), and lipoamide dehydrogenase (Lpd), and the four enzymes together work as peroxydases and peroxynitroreductases and peroxynitroreductasesdependant of NADH [81]. The dual functionality of these enzymes is interesting as potential targets for the development of anti-TB active principles.

Moreover, the DosRsystem, discovered 15 years ago, regulates the development of a form of non-eplicative survival without morphologic differentiation in *M. tuberculosis* (known as latency state). This state of physiologic quiescence maintained viable the microorganism for long periods of time, contributing with two key characteristicsof TB: the symptom-free latent infection state and the persistence of the active disease of the tubercular infection in spite of the prolonged therapy time. Due to the importance of the bacilli latency state in the pathophysiology and chemotherapy of the disease, researchers have set their interest in the DosR system. Drugs that attack the latent state of the bacterium not only would be the key for eradication of the latent infection, but also shortening the time of treatment of active infection [84].

9. A new approach to research processes

Traditionally, the focus of research is the evaluation of a single drugin extensive and costly trials. This process may take a lot of time and reduces the possibility of developing a combination of new drugs that is effective. For this reason, a new approach to research has been led by the Critical Path to TB Drug Regimens (CPTR) organization created in March 2010 by the Bill & Melinda Gates Foundations, the Critical Path Institute, and the TB Alliance. This strategic partnership has the strength of reducing the time necessary to develop a new TB treatment scheme, as well as reducing significantly the research costs. This initiative has been endorsed by the US Food and Drug Administration and other regulatory entities, as well as the World Health Organization [67].

As a result of the 41st Union World Conference in Berlin, Germany, on November 2010, the TB Alliance announced the launch of the first clinical phase to test a new TB treatment scheme which expedites the treatment of patients. The combination of three drugs has been promising for the treatment of drug sensitive (DS-TB) and MDR-TB, thus changing the course of the TB

http://dx.doi.org/10.5772/54278

pandemics through simplification and shortening in the treatment time of the disease worldwide. The combination is currently in phase II of clinical trials and contains PA-824 and moxifloxacin together with PZA. Researchers have reported that preclinical data reveal a decrease in the treatment time both for DS-TB and MDR-TB patients, and possibly XDR-TB ones with a simple three-drug treatment scheme [67].

10. Nano-particles: A projection towards the future

Nanoparticles can create new directions in the diagnosis, treatment, and prevention of TB. A significant application in the progress of this technology is using drug carriers.This system has been found to be advantageous, as it gives high stability of the drug, high load capacity (many molecules of the medication can be incorporated in the matrix of the particle), easiness to incorporate hydrophilic and hydrophobic substances, possibility of being administered orally or via inhalation. Perhaps more importantly, the anti-TB drug release in a controlled manner from the matrix enables to improve the bioavailability and reduction of the doses frequency. Load or delivery systems such as liposome or microspheres have been developed for the sustained release of anti-TB drugs, and better chemotherapeutical efficacy has been found when the system is researched in animal models (e.g. mice) [85,86].In 2005, the efficacy of nanoparticles was assessed in the distribution of anti-TBdrugs administered every 10 days versus the non capsulated form of aerosol administration of drugs against *M. tuberculosis* in guinea pigs; in both cases the treatments reduced the bacteria count. These findings suggest that the distribution of drugs by nanoparticles has a great potential in the treatment of TB [86].

11. Conclusions

Currently, devastating diseases in the world such as tuberculosis get the attention of authorities with the aim of supporting breakthroughs which provide alternatives for their control.

The development of active principles against *M. tuberculosis* is nowadays a worldwide priority due to the appearance of strains resistant to medications used in current therapeutic schemes, thus existing the need to articulate in an expedite manner the basic research looking for new therapeutic choices, along with clinical research and its articulation with the industry in order to guarantee a quick production of novel alternatives which overcome the limitations of current treatment schemes.

The concern in many sectors devoted to tuberculosis control is that there are not sufficient alternatives that can join rapidly the treatment against tuberculosis, and they convey discouraging estimations regarding the degree of resistance that each one of these molecules will have at the moment of entering the therapeutic scheme deduced from natural resistant bacilli. These justifications have promoted research around the world towards finding new molecules, based on investigations of natural sources such as plants, insects, marine microorganisms, synthetic molecules deduced from the modification or substitutions made on the structure of already

existing molecules with the aim of potentiate their effect; or from new sources such as nanoparticles, computing studies, among many others.

The results expected at the end of each process producing a new alternative of treatment against tuberculosis is that these drugs may shorten the duration of the current treatment, be active against resistant strains and non-replicative conditions of *M. tuberculosis* as well as not interfering with HIV antiretroviral therapy, reduce adverse side effects, and that it is of easy administration to facilitate the patient's compliance. For the management of tuberculosis as a public health event worldwide, these new drugs must be produced easily in large amounts; they must be stable under minimum storage conditions, and they must be of low cost so that all governments may guarantee access of all the diseased population.

For these expectations to become true in the short term, more basic and applied research is required to generate new ideas in the development of anti-tuberculosis drugs, as well as stronger financial support in research and greater commitment from the pharmaceutical industry and public health entities.

Acknowledgements

Magda Lorena Orduz and Hernando YesidEstupiñan for the figures and technical contribution to manuscript.

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