Review Article

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New Strategies for the Management of Tuberculosis: An Overview

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Abstract: Tuberculosis is today amongst the worldwide health threats and continues to be one of the most important global infectious causes of morbidity and mortality. This contagious disease, caused by the bacterium Mycobacterium tuberculosis has increased around the world due to AIDS, poor socioeconomic conditions, immigration, the lack of new drugs on the market, the appearance of multi-drug resistant bacteria, and more recently the emergence of extensively drug-resistant bacteria, commonly defined as strains resistant to all the current first-line, as well as some second-line drugs. A number of drug targets from cell wall biosynthesis, nucleic acid biosynthesis, and many other biosynthetic pathways are being unraveled throughout the world and are being utilized for drug development. In this review, socioeconomic problems in developing countries, efforts to manage this disease in different individuals, the targets (known already and newly discovered), and existing anti-tubercular agents including natural products and lead molecules, and the future prospects to develop new anti-TB agents are described.

Keywords: tuberculosis, anti-tuberculosis drugs, MDR TB, XDR TB.

INTRODUCTION:

Tuberculosis (TB), caused by Mycobacterium tuberculosis, a slow growing bacterium, evolved from soil bacterium more than 10,000 years ago. It is a respiratory transmitted disease affecting nearly 32% of the world's population, more than any other infectious disease. At the present time, nearly two billion people worldwide are infected with the tubercle bacillus, and the prevalence of active TB is increasing. [1-3] Among HIV-infected people with weakened immune system, TB is a leading killer epidemic. Every year about two million people living with HIV/AIDS die from TB. [4] Furthermore, in recent times the appearance of multi drug-resistant TB (MDR-TB), a form of TB that does not respond to the standard treatments using first-line drugs is a serious threat to TB control and treatment. It's a shocking revelation that MDR-TB is present in almost all countries as per the recent survey, made by the World Health Organization (WHO) and its partners. A recent estimation by WHO has revealed that within next 20 years approximately 30 million people will be infected with the *bacillus*. [5] Keeping in view of the above statistics, WHO declared TB as a global health emergency and aimed at saving 14 million lives between 2006 and 2015. [6]

India having 2% of the land area and 15% of the total population of the world accounts a disproportionately high 30% of the TB burden. In India, TB kills 14 times more people than all tropical diseases. TB is one of the leading causes of mortality in India, killing 2 persons every three

minute, nearly 1,000 every day. However, in terms of absolute numbers, India accounts for one fifth of the global Tuberculosis burden. Every year 1.9 million people in India develop tuberculosis (TB), of which 0.8 million are sputum positive cases that are infectious. [7-9]

The history of tuberculosis is very old. [10] Fragments of the spinal column from Egyptian mummies 2400 BCE show definite pathological signs of tubercular decay. Exact pathological and anatomical description of the disease appeared in the 17th century. In 1882, Robert Koch's scientific brilliance led to the discovery of M. tuberculosis as the causative agent of the disease. Different means of TB curtailment were developed from time to time. In the 19th century, a French bacteriologist Calmette together with Guerin created the Bacilli Calmette-Guerin (BCG) vaccine.

The history of TB in India [11, 12] also dates back to 600BC where in a Sushruta Samhita, the disease is known as Kshaya, 'Wasting disease or RajaYakshmaa', 'the king of diseases.' The four causes of the disease proposed were overstrain, suppression of natural urges, wasting (e.g., because of grief, anxiety, or longing), and a promiscuous diet, any of which could cause the three morbid humors Vata, Pitta, and Kapha to flare up. A treatment based on the principles of Ayurveda, the classical Indian system of health and healing was provided. In addition, medicines and dietary prescriptions were detailed. Alcohol in moderate quantities, the flesh of birds and animals, which inhibit dry areas, and goat's milk were among the items recommended. TB was rare until the second half of the 19th century. Concomitant with the growing population density caused by industrialization, its incidence has increased progressively since then. [13, 14]

Pathgogenesis: Tubercular bacilli is the main causative agent of TB infection spread in the environment by coughing, sneezing, shouting, and singing of a patient with active TB, the air is contaminated with these bacilli. Inhaled bacilli in a person are inoculated into his respiratory bronchioles and alveoli, usually towards the apex of the lung. When the inhaled microorganisms multiply to a sufficient extent, an antigen–antibody interaction is evoked by the cell-mediated T-lymphocytes. Tubercles are then formed because of accumulation of macrophages at the site of infection. [15–17] This may lead to permanent suppression of infection or some microbes may survive in the focii and may become the source of post primary infection when these focii break down under the conditions of weak host defense mechanisms. [18, 19] This may happen immediately or months or years later. The hilar lymph nodes may get easily infected because of the spreading of infected macrophages having active bacilli.

The released microorganisms are circulated through lymph and blood vessels to different parts of the body and infect (i) Reticuloendothelial system (e.g. liver, spleen, and lymph nodes) (ii) Serosal surfaces and sites with high oxygen pressure (apices of lungs, renal cortex, and epiphyses of growing bones). Because of multiplication of organisms at these sites, numerous small focii develop throughout the body. [20, 21] This type of wide-spread of infection is known as milliary TB. Infection of the oropharynx, larynx, and tracheobronchial tree respond fairly well to anti-TB drugs, whereas infections in the gastrointestinal tract, urinary tract, or lymph nodes respond partially to the treatment. [22, 23]

Multi Drug Resistance TB and HIV: The concomitant resurgence of TB with the MDR- or XDR-TB and HIV/AIDS pandemic has exposed the frailties of the current drug armatorium. The emergence of

MDR-TB and extremely drug resistant TB, first reported in November 2005 [24] has created new challenges to control and defeat the disease.

An estimated one-third of the 42 million people living with HIV/AIDS worldwide are co-infected with TB. [25] As per WHO reports, approximately 90% of patients having both TB and HIV died within a few months after clinical symptoms appeared for the disease. Therefore, WHO warned the world for ‘‘even greater TB/HIV crisis’’ and called for wide availability of free anti-TB drugs to those living with HIV. As per WHO, HIV is spreading rapidly in the country with the largest number of TB cases in the world. [26–30] In India, already 180,000 people living with HIV are co-infected with TB. The emergence of multiple drug resistant (MDR) TB has focused the attention of scientific community throughout the world on the urgent need for new anti-tubercular drugs. Resistance has been developed against almost every front-line drug. [31–38] Different mechanisms have been put forward for the causes of the development of resistance against existing drugs, such as impermeability of the highly hydrophobic cell envelop to many drugs, [39] a well-developed drug–efflux system, [40] production of certain enzymes to inactivate the drugs (b-lactamases, aminoglycoside acyl transferase), [41, 42] and at the molecular level acquisition of resistance in M. tuberculosis because of mutational events in the chromosomes. [43]

TREATMENT OF TUBERCULOSIS:

A. Vaccines: Active immunization is one of the essential components to control TB. Many countries use BCG vaccine as part of their TB control programme, especially for infants.
This was the first vaccine for TB and developed at the Pasteur Institute in France between 1905 and 1921. [44] Until now one billion people have been vaccinated with BCG. In general, the efforts to make new four classes of vaccine candidates are being worked on: the rationally attenuated strains of M. tuberculosis, BCG vaccine, protein subunit vaccines, and nucleic acid vaccines.

1. Rationally Attenuated Strains of Mycobacterium tuberculosis: The manipulation of M. tuberculosis chromosome generates bacterial strains, which lack the pathogenecity but elicit a protective immune response. It has been achieved by several techniques. [45–48] A library of mutants can be generated using transposons, mobile genetic elements that can ‘hop,’ disrupting genes. [49, 50]

2. Bacilli Calmette-Guerin Vaccine: An advance in the discovery and characterization of genes and antigens of M. tuberculosis has led to substantial progress toward the development of improved vaccines since BCG vaccine was first used successfully. The cheap BCG vaccine with minor side effects can safely be given to children. It is proven now that BCG does have some protective effects in children and is effective against meningitis.

TB, but it does not prevent the emergence of pulmonary TB, particularly in the adulthood. Variation in the efficacy of BCG vaccine has been because of interference of BCG take because of previous exposure to environmental Mycobacteria. M. tuberculosis genes encoding antigens which have high reactivity to memory immune T-cells, but were deleted from BCG in the mutations led to attenuation. [51, 52] Alternatively, the immune responses to purified protein derivative have been enhanced in mice by injecting BCG clones expressing various immune stimulating protei.

3. Protein Subunit Vaccines: Purified proteins as vaccine candidates have several advantages over attenuated organisms. They are inherently safe and have no propensity to cause disease, which is an important consideration when vaccinated individuals have been exposed to HIV. The efficacy of such vaccines has been demonstrated in mice and guinea pigs. [53, 54]

4. Nucleic Acid Vaccines: The hypothesis that ''Naked DNA vaccines'' and DNA-encoding influenza nucleoprotein lend immunity to influenza in mice [55] has been applied to M. tuberculosis also. Vaccination with DNA encoding either the M. leprae 65 kDa heat shock protein [56] or the M. tuberculosis antigen 85A protein [57] protect from subsequent infection with virulent M. tuberculosis. Many nucleic acid vaccines have shown efficacy in experimental TB. [58–60]

B. Genomics: The most significant developments in the area of TB are perhaps the sequencing of the mycobacterial genome. Sequencing of the H37Rv strains of M. tuberculosis and a highly virulent clinical isolate is well documented. [61] Among the estimated 4,500 genes, every drug target and every antigen, or protein elicits an immune response. Among these precise biochemical functions, only 40% of the above is known, another 44% have some sequence homology, whereas functions of 16% are completely unknown, and they may account for specific mycobacterial functions. Comparision of the two Mycobacterium genome sequences M. tuberculosis and M. leprae has been shown to be useful in identifying genes associated with virulence, [62] this will give new insight in identifying the targets and development of new control strategies.

C. Immunotherapy: Immunotherapy is a therapeutic means whereby the immune system is stimulated by the injection of inactivated Mycobacteria, resulting in the activation of TH1cells and inactivation of TH2 cells, the two kinds of helper cells. It represents an important adjunct to modern chemotherapy to overcome the problems of non-compliance and drug resistance to many drugs, as well as improving cure rates and reducing mortality. Cell mediated immune (CMI) and delayed type of hypersensitive (DTH) responses play very important roles in the host during M. tuberculosis infection. The CD4 T lymphocyte is divided into TH1 and TH2 subsets depending on the type of cytokines produced. TH1 cells produce the cytokines interferon-gamma and interleukin-2, an important event for activation of anti-mycobacterial activities of many drugs and essential for the DTH response. [63] Several studies on the effectiveness of Mycobacterium vaccine, as an immunotherapeutic agent for TB control, have recently been carried out. [64–66] It is thought to be a powerful TH1 adjuvant and has a beneficial effect with enough evidence of its use as an immunotherapeutic agent. Its application as an adjunct to chemotherapy in the treatment of TB, especially in MDR TB and cases where chemotherapy was incomplete or intermittent in many parts of the developing world, has been reported. [67]

D. Chemotherapy: Chemotherapy of TB started in the 1940s. In 1943, anti-TB research resulted in discovery of the active anti-TB agents, and strategies have been devised to treat TB from time to time. [68–69] A number of agents have been discovered since then, including para-aminosalicylic acid (PAS), isoniazid (INH), pyrazinamide (PZA), cycloserine, ethionamide, rifampicin (RMP), and ethambutol. The majority of these drugs were discovered through broad random screening. Very little optimization was undertaken with insignificant to the targets of drug action, as no biochemical tools for these studies were known at that time.

RECENTLY DISCOVERED MOLECULES FOR THE MANAGEMENT OF TUBERCULOSIS:

Tryptanthrin: Tryptanthrin **(Figure 1)** is a potent structurally novel indoloquinazolinone alkaloid, active against MDRTB with an MIC of 0.5–1.0 mg/mL. But *in vivo* data and in vitro toxicity are needed before this structural prototype is applied in MDR TB. [70]

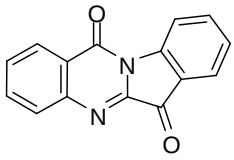
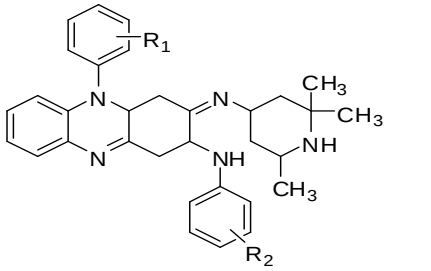


Figure 1: Tryptanthrin

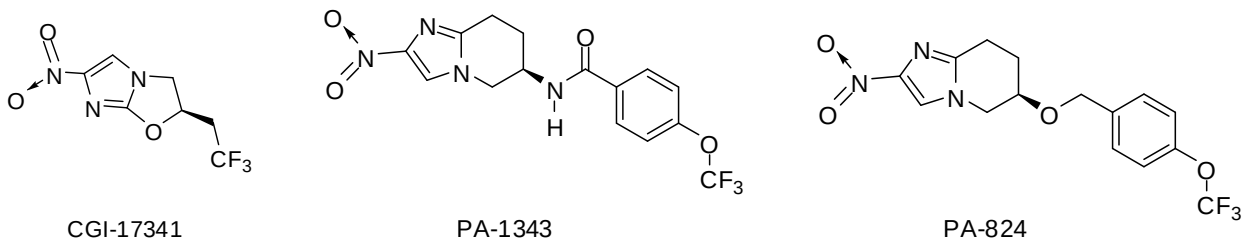
Few of the tetramethyl piperidine substituted phenazines (TMP phenazines) were found to possess significantly more activity against M. tuberculosis, including MDR clinical strains than clofazimines analogs **(Figure 2)** and the intracellular accumulation in mononuclear phagocytic cells, anti-inflammatory activity, a low incidence of drug resistance and slow metabolic elimination rate, make clofazimines attractive candidate for the treatment of mycobacterial infections. The compounds of this series are active *in vivo* also. [71- 73]



B4121, R_1 & R_2 = 3, 5 dIchloro
B4125, R_1 & R_2 = 2 chloro
B4128, R_1 & R_2 = 2,4 dichloro
B4169, R_1 & R_2 = 3,4, 5 trichloro

Figure 2: Clofazimine analogs

Nitroimidazofurans and Nitroimidazopyrans (Figure 3): Nitroimidazofurans, originally used as a radio sensitizer in cancer chemotherapy, have been reported to possess in vivo anti-tubercular activities. However, because of mutagenic side effects, this series of compounds could not enter into clinics for the treatment of TB. [74, 75]



CGI-17341 PA-1343 PA-824

Figure 3: Nitroimidazofuran and nitroimidazopyran derivatives

Thiolactomycin: Thiolactomycin **(Figure 4)** (TLM) (7) [(4R) (2E, 5E) 2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide], belonging to a small group of thioteronic acid anti-bacterial, is a unique thiolactone exhibiting anti-TB activity by inhibiting mycolic acid biosynthesis. It inhibits FAS-II of plant and bacterial origin but not of mammalian or yeast type FAS-I. [76]

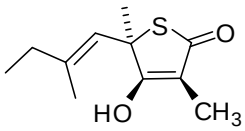
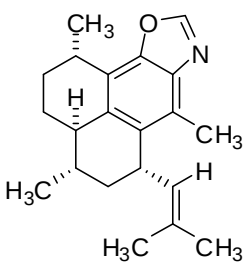
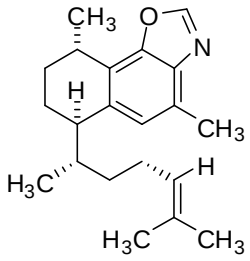


Figure 4: Thiolactamycin

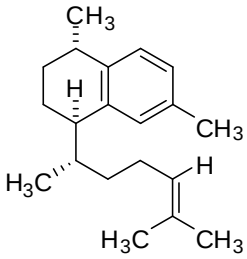
Diterpenoids: Diterpenoid **(Figure 5)** compounds known for various medicinal values have recently been screened for anti-TB activities against M. tuberculosis. Benzooxazole alkaloids isolated from the Indian sea whip Pseudopterogorgia elisabethae were tested against the bacterium, and it was found that pseudopteroxazole has potent inhibitory activity (97%) at 12.5 mg/mL in M. tuberculosis H37 Rv strain. [77-79]



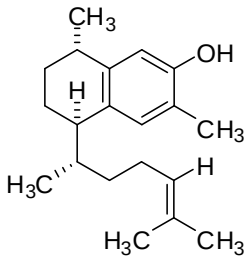
Pseudoterpteroxazole



Seco-pseudopteroxazole



Erogorgiaene

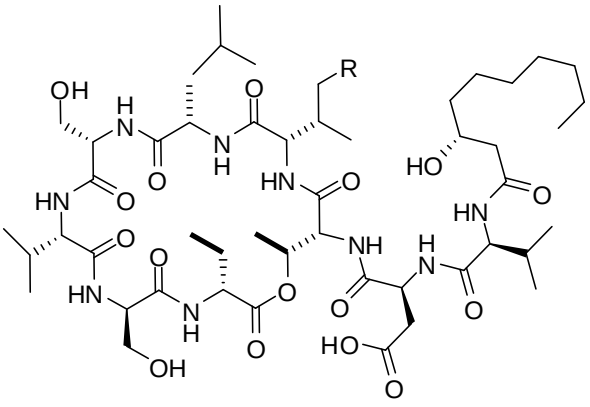


7- Hydroxy Erogorgiaene

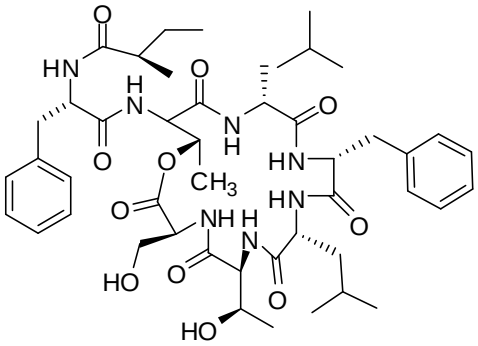
Figure 5: Diterpenoids

Marine Natural Products: Massetolide A and Viscosin B, cyclic depsipeptides isolated from cultures of two pseudomonans, a marine alga and tune worm, were tested against M. avium-intracellulare and showed MIC values of 2.5–5 and 5–10 mg/mL, respectively. [80a] Kahalalides A, isolated from the

Sacoglossan mollusk Elysia rufescens, [80b, c] inhibited the growth of M. tuberculosis H37 Rv (83%) at 12 mg/mL. **(Figure 6)**



R= CH3,Massetoide A
R= H, Viscosin



Kahalalide A

Figure 6: Marine natural products

Chalcones and flavones (Figure 7): Twenty-five chalcones molecules as antimycobacterial agents were synthesized, activity was evaluated and quantitative structure–activity relationship (QSAR) was developed by P.M. Sivkumar et al. [81a] Yuh-Meei Lin et al synthesized a series of flavonoids, chalcones and chalcone-like compounds and evaluated for inhibitory activity against Mycobacterium tuberculosis H37Rv. Among them, eight compounds exhibited >90% inhibition on the growth of the bacteria at a concentration of 12.5 µg/mL. [81b] B.S. Vatkar et al synthesized various analogs of flavanone by oxidative cyclization of chalcones with satisfactory yield and purity. All analogues of 2, 3-dihydro-2-phenylchromen-4-one have shown moderate to good antimicrobial and antifungal property. [81c] Kozmik Vaclav et al synthesized azachalcone derivatives and their bis substituted analogs as novel antimycobacterial agents. [81d]

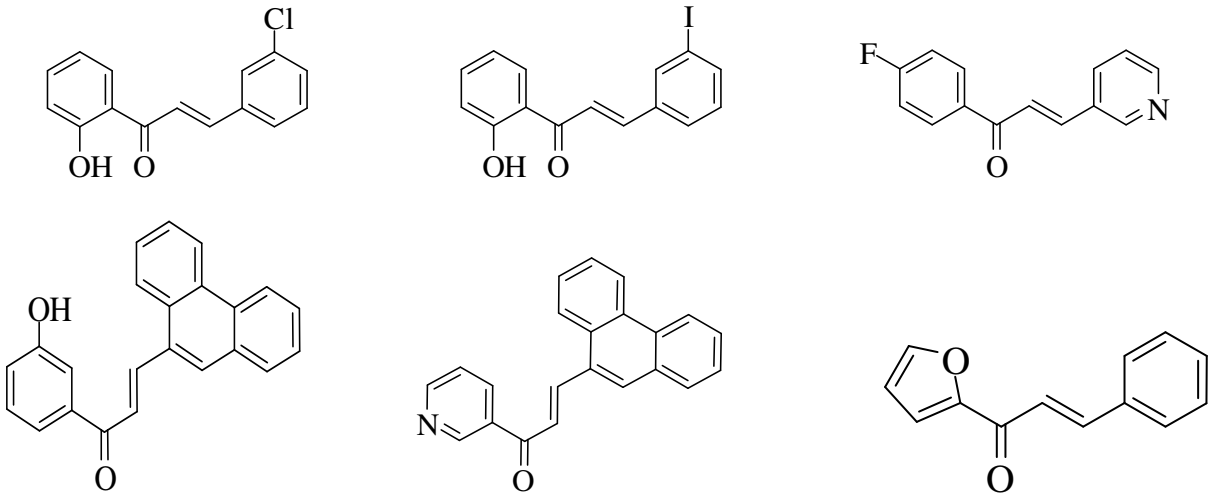


Figure 7: Chalcone derivatives

FUTURE PERSPECTIVE:

The present knowledge in TB has revealed that XRDTB, MDRTB, HIV-TB co-infection and transmission of drug-resistant strains are the major challenges to TB control programs. In the last few years, there has been considerable progress in understanding the biochemistry of M. tuberculosis, the disease process, and the mechanism of drug resistance, and in establishing the value of DOTS in preventing treatment failure. However, only a limited effort has been made to develop new active chemical entities or rapid diagnostic tests, and their relevance to the global TB control has been matter of concerned. This decade has seen dramatic advances in understanding the biology, intracellular lifestyle, and detailed biochemistry of the mycobacterium, generating a wealth of information to undertake different targets for new drug development even against MDRTB. The new validated and selective targets compatible to high throughput assays against all forms of TB should be developed for drug susceptibility testing, baseline screening to identify new chemical entities and early drug resistance, and to guide retreatment efforts. Development of combinatorial and virtual libraries and in silico screening of the compounds should be prioritized to get an early lead for developing new drugs. For this, enormous funding from the developed countries and voluntary organizations is needed.

This review has summarized the global disease burden of TB, approaches for its control, and drug combinations used for the treatment of TB in different set of individuals. The existing targets and new targets identified by different workers as a result of genetic and biochemical engineering of the cell wall has been presented in short. Efforts to control latent TB and the role of isocitrate lyase in developing new drugs against persistent infection have been elucidated. Last, the future prospect of the strategies to control TB has been discussed.

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