# Mycobacterial lipids as potential drug target to combat Tuberculosis

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*Mycobacterium tuberculosis* (MTB) is the etiological agent of Tuberculosis (TB), which causes mortality and morbidity throughout the world. Moreover, the currently available drug regimens that are used for the treatment of TB are associated with adverse side effects on patients, high medical costs and emergence of multidrug resistance (MDR). Hence novel drug targets are urgently needed to address the issue. MTB cell wall contain large amount of unique lipids such as mycolic acid, Phosphatidyl Inositol Mannosides (PIM), phthiocerol dimycocerate, LAM (lipoarabinomannan) trehalose monomycolate and dimycolate (TMM, TDM), phthiocerol dimycocerosate (PDIM), cord factor, sulfolipids and wax-D that are involved in pathogenesis. Therefore interest in the discovery of developing new drugs either from natural or synthetic sources that targets lipid metabolic pathway of MTB has emerged as significant area of research in recent times. This chapter covers the gist of drugs from natural and synthetic sources along with known anti-TB drugs that target lipid machinery of MTB.

**Keywords:** *Mycobacterium*; natural; synthetic; lipid; mycolic acid; traditional medicine

## 1. Introduction

Tuberculosis (TB) still remains a major pandemic with enormous public health concern that affected 10.4 million people [1]. *Mycobacterium tuberculosis* (MTB) (rod shape, aerobic bacteria) is the causal organism of TB. Owing to widespread resistance due to inadequate compliance of current anti-TB drug, new drugs are desperately needed to control this disease [2]. Traditional medicines and natural products owing to their diversity have become renewed source of interest nowadays. The mycobacterial cell wall is one of the major targets for most of the known anti-TB drug due to the presence of complex lipid that reduces the permeability of antimycobacterial drug. Among diverse lipids mycolic acid, trehalose monomycolate and dimycolate (TMM, TDM), phthiocerol dimycocerosate (PDIM), sulfolipid-1 (SL-1), diacyl trehalose (DAT), lipoarabinomannan (LAM), Phosphatidyl inositol mannosides (PIM) are responsible for pathogenesis [3-6].

Mycolic acids are the major lipid components found in mycobacterial cell wall responsible for the protection of the MTB from environmental stress and contribute to disease persistence during infection. In *Mycobacterium* cell wall, the innermost monolayer contains mycoloyl residues which are covalently linked to the arabinogalactan connecting with peptidoglycans whereas, the outermost monolayer is composed of various glycolipids, including TDM and TMM [7]. The composition and amounts of mycolic acids determine the virulence, growth rate and permeability of MTB [8]. The biosynthesis of mycolic acids has been the focus of intense research for a number of years, primarily because of the presumption that enzymes involved in the synthesis of this unusual lipid are attractive targets for the development of novel chemotherapeutic agents [9]. Therefore, this chapter highlights the potential of lipid pathways of MTB to be targeted for searching new drugs either from natural, synthetic or traditional origin “Fig. 1”.

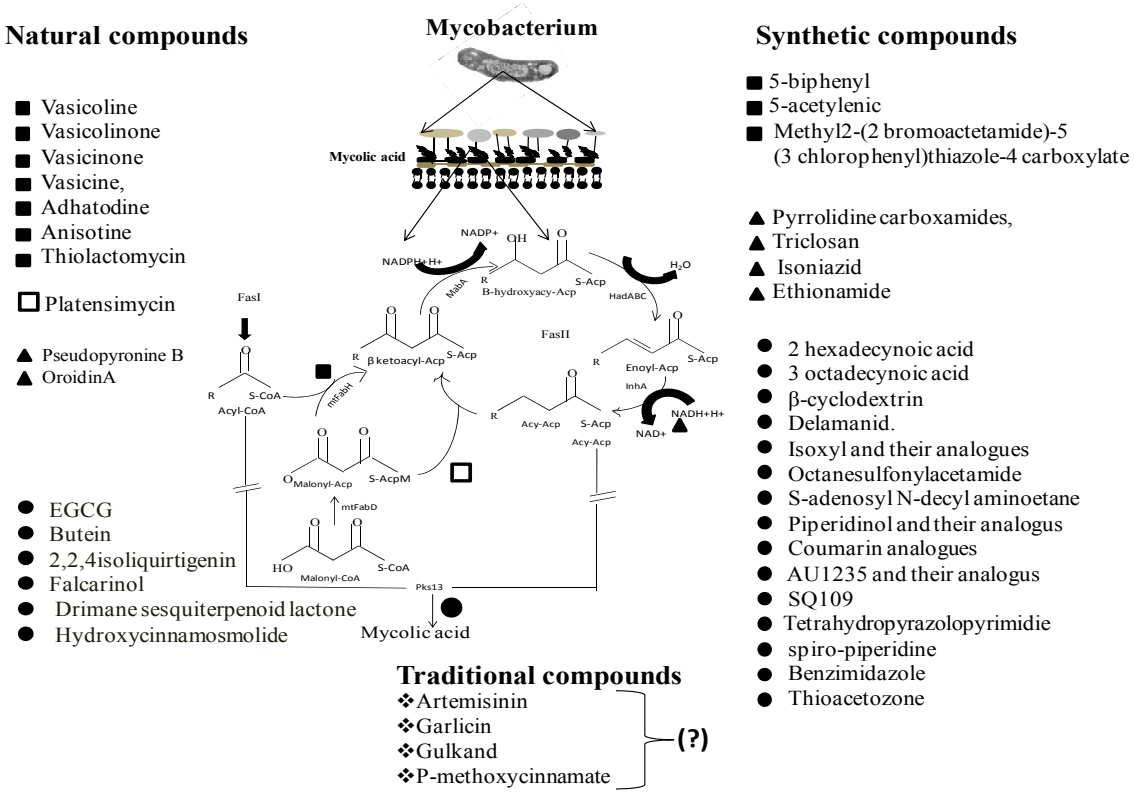


**Fig.1**. Natural, synthetic and traditional compounds possessing antimycobacterial activity. Symbols show ( ■ □ ▲● ) site of action and (?) depicts unknown targets.

## 2. Emerging role of Lipidomics

Role of lipids in the cell, tissue and organ physiology has been demonstrated by a large number of human diseases and by various genetic diseases that involves disruption of lipid metabolic enzymes and pathways. Some examples of such diseases are obesity, diabetes, Alzheimer's disease, atherosclerosis, cancer, fungal and bacterial infections including TB [10, 11]. Lipidomics is an emerging field involved in the study of pathway and network of cellular lipid as well as their interactions with other lipids, protein and metabolites. With the development of recent advancements in novel analytical approaches such as liquid chromatography (LC), gas chromatography (GC) in combination with mass spectrometry (MS) technologies, quantitative analysis of lipid along with defining new roles to lipid has emerged. The MS based lipids analysis strategies help in understanding the diverse biological role of lipid and provide a powerful tool for elucidating the mechanism of lipid based disease and biomarker development [12].

## 3. Natural compounds targeting MTB lipids

The natural compound is a chemical compound or substance derived from living organisms such as plants, micro organisms and marine organism, having potential to target different diseases including infectious disease. Previous literature has been reported that natural compounds and their analogs exhibited inhibitory activity towards MTB. For instance, exocarpic acid isolated from the stem of *Exocarpus latifolius* have shown antimycobacterial activity against MTB. In the preliminary analysis, exocarpic acid upregulates the lipid metabolism and channel transporter gene in MTB. Hence the authors suggested that it may interfere with the fatty acid acid metabolism in *Mycobacterium* [13]. Furthermore another study has reported inhibition of mycolic acid biosynthesis in the presence of exocarpic acid, which was determined by microarray analysis [14]. Similarly, thiolactomycin is a known antibiotic, obtained from *Nocardia* species has ability to inhibit mycolic acid synthesis pathway [15]. In the previous study, an antitubercular activity of thiolactomycin was tested against type II fatty acid synthase (FAS-II), an enzyme responsible for the building block of bacterial cell walls. In addition, it also exhibited inhibitory activity against beta-ketoacyl-acyl carrier protein synthases (Kas A and B), which are responsible for chain elongation of meromycolic acids in the FAS-II system of MTB. Thiolactomycin containing thiotetronic acid has shown potent activity against Kas A and B over human FAS I system as well [16, 17]. Jha et al isolated alkaloids such as vasicolinone, vasicinone, vasicoline, vasicine, adhatodine and anisotine from leaves of *Justicia adhatoda*, and observed antimycobacterial activity against MTB. These alkaloids were found responsible for inhibiting β-ketoacylacyl- carrier protein synthase III (FabH) that plays a very important link between the fatty acid synthase-I and fatty acid synthase-II [18]. Similarly, platensimycin, a natural product produced

by *Streptomyces platensis* represents a new chemical class of antibacterial agent against gram positive bacteria. This compound showed inhibitory activity against beta ketoacyl-acyl protein synthase I/II, involved in lipid biosynthesis, by interacting with the target protein acyl enzyme intermediate. Platensimycin was also found to have antimycobacterial activity against *M.smegmatis* and MTB. MTB genome encodes three unique beta ketoacyl-acyl protein synthase enzyme *viz*. Kas A, Kas B and Fab H [19]. Moreover in vitro assays showed that it can be a good inhibitor of Kas A and Kas B with no effect on FabH in MTB [20].

*Pseudopmonas* species F92591 produce pseudopyronines A and B, which have been reported to have antimycobacterial activity against MTB; but among these two, only pseudopyronines B showed inhibition against enoyl-acyl carrier protein reductase which is involved in fatty acid metabolism [21]. Likewise, cerulenin, isolated from *Cephalosporium caerulens*, is a potent inhibitor of fatty acid synthase (FAS) in a variety of prokaryotic and eukaryotic cells. It exhibited inhibitory activity against several species of mycobacteria, including MTB and *M. bovis,* as well as several non-tuberculous species. They pointed out the inhibition of phospholipids and mycolic acids in *M.bovis* as compare to controls. In addition, they also observed decrease in the quantity of long-chain extractable lipids (intermediate in polarity), triglycerides and glycopeptidolipids after the treatment of cerulenin all three species of mycobacteria [22]. The metabolite, 3-nitropropionic acid of several strains of the endophytic fungus, belonging to genus *Phomopsis*, also exhibited inhibitory activity towards the growth MTB H37Ra (MIC 0.4 μg/ml). Although it has been well known for its potent neurotoxic activity which creates hindrance to its use as a pharmaceutical ingredient and it could be used as a model for the synthesis of new inhibitors of isocitratelyase, an enzyme involved in the catabolism of fatty acids and virulence of MTB [23]. Epigallocatechin gallate (EGCG), a catechin found in green tea, inhibits the enoyl-ACP reductase of MTB. Furthermore, Sun et al demonstrated that EGCG can initiate changes in cell envelope structure and the morphological character, which might be related to defects in the biosynthesis of mycolic acid [24]. In the same way oroidin produced by Turkish sponge *Agelas oroides*, inhibits enoyl-ACP reductase enzyme involved in the fatty acid pathway of several pathogenic microorganisms viz. MTB, *Plasmodium falciparum*, and *Escherichia coli* [25]. Butein, isolated from *Rhus verniciflua* belonging to Anacardiaceae, fisetin from *Rhus cotinus*, isoliquirtigenin and 2,2′,4′-trihydroxychalcone from *Dalbergia odorifera* showed inhibition towards *M.bovis* BCG. Furthermore, these flavonoids showed in vitro inhibition against mycolic-acid-producing fatty acid synthase II (FAS-II) of *M.smegmatis.* Based on bioinformatic approach, they revealed that product of Rv0636, a reputed dehydratase, can be used as a promising target for these flavonoids. This study demonstrated that the overexpression of this gene in *M.bovis* BCG restricted the growth inhibition by butein and isoliquirtigenin, and prevented inhibition of fatty acid and mycolic acid biosynthesis in vivo. Moreover FAS-II was found to be less sensitive to these inhibitors in vitro, after overexpression of Rv0636 in *M. Smegmatis* [26]. Similarly, the extract of *Warburgia salutaris* and pure compound, a novel drimane sesquiterpenoid lactone, 11alpha-hydroxycinnamosmolide, showed inhibition of arylamine N-acetyltransferase (NAT) an enzyme responsible for the transfer of an acetyl group to an arylamine, via the hydrolysis of acetyl and help in the cell wall lipid synthesis. In this study, they also showed anti-mycobacterial activity of the above compounds against MTB and *M.bovis* BCG. Whereas neither extract nor pure compound showed inhibitory activity against NAT deleted strain of *M.bovis* BCG, suggesting that NAT may indeed be a target within the mycobacterial cell [27]. Li et al isolated and identified two C17 polyacetylenes from *Aralia nudicauis,* namely falcarinol and panaxydol, and demonstrated their antimycobacterial activity against MTB [28]. The study established that falcarinol affected the amino acid biosynthesis and fatty acid biosynthesis pathways, such as methionine biosynthesis and cyclopropane fatty acids biosynthesis whereas panaxydol affect unusual fatty acid biosynthesis [29].

## 4. Synthetic compounds targeting MTB lipids

Apart from the natural products a number of known synthetic compounds also have been reported to possess significant antimycobacterial activity. For instance, two alkyonic acids, 2-Hexadecynoic acid and 2 octadecynoic acids have antimycobacterial activity against *M.smegmatis* and *M.bovis.* It has been reported that 2 and 3 hexa/ octadecynoic acid is responsible for inhibition of fatty acid degradation, fatty acid biosynthesis and mycolic acid biosynthesis [30]. PA-824 (Pretomanid) is a 3-substituted nitroimidazopyran showing inhibitory activity against MTB and is recommended as safe for oral delivery. Additionally, PA-824 has potent inhibitory activity towards protein and lipid synthesis; it also has ability to inhibit both actively replicating and static MTB as well as MDR-TB. Hence, PA-824 may pose as a significant lead compound with a potency to shorten the duration of therapy against the active and MDR-TB [31]. Previous studies showed beta cyclodextrins to be responsible in enhancing sterol conversion to 4-androstene-3,17-dione (AD) and 1,4-androstadiene-3,17-dione (ADD) in *Mycobacterium* species. Donova et al observed that methylated cyclodextrins is responsible for cell wall exfoliation and accumulation of membrane-like structures outside the cells which was visualized by electron microscopy. Additionally, they also found that this compound enhanced the content of mycolic and fatty acids outside the cells; but there was no alteration in polysaccharide of cell wall, whereas the overall proportion of the carbohydrates in the cell wall increased after the exposure of methylated cyclodextrins [32]. Antimycobacterial activity of delamanid ([2R]-2-methyl-6-nitro-2-[(4-{4-[4-(trifluoromethoxy)phenoxy]-1-piperidinyl}phenoxy)methyl]-2,3-dihydroimidazo[2,1-b][1,3]oxazole), derived from dihydro-imidazooxazole, has been reported against MTB and murine TB model [33]. They also suggested that it may be involved in the inhibition of

mycolic acid synthesis pathway by showing interference in the production of both methoxymycolic acid and ketomycolic acid. In addition delamanid has shown reported inhibitory activity against both growing and nongrowing mycobacteria [34]. Similarly, another study established that pyrrolidine carboxamides is a novel class of inhA inhibitor that encodes enoyl acyl carrier protein reductase, which is an effective antimicrobial target identified by the help of high throughput screening methods. Pyrrolidine carboxamide is a potent inhibitor with novel scaffold which was determined by in situ activity screening. The resolution of racemic mixture clearly showed that one enantiomer is responsible to inhibit inhA of *Mycobacterium* [35]. In the same way, pthalazinyl derivatives are involved in the inhibition of isocitrate lyase enzyme, which plays an important role in glyoxylate cycle when tricarboxylic acid cycle (TCA) is down regulated upon oxygen and nutrient depletion [36]. Various 3-nitropropionamides were synthesized and their activity was observed against log and starved phase culture of *Mycobacterium* species, and was found to have good inhibitory activity against isocitrate lyase (ICL) of MTB [37]. Isoxyl (ISO, 4,4'-diisoamyloxydiphenylthiourea, 4,4' diisoamyloxythiocarbanilide, thiocarlide ) is a diaryl thiourea, reported to inhibit fatty acid and mycolic acid synthesis in *M.bovis* after exposure to a concentration of 10µg/ml. They also observed antimycobacterial activity of ISO at 2mg/ml against MTB; and the ISO derivatives substituted in the para and para' positions by alkyl, alkoxy, or sulfur functional groups were found in the range of < 0.1 to > 20 mg/ml. Additionally, in vivo studies pointed out the inhibition of synthesis of both short chain fatty acid and mycolic acid (a-mycolates, 91.6%; methoxymycolate, 94.3%; and ketomycolate 91.1%) in the presence of ISO [38]. 5-biphenyl or 5-acetylenic analogues of thiolactomycin, have excellent inhibitory activity against MTB β-ketoacyl-ACP synthase mtFabH condensing enzyme [39, 40]. Triclosan (2,4,4'-trichloro-2'-hydroxyphenyl ether) is a broad spectrum antimicrobial agent that poses as a competitive inhibitor of bacterial enzyme, enoyl ACP reductase [41]. Another study revealed that EGCG enhances the inhibitory activity of triclosan towards inhA in MTB [42]. Further studies reported anti-tubercular activities of triclosan analog and showed their interaction with enoyl ACP reductase [43, 44]. Parrish et al, displayed that N-Octanesulfonylacetamide (OSA) is a b-sulfonyl carboxamide,is responsible for inhibition of fatty acid elongation. The study reported that antimycobacterial activity of OSA against different species of mycobacteria including MTB ranging from 6.25 to 12.5 (mg/ml). Additionally OSA showed inhibition of mycolic acids in *M.bovis* BCG commonly which was revealed by two-dimensional thin layer chromatography. The inhibition of INH resistant MTB displayed inhibited mycolic acid biosynthesis after exposure of OSA and suggested that it may be responsible for interaction with an enzymatic target which is different from INH [45]. Brian et al identified two compounds V-13-011503 and V-13-012725 which are involved in cholesterol metabolism pathway by inhibiting HsaAB enzyme complex responsible for the complete degradation of the A/B ring of cholesterol against *Mycobacterium* [46]. Furthermore, Vaubourgeix et al reported that S-adenosyl N-decyl aminoethane, is the analog of AdoHcy, exhibited antimycobacterial activity against MTB. They observed that above compound block the epoxy-keto and hydroxyl mycolic acid biosynthesis [47]. Similarly piperidinol is a inhibitor of aNAT ( arylamine N-acetyltransferase (aNAT), a cytosolic enzyme involved in transfer of acetyl group to an arylamine, by hydrolysis of acetyl-CoA showed good potency against MTB and *M. marinum* aNAT enzymes [48, 49]. But the addition of chlorines in 4th position of phenyl ring increased potency against MTB and *M. marinum* aNAT enzymes. Furthermore substituting the N-methyl group significantly reduce the potency against aNAT in MTB. But the addition of functional group to this compound enhanced the activity of potent anti-TB compound both enzymatically and against whole MTB cells [49]. Stanley et al showed antimycobacterial activity of coumarin against MTB which was observed by high throughput screening of 20502 compounds. They observed aniline substituted coumarin is the most potent compound against MTB and five clinical isolates of MTB. This potent compound showed point mutation in FadD32 gene which was determined by genetic analysis of resistant mutants [50]. Another compound Methyl 2-(2-bromoa-cetamido)-5-(3-chlo-rophenyl) thiazole-4-carboxylate, is the derivative of 2-aminothiazole-4-carboxylate exhibited inhibitory activity against beta-ketoacyl-ACP synthase mtFabH in MTB [51]. Similarly compound AU1235, 1-(2-adamantyl)-3-(2,3,4-triflurophenyl) urea is responsible for decreased TDM formation and cell wall bound α-methoxy and keto-mycolic acid [52]. The derivates of AU1235 also observed inhibition of TDM and increase of TMM in MTB as well as inhibited MmpL3 membrane transporter [53]. Another study demonstrated that SQ109 and Ethambutol (EMB) although have similar diamine core but have different mechanism of action. EMB is responsible to inhibit arabinogalactan whereas SQ109 inhibit MmpL3 transporter (membrane mycolic acid transporter). Furthermore macromolecular effect of SQ109 displayed inhibition of binding of mycolic acid to cell wall. They also found accumulation of TMM and depletion of TDM after exposure of SQ109 [54]. Likewise two compound tetrahydropyrazolopyrimidie and spiro-piperidine showed inhibitory activity against MmpL3 transporter [55, 56]. Stanley et al identify a benzimidazole inhibitor of MmpL3 by using high throughput screen against MTB [57]. Similarly analogues of sansinamycin showed inhibition of MTB phospho-MurNAc-pentapeptide translocase, the enzyme responsible for the synthesis of lipid I in mycobacteria [58]. Isoniazid is a first line drug having the ability to inhibit mycobacterial enoyl reductase InhA, which is required for the biosynthesis of mycolic acids, the dominant feature of the outer mycobacterial cell wall that is essential for growth and virulence [59, 60]. Ethionamide (ETH) was found to be the most potent second-line therapy involved in the treatment of MDR-TB. Both, ETH and INH have found inhibitory effects on the synthesis of mycolic acid [9]. The target of isoniazid, inhA-encoded enoyl reductase had been suggested to also be a target of ethionamide due to a single mutation in the inhA gene and provides resistance to ETH and INH [9]. Thioacetozone (TAC) is a widely used second line anti-TB drug often used in

combination of other drug, such as INH. The studies showed that TAC is responsible to inhibit mycolic acid cyclopropanation [9]. Pyrazinamide is a pro drug which requires amide hydrolysis by mycobacterial pyrazinamidase to pyrazionic acid. They suggested that pyrazinoic acid may inhibit FAS I which is involved in fatty acid synthase pathway [61].

## 5. Antimycobacterial activity of traditional medicines

Traditional medicine is sum of total of knowledge based on the theories, experiences and beliefs indigenous to different culture which are used to treat, diagnose, improve and prevent illnesses or maintain well-being. *Centella asiatica* is a medicinal plant of prehistoric times that has been mainly used for wound healing, burns, ulcers, leprosy, lupus, skin diseases, eye diseases, fever, inflammation, asthma, hypertension, rheumatism, syphilis, epilepsy, diarrhea, and mental illness and is also eaten as a vegetable or used as a spice. Interestingly, in addition it also shows antitubercular activity against MTB [62]. *Cyclopia genistoides (*honeybush) is a traditional herbal tea of South Africa and it contains a polyphenolic compound that shows health-promoting properties. Furthermore a concentrated extract of honey bush was used as an expectorant and restorative for the treatment of pulmonary tuberculosis [62]. *Pelargonium sidoides* is medicinal herb native to the coastal regions of South Africa. The root extract is very effective for the treatment of acute respiratory infections [63]. In a study, Taylor et al showed hexane extract of root *P. reniforme* and *P. sidoides* has shown antimycobacterial activity [64]. However contrasting views according to Gödecke et al were reported which shows extract and fraction of *P.sidoides* have shown no significant activity against *Mycobacterium* [65]. An assumption was made that antitubercular effect can thus be achieved by indirect stimulation of immune response which was later supported by Mativandlela et al and they stated that above extracts and fraction have no significant effect on MTB [66]. Artemisinin, an ancient Chinese medicine isolated from Chinese herb *Artemisinin annua*, is being used as first line drug for treatment of malaria. Furthermore his compound has ability to inhibit growth of mycobacteria under the dormant stage, a non-replicating persistence which is characterized by a low metabolic activity and phenotypic drug resistance when encountering any stress from the host immune system including hypoxia, acidic pH or starvation [67]. *Ligularia atroviolacea*, is a herbaceous perennial plant and has been traditionally used as herbal medicine to treat hepatitis B, asthma, hemoptysis, and pulmonary tuberculosis [68]. Liu et al have reported that garlicin is a chinese traditional medicine which cannot only inhibit MTB protein synthesis but is also responsible to inhibit bacterial rotamase, thus preventing DNA replication and degradation resulting in MTB death [69]. *Adhatoda vasica nees* has been widely used in traditional Ayurveda and is found in different region of India and throughout the world. The two widely used mucolytics i.e bromhexine and ambroxol are the chemical constituents produced by Adhatoda alkaloids and both chemical have a pH-dependent growth inhibition on MTB. Moreover Adhatoda also has indirect effect that includes increased level of rifampicin and lysozyme in bronchial secretions, lung tissues and sputum and suggesting that it may play important role as an adjunctive in TB treatment [70, 71]. Gulkand (a preparation made from vasaka flowers) is another compound, used to treat tuberculosis [72]. *Alpinia galangal* is found in South-east Asian countries such as Philippines, Indonesia, Thailand, India, and China. It is widely used in diets as well as in the traditional systems of medicine viz., Thai, Ayurveda, Unani and Chinese folk medicine [73]. Gupta et al observed antimycobacterial activity of extracts under microaerophilic and anaerobic conditions therefore it could be effective in treating of dormant and non-replicating bacteria of latent TB [73]. Similarly Lakshmanan et al purified active molecule ethyl p-methoxycinnamate from rhizome of *kaempferia galanga* which exhibited inhibitory activity against drug susceptible as well as MDR-MTB [74]. Shashidhar et al has reported some traditional herbs such as *Allium cepa, Aloe vera, Trichosanthes dioica, Prunus armeniaca, ocimum sanctum, cansora decussate, clavija procera, cryptocarya latifolia, colebrookea oppositifolia, kalanchoe integra, mallotus philippensis, flacourtia ramontchii, leysera gnaphalodes, morinda citrifolia* which exhibited antimycobacterial activity [75].

## 6. Clinical implications of novel drugs

Bedaquiline, a novel diarylquinoline compound works by blocking the proton pump of adenosine triphosphate (ATP) synthase in MTB [76]. According to previous studies, bedaquiline is recommended for adult patients with pulmonary MDR-TB when the patients fail to respond to an effective regimen due to intolerance, resistance, or an unavailability of other TB drugs. Delamanid is a novel nitroimidazole compound that has very low MIC value (0.006 to 0.024 μg/mL) against drug sensitive and drug resistant strain of MTB than those of rifampin, isoniazid, ethambutol, streptomycin and pretomanid. The mechanism of action of this drug involves the target of cell wall by interfering in the synthesis of methoxy- mycolic and keto-mycolic acids [77]. Pretomanid is a novel bicyclic nitroimidazo-oxazole currently being developed by the TB Alliance. It has potent anti-mycobacterial activity against both drug sensitive and drug resistant TB. It is responsible for the disruption of mycolic acid acid synthesis pathway. In addition it has also shown bacteriocidal activity against both replicating and hypoxic state of MTB. Based on the previous studies this regimen showed good efficacy and safety [77]. Sutezolid (PNU-100480) is a linezolid with high efficacy against MTB. It has shown better antimycobacterial activity than linezolid against sensitive and resistant strain of MTB. SQ109 and bedaquiline are the first new anti-TB drugs which were approved by the FDA, are apparently the two

best drugs to pair with sutezolid in a new TB regimen for the treatment of drug susceptible and drug resistant TB [77].

## 7. Conclusion

Mycolic acids are the unique component of the Mycobacterium cell wall and the main target of anti-TB drugs. Considering the fact that bulk of the MTB genome codes for lipid metabolism genes, compounds targeting lipid biosynthetic pathways must be studied in intricate details. Diverse number of natural, synthetic and traditional compounds has been reported for antimycobacterial activity with lipid metabolism disruption as prime mode of action. Together, the antimycobacterial effect of these compounds not only provides a platform to improve therapeutic strategies but also will help to unravel novel regulatory circuitry that impacts MDR-TB.

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