Overview on Antitubercular Agent Thiolactomycin and its Analogues

Mohammad asif

Department of pharmacy, GRD(PG)IMT, Dehradun India

Abstract

Various antitubercular agents are used the control and treatment of tuberculosis (TB). Currently, various works is going on in the research and development of new antiTB drugs for less side effects, more potent, short period of therapy and more importantly active against multi drug resistant strains of Mycobacterium. In this continuation we study here thiolactomycin (TLM) and its analogues as anti-TB agent and effect of various substituents on TLM for development of more effective molecule with considerable potential. TLM is naturally occurring and exhibited potent in-vivo activity against many pathogenic bacteria including Mycobacterium strains. TLM inhibits bacterial and plant type-II fatty acid synthases (FAS-II) but not effective in mammalian or yeast type-I fatty acid synthases (FAS-I). This review provides an overview on the TLM derivatives and their structure activity relationship.

Keywords Antitubercular agents, Mycobacterium tuberculosis, thiolactomycin, fatty acid synthases

Introduction

Tuberculosis (TB) is a respiratory transmitted disease caused by slow growing Mycobacterium species, such as M. tuberculosis, M. bovis, M. africanum, M. microti, M. avium, and M. leprae. TB is affecting nearly 32% of the world’s population that is more than any other infectious disease. Mycobacterium species are more susceptible in the HIV/AIDS patient so that one-third of people living with HIV/AIDS worldwide is co-infected with TB. One of most common problems related to TB therapy is drug multi drug (MDR) and extensively drug (XDR) resistance strains of Mycobacterium. Other complications are high toxicities and long duration of therapy for the current antiTB drugs (Dye et al., 1999; Dony et al., 2004; Turenne et al., 2001). Chemotherapy of TB of currently used the five first-line antiTB drugs (streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide) and less commonly used some second line anti-TB drugs [aminoglycosides (amikacin, kanamycin), polypeptides (capreomycin, viomycin), fluoroquinolones (ciprofloxacin, moxifloxacin), thioamides (ethionamides, prothioamide), cycloserine and p-aminosalicylic acid] (Tomasz. 1994; WHO, 2008). They may be classified as a second-line because of following reasons: it may be less effective or it may have high toxic effects than the first-line drugs. Clarithromycin is an antibiotic used in HIV infected TB patients to treat the M. avium complex (MAC) (Asif et al., 2011; Asif. 2011; Center for Disease Control. 2006). The MDR-TB are refers to simultaneous resistance to at least two or more of the five first-line anti-TB drugs. Treatment for MDR-TB is long lasting, less effective, costly, and poorly tolerated. XDR-TB is resistance to at least isoniazid and rifampicin in addition to any quinolone and at least one second-line drug like capreomycin, amikacin, and kanamycin. MDR-TB are treated with a combination of drugs containing second line drugs that are less effective, more expensive and higher toxicity. Hence there is an urgent need for novel antiTB drugs that are active against Mycobacterium species (Walters et al., 2006; Slayden et al., 2002; Clatworthy et al., 2007; Espinal. 2003; Amalio and Michael. 2000).
**Thiolactomycin:** Thiolactomycin (TLM) (1) is a naturally occurring molecule that exhibit potent *in-vivo* antimicrobial activity against many pathogenic Gram-positive bacteria, Gram-negative bacteria and *Mycobacterium* species particularly *Mtb* (Noto et al., 1982; Miyakawa et al., 1982). TLM is a thiolactone antibiotic obtained from fermentation broth of *Nocardia* species, a strain of actinomycetes. It shows complete inhibition of growth of the virulent strain *Mtb* Erdmann at 25μg/mL. Various TLM derivatives are also considered as potential anti-TB agents. Thiolactomycin (TLM) possesses *in-vivo* anti-TB activity against the saprophytic strain *M. smegmatis* (*Msg*) and the virulent strain *M. tuberculosis* (*Mtb*) and inhibition of growth at 75 and 25 mg/ml, respectively. The TLM was shown to inhibit the synthesis of both fatty acids and mycolic acids. However, synthesis of the shorter-chain mycolates of *Msg* was not inhibited by TLM, whereas the longer-chain mycolates and epoxymycolates were almost completely inhibited at 75 mg/ml. The use of *Msg* cell extracts demonstrated that TLM inhibited the mycobacterial acyl carrier protein-dependent type II fatty acid synthase (FAS-II) but not the multifunctional type I fatty acid synthase (FAS-I) (Hayashi et al., 1983). The selective inhibition of long-chain mycolate synthesis by TLM was recognized in a dose-response manner, cell wall-containing extracts of *Msg* cells. The mechanism of TLM resistance in *E. coli* suggest that two distinct TLM targets exist in mycobacterial. In *Escherichia coli*, it inhibits both β-ketoacyl-acyl carrier protein (ACP) synthase-I to III and acetyl coenzyme A (CoA): ACP transacylase activities in *in-vivo* and *in-vitro* conditions (Heath et al., 2001; Tsay et al., 1992). The β-ketoacyl-acyl carrier protein synthases involved in FAS-II and the elongation steps leading to the synthesis of the mycolates and oxygenated mycolates. The efficacy of TLM against *Msg* and *Mtb* offered the scenario of identifying fatty acid and mycolic acid biosynthetic genes and revealing a novel range of anti-infective agents against *Mtb* (Slayden et al., 1996). Consequently, an understanding of the mode of action of TLM is important in the development of more effective antibiotics that exhibit selective action against bacterial FAS-II.

![Thiolactomycin](image)

**Thiolactomycin and its Analogs:**
Thiolactomycin [(4R)(2E,5E)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide] (TLM) is a first naturally occurring unique thiolactone antibiotic isolated initially from a soil *Nocardia* sp., a strain of actinomycetes which exhibits potent *in-vivo* and moderate *in-vitro* activity against broad spectrum of pathogens including *Mtb* (Miyakawa, et al., 1982; Hamada et al., 1990). TLM has a unique structure without any chemical relation to any other group of known antibiotics (Chambers and Thomas. 1997; Sasaki et al., 1982; Oishi et al., 1982). It shows complete inhibition of growth of the virulent strain *Mtb* at 25μg/mL (Slayden et al., 1996). TLM is well absorbed orally with an LD₅₀ of 1.689g/kg reported in rodents (Noto et al., 1982). TLM also showed encouraging antimalarial activity by inhibition of type II fatty acid biosynthetic pathway in apicoplasts (Heath, 2001; Nie et al., 2005). TLM has non-toxic to mice and affords significant protection in urinary tract and intraperitoneal bacterial infections (Hayashi et al., 1983). TLM inhibits type-II dissociable fatty acid synthases (Jones et al., 2000), it also inhibits the biosynthesis of both fatty acids and mycolic acids in *Mycobacterium* (Minnikin et al., 1982; Jackowski et al., 2002).

**Mechanism of action:**
A rational approach for development of new antibacterial agents is targeting fatty acid biosynthesis (FAB) pathway (Payne et al., 2001; Khandekar et al., 2003), because FAB in bacteria, plants and animals is carried out by the ever-present fatty acid synthase (FAS) system (Jayakumar et al., 1995). FAB is an essential metabolic process for prokaryotic organisms and is required for cell viability and growth. In the type I system of animals, including humans, FAS is a homodimer of two large polypeptides, each composed of several distinct enzyme domains and is an integral acyl carrier protein (ACP) (Heath, 2001). In the type II system of bacteria, plants and protozoa, the FAS components, including the ACP, exist as discrete proteins (Chirala et al., 2001). The corresponding enzymes of the type I and II FAS are related in structure and function but generally lack overall sequence homology. Large multifunctional proteins termed type-I FAS catalyze these essential reactions in eukaryotes. In contrast, the bacterial use of multiple enzymes to accomplish the same goal is referred to as type II, or dissociated FAS (Clough et al., 1992). The bacterial system and proteins bear little homology to the human system and therefore represent a set of attractive target proteins for antibacterial development. Since many bacterial infections are resistant to several of the available antibiotics so compounds that targeting the FAB pathway could fill a serious medical need (Waller et al., 1998). A key enzyme responsible for the initiation of bacterial FAB has not yet received attention. FabH, a β-ketoacyl–acyl carrier protein synthase, is the bacterial condensing enzyme in that initiates the cycle by catalyzing the first condensation step between acetyl–CoA and malonyl–ACP (Nie et al., 2005). Mycolic acid is a fatty acids, is a vital cell wall component of the *Mtb* strain. Biosynthesis of the fatty acyl chain involves two fatty acid synthetic systems, fatty acid synthase I (FAS I), which catalyzes *de novo* fatty acid synthesis and fatty acid synthase II (FAS II), which consists of monofunctional enzymes that elongate FAS I products into long chain mycolic acid precursors. Three enzymes, β-ketoacylacyl carrier protein synthase (Kas) A and B and the condensing enzyme FabH, have all been identified as FAS II enzymes.
involved in mycolic acid and fatty acid biosynthesis. TLM inhibits fatty acid synthesis by inhibiting FabH, KasA and KasB. TLM inhibited KasA and KasB displaying IC50 values of 20μM and 90μM respectively (Schaeffer et al., 2001). The use of Msg cell extracts confirmed that TLM specifically inhibited the mycobacterial acyl–carrier-protein-dependent type II fatty acid synthase (FAS-II), but it did not inhibit the multifunctional type I fatty acid synthase (FASI). Analysis of the in vivo and in vitro data has suggested two separate sites of action for TLM, the β-ketoacyl carrier protein synthase in FAS-II and the elongation step involved in the synthesis of α-mycocytes and oxygenated mycolates. The studies have shown that the enzymes targeted by TLM are KasA and KasB, which are involved in fatty acid and mycolic acid biosynthesis in Mtb. The in vitro activity of TLM is substantial against a wide range of Mtb strains, including those that are resistant to MDR and XDR-TB, however this is at somewhat higher concentration (Kremer et al., 2000; Morita et al., 2000).

**STRUCTURE ACTIVITY RELATIONSHIP**

**Modifications at C-5:** Various analogs of TLM at the C-5 position and showed their activity against whole cells of Mtb H37Rv as well as mycolic acid biosynthesis in cell extracts of Msg (Douglas et al., 2002). The analog with a 5-tetrahydrogeranyl substuent showed the highest biological activity with an MIC 90 of 29 μM for Mtb and 92% mycolate inhibition in extracts of Msg, as compared to 125 μM and 54% respectively, for TLM. Furthermore, the trans-geranyl analogue (2) was inactive against Mtb H37Rv, but the sequential saturation of one or two double bonds (3b or 4) showed significant activities. Increasing the length of the side chain to trans-trans-farnesyl (5) enhanced activity. Various other TLM analogs with biphenyl and acetylene side-chains at the C-5 position of the thiolactone ring such as compounds (6) and (7) have been derivatized with biphenyl ring and compounds 8-10 with acetylene side chains (Senior et al., 2003; Senior et al., 2004). These compounds exhibited moderate in-vitro inhibitory activity against the Mtb. Recently, there was another report on C5 analogues of TLM have been assessed for their mtFabH and whole cell M. bovis BCG activity, respectively (Bhowruth et al., 2007). Amongst them three analogues (11-13) exhibited a significant enhancement in the in-vitro activity against mtFabH assay. The analogue 17 (5-(4-methoxyacarbonyl–biphenyl-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one) showed an IC50 value of 3 μM compared to 75 μM for the parent drug TLM against mtFabH. Finally, they reaffirm the requirement for a linear π-rich system containing hydrogen bond accepting substituents attached to the paro positions of the CS biphenyl analogue to generate compounds with enhanced activity. Other reports have showed, C-5 analogues of TLM and suggesting that an intact (5R)-isoprene unit is necessary for the activity against condensing enzymes FabH, KasA and KasB from Mtb (Kim et al., 2006). The modifications at C-5 position, like increasing the alkyl chain length 14A-14F and reduction of one or both double bond of isoprene unit 15-17, resulted in markedly reduced activity. There is no significant difference in activity for the analogues 18, 19 obtained by addition and removal of methyl group. However, C5-biphenyl TLM with increased activity against mtFabH condensing enzyme, thus supporting C5-derivatization of TLM scaffold (Bhowruth et al., 2007). The new structural analogs of TLM (20-22) at C-5 position have exhibited weak to moderate activity against Type-I fatty acid synthase (McFadden et al., 2005). However, analogs as enantiomerically pure molecules (Chambers and Thomas, 1997) and racemic mixtures, like compounds (4) and (5), which have greater activity than the parent in inhibiting Mtb H37Rv in-vitro.

**Modifications at C-4:**
A series of C-4 analogues (23-26) of TLM molecules have been evaluated against four different *Mycobacterium* species namely Mtb H37Rv ATCC 27294, Mtb clinical isolates (sensitive and resistant), *M. avium* ATCC 49601 and *M. intracellulare* ATCC 13950 (Kamal et al., 2005). The compound 24e, having spacer of eight methylene groups and linked to methyl thioglycolate proved to be the most active compound with an MIC value 1.0-4.0 μg/mL against drug sensitive and resistant strain of Mtb.

**Modifications at C-3 and C-5:**
A series of analogues of naturally occurring TLM and evaluated their ability to inhibit the growth of malaria parasite *Plasmodium falciparum* (Jones et al., 2004; Jones et al., 2005). Compounds 27-30 with substitutions at the C-3 and C-5 positions with long chain alkane residues show improved activity against *P. falciparum*, blood streams of *Trypanosoma brucei* and *T. cruzi* amastigotes cultured intracellularly. This shows that the analogues of TLM not only have potential in the development of new anti-TB compounds but may be useful as new antimalarial agents. 3-acetyl analogues of TLM and profiled their activity against live stock pathogens (Sakaya et al., 2001). Compounds 31-33 have shown improved activity over TLM, against *Staphylococcus aureus* and moderate to comparable activity against *Pasteurella multocida*.

It inhibits MtFabII through inhibition of β-ketoacyl-ACP synthase condensing enzymes, mtFabH and KasA, in vitro and in vivo leading to inhibition of cell wall mycolic acid biosynthesis and to cell death. In this concept, initially synthesized TLM analogues with biphenyl-based 5-substituents and found to have excellent in vitro inhibitory activity against the recombinant Mtb β-ketoacyl-ACP synthase mtFabH condensing enzyme. In particular, 5-(4’-benzoxoxy-biphen-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one (34) exhibited approximately a 4-fold (IC50=17 μM) increased potency compared to TLM (Senior et al. 2003). Whereas in a series of TLM with acetylenebased side chains, 5-[3-(4-acetyl-
phenyl)-prop-2-ynyl]-4-hydroxy-3,5-dimethyl-5'Hthiophen-2-one (35) exhibited more than an 18-fold (IC50=4 μM) increased potency, compared to TLM against same enzyme (Senior et al. 2004). While in another series, 5-(4-methoxy carbonyl-biphenyl-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5'H-thiophen-2-one (36) gave an IC50 value of 3 μM compared to the parent drug TLM (75 μM) against mtFabH (Bhowruth et al., 2007). The biological analysis of this library reaffirms the requirement for a linear π-rich system containing hydrogen bond accepting substituents attached to the para-position of the C5 biphenyl analogue to generate compounds with enhanced activity. In the same direction, synthesized new TLM series was identifying as most potent TLM derivative (37) with a MIC of 1 μg/mL against Mtb H37Rv. This derivative also exhibited potency against MDR-TB strains (Kamal et al., 2005). In a different approach, a series of 2-amino-5-arylthieno[2,3-b]thiophenes exhibited in-vitro activity against Mtb H37Rv and MDR-TB strains (Balamurugan et al., 2009).

Thiolactamycin Derivatives:
The TLM Analogs have been synthesized to enhance the activity against pathogenic strain of Mycobacterium (Douglas et al., 2002). TLM analogs act through the inhibition of the mycolate synthase, an enzyme involved in the biosynthesis of the cell wall of Mycobacterium species. TLM selectively inhibits the mycobacterial acyl carrier protein-dependent type-II fatty acid synthase (FAS-II) which provide essential building blocks for the bacterial cell wall but not the multifunctional type-I fatty acid synthase (FAS-I) present in mammals or yeast. It also has been shown to block long-chain mycolate synthesis, cell wall-containing extracts of Msg (Slayden et al. 1996). TLM is believed to exert its overall effect by inhibition of the β-keto acyl-ACP-synthases, key condensing enzymes involved in the chain elongation in FAS-II. TLM is a considerable interest because of its selective activity in disrupting essential fatty acid synthesis in bacteria, plants and some protozoa, but not in eukaryotes. The inhibitors of the TLM target enzyme, FAS-II, are of potential value in the treatment of malaria (Wallner, et al. 1998), trypanosomiasis or sleeping sickness (Morita, et al., 2000) and various bacterial infections including TB (Kremer et al., 2000). Consequently, TLM is active in-vitro against a wide range of strains of Mtb, including those resistant to isoniazid, albeit at somewhat high concentrations. Complete inhibition of growth on solid media of the virulent strain Mtb is seen at 25μg/ml. In rodents, TLM is well absorbed orally with an LD50 of 1.68g/kg. The activity against bacterial infections in mice by both oral and sub-cutaneous route have EDS0’s >70mg/kg (Miyakawa et al. 1982). The above activity is interesting for the progression of TLM analogs as an anti-TB agent.

TLM analogues with biphenyl-based 5-substituents have excellent in vitro inhibitory activity against the recombinant Mtb β-ketoacyl-ACP synthase mtFabH condensing enzyme. In particular, 5-[(4'-benzoxyl-biphenyl-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5'H-thiophen-2-one (11) exhibited approximately a 4-fold (IC50=17 μM) increased potency compared to TLM. Whereas in a series of TLM with acetylenebased side chains, 5-[3-(4-acetyl-phenyl)-prop-2-ynyl]-4-hydroxy-3,5-dimethyl-5'Hthiophen-2-one (9) exhibited more than an 18-fold (IC50=4 μM) increased potency, compared to TLM against same enzyme. While in another series, 5-[(4-methoxy carbonyl-biphenyl-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5'H-thiophen-2-one (17) gave an IC50 value of 3 μM compared to the parent drug TLM (75 μM) against mtFabH [43]. The biological analysis for a linear π-rich system containing hydrogen bond accepting substituents attached to the para position of the C5 biphenyl analogue to generate compounds with enhanced activity. In the same direction, new TLM series, where identifying most potent TLM derivative (24e) with a MIC of 1 μg/mL against Mtb H37Rv. This derivative also exhibited potency against resistant strains of mycobacterium. The susceptibilities of Msg and Mtb to various concentrations of TLM (1 to 150 mg/ml) were determined compared to values for INH and ethionamide. The MICs of TLM against Mtb and Msg were 25 and 75mg/ml, respectively, compared to the 10mg/ml for ethionamide against both species and 0.2 and 5mg/ml for INH (Banerjee, et al., 1994), respectively. The MICs reported for TLM against Msg and Mtb may be substantially lower, since a racemic mixture of TLM was used (Jackowski, et al., 1989; Hayashi, et al., 1983; Sasaki, et al., 1982; Wang and Salvino 1984). Effect of TLM on the growth of mycobacteria within bone marrow macrophages: Addition of TLM to macrophage cultures containing Mtb resulted in bacterial killing in a dose-dependent manner (Noto, et al., 1982). Whether TLM is directly responsible for the observed bactericidal activity is unclear, since it could compromise the integrity of the bacterial envelope, thereby potentiating macrophage bactericidal activity. Mycobacteria are unusual in that they possess two FAS systems, multifunctional mycobacterial FAS-I and dissociated mycobacterial FAS-II (Bloch. 1975; 4. Bloch. 1977). Since TLM is a known inhibitor of only FAS-II b-ketoacyl-ACP synthases in E. coli, we postulated that the observed inhibition of fatty acid synthesis during the labeling of whole mycobacteria cells with [1,2-14C]acetate is due to selective inhibition of the dissociated FAS-II b-ketoacyl-ACP synthase. To test this hypothesis, the ability of TLM to selectively inhibit mycobacterial FAS-I in cell extracts from M. smegmatis was examined. The multifunctional mycobacterial FAS-I system was insensitive to TLM in a dose-dependent manner at up to 0.75 mg/ml. However, a corresponding dose-response curve against the dissociated mycobacterial FAS-II system demonstrated 60% inhibition of the FAS-II synthase at 0.75 mg/ml, presumably through its effect on the FAS-II b-ketoacyl-ACP synthase. Interestingly, formation of the medium-chain (C24 to C30) fatty acids produced by FAS-II was much more sensitive to TLM inhibition in the in vitro [2-14C]malonyl-CoA assay than in the in vivo labeling experiments using [1,2-14C]acetate. A corresponding effect was also observed by Tsay et al. (Tsai, et al., 1992) during
their work on the characterization of b-ketoacyl-ACP synthase I and the mechanism of TLM resistance in *E. coli* K-12. For the effects of TLM on mycolic acid synthesis, we postulated a similar mechanism of action and an identical target. The synthesis of the long-chain a-mycocytes and oxygenated mycolates probably occurs via a series of elongation steps from the intermediate a9-mycocyte, involving, among other enzymes, a type II b-ketoacyl-ACP synthase. The introduction of oxygen functions probably occurs late in the pathway, and as a consequence, inhibition by TLM of the elongation steps leading to the a-mycocytes would lead to subsequent inhibition of oxygenated mycolic acid synthesis. To test this possibility, cell-free, particulate cell wall enzyme (P60) extracts, which preferentially catalyze the synthesis of mycolates, were analyzed in a dose-response manner against TLM. As predicted, synthesis of the a9-mycocytes was insensitive to TLM at up to 1.0 mg/ml, whereas that of the a (both a1 and a2)-mycolates was strongly inhibited, resulting in approximately 65% inhibition at 1.0 mg/ml. Medium-chain (C16 to C24) fatty acids produced within the in vitro mycolate assay were not inhibited by TLM (results not shown). It should be noted that MAME formation was much more sensitive to TLM than in the in vivo labeling experiments described earlier.

Extensive studies on fatty acid biosynthesis in both prokaryotes and eukaryotes have resulted in the definition of two distinct classes of enzyme activities necessary for the synthesis of long-chain fatty acids (Jackowski, et al., 1991; Magnuson, et al., 1993; Tsukamoto, et al., 1983). Many bacteria and plants possess a dissociable FAS-II, where each individual reaction is catalyzed by a discrete monofunctional peptide. In contrast, yeast and mammalian systems possess a multifunctional FAS-I. The extensive work of Bloch demonstrated that Msg is unique in that it possesses both the multifunctional mycobacterial FAS-I and the monofunctional mycobacterial polypeptide FAS-II systems (*Bloch* 1975; *Bloch* 1977). The primary products produced by the de novo FAS-I system were C16 to C18 and C24 to C26 fatty acyl-CoA derivatives, and the distribution of these products was bimodal and influenced by the presence of the mycobacterial 6-O-methylglucose- and 3-O-methylmannose-containing polysaccharides (Peterson and Bloch 1977). The type I complex appears to have limited elongation capacity (*Bloch* 1975; *Bloch* 1977) and is apparently not capable of extending fatty acids to the meromycolate level. The ACP-requiring FAS-II system was regarded as an elongation mechanism devoted primarily to the elongation of C16 fatty acid primers to C24 to C30 fatty acyl-ACPs, but again, this elongation apparently did not extend to the meromycolate level. Thus, it appears that the synthesis of mycolates, or at least meromycolates, requires a separate synthase. Qureshi et al. (Qureshi, et al., 1984) did demonstrate elongation of a C24 fatty acid to C52 meromycolate, but not to mycolate, by a cell extract of *Mtbc* H37Ra. More recently, a particulate cell-free system of *M. aurum* and Msg was developed which allowed the synthesis of whole mycolic acids from acetate rather than malonate (Lacave, et al., 1990; Wheeler, et al., 1993) and also from a C24:1DS fatty acid intermediate. The issues that are most relevant in the present context concern the mode of action of TLM in terms of the mycobacterial FAS-I and FAS-II synthases and how this relates to the action of TLM, e.g., on the *Escherichia coli* FAS-II synthase (Tsay, et al., 1992), and also the question of the utility of TLM as an example of a new generation of antagonists targeted against mycolic acid synthesis and mycobacteria. In general terms, there are two accepted pathways for the initiation of fatty acid biosynthesis in *E. coli* (Jackowski, et al., 1991; Magnuson, et al., 1993). Firstly, acetyl-CoA is transacylated to ACP via the acetyl-CoA:ACP transacylase and then condensed by either b-ketoacyl-ACP synthase I or II with malonyl-ACP to form acetoacetyl-ACP. The second route to acetoacetyl-ACP is the condensation of acetyl-CoA with malonyl-ACP catalyzed by synthase III. In *E. coli*, TLM has long been considered to disrupt fatty acid biosynthesis by inhibiting the acetyl transacylase step (Jackowski, et al., 1989) and all three b-ketoacyl-ACP synthases (Nishida, et al., 1986). However, a recent communication by Lowe and Rhodes (Lowe, and Rhodes. 1988) which described the purification of the acetyl-CoA:ACP transacylase to homogeneity demonstrated that this activity was not inhibited by TLM, and the extensive work of Rock and colleagues (Magnuson, et al., 1993; Tsay, et al., 1992) suggests that the b-ketoacyl-ACP synthase I may be the only activity required for the initiation of fatty acid biosynthesis and is the primary site of action of TLM in *E. coli*. The monofunctional mycobacterial polypeptide FAS-II is unusual in that it functions primarily as an elongating system in mycobacteria (*Bloch* 1975; *Bloch* 1977). The initial steps involved within this synthase, which are presumably analogous to the initiation steps in *E. coli* (Jackowski, et al., 1989; Magnuson, et al., 1993), involve a palmitoyl-CoA:ACP transacylase to afford palmitoyl-ACP, which is then condensed with malonyl-ACP to yield the corresponding b-ketoacyl-ACP. The next step involves reduction of the b-keto ester-ACP by an NAD(P)H-dependent b-ketoacyl-ACP reductase, followed by dehydration (b-hydroxyacyl-ACP dehydratase) to the trans-2-unsaturated acyl-ACP, which is then reduced by an NADH-dependent enoyl reductase to form the C18 ACP, which can then serve as a substrate for additional rounds of elongation. The in vitro FAS-I–FAS-II data presented in this communication demonstrate that TLM is a potent inhibitor of the monofunctional mycobacterial polypeptide FAS-II system, whereas the multifunctional mycobacterial FAS-I system is insensitive. This is consistent with other reports concerning the mode of action of TLM against mammalian and yeast FAS-I systems (Hayashi, et al., 1983) and bacterial and plant FAS-II systems (Nishida, et al., 1986). In addition, through
precedence with the FAS-II system of *E. coli*, the presumed target within the ACP-dependent monofunctional mycobacterial polypeptide FAS-II system would be a b-ketoacyl-ACP synthase (Tsay, et al., 1992). Interestingly, the related actinomycete species *C. matruchotti*, which possess only a closely related multifunctional FAS-I system (Bloch. 1977) and synthases which are responsible for the synthesis of corynomycolates (C28 to C36), was unsusceptible to TLM on solid media. This is not surprising, since this study has defined the primary targets of TLM as two distinct b-ketoacyl-ACP synthases within mycobacteria, firstly, the ACP-dependent mycobacterial FAS-II system, and secondly, the synthases responsible for long-chain mycolate synthesis, neither of which is present in corynебacteria.

On the other hand, the observed insensitivity of corynebacteria to TLM may be due to other resistance mechanisms. A report suggested that InhA, which is a long-chain (C12 to C24) enoyl-ACP dependent reductase, is part of the type II ACP-dependent FAS-II system described by Bloch (3, 4). However, in our studies, INH in the presence of catalase-peroxidase activity (Zhang et al., 1992) and crude cell extracts of *Msg*, failed to inhibit the ACP-dependent FAS-II system described by Bloch (Bloch. 1975; Bloch. 1977). The relevance of this observation to the present study becomes more obvious when the mode of action of TLM is considered in the context of FAS-II and the synthases responsible for mycolate synthesis. If the ACP-dependent FAS-II system were involved in the early stages of mycolic acid synthesis as suggested (Quemard, et al., 1995), then TLM, which has been shown to be a potent inhibitor of this system, would lead to the subsequent inhibition of all types of mycolic acids. However, this is not the case, since the a9-mycolates of *Msg* are still synthesized, while TLM exhibits selective inhibition of the ACP-dependent FAS-II system and an elongation step specific to the synthesis of the long-chain a-mycolates and oxygenated mycolates of *Msg* and *Mtb*. This suggests an alternative mechanism, presumably involving a membranebound ACP-dependent FAS-II system, of which InhA is a part, which performs de novo synthesis and provides fatty acid primers used exclusively for mycolate synthesis. In addition, this specialized type II mycolic acid synthase is particulate in nature, as demonstrated by its location within P60 extracts, and is clearly distinct from the soluble-ACP-dependent FAS-II system. Indeed, addition of INH to P60 extracts in the presence of catalase-peroxidase activity results in strong (.85%) inhibition of mycolate synthesis in *Msg* at 100 mg/ml. The other potential TLM target identified in this study was a key elongation enzyme, leading from either the C24:1DS intermediate or from the short-chain a9-mycolates to the longchain a-mycolates and oxygenated mycolates of *Msg* and *Mtb*, presumably another b-ketoacyl-ACP synthase. The findings presented within this study, along with the isolation of InhA (Banerjee, et al., 1994; Quemard, et al., 1995), suggest that multiple ACP-dependent FAS-II systems exist involving multiple, unique b-ketoacyl-ACP synthases, b-ketoacyl-ACP reductases, b-hydroxyacyl-ACP dehydratases, and b-enoyl-ACP reductases involved in both fatty acid and mycolic acid synthesis. Each isozyme is apparently based on individual substrate specificities and therefore makes a unique contribution to the regulation and distribution of products from each pathway. However, direct evidence for their existence is not available because of a lack of adequate biochemical and genetic tools. Nevertheless, the identification of two TLM targets within *Msg* allows the generation of mutants and transformants capable of conferring TLM resistance either by enzyme overproduction or via point mutation leading to the identification of the genes involved in TLM resistance and hence fatty acid and mycolic acid biosynthesis. Finally, it should be noted that other mechanisms of TLM resistance may exist in mycobacteria. For instance, in *E. coli*, disruption of the emrB gene restored sensitivity to TLM-resistant strain *E. coli* CDM5 and we have demonstrated that overexpression of the emrAB operon confers TLM resistance on susceptible *E. coli* strains (Furukawa, et al., 1993).

**Discussion**

TLM inhibits mycobacterial fatty acid synthase and the elongation steps of mycolic acid biosynthesis, with negligible toxicity and thus structures based on this lead could provide a new class of drug against TB. Hence TLM is a useful tool for studying the mechanisms of underlying infections diseases (Kamal et al., 2008). The TLM derivatives have proven to be of considerable interest with its unique thiolactone moiety and because of its broad antibacterial spectrum. The favourable physical and pharmacokinetic properties of TLM, like low toxicity profile and good activity against several strains of *Mycobacterium* which are resistant to the other drugs has made it an attractive lead molecule for the development of a drug against the treatment of *tuberculosis*. In view of the persistent drug-resistant TB problem, it is important that new drugs should address different targets, as those of currently used drugs including the shortening of TB therapy. The unique structure of the mycobacterial cell wall makes it a useful target for drug development and studies can be directed to specific sites like cell wall biosynthetic pathways (Heath and Rock, 2004).

**Conclusion**

In spite of the availability of the BCG vaccine and some chemotherapeutic agents, TB remains a leading infectious killer worldwide, mainly due to the lack of new drugs, particularly for effective against the MDR-TB and XDR-TB strains. Therefore, there is an urgent need for the development of new anti-TB drugs with lesser side-effects, improved pharmacokinetic properties and new target mechanism to be effective against bacteria including the resistant strains and reduce the overall duration of TB treatment. Hence, new molecules
that could provide valuable therapeutic options for the therapeutic application of drug resistance. TLM inhibits bacterial as well as plant type II fatty acid synthases (FAS-II), which provide essential building blocks for the bacterial cell wall. TLM is believed to exert its overall effect by inhibition of the β-keto acyl-ACP-synthases ( Kas), key condensing enzymes involved in the chain elongation in FAS-II. Therefore, developing structurally modified compounds based on TLM or thiolactone ring could provide novel anti-TB drugs. Based on its potential as an attractive lead molecule, efforts are being made to structurally modify this natural product for the development of new anti-TB agents.

References


AUTHORS’ CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

Structures of some thiolactomycin analogues