International Journal of Pharmacy Teaching & Practices 2013, Vol.4, Issue 2, 587-597.

Overview on Antitubercular Agent Thiolactomycin and its Analogues

Mohammad asif

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

Research Article

Please cite this paper as: Mohammad asif. Overview on antitubercular agent thiolactomycin and its analogues. IJPTP, 2013, 4(2), 587-597.

Corresponding Author:

Mohammad asif

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

Abstract

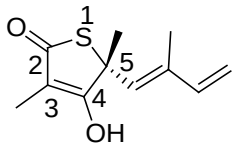
Vrious antitubercular agents are used the control and treatment of tuberculosis (TB). Currently, various works is going on in the research and development of new antiTB drugs for less side effects, more potent, short period of therapy and more importantly acive against multi drug resistant strains of *Mycobacterium*. In this continuation we study here thiolactomycin (TLM) and it 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 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 suceptble 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 commomnly 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 *Mycobacrium* 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 *Mycobaterium* 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* Erdmman 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 (**1**)

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 LD_{50} of 1.689g/kg repoted 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 specially 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-mycolates and oxygenated mycolates. The studied 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 substiuent 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 *mt*FabH 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 *mt*FabH assay. The analogue **17** (5-(4-methoxycarbonyl–biphenyl-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5*H*-thiophen-2-one) showed an IC50 value of 3 µM compared to 75 µM for the parent drug TLM against *mt*FabH. Finally, they reaffirm the requirement for a linear π-rich system containing hydrogen bond accepting substituents attached to the *para* positions of the C5 biphenyl analogue to generate compounds with enhanced activity. Other reports have showed, C-5 analogues of TLM and suggesting that an intact (5*R*)-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 is 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 intracelluarly. 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 *Stapylococcus aureus* and moderate to comparable activity against *Pasteurella multocida*.

It inhibits *Mtb* FASII 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'-benzyloxy-biphen-4-ylmethyl)-4-hydroxy-3,5- dimethyl-5*H*-thiophen-2-one (3**4**) 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*H*thiophen-2-one (**35**) exhibited more than an 18-fold (IC50=4 µM) increased potency, compared to TLM against same enzyme (Senior e*t al.* 2004). While in another series, 5-(4-methoxycarbonyl-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 (Waller, et al. 1998), trypanosomiasis or sleeping sickness (Morita et al., 2000) and various bacterial infectionds 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 1.689g/kg. The activity against bacterial infections in mice by both oral and sub-cutaneous route have ED50'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'-benzyloxy-biphen-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*H*thiophen-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-methoxycarbonyl-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. (Tsay, 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-mycolates and oxygenated mycolates probably occurs via a series of elongation steps from the intermediate a9-mycolate, 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-mycolates would lead to subsequent inhibition of oxygenated mycolic acid synthesis. To test this possibility, cellfree, 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-mycolates 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 in the in vitro mycolate assay 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*-methylmannosecontaining 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 *Mtb* 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 C24:1D5 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 *E. 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-ketoester-ACP by an NADPHdependent 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 corynebacteria.

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 C24:1D5 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

Inspite 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 bacterias 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

Amalio T, Michael I. (2000) Drug-Resistant Tuberculosis: What Do We Do Now? *Drugs* 59: 171-179.

Asif M, Anita Singh A, Ratnakar L. (2011) Antimicrobial Agents: Brief Study of Pyridazine Derivatives against Some Phathogenic Microrganisms. *Journal of Pharmacy Research,* 4(3): 664-667.

Asif M. (2011) Study of clinically used and recently developed antimycobacterial agents. *Orient Pharm Exp Med,* DOI 10.1007/s13596-011-0020-8.

Balamurugan K, Perumal S, Reddy ASK, Yogeeswari P, Sriram D. A facile domino protocol for the regioselective synthesis and discovery of novel 2-amino-5-arylthieno-[2,3-b]thiophenes as antimycobacterial agents. *Tetrahedron Lett.* 50(45), 6191-6195 (2009).

Banerjee, A., E. Dubnau, A. Quemard, V. Balasubramanian, K. S. Um, T. Wilson, D. Collins, G. DeLisle, and W. R. Jacobs, Jr. 1994. *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. Science 263:227–230.

Besra, G. S., K.-H. Khoo, M. R. McNeil, A. Dell, H. R. Morris, and P. J. Brennan. 1995. A new interpretation of the structure of the mycolyl-arabinogalactan complex of *Mycobacterium tuberculosis* as revealed through the characterization of oligoglycosyl alditol fragments by fast-atom bombardment mass-spectrometry and 1H-nuclear magnetic resonance spectroscopy. Biochemistry 34:4257–4266.

Bhowruth V, Brown AK, Senior SJ, Snaith JS, Besra GS. (2007) Synthesis and biological evalution of a C5-biphenyl thiolactomycin library. Bioorg Med Chem Lett 17: 5643-5646.

Bloch, K. 1975. Fatty acid synthases from *Mycobacterium phlei*. Methods Enzymol. 35:84–90.

Bloch, K. 1977. Control mechanisms for fatty acid synthesis in *Mycobacterium smegmatis*. Adv. Enzymol. 45:1–84.

Center for Disease Control. (2006) "Emergence of Mycobacterium tuberculosis with Extensive Resistance to Second-Line Drugs-Worldwide, 2000–2004". MMWR Weekly 55: 301–305.

Chambers MS, Thomas EJ. (1997) Asymmetric synthesis of 5,5-disubstituted thiotetronic acids using an allyl xanthate to dithiocarbonate rearrangement: total synthesis of (5S)-thiolactomycin with revision of the absolute configuration of the natural. *Journal of the Chemical Society, Perkin transactions 1*, 1:417-432.

Chirala SS, Huang WY, Jayakumar A, Sakai K, Wakil SJ. (2001) Human fatty acid synthase: Role of interdomain in the formation of catalytically active synthase dimmer. Proc Natl Acad Sci USA 98: 3104-3108.

Choi KH, Kremer L, Besra GS Rock CO. (2000) Identification and substrate specificity of beta ketoacyl (acyl carrier protein) synthase III (mtFabH) from mycobacterim tuberculosis. J Biol Chem 275: 28201-28207.

Clatworthy AE, Pierson E, Hung DT. (2007) Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol 3: 541-548.

Clough RC, Matthis AL, Barnum SR, Jaworski JG. (1992) Purification and characterization of 3-ketoacyl-acyl carrier protein synthase III from spinach. A condensing enzyme utilizing acetylcoenzyme A to initiate fatty acid synthesis. J Biol Chem 267**:** 20992-20998.

Cohn DL, Bustreo F, Raviglione MC. (1997) Drug resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD Global Surveillance Project. Clin Infect Dis, 24: S121-S130.

Cole ST, Phillip W, Heym B. (1996) Mechanisms of drug resistance in Mycobacterium tuberculosis. Curr Top Microbiol Immunol, 215: 49-69.

Dony JF, Ahmad J, Khen TY. (2004) Epidemiology of tuberculosis and leprosy, Sabah, Malaysia. Tuberculosis 84: 8-18.

Douglas JD, Senior SJ, Morehouse C, Phetsukiri B, Campbell IB, Besra GS, Minnikin DE. (2002) Analogues of thiolactomycin: Potential drugs with enhanced anti-mycobacterial activity. Microbiology 148: 3101-3109.

Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. (1999) Global Burden of Tuberculosis. J Am Med Assoc 282: 677.

Espinal MA. (2003) The global situation of MDR-TB. Tuberculosis 83: 44-51.

Furukawa, H., J. T. Tsay, S. Jackowski, Y. Takamura, and C. O. Rock. 1993. Thiolactomycin resistance in *Escherichia coli* is associated with the multidrug resistance efflux pump encoded by *emrAB*. J. Bacteriol. 175:3723–3729.

George, K. M., Y. Yuan, D. R. Sherman, and C. E. Barry III. 1995. The biosynthesis of cyclopropanated mycolic acids in *Mycobacterium tuberculosis*. Identification and functional analysis of CMAS-2. J. Biol. Chem. 270:27292–27298.

Global Tuberculosis Control: Surveillance, Planning, Financing WHO REPORT 2008, 51-54.

Hamada S, Fujiwara T, Shimauchi H, Ogawa T, Nishihara T, Koga T, Nehashi T, Matsuno T. (1990) Antimicrobial activities of thiolactomycin against gram-negative anaerobes associated with periodontal disease. Oral Microbiol Immunol 5: 340-345.

Hayashi T, Yamamoto O, Sasaki H, Kawaguchi A, Okazaki H. (1983) Mechanism of action of the antibiotic thiolactomycin inhibition of fatty acid

synthesis of *Escherichia coli*. Biochem Biophys Res Commun 115: 1108-1113.

Hayashi T, Yamamoto O, Sasaki H, Okazaki H, Kawaguchi A. (1984) Inhibition of fatty acid synthesis by the antibiotic thiolactomycin. J Antibiot 37: 1456-1461.

Heath RJ, Rock CO. (2004) Fatty acid biosynthesis as a target for novel antibacterials. *Curr Opin InVest Drugs*, 5: 146-153.

Heath RJ, White SW, Rock CO. (2001) Lipid biosynthesis as a target for antimicrobial agents. rog Lipid Res 40: 467-497.

Heath RJ. (2001) Bacterial fatty-acid biosynthesis: An antibacterial drug target waiting to be exploited Drug Discovery Today 6: 715.

Jackowski S, Zhang YM, Price AC, White SW, Rock CO. (2002) A missense mutation in the fabB (β-ketoacyl-acyl carrier protein synthase I) gene confers thiolactomycin resistance to *Escherichia coli.* Antimicrob Agents Chemother 46: 1246-1252.

Jackowski, S. J., C. M. Murphy, J. E. Cronan, Jr., and C. O. Rock. 1989. Acetoacetyl-acyl carrier protein synthase. A target for the antibiotic thiolactomycin. J. Biol. Chem. 264:7624–7629.

Jaworski, J. G., R. C. Clough, S. R. Barnum, D. Post-Beittenmiller, and J. B. Ohlrogge. 1990. Initial reactions of fatty acid synthesis and their regulation, p. 97–104. *In* P. J. Quinn and J. L. Harwood (ed.), Plant lipid biochemistry, structure and utilization. Portland Press, London.

Jayakumar A, Tai MH, Huang WY, Al Feel W, Hsu M, Abu-Elheiga L, Chirala SS, Wakil SJ. (1995) Human fatty acid synthase: Properties and molecular cloning. Proc Natl Acad Sci USA 92: 8695-8699.

Johnsson, K., and P. G. Schultz. 1994. Mechanistic studies of the oxidation of isoniazid by the catalase-peroxidase from *Mycobacterium tuberculosis*. J. Am. Chem. Soc. 116:7425–7426.

Johnsson, K., D. S. King, and P. G. Schultz. 1995. Studies on the mechanism of action of isoniazid and ethionamide in the chemotherapy of tuberculosis. J. Am. Chem. Soc. 117:5009–5010.

Jones AL, Herbert D, Rutter AJ, Dancer JE, Harwood JL. (2000) Novel inhibitors of the condensing enzymes of the type II fatty acid synthase of pea (*pisum sativum*). Biochem J 347: 205-209.

Jones PB, Parrish NM, Houston TA, Stapon A, Bansal NP, Dick JD, Townsend C A. (2000) A new class of antituberculosis agents. J Med Chem 43: 3304-3314.

Jones SM, Urch JE, Brun R, Harwood JL, Berry C, Gilbert IH. (2004) Analogues of thiolactomycin as potential anti-malarial and anti-trypanosomal agents. Bioorg. Med. Chem., 12: 683-692.

Jones SM, Urch JE, Kaiser M, Brun R., Harwood JL, Berry C, Gilbert IH. (2005) Analogues of thiolactomycin as potential antimalarial agents. J Med Chem 48: 5932-5941.

Kamal A, Azeeza S, Malik MS, Shaik AA, Rao MV. (2008) Efforts Towards the Development of New Antitubercular Agents: Potential for Thiolactomycin Based Compounds. J Pharm Pharmaceut Sci 11 (2): 56s-80s.

Kamal A, Shaik AA, Sinha R, Yadav JS, Arora SK. Antitubercular agents. Part 2: New thiolactomycin analogues active against Mycobacterium tuberculosis. *Bioorg. Med. Chem. Lett.* 15(7), 1927-1929 (2005).

Khandekar SS, Daines RA, Lonsdale JT. (2003) Bacterial -ketoacyl-Acyl Carrier Protein Synthases as Targets for Antibacterial Agents. Curr. Protein and Peptide Sci. 4: 21-29.

Kim P, Zhang Y, Shenoy G, Nguyen Q, Boschoff HI, Manjunath UH, Goodwin MB, Lonsdale J, Price AC, Miller DJ, Duncan K, White SW, Rock CO, Barry III CE, Dowd CS. (2006) Structure-Activity Relationships at the 5-Position of thiolactomycin: An intact (5*R*)-isoprene unit is required for activity against the condensing enzymes from mycobacterium tuberculosis and Escherichia coli. J Med Chem 49: 159-171.

Kremer L, Douglas JD, Baulard AR.and 9 other authors. Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting KasA and KasB condensing enzymes in *Mycobacterium tuberculosis*. J Biol Chem 275: 16857-16864, 2000.

Lacave, C., M.-A. Laneelle, and G. Laneelle. 1990. Mycolic acid synthesis by *Mycobacterium aurum* cell-free extracts. Biochim. Biophys. Acta 1042:315–323.

Lowe, P. N., and S. Rhodes. 1988. Purification and characterization of [acylcarrier-protein]acetyltransferase from *Escherichia coli*. Biochem. J. 250:789–796.

Magnuson, K., S. Jackowski, C. O. Rock, and J. E. Cronan, Jr. 1993. Regulation of fatty acid biosynthesis in *Escherichia coli*. Microbiol. Rev. 57:522–542.

McFadden JM, Medghalchi SM, Thupari JN, Pinn ML, Valamudi A, Miller KI, Kuhajda FP, Townsend CA. (2005) Application of a flexible synthesis of (5*R*)-thiolactomycin to develop new inhibitors of type I fatty acid synthase. J Med Chem 48: 946-971.

McNeil, M. R., M. Daffe, and P. J. Brennan. 1991. Location of the mycolyl ester substituents in the cell walls of mycobacteria. J. Biol. Chem. 266:6734–6743.

Minnikin DE, Minnikin SM, Goodfellow M, Stanford JL. (1982) The mycolic acids of mycobacterium chelonei. J Gen Microbiol 128: 817-822.

Minnikin, D. E. 1992. Lipids: complex lipids, their chemistry, biosynthesis and roles, p. 95–184. *In* C. Ratledge and J. Stanford (ed.), The biology of the mycobacteria, vol. 1. Academic Press, London.

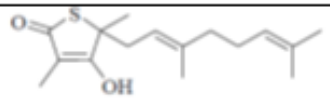
Miyakawa S, Suzuki K, Noto T, Harada Y, Okazaki H. (1982) Thiolactomycin, a new antibiotic. IV. Biological properties and chemotherapeutic activity in mice. J Antibiot (Tokyo) 35: 411–419.

Morita YS, Paul KS, Englund PT. (2000) Specialized fatty acid synthesis in African trypanosomes: myristate for GPI anchors. Science 288: 140-143.

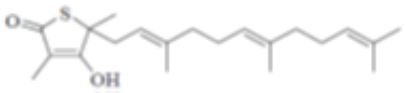
Nie Z, Perretta C, Lu J, Su Y, Margosiak S, Gajiwala KS, Cortez J, Nikulin V, Yager KM, Appelt K, Chu S. (2005) Structure-based design, synthesis, and study of potent inhibitors of β- ketoacyl-acyl carrier

protein synthase III as potential antimicrobial agents. J Med Chem 48: 1596-1609.
Nishida, I., A. Kawaguchi, and M. Yamada. 1986. Effect of thiolactomycin on the individual enzymes of the fatty acid synthase system in *Escherichia coli*. J. Biochem. 99:1447–1454.
Noto T, Miyakawa S, Oishi H, Endo H, Okazaki H. (1982) Thiolactomycin, a new antibiotic. III. In vitro antibacterial activity. J Antibiot (Tokyo) 35: 401–410.
Oishi H, Noto T, Sasaki H, Suzuki K, Hayashi T, Okazaki H, Ando K, Sawada M. (1982) Thiolactomycin, a new antibiotic. I. Taxonomy of the producing organism, fermentation and biological properties. J Antibiot (Tokyo) 35: 391-395.
Pablos-Mendez A, Raviglione MC, Laszlo A, Binkin N, Rieder HL, Bustreo F, Cohn DL, Lambregts-van Weezenbeek CS, Kim SJ, Chaulet P, Nunn P. (1998) Global Surveillance for Antituberculosis-Drug Resistance 1994–1997. N Engl J Med 338: 1641-1649.
Payne DJ, Warren PV, Holmes DJ, Ji Y, Lonsdale JT. (2001) Bacterial fatty-acid biosynthesis: A genomics-driven target for antibacterial drug discovery. Drug Discovery Today 6: 537-544.
Peterson, D. O., and K. Bloch. 1977. *Mycobacterium smegmatis* fatty acid synthetase: long chain transacylase chain length specificity. J. Biol. Chem. 252:5735–5739.
Quemard, A., J. C. Sacchettini, A. Dessen, C. Vilcheze, R. Bittman, W. R. Jacobs, Jr., and J. S. Blanchard. 1995. Enzymatic characterization of the target for isoniazid in *Mycobacterium tuberculosis*. Biochemistry 34:8235–8241.
Quemard, A., S. Mazeres, A. Sut, G. Laneelle, and C. Lacave. 1995. Certain properties of isoniazid inhibition of mycolic acid synthesis in cell-free systems of *M. aurum* and *M. avium*. Biochim. Biophys. Acta 1254:98–104.
Qureshi, N., N. Sathyamoorthy, and K. Takayama. 1984. Biosynthesis of C30 to C56 fatty acids by an extract of *Mycobacterium tuberculosis* H37Ra. J. Bacteriol. 157:46–52.
Sakaya SM, Contreras MS, Dirlam JP, O'Connell TN, Hayashi SF, Santoro SL, Kamicker BJ, George DM, Ziegler CB. (2001) Synthesis and structure–activity relationships of thiotetronic acid analogues of thiolactomycin. Bioorg Med Chem Lett 11: 2751-2754.
Sasaki H, Oishi H, Hayashi T, Noto T, Ando K, Sawada M. (1982) Thiolactomycin, A new antibiotic II. Structure elucidation. J Antibiot (Tokyo) 35: 396-400.
Schaeffer ML, Agnihotri G, Volker C, Kallender H, Brennan PJ, Lonsdale JT. (2001) Purification and biochemical characterization of the mycobacterium tuberculosis β-ketoacyl-acyl carrier protein synthases KasA and KasB. J Biol Chem 276: 47029-47037.
Senior SJ, Illarionov PA, Gurcha SS, Campbell IB, Schaeffer ML, Minnikin DE, Besra GS. (2003) Biphenyl-based analogues of thiolactomycin, active against mycobacterium tuberculosis mtFabH fatty acid condensing enzyme. Bioorg Med Chem Lett, 13 (21): 3685-3688.
Senior SJ, Illarionov, PA, Gurcha SS, Campbell IB, Schaeffer ML, Minnikina DE Besra GS. (2004) Acetylene-based analogues of thiolactomycin, active against mycobacterium tuberculosis mtFabH fatty acid condensing enzyme. Bioorg Med Chem Lett 14 (2): 373-376.
Slayden RA, and Barry CE. (2002) The role of KasA andKasB in the biosynthesis of meromycolic acids and isoniazid resistance in *Mycobacterium tuberculosis*. Tuberculosis 82: 149–160.
Slayden RA, Lee RE, Armour JW, Cooper AM, Orme IM, Brennan PJ, Besra GS. (1996) Antimycobacterial action of thiolactomycin: An inhibitor of fatty acid and mycolic acid synthesis. Antimicrob Agents Chemother 40 (12): 2813-2819.
Tomasz A. (1994) Multiple-antibiotic-resistant pathogenic bacteria. A report on the Rockefeller University Workshop. N Eng J Med 330: 1247- 1251.
Tsay JT, Rock CO, Jackowski S. (1992) Overproduction of beta-ketoacyl-acyl carrier protein synthase I imparts thiolactomycin resistance to Escherichia coli K-12. J Bacteriology 174: 508-513.
Tsukamoto, Y., H. Wong, J. S. Mattick, S. J. Wakil. 1983. The architecture of the animal fatty acid synthetase complex. J. Biol. Chem. 258: 15312–15322.
Turenne CY, Tschetter L, Wolfe J, Kabani A. (2001) Necessity of quality-controlled 16S rRNAgene sequence databases: identifying nontuberculous *Mycobacterium* species. J Clin Microbiol 39: 3637-3648.
Waller RF, Keeling PJ, Donald RG, Striepen B, Handman E, Lang-Unnasch N, Cowman AF, Besra GS, Roos DS, McFadden GI. (1998) Nuclearencoded proteins target to the plastid in Toxoplasma gondii and *Plasmodium falciparum.* Proc Natl Acad Sci USA 95: 12352-12357.
Walters SB, Dubnau E, Kolesnikova I, Laval F, Daffe M, Smith I. (2006) The *Mycobacterium tuberculosis* PhoPR two-component system regulates genes essential for virulence and complex lipid biosynthesis. Molecular Microbiol. 60: 312–330.
Wang, C.-L. J., and J. M. Salvino. 1984. Total synthesis of (6)-thiolactomycin. Tetrahedron Lett. 25:5243–5246.
Wheeler, P. R., G. S. Besra, D. E. Minnikin, and C. Ratledge. 1993. Stimulation of mycolic acid biosynthesis by incorporation of *cis*-tetracos-5-enoic acid in a cell wall preparation from *Mycobacterium smegmatis*. Biochim. Biophys. Acta 1167:182–188.
Winder, F. G. 1982. Mode of action of the antimycobacterial agents and associated aspects of the molecular biology of mycobacteria, p. 354–441. *In* C. Ratledge and J. Stanford (ed.), The biology of the mycobacteria, vol. 1. Academic Press, London.
Yamada, M., M. Kato, I. Nishida, K. Kawano, A. Kawaguchi, and T. Ehara. 1987. Modulation of fatty acid synthesis in plants by thiolactomycin, p. 447–454. *In* P. K. Stumpf, J. B. Mudd, and W. D. Nes (ed.), The metabolism, structure and function of plant lipids. Plenum Press, New York.
Zhang, Y., B. Heym, B. Allen, D. Young, and S. Cole. 1992. The catalaseperoxidase gene and isoniazid

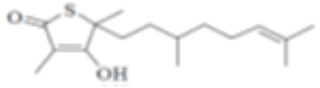
resistance of *Mycobacterium tuberculosis*. Nature (London) 358:591–593.



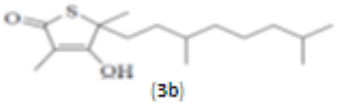
(2)



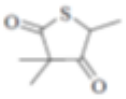
(5)



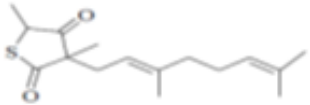
(3a)



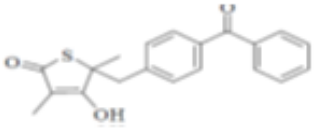
(3b)



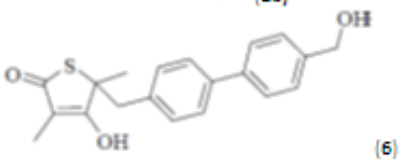
(1b)



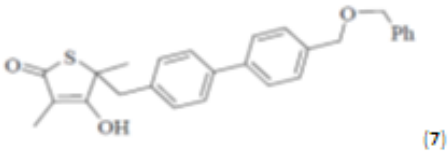
(3c)



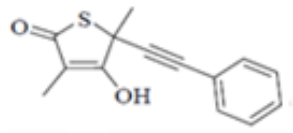
(4)



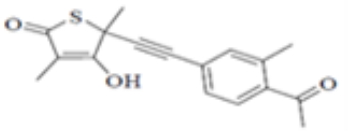
(6)



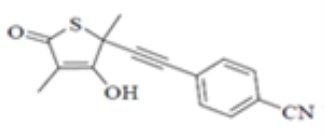
(7)



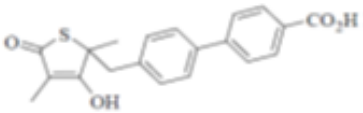
(8)



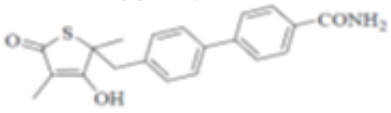
(9) aR=H, bR=CH_3



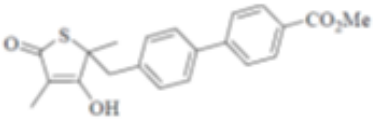
(10)



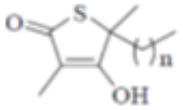
(11)



(12)

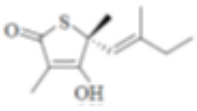


(13)

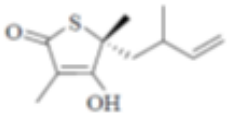


	a	b	c	d	e	f
n =	4	5	7	9	11	12

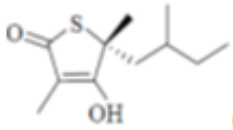
(14A-14F)



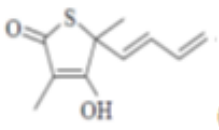
(15)



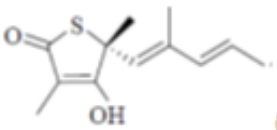
(16)



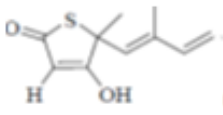
(17)



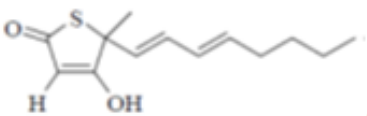
(18)



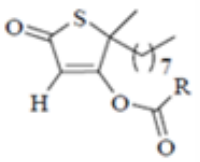
(19)



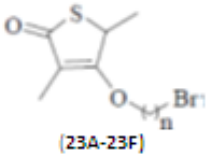
(20)



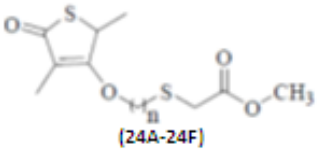
(21)



(22)



(23A-23F)

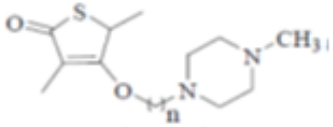


(24A-24F)

	a	b	c	d	e	f
n=	3	4	5	6	8	10

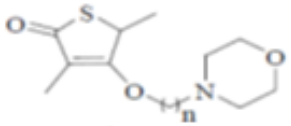
a R = -OCH_3 b R = -OCH_2CH_3

c R = -$OCH_2CH{=}CH_2$



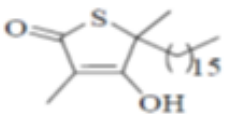
(25A-25F)

	a	b	c	d	e	f
n=	3	4	5	6	8	10

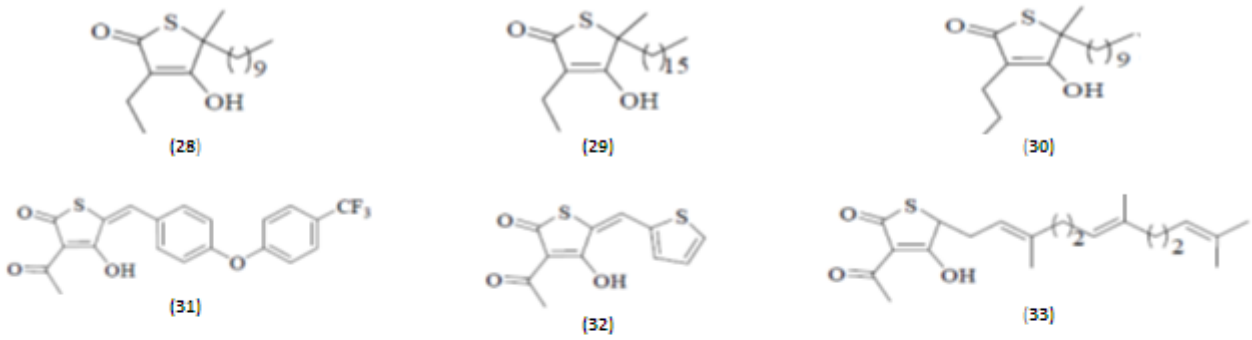


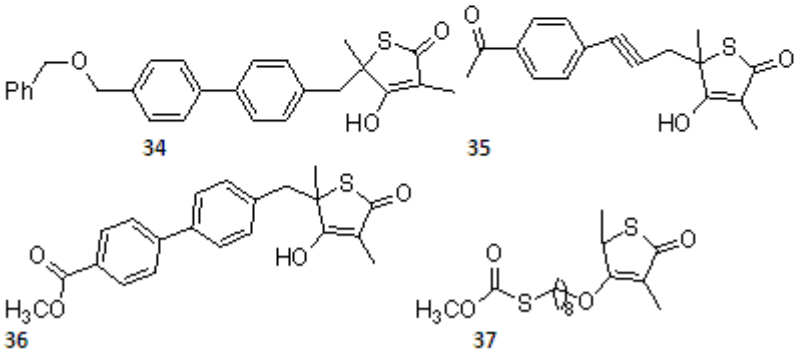
(26A-26F)

	a	b	c	d	e	f
n=	3	4	5	6	8	10



(27)





Structures of some thiolactomycin analogues

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.