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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*

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Abstract

Thiolactomycin inhibits bacterial cell growth through inhibition of the β -ketoacyl-ACP synthase activity of type II fatty acid synthases. The effect of modifications of the 5-position isoprenoid side chain on both IC₅₀ and MIC were determined. Synthesis and screening of a structurally diverse set of 5-position analogues revealed very little tolerance for substitution in purified enzyme assays but a few analogues retained MIC, presumably through another target. Even subtle modifications such as reducing one or both double bonds of the diene were not tolerated. The only permissible structural modifications were removal of the isoprene methyl group or addition of a methyl group to the terminus. Co-crystallization of these two inhibitors with the condensing enzyme from *E. coli* revealed that they retained the TLM binding mode at the active site with reduced affinity. These results suggest a strict requirement for a conjugated, planar side chain inserting within the condensing enzyme active site.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is one of the most deadly infectious diseases, infecting more than 8 million and killing more than 2 million people each year.¹ Multidrug-resistant strains of TB and co-infection with TB and HIV have made treating TB even more difficult. The most widely used TB therapy is a combination of isoniazid, rifampicin, pyrazinamide, and ethambutol.^{2,3} Even such four-drug regimens still require treatment times of at least 6 months, decreasing TB patient compliance. New TB therapies are desperately needed that will shorten the treatment period and combat multidrug-resistant TB.

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Supporting Information Available: Physical and biological data for the complete set of analogues plus elemental analyses for key compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Cell wall biosynthesis is a proven broad-spectrum antibacterial drug target that has been historically very efficacious.⁴ In the treatment of TB in particular two of the four agents that constitute front-line therapy (isoniazid and ethambutol) target different aspects of cell wall biosynthesis. Although recent analysis suggests that interrupting cell wall synthesis may have little potential to significantly shorten the duration of therapy, new agents active against cell wall targets may offer valuable therapeutic options for the management of cases of drug-resistant disease.⁵ Thiolactomycin (TLM, **1**) is a thiotetronic acid-containing natural product that inhibits bacterial and plant type II fatty acid synthases (FASII), which provide essential building blocks for bacterial cell walls.⁶⁻⁸ Although fatty acid synthases are also present in higher eukaryotes, these type I systems are structurally distinct, and the presence of a 3-position methyl group on thiotetronic acids has been shown to confer high selectivity for type II over type I systems.⁶⁻⁹ TLM exerts its effect via inhibition of the β -ketoacyl-ACP synthases (KAS), key condensing enzymes involved in chain elongation in FASII.¹⁰⁻¹² The core thiotetronic acid structure is believed to structurally mimic the transition state adopted by the thiomalonate intermediate in chain elongation. But there are more subtle specific interactions, since even closely related condensing enzymes such as FabH, which initiates chain formation from acetyl-CoA and malonyl-ACP, are only weakly inhibited by TLM and are not physiologically relevant targets for the antibiotic.¹³⁻¹⁵ In Mtb, for example, TLM inhibits KasA and KasB, two KAS enzymes that are components of the specialized FAS II system involved in synthesis of the very long-chain meromycolic acids (*ca.* C₅₀), but is only a weak inhibitor of the initiating condensing enzyme mtFabH.^{11,16} TLM has only modest activity against laboratory strains of Mtb, but it retains this activity against several strains of Mtb that are resistant to other drugs.^{11,17}

TLM has several characteristics that make it an attractive lead molecule for TB.¹⁸ TLM has low toxicity in mice,¹⁹ low molecular weight (MW = 210 g/mol), high water solubility, and drug-appropriate lipophilicity (log P = 3). TLM is well absorbed orally and has shown chemotherapeutic efficacy against *Serratia marcescens* and *Klebsiella pneumoniae* in a mouse infection model.¹⁹ Thus, derivatization of TLM to improve potency for various pathogens has been the focus of many recent investigations. For reasons of synthetic accessibility, the 5-position of the molecule has received the most attention. Many groups have reported a wide variety of substitutions at the 5-position and evaluated whole-cell activity against plants, malaria, trypanosomes, and livestock pathogens, or have reported activity in complex, partially purified fatty acid synthase systems with varying degrees of success.^{10,20,21} 5-position analogues have been reported with improvement in MIC against whole cells of Mtb over TLM but no attempt was made to measure IC₅₀ against the relevant condensing enzymes and to correlate it to MIC.¹¹ Other reports have shown that 5-biphenyl or 5-acetylenic analogues of TLM were able to inhibit the related Mtb FabH, however, no Mtb MIC values were provided, and FabH is not essential in Mtb suggesting these would not be expected to have whole cell potency.^{16,22,23} Because nearly all of these studies fail to report IC₅₀ values against the relevant purified condensing enzymes, evaluation of the resulting SAR has been impossible. Moreover the presence of multiple condensing enzymes, including both type I and type II in addition to condensing enzymes present to produce essential polyketide components of the cell envelope of Mtb has made it especially difficult to interpret the wide variety of structures that have been produced.

The *E. coli* model system has been instrumental in elucidating the mechanism of action of TLM. The FabB condensing enzyme appears to be the relevant physiological target since cells that overexpress FabB, but not FabH, are resistant to TLM.¹³ Likewise cells of Mtb that overexpress KasA acquire TLM resistance.²⁴ In addition, selection of drug-resistant *E. coli* has shown that the F390V mutation in FabB confers TLM resistance.¹⁵ The co-crystal structure of TLM bound to *E. coli* FabB provides a detailed view of how this molecule interacts with the active site.¹² TLM binds to the malonyl-ACP side of the active site with the 2-position

carbonyl oxygen of TLM forming tight hydrogen bonds with His-298 and His-333. The 4-position hydroxyl group forms hydrogen bonds with a series of water molecules which in turn interact with the backbone of the polypeptide chain. These water molecules lie within the portion of FabB that forms the binding site for acyl carrier protein-bound phosphopantetheine. The sulfur atom of the thiolactone does not make any specific contacts but lies adjacent to the active site cysteine. The isoprene side chain of TLM slips into a narrow lipophilic pocket between two peptide bonds and this conjugated aliphatic chain interacts with the peptide via tight van der Waals interactions. A hydrophobic pocket opens up beyond the isoprene binding slot, an observation that led to an early conclusion that this binding site is not “optimally” filled by TLM and that hydrophobic 5-position analogues may improve the binding affinity. To attempt to improve the binding of the TLM isoprene side chain, we began to explore the SAR of the 5-position of TLM for the condensing enzymes of the type II FAS system.

Chemistry

Synthetic routes to both racemic and chiral TLM have been reported.²⁵⁻²⁷ 3,5-Dimethylthiotetronic acid **2** was prepared using the method of Wang and Salvino.²⁷ Using a method similar to that previously reported,²⁸ the dianion of **2** was generated using LiHMDS at 0 °C (Scheme 1). Subsequent addition of alkyl or aryl halides gave racemic 5-substituted TLM derivatives (**3-31**) in moderate to good yields (30-90%). In cases where the isolated purity was below 80% (by LC/MS), the compound was purified by flash chromatography. In general, bulky halides or alkyl and aryl chlorides afforded lower yields. The compounds were made in parallel using the Radley's carousel reaction system. In all, 77 compounds were prepared by this method and were examined for biological activity. Compounds **3-31** are a representative set. (For a complete list, see Supplementary Information.)

To investigate the relative importance of each double bond in the isoprenoid side chain, compounds **32** and **33**, where each double bond was reduced individually, as well as compound **34**, the fully saturated analogue, were prepared. Chiral compounds **32-34** were prepared from fermentation-derived TLM, preserving the stereochemistry of the 5-position.

While catalytic hydrogenation attempts using either 5% Pd/C or 5% Rh/Al₂O₃ gave only trace amounts of **32** or **34**, an excess of diimide²⁹ from NH₂NH₂·H₂O and 30% H₂O₂ (aq) generated these two compounds readily from TLM (Scheme 2). To obtain compound **33**, the terminal double bond of the TLM isoprene side chain was first masked. Hydroboration of TLM with 9-BBN and subsequent oxidation were used to produce terminal alcohol **35** in 88% yield.³⁰ The internal double bond of **35** was then reduced with an excess of diimide, resulting in compound **36**. Mesylation using MsCl and Et₃N then furnished dimesylate **37** in modest yield.³¹ Unfortunately, attempted elimination of mesylate **37** with DBU in toluene under reflux did not afford the desired terminal double bond of **33** but instead generated two compounds having the same molecular weight as **33** but that were far less polar than the desired target molecule.³² Functional group transformation from mesylate **37** to iodide was successful using NaI in acetone to afford compound **38** in 66% yield.³³ Dehydroiodination and C4-mesylate deprotection of **38** were accomplished in one pot using an excess of *t*-BuOK (20 equiv.) in CH₂Cl₂ to give compound **33** in 50% yield.³⁴ Use of fewer equivalents or slow addition of *t*-BuOK led to the same nonpolar compounds as above, most likely by intramolecular nucleophilic attack of the demesylated C4-oxygen onto the 5-position side chain. Several other elimination attempts such as DBU, TBAF, and *t*-BuOK in THF gave similar results. The diastereomers of compounds **33** and **36-38** were not separated.

To investigate the importance of the methyl branch on the isoprene side chain, the simple butadiene analogue of TLM (*i.e.*, desmethyl-TLM, **47**) was prepared (Scheme 3). The racemic 5-position aldehyde was selected as the initial step from 3,5-dimethylthiotetronic acid **2**,

anticipating that simple olefination would afford the desired butadiene. However, direct formylation of **2** was unsuccessful, therefore, the dianion of **2** was treated with paraformaldehyde to afford the 5-hydroxymethyl compound **39**.³⁵ Unfortunately, several oxidation conditions (PCC, PDC, TPAP/NMO, Dess-Martin periodinane, and Swern oxidation) were unsuccessful at converting **39** to aldehyde **40**. Unsuccessful oxidation attempts of a primary alcohol at the 5-position of either tetronic or thiotetronic acid without first protecting the 4-hydroxyl group have been previously reported.^{26,36} Protection of the 4-hydroxyl group of **2** by treatment with MOMCl and DIPEA provided compound **41**.³⁷ 5-Hydroxymethylation of **41** then furnished **42** in 78% yield.³⁵ Oxidation of **42** using Dess-Martin periodinane gave the desired aldehyde **43** (43% from **2**).³⁸ When **43** was subjected to Horner olefination conditions using diethyl allylphosphonate,³⁹ the reaction generated **41** as the major product via deformylation, in addition to trace amounts of the 5-diene analogue **46**.

From aldehyde **43**, a Horner-Wadsworth-Emmons reaction with a stabilized ylide diethyl 2-oxopropylphosphonate, gave *trans*-enone **44** in 48% yield in addition to the deformylation product **41** (18%).^{39,40} The ketone of **44** was then successfully reduced with NaBH₄ and CeCl₃·7H₂O to afford allyl alcohol **45** in 64% yield.⁴¹ Subsequent dehydration of **45** in the presence of the Burgess reagent⁴² furnished butadiene **46**. A variety of conditions were employed to deprotect compound **46**, most yielding only nonpolar products.³² The most successful condition was the very mild NaHSO₄·SiO₂⁴³ at room temperature. These conditions furnished the desired deprotected product **47** in 75% yield from compound **46**.

Finally, we prepared analogue **50**, containing an additional methyl group at the terminus of the isoprenoid side chain of TLM (Scheme 4). The 4-position hydroxyl group of TLM was first protected using MOMCl to give compound **48** in nearly quantitative yield. Olefin cross-metathesis,⁴⁴ using Grubbs' second generation catalyst in the presence of *trans*-2-butene, furnished (1*E*,3*E*)-diene **49** in good yield. The optimal deprotection condition for compound **49** was found to be polymer-bound TsOH stirred with silica gel at room temperature.⁴⁵ This condition yielded desired compound **50** in 75% yield.

Results and Discussion

Effect of 5-position stereochemistry on biological activity of TLM

Because many of the 5-substituted TLM analogues to be evaluated were racemic, it was important to establish whether the presence of the opposite enantiomer of this series of molecules would antagonize activity of chiral TLM, isolated from fermentation of *Nocardia* *sp.* 2-200. Therefore, we evaluated the activity of racemic TLM (Sigma-Aldrich) in our biological assays. The IC₅₀ of racemic TLM against *E. coli* FabB was 20 μM, exactly 2-fold higher than that of TLM (Figure 1A). This result indicates that the enantiomer of TLM is inactive and non-antagonistic, consistent with the crystal structure of the FabB-TLM complex in which the shape and hydrogen bonding network of the TLM binding pocket is only able to accommodate the natural stereoisomer. Similarly, the MIC of racemic TLM against *E. coli* strain ANS1 was 12 μM, 2-fold higher than that of TLM (Figure 1B). These results suggested that racemic TLM analogues would likely result in only an apparent 2-fold decrease in potency compared with TLM itself. Since our goal was to obtain enhanced inhibition of Mtb KasA and KasB, the activity of TLM was also measured in a KasA (or KasB) extension assay using palmitoyl-ACP (from *E. coli*) with [¹⁴C]-malonyl-CoA in the presence of FabD and reduced ACP. Reaction products were reduced with NaBH₄ and organic soluble radioactivity then provided a measure of enzyme activity. Using this assay, the IC₅₀ of TLM for Mtb KasA and KasB was found to be 5 μM (Figure 1C). TLM showed little selectivity between the enzymes.

Biological activity of structurally diverse 5-position analogues of TLM

The biological assay results for compounds **3-31** are shown in Table 1. Enzyme inhibition assays were performed at 100 μM against *E. coli* FabB/H and Mtb KasA/B and FabH. When no inhibition was seen at 100 μM , the compound was not tested further for enzyme inhibitory activity. When the compound showed greater than 30% inhibition of the enzyme, a discrete IC_{50} was measured as shown in Figure 1. Inhibition between 0 and 30% was recorded as an IC_{50} value of $>100 \mu\text{M}$. The compounds were also evaluated in MIC assays against *E. coli* strain ANS1 and Mtb strain H37Rv.

As a set, the simple 5-alkyl, alkenyl and aryl substituted thiotetronic acid analogues lacked significant activity in the condensing enzyme assays. Straight-chain alkyl groups showed very modest activity against *E. coli* and Mtb FabH enzymes with longer aliphatic chains correlated with improved activity. A relationship between chain length and FabH activity was shown in compounds **4-9**. Increasing chain length resulted in improved activity against Mtb FabH, with the maximal inhibition by compound **7** (C_{12}). Longer chain lengths led to less activity against FabB. These compounds, however, were inactive against FabB, KasA, and KasB and did not have enhanced MIC values against Mtb. Likewise, they showed only modest MICs against *E. coli*. These data are consistent with the report that in Mtb FabH is not essential.²³ In some cases, growth inhibition towards an organism (**7**, *E. coli*) did not necessarily translate into inhibition of a known condensing enzyme.

One striking observation was that compound **14**, corresponding to a simple saturated version of the racemic form of TLM completely lacked activity. We were surprised to note that this comparatively simple modification abolished activity against the condensing enzymes and failed to show any observable MIC against the intact organisms relative to that of TLM given the apparent permissive nature of tolerated substitutions at this position reported by other authors. Similar results were found with racemic compound **23**, where the proximal double bond was reduced. The geranyl-substituted analogue **25** had low activity in all assays employed, which was also contrary to prior reports.¹¹

Because of the lack of activity displayed by compounds **3-31**, it became clear that the intact isoprene side chain of TLM was absolutely critical to its activity. Our focus then shifted to determining the precise structural features within the isoprene unit that were responsible for the binding of TLM.

The relative importance of each olefin as a binding determinant of the isoprene side chain

Using chiral, fermentation-derived TLM allowed us to systematically explore the contributions of each double bond in the TLM side chain independently using compounds **32-34**. Racemic **47** allowed us to explore the contribution of the methyl branch in the isoprenoid side chain. The results of *E. coli* and *M. tuberculosis* β -ketoacyl-ACP synthase activity assays and MIC determinations are shown in Table 2. Surprisingly, reduction of either double bond, independently or both together, completely ablated both enzyme inhibition and whole-cell growth-inhibitory activity. Therefore, the presence of two conjugated double bonds adjacent to the thiolactone ring appears to be a formal requirement of this mode of binding to the condensing enzyme active site. Compound **47**, corresponding to the racemic desmethyl analogue of TLM, maintained full activity towards KasA (the measured IC_{50} is for the racemate so presumably the correct enantiomer has an IC_{50} of half this concentration). Despite this potent activity against KasA, this molecule was totally inactive against FabB and KasB and lacked whole cell MIC activity against both organisms as well.

To extend our understanding of the structural requirements for binding beyond the essentiality of the conjugated diene, we also examined the effect of elongation of the side chain by addition

of an extra methyl group to the terminus of the isoprenoid unit. The FabB-TLM co-crystal structure suggested that this should be tolerated because the binding pocket extends beyond the TLM side chain toward the interior of the protein.¹² Compound **50**, methyl-TLM, retains activity as an inhibitor of only KasA (Table 2). Compound **50** showed no activity against FabB or KasB, and, similar to desmethyl-TLM, did not show inhibition of growth of either organism.

The compounds in Table 2 were also examined for their inhibitory effects against human FASI isolated from breast cancer cell line ZR-75-1 and found to be inactive. These results correlate well with the recent report that the presence of a 3-position methyl group is a key structural determinant of the observed specificity for FASII compared with FASI.⁹

Molecular modeling of TLM and its analogues

We employed a virtual docking strategy using the FlexX module within the Tripos drug discovery software suite to gain insights into how the shape and double-bond arrangement of the isoprenoid side chain might affect TLM binding affinity. To verify the validity of the approach, FlexX was first used to dock TLM into the FabB active site. FlexX was not able to dock TLM into the native FabB structure, but it did succeed in docking TLM into the FabB-TLM complex structure from which TLM had been removed. This implies that a small but significant conformational change occurs within the FabB active site upon TLM binding¹² that is absolutely required for FlexX to reproduce the observed binding. Analogues lacking one or both double bonds (**32**, **33**, **34**) and desmethyl TLM (**47**) were docked to the FabB structure from which TLM had been extracted. ChemScore was used⁴⁶ to rank the resulting solutions because it placed TLM at the top of the rankings when used as an internal control. In general, none of the analogues docked with scores comparable to that of native TLM. To achieve a realistic docking result, it was necessary to lock the thiolactone ring within the active site according to the crystal structure. Generally, variants that contained the branch methyl group in the isoprene side chain were ranked higher than those lacking it, and the presence or absence of the isoprene double bonds were of secondary importance. Since this result is contrary to the observed biochemical outcome, it is apparent that FlexX significantly underestimates the importance of the van der Waals interactions of the conjugated diene system.

Examination of the binding mode of desmethyl- and methyl-TLM with *E. coli* FabB

From the IC₅₀ values of the compounds shown in Table 2, compounds **47** and **50** displayed a clear selectivity for KasA over KasB and FabB. In order to attempt to understand these differences and to verify that **47** and **50** bind to the active site in a similar mode as TLM, ligand binding was directly visualized using X-ray crystallography. Since KasA crystals remain elusive despite considerable effort, *E. coli* FabB was used for these studies even though the IC₅₀ values suggested that this might be problematic. Co-crystallization was performed using a 3-fold molar excess of ligand, and data were collected for **47** and **50** at 2.2 Å and 2.3 Å, respectively. The structures were solved by molecular replacement and refined, and pertinent statistics for both FabB complexes are shown in Table 3. Electron density for both compounds was present in the co-crystal structures (Figure 3) but was significantly lower than the surrounding protein, indicating partial occupancy, as was expected from the IC₅₀ values. To avoid model bias, the electron densities shown in Figure 3 correspond to simulated annealing omit maps in which the compounds were not included in the refinement. Despite the poor ligand electron densities, the placement of **47** and **50** in each active site was straightforward and unambiguous. By maintaining a constant ligand temperature factor during refinement corresponding to the average value of the active site residues, it was possible to estimate the ligand occupancies as 60% for **47** and 75% for **50**. These two values are consistent with the relative quality of the ligand densities where that of **50** is clearly superior. The concentration

of each compound which allowed for co-crystallization was only 2-fold above the IC₅₀ value, but attempts to use higher concentrations to increase occupancy compromised crystallization.

Compounds **47** and **50** dock into the active site in the same orientation as TLM. For comparison, the **47** and **50** active-site complexes are shown in Figure 3 (left and right panels, respectively) in the same orientation as the TLM complex shown in Figure 2. Although FabB does not undergo any large conformational changes upon binding TLM, His298 and Phe392 have altered positions to accommodate the inhibitor,¹² and this is also the case with compounds **47** and **50**. The 50-fold loss in activity towards FabB of **47** and **50** relative to TLM is not easily understood from structural comparisons alone. However, two points are worth noting. Compound **47** does not contain the branch methyl group of TLM, and it may bind more weakly than TLM due to the loss of van der Waals interactions with residues Phe390 and Pro272. In the case of compound **50**, the problem may be steric in nature. The extra methyl group extending distally from the isoprene group sits in a small cavity bounded by the backbone carbonyl oxygens of Asp268 and Ala271, and the amide nitrogen of Gly394. Using the SegMod algorithm,⁴⁷ we have constructed homology models of KasA and KasB based on the FabB structure to identify any obvious differences in the active sites of the Mtb enzymes. The active sites are extremely similar, and the molecular basis for the selectivity of **47** and **50** for KasA is not immediately apparent.

Conclusion

This work suggests a conceptual shift is necessary for evaluating 5-position substitutions in the development of more effective TLM analogues for bacterial condensing enzymes. Far from being permissive to replacement and substitution, the isoprenoid side chain appears to be absolutely required to maintain activity against KasA, KasB and FabB. Unfortunately, this side chain also provides one of the most serious obstacles to the exploration of analogues of this structural class because of the difficult synthetic accessibility of this moiety. Despite the appearance of several reports in the literature suggesting that significant substitutions in this position would be tolerated, we found that selective removal of either double bond of TLM resulted in a dramatic drop in the ability of this molecule to inhibit the elongation catalyzed by the condensing enzyme reaction and the resulting growth-inhibitory activity of the analogue.

Derivatives that had improved activity against whole cells were obtained but the effect on whole cells did not correlate with inhibition of enzyme activity, suggesting that the mode of action of these molecules does not involve these specific condensing enzymes. Compound **7**, for example, had an MIC against *E. coli* of 25 μ M, but has no activity against FabB, the known TLM target. We observe weak activity of compound **7** against FabH, but the IC₅₀ of 100 μ M is higher than the MIC, ruling out FabH as the likely cellular target unless significant accumulation occurs *in vivo*. Furthermore, compound **7** has a lower IC₅₀ against Mtb FabH compared with TLM, however, **7** does not inhibit Mtb *in vivo*, a result congruent with the non-essentiality of this gene.²³ These data demonstrate that the activity of such TLM analogues against Mtb cannot be casually ascribed to inhibition of the mycolic acid biosynthetic pathway without solid biochemical corroboration. This result is perhaps not too surprising since TLM is thought to act as a transition-state mimic for the β -ketoacyl-ACP synthase elongation enzymes and there are no fewer than six essential enzymes that catalyze this reaction in Mtb.²³ Analogues of TLM with appreciable Mtb MICs and without KasA or KasB activity may well inhibit another condensation enzyme, or they may interact with a different target altogether. This complexity dramatically reduces the utility of developing an SAR based upon simple whole-cell activity for any analogues of this class of molecules.

Although space-filling models of the active site suggested that larger hydrophobic molecules may fit in the cavity beyond the slot occupied by the isoprene unit, our data show that the

presence of the planar, conjugated diene structure of the side chain within that slot is critical for high affinity binding. The narrow opening occupied by this side chain in the active site favors a planar geometry that predetermines the position of the entire side chain relative to the thiolactone ring. The co-crystal structure clearly reveals the rigidity of the pocket and the extremely tight fit of the isoprenoid unit (Figure 2). It is clear that the presence of a conjugated diene at the 5-position of TLM, structurally and stereochemically, cannot be altered while retaining binding mode and activity against the elongation condensing enzymes.

One intriguing finding from this study is the differential activity of compounds **47** and **50** against various condensing enzymes. From these two examples, it would appear that inhibition of mtKasA alone is not sufficient for killing the whole organism, despite strong genetic evidence that this enzyme is essential for viability. The most likely possibility is that these two compounds are effluxed or metabolized more efficiently. The disparity between the IC₅₀ of TLM (14 μM) and the MIC (63 μM) suggests that penetration, metabolism, or efflux play a role in determining the susceptibility of Mtb to this agent. The other possibility is that KasB compensates for the loss of elongating activity of KasA. This possibility seems less likely since the biological substrates for these two enzymes are very different, KasA operating to extend palmitoyl-CoA to products that are approximately C₄₀ while KasB extends the products of KasA to products of an average size of C₅₄.⁴⁸ Both enzymes are capable, however, of extension of shorter acyl-CoAs so this possibility cannot be excluded.

These results support the notion that TLM analogues must be evaluated with respect to both their activity in terms of IC₅₀ against a specific condensing enzyme as well as in terms of growth-inhibitory potential in order to avoid confusing SAR resulting from effects on multiple enzyme targets. The tight fit of the isoprenoid side chain to a narrow groove adjacent to the enzyme active site of KasA, KasB and FabB drastically limits the ability to simplify TLM to a simple thiotetronic acid skeleton upon which variation may be induced. Because of the synthetic complexity introduced by the chiral conjugated diene at the 5-position, future efforts at semisynthetic analogues at the 4-position enol, which extends in the direction of the binding tunnel for the phosphopantetheine prosthetic group, may be a profitable area for exploration.

Experimental Section

Anhydrous solvents and reagents were purchased from Sigma-Aldrich and used as received unless otherwise stated. Melting points were obtained on an Electrothermal 9100 apparatus and are uncorrected. LC/MS analysis was conducted on an Agilent 1100 series HPLC with attached Agilent quadrupole mass analyzer model G1946 D SL with electrospray ionization in positive ion mode. LC chromatography used a Phenomenex Luna C₁₈(2) column (2 × 50 mm, 3 μm) with a water/acetonitrile (each with 0.1% (v/v) formic acid) gradient using a flow rate of 0.3 mL/min. UV detection was with an Agilent Diode Array Detector model G1315A spectrometer at 270 and 310 nm. Proton (¹H) and carbon (¹³C) NMR spectra were recorded at 300 and 75.5 MHz, respectively, on a Varian Gemini spectrometer, using TMS or the solvent peak as an internal standard. Column chromatography was conducted using either silica gel (Geduran, 60, mesh 40-63 μm) or prepacked RediSep columns (Teledyne Isco, Inc., Lincoln, NE USA) on an Isco CombiFlash Optix10 instrument. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA USA). HRMS analyses were performed at the W.M. Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, CT USA).

General procedure for compounds 3-31

At 0 °C and under an argon atmosphere, LiHMDS (1 M in THF, 4 mmol) was added to a stirring solution of 3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**2**) (0.25 g, 1.73 mmol) in anhydrous THF (6 mL). The reaction mixture was allowed to stir at 0 °C for 30 min during which time

the mixture turned from clear to opaque yellow. The alkyl halide (1.73 mmol) in anhydrous THF (10 mL) was added and the reaction mixture was allowed to come to rt overnight. Saturated NH_4Cl (aq) solution was added to quench the reaction and the mixture was extracted between H_2O and EtOAc (3×25 mL). The combined EtOAc layers were dried with anhydrous MgSO_4 , filtered, and evaporated *in vacuo* to yield the crude product. Reaction products less than 80% pure (by LC/MS) were purified by column chromatography. All compounds with purity above 80% were characterized by mass spectrometry and ^1H NMR, and assayed for biological activity. Yields and spectral data are given for selected compounds. Physical and biological data for the entire compound set can be found in Supplementary Information.

3,5-Dimethyl-4-hydroxy-5-pentyl-5*H*-thiophen-2-one (**3**). Yield 61%. ^1H NMR (acetone- d_6) δ 0.87 (t, $J = 6.9$ Hz, 3H), 1.26-1.31 (m, 6H), 1.62 (s, 3H), 1.65 (s, 3H), 1.84-1.88 (m, 2H), 2.92 (bs, 1H). LC/MS: 215.0 (M+1), 237.1 (M+Na $^+$).

3,5-Dimethyl-5-hexyl-4-hydroxy-5*H*-thiophen-2-one (**4**). Yield 42%. ^1H NMR (CDCl_3) δ 0.85-0.90 (m, 3H), 1.26-1.34 (m, 8H), 1.66 (s, 3H), 1.73 (s, 3H), 1.81-1.87 (m, 2H), 5.68 (bs, 1H). LC/MS: 229.1 (M+1), 251.0 (M+Na $^+$).

3,5-Dimethyl-4-hydroxy-5-octyl-5*H*-thiophen-2-one (**5**). Yield 79%. ^1H NMR ($\text{DMSO}-d_6$) δ 0.85 (t, $J = 7.2$ Hz, 3H), 1.02-1.38 (m, 12H), 1.48 (s, 3H), 1.51 (s, 3H), 1.66-1.74 (m, 2H). LC/MS: 257.1 (M+1), 279.0 (M+Na $^+$).

5-Decyl-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**6**). Yield 99%. Mp 70-72 $^\circ\text{C}$. ^1H NMR (CDCl_3) δ 0.88 (t, $J = 6.3$ Hz, 3H), 1.25 (bs, 16H), 1.66 (s, 3H), 1.73 (s, 3H), 1.81-1.87 (m, 2H). LC/MS: 285.1 (M+1), 307.1 (M+Na $^+$).

3,5-Dimethyl-5-dodecyl-4-hydroxy-5*H*-thiophen-2-one (**7**). Yield 72%. ^1H NMR (acetone- d_6) δ 0.88 (t, $J = 6.3$ Hz, 3H), 1.28 (bs, 20H), 1.63 (s, 3H), 1.66 (s, 3H), 1.84-1.91 (m, 2H). GC/MS: 312 (M $^+$).

3,5-Dimethyl-4-hydroxy-5-tridecyl-5*H*-thiophen-2-one (**8**). Yield 69%. ^1H NMR (CDCl_3) δ 0.88 (t, $J = 6.3$ Hz, 3H), 1.25-1.33 (m, 22H), 1.66 (s, 3H), 1.73 (s, 3H), 1.81-1.86 (m, 2H). LC/MS: 327.2 (M+1).

3,5-Dimethyl-4-hydroxy-5-tetradecyl-5*H*-thiophen-2-one (**9**). Yield 96 %. ^1H NMR (CDCl_3) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.25-1.33 (m, 24H), 1.66 (s, 3H), 1.73 (s, 3H), 1.81-1.86 (m, 2H). LC/MS: 341.2 (M+1).

3,5-Dimethyl-4-hydroxy-5-isobutyl-5*H*-thiophen-2-one (**10**). Yield 38%. ^1H NMR (acetone- d_6) δ 0.86 (d, $J = 6.3$ Hz, 3H), 0.92 (d, $J = 6.6$ Hz, 3H), 1.54 (s, 3H), 1.59 (s, 3H), 1.76-1.80 (m, 2H), 2.07-2.09 (m, 1H). LC/MS: 201.1 (M+1), 223.1 (M+Na $^+$).

3,5-Dimethyl-5-(2-ethylbutyl)-4-hydroxy-5*H*-thiophen-2-one (**11**). Yield 45%. ^1H NMR (CDCl_3) δ 0.77-0.90 (m, 6H), 1.20-1.44 (m, 5H), 1.65 (s, 3H), 1.73 (s, 3H), 1.78-1.82 (m, 2H). LC/MS: 229.1 (M+1), 251.1 (M+Na $^+$).

3,5-Dimethyl-4-hydroxy-5-isopropyl-5*H*-thiophen-2-one (**12**). Yield 61%. ^1H NMR (acetone- d_6) δ 0.81 (d, $J = 6.6$ Hz, 6H), 1.61 (m, 6H), 2.07-2.10 (m, 1H). LC/MS: 187.0 (M+1), 209.1 (M+Na $^+$).

3,5-Dimethyl-4-hydroxy-5-(1-methylpropyl)-5*H*-thiophen-2-one (**13**). Yield 36%. ^1H NMR (acetone- d_6) δ 0.82-0.97 (m, 5H), 1.03 (d, $J = 6.6$ Hz, 3H), 1.67 (s, 3H), 1.69 (d, $J = 3.9$ Hz, 3H), 1.93-2.01 (m, 1H). LC/MS: 200.9 (M+1), 223.0 (M+Na $^+$), 423.1 (2M+Na $^+$).

3,5-Dimethyl-4-hydroxy-5-((*S*)-2-methylbutyl)-5*H*-thiophen-2-one (**14**). Yield 29%. ¹H NMR (CDCl₃) δ 0.81-0.88 (m, 4.5H), 0.96 (d, *J* = 6.9 Hz, 1.5H), 1.08-1.50 (m, 3H), 1.60 (s, 3H), 1.68-1.80 (m, 1H), 1.74 (s, 3H), 1.86-1.97 (m, 1H). LC/MS: 215.2 (M+1), 237.1 (M+Na⁺).

5-Cyclopropylmethyl-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**15**). Yield 71%. ¹H NMR (CDCl₃) δ 0.11-0.20 (m, 2H), 0.39-0.54 (m, 2H), 0.75-0.85 (m, 1H), 1.70 (s, 3H), 1.75 (s, 3H), 1.96 (d, *J* = 6.0 Hz, 1H), 2.01 (d, *J* = 6.3 Hz, 1H). LC/MS: 199.0 (M+1), 221.1 (M+Na⁺).

5-Cyclopentyl-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**16**). Yield 57% ¹H NMR (acetone-*d*₆) δ 1.49-1.71 (m, 8H), 1.65 (s, 3H), 1.67 (s, 3H), 1.92-1.97 (m, 1H). LC/MS: 213.1 (M+1), 235.0 (M+Na⁺).

5-Cyclohexyl-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**17**). Yield 9%. ¹H NMR (CDCl₃) δ 1.02-1.36 (m, 4H), 1.43-1.86 (m, 6H), 1.68 (s, 3H), 1.71 (s, 3H), 1.92-2.00 (m, 1H). LC/MS: 227.1 (M+1), 248.9 (M+Na⁺).

5-Allyl-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**18**). Yield 95%. ¹H NMR (CDCl₃) δ 1.66 (s, 3H), 1.73 (s, 3H), 2.63 (m, 2H), 5.11-5.20 (m, 2H), 5.75 (ddt, *J* = 17.1, 10.2, 7.2 Hz, 1H). LC/MS: 185.1 (M+1), 206.9 (M+Na⁺).

5-((*E*)-But-2-enyl)-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**19**). Yield 96%. ¹H NMR (acetone-*d*₆) δ 1.58 (s, 3H), 1.61-1.64 (m, 6H), 2.46-2.62 (m, 2H), 5.35-5.45 (m, 1H), 5.50-5.59 (m, 1H). LC/MS: 199.0 (M+1), 221.1 (M+Na⁺).

3,5-Dimethyl-4-hydroxy-5-(3-methylbut-2-enyl)-5*H*-thiophen-2-one (**20**). Yield 77%. ¹H NMR (acetone-*d*₆) δ 1.61-1.68 (m, 12H), 2.43-2.53 (m, 1H), 2.64-2.71 (m, 1H), 5.11-5.15 (m, 1H). LC/MS: 213.1 (M+1), 235.0 (M+Na⁺).

3,5-Dimethyl-4-hydroxy-5-(2-methylallyl)-5*H*-thiophen-2-one (**21**). Yield 48%. ¹H NMR (acetone-*d*₆) δ 1.63 (s, 3H), 1.66 (s, 3H), 1.78 (s, 2H), 2.60-2.72 (m, 3H), 4.80 (bs, 1H), 4.88 (bs, 1H). LC/MS: 199.1 (M+1), 221.0 (M+Na⁺).

3,5-Dimethyl-4-hydroxy-5-(pent-4-enyl)-5*H*-thiophen-2-one (**22**). Yield 77%. ¹H NMR (acetone-*d*₆) δ 1.24-1.34 (m, 2H), 1.64 (s, 3H), 1.67 (s, 3H), 1.85-1.92 (m, 4H), 4.90-4.95 (m, 1H), 4.96-5.03 (m, 1H), 5.79 (ddt, *J* = 17.1, 10.5, 6.6 Hz, 1H). LC/MS: 213.1 (M+1).

3,5-Dimethyl-4-hydroxy-5-(2-methylbut-3-enyl)-5*H*-thiophen-2-one (**23**): To a solution of **3** (500 mg, 3.47 mmol) in THF (25 mL) was added dropwise 1.0 M LiHMDS (7.3 mL, 7.3 mmol) in THF at 0 °C under an argon atmosphere. The reaction mixture was stirred at 0 °C for 45 min. A solution of 2-methyl-3-butenyl methanesulfonate^{49,50} (854 mg, 5.20 mmol) and NaI (779 mg, 5.20 mmol) in THF (5 mL) was prepared separately. To this solution, the above prepared dianion solution of **2** was added by canula at 0 °C. The reaction mixture was stirred at rt for 1 h and then heated at 40-50 °C overnight. The reaction mixture was quenched with H₂O, acidified with 1 N HCl (aq), extracted with EtOAc (1x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1:1) to give the title compound as a colorless oil (60 mg, 0.28 mmol, 8%) in addition to recovered **3** (42 mg, 0.29 mmol). ¹H NMR (CDCl₃) δ 1.00 (d, *J* = 6.6 Hz, 1.5H), 1.01 (d, *J* = 6.6 Hz, 1.5H), 1.66 (s, 1.5H), 1.68 (s, 1.5H), 1.69 (s, 1.5H), 1.76 (s, 1.5H), 1.85-2.09 (m, 2H), 2.25-2.43 (m, 1H), 4.83-4.98 (m, 2H), 5.58-5.82 (m, 1H). LC/MS: 213.1 (M+1), 235.0 (M+Na⁺).

3,5-Dimethyl-5-(hex-5-enyl)-4-hydroxy-5*H*-thiophen-2-one (**24**). Yield 84%. ¹H NMR (acetone-*d*₆) δ 1.19-1.57 (m, 6H), 1.63 (s, 3H), 1.65 (s, 3H), 1.83-1.95 (m, 2H), 4.90 (m, 1H), 4.98 (m, 1H), 5.79 (ddt, *J* = 17.4, 10.5, 6.6 Hz, 1H). LC/MS: 227.1 (M+1), 249.1 (M+Na⁺).

3,5-Dimethyl-5-((2*E*,6*E*)-3,7-dimethylocta-2,6-dienyl)-4-hydroxy-5*H*-thiophen-2-one (**25**). Yield 89%. ¹H NMR (CDCl₃) δ 1.66 (m, 15H), 1.98-2.12 (m, 4H), 2.51 (dd, *J* = 13.2, 6.6 Hz, 1H), 2.67 (dd, *J* = 15.9, 8.1 Hz, 1H), 5.00-5.13 (m, 1H), 5.14-5.20 (m, 1H). LC/MS: 281.1 (M+1), 303.0 (M+Na⁺).

3,5-Dimethyl-4-hydroxy-5-((2*E*,6*E*,10*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)-5*H*-thiophen-2-one (**26**). Yield 97%. ¹H NMR (acetone-*d*₆) δ 1.56-1.68 (m, 18H), 1.97-2.09 (m, 10H), 5.08-5.19 (m, 3H). LC/MS: 349.1 (M+1), 371.2 (M+Na⁺).

5-Benzyl-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**27**). Yield 64%. ¹H NMR (acetone-*d*₆) δ 1.60 (s, 3H), 1.67 (s, 3H), 3.20 (s, 2H), 7.22-7.27 (m, 5H). LC/MS: 235.1 (M+1), 257.0 (M+Na⁺).

3,5-Dimethyl-4-hydroxy-5-((*E*)-3-phenylallyl)-5*H*-thiophen-2-one (**28**). Yield 60%. ¹H NMR (CDCl₃) δ 1.71 (s, 3H), 1.72 (s, 3H), 2.70-2.85 (m, 2H), 6.15 (dt, *J* = 15.6, 7.5 Hz, 1H), 6.51 (d, *J* = 15.6 Hz, 1H), 7.22-7.34 (m, 5H). LC/MS: 261.1 (M+1), 283.0 (M+Na⁺).

5-(2,4-Dichlorobenzyl)-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**29**). Yield 31%. ¹H NMR (acetone-*d*₆) δ 1.65 (s, 3H), 1.77 (s, 3H), 3.30 (d, *J* = 14.1 Hz, 1H), 3.54 (d, *J* = 14.1 Hz, 1H), 7.26-7.35 (m, 2H), 7.46 (d, *J* = 1.8 Hz, 1H). LC/MS: 304.0 (M+1), 325.9 (M+Na⁺).

3,5-Dimethyl-4-hydroxy-5-(4-trifluoromethylbenzyl)-5*H*-thiophen-2-one (**30**). Yield 69%. ¹H NMR (CDCl₃) δ 1.58 (s, 3H), 1.72 (s, 3H), 3.18-3.26 (m, 1H), 3.37-3.47 (m, 1H), 7.27-7.36 (m, 2H), 7.49-7.61 (s, 2H). LC/MS: 303.0 (M+1), 325.0 (M+Na⁺).

5-(3,4-Dichlorobenzyl)-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**31**). Yield 39%. ¹H NMR (acetone-*d*₆) δ 1.59 (s, 3H), 1.73 (s, 3H), 3.19 (d, *J* = 13.5 Hz, 1H), 3.29 (d, *J* = 13.8 Hz, 1H), 7.23 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.44-7.46 (m, 2H). LC/MS: 304.0 (M+1), 325.9 (M+Na⁺).

(*R*)-3,5-Dimethyl-4-hydroxy-5-((*E*)-2-methylbut-1-enyl)-5*H*-thiophen-2-one (**32**): To a solution of TLM (55 mg, 0.26 mmol) in EtOH (3 mL) was added NH₂NH₂·H₂O (0.50 mL, 10 mmol) at 0 °C. To this mixture 30% H₂O₂ (aq) (1.0 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 1.5 h, and acidified with 6 N HCl (aq) at 0 °C to pH 2. The reaction mixture was diluted with H₂O, and extracted with EtOAc (3x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. Chromatographic separation on silica gel (EtOAc/hexanes = 1/9 to 1/6) gave the title compound (18 mg, 0.085 mmol, 33%) as a white solid in addition to a 5:1 (in LC/MS) mixture of **32** and **34** (9.3 mg, 0.037 mmol, 14%). [α]_D²⁰ = +63 (c 0.72, MeOH). mp 103-105 °C. ¹H NMR (CDCl₃) δ 1.00 (t, *J* = 7.5 Hz, 3H), 1.60 (d, *J* = 1.2 Hz, 3H), 1.76 (s, 3H), 1.81 (s, 3H), 2.03 (q, *J* = 7.5 Hz, 2H), 5.27 (q, *J* = 1.2 Hz, 1H), 7.56 (bs, 1H). HRMS (ESMS) calcd for C₁₁H₁₆O₂S [M + Na⁺] 235.0769, found 235.0765.

(*R*)-3,5-Dimethyl-4-hydroxy-5-(2-methylbutyl)-5*H*-thiophen-2-one (**34**): To a solution of TLM (52 mg, 0.24 mmol) in EtOH (3 mL) was added NH₂NH₂·H₂O (3.0 mL, 60 mmol) at 0 °C. To this mixture 30% H₂O₂ (aq) (6.0 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 7 h. To this reaction mixture was added again NH₂NH₂·H₂O (3.0 mL, 60 mmol) and 30% H₂O₂ (aq) (6.0 mL) at 0 °C. The reaction mixture was stirred at rt overnight, and acidified with 6 N HCl (aq) at 0 °C to pH = 2. The reaction mixture was diluted with H₂O, and extracted with EtOAc (3x). The combined organic layers were dried with anhydrous

MgSO₄, filtered, and concentrated. Chromatographic separation on silica gel (EtOAc/hexanes = 1/9 to 1/7) gave the title compound (10 mg, 0.048 mmol, 20%) as a slightly yellow oil in addition to a 1:1 (in LC/MS) mixture of **32** and **34** (12 mg, 0.028 mmol, 12%). ¹H NMR (CDCl₃) δ 0.80-0.88 (m, 4.5H), 0.95 (d, *J* = 6.6 Hz, 1.5H), 1.09-1.50 (m, 3H), 1.66 (s, 3H), 1.69-1.80 (m, 1H), 1.74 (s, 3H), 1.86-1.97 (m, 1H). HRMS (ESMS) calcd for C₁₁H₁₈O₂S [M + H⁺] 215.1106, found 215.1103.

(*R*)-3,5-Dimethyl-4-hydroxy-5-((*E*)-4-hydroxy-2-methylbut-1-enyl)-5*H*-thiophen-2-one (**35**): To a solution of TLM (503 mg, 2.39 mmol) in THF (50 mL) was added dropwise 0.5 M 9-BBN (40.0 mL, 20.0 mmol) in THF for 50 min. The reaction mixture was stirred at rt for 6 h. To this mixture 3 M NaOH (aq) (8.4 mL) and 30% H₂O₂ (aq) (4.2 mL) were added sequentially at 0 °C. The reaction mixture was stirred at rt for 10 min, concentrated. Chromatographic separation on silica gel (EtOAc/hexanes = 2/1 to MeOH/EtOAc = 1/10) gave the title compound as a colorless oil (478 mg, 2.10 mmol, 88%). [α]_D²⁰ = +70 (c 0.56, MeOH). ¹H NMR (MeOH-*d*₄) δ 1.60 (d, *J* = 0.9 Hz, 3H), 1.66 (s, 3H), 1.73 (s, 3H), 2.22 (t, *J* = 6.6 Hz, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 5.37 (q, *J* = 1.2 Hz, 1H). ¹³C NMR (MeOH-*d*₄) δ 8.0, 16.8, 30.7, 44.7, 57.1, 61.1, 108.6, 127.2, 140.1, 187.6, 198.1. Anal. (C₁₁H₁₆O₃S·0.5H₂O) C, H.

(*R*)-3,5-Dimethyl-4-hydroxy-5-(4-hydroxy-2-methylbutyl)-5*H*-thiophen-2-one (**36**): To a solution of **35** (370 mg, 1.62 mmol) in EtOH (50 mL) was added NH₂NH₂·H₂O (75 mL, 1.5 mol) at rt. To this mixture 30% H₂O₂ (aq) (130 mL) was added dropwise at 0 °C for 50 min. The reaction mixture was stirred at rt overnight, concentrated to a half, and acidified with 6 N HCl (aq) (90 mL) at 0 °C. The reaction mixture was extracted with EtOAc (3x). The combined organic layers were dried with anhydrous MgSO₄, concentrated. Chromatographic separation on silica gel (EtOAc/hexanes = 2/3 to 1/1) gave the title compound as a colorless oil (87.3 mg, 0.378 mmol, 23%) in addition to a 1:10 (in LC/MS) mixture of **35** and **36** (98.4 mg, 0.428 mmol, 26.4%). ¹H NMR (CDCl₃) δ 1.13 (d, *J* = 6.6 Hz, 3H), 1.37-2.07 (m, 4H), 1.77 (s, 3H), 1.85 (s, 3H), 2.28 (dd, *J* = 14.7, 5.1 Hz, 1H), 3.75-3.97 (m, 2H). HRMS (ESMS) calcd for C₁₁H₁₈O₃S [M + H⁺] 231.1049, found 231.1052.

(*R*)-2,4-dimethyl-2-(4-methanesulfonyloxy-2-methylbutyl)-5-oxo-2,5-dihydrothiophen-3-yl methanesulfonate (**37**): To a solution of **36** (35.3 mg, 0.153 mmol) and Et₃N (0.10 mL, 0.72 mmol) in CH₂Cl₂ (5 mL) was added MsCl (0.050 mL, 0.65 mmol) at 0 °C. The reaction mixture was stirred at rt for 3 h, acidified with 1 N HCl (aq), and extracted with EtOAc (2x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. Chromatographic separation on silica gel (EtOAc/hexanes = 3/1) gave the title compound as a colorless oil (25 mg, 0.064 mmol, 42%). ¹H NMR (CDCl₃) δ 0.98 (d, *J* = 6.3 Hz, 3H), 1.56-2.10 (m, 5H), 1.69 (s, 3H), 1.93 (s, 3H), 3.01 (s, 3H), 3.38 (s, 3H), 4.18-4.33 (m, 2H). HRMS (ESMS) calcd for C₁₃H₂₂O₇S₃ [M + Na⁺] 409.0425, found 409.0426.

(*R*)-2,4-dimethyl-2-(4-iodo-2-methylbutyl)-5-oxo-2,5-dihydrothiophen-3-yl methanesulfonate (**38**): A solution of **37** (36.9 mg, 0.0955 mmol) and NaI (79.0 mg, 0.527 mmol) in acetone (10 mL) was refluxed for 4 h. The reaction mixture was filtered, concentrated, and purified by preparative TLC (EtOAc/hexanes = 1/2) to give the title compound as a slightly yellow oil (26.2 mg, 0.0626 mmol, 65.6%). ¹H NMR (CDCl₃) δ 0.99 (d, *J* = 6.3 Hz, 3H), 1.52-1.96 (m, 5H), 1.71 (s, 3H), 1.93 (s, 3H), 3.07-3.15 (m, 1H), 3.20-3.28 (m, 1H), 3.38 (s, 3H). HRMS (ESMS) calcd for C₁₂H₁₉IO₄S₂ [M + H⁺] 418.9848, found 418.9847.

3,5-Dimethyl-4-hydroxy-5-(2-methylbut-3-enyl)-5*H*-thiophen-2-one (**33**): To a solution of **38** (20 mg, 0.048 mmol) in CH₂Cl₂ (3 mL) was added 1.0 M *t*-BuOK (1.0 mL, 1.0 mmol) in THF at rt. The reaction mixture was stirred at rt for 30 min, quenched with 1 N HCl (aq), and extracted with EtOAc (2x). The combined organic layers were dried with anhydrous MgSO₄,

filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1/2) to give the title compound as a colorless oil (5.0 mg, 0.024 mmol, 50%). ¹H NMR (CDCl₃) δ 1.00 (d, *J* = 6.6 Hz, 1.5H), 1.01 (d, *J* = 6.6 Hz, 1.5H), 1.65 (s, 1.5H), 1.66 (s, 1.5H), 1.67 (s, 1.5H), 1.74 (s, 1.5H), 1.80-2.17 (m, 2H), 2.28-2.43 (m, 1H), 4.82-5.00 (m, 2H), 5.57-5.83 (m, 1H). HRMS (ESMS) calcd for C₁₁H₁₆O₂S [M + H⁺] 213.0949, found 213.0946.

3,5-Dimethyl-4-hydroxy-5-hydroxymethyl-5H-thiophen-2-one (39): To a solution of **2** (1.0 g, 6.9 mmol) in THF (60 mL) was added 1.0 M LiHMDS (14.6 mL, 14.6 mmol) in THF at 0 °C under an argon atmosphere. The reaction mixture was stirred for 30 min at 0 °C. To this reaction mixture (CH₂O)_n (329 mg, 10.4 mmol) was added at 0 °C. The reaction mixture was stirred overnight at rt, quenched with H₂O, acidified with 6 N HCl (aq), and extracted with EtOAc (2x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 2/1) to give the title compound as a white solid (360 mg, 2.07 mmol, 30%). mp 136-138 °C. ¹H NMR (MeOH-*d*₄) δ 1.60 (s, 3H), 1.68 (s, 3H), 3.77 (s, 2H). LC/MS: 175.0 (M+1).

3,5-Dimethyl-4-methoxymethoxy-5H-thiophen-2-one (41): To a solution of **2** (5.0 g, 35 mmol) in CH₂Cl₂ (150 mL) was added DIPEA (7.3 mL, 41 mmol) at rt. To this solution MOMCl (3.2 mL, 42 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, quenched with water, and concentrated. The aqueous layer was extracted with EtOAc (2x). The combined organic layers were washed with brine, dried with anhydrous MgSO₄, filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1/2) to give the title compound as a slightly yellow oil (5.5 g, 29 mmol, 84%). ¹H NMR (CDCl₃) δ 1.59 (d, *J* = 7.2 Hz, 3H), 1.83 (d, *J* = 1.2 Hz, 3H), 3.51 (s, 3H), 4.23 (qq, *J* = 7.2, 1.2 Hz, 1H), 5.12 (d, *J* = 6.6 Hz, 1H), 5.30 (d, *J* = 6.3 Hz, 1H). ¹³C NMR (CDCl₃) δ 8.8, 20.0, 42.1, 57.2, 95.5, 117.0, 176.2, 196.1. HRMS (ESMS) calcd for C₈H₁₂O₃S [M + Li⁺] 195.0662, found 196.0669.

3,5-Dimethyl-5-hydroxymethyl-4-methoxymethoxy-5H-thiophen-2-one (42): To a solution of **41** (1.88 g, 9.99 mmol) in THF (50 mL) was added 1.0 M LiHMDS (11.2 mL, 11.2 mmol) in THF at 0 °C under an argon atmosphere. The reaction mixture was stirred for 30 min at 0 °C. To this reaction mixture (CH₂O)_n (377 mg, 11.9 mmol) was added at 0 °C. The reaction mixture was stirred for 30 min at rt, quenched with H₂O, and extracted with EtOAc (1x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 3/2) to give the title compound as a colorless oil (1.70 g, 7.79 mmol, 78%). ¹H NMR (CDCl₃) δ 1.55 (s, 3H), 1.86 (s, 3H), 2.82 (bs, 1H), 3.50 (s, 3H), 3.63-3.85 (m, 2H), 5.23 (d, *J* = 6.3 Hz, 1H), 5.29 (d, *J* = 6.3 Hz, 1H). ¹³C NMR (CDCl₃) δ 9.4, 22.0, 57.3, 59.8, 67.0, 96.2, 114.3, 175.7, 195.5. HRMS (ESMS) calcd for C₉H₁₄O₄S [M + Li⁺] 225.0768, found 225.0774. Anal. (C₉H₁₄O₄S) C, H.

2,4-Dimethyl-3-methoxymethoxy-5-oxo-2,5-dihydrothiophene-2-carbaldehyde (43): To a solution of **42** (352 mg, 1.61 mmol) in CH₂Cl₂ (10 mL) was added Dess-Martin periodinane (852 mg, 1.95 mmol) at rt. The reaction mixture was stirred for 30 min at rt, filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1/3 to 1/1) to give the title compound as a colorless oil (225 mg, 1.04 mmol, 65%). ¹H NMR (CDCl₃) δ 1.70 (s, 3H), 1.95 (s, 3H), 3.45 (s, 3H), 5.23 (dd, *J* = 2.4, 0.6 Hz, 2H), 9.02 (s, 1H). ¹³C NMR (CDCl₃) δ 9.8, 16.7, 57.4, 63.4, 96.3, 118.0, 172.1, 189.6, 193.5. HRMS (ESMS) calcd for C₉H₁₂O₄S [M + Li⁺] 223.0611, found 223.0618.

3,5-Dimethyl-4-methoxymethoxy-5-((*E*)-3-oxobut-1-enyl)-5H-thiophen-2-one (44): To a solution of LiCl (49 mg, 1.2 mmol) in CH₃CN (5 mL) was added diethyl (2-oxopropyl) phosphonate (0.23 mL, 1.2 mmol) and DIPEA (0.17 mL, 0.97 mmol) at rt. To this mixture was added a solution of **43** (209 mg, 0.966 mmol) in CH₃CN (5 mL) at rt. The reaction mixture

was stirred for 3 h at rt and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1/6) to give the title compound as a colorless oil (120 mg, 0.47 mmol, 48%) in addition to **41** (33 mg, 0.18 mmol, 18%). ¹H NMR (CDCl₃) δ 1.79 (s, 3H), 1.93 (s, 3H), 2.26 (s, 3H), 3.48 (s, 3H), 5.25 (dd, *J* = 8.7, 6.3 Hz, 2H), 6.23 (d, *J* = 15.9 Hz, 1H), 6.75 (d, *J* = 15.9 Hz, 1H). ¹³C NMR (CDCl₃) δ 9.6, 23.4, 27.7, 56.1, 57.5, 96.2, 114.0, 130.0, 146.5, 175.3, 194.2, 198.1. HRMS (ESMS) calcd for C₁₂H₁₆O₄S [M + Na⁺] 279.0662, found 279.0670.

3,5-Dimethyl-5-((*E*)-3-hydroxybut-1-enyl)-4-methoxymethoxy-5*H*-thiophen-2-one (**45**): To a solution of CeCl₃·7H₂O (212 mg, 0.569 mmol) in MeOH (5 mL) was added a solution of **44** (80.9 mg, 0.316 mmol) in MeOH (5 mL) at rt. To this mixture was added NaBH₄ (14.0 mg, 0.363 mmol) at rt. The reaction mixture was stirred for 30 min at rt, quenched with water, extracted with EtOAc (2x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1/2) to give the title compound, a mixture of diastereomers, as a colorless oil (52.4 mg, 0.203 mmol, 64%). ¹H NMR (CDCl₃) δ 1.28 (d, *J* = 6.6 Hz, 3H), 1.69 (bs, 1H), 1.76 (s, 3H), 1.91 (s, 3H), 3.51 (dd, *J* = 1.8, 0.6 Hz, 3H), 4.34 (quint, *J* = 5.4 Hz, 1H), 5.25 (m, 2H), 5.79 (m, 2H). ¹³C NMR (CDCl₃) δ 9.47, 9.48, 23.45, 23.48, 24.26, 24.32, 56.7, 57.36, 57.39, 68.2, 96.1, 113.4, 113.6, 130.8, 130.9, 135.17, 135.21, 176.6, 195.4. HRMS (ESMS) calcd for C₁₂H₁₈O₄S [M + Na⁺] 281.0818, found 281.0824.

5-((*E*)-Buta-1,3-dienyl)-3,5-dimethyl-4-methoxymethoxy-5*H*-thiophen-2-one (**46**): To a solution of **45** (46.8 mg, 0.181 mmol) in toluene (5 mL) was added the Burgess reagent (137 mg, 0.558 mmol) at rt. The reaction mixture was stirred at rt for 16 h, quenched with H₂O, and then extracted with EtOAc (2x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. The crude mixture was chromatographed by preparative TLC (EtOAc/hexanes = 1/5) to give the title compound as a colorless oil (7.0 mg, 0.029 mmol, 16%). ¹H NMR (CDCl₃) δ 1.80 (s, 3H), 1.93 (s, 3H), 3.50 (s, 3H), 5.12-5.15 (m, 1H), 5.21-5.31 (m, 1H), 5.26 (s, 2H), 5.76-5.85 (m, 1H), 6.25-6.40 (m, 2H). HRMS (ESMS) calcd for C₁₂H₁₆O₃S [M + Na⁺] 263.0712, found 263.0719.

5-((*E*)-Buta-1,3-dienyl)-3,5-dimethyl-4-hydroxy-5*H*-thiophen-2-one (**47**): To a solution of **46** (8.6 mg, 0.036 mmol) in CH₂Cl₂ (1 mL) was added NaHSO₄·SiO₂ (50 mg) at rt. The reaction mixture was stirred at rt for 3 h, filtered, and concentrated. The crude mixture was chromatographed by preparative TLC (MeOH/EtOAc = 1/7) to give the title compound as a colorless oil (5.3 mg, 0.027 mmol, 75%). ¹H NMR (CDCl₃) δ 1.59 (s, 3H), 1.63 (s, 3H), 5.00 (dd, *J* = 9.9, 2.1 Hz, 1H), 5.16 (dd, *J* = 16.2, 2.7 Hz, 1H), 5.79 (d, *J* = 14.4 Hz, 1H), 6.19-6.40 (m, 2H). HRMS (ESMS) calcd for C₁₀H₁₂O₂S [M + Li⁺] 203.0713, found 203.0720.

(*R*)-3,5-Dimethyl-4-methoxymethoxy-5-((*E*)-2-methylbuta-1,3-dienyl)-5*H*-thiophen-2-one (**48**): To a solution of TLM (80 mg, 0.38 mmol) in CH₂Cl₂ (3 mL) was added DIPEA (0.24 mL, 1.4 mmol) at rt. To this solution MOMCl (0.12 mL, 1.6 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 3 h, quenched with water, and extracted with CH₂Cl₂ (2x). The combined organic layers were dried with anhydrous MgSO₄, filtered, and concentrated. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1/2) to give the title compound as a colorless oil (92 mg, 0.36 mmol, 95%). ¹H NMR (CDCl₃) δ 1.74 (s, 3H), 1.82 (s, 3H), 1.94 (s, 3H), 3.50 (s, 3H), 5.04 (d, *J* = 10.8 Hz, 1H), 5.22 (d, *J* = 17.4 Hz, 1H), 5.28 (s, 2H), 5.63 (s, 1H), 6.29 (dd, *J* = 17.4, 10.8 Hz, 1H). ¹³C NMR (CDCl₃) δ 9.4, 12.3, 30.2, 55.2, 57.3, 96.0, 113.0, 113.5, 131.0, 139.2, 141.1, 177.5, 195.8. HRMS (ESMS) calcd for C₁₃H₁₈O₃S [M + Na⁺] 277.0869, found 277.0859.

(*R*)-3,5-Dimethyl-4-methoxymethoxy-5-((1*E*,3*E*)-2-methylpenta-1,3-dienyl)-5*H*-thiophen-2-one (**49**): To a solution of **48** (44 mg, 0.17 mmol) and Grubbs' II catalyst (9.1 mg, 6 mol%) in

CH₂Cl₂ (3 mL) was added *trans*-2-butene (105 mg) at −78 °C in a sealed tube. The reaction mixture was stirred at 45 °C for 1 day. The crude mixture was chromatographed on silica gel (EtOAc/hexanes = 1/10 to 1/4) to give the title compound as a colorless oil (31 mg, 0.12 mmol, 66%). ¹H NMR (CDCl₃) δ 1.72 (s, 3H), 1.76 (d, *J* = 6.6 Hz, 3H), 1.94 (s, 3H), 3.51 (s, 3H), 5.28 (s, 2H), 5.48 (s, 1H), 5.72 (dq, *J* = 15.9, 6.6 Hz, 1H), 6.01 (m, 1H). HRMS (ESMS) calcd for C₁₄H₂₀O₃S [M + Na⁺] 291.1025, found 291.1019.

(*R*)-3,5-Dimethyl-4-hydroxy-5-((1*E*,3*E*)-2-methylpenta-1,3-dienyl)-5*H*-thiophen-2-one (**50**): To a solution of **49** (24 mg, 0.17 mmol) in MeOH (2 mL) was added silica gel (180 mg) and polymer-bound TsOH (46 mg, 0.092 mmol) at rt. The reaction mixture was stirred at rt overnight. The reaction mixture was filtered and concentrated to give the title compound as a colorless oil (15 mg, 0.067 mmol, 75%). [α]_D²⁰ = +117 (*c* 0.59, MeOH). ¹H NMR (CDCl₃) δ 1.68 (d, *J* = 1.2 Hz, 3H), 1.69 (s, 3H), 1.77 (dd, *J* = 6.6, 1.2 Hz, 3H), 1.79 (s, 3H), 5.46 (s, 1H), 5.77 (dq, *J* = 15.3, 6.6 Hz, 1H), 6.07 (m, 1H). HRMS (ESMS) calcd for C₁₂H₁₆O₂S [M + Na⁺] 247.0763, found 247.0772.

Condensing enzyme assays

Every compound was tested against 5 condensing enzymes, FabH and FabB from *E. coli* (ecFabH and ecFabB); FabH, KasA and KasB from *M. tuberculosis* (mtFabH, KasA and KasB). The compounds were dissolved in 20% DMSO and diluted 10 fold in the reaction to the indicated concentrations. Enzymatic activity of the reactions treated with the solvent was used as controls.

ecFabH assay—The coupled assay of FabH, as described previously,⁵¹ contained 25 μM ACP, 1 mM β-mercaptoethanol, 65 μM malonyl-CoA, 45 μM [1-¹⁴C]-acetyl-CoA (specific activity 60 μCi/μmole), 1 μg purified FabD, 0.1 M sodium phosphate buffer, pH 7.0, and 10 ng FabH protein in a final volume of 40 mL. The FabD protein was present to generate the malonyl-ACP substrate for the reaction. The ACP, β-mercaptoethanol and buffer were pre-incubated at 37 °C for 30 minutes to ensure the complete reduction of ACP. The reaction was initiated by the addition of FabH. After incubation at 37 °C for 15 minutes, 35 μL of reaction mixture was removed and dispensed onto a paper filter disc (Whatman No. 3MM filter paper). The disc was washed successively with ice cold 10%, 5%, and 1% trichloroacetic acid with 20 minutes for each wash and 20 mL of wash solution per disc. The filter discs were dried and counted for ¹⁴C-isotope in 3 mL of scintillation fluid.

mtFabH Assays—The filter disc assay developed for ecFabH⁵¹ was modified to measure the activity of mtFabH with [1-¹⁴C]-lauroyl-CoA (specific activity, 55 μCi/μmol). The assays contained 50 μM ACP, 1 mM β-mercaptoethanol, 0.1 M sodium phosphate buffer pH 7.0, 50 μM malonyl-CoA, 12.5 μM [1-¹⁴C]-lauroyl-CoA, 1 μg *E. coli* FabD and 40 ng mtFabH in a final volume of 40 μL. ACP, β-mercaptoethanol and the buffer was incubated at 37 °C for 30 min to ensure complete reduction of ACP before the remaining components (except mtFabH) were added. The mixture was then aliquoted into the assay tubes, and the reaction was initiated by the addition of mtFabH. After incubation at 37 °C for 40 min, 35 μL of the reaction mix was removed and deposited on a Whatman DE-81 filter disc. The discs were washed three times (20 minutes for each wash, 20 mL wash solution for each disc) with ice-cold chloroform/methanol/acetic acid (2:5:3, v/v/v) containing 0.2 M LiCl. The filters were dried and ¹⁴C-isotope was counted in 3 mL of scintillation fluid.

ecFabB, KasA and KasB Assay—The condensation assay was essentially the same as described previously.¹⁵ Briefly, condensing enzyme assays contained 45 μM acyl-ACP (myristoyl-ACP for ecFabB, palmitoyl-ACP for KasA and KasB), 50 μM [2-¹⁴C]-malonyl-CoA (specific activity, 52 μCi/μmol), 100 μM ACP, 1 μg FabD and condensing enzyme (25

ng ecFabB, 500 ng KasA, or 500 ng KasB) in a final volume of 20 μ L. ACP was reduced by 0.3 mM DTT before the other reaction components were added. The reaction was initiated by the addition of the enzyme. After incubation at 37 °C for 15 min, the reaction was stopped by adding 0.4 mL of the reducing reagent containing 0.1 M K_2HPO_4 , 0.4 M KCl, 30% tetrahydrofuran and 5 mg/mL $NaBH_4$. The reaction contents were vigorously mixed after the addition of the reducing reagent and incubated at 37 °C for 40 minutes before being extracted into 0.4 mL toluene. The ^{14}C -isotope in the upper phase was quantitated by scintillation counting.

***M. tuberculosis* MIC Assay**

A stock culture of *M. tuberculosis* H37Rv was grown to OD 0.5 in Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween 80, 0.2% glycerol and albumin/NaCl/glucose (ADC) complex. The culture was diluted 1:1000 in 7H9-based medium before aliquoting 50 μ L into each well of a 96-well plate. The inhibitors were dissolved in DMSO to make stock solutions of 50 μ mol/mL. Inhibitors were added to the first row of wells of the 96-well plate with 100 μ L 7H9-based medium. After pipette mixing and using a multi-channel pipette, 50 μ L was removed from each well in the first row and added to the second row. 2-Fold dilution in this manner was carried out to give 8 dilutions of each inhibitor (1000 μ M-7.8 μ M). The plates were incubated for 2 weeks at 37 °C and were read macroscopically using an inverted plate reader. Each measurement was made three independent times.

***E. coli* MIC Assay**

Strain ANS1 (*metB1 relA1 spoT1 gyrA216 tolC::Tn10 λ^- λ^R F $^-$*) was defective in TolC-dependent efflux pumps and was used to determine the minimal inhibitory concentrations.¹⁵ The MICs of the test compounds against *E. coli* strain ANS1 were determined by a broth microdilution method. ANS1 was grown to mid-log phase in 1% tryptone broth and then diluted 30,000-fold in the same medium. A 10 μ L aliquot of the diluted cell suspension (3,000 to 5,000 colony forming units) was used to inoculate each well of a 96-well plate (U-bottom with low evaporation lid) containing 100 μ L of tryptone broth with the indicated concentration of inhibitors. The plate was incubated at 37 °C for 20 hours before being read with a Fusion™ Universal Microplate Analyzer (Packard, Canada) at 600 nm. The absorbance was normalized to the solvent treated control which was considered as 100%.

Crystallization and data measurement

N-terminal His-tagged FabB was expressed, purified, and crystallized as previously described.¹² For co-crystallization, 14 mg/mL of protein was incubated with 1 mM of compound **47** or **50**, and drops were set up at 18 °C over a well solution containing 1.9 M ammonium sulfate, 0.1 M HEPES pH 7.5, and 2% PEG 400. For compound **47**, X-ray data were measured with a Bruker-Nonius CCD, integrated with SAINT (Bruker AXS), and scaled using PROSCALE (Bruker AXS). For compound **50**, data were measured at the Southeastern Regional Collaborative Access Team (SER-CAT) beamline 22ID at the Advanced Photon Source, integrated using MOSFLM⁵² and scaled using SCALA.⁵³

Model building and refinement

Both **47**-FabB and **50**-FabB structures were solved by molecular replacement using AMoRe.⁵⁴ The protein model from the known TLM-FabB crystal complex (PDB code 1FJ4) was used as the search model. Iterative rounds of model building using O⁵⁵ and refinement using REFMAC⁵⁶ were performed. CNS-1.1⁵⁷ was used for the B-factor and occupancy refinements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.
5-position TLM derivatives

Table 2.
Analogues of TLM examining structural features of the isoprene side chain

Scheme 1.
Synthesis of simple 5-position analogues

Scheme 2.

Synthesis of analogues to examine importance of isoprene double bonds in TLM, **32-34**^a

^aReagents and conditions: (a) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ (39 equiv), 30% H_2O_2 (aq), EtOH, 0 °C $\rightarrow$ rt, 1.5 h; (b) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ (500 equiv), 30% H_2O_2 (aq), EtOH, 0 °C $\rightarrow$ rt, overnight; (c) i) 9-BBN, THF, rt, 6 h; ii) 3M NaOH (aq), 30% H_2O_2 (aq), 0 °C $\rightarrow$ rt, 10 min; (d) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$ (935 equiv), 30% H_2O_2 (aq), EtOH, 0 °C $\rightarrow$ rt, overnight; (e) MsCl, Et_3N , CH_2Cl_2 , rt, 3 h; (f) NaI, acetone, reflux, 4 h; (g) *t*-BuOK in THF, CH_2Cl_2 , rt, 30 min.

Scheme 3.

Syntheses of 5-alkylidene analogues and diolefinic desmethyl-TLM^a

^aReagents and conditions: (a) i) LiHMDS, THF, 0 °C; ii) paraformaldehyde, 0 °C → rt; (b) MOMCl, DIPEA, CH₂Cl₂, rt; (c) Dess-Martin periodinane, CH₂Cl₂, rt; (d) AcCH₂P(=O)(OEt)₂, DIPEA, LiCl, CH₃CN, rt; (e) NaBH₄, CeCl₃·7H₂O, MeOH, rt; (f) Burgess reagent, toluene, rt; (g) NaHSO₄·SiO₂, CH₂Cl₂, rt.

Scheme 4. Synthesis of compound **50**^aReagents and conditions: (a) MOMCl, DIPEA, CH₂Cl₂, rt; (b) Grubbs' II catalyst, *trans*-2-butene, sealed tube, CH₂Cl₂, 45 °C; (c) silica gel, polymer-bound TsOH, CH₂Cl₂, rt.

Figure 1.
(A) IC₅₀ of TLM (open circles) and racemic TLM (closed circles) at *E. coli* FabB. (B) MIC of TLM (open circles) and racemic TLM (closed circles) in *E. coli* strain ANS1. (C) IC₅₀ of TLM against *M. tuberculosis* KasA (open circles) and KasB (closed circles).

Figure 2.

Detail of TLM-FabB co-crystal highlighting placement of isoprene side chain. Left panel: TLM binds on the malonyl-ACP side of the active site and forms two strong hydrogen bonds with both active site histidines. The isoprene side chain inserts between the peptide bond of residues 271-272 and 391-392. Right panel: Space filling model of TLM in the same orientation as shown at left. The isoprene chain interacts with the peptide backbone through van der Waals interactions that leave no room for non-planar or bulky substituents at the 5-position.

Figure 3.

Compound **47**-FabB (left, PDB code 2AQB) and **50**-FabB (right, PDB code 2AQ7) co-crystal complexes. Simulated annealing omit maps are shown in red for each ligand, and the docked ligands are shown in cyan. Both compounds bind to the active site in a similar way to TLM, and this is emphasized by preserving the orientation of the active site that is shown for the TLM-FabB complex in Figure 2. The maps are contoured at 2.5σ .

Table 3
Data measurement and refinement statistics for **47-FabB** and **50-FabB** co-crystal complexes

Crystal Details	48-FabB	51-FabB
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Cell: $\alpha, \beta, \gamma=90.0^\circ$	a= 59.10, b=139.19, c=212.33	a= 59.12, b= 139.00, c= 212.46
Processing		
Wavelength (Å)	1.54169	0.97926
Resolution limit (Å)	2.2	2.3
Reflections (unique)	612,686 (90,238)	519,111 (75,836)
% complete (last shell)	99.5 (98)	96.5 (87.2)
I/ σ (last shell)	18.6 (5)	6.0 (4.6)
% R _{merge} (last shell)	7.7 (18.2)	9.2 (15.2)
Refinement		
Resolution (Å)	40-2.2	40-2.3
No. reflections	90,238	75,757
% R _{work} (% R _{free})	19.6 (22.5)	18.4 (22.8)
Rmsd	bonds 0.008 Å, angles 1.2°	bonds 0.008 Å, angles 1.2°
No. protein residues	1615 of 1624	1615 of 1624
No. other molecules	537 waters, 3 of compound 48	538 waters, 3 of compound 51
Mean B factor (Å ²)	15.5	18.2