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# Design and synthesis of novel antimicrobials with activity against Gram-positive bacteria and mycobacterial species, including *M. tuberculosis*

V. V. N. Phani Babu Tiruveedhula [a], Christopher M. Witzigmann [a], Ranjit Verma [a], M. Shahjahan Kabir [a], Marc Rott [c], William R. Schwan [c], Sara Medina-Bielski [c], Michelle Lane [c], William Close [c], Rebecca L. Polanowski [c], David Sherman [d], Aaron Monte [b], Jeffrey R. Deschamps [e], James M. Cook [a,*]

[a] Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA
[b] Department of Chemistry and Biochemistry, University of Wisconsin-La Crosse, La Crosse, WI 54601, USA
[c] Department of Microbiology, University of Wisconsin-La Crosse, La Crosse, WI 54601, USA
[d] Seattle Biomedical Research Institute and Department of Global Health, University of Washington, Seattle, WA 98109, USA
[e] Center for Bimolecular Science and Engineering, Naval Research Laboratory, Code 6930, Washington, DC 20375, USA

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ABSTRACT

The alarming increase in bacterial resistance over the last decade along with a dramatic decrease in new treatments for infections has led to problems in the healthcare industry. Tuberculosis (TB) is caused mainly by *Mycobacterium tuberculosis* which is responsible for 1.4 million deaths per year. A world-wide threat with HIV co-infected with multi and extensively drug-resistant strains of TB has emerged. In this regard, herein, novel acrylic acid ethyl ester derivatives were synthesized in simple, efficient routes and evaluated as potential agents against several *Mycobacterium* species. These were synthesized via a stereo-specific process for structure activity relationship (SAR) studies. Minimum inhibitory concentration (MIC) assays indicated that esters **12**, **13**, and **20** exhibited greater in vitro activity against *Mycobacterium smegmatis* than rifampin, one of the current, first-line anti-mycobacterial chemotherapeutic agents. Based on these studies the acrylic ester **20** has been developed as a potential lead compound which was found to have an MIC value of 0.4 μg/mL against *Mycobacterium tuberculosis*. The SAR and biological activity of this series is presented; a Michael-acceptor mechanism appears to be important for potent activity of this series of analogs.



## 1. Introduction

Surprisingly, tuberculosis (TB) is the second leading lethal infectious disease in the world, following human immunodeficiency virus (HIV). According to a recent global tuberculosis report by the World Health Organization (WHO), TB caused 1.4 million deaths in 2011 and 9 million newly infected cases are reported each year.[1] TB is a bacterial infection caused by the acid-fast bacillus *Mycobacterium tuberculosis.* TB mainly infects the lungs (pulmonary TB), although it can affect most organs in the body (extra pulmonary TB) including the liver, brain and kidney.[2] The traditional current first-line treatment of drug-sensitive TB infections consists of a four-drug regimen that includes rifampin, isoniazid, pyrazinamide, and ethambutol.[3,4] This treatment requires a minimum of 6 months to be effective.[5] Due to the extended time course of treatment many patients stop taking the medication as soon as their symptoms decrease long before the infection has been eradicated, allowing the bacteria to develop drug resistance, potentially leading to multidrug-resistant (MDR) and extensively drug-resistant (XDR) forms of TB. Treatment of these infections may extend to 18–20 months.[2] The ability to treat TB is further confounded by co-infection with HIV leading to treatment failures as well as a rise in transmission rates and mortality due to TB. Without improvements one billion people will be newly infected, there will be around 125 million people get sick, and 14 million will die in the next 10 years.[6–11] Consequently, the development of new chemotherapeutic combinations for TB that eradicate the disease quicker as well as are less complex, cheaper and have fewer side effects are essential for the future.

In our continued efforts to develop new anti-mycobacterial agents, a novel class of acrylic esters was synthesized.[12–14] In early efforts to increase the molecular diversity in this series of antimicrobial agents, certain acrylic acid ethyl esters such as **1** were synthesized.[15a] This initial lead compound exhibited a promising MIC of 16 μg/mL against *Mycobacterium smegmatis*, a safer surrogate of

* Corresponding author. Tel.: +1 (414) 229 5856; fax: +1 (414) 229 5530.
*E-mail address:* capncook@uwm.edu (J.M. Cook).

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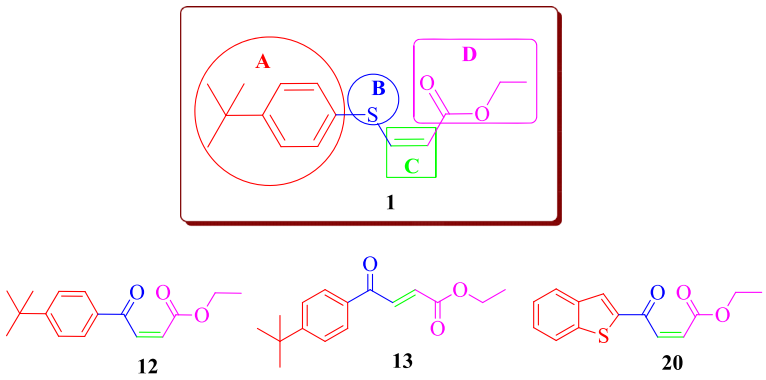



**Figure 1.** Lead compounds.

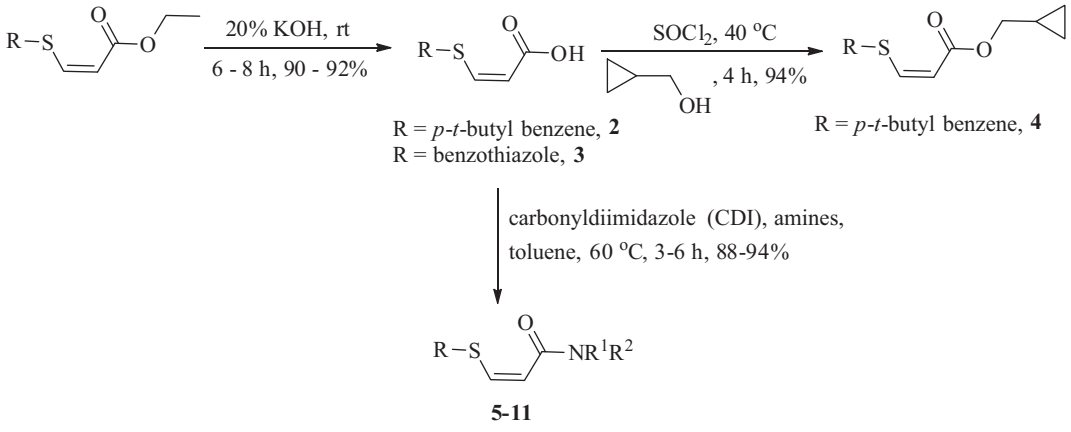
the clinically significant TB causing mycobacteria. Consequently, **1** was assayed against the more virulent strain, *M. tuberculosis*, resulting in an MIC value of 25 μg/mL.[12]

## 2. Results

The structure–activity relationship (SAR) study of lead compound **1** provided valuable information regarding the basic structural requirements for anti-mycobacterial activity. In addition, manipulations of the basic unit led to increased potency and stability.[15b] In order to evaluate the effect of structural changes on anti-mycobacterial activity, the esters of **1** at positions **A**, **B**, **C**, and **D** were altered. First, in order to increase the hydrophobic interactions of ester **1** with bacteria the ethyl ester was replaced with a methyl cyclopropyl ester to give **4** (Scheme 1) at position **D** in **1**. To increase the stability as well as the water solubility of the ester **1**, the acids **2** and **3** (Scheme 1) were prepared. This increased the hydrophilic character of the molecule and the *C*Log*P* value went from 5.7 to 4.7 in agreement with Lipinski's rules and the classic QSAR studies of Hansch.[16] Various amides (**5**–**11**) were synthesized to increase stability and to evaluate steric and electronic effects on the bioavailability and potency (Scheme 1) of **1**.

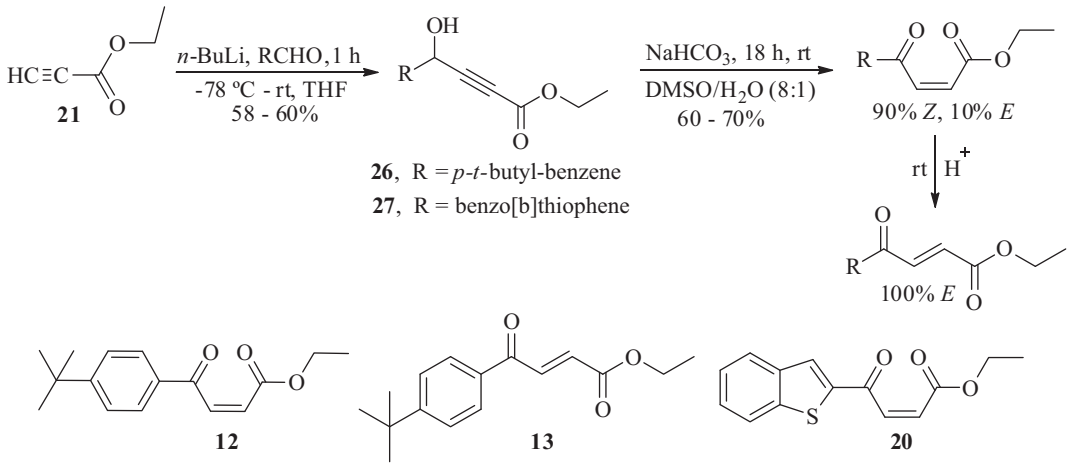
Further SAR studies on these compounds were carried out with ligands which contained similar functionality. Hence, the sulfur atom in **1** was replaced with the keto group at position **B** to furnish ketones **12** and **13** (Scheme 2).[17] This altered the electronic character of the double bond of analog **1**. These 4-oxo substituted acrylic esters exhibited increased activity against *Mycobacterium smegmatis* and *M. tuberculosis* (see Tables 1 and 2).

Presumably, the *trans* ester **13** is more stable in vivo than the *cis* ester **12**. Accordingly, a series of analogs were prepared to study the importance of the double bond in regard to the increased potency of **13**. To evaluate the importance of the electronic character of the double bond in keto ester **13**, the saturated compounds **14** and **15** (Scheme 3) were synthesized as well as **19**, **28**, and **29**, with a benzene, cyclopropyl and epoxide ring in place of the double bond, as illustrated in Scheme 4. To increase the hydrophobic character of the molecule **13**, a prenyl group was substituted for the ethyl function (see Ref. 31 for a precedent) to provide alkyl ester **17** (Scheme 3). The hydrogen bond acceptor properties of the




R = benzothiazole; $R^1 = R^2$ = isopropyl: **5**
R = *p*-*t*-butyl benzene; $R^1$ = H; $R^2$ = phenyl: **6**
R = *p*-*t*-butyl benzene; $R^1$ = methyl; $R^2$ = phenyl: **7**
R = *p*-*t*-butyl benzene; $R^1$ = H; $R^2$ = methyl: **8**
R = *p*-*t*-butyl benzene; $R^1 = R^2$ = methyl: **9**
R = *p*-*t*-butyl benzene; $R^1 = R^2$ = isopropyl: **10**
R = *p*-*t*-butyl benzene; $R^1$ = H; $R^2$ = cyclopropyl: **11**

**Scheme 1.** Synthesis of *cis* acrylic acids, amides and ester.




**Scheme 2.** Synthesis of 4-oxo substituted acrylic acid ethyl esters[a]. [a]*Z* and *E* isomers were separated by flash chromatography on silica gel.

**Table 1**
Minimum inhibitory concentrations (MIC) of acrylic acid ethyl ester analogs against common bacterial species (μg/mL)

| Compound | *M. smegmatis* | *S. aureus* ATCC 29213 | *B. cereus* | *E. coli* ATCC 29522 |
|---|---|---|---|---|
| **1** | 16 | >128 | >128 | >128 |
| **2** | >128 | >128 | 128 | >128 |
| **3** | >128 | >128 | >128 | >128 |
| **4** | 16 | >128 | 128 | >128 |
| **5** | >128 | >128 | >128 | >128 |
| **6** | >128 | >128 | >128 | >128 |
| **7** | 32 | >128 | >128 | >128 |
| **8** | >128 | >128 | 128 | >128 |
| **9** | 64 | >128 | 16 | >128 |
| **10** | 64 | >128 | >128 | >128 |
| **11** | >128 | >128 | >128 | >128 |
| **12** | **8** | **4** | **8** | **>128** |
| **13** | **8** | **2** | **4** | **>128** |
| **14** | >128 | >128 | ND[b] | >128 |
| **15** | 64 | >128 | ND[b] | >128 |
| **16** | >128 | 32 | ND[b] | >128 |
| **17** | >128 | 0.5 | ND[b] | >128 |
| **18** | >128 | 16 | ND[b] | >128 |
| **19** | >128 | >128 | ND[b] | >128 |
| **20** | **4** | **1** | ND[b] | **128** |
| **24** | >128 | >128 | >128 | >128 |
| **25** | >128 | >128 | >128 | >128 |
| **28** | 16 | 64 | ND[b] | >128 |
| **29** | 128 | 32 | ND[b] | >128 |
| Tetracycline[a] | ND[b] | 0.25 | ND[b] | 1 |
| Rifampin[a] | 64 | ND[b] | ND[b] | ND[b] |

[a] Positive control.
[b] ND = not determined.

olefin in **13** were decreased via synthesis of an α,β-unsaturated ester **18** (Scheme 4). To alter both the geometry of the molecule and the Michael acceptor properties, the alkyne **25** was synthesized (Scheme 4). It is well-known that acetylenic ketones do not undergo Michael additions as rapidly as olefinic ketones or esters.[18–21]

## 2.1. Chemistry

To study the SAR and establish the pharmacophoric unit of **1**, as mentioned earlier, the molecule was divided into four areas **A**, **B**, **C**, and **D** (Fig. 1). To alter area **D**, the two esters represented by structure **1** were saponified to provide the corresponding carboxylic acids **2** and **3** in excellent yields (91% and 92%), respectively, using an aqueous solution of 20% KOH (Scheme 1). The stereochemistry of acid **2** was assigned by $^1$H NMR. The characteristic olefinic hydrogens appeared with a value of the coupling constant of 10.1 Hz and were readily correlated with the *cis* isomer with the help of the available literature on acrylic esters and also confirmed by X-ray crystallographic analysis (Fig. 2).[15a,22,23] In the case of acid **3**, the starting moiety in area **A** had been altered from *t*-butyl benzene to benzothiazole. Due to the presence of the keto group adjacent to the double bond in keto ester **13**, a similar hydrolysis reaction was attempted with 20% KOH but resulted in the disappearance of the alkene protons. The hydrolysis conditions were modified and **16** was prepared from keto ester **13** in 90% yield using $K_2CO_3$ in refluxing aqueous methanol, as illustrated in Scheme 3. The cyclopropyl methyl ester **4** was prepared from the acid using thionyl chloride and then addition of cyclopropylmethyl alcohol in excellent yield 93% (Scheme 1).

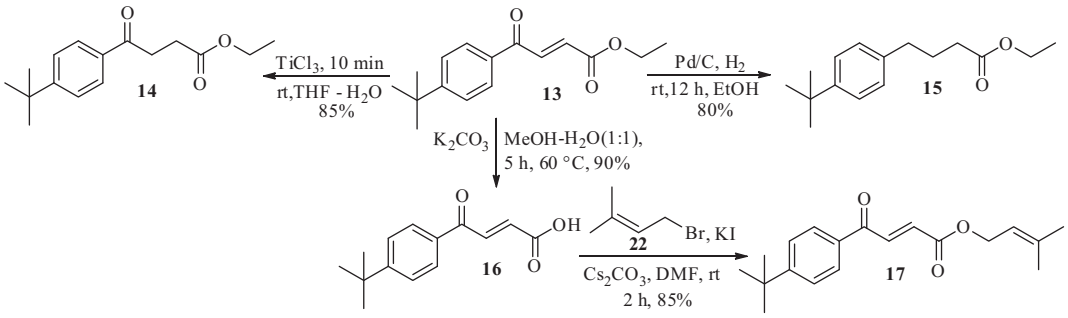
Additional alteration of the ester moiety in **1** (area **D**) was accomplished using carbonyldiimidazole (CDI) and the corresponding amines in toluene at 60 °C giving amides **5**–**11** (Scheme 1) in good to excellent yields 88–93%. The SAR of area **B** of **1** was explored by introduction of the keto group in place of the sulphur atom in **1** to furnish ketones **12** and **13** (individually). In order to do this the 4-hydroxy-2-alkynoates **26** and **27** were prepared first by the addition of *n*-butyllithium to propynoic acid ethyl ester at low temperature −78 °C and the alkynic anion, which resulted, rapidly added to the corresponding aldehydes (Scheme 2) in 58–60% yield.[24] Treatment with sodium bicarbonate as a catalyst for the required isomerization gave a mixture of *cis* and *trans* isomers **12** and **13** (9:1) which were readily separated by flash column chromatography (overall yield 70%). Treatment of the mixture of **12** and **13** with anhydrous HCl(g) in ether gave complete conversion of *cis* **12** into *trans* **13** in excellent yield. In the presence of the benzothiophene heterocyclic ring in **20**, the yield decreased to 60% (Scheme 2).[25]

Alteration of area **C** of **13** from alkene to alkane was slightly more challenging. The classic route using Pd/C in EtOH with hydrogen gas (pressure at 20 psi) furnished saturated analog **15** instead of the desired ketone **14** because the ketone was both benzylic and allylic. However, the reduction procedure of *trans* ester **13** with $TiCl_3$[26] gave ketone **14** in good yield 85% (Scheme 3). In the case of thioalkyl **24**, the standard Pd/C ($H_2$) reduction was readily executed (Scheme 4). Prenyl ester **17** was prepared by alkylation of acid **16** with prenyl bromide **22** with cesium carbonate as the base in DMF in 85% yield (Scheme 3). The synthesis of **25** was accomplished using the Dess–Martin reagent on propargylic alcohol **26** in good yield (85%, Scheme 4). The α,β-unsaturated analog **18** was synthesized by a Suzuki palladium catalyzed cross coupling reaction (Scheme 4) with the allylic bromide **23** and the

**Table 2**
Minimum inhibitory concentrations (MIC) of select compounds against additional *mycobacterial* species (μg/mL)

| Compound | *M. tuberculosis* | *M. fortuitum* | *M. kansasii* | *M. chelonae* | *M. avium* | *M. intracellulare* |
|---|---|---|---|---|---|---|
| **12** | **0.8** | **8** | **64** | **16** | **32** | **8** |
| **13** | **0.8** | **4** | **32** | **8** | **16** | **4** |
| **14** | ND[b] | >128 | >128 | >128 | >128 | >128 |
| **15** | ND[b] | 32 | 128 | 32 | >128 | >128 |
| **16** | ND[b] | >125 | ND[b] | ND[b] | ND[b] | ND[b] |
| **17** | ND[b] | 64 | >128 | 32 | >128 | >128 |
| **18** | ND[b] | >128 | >128 | >128 | >128 | >128 |
| **19** | ND[b] | >128 | >128 | >128 | >128 | >128 |
| **20** | **0.4** | **16** | **8** | **8** | **16** | **4** |
| **24** | ND[b] | >128 | >128 | >128 | >128 | >128 |
| **25** | ND[b] | >128 | >128 | >128 | >128 | >128 |
| **28** | ND[b] | 64 | ND[b] | ND[b] | ND[b] | ND[b] |
| **29** | ND[b] | >128 | ND[b] | ND[b] | ND[b] | ND[b] |
| Ethambutol[a] | 1.2 | ND[b] | ND[b] | ND[b] | ND[b] | ND[b] |
| Isoniazid[a] | 0.25 | ND[b] | ND[b] | ND[b] | ND[b] | ND[b] |
| Rifampin[a] | <0.03 | 32 | 0.5 | 32 | 2 | 1 |

[a] Positive controls.
[b] ND = not determined.




**Scheme 3.** Synthesis of acrylic acid ester derivatives.

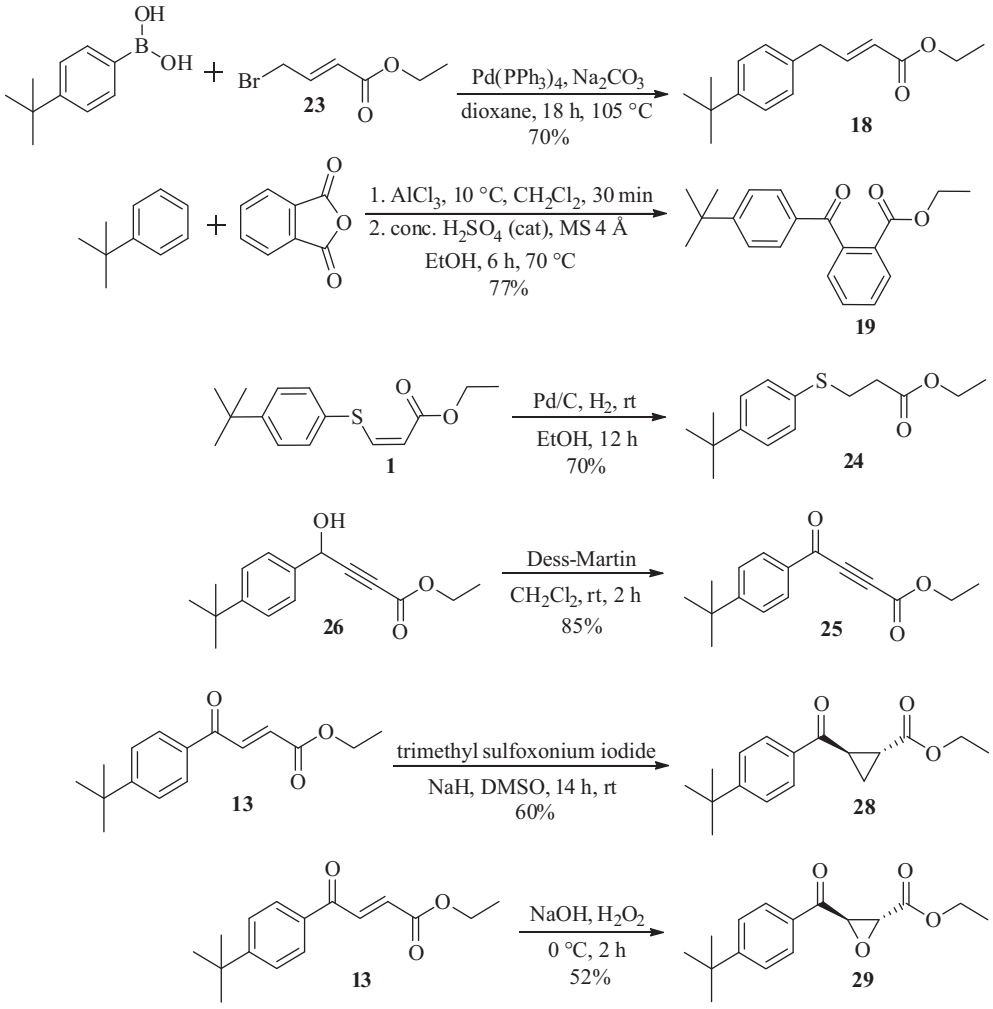
appropriate phenyl boronic acid.[27] The benzene substituted compound **19** was synthesized using a Friedel–Crafts acylation reaction between phthalic anhydride and *p*-*t*-butyl benzene in the presence of a Lewis acid ($AlCl_3$).[28] The subsequent acid was converted into the ethyl ester with EtOH in the presence of a catalytic amount of $H_2SO_4$ at 70 °C; activated molecular sieves (MS 4 Å) were used for removal of water. Cyclopropanation of **13** to **28** was achieved by the use of dimethylsulfoxonium methylide and *trans* epoxide **29** was prepared by the epoxidation of the *trans* olefin **13** with alkaline hydrogen peroxide.[29,30]

## 2.2. Biology

Structure–activity relationship (SAR) studies based on antimicrobial activity in a standard minimum inhibitory concentration (MIC) assay, indicated 42% (10 of 24) of analogs tested showed equal (3 of 24) or greater (7 of 24) potency than the positive control rifampin against *M. smegmatis* (MIC ⩽ 64 μg/mL, Table 1). Anti-mycobacterial activity of **1** was abolished by alteration of position **D** to a carboxylic acid (see MIC values for **2** and **3**). Anti-mycobacterial activity of **1** was retained when position **D** was altered to a cyclopropane (**4**), however, anti-mycobacterial activity was abolished by the larger prenyl group (**17**). However, **17** showed potent anti-staphylococcal activity (MIC = 0.5 μg/mL) which is exciting via another study. An amide in position **D** either abolished (**5**, **6**, **8**, and **11**) or decreased (**7**, **9**, and **10**) anti-mycobacterial activity.

At position **B**, replacement of the sulfur atom with a keto group (**12** and **13**) doubled the potency of **1** and extended the activity to include the Gram-positive species tested. However, replacement of the ethyl ester (**13**) with a carboxylic acid (**16**) in position **D** again destroyed anti-mycobacterial activity although some anti-staphylococcal activity was retained. In the *cis* keto compound **12**, replacement of the *p*-*t*-butyl phenyl group (**12**) with a benzo[*b*]thiophene moiety (**20**) in position **A** increased antibacterial potency for all bacteria tested except *Mycobacterium fortuitum* (Table 2). Saturation of the alkene bond in position **C** of the keto esters (**12** and **13**) abolished all antibacterial activity (see **14** and **24**). Reduction of the $C_1$ keto function (**15**) partially restored anti-mycobacterial activity. However, anti-mycobacterial activity was again abolished in the unsaturated alkene with the $C_1$ keto group fully reduced (**18**). When the alkene was replaced by either benzene (**19**) or an alkyne (**25**) activity was abolished. Whereas, the cyclopropyl analog **28** showed decreased potency (MIC = 16 μg/mL, Table 1) on *M. smegmatis* and epoxide ester **29** showed moderate anti-staphylococcal activity but no activity against mycobacterial species.

Of the compounds active against *M. smegmatis*, the three most potent (**12**, **13**, and **20**) were also active against both the Gram-positive bacteria tested (Table 1) as well as the other mycobacterial species that were tested (Table 2), including *M. tuberculosis*. In fact, the in vitro sub-μg/mL anti-mycobacterial activity of **12**, **13**, and **20** against *M. tuberculosis* indicated an increase in potency over the lead compound **1** of 32- and 64-fold, respectively. Furthermore, both **12** and **13** were 1.5-fold more active than ethambutol against *M. tuberculosis*, whereas **20** was threefold more potent than this current first line anti-tuberculosis drug.




**Scheme 4.** Synthesis of acrylic acid ethyl ester derivatives.

## 3. Discussion

The SAR studies clearly show that the structure of the most potent compounds, **12**, **13**, and **20** contain an aromatic ring in area **A** with a Michael acceptor scaffold in areas **B**, **C**, and **D**. In order to study this effect, the saturated analogs (**14**, **15**, **18**), prenyl ester **17**, benzene compound **19**, keto analog **20**, alkyne **25**, cyclopropyl ester **28** and epoxide ester **29** were prepared. Alkyne **25** failed to behave as a Michael acceptor because of the *sp* character in area **C** as compared to the $sp^2$ character in olefin **13**. It is also possible that the geometry of the molecule plays some role in activity from $sp^2$ hybridization to *sp* hybridization. To mimic the double bond nature of the active compound **13**, but limit the Michael acceptor properties, the olefin in **13** was replaced by the benzene ring in analog **19**, cyclopropyl ring in analog **28** and epoxide ring in analog **29**. The benzene analog **19** was inactive, presumably because the Michael acceptor properties were decreased because of resonance stabilization. However, the cyclopropyl ethyl ester **28** demonstrated weak activity similar to the thio ester **1** on *M. smegmatis*, whereas epoxide ester **29** showed moderate activity on *Staphylococcus aureus* ATCC29213 (MIC = 32 μg/mL, Table 1) but no activity against mycobacterial species. Saturated analogs **14**, **15**, and **18**, devoid of a keto function, were also prepared to examine the importance of the Michael acceptor scaffold (area **B**–**C**) for activity. The saturated analogs **14** and **15** were not active and loss of the ketone in olefin **18** completely eliminated activity. When the ethyl ester was transformed into the prenyl ester to give the lipophilic **17**, activity against *M. smegmatis* was completely eliminated, although **17** was nearly as potent (MIC = 0.5 μg/mL, Table 1) against *S. aureus* ATCC29213 as compared to the standard tetracycline (MIC = 0.25 μg/mL, Table 1) which is of interest in other studies.[12] It is not clear why the prenyl ester is not active since it has been previously demonstrated that hydrophobicity was important for very potent activity.[31] Since mycolic acid surrounds the mycobacterial cell, it is possible that the prenyl group of olefin **17** adheres to the mycolic acid bilayer and does not penetrate the cell. Further work to explore this result is required. The methyl cyclopropyl ester **4** was still active against *M. smegmatis* (MIC = 16 μg/mL, Table 1), but not active on other strains.

It is clear the Michael acceptor property of the active keto targets **12**, **13**, and **20** is very important. In support of this, the acrylic ester amides **5**–**11** were not active, presumably, because the Michael acceptor properties of the olefin (area **B**) were decreased. In modern medicine, many Michael acceptors are employed in the clinic including several corticosteroids, antibiotics, antiviral and anticancer drugs.[32–39] Some Michael acceptor scaffolds have been developed by accident to impart structural rigidity, for example, corticosteroids, while others require Michael acceptors due to the desired mechanism of action for some chronic diseases, such as those used in antiviral and anticancer therapies. In drug discovery, Michael acceptors are used to trap an active intermediate in the biological cycle. One important component of such an intermediate

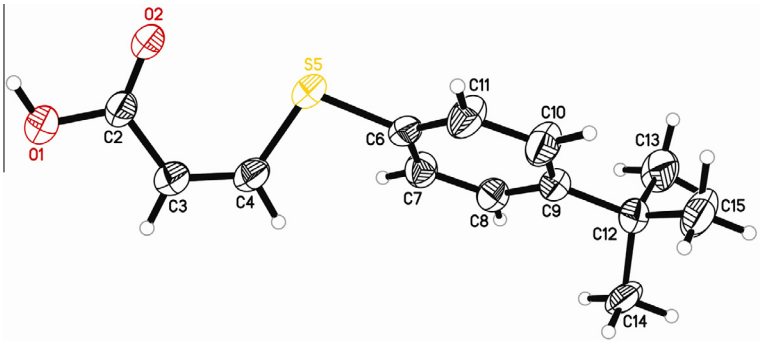



**Figure 2.** ORTEP view of the crystal structure of acrylic acid **2**.

can be a free thiol. An example of this can be found in cysteine protease inhibitors, which can be employed to help treat and prevent many diseases including emphysema, stroke, viral infections, cancer, Alzheimer's disease, inflammation and arthritis.[40–42] Acifran,[43] affinin,[44] amcinonide,[45] betamethasone,[46] dexamethasone,[47] are a few examples of Michael acceptor drugs used clinically. Rifampin is also a Michael acceptor and, as mentioned, is one of the current first line drugs in the tuberculosis treatment regimen. These results suggest that Michael acceptor acrylates **12**, **13**, and **20** could potentially be developed into viable anti-mycobacterial agents providing alternatives to current front line therapies used in the treatment of TB, MRSA, and other less common infections caused by other *Mycobacterium* species. In unpublished work, Schwan and co-workers have given mice a 300 mg/kg dose of **20** and saw no overt toxic effects, showing that such compounds may not be overtly cytotoxic in vivo. Much work must be done to follow up these results and gain a clear understanding of the mechanism of action for these extremely active compounds. It is important to note that **12**, **13**, and **20** were not active toward *Escherichia coli* or *Pseudomonas aeruginosa* indicating their mode of action is not an indiscriminate interaction with bacteria.

## 4. Conclusion

A new series of acrylic acids, including various amides, prenyl and ethyl esters were synthesized by simple, cheap and efficient synthetic routes as compared to those agents employed in first-line therapies for TB, including rifampin.[48–50] Due to their simple, unique, and novel scaffold, these analogs have been evaluated and demonstrated antimicrobial activity against a range of Gram-positive bacteria including *M. smegmatis* and the pathogenic *M. tuberculosis*. Keto analogs **12**, **13**, and **20** exhibited the most potent antimicrobial activity; keto olefins **12** and **13** demonstrated an eightfold greater activity against *M. smegmatis* than rifampin, one of the primary anti-mycobacterial agents currently used to treat TB (Table 1). Accordingly, **12** and **13** were assayed against other, more virulent mycobacteria species, including *M. tuberculosis*. Both analogs exhibited an MIC value of 0.8 μg/mL against *M. tuberculosis* (Table 2), indicating less potency than isoniazid (MIC = 0.25 μg/mL, Table 2) or rifampin (MIC = <0.03 μg/mL, Table 2) but greater potency than ethambutol (MIC = 1.2 μg/mL, Table 2), all three of which are part of the current first-line drug regimen for TB. Agents which exhibit MIC values of less than 10 μg/mL are generally considered clinically significant for further study.[51] This activity may signify a new mechanism of action for these readily available small molecules. Analog **20** is unique in that it exhibits an MIC value of 4 μg/mL against *M. smegmatis* with 16-fold greater potency than rifampin against this strain and at the same time is very potent (1 μg/mL) against *S. aureus* ATCC2913. The benzothiophene analog **20** exhibited excellent activity against *M. tuberculosis* with an MIC value of 0.4 μg/mL, a 64-fold increase in activity over lead compound **1** and threefold increase in potency over ethambutol. Although the mechanism of action of the acrylic ethyl esters **12**, **13**, and **20** is not known at this time, experiments are underway to see which biochemical pathway (if any) in the biogenesis of TB is disrupted. These simple scaffolds warrant further study to treat drug resistant antimicrobial strains including those related to *M. tuberculosis*.[52]

These small molecules are easily and inexpensively synthesized, even in multi-gram quantities, in comparison to other front-line treatments. Further SAR studies to obtain greater potency, in addition to elucidation of the mode of action of the active compounds are ongoing in our laboratories.

## 5. Experimental

### 5.1. Chemistry

All reactions were performed in oven-dried round-bottom flasks under an argon atmosphere unless the reaction conditions were supposed to contain water. Stainless steel syringes were used to transfer air-sensitive liquids. Organic solvents were purified when necessary by standard methods[53] or purchased from Sigma–Aldrich.™ All chemicals purchased from Sigma–Aldrich™ were employed as is, unless stated otherwise in regard to purification. Silica gel (Dynamic Adsorbents, 230–400 mesh) for flash chromatography was utilized to purify the analogues. The $^{1}H$ and $^{13}C$ NMR data were obtained on Bruker Spectrospin 300 MHz and GE 500 MHz instruments with chemical shifts in $\delta$ (ppm) reported relative to TMS. The HRMS and GC/MS spectral data were determined by the laboratory for mass spectrometry, University of Kansas, Lawrence, KS 66045-7582, USA. Melting points were taken on a Stuart melting point apparatus SMP3 manufactured by Barloworld Scientific US Ltd. X-ray crystallographic studies were performed at the Naval Research Laboratory, Code 6930, Washington, D. C. 20375, USA.

### 5.2. General method for the synthesis of acids 2 and 3

To the ester **1** (0.1 mmol) was added 20% aq KOH (20 mL) and the mixture was stirred at rt. The reaction progress was monitored by TLC on a silica gel plate (10% EtOAc in hexane). After 6–8 h the starting ester had disappeared on TLC and the mixture was cooled to 0 °C. The acid was precipitated from the solution by addition of cold aq 5% hydrochloric acid until the pH of the solution reached 1.5–2. The slurry which resulted was allowed to stir for 30 min and the acid was filtered off under vacuum. The acid was dissolved in a saturated aq solution of $Na_2CO_3$ (10 mL) and the aq layer was extracted twice with DCM (15 mL) to remove impurities. The aq layer was cooled to 0 °C while adjusting the pH to 1.5–2. The pure acid precipitated and was filtered and dried in the air with yields ranging from 90% to 92%.

#### 5.2.1. (*Z*)-3-(4-(*tert*-Butyl)phenyl)thio)acrylic acid (2)

The general method above was followed using ester **1** (215 mg, 0.1 mmol) which yielded 171 mg (92%) of acid **2** as a white powder. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.47–7.40 (m, 5H), 5.96–5.93 (d, $J$ = 10.1 Hz, 1H), 1.33 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 170.5, 149.1, 146.4, 132.1, 129.7, 125.4, 112.3, 40.7, 31.1. HRMS (ESI) $(M+H)^+$ calcd for $C_{13}H_{17}O_2S$ 237.0949; found 237.0942.

#### 5.2.2. (*Z*)-3-(Benzo[*d*]thiazol-2-ylthio)acrylic acid (3)

The general method above was followed using benzothiazole acrylic acid ethyl ester (266 mg, 0.1 mmol) which yielded 215 mg (91%) of acid **3** as an off white powder. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.27–8.23 (d, $J$ = 9.75 Hz, 1H), 8.12–8.09 (d, $J$ = 7.5 Hz, 1H), 7.99–7.96 (d, $J$ = 7.95 Hz, 1H), 7.56 (t, $J$ = 6.12 Hz, 1H), 7.47 (t, $J$ = 7.5 Hz, 1H), 6.30–6.27 (d, $J$ = 9.75 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 167.8, 167.6, 152.4, 140.3, 135.4, 127.3, 125.8, 122.7, 121.1, 117.6. HRMS (ESI) $(M+H)^+$ calcd for $C_{10}H_8NO_2S_2$ 237.9996; found 237.9989.

### 5.3. (*Z*)-Cyclopropylmethyl 3-((4-(*tert*-butyl)phenyl)thio)acrylate (4)

To a stirred suspension of the acid **1** (236 mg, 0.1 mmol) in DCM (10 mL) was added thionyl chloride (0.1 mL, 0.15 mmol). The reaction mixture was allowed to heat to reflux for 2 h. The reaction mixture was cooled to rt and the appropriate alcohol (0.2 mmol) was added with stirring. The reaction was again heated at reflux for 2 h. The reaction progress was monitored by TLC (silica gel). After complete conversion of the starting acid into the ester, the reaction solution was cooled to 0 °C and water (5 mL) was added slowly. The reaction mixture was stirred further for 15 min and the layers separated. The aq layer was extracted again with DCM (3 × 10 mL). The combined organic extracts were washed with brine (2 × 15 mL) and this was followed by cold water (10 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under vacuum to yield the crude ester **4**. This material was further purified by flash column chromatography (silica gel). The pure ester **4** (270 mg) was obtained in 93% yield as an oil. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.64–7.61 (m, 4H), 7.58 (d, $J$ = 10.38 Hz, 1H), 6.11 (d, $J$ = 10.38 Hz, 1H), 4.12 (d, 2H), 1.37 (s, 9H), 0.91–0.89 (m, 1H), 0.38–0.34 (m, 2H), 0.12–0.07 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 168.1, 157.7, 141.4, 132.2, 126.4, 117.9, 114.9, 54.2, 34.2, 28.3, 11.3, 2.7. HRMS (ESI) $(M+H)^+$ calcd for $C_{17}H_{23}O_2S$ 291.1419; found 291.1428.

### 5.4. General method for the synthesis of amides 5–11

Acid (**2** or **3**, 0.1 mmol) and toluene (10 mL) were suspended in a clean dry flask. The suspension was allowed to stir and warmed to 60 °C under an inert atmosphere. The CDI (0.178 g, 0.11 mmol) was then added and the mixture allowed to stir for 15 min which yielded a clear solution. The heating was discontinued and the reaction solution was allowed to cool to rt under an inert atmosphere. The appropriate amine (0.11 mmol) was dissolved in dry toluene (5 mL) and transferred to the reaction flask. After completion of the addition, the reaction mixture was stirred for 15 min at rt. The reaction mixture was then heated to 45–60 °C and this temperature was maintained for 3–6 h. The progress of the reaction was followed using TLC (silica gel). On completion by analysis of the mixture by TLC, the reaction mixture was cooled to rt and water (5 mL) was added slowly. The reaction solution was allowed to stir for 10 min and then diluted with EtOAc (10 mL). The layers were separated and the aq layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine (2 × 15 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure. Further purification was carried out by flash column chromatography (silica gel) to yield a pure amide. The yield was typically 88–94% depending on the amine.

#### 5.4.1. (*Z*)-3-(Benzo[*d*]thiazol-2-ylthio)-*N*,*N*-diisopropylacrylamide (5)

The general method above was followed using acid **3** (237 mg, 0.1 mmol) and diisopropylamine (111 mg, 0.11 mmol) yielding 285 mg (89%) of amide **5**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.51 (d, $J$ = 9.82 Hz, 1H), 8.12 (d, $J$ = 8.2 Hz, 2H), 7.38 (d, $J$ = 7.8 Hz, 2H), 6.37 (d, $J$ = 9.82 Hz, 1H), 3.93 (m, 2H), 1.27 (s, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 161.7, 156.8, 153.0, 145.3, 136.2, 125.3, 124.9, 121.6, 121.3, 116.8, 47.1, 22.3. HRMS (ESI) $(M+H)^+$ calcd for $C_{16}H_{21}N_2OS_2$ 321.1095; found 321.1088.

#### 5.4.2. (*Z*)-3-((4-(*tert*-Butyl)phenyl)thio)-*N*-phenylacrylamide (6)

The general method above was followed using acid **2** (236 mg, 0.1 mmol) and aniline (102 mg, 0.11 mmol) yielding 289.5 mg (93%) of amide **6**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.69–7.03 (m, 9H), 7.26 (d, $J$ = 10.17 Hz, 1H), 5.88 (d, $J$ = 10.17 Hz, 1H), 1.29 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 169.7, 146.8, 145.1, 134.4, 132.0, 129.6, 129.3, 129.1, 125.6, 125.2, 121.7, 121.1, 112.5, 39.9, 31.4. HRMS (ESI) $(M+H)^+$ calcd for $C_{19}H_{22}NOS$ 312.1422; found 312.1411.

#### 5.4.3. (*Z*)-3-((4-(*tert*-Butyl)phenyl)thio)-*N*-methyl-*N*-phenylacrylamide (7)

The general method above was followed using acid **2** (236 mg, 0.1 mmol) and N-methylaniline (118 mg, 0.11 mmol) yielding 296 mg (91%) of amide **7**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.61-–7.00 (m, 9H), 7.28 (d, $J$ = 10.05 Hz, 1H), 5.90 (d, $J$ = 10.05 Hz, 1H), 2.77 (s, 3H), 1.29 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 161.7, 148.1, 145.7, 134.2, 132.3, 129.9, 129.4, 129.1, 125.4, 125.1, 121.7, 121.3, 112.6, 39.2, 31.7, 30.2. HRMS (ESI) $(M+H)^+$ calcd for $C_{20}H_{24}NOS$ 326.1579; found 326.1586.

#### 5.4.4. (*Z*)-3-((4-(*tert*-Butyl)phenyl)thio)-*N*-methylacrylamide (8)

The general method above was followed using acid **2** (236 mg, 0.1 mmol) and N-methylamine (34 mg, 0.11 mmol) yielding 220 mg (88%) of amide **8**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.51–7.35 (m, 4H), 7.28 (d, $J$ = 10 Hz, 1H), 5.90 (d, $J$ = 10 Hz, 1H), 2.84 (s, 3H), 1.29 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 163.9, 151.5, 150.4, 133.7, 131.2, 125.1, 114.5, 60.2, 34.5, 27.0. HRMS (ESI) $(M+H)^+$ calcd for $C_{14}H_{20}NOS$ 250.1266; found 250.1259.

#### 5.4.5. (*Z*)-3-((4-(*tert*-Butyl)phenyl)thio)-*N*,*N*-dimethylacrylamide (9)

The general method above was followed using acid **2** (236 mg, 0.1 mmol) and dimethylamine (50 mg, 0.11 mmol) which yielded 243.5 mg (92.5%) of amide **9**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.67–7.28 (m, 4H), 7.28 (d, $J$ = 10.1 Hz, 1H), 5.90 (d, $J$ = 10.1 Hz, 1H), 2.77 (s, 6H), 1.32 (s, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 162.6, 151.2, 150.9, 133.3, 131.7, 125.0, 114.6, 60.7, 34.1, 27.6. HRMS (ESI) $(M+H)^+$ calcd for $C_{15}H_{22}NOS$ 264.1422; found 264.1429.

#### 5.4.6. (*Z*)-3-((4-(*tert*-Butyl)phenyl)thio)-*N*,*N*-diisopropylacrylamide (10)

The general method above was followed using acid **2** (236 mg, 0.1 mmol) and diisopropylamine (111 mg, 0.11 mmol) which yielded 300 mg (94%) of amide **10.** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.46–7.39 (m, 4H), $\delta$ 7.28 (d, $J$ = 10.1 Hz, 1H), 5.90 (d, $J$ = 10.1 Hz, 1H**),** 3.93 (m, 2H), 1.36 (s, 9H), 1.12 (s, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 161.2, 151.4, 150.9, 132.1, 131.8, 125.3, 114.4, 45.6, 40.7, 34.5, 31.1, 21.4. HRMS (ESI) $(M+H)^+$ calcd for $C_{19}H_{30}NOS$ 320.2048; found 320.2040.

#### 5.4.7. (*Z*)-3-((4-(*tert*-Butyl)phenyl)thio)-*N*-cyclopropylacrylamide (11)

The general method above was followed using acid **2** (236 mg, 0.1 mmol) and cyclopropylamine (63 mg, 0.11 mmol) which yielded 248 mg (90%) of amide **11**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.07 (s, 1H), 7.46–7.39 (m, 4H), 7.28 (d, $J$ = 10 Hz, 1H), 5.90 (d, $J$ = 10 Hz, 1H), 2.32 (m, 1H), 1.56 (s, 9H), 0.38–0.34 (m, 2H), 0.12–0.07 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 165.4, 146.8, 145.1, 132.0, 129.5, 129.1, 116.7, 40.6, 31.4, 24.2, 7.4. HRMS (ESI) $(M+H)^+$ calcd for $C_{16}H_{22}NOS$ 276.1422; found 276.1424.

### 5.5. General method for the preparation of propargylic alcohols 26 and 27

A round bottom flask was charged with anhydrous THF (5 mL) and propynoic acid ethyl ester **21** (100 mg, 1.02 mmol) after which it was cooled to −78 °C. Then *n*-BuLi (1.6 M of 0.8 mL, 1.22 mmol) was added dropwise. After the addition the mixture which resulted was stirred for 5 min and the appropriate aldehyde (1.02 mmol) was added slowly. The solution which resulted was stirred for 1 h at −78 °C and then allowed to warm to rt. The reaction mixture was then quenched with a saturated aq solution of $NH_4Cl$, extracted with EtOAc (2 × 10 mL) and then washed with brine. The combined organic extracts were dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude oil was purified by silica gel flash column chromatography (10–20% EtOAc in hexanes) to yield pure alcohols.

#### 5.5.1. Ethyl 4-(4-(*tert*-butyl)phenyl)-4-hydroxybut-2-ynoate (26)

The general method above was followed using *t*-butyl benzaldehyde (0.17 mL, 1.02 mmol) which yielded 169 mg (60%) of alcohol **26**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.44 (m, 4H), 5.57 (s, 1H), 4.24 (q, $J$ = 6.9 Hz, 2H), 1.33 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 153.3, 152.2, 135.6, 126.5, 125.9, 86.04, 77.2, 64.2, 62.3, 34.7, 31.3, 14.0. HRMS (ESI) $(M+Na)^+$, calcd for $C_{16}H_{20}O_3Na$ 283.1310; found 283.1309.

#### 5.5.2. Ethyl 4-(4-(benzo[*b*]thiophen-2-yl)-4-hydroxybut-2-ynoate (27)

The general method above was followed using benzothiophene-2-carboxaldehyde (165 mg, 1.02 mmol) which yielded 154 mg (58%) of alcohol **27**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.77 (m, 2H), 7.40 (s, 1H), 7.36 (m, 2H), 5.85 (d, $J$ = 5.7 Hz, 1H), 4.28 (q, $J$ = 7.2 Hz, 2H), 1.33 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 155.8, 142.4, 140.1, 139.0, 125.1, 124.7, 124.4, 124.1, 122.8, 84.2, 77.9, 62.5, 60.9, 14.0. HRMS (ESI) $(M+H)^+$, calcd for $C_{14}H_{13}O_3S$ 283.0585; found 283.0572.

### 5.6. The method for the preparation of enones 12 and 13

A round bottom flask was charged with propargylic alcohol **26** (100 mg, 0.3618 mmol), DMSO: $H_2O$ (8:1; 1.25 mL) and then a solution of 0.01 M of hydroquinone in DMSO (0.36 mL, 0.0036 mmol) was added at 23 °C. Subsequently, solid $NaHCO_3$ (6 mg, 0.0723 mmol) was added in one portion. After the addition the solution which resulted was stirred for 18 h at 23 °C. The reaction mixture was then diluted with $H_2O$ and brought to pH 3 [to obtain pH 3 the phosphate buffer which was employed was pH 7.2 phosphate buffer and an aq solution of HCl (the solution of 1 N HCl was used to reduce the pH 7.2 to pH 3)]. The solution which resulted was extracted with diethyl ether (2 × 10 mL) and the ether layer washed with brine. The combined organic extracts were dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude oil was purified by silica gel flash column chromatography (5% EtOAc in hexanes) to afford pure *cis* ester **12** (59 mg, 63%) and *trans* ester **13** (6.5 mg, 7%). *cis* Ester **12** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.89 (d, $J$ = 8.4 Hz, 2H), 7.50 (d, $J$ = 8.4 Hz, 2H), 6.89 (d, $J$ = 12.3 Hz, 1H), 6.27 (d, $J$ = 12 Hz, 1H), 4.06 (q, $J$ = 7.2 Hz, 2H), 1.35 (s, 9H), 1.08 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 189.1, 165.7, 157.8, 136.6, 134.1, 132.2, 128.9, 125.9, 61.3, 35.3, 31.3, 14.2. HRMS (ESI) $(M+Na)^+$, calcd for $C_{16}H_{20}O_3Na$ 283.1310; found 283.1334. *trans* Ester **13** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.96 (d, $J$ = 8.4 Hz, 2H), 7.93 (d, $J$ = 15.6 Hz, 1H), 7.55 (d, $J$ = 8.4 Hz, 2H), 6.90 (d, $J$ = 15.6 Hz, 1H), 4.32 (q, $J$ = 7.2 Hz, 2H), 1.38 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 189.0, 165.6, 157.8, 136.6, 134.1, 132.2, 128.9, 125.8, 61.3, 35.2, 31.0, 14.2. HRMS (ESI) $(M+Na)^+$, calcd for $C_{16}H_{20}O_3Na$ 283.1310; found 283.1348. When the mixture of **12** and **13** was stirred with anhydrous HCl(g) in ether it was completely converted into trans **13** with no formation of the corresponding acid 16.

#### 5.6.1. (*Z*)-Ethyl 4-(benzo[*b*]thiophen-2-yl)-4-oxobut-2-enoate (20)

The procedure (Section 5.6) was followed. The mixture of 4-benzo[*b*]thiophen-2-yl-4-hydroxy-but-2-ynoic acid ethyl ester **27** (157 mg, 0.6031 mmol), a 0.01 M solution of hydroquinone in DMSO (0.6 mL, 0.0060 mmol) and $NaHCO_3$ (10 mg, 0.1206 mmol) in DMSO: $H_2O$ (8:1; 2 mL) was allowed to stir. Flash column chromatography on silica gel (2% EtOAc in hexane) provided enoate **20** (94 mg, 60% yield). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.89 (m, 3H), 7.47 (m, 2H), 6.95 (d, $J$ = 12.3 Hz, 1H), 6.36 (d, $J$ = 12 Hz, 1H), 4.12 (q, $J$ = 6.9 Hz, 2H), 1.14 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 187.2, 164.8, 143.0, 142.8, 138.9, 138.3, 131.0, 127.8, 127.6, 126.2, 125.3, 123.1, 61.3, 13.8. HRMS (ESI) $(M+Na)^+$, calcd for $C_{14}H_{12}O_3SNa$ 283.0405; found 283.0432.

### 5.7. Ethyl 4-(4-(*tert*-butyl)phenyl)-4-oxobut-2-ynoate (25)

To alcohol **26** (0.5 g, 1.8 mmol) in dry $CH_2Cl_2$ (10 mL) was added the Dess–Martin periodinane reagent (0.77 g, 1.8 mmol) at rt and the reaction mixture was allowed to stir for 2 h. The volume of the reaction mixture was increased by the addition of $CH_2Cl_2$ (10 mL). An aq solution (20 mL) containing sodium thiosulfate (100 g/L) and sodium bicarbonate (100 g/L) was added and the mixture which resulted was allowed to stir for 10 min The organic phase was separated and washed with $H_2O$ (30 mL) and dried ($Na_2SO_4$). The solvent was removed in vacuo. The residue was purified by flash column chromatography on silica gel (10% ethyl acetate in hexane) to afford ketone **25** (0.39 g, 85%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.07 (d, $J$ = 8.7 Hz, 2H), 7.55 (d, $J$ = 8.4 Hz, 2H), 4.37 (q, $J$ = 7.2 Hz, 2H), 1.39 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 175.8, 159.4, 152.4, 133.2, 129.8, 125.9, 80.1, 80.0, 62.9, 35.4, 31.0, 13.9. HRMS (EI) $(M)^+$, calcd for $C_{16}H_{18}O_3$ 258.1256; found 258.1243.

### 5.8. Ethyl 4-(4-(*tert*-butyl)phenyl)-4-oxobutanoate (14)

The *trans* ester **13** (40 mg, 0.153 mmol) was dissolved in acetone (5 mL) and cold 20% $TiCl_3$ solution (0.15 ml, 0.306 mmol) was added dropwise with a syringe and the mixture was allowed to stir for 10 min at rt. The solution was then poured into brine (20 mL) and extracted with diethyl ether (2 × 10 mL). The combined extracts were dried ($Na_2SO_4$) and the solvent removed under reduced pressure. The crude oil was purified by silica gel flash column chromatography (10% EtOAc in hexanes) to afford saturated analog **14** (34 mg, 85%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.95 (d, $J$ = 8.4 Hz, 2H), 7.50 (d, $J$ = 8.4 Hz, 2H), 4.18 (q, $J$ = 7.2 Hz, 2H), 3.31 (t, $J$ = 6.9 Hz, 2H), 2.77 (t, $J$ = 6.6 Hz, 2H), 1.36 (s, 9H), 1.28 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 197.8, 173.0, 156.9, 134.0, 128.0, 125.5, 60.6, 35.1, 33.2, 31.1, 28.3, 14.2. HRMS (ESI) $(M+H)^+$, calcd for $C_{16}H_{23}O_3$ 263.1647; found 263.1631.

### 5.9. Ethyl 4-(4-(*tert*-butyl)phenyl)butanoate (15)

A Parr hydrogenation bottle (50 mL) was charged with dry Pd/C (10% by wt, 400 mg, 0.38 mmol) and the *trans* ester **13** (100 mg, 0.38 mmol) in ethanol (3 mL) was added. The mixture was degassed under reduced pressure at rt and back filled with $H_2$ (3 times) and then flushed with $H_2$ and pressurized to the desired pressure (20 psi) and stirred with $H_2$ overnight at rt. The catalyst was removed by filtration (celite) and the solid which remained was washed with ethanol ($3 \times 10$ mL). The combined organic layers were concentrated under reduced pressure to give an oil. This oil was purified by silica gel flash column chromatography (2% EtOAc in hexanes) to afford ester **15** (76 mg, 80%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.33 (d, $J$ = 8.1 Hz, 2H), 7.13 (d, $J$ = 8.1 Hz, 2H), 4.14 (q, $J$ = 7.2 Hz, 2H), 2.64 (t, $J$ = 7.5 Hz, 2H), 2.35 (t, $J$ = 7.5 Hz, 2H), 1.97 (m, 2H), 1.33 (s, 9H), 1.27 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.6, 148.7, 138.3, 128.1, 125.3, 60.2, 34.6, 34.4, 33.8, 31.4, 26.5, 14.2. HRMS (ESI) $(M+Na)^+$, calcd for $C_{16}H_{24}O_2Na$ 271.1674; found 271.1657.

### 5.10. (*E*)-4-(4-(*tert*-Butyl)phenyl)-4-oxobut-2-enoic acid (16)

To the *trans* ester **13** (105 mg, 0.4 mmol) in $CH_3OH$ (5 mL) was added $K_2CO_3$ (0.279 mg, 2 mmol) in $H_2O$ (5 mL). The reaction mixture was allowed to reflux for 5 h and then the $CH_3OH$ was removed under reduced pressure. The residue was then cooled to 0 °C and brought to pH 2 with a solution of cold aq HCl (1 M). The mixture, which resulted, was extracted with diethyl ether ($2 \times 15$ mL). The combined extracts were washed with brine (20 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure to furnish a pale green solid acid **16** (84.3 mg, 90%). Mp 118–121 °C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.03 (d, $J$ = 15.6 Hz, 1H), 7.98 (d, $J$ = 7.97 Hz, 2H), 7.56 (d, $J$ = 7.97 Hz, 2H), 6.91 (d, $J$ = 15.3 Hz, 2H), 1.38 (S, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 188.8, 170.5, 158.2, 138.6, 133.8, 131.1, 128.9, 125.9, 35.3, 31.0. HRMS (ESI) $(M+H)^+$, calcd for $C_{14}H_{17}O_3$ 233.1178; found 233.1155. This material was employed directly in the next experiment.

### 5.11. (*E*)-3-Methylbut-2-en-1-yl 4-(4-(*tert*-butyl)phenyl)-4-oxobut-2-enoate (17)

The acid **16** (70 mg, 0.3 mmol) was dissolved in anhydrous DMF (1 mL) and cesium carbonate (200 mg, 0.6 mmol) and KI (50 mg, 0.3 mmol) were added. The mixture which resulted was stirred for 5 to 10 min at rt and then a solution of 1-bromo-3-methylbut-2-ene (**22**) in DMF (0.5 mL) was added with a syringe under a positive pressure of argon. After the addition the mixture, which resulted, was stirred for 2 h at rt. The reaction mixture was then quenched with $H_2O$ and extracted with diethyl ether ($2 \times 10$ mL) as well as washed with brine ($2 \times 30$ mL). The combined organic extracts were dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude oil was purified by flash column chromatography (5% EtOAc in hexanes) on silica gel to afford prenyl ester **17** (76.5 mg, 85%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.96 (d, $J$ = 7.5 Hz, 2H), 7.93 (d, $J$ = 15.3 Hz, 1H), 7.54 (d, $J$ = 8.7 Hz, 2H), 6.90 (d, $J$ = 15.6 Hz, 1H), 5.43 (t, $J$ = 7.5 Hz, 1H), 4.76 (d, $J$ = 7.5 Hz, 2H), 1.79 (d, $J$ = 9.9 Hz, 6H), 1.37 (S, 9H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 189.1, 165.7, 157.8, 139.9, 136.6, 134.1, 132.2, 128.9, 125.9, 118.1, 62.2, 35.3, 31.0, 25.8, 18.1. HRMS (ESI) $(M+Na)^+$, calcd for $C_{19}H_{24}O_3Na$ 323.1623; found 323.1627.

### 5.12. (*E*)-Ethyl 4-(4-(*tert*-butyl)phenyl)but-2-enoate (18)

To a solution of ethyl 4-bromocrotonate **23** (0.9 mL, 5.18 mmol) in anhydrous dioxane (10 mL), palladium triphenyl phosphine tetrakis (300 mg, 0.26 mmol) was added to the round bottom flask. The flask was then evacuated three times at rt and backfilled with argon. The reaction mixture was allowed to stir for 15 min at rt then phenyl boronic acid (1.84 g, 10.36 mmol) and $Na_2CO_3$ (2.75 g, 25.9 mmol) were added under a positive pressure of argon. The reaction mixture which resulted was heated at reflux for 18 h and then cooled to rt. It was then passed through a short bed of celite. The celite bed was washed with EtOAc (50 mL) and the combined organic layers were washed with water (50 mL) and brine (30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude oil was purified by flash column chromatography (5% EtOAc in hexanes) on silica gel to afford ester **18** (893 mg, 70%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.37 (d, $J$ = 8.1 Hz, 2H), 7.14 (d, $J$ = 7.8 Hz, 2H), 7.12 (d, $J$ = 15.6 Hz, 1H), 5.85 (d, $J$ = 15.3 Hz, 1H), 4.21 (q, $J$ = 7.2 Hz, 2H), 3.52 (d, $J$ = 6.9 Hz, 2H), 1.35 (S, 9H), 1.31 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 166.5, 149.6, 147.5, 134.7, 128.5, 125.6, 122.2, 60.2, 38.0, 34.4, 31.4, 14.3. HRMS (ESI) $(M+Na)^+$, calcd for $C_{16}H_{22}O_2Na$ 269.1518; found 269.1512.

### 5.13. Ethyl 2-(4-(*tert*-butyl)benzoyl)benzoate (19)

A round bottom flask was charged with *tert*-butyl-benzene (1.04 mL, 6.7 mmol), phthalic anhydride (1 g, 6.7 mmol), anhydrous $CH_2Cl_2$ (10 mL) and cooled to 10 °C. Then $AlCl_3$ (1.8 g, 13.4 mmol) was added portionwise under a positive pressure of argon. The reaction mixture which resulted was then stirred for 15 min at 10 °C. The mixture was poured into an excess of ice-water (100 mL) and the aq phase was extracted with $CH_2Cl_2$ ($2 \times 30$ mL). It was then washed with brine (50 mL). The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to furnish an acid (1.62 g, 85%) intermediate. The acid was then dissolved in EtOH (10 mL) and a catalytic amount of $H_2SO_4$ as well as activated MS (4 Å) were added. The mixture was allowed to stir for 6 h. The EtOH was removed under reduced pressure and the residue dissolved in EtOAc (30 mL). This organic solution was washed with $H_2O$ (50 mL), brine (50 mL), dried ($Na_2SO_4$) and concentrated under reduced pressure. The crude oil was purified by silica gel flash column chromatography (10% EtOAc in hexanes) to afford the benzoate **19** (1.9 g, 90%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.06 (m, 1H), 7.71 (d, $J$ = 8.4 Hz, 2H), 7.58 (m, 2H), 7.45 (d, $J$ = 8.4 Hz, 2H), 7.38 (m, 1H), 4.10 (q, $J$ = 6.9 Hz, 2H), 1.33 (S, 9H), 1.06 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 196.7, 166.0, 156.9, 142.0, 134.6, 132.2, 130.1, 129.4, 127.7, 125.4, 61.4, 35.1, 31.1, 13.6. HRMS (ESI) $(M+H)^+$, calcd for $C_{20}H_{23}O_3$ 311.1647; found 311.1649.

### 5.14. 3-(4-*tert*-Butyl-phenylsulfanyl)-propionic acid ethyl ester (24)

The Parr hydrogenation bottle (500 mL) was charged with dry Pd/C (10% by wt, 400 mg, 0.38 mmol), and thio ester **1** (100 mg, 0.38 mmol) in ethanol (3 mL).The mixture which resulted was degassed under reduced pressure at rt and back filled with $H_2$ (3 times) and then flushed with $H_2$. It was pressurized to the desired pressure (20 psi) with $H_2$ and stirred overnight at rt. The catalyst was removed by filtration (celite) and washed with ethanol ($3 \times 10$ mL). The solvent was removed under reduced pressure. The crude compound was purified by flash column chromatography (2% EtOAc in hexanes) on silica gel to afford ester **24** (70 mg, 70%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.34 (s, 4H), 4.15 (q, $J$ = 7.2 Hz, 2H), 3.15 (t, $J$ = 7.5 Hz, 2H), 2.63 (t, $J$ = 7.5 Hz, 2H), 1.33 (s, 9H), 1.27 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 171.9, 150.0, 131.5, 130.5, 126.1, 60.7, 34.6, 34.5, 31.3, 29.5, 14.2. HRMS (ESI) $(M+Na)^+$, calcd for $C_{15}H_{22}O_2SNa$ 289.1238; found 289.1227.

### 5.15. (1*R*,2*R*)-Ethyl 2-(4-(*tert*-butyl)benzoyl)cyclopropanecarboxylate (28)

Trimethylsulfoxonium iodide (40 mg, 0.18 mmol) was added portionwise to a slurry of sodium hydride (5 mg, 0.2 mmol) in DMSO (1 mL). The mixture was stirred at rt until a completely clear solution was obtained. The ester **13** was added dropwise and the reaction mixture was then stirred for 14 h at rt. After completion, the reaction mixture was poured on crushed ice (50 g) and the oily product extracted with diethyl ether (2 × 10 mL). The combined organic extracts were washed with brine (2 × 15 mL). The organic layer was dried over $MgSO_4$ and concentrated under vacuum to yield the crude cyclopropyl ester. The crude compound was purified by flash column chromatography (5% EtOAc in hexanes) on silica gel to afford *trans* cyclopropyl ester **28** (25.2 mg, 60%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.99 (d, $J$ = 8.4 Hz, 2H), 7.53 (d, $J$ = 8.4 Hz, 2H), 4.20 (q, $J$ = 7.2 Hz, 2H), 3.20 (ddd, $J$ = 5.7, 5.7, 9.3 Hz, 1H), 2.37 (ddd, $J$ = 5.7, 5.7, 9.6 Hz, 1H), 1.57 (m, 2H), 1.37 (s, 9H), 1.31 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 196.6, 172.5, 157.2, 134.5, 128.3, 125.6, 61.1, 35.2, 31.1, 25.9, 24.5, 17.7, 14.2. HRMS (ESI) $(M+H)^+$, calcd for $C_{17}H_{23}O_3$ 275.1647; found 275.1639.

### 5.16. (2*R*,3*R*)-Ethyl 3-(4-(*tert*-butyl)benzoyl)oxirane-2-carboxylate (29)

A 6 N NaOH (0.1 mL) solution was added dropwise into a solution of 13 (100 mg, 0.38 mmol) and 30% $H_2O_2$ (0.05 mL) in EtOH (5 mL) at 0 °C. The reaction mixture which resulted, was stirred for 2 h at the same temperature after which water was added to the reaction mixture and it was extracted with $CH_2Cl_2$ (2 × 10 mL). The combined organic extracts were washed with brine (2 × 15 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum to yield the crude epoxide ethyl ester. The crude compound was purified by flash column chromatography (10% EtOAc in hexanes) on silica gel to afford epoxide ethyl ester **29** (55 mg, 52%). $^1$H NMR (300 MHz, $CDCl_3$)**:** $\delta$ 7.99 (d, $J$ = 8.4 Hz, 2H), 7.55 (d, $J$ = 8.4 Hz, 2H), 4.46 (d, $J$ = 1.8 Hz, 1H), 4.32 (m, 2H), 3.70 (d, $J$ = 1.8 Hz, 1H), 1.38 (m, 12H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 191.3, 167.31, 158.5, 132.5, 128.6, 126.0, 62.3, 55.2, 53.0, 35.3, 31.0, 14.1. HRMS (ESI) $(M+Na)^+$, calcd for $C_{16}H_{20}O_4Na$ 299.1259; found 299.1248.

### 5.17. Microbiology

#### 5.17.1. MIC determinations

In vitro minimum inhibitory concentration (MIC) determinations were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* (University of Wisconsin-La Crosse culture collection), and *Escherichia coli* ATCC 29522.[54] Tetracycline was used as a control antibiotic and correlated with established MIC values.

All anti-mycobacterial activity evaluations (except for the *M. tuberculosis* assays) were performed using MIC assays in Middlebrook 7H9 broth with 10% oleic acid albumin dextrose complex (OADC) as previously described.[12] The following mycobacterial species were tested: *Mycobacterium avium*, *Mycobacterium chelonae*, *M. fortuitum*, *Mycobacterium intracellulare and Mycobacterium kansasii.* All of the mycobacterial species that were used were from the University of Wisconsin-La Crosse culture collection. Rifampin was used as the positive control for the mycobacterial MICs. All MIC values reported were a compilation of the geometric means from three separate runs.

For *M. tuberculosis* MIC determinations, *M. tuberculosis* strain H37Rv was used. Briefly, black, clear-bottom, 384-well microtiter plates and Middlebrook 7H12 (7H9 broth supplemented with 0.1% casitone, 5.6 μg/mL palmitate, 0.5% bovine serum albumin and 4 μg/mL catalase) broth were used. The compounds were diluted in assay media to 2× the final test concentration and 25 μL of these diluted compounds were transferred to 384-well plates. Amikacin was included in the positive control wells in every assay plate. Plates containing test compounds and positive control compounds were transferred into the BSL3 facility for bacteria addition and incubation. The bacterial stock was diluted to 1-2 × $10^5$ CFU/mL in the assay medium, Middlebrook 7H12 broth and 25 μL was plated over the compounds using a Thermo Scientific Matrix WellMate, inside a Class 2A Biological Safety Cabinet. Positive and negative control wells were included in each plate. Plates were placed in stacks of two and incubated for 7 days at 37 °C with approximately 95% humidity. After 7 days of incubation, autofluorescence of any test compounds was determined by pre-reading the high dose plate by a bottom read for fluorescence using a Perkin Elmer Envision plate reader at 535 nm excitation and 590 nm emission. The assay plates were removed from the incubator and allowed to equilibrate to room temperature. Twenty-five microliters of Promega BacTiter-Glo™ Microbial Cell Viability (BTG) reagent, one third of the final volume of the well, was added using a WellMate. The plates were incubated for 20 min at room temperature, sealed with a Perkin Elmer clear Top-Seal A and read from the top using luminescence on a Perkin Elmer Envision. Rifampin, ethambutol, amikacin, isoniazid, and pyrimethamine were used as positive controls.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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.10.011.

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